Comprehensive Textbook of MEDICAL PHYSIOLOGY

Volume ONE

Highlights
- Learning Objectives
- Scientist Contributed
- Application and Clinical Box
- Important Note
- Over 1,300 schematic diagrams and graphs
- Structured presentation and flowcharts
- Chapter summary

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Comprehensive Textbook of Medical Physiology
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Vol 1

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Dedicated at the feet of
Sreema, the Divine Mother
and
Sri Aurobindo, the Divine Master

All Nature dumbly calls to her alone
To heal with her feet the aching throb of life
And break the seals on the dim soul of man
And kindle her fire in the closed heart of things.
All here shall be one day her sweetness’ home,
    All contraries prepare her harmony;
Towards her our knowledge climbs, our passion gropes;
    In her miraculous rapture we shall dwell,
    Her clasp shall turn to ecstasy our pain.
Our self shall be one self with all through her.
In her confirmed because transformed in her,
    Our life shall find in its fulfilled response
Above, the boundless hushed beatitudes,
Below, the wonder of the embrace divine.

Sri Aurobindo (in ‘SAVITRI’)
Physiology is the key subject in medicine. Starting from the knowledge of body functions, physiology provides the concept of dysfunctions, the basis of understanding the disease processes and the insight into disease management and prevention. Physiology is the core of medical wisdom. Due to its enormous contribution to the growth of medical knowledge, the Nobel Prize in health sector has been designated as Nobel Prize in Physiology and Medicine. Physiology as a subject in medical science has changed over the years from its nonclinical to preclinical and then to the current proclinical format with the incorporation of Applied and Clinical Physiology as the essential components in its core curriculum. Physiology is the foundation of medical practice. Many clinical investigations related to neurological disorders, autonomic dysfunctions, cardiovascular and respiratory diseases, endocrinal, renal, reproductive and metabolic problems are carried out in the well-equipped laboratories of physiology departments. Further, many research investigations are conducted in physiology laboratories. Sooner, the superspecialty course in Clinical Physiology will be a reality.

In India, Physiology as a subject in medical curriculum has changed immensely over decades. With the introduction of the new Medical Council of India (MCI) guidelines in 1997, the duration of first MBBS course was reduced from its original one-and-half years to one year. With subsequent modifications by MCI, directing physiology to become a more clinically oriented subject, a need aroused in reshaping the subject, integrating it with subjects of paraclinical and clinical medicine and orienting physiology knowledge for application-based learning. Therefore, in the present textbook, we have made all our sincere efforts without diluting the core concepts of physiology that includes regulation and integration of body functions, to amalgamate the knowledge in physiology with other subjects for its application in medicine.

After the publication of our Textbook and Practical Book of Physiology, the students and teachers in Physiology across the globe have been requesting to write a comprehensive book in Physiology that can offer a holistic concept of functions, integration, dysfunctions of body systems, and physiological basis of management and prevention of diseases. With all their wishes and blessings, finally this book has been made available to them. We hope this book will fulfill the aspiration of the readers in acquiring and applying the knowledge of physiology in clinics. Nevertheless, this is a project in evolution, and needs inputs, support and encouragement from our readers for its endless progression.

Gopal Krushna Pal
Pravati Pal
Nivedita Nanda

Oh India, land of light and spiritual knowledge!
Wake up to your true mission in the world,
Show the way to union and harmony.

The Mother (of Sri Aurobindo Ashram, Puducherry, India)
Acknowledgments

Let us work as we pray. For indeed work is the body’s best prayer to the Divine.

The Mother (of Sri Aurobindo Ashram, Puducherry, India)

With pride and privilege, we acknowledge the contribution of all our past teachers, especially the professors of VSS Medical College, Burla, Odisha for educating us acquire the principle and practice of clinical medicine. We also gratefully acknowledge our past physiology teachers at Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry, India for having guided us learn the essentials of physiology, and notable among them are Dr DP Thombre, Dr V Srinivasan, and Dr (Late) DB Koner.

We sincerely acknowledge the contribution of Shri Jitendar P Vij, Group Chairman, Jaypee Brothers Medical Publishers Pvt Ltd, New Delhi for personally coming to Puducherry, and motivating and inspiring us to take up this special responsibility of writing such a wonderful book. For preparing the manuscript of the present book, we are especially thankful to Ms Chetna Malhotra Vohra (Associate Director - Content Strategy) and Ms Angima Shree (Senior Development Editor) for their constant support and timely help. The contribution of Ms Angima Shree is immense and praiseworthy. We also thank the other editors and designers of the Jaypee group who helped in the preparation of this book. We acknowledge Mr Narendra Singh Shekhawat (Delhi, India), Mr Venugopal (Bengaluru, Karnataka, India), and Mr Muralidharan (Puducherry, India) of the Jaypee group for their support. We are also thankful to Jaypee Brothers Medical Publishers for providing us many of the pictures and materials of their medical publications.

We are thankful to all our colleagues and students across the globe for reading our book and providing us their inputs for its further improvement. We thank all our colleagues and residents of JIPMER for their constant inspiration and support.

Auroprajna and Auroprakash, the divine children gifted to us, have been the constant support to us in all our endeavors. We shall fail in our duty if we do not appreciate the contribution of our sister Sabita Nanda, who has been constantly taking care of all our family requirements. We always keep in high esteem our parents Dr (Late) Artatran Nanda, Smt Anupama Nanda, Sri Mrutyunjay Pal and Srimati Malatimani Pal for showering on us their love and blessings and providing us everything to come to the greater heights in our life. We take this opportunity to express our heartfelt obeisance to Ms Kumud Ben of Sri Aurobindo Ashram, who is no more physically on this earth but lives in our hearts forever.
**Special Features of the Book/How to use it Best**

All sincere prayers are granted, every call is answered. With my Blessings.

The Mother (of Sri Aurobindo Ashram, Puducherry, India)

This *Comprehensive Textbook of Medical Physiology* has the following special features. These tips are meant for the readers to best use the book.

**Learning Objectives:** The topics start with ‘Learning Objectives’. By reading the learning objectives, a student will know the gross content of the topic, and how much he should acquire from it after reading the topic. The objectives have been divided into ‘Must Know’ criteria that a student should minimum acquire, and ‘May Know’ criteria that a student is desirable to acquire. These ‘Must Know’ and ‘May Know’ criteria will help a teacher prepare the content of his lecture class and to focus more on the major criteria.

**Scientists Contributed:** Invariably, important topics start with the contributions of great scientists in the concerned field, especially those who have received the Nobel Prize and/or are popular for their contributions in that field. Often, examiners ask to name the scientists who have invented/discovered the concepts or profoundly contributed to the development of the subject. This will not only give the information of the history of medicine, but will also inspire students and teachers to take up research in physiology and medicine. This part the readers should not miss!

**Application Box:** The concepts of Physiology have lot of applications in daily life and medical practice. Therefore, a major component of physiology is ‘Applied Physiology’. These important concepts and applied aspects of the topic are depicted in the ‘Application Box’ and highlighted by green-colored boxes. If a student will miss to read these boxes, he/she will miss the core concepts in physiology.

**Clinical Physiology:** Presently, the learning in physiology is oriented to understand the etiology of the disease, and the physiological basis of management. Therefore, the major part of physiology is devoted for ‘Clinical Physiology’. The Clinical Physiology has been depicted in this book in the following formats:

- At the end of each topic, a description has been given for the common dysfunctions and disorders or diseases. A note has been given to explain the pathophysiology of the disease process and the physiological basis of the management. This is primarily to sensitize the 1st MBBS student for learning medicine, and to highlight the importance of physiology in learning medicine.

- Constructed pictures or original photos of the common diseases have been shown at the end of the topics. This is to create interest in the mind of the 1st MBBS student for clinical medicine. It also helps to understand and memorize Clinical Physiology.

- The core concepts related to diseases and patient management have been highlighted in ‘Clinical Box’. This provides the core concepts of understanding clinical medicine. The clinical boxes are highlighted with pink-colored bars.

The Clinical Physiology part is the uniqueness of this book, which is not given in any other textbook in this structured format. A student should never miss to read these clinical components in physiology.

**Important Note:** Some important and useful facts that are not covered in application or clinical boxes, are depicted as ‘Important Note’. These are useful information that may be asked in examinations, especially in viva voce.

**Structured Presentation:** Every chapter is divided into various parts by different headings and subheadings with different fonts and colors. Further, all important and complex mechanisms are structured and presented in a *point-wise description*. This structured presentation will help the student easily grasp the topic and memorize it. Further, this will ensure that a student does not miss any of the relevant points.
Flowcharts: All major concepts are simplified and summarized in ‘Flowcharts’. Not only it helps to memorize and recap the topic, but also, presenting the text along with flowcharts in examination helps the examiner easily assess the knowledge of the student. Usually, presentation with flowcharts in examination is more marks-fetching. The presentation of many flowcharts is a special feature of this book. A student must read and remember these flowcharts.

Schematic Diagrams and Graphs: All the relevant and significant mechanisms, theories and concepts are described in this book with the help of schematic diagrams and graphs. If a student is able to draw a labeled schematic diagram, it is always considered that a student has understood the topic. Especially in an examination, due to shortage of time, if a student draws a good schematic diagram and gives a brief answer with the help of flowcharts, even if he fails to give a descriptive answer, he gets good marks invariably. Therefore, the student should never miss to understand the diagrams and figures.

Tables: All important data, special concepts and lengthy information that a student needs to remember have been presented in structured tabular format. Reading the tables helps to revise and remember these facts quickly.

Histological Pictures: All mechanisms and manifestations of a disorder that require structural knowledge of a tissue or organ to comprehend the concept of the disease have been identified with appropriate histological pictures. For example, when a student sees the blood cells of an anemia in a blood smear, he understands and remembers better. A student must see these histological pictures.

Chapter Summary: All topics end with a ‘Chapter Summary’ that has been divided into two parts:
1. The first part is the ‘Key Concepts’, that depicts the central theme or the major take-home message of the topic. This is not the chapter summary, rather the summary of the main concepts.
2. The second part is the ‘Important to Know (Must Read)’ that provides all the probable long questions and short questions that usually come in theory examinations. Also, the questions that are usually asked in oral (viva) examination, are listed in this section. Students will definitely find it very useful. This will also help teachers to frame questions for the examination. A student should never miss this part.

Thus, this book is a comprehensive textbook that has incorporated all the requirements of a medical student for imparting the knowledge and skill of the subject, for acquiring all the ingredients needed to appear in the examination, and to complete the course with the best results.
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“All the world possibilities in man
Are waiting as the tree waits in its seed:
His past lives in him; it drives his future’s pace;
His present’s acts fashion his coming fate.
The unborn gods hide in his house of Life.”

Sri Aurobindo (in ‘SAVITRI’)
Homoeostasis is the state of internal harmony among the body systems. A disease is an expression of functional disharmony of the systems that usually occurs due to prolonged disturbances of any kind. Understanding the nature of disturbance is critical to comprehend the pathophysiology of a disease and the physiological basis of management of the disease. Therefore, to learn any branch of medicine, it is essential to acquire the basic knowledge of systemic physiology related to the specialty. The concept in physiology helps understand, diagnose and treat the disease. For example, for management of a case of acute myocardial infarction (AMI), the physician should know the physiology of coronary blood flow, electrophysiology of cardiac myocytes, homeostasis of myocardial perfusion, principle of regulation of coronary circulation, and the factors that disturb coronary hemodynamics to produce myocardial ischemia. All these physiology concepts help the physician to understand the mechanism of genesis and spread of pain of AMI, to appreciate the nature of radiation of myocardial ischemic pain to left upper arm, to interpret ECG abnormalities to diagnose AMI, and to prescribe appropriate drugs that improve coronary blood flow, re-establish myocardial perfusion and prevent progression of infarction. For many diseases, knowledge in physiology facilitates proper diagnosis and effective treatment. Therefore, the great Physiologist EH Starling had said “Physiology of Today is the Medicine for Tomorrow”.

Physiology is the study of body functions, their mechanisms and regulations in all living organisms. Human physiology is the study of functions of various cells, organs and organ systems of human body. It encompasses the integration and control of organ systems that help in smooth functioning of body systems as a single unit. Medical physiology is the application of knowledge of human physiology in the management of dysfunctions and diseases in human beings.

In essence, human physiology is similar to the physiology of other mammals and vertebrates. However, as human being is the highest creation in the hierarchy of nature’s evolution, the physiological processes that govern human life are more refined and developed. Nevertheless, the intricacies of human mind and vital and their profound influence on body functions have made human physiology more complex. Therefore, treatment of human diseases not only requires knowledge in physiology and medicine, but also the understanding of human behavior.

**Physiology for Learning Medicine**

Physiology provides the essential knowledge to understand the process of homeostasis, which is defined as the constancy of the internal environment of the body. Homeostasis is the state of internal harmony among the body systems. A disease is an expression of functional disharmony of the systems that usually occurs due to prolonged disturbances of any kind. Understanding the nature of disturbance is critical to comprehend the pathophysiology of a disease and the physiological basis of management of the disease. Therefore, to learn any branch of medicine, it is essential to acquire the basic knowledge of systemic physiology related to the specialty. The concept in physiology helps understand, diagnose and treat the disease. For example, for management of a case of acute myocardial infarction (AMI), the physician should know the physiology of coronary blood flow, electrophysiology of cardiac myocytes, homeostasis of myocardial perfusion, principle of regulation of coronary circulation, and the factors that disturb coronary hemodynamics to produce myocardial ischemia. All these physiology concepts help the physician to understand the mechanism of genesis and spread of pain of AMI, to appreciate the nature of radiation of myocardial ischemic pain to left upper arm, to interpret ECG abnormalities to diagnose AMI, and to prescribe appropriate drugs that improve coronary blood flow, re-establish myocardial perfusion and prevent progression of infarction. For many diseases, knowledge in physiology facilitates proper diagnosis and effective treatment. Therefore, the great Physiologist EH Starling had said “Physiology of Today is the Medicine for Tomorrow”.

**Learning Objectives**

On completion of study of this chapter, the student **must** be able to:
1. Understand the importance of physiology in learning various aspects and branches of medicine.
2. Appreciate the role of physiology in understanding the principles of medical practice.
3. Realize the value of physiology knowledge for conducting medical research.

The student **may** also be able to:
1. Learn the role of physiology in health promotion and disease prevention.
2. Comprehend that physiology knowledge facilitates integral progress of human being.
For Learning Medical Subjects

A medical student with ample knowledge in physiology can easily learn pharmacology and pathology. In principle, pharmacology is extension of physiology. For example, while learning pharmacotherapy for bronchial asthma, the student learns the use of catecholaminergic agents, especially β-receptor agonists that cause bronchodilation and provide immediate relief. It is easy for the student to understand pharmacotherapeutics of asthma as he has studied in physiology that sympathetic stimulation causes relaxation of bronchial smooth muscle by releasing norepinephrine, acting through β-receptors. Similarly, action of all of drugs has physiological basis; and therefore, a fundamental knowledge in physiology is essential for learning pharmacology.

Physiology is useful in learning pathology. For example, in physiology the student learns neutrophil as the first line of defence against acute infections and monocyte as the defence for chronic infections. Therefore, in histology slide when he finds abundant neutrophils in the microscopic field, he comprehends the infection or inflammation is of acute nature, and presence of abundant monocytes or macrophages makes him realize the infection or inflammation is of chronic nature. Similarly, understanding pathological basis of diseases requires the basic knowledge in physiology. In fact, all the diseases have a pathophysiological basis.

Likewise, the student utilizes his knowledge in physiology for learning all branches of medicine. Etiopathogenesis, clinical manifestation, diagnosis and management of all diseases have the physiological basis. Without basic concepts in physiology, a student will not be able to understand the principle of medicine. Hence, all the chapters in medicine textbooks start with an introduction to relevant clinical physiology.

In surgery textbooks, there are portions for surgical physiology. In all branches of medicine, such as gynecology, ophthalmology, otorhinolaryngology, pediatric medicine, endocrinology, oncology, gastroenterology, pulmonology, neurology, cardiology and so on, a considerable portion is devoted to clinical physiology of the topic that helps reader comprehend the physiological basis of the management.

The knowledge in physiology guides a physician to understand the principles of medical practice and the rationality of disease management. The present-day physician often tends to provide prompt symptomatic relief by prescribing a list of medicines starting from analgesics to antibiotics. This is because the modern man is in a hurry and wants immediate relief from the sufferings. There is no time, interest and knowledge for an assisted-natural remission of the disease. The physician wants to earn his money and reputation of being a good physician by providing a quick relief. But, this type of treatment does not help the person in the long run, as often medicine suppresses immunity. Although, few diseases require prompt and aggressive treatment by modern therapeutics such as antibiotics, many diseases are cured by assisted-natural remission with minimum medicines. For example, for management of hypertension in the early stage, a good physician can advise reduction in salt intake and stress level, practice of mental relaxation, regular physical exercises and adaptation to a healthy diet and lifestyle. Usually, these nonpharmacological practices are known to decrease blood pressure. Therefore, the physician should motivate his patient to practice natural measures, and should not prescribe a list of blood pressure reducing drugs that have known side effects. Medicines should be prescribed only when these natural means fail to show desirable results.

Physiology educates physicians that effective treatment of many diseases requires improvement of immunity of the subject as enhancement of immunity halts the spread of diseases and slowly discards the disease from the body. For, example, fever due to acute inflammation is a natural manifestation of the disease and part of the defense mechanisms of the body. Therefore, instead of immediately prescribing antibiotics, physician can reassure the patient that the body heals itself or at best he can prescribe mild antipyretic-analgesic. Administration of higher antibiotics and analgesics suppresses the immunity of the patient that exposes him to various infections. The doctor should explain to the patient that fever is a natural defence mechanism against infection, as rise in body
temperature kills the organisms. Moreover, prescribing antibiotics in the early part, the course and usual manifestation of the disease is missed, and therefore the diagnosis of the disease is overlooked. Another example, a simple boil (cellulitis), which heals by itself, does not warrant prescription of antibiotics. A doctor should assure the patient about the natural course of the boil and may advise him for warm fomentation on the part that improves blood flow to the region and facilitates the process of healing. Treatment by antibiotic from the beginning may halt the healing process of the boil and in some cases may produce antibioma (tumor due to antibiotics). A doctor should know that frequent use of antibiotics suppresses immune system of the body and predisposes the body to other infections. A physician with ample knowledge in physiology helps patient in facilitating the natural remission and recovery without prescribing higher medicines. Hence, a good physician is a physiologist.

For Disease Prevention and Health Promotion

Pathophysiology elucidates the physiological basis of pathology. A physician with physiology knowledge understands the measures that prevent occurrence of diseases. Presently, India is the epicenter of metabolic disorders and cardiovascular risks. A physician knows that regular physical exercises with a good eating habit retard the process of atherosclerosis and oxidative stress that prevents occurrence of cardiovascular and cerebrovascular accidents, many metabolic diseases, and retards degeneration and decay. A physiologist not only practices healthy lifestyle himself for his personal health but also encourages others to adapt to such a life. This promotes the community health. Therefore, a good physiologist is a physician.

We give emphasis on preventive and promotive aspects of health, which will be more promulgated by physiologists and will be established as preventive and promotive physiology.

Physiology is the core subject of medical research. The inventions and discoveries in medical science and advances in medicine are mainly due to research contributions from physiology. Therefore, from its inception in 1901, the Nobel Prize in the field of medical sciences has been designated as ‘Nobel Prize in Physiology’ or ‘Physiology and Medicine’. Many Nobel Prize winners in medicine starting from Emil Adolph Von Behring (1901), Sir Ronal Ross (1902), Prof. Finsen NR (1903), Prof. Ivan Petrovich Pavlov (1904) to Dr. Robert G. Edward (2010), John O’Keefe (2014) are physiologists or physicians who have worked in medical physiology or medical fields linked to physiology. Etiopathogenesis of the diseases and physiological basis of management are elucidated through research discoveries in physiology.

**Physiology for Medical Research**

Research in basic and clinical physiology has contributed to enormous growth of clinical medicine. Basic research reveals the mechanism and pathophysiology of diseases, whereas clinical research unravels the management and prevention of diseases. Usually clinicians will not have enough interest and time for research. The collaborative research between physiology and clinical departments creates avenues for clinical research, ignites research interest in clinicians, improves research outlook, attracts extramural financial support from various funding agencies and yields more productive results. The ultimate objective of a medical research should be to reduce suffering, facilitate healing, cure the ailment, prevent the occurrence of disease and promote health. Physiologists
should make efforts for larger participation of physician in research and ensure research to be more applied and clinical.

**PHYSIOLOGY FOR INTEGRAL PROGRESS**

**Physiology for Ethics**

Physiology teaches the ethics and values of life. Appropriate judgment and application of knowledge for healing the human suffering is true physiology. Ethics of medical practice was devised by Hippocrates, an ancient Greek physician. Hippocrates was a teacher at the School in the Health Temple at Cos, Greece. Writings of the group are distinguished by a high ethical tone, keen observation, logical application of existing ideas and cautious judgment. Hippocrates established the popular ethics of medical practice for physicians, which is commonly known as ‘Hippocratic Oath’ that all physicians solemnly pledge at the time of obtaining their degrees to start medical practice [Reference: Jones WHS and Withington ET. The works of Hippocrates. 4 volumes. London, Heinemann: 1923].

**Physiology for Integral Knowledge**

Physiology provides the foundation for medical practice. Understanding physiology is central to obtain and maintain perfect health. Physiology is the key subject in Medicine. Knowledge in physiology is fundamental in understanding all other subjects in medicine. Physiology is the core of medical research. Learning physiology is crucial to the integral progress.

**CHAPTER SUMMARY**

Physiology is the key subject in Medicine. Knowledge in physiology is fundamental in understanding all other subjects in medicine. Physiology provides the foundation for medical practice. Understanding physiology is central to obtain and maintain perfect health. Physiology is the basis for prevention of diseases and promotion of health. Physiology is the core of medical research. Learning physiology is crucial to the integral progress.
CHAPTER 2

Functional Organization of Human Body

LEARNING OBJECTIVES

On completion of study of this chapter, the student WILL be able to:
1. Understand the physiological organization of different systems of the body.
2. Say the general functions of each system.
3. Conceptualize the general integration of body systems.

NATURE OF ORGANIZATION

The organization of human body was studied first time systematically by Leonardo da Vinci, the great artist-philosopher-scientist of all the times.

Scientist contributed

Leonardo da Vinci
(1452–1519)

This picture of Leonardo has a very close resemblance with Sri Aurobindo and once he had commented on him as one of his past incarnation.

Leonardo left extraordinary drawings of physiological interest on body functions, muscle actions, and on heart valves, papillary muscles of ventricle and hydraulic operations in the cardiovascular systems. He had adequate knowledge on functioning of the human body. He had many paintings that are widely acknowledged and appreciated. Among them, commonly known are the paintings of Mona Lisa, The Last Supper and The Great Lady Anatomy. The painting of ‘great lady anatomy’ depicting the internal anatomical structures with details of some of the internal organs in a female (given below), is the first kind of scientific picture on the human anatomy and physiology.


Unicellular vs Multicellular Organisms

Unicellular Organisms

In unicellular organisms such as ameba, the processes that sustain life are carried out by a single cell interacting with the environment around it. Therefore, unicellular organisms have simpler organization:
1. As they are surrounded by fresh water, the O₂ and soluble nutrients enter the cell by simple diffusion, and macromolecules of nutrients are ingested by phagocytosis.
2. Metabolic waste products, including CO\(_2\) diffuse out of the cell into water, and undigested materials are removed by exocytosis.
3. The presence of contractile proteins in the cell enables it to move by pseudopodia away from noxious stimuli or towards food, and facilitates the movements in phagocytosis and exocytosis.
4. Reproduction is achieved by simple cell division, and no meeting of sexes is involved.

**Multicellular Organisms**

In multicellular organisms such as animal and man, the cells are organized into tissues (nerves, muscles, connective tissue, lymphoid tissue, epithelial tissue, blood etc.); and the tissues are organized into larger units known as organs (e.g. kidney, liver etc.). The tissues and organs are further organized into functional systems meant for performing various body functions.

**Major Physiological Systems**

The body systems can be broadly divided into:
1. The **system for supplying nutrition to the body**, which is achieved by the gastrointestinal (GI) system.
2. The **systems to support the body for locomotion and external works**. The movements are performed by the muscles and bones (the musculoskeletal system), and the nerves supplying the skeletal muscles.
3. The **systems for internal communication, integration and regulation**: The communication between the cells and organs and control of organ systems is achieved by the circulating blood, gaseous exchange at tissue level through respiration, the hormones secreted from endocrine glands, and the neurons innervating the tissues.
4. The **systems for reproduction**: Reproduction is accomplished by the reproductive organs.
5. The **systems for excretion**: Though kidney is the major organ of excretion, lower parts of GI tract (colon, rectum, and anal canal) and skin also contribute to excretion of wastes from the body.

Thus, the major body systems are:
- **Musculoskeletal system**: This system enables the body to adopt posture against gravity, and move about in the environment. This is mainly for execution of works.
- **Gastrointestinal system**: The GI system serves to ingest, digest, and absorb nutrients, water and electrolytes, and excrete undigested or unabsorbed waste matter.
- **Respiratory system**: The respiration is responsible for delivering atmospheric oxygen to the blood, and excreting CO\(_2\) produced by the cells of the body.
- **Blood**: The blood serves as transport medium for conveying nutrients and oxygen to all the cells, and for delivering waste products from tissues to the organs that excrete them (the lungs and kidneys). Blood contains cells that take part in body defence mechanisms.
- **Cardiovascular system**: The heart is the central pump that generates pressure for flow of blood in the blood vessels that serve as vehicular system for blood to flow.
- **Urinary system**: The urinary system consisting mainly of kidneys, ureter and urinary bladder excretes waste products such as urea, uric acid and creatinine, and plays a major role in maintaining the constancy of the internal milieu.
- **Endocrine system**: Hormones secreted by various endocrine glands serve mainly regulatory functions. They control metabolism, growth, development and reproductive functions.
- **Reproductive system**: Reproductive organs and genital structures ensure maintenance of progeny and the species.
- **Nervous system**: The nervous system is mainly for coordination, communication and regulation of body functions.

**CHAPTER SUMMARY**

The human body consists of various systems that are organized to perform their respective functions independently and interdependently. The major objectives of this systemic organization are to provide nutrition and energy for carrying out external works, provide support to the body for locomotion, execution and protection from environment, to control body activities, and to reproduce the offspring for continuation of the species.
Chapter 3

Principles of Homeostasis

Learning Objectives

On completion of study of this chapter, the student **MUST** be able to:

1. Define homeostasis.
2. Name the scientist who gave the concept of *milieu intérieur*, and the scientist who coined the term homeostasis.
3. Explain the feedback mechanisms of homeostatic regulation.
4. Give the examples of homeostatic regulations.
5. Understand that dysfunctions are mainly due to failure of homeostatic regulations.

The student **MAY** also be able to:

1. Describe the details of negative and positive feedback mechanisms.
2. Give a note on intracellular homeostasis.

Concept of Homeostasis

Homeostasis is **defined** as the maintenance of constancy of the internal environment of the body. In the nineteenth century, **Claude Bernard**, a French physiologist, was first to introduce the concept of *milieu intérieur*, which means the internal environment of the body. He had the following remarkable observations:

1. The volume and composition of the fluid are maintained constant, independent of the changes in the environment in which the animal lives.
2. He designated the fluid (ECF) in the body as the internal environment, to differentiate it from the external environment of the body.
3. He suggested that the ability to regulate internal environment is the major reason for humans and animals to live a normal life in spite of changes and challenges imposed on them by the external environment.
4. He noted the difference between intracellular and extracellular fluid and had proposed that the extracellular fluid is the internal environment of the body.

Extracellular Fluid as the Internal Environment

About 60% of the total body weight is water, which means a man weighing 70 kg has a total water content of about 42 liters, of which 28 liters is present inside the cells and 14 liters is present outside the cell. The electrolyte composition of the extracellular fluid roughly resembles that of seawater, which is rich in sodium and chloride, and poor in potassium. The intracellular fluid contains more potassium and less sodium-chloride.

Scientist contributed

**Claude Bernard**, a student of François Magendie (1783–1855) was a genius and great French physiologist of all time. He was the first scientist to introduce the concept of *milieu intérieur*, preservation of the internal stability despite changes in the external environment. This concept was later utilized to understand the mechanism of 'homeostasis'. He emphasized the concept of integrated interrelation between the parts of a living being. He demonstrated the glycogenic function of the liver, manifold digestive action of pancreatic juice, vasomotor mechanism (sympathetic control) for regulation of blood flow. Many important practical concepts have been derived from his studies on CO poisoning, asphyxia and anesthesia. He was the first to provide satisfactory explanation for the mechanism of action of any drug.


In 1929, an American physiologist **Walter Cannon** coined the term 'homeostasis' (*homoios* means 'like')
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Flowchart 3.1: Feedback mechanism of homeostatic regulation. Note, failure of feedback control leads to the dysfunctions or diseases.

Feedback Mechanisms of Homeostatic Regulations

Homeostatic regulation is mainly achieved through the feedback mechanisms that operate to safeguard a set point already set for the physiological variable. The stimulus for feedback control is a change in the level of the variable, which is detected by the sensor (the receptors) that activate the feedback system which in turn triggers a response to bring the variable back to the normal range of the set point and restores homeostasis (Flowchart 3.1). There are two types of feedback regulations: The negative feedback and the positive feedback.

Negative Feedback

The negative feedback mechanism is the usual mechanism of homeostatic regulation. When the variable is raised above the set point, the negative feedback mechanism triggers processes that inhibit the formation of the variable (Flowchart 3.2) and when the variable is lowered below the set point, negative feedback system withdraws the inhibition to allow production of the variable. Thus, the negative feedback system provides the physiological basis for homeostatic regulation (detailed description is given below).

Positive Feedback System

In a positive feedback system, increase in the variable triggers processes that further increase the variable. Therefore, this control mechanism does not operate to provide homeostasis. Rather, positive feedback mechanism is a vicious cycle that terminates only when the stimulus applied to trigger is withdrawn or the process itself is self-terminated. Examples of positive feedback regulation are:

1. The parturition reflex initiated by oxytocin: Towards term, when the head of the matured fetus presses on the uterine cervix, the cervical distension sends signal to posterior pituitary to release oxytocin.

Flowchart 3.2: Components of negative feedback regulation system.

HOMEOSTATIC MECHANISMS

Homeostasis is the stability of the internal environment. It is not only the equilibrium of the internal environment as a whole, but also the balance of the composition and components of the environment and the physiological variables that influence the environment. Homeostasis of the composition of internal environment, especially of the body fluids, and various other body parameters that influence the environment, is the minimum requirement for smooth functioning of the body. Abnormal deviation from these homeostatic processes leads to bodily dysfunctions. Therefore, it is fundamental to know how the homeostasis of different physiological variables is achieved and how the abnormalities in these homeostatic regulations lead to various dysfunctions.
increases the excitability of myometrium and causes uterine contraction. Contraction of uterus further pushes the fetus onto the cervix, and cervical distension further increases oxytocin release that promotes uterine contraction, and the vicious cycle continues till the baby is delivered.

2. **LH surge**: This is increased luteinizing hormone (LH) secretion that leads to ovulation. Normally, estrogen inhibits LH secretion. But, before ovulation, estrogen provides positive feedback to LH secretion, which results in LH surge.

3. **The Hodgkin’s cycle**: Sodium influx that causes upstroke (depolarization) of nerve or muscle action potential (Flowchart 3.3). Opening of few set of sodium channel provides positive feedback for opening of other sodium channels that results in massive sodium influx causing depolarization.

4. **Activation of digestive enzymes**: The activation of digestive enzymes pepsinogen and trypsinogen by pepsin and trypsin respectively.

5. **Enzyme cascade hypothesis of coagulation**: Activation of one clotting factor, which acts as an enzyme to activate the next clotting factor in the coagulation cascade.

**Negative Feedback System**

Negative feedback control system requires a sensor that detects the change in variable, a control center that receives input from the sensor and initiates command signal, and an effector that brings in responses according to the command signal directed from the control center (see Flowchart 3.2). Physiological variables sometimes require more than one homeostatic mechanism for their regulation. For example, control of arterial pressure involves pressure monitoring system, volume monitoring system, hormonal mechanisms, reflex regulation, autonomic control, etc.

The **sensor**: The sensor contains receptors that monitor the change of the variable and provide sensory signals to the control center of the changes detected. The examples are carotid sinus and aortic arch that contain baroreceptors in their wall and detect change in pressure in their lumen. They send signals to the control centers located in the medullary cardiovascular centers.

The **control center**: Usually, control centers are located in the central nervous system, especially in the brain. For example, centers for blood pressure regulation are located in medulla and the hypothalamus.

The **effector**: The effector is the target organ that carries out the command of the control center to achieve an effective response. For example, blood vessels and heart are the effector organs for blood pressure regulation. Depending on the rate of sympathetic and vagal discharges, the effector organs change their activities to achieve the target effect. When the deviation is the increase in the variable above the set point, the effector will be inhibited and inhibition continues until the variable is reduced to the normal set point. Reverse process is initiated when the variable is reduced below the set point (Application Box 3.1).

**Application Box 3.1**

**Priorities in homeostatic controls**: Life-saving regulatory mechanisms normally take precedence over those that are activated routinely. For example, regulation of body temperature on exposure to extreme weather gets priority over the control of water content of the body in natural conditions. Similarly, activation of withdrawal reflex in response to a noxious stimulus, which protects the body from injury, takes precedence over other somatic reflex and visceral reflexes.

**Kidney Plays a Vital Role**

The kidneys are important organ for the homeostasis of many physiological variables such as regulation of blood volume and blood pressure, pH balance, electrolyte composition of body fluids, and osmolarity of fluid compartments.

**Examples of Homeostatic Regulations**

Important examples of homeostatic regulations are control of pH, osmolarity, and water and electrolyte composition of the body fluids, regulation of solutes in the blood like blood sugar, balance of body weight, etc. Another example is the myotatic or stretch reflex that seeks to maintain muscle length. Details of few examples are given below:

Body temperature regulation: The temperature of the body is maintained within a narrow range of 96°F to 98.4°F. Increased temperature above normal is called as fever and below is called as hypothermia. When temperature is above normal, the body activates controlling mechanisms to increase heat loss through cutaneous vasodilation.
and sweating. When hypothermia develops, the body responds by decreasing heat loss through vasoconstriction and by increasing heat production through shivering.

**Blood pressure regulation:** The normal systolic pressure in adult is kept constant between 100 and 140 mm Hg and diastolic pressure between 60 and 85 mm Hg. Sustained rise in pressure is called hypertension, and fall in pressure is called hypotension that initiates many neural and humoral mechanisms to restore blood pressure (for details refer “Regulation of blood pressure”).

**Regulation of hormone secretion:** Secretion of many hormones such as thyroxine, cortisol, etc. is regulated mainly by negative feedback mechanisms. Increase in hormone concentration in plasma inhibits their production and decrease in concentration facilitates their production mainly by altering the secretion of their regulating trophic hormones. This forms the basis of diagnosis of these hormonal disorders.

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**CHAPTER SUMMARY**

Homeostasis is **defined** as the maintenance of the constancy of the internal environment of the body. Homeostatic regulation is achieved by two feedback mechanisms: The negative feedback and the positive feedback.

**Negative Feedback**

The negative feedback mechanism is the general mechanism of regulations in which if the variable is raised above the set point, the feedback mechanism triggers processes that inhibit the formation of the variable and if the variable is lowered below the set point, the feedback system withdraws the inhibition to allow production of the variable. Regulation of hormone secretion is the common examples.

**Positive Feedback**

In a positive feedback system, increase in the variable triggers the processes that further increase the variable. This process triggers a vicious cycle that terminates only when the stimulus applied to trigger is withdrawn or the process itself is self-terminated. Important examples of positive feedback regulation are parturition reflex initiated by oxytocin, LH surge during ovulation, The Hodgkin's cycle of sodium influx during depolarization of action potential, and enzyme activation in coagulation cascade.

**Important to Know (Must Read)**

1. Usually no Long Questions are asked from this chapter.
2. Homeostasis, Feedback mechanisms, Negative feedback system, may be the Short Questions.
3. In Viva, examiner may ask… Define homeostasis, Give examples of homeostasis, and What are the feedback mechanisms and give their examples.

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**INTRACELLULAR HOMEOSTASIS**

Though, traditionally homeostasis (constancy of internal environment) refers to the stability of extracellular fluid volume and composition, it is also applicable to intracellular fluid and intracellular conditions. Rather, the primary objective of homeostasis is to maintain intracellular homeostasis that promotes cell or tissue (organ) functions. Homeostasis is greatly affected by intracellular activities. For example, pH homeostasis, temperature homeostasis, Na⁺ homeostasis, K⁺ homeostasis, etc. depend mainly on intracellular enzyme activities and cell metabolism. Similarly, intracellular milieu is largely influenced by changes in the ECF volume and composition. Hence, homeostasis is mostly achieved by the coordinated and balanced interaction between intracellular and extracellular environments of the body.
CHAPTER 4

Cellular Organization and Intercellular Connections

Learning Objectives

On completion of study of this chapter, the student MUST be able to:

1. Give the structure and list the functions of cell membrane, organelles and nucleus.
2. Name various cytoskeletons and molecular motors of cell and give their functions.
3. Classify intercellular junctions and give their functions.
4. Describe the importance of gap junction in health and disease.
5. Classify cell adhesion molecules and give their functions.

The student MAY also be able to:

1. Give the details of composition of cell membrane and function of each constituent.
2. Describe the details of the structure, functions and dysfunctions of each organelle of the cell.
3. Explain the detailed structure, functions and dysfunctions of microfilaments, cellular motors and cell adhesion molecules.
4. Give the structure, functions and dysfunctions of intercellular junctions.

Cellular Organization

The cell is the structural and functional unit of tissues. A cell consists of three fundamental structures: cell membrane, cytoplasm and nucleus (Fig. 4.1).

1. The cell membrane or the plasma membrane is the boundary in all animal cells that surrounds the cytoplasm, the fluid medium containing a variety of organelles.

2. In cytoplasm, organelles are bound by membranes similar to the structure of cell membrane. The organelles usually present in animal cells are mitochondria, ribosome, peroxisome, lysosomes, centrioles, endoplasmic reticulum and Golgi apparatus. Cytoplasm also contains filamentous cytoskeletal structures such as microfilaments, intermediate filaments and microtubules.

3. The nucleus is present at the center of the cell. In addition, there are many other proteins in the cells like actin and myosin that provide strength and mobility to the cell and also the mechanisms for adhesion to other cells.

Structure of Cell Membrane

The cell membrane is basically a double layer of lipid molecules having thickness of 7–10 nm, into which are inserted...
or attached various protein molecules (Fig. 4.2). Lipids constitute about 45% of the dry weight of the membrane and protein constitutes about 50% and carbohydrate constitutes 5%.

**Fluid-Mosaic Model of the Membrane**

Though, many models for cell membrane have been described in the past, the widely accepted one is *Fluid-Mosaic Model* described by Singer and Nicolson in 1972.

**Special features** of this model are:

2. The model is called fluid mosaic model as the membrane lipids are present in the fluid form that allows the flexibility of the membrane without disturbing the structural integrity.
3. The membrane proteins are loosely attached and float in the fluid phospholipid bilayer. Rapid and random redistribution of integral proteins occurs in the membrane.
4. Also, phospholipids undergo rapid redistribution in the plane of the membrane.
5. This type of diffusion within the plane of the membrane is called **translational diffusion**. This occurs rapidly for phospholipids, which can move several micrometers per second.
6. The fluidity of the membrane is mainly dependent on the lipid composition of the membrane.

**Effect of temperature**: In a lipid bilayer, the hydrophobic chains of fatty acids are highly aligned or arranged orderly to provide a rather stiff structure. When temperature increases, the hydrophobic side chains undergo a transition from its ordered gel or crystalline state to a more disordered liquid state. The temperature at which this disorder or melting occurs is called **transition temperature** (Tm). When the fatty acid chains are longer and more saturated, they interact more strongly with each other and cause higher values of Tm. As a result, higher temperature is required to increase the fluidity of the lipid bilayer having long chain and saturated fatty acids. Thus, **degree of unsaturation** determines the fluidity of the membrane.

**Lipid Bilayer**

The major lipids in the cell membrane are phospholipids, glycolipids and cholesterol.

1. The **phospholipids** are phosphatidylcholine, sphingomyelin, phosphatidyserine and phosphatidyl ethanolamine.
2. **Glycolipids** are generally found in the outer layer.

Lipids are **amphipathic** ("amphi" means both) molecules as their head or polar region is hydrophilic and tail or nonpolar region is hydrophobic (Fig. 4.3). The globular or the head end contains phosphate or hydroxyl moieties that are positively charged and soluble in water. The tail end contains two chains of fatty acids that are insoluble in water. The arrangement is such that the hydrophobic tail ends are directed toward the center and the hydrophilic head is located to periphery of the membrane (as depicted in Figure 4.2). Thus, hydrophilic head of lipid molecules faces the aqueous phase from both inside and outside the cell. A similar bilayer arrangement is found in bile salts where they form spherical micelles.

3. **Cholesterol** is incorporated into the hydrophobic regions of the membrane, and serves to reinforce the lipid permeability barrier.

**Application Box 4.1**

**Determinant of the fluidity of membrane**: The fluidity of a membrane depends on the composition of lipids and the degree of unsaturation. The major determinant is its **cholesterol-phospholipid ratio**. In eukaryotes, the ratio is about 1:1. Higher cholesterol content reduces the fluidity of the membrane.
Chapter 4: Cellular Organization and Intercellular Connections

The individual phospholipid molecules can move freely within the specific layer (but not from one layer to another). Hence, the cell membrane is said to be fluid in nature (Application Box 4.1).

**Functions of the Lipid Bilayer**

The main function of the lipid bilayer of a cell membrane is to create a permeability barrier between the interstitial fluid and the cytoplasm. The permeability of a substance depends on whether it is lipid-soluble or water-soluble. Lipid soluble substances like oxygen and alcohol can pass easily through the cell membrane, whereas water soluble substances like urea and glucose cannot pass easily. Thus, lipid bilayer makes the membrane semipermeable.

**Membrane Proteins**

The protein content of biological membrane depends on the function of the membrane. For example, in the Schwann cell, the cell membrane is concerned with insulating the nerve axon, which is mainly the function of lipids. Therefore, protein constitutes less than 25 percent of the membrane. In membrane of mitochondria, which is involved in cell metabolism, 75 percent of the membrane is protein. However, average protein content of membrane is 50–60% of the membrane mass.

Membrane proteins are of two types: Integral proteins and peripheral proteins (Fig. 4.4).

**Integral Proteins**

Some membrane proteins that span the entire thickness of the membrane are known as integral proteins. They are also called transmembrane proteins. Some integral proteins penetrate only a portion of the membrane (Fig. 4.4), so that they are exposed either to the external environment of the cell (interstitial fluid) or to the cytoplasmic surface of the membrane (intracellular fluid). Some membrane proteins also move laterally within the membrane (e.g. membrane receptors can move to sites of endocytosis).

The **functions of integral membrane proteins** are as follows:

1. **Serve as channel proteins**: Channels or pores are integral proteins through which water-soluble substances can diffuse across the cell membrane.
2. **Act as carriers**: Carrier proteins transport substances across the cell membrane by facilitated diffusion; for example, transport of glucose through glucose transporter.
3. **Serve as ion pumps**: Membrane proteins serve as pumps for active transport of ions across the membrane; for example, Na⁺–K⁺ ATPase that pumps K⁺ into the cell and Na⁺ out of the cell against their concentration gradient.
4. **Serve as receptor and enzyme proteins**: Integral proteins that are present toward the outer half of the membrane usually serve as receptors and usually those on the inner half serve as enzymes. Membrane G proteins belong to this category of proteins.
5. **Antigenic functions**: Complex membrane proteins such as glycoproteins act as antigens on the surface of the cell; for example, blood group antigens in the membrane of red cells.

**Peripheral Proteins**

Some protein molecules are inserted lightly in the outer or inner border of the membrane or are just bound to the surface of the membrane. Such proteins are called peripheral proteins.

They are of two types: Intrinsic and extrinsic proteins. **Intrinsic proteins**: They are present on the inner surface of the membrane. They usually serve as enzymes or anchor proteins for cytoskeleton and other microfilaments that maintain cell shape.

**Extrinsic proteins**: They are present on the outer surface of the membrane. They serve as cell adhesion molecules for anchoring cells with basal lamina and with neighboring cells. They can be removed without disrupting the membrane.

**Membrane Carbohydrates**

The external surface of the cell membrane is loosely covered by a carbohydrate layer known as the cell coat or glyocalyx. These carbohydrates are usually oligosaccharides that are covalently linked to membrane proteins forming glycoproteins or lipids forming glycolipids. Some are also polysaccharide chains of proteoglycans, the integral membrane protein. Thus, the outer surface of the lipid bilayer is covered by a layer of glycoproteins and glycolipids.

**Functions of the Glyocalyx**

1. Glyocalyx serves as a protective coat. Carbohydrate is negatively charged and therefore prevents negative particles like protein molecules to interact between cells.
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2. Some of the transmembrane glycoproteins like selectins recognize and bind with specific oligosaccharides on other cell membranes, and therefore, permit temporary cell-to-cell adhesion. Such temporary adhesion occurs between neutrophils and endothelial cells at the site of inflammation. Stronger adhesion between cells is formed by integral membrane proteins such as integrins.

3. Some carbohydrate molecules serve as receptors.

Functions of Cell Membrane

1. Cell membrane maintains a constant and distinctive intracellular environment, which is essential for functioning of the organelles. For example, the intracellular fluid has lower concentration of sodium and chloride, low pH, but higher concentration of potassium, organic phosphates and magnesium.

2. Cell membrane maintains cell volume by actively transferring ions across it, especially by pumping sodium ions out of the cell.

3. In neurons and muscle cells, it maintains a potential difference between intracellular and extracellular surfaces, which enables cells to respond to various stimuli.

4. Cell membrane helps in recognizing foreign cells or antigens so that they can be destroyed by phagocytes.

Special Features of RBC Membrane

The red cell membrane is most extensively studied membrane. In addition to the general features of membrane described above, red cell membrane contains some special proteins such as integral and peripheral proteins.

Integral Proteins

Two special types of integral proteins are found in red cell membrane (Fig. 4.5). These are glycophorins and band-3 proteins.

Glycophorins: Glycophorins are glycoproteins that contain 60% carbohydrate and 40% protein. The carbohydrate component is oligosaccharide and the polypeptide component contains 131 amino acids. Oligosaccharides of glycophorins serve as antigen for MN blood group.

Band-3 proteins: Band-3 protein is a dimeric protein with molecular weight 93,000 that traverses membrane about 12 times. It acts as “pore” or transport protein that exchanges bicarbonate ions in the capillaries of lungs.

Peripheral Proteins

The inner surface of red cell membrane contains two special proteins that are linked to the cytoskeleton and are essential for stabilization of membrane and biconcave shape of the cell. These are spectrin and ankyrin.

Spectrin: Spectrin is a fibrous protein that contains an α-chain with molecular weight of 240,000 and a β-chain with molecular weight of 220,000. Along with ankyrin it is attached to cytoskeletal protein that maintains membrane integrity and cell shape.

Ankyrin: The cytoskeletal proteins are attached to spectrin through ankyrin. Ankyrin has molecular weight of 200,000. It has two domains: one bind with spectrin and other with N-terminal region of band-3 protein that extends into cytoskeleton (Application Box 4.2).

Application Box 4.2

Diseases due to membrane protein defects: Disorders of red cells such as hereditary spherocytosis and elliptocytosis occur due to defects in the membrane proteins (for details, refer “Red Blood Cell”).

Cell Organelles

The usually occurring organelles in animal cells are mitochondria, endoplasmic reticulum, Golgi apparatus, ribosome, peroxisome, lysosome and centriole. The red blood cell is the only living cell, which loses nucleus and most of its organelles such as mitochondria, ribosome and lysosome during maturation.

Mitochondria

Mitochondria are the “power house” of the cell. They are cigar-shaped organelles whose shape, size and number vary in different tissues of the body. They are most abundant in cells that have high rate of metabolism, as in liver, cardiac muscle, etc.

Structure

Mitochondria consist of two important components: Membranes and cristae.

Mitochondrial Membranes

Electron microscopy shows that each mitochondrion has two layers of membranes: the outer and inner membranes.

Outer Mitochondrial Membrane: This forms a continuous envelop of the organelle. Outer membrane consists mostly of phospholipids and cholesterol, and contains a specific membrane protein that forms “porin”. Porins are channels that permit substances with molecular weight of less than 10,000 to diffuse freely across the outer membrane.
**Inner Mitochondrial Membrane:** It is rich in proteins, and the ratio of lipid to protein is 0.27 to 1. Hence, it is virtually impermeable to polar and ionic substances. The inner membrane is folded into multiple incomplete septa like structures called **cristae** (Fig. 4.6), which is rich in many enzymes like cytochromes b, C, a and a3, NADH dehydrogenase, succinate dehydrogenase, electron transferring flavoproteins, carnitine-palmitoyl transferase, etc.

**Mitochondrial Matrix**

The region enclosed by the inner membrane is called **matrix**. An amorphous material fills the matrix, which contains enzymes involved in the Kreb’s citric acid cycle and fatty acid oxidation (Table 4.1). Matrix also contains several strands of DNA, ribosomes and enzymes for synthesis of proteins coded in the mitochondrial genome.

**Functions**

The inner membrane contains the cytochromes of the electron transport system and the associated enzymes for oxidative phosphorylation. The TCA cycle takes place in the matrix. The initial breakdown of proteins, carbohydrates and fats occurs in the cytoplasm of the cell, and the final end product that enters the mitochondria as acetyl-CoA combines with oxaloacetate to form citric acid. The end products of the citric acid cycle (Kreb’s cycle) are CO₂, H₂O and ATP.

Like most components of the cell, mitochondria have a short lifespan and are constantly renewed. As they have strands of DNA they are capable of self-replication as well as protein synthesis (Clinical Box 4.1).

**Clinical Box 4.1**

**Mitochondrial diseases:** The disease that affects mitochondrial energy transduction is called **Leuf’t’s disease.** The abnormality of mitochondrial DNA leads to cellular dysfunction called as **mitochondrial cytopathy syndrome,** which manifests with muscle weakness, degenerative lesions of the brain and high levels of lactic acid in blood. Mitochondria are also damaged by free radicals and affected in age-related degenerations.

---

**Table 4.1: Mitochondrial enzymes.**

<table>
<thead>
<tr>
<th><strong>A. Membrane enzymes</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Cytochrome b₅ and b₅ reductase</td>
</tr>
<tr>
<td>2. Fatty acid CoA synthase</td>
</tr>
<tr>
<td>3. Phospholipase A</td>
</tr>
<tr>
<td>4. Nucleoside diphosphokinase</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>II. Enzymes of inner membrane</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Cytochrome b, C, a and a₃</td>
</tr>
<tr>
<td>2. NADPH dehydrogenase</td>
</tr>
<tr>
<td>3. Succinate dehydrogenase</td>
</tr>
<tr>
<td>4. Electron transferring flavoproteins</td>
</tr>
<tr>
<td>5. β-OH-butyrate dehydrogenase</td>
</tr>
<tr>
<td>6. Carnitine palmitoyltransferase</td>
</tr>
<tr>
<td>7. All translocases</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>B. Enzymes in inter-membrane space</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Adenylate kinase</td>
</tr>
<tr>
<td>2. Nucleoside diphosphokinase</td>
</tr>
<tr>
<td>3. Sulphite oxidase</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>C. Enzymes in the matrix</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Pyruvate dehydrogenase complex</td>
</tr>
<tr>
<td>2. Citrate synthase</td>
</tr>
<tr>
<td>3. Isocitrate dehydrogenase</td>
</tr>
<tr>
<td>4. Malate dehydrogenase</td>
</tr>
<tr>
<td>5. Fatty acid oxidation system</td>
</tr>
<tr>
<td>6. Ornithine transcarbamylase</td>
</tr>
<tr>
<td>7. α-oxoglutarate dehydrogenase</td>
</tr>
<tr>
<td>8. Aconitase</td>
</tr>
</tbody>
</table>

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**Endoplasmic Reticulum**

The endoplasmic reticulum (ER) consists of a network of anastomosing membranous **tubules, vesicles,** and flattened **cisternae.** The membranes of ER are continuous with the outer membrane of nucleus and are also connected with Golgi apparatus (Fig. 4.7). ER is the site of the synthesis of proteins and lipids for the membrane of the cell and organelles, and secretory vesicles of the cytoplasm. They are of two types: rough ER and the smooth ER.

**Rough Endoplasmic Reticulum**

When surface of ER is studded with ribosome, the organelle is called rough ER (RER) or **granular ER** as it gives a “rough” or “granular” appearance to it. In some cells such as red blood cells, the ribosomes lie free in the cytoplasm. RER are present in more number in cells that are actively involved in protein synthesis like acinar cells of pancreas and neurons. In neurons, the **Nissel granules** are modified RER.

**Functions of RER**

1. It is concerned with **protein synthesis.** Therefore, it is abundant in cells of endocrine glands and cells secreting digestive enzymes.
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2. It also plays some role in the conjugation of carbohydrates with proteins to form glycoproteins, a function which it shares with the Golgi apparatus.

Smooth Endoplasmic Reticulum

When ribosomes are not attached to the surface of ER, the organelle is called smooth ER (SER) or agranular ER as its surface has “smooth” or “agranular” appearance.

Functions of SER

1. It is concerned with the synthesis of lipids. Hence it is abundant in cells that synthesize cholesterol, steroid hormones and phospholipids.
2. In muscles, it is called sarcoplasmic reticulum, which is specialized for the storage of calcium ions that is released during excitation-contraction coupling.
3. SER is part of intracellular transport system as it is continuous with the RER and Golgi apparatus.
4. It is also the site for the detoxification or neutralization of hormones and toxic substances.

Golgi Apparatus

The Golgi apparatus (GA) is also known as Golgi complex or dictyosome. It is present in all cells and generally is located close to the nucleus. It appears as flat discs that are dilated peripherally and stacked together in a bunch as stack of cisternae (Fig. 4.8). Anatomically and functionally it is closely associated with endoplasmic reticulum. Functionally GA two main regions: cis and trans.

The region nearer to the nucleus is the cis face (cis Golgi) and the region close to the membrane is the trans face (trans Golgi) (Fig. 4.7).

Major Functions of GA

1. It is the site for the packaging of secretory products into the secretory granules. Materials produced in rough ER travel through the lumen of smooth ER. Vesicles budding off from smooth ER transport material to cis-face of Golgi complex. Some proteins are phosphorylated here, and then pass to the trans-face, where they are packaged into the secretory vesicles (Fig. 4.7).
2. It is the site for the incorporation of carbohydrates into the newly synthesized proteins to form glycoproteins.
3. Lysosomal enzymes are formed in GA.
4. Transports material to other organelles and cell surface.

Lysosomes

Lysosomes are membrane-bound spherical organelles that contain a variety of hydrolytic enzymes meant for intracytoplasmic digestion. More than 40 different lysosomal enzymes (lysozymes) have been isolated. Some important lysozymes are listed in Table 4.2. Lysosomes are found in almost all animal cells except erythrocytes. They are particularly abundant in cells having high phagocytic activity such as neutrophils and monocytes.

Important Note

Absence of lysosomes: Lysosomes are present in all cells except RBC. They are prominently present in neutrophils.

In granulocytes, the lysosomes appear as cytoplasmic granules. Lysosomal activity of a cell tissue can be determined (Application Box 4.3).

Application Box 4.3

Marker for lysosomal activity: Acid phosphatase is used as a marker for lysosomal activity.

Fig. 4.7: Structure of endoplasmic reticulum (ER). Note that the inner part of ER is in continuity with the nuclear membrane and the outer part interacts with Golgi complex. Rough ER has numerous ribosomes on the membrane, and smooth ER has no ribosome on the surface.

Fig. 4.8: Structure of Golgi complex. Note the structure mainly consisting of stack of cisternae.
During the process of phagocytosis, the **phagosome** that is formed by the cytoplasmic pseudopodia containing foreign body such as bacteria, viruses, etc. fuse with the lysosomes to form **phagolysosome** (Fig. 4.9) and lysosomal enzymes later digest these foreign organism. Therefore, lysosomes are known as **autophagosomes**.

The interior of lysosome is acidic (pH is about 5) compared to its cytosolic exterior (pH of cytoplasm is about 7.2). Acidic interior of this organelle is due to the action of proton pump, the H⁺-ATPase, present on the lysosomal membrane that pumps protons (H⁺) from cytosol into the lysosomal interior against its electrochemical gradient. The lysosomal enzymes are **acid hydrolases** as they function best in acidic pH.

**Types of Lysosomes**

There are three forms of lysosomes: Primary lysosomes, secondary lysosomes (endolysosome) and tertiary lysosomes (phagolysosome) (Flowchart 4.1).

Primary lysosomes: They are formed with hydrolytic enzymes synthesized by rough endoplasmic reticulum and packaged in the Golgi apparatus. They are generally referred to as **storage vacuoles**.

Secondary lysosomes: These are lysosomes fused with endosomes. Hence they are called **endolysosome**.

Tertiary lysosomes: These are **autophagic vacuoles** or **autophagosomes**. They are formed by fusion of phagocytic vacuoles with primary lysosomes. Hence they are called **phagolysosome**.

### Table 4.2: Important lysosomal enzymes.

<table>
<thead>
<tr>
<th>A. Proteolytic enzymes</th>
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</thead>
<tbody>
<tr>
<td>1. Cathepsins (proteinases)</td>
<td></td>
<td></td>
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<tr>
<td>2. Collagenases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Elastase</td>
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<table>
<thead>
<tr>
<th>B. Lipolytic enzymes</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Lipases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Phospholipases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Fatty acyl esterases</td>
<td></td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>C. Carbohydrate splitting enzymes</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. α-glycosidase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. β-galactosidase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Hyaluronidase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Arylsulfatase</td>
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<table>
<thead>
<tr>
<th>D. Nucleic acid hydrolyzing enzymes</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Ribonuclease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Deoxyribonuclease</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>E. Other enzymes</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Acid phosphatases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Catalase</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Functions of Lysosome**

1. Lysosomes contain many enzymes essential for **intra-cellular digestions**. Absence of these enzymes leads to various storage diseases (Clinical Box 4.2), as listed in Table 4.3.
2. Lysosomes kill and remove infective organisms and foreign bodies.
3. Acrosome, located on the head of spermatozoa is a specialized lysosome that contains many hydrolytic enzymes. Acrosome plays an important role in penetration of ovum by sperm.
4. **Autolysis**: When a cell is damaged or ruptured, the enzymes liberated within the cytoplasm digest the cytoplasmic contents, a process known as **autolysis**. The autolytic enzymes are mainly lysosomal enzymes.
5. Lysosomes remove intracellular products of metabolism from the cell.
Cutaneous angiokeratomas and hypohydrosis.

Mental retardation, coarse facies, CVS involvement.

Unique features

Hepatosplenomegaly, and skeletal dysplasia.

Mental retardation, macrocephaly and hypercusis in infants.

Proliferation of peroxisome is caused by many syntheses. Peroxisomes are small spherical organelles having diameter of about 0.5 µ, and therefore denoted as microbodies. Specialties of peroxisomes:

1. They are formed by budding from or by division of smooth endoplasmic reticulum. They are referred to as subcellular respiratory organelles. But, they do not have energy-coupled electron transport system.

2. They contain oxidases that promote oxidation of lipids (especially β-oxidation of long chain fatty acids) forming acetyl-CoA and hydrogen peroxide (H₂O₂), and catalases that liberate oxygen from H₂O₂. They protect the tissue from oxidative stress (Application Box 4.4)

3. With the help of peroxins, the protein chaperons, various proteins with specific signal sequence are directed to peroxisome. The membrane of peroxisome contains a number of specific proteins that transport substances between peroxisome matrix and cytosol.

4. Peroxisome matrix contains more than 50 enzymes that are involved in many metabolic reactions of lipids (long chain fatty acids, plasmalogens, cholesterol, bile acids) purines, amino acids and H₂O₂. Deficiency of enzymes leads to many diseases (Clinical Box 4.3)

5. Proliferation of peroxisome is caused by many synthetic products that act on receptors in the nuclei of cells, known as peroxisome proliferation activated receptors (PPARs). PPARs are members of nuclear receptor superfamily those on activation bind to DNA and produce changes in mRNA production. PPARs have extensive physiologic effects and affect many tissues and organs.

Lysosomal storage diseases: Congenital absence of lysosomal enzymes leads to accumulation of materials in the lysosome that are degraded by lysosomes, causing lysosomal storage diseases. Especially, elimination of certain substances from the cells of the body, e.g. glycogen, cerebrosides, gangliosides and sphingomyelin mainly depend on the activity of lysosomal enzymes. Therefore, in the absence of active lysosomes, intracellular accumulation of these substances interferes with normal cell function and produces diseases. Accumulation of partly digested cellular material is called inclusion bodies.

Ribosomes

Though ribosomes are usually present on the surface of endoplasmic reticulum, they are also present as free organelles in the cytoplasm. They contain about 85% of RNA of the cell. They are the major site of protein synthesis. RNA is also present in nucleus, mitochondria and cytoplasm, and consequently protein synthesis occurs in these structures too. They may be present singly (monoribosome) or in groups (polyribosomes)

Peroxisomes

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Application Box 4.4

Peroxisomes protect from oxidative stress (OS): H₂O₂, a potent oxidant produced during metabolic reactions is one of the agents for oxidative stress. H₂O₂ is destroyed by catalase, a peroxisomal enzyme, which is designated as an antioxidant for combating OS. Thus, peroxisomes protect cells from the toxic effects H₂O₂. Hence, increased catalase activity is one of the markers of OS.

Clinical Box 4.3

Diseases of peroxisomes: Peroxisomes may be abnormal or absent in inherited disorders

1. Zellweger syndrome, which occurs due to mutations in the gene encoding certain proteins such as peroxins or peroxisomal enzymes. This syndrome is characterized by profound neurologic impairment, accumulation of very-long chain fatty acids, abnormalities in synthesis of bile acids and marked reduction of plasmalogens. The child usually dies within a year in this disorder.

2. Another similar genetic disorder is infantile Refsum disease, which is less severe and only few proteins are affected.

3. Brown-Schilder’s disease (Adenoleukodystrophy) occurs due to insufficient oxidation of very long chain fatty acids by peroxisomes. This is an autosomal recessive disease manifests with progressive degeneration of liver, kidney and brain.

4. Primary hyperoxaluria occurs due to defective peroxisomal metabolism of glycosylate derived from glycine.

Centrosomes

Centrosome is located close to the nucleus in eukaryotic animal cells. It is formed by two centrioles placed at right angle to each other that are present within the amorphous pericentriolar material. Centrioles are short cylindrical structures made up of microtubules placed in group of three (triplets) that run longitudinally in the wall of centrioles. There are nine sets of triplets arranged at regular intervals in the wall of each centriole (Fig. 4.10). The subunits of microtubules in centrosome are γ-tubulins. Centrosomes are microtubule-organizing center (MTOCs).

Functions

Centrosomes regulate chromosome movement during cell division. They duplicate themselves and move apart...
from each other to the poles of mitotic spindle to monitor the process of cell division.

**Cytoskeleton**

Cytoskeleton is an intracellular system of fibers that not only maintains the structural integrity of the cell, but also allows appropriate change in cell shape for cell mobility and participation of cell in various physiological activities. It consists of microfilaments, microtubules and intermediate filaments. These cytoskeletal elements are made up of different cell proteins (Table 4.4).

**Microfilaments**

All eukaryotic cells contain microfilaments that contribute to the maintenance of and change in cell shape and regulation of cell functions. These are long solid filaments (Fig. 4.11A) having diameter of about 7 nm. They are made up of actin. Actin is the most common cell protein that accounts for about 15% of total protein in the cells.

Actin in the globular form is called \( G \text{actin} \), which is the unpolymerized actin subunit. \( G \text{actin} \) subunits polymerize to form the filamentous actin, referred to as \( F \text{actin} \). In vivo, actin filaments polymerize and depolymerize. Also, polymerization occurs at one end of actin filament and depolymerization occurs at the other end.

**Special characteristics:**
1. Though microfilaments are well developed in muscle cells, they are present in almost all cells and better organized in cells that secrete granular content by exocytosis. For example, the developed contractile system in platelets consists of microtubules and extensive network of microfilaments.
2. Microfilaments help platelets to change shape and move granules from interior of cytoplasm to canaliculi for release of chemicals (release reaction of platelet).
3. In cells with microvilli on their epithelial surface, microfilaments extend into the microvilli.

**Functions**

1. Microfilaments are the major contractile fibers of the cell that help in change in cell shape and cell movement.
2. As microfilaments exhibit contractile phenomena within the cytoplasm, they help in phagocytosis, transport and secretion of cellular materials, cell contraction, cell discharge, movement of secretory granules, etc.

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**Table 4.4: Protein subunit and diameter of cytoskeletal elements of cell.**

<table>
<thead>
<tr>
<th>Microfilament</th>
<th>Intermediate filament</th>
<th>Microtubule</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. Shape</strong></td>
<td>Double stranded helical arrangement</td>
<td>Tubular hollow</td>
</tr>
<tr>
<td><strong>2. Diameter</strong></td>
<td>7 nm</td>
<td>10 nm</td>
</tr>
<tr>
<td><strong>3. Basic protein units</strong></td>
<td>Actin</td>
<td>Various proteins</td>
</tr>
<tr>
<td><strong>4. Location in cell</strong></td>
<td>– Forms a network adjacent to cell</td>
<td>– Extend across cytoplasm connecting desmosome and hemidesmosome</td>
</tr>
<tr>
<td></td>
<td>– Core of microvilli</td>
<td>– The nuclear lamina</td>
</tr>
<tr>
<td></td>
<td>– Contractile elements of muscle</td>
<td>– In skin epithelium as keratin</td>
</tr>
<tr>
<td><strong>5. Major functions</strong></td>
<td>Essential element of contractile element of muscles</td>
<td>Provide mechanical strength and link cells together</td>
</tr>
</tbody>
</table>

---

**Fig. 4.10:** Transverse section across a centriole. Note nine groups of tubules, each group having three microtubules.

**Figs. 4.11A to C:** Structure of microfilaments (A), intermediate filaments (B) and microtubules (C).
3. Filamentous actin helps in movement of chromosome and cell division.
4. Through anchoring proteins, F actin fibers attach to various cytoskeletal structures and interact with membrane bound proteins.
5. Microfilaments project into the microvilli present on the epithelial surface such as on the intestinal mucosa, and extend up to the tip of microvilli. They help in microvilli movement.
6. Microfilaments in the lamellipodia (lamellar extensions from cell that occur at the time of cell crawling) help in cell movement on a surface.
7. Actin filament interacts with integrin receptors to form focal adhesion complexes (FAC). FAC serve as points of traction with the surface over which cell pulls itself.
8. Microfilaments are also used by some molecular motors as tracks.

**Microtubules**

Microtubules are long hollow tubular structures (Fig. 4.11C) having diameter of about 25 nm, including the wall thickness. The inner cavity diameter of microtubules is about 15 nm. They are made up of subunits of globular proteins called tubulins that are arranged in a closely packed helical manner.

Tubulins in microtubules are of two types: α-tubulin and β-tubulin, except the tubulins in centrioles that are γ-tubulins. The α and β-tubulin subunits form heterodimers that aggregate to make tubular structures (protofilaments) of stacked rings. Each ring in microtubule usually contains 13 subunits (13 protofilaments) (Fig. 4.12).

**Special characteristics:**
1. A unique property of tubulin subunits is their property of disaggregating (disassembly) and re-aggregating (assembly). Therefore, microtubules form a dynamic cytoskeletal framework of the cell. Microtubules are polar in nature with assembly predominating end (+ve end) and disassembly predominating end (–ve end). The process of assembly and disassembly may occur simultaneously.
2. Many drugs act by inhibiting microtubule assembly (Clinical Box 4.4)
3. Interaction with GTP facilitates microtubule formation.
4. Microtubule growth is temperature sensitive with cold conditions favoring disassembly.
5. In cells with cilia and flagella, microtubules extend into these structures.
6. Kinesin and dynein are microtubule-based molecular motors.

**Functions**

1. Microtubules serve as cytosolic guide rails for transport of substances within the cytoplasm or transport of secretory granules to the cell membrane. They help in transport of vesicles and organelles from one part of the cell to another.
2. They contribute to the maintenance of cell strength and cell shape.
3. They help in the formation of spindles that move chromosomes during mitotic cell division.
4. They are the pillars for structure and function of cilia.
5. They contribute to cell motility.

**Cilia:** Cilia are hair-like outgrowths of cells of the epithelial membrane that characteristically beat in a synchronous whip-like fashion. Therefore, cilia allow a directional flow. Each cilium is an outgrowth of a basal body situated beneath the cell membrane, which has the property of reproducing itself. In each cilium, there is an axoneme that typically consists of nine doublets of microtubules that run circumferentially from base to the tip while a single pair of microtubules runs centrally (9 + 2 arrangement).

Clinical Box 4.4

Cytotoxic drugs act by inhibiting microtubular functions: Many cytotoxic drugs act by inhibiting the microtubular activity. For example, anticancer chemotherapeutic agents such as vincristine and vinblastine promote disassembly of microtubules. Paclitaxel, another anticancer drug binds with microtubules and stabilize them against depolymerization. The stabilized microtubules function abnormally and do not undergo dynamic changes necessary for cell cycle completion. It prevents formation of mitotic spindles, and cells die. Colchicine inhibits microtubule assembly.
Intermediate Filaments

These are filamentous structures made up of various sub-units (Fig. 4.11B). The average diameter of these filaments varies from 8 to 14 nm.

**Functions:**
1. They connect the nuclear membrane to the cell membrane and also membranes of cell organelles. Therefore, the major function of these filaments is to integrate the organelles within the cytoplasm.
2. They form supple skeletal network for the cell and resist rupture of cell from external pressure. In the absence of microfilaments, cells easily rupture. Blister formation in skin is common in humans when these filaments are absent or abnormal.
3. They are used as cell markers (Clinical Box 4.5)

**Clinical Box 4.5**

*Intermediate filament proteins are cell markers:* Proteins of intermediate filaments are cell specific. Therefore, they are frequently used as cellular markers. For example, cytokeratin is the marker of epithelial cells, whereas vimentin is the marker of fibroblast.

Molecular Motors

Molecular motors help in the movement of various cell parts, proteins and organelles within the cell cytoplasm. They are 100-kDa ATPases. They have two domains: The domain that attaches with cargo (the cell part to be moved) and the other domain attaches with microtubules or actin filaments. The domain attaching with microtubules or actin is the head part that contains ATPase, which causes hydrolysis of ATP to provide energy for movement of molecules. Molecular motors can be broadly divided into two categories: Microtubule-based and actin-based.

**Microtubule-based Molecular Motors**

Microtubule-based molecular motors make movement of molecules along the microtubules. They are mainly kinesin and dynein.

**Kinesin**

The *conventional kinesin* is a double-headed molecule that transports its cargo toward the negative terminal of microtubules. One head attaches with microtubule and the other head with the cargo. Some kinesins move cargo toward the positive terminal of microtubules. Other kinesins are involved in cell divisions such as mitosis and meiosis.

**Dynein**

Dynein is also a double-headed molecule. There are two types of dynein: Cytoplasmic form and axonemal form.

**Cytoplasmic Dynin:** It functions like that of conventional kinesin that moves cargo toward the negative terminals of microtubules.

**Axonemal Dynein:** Cilia and flagella contain dynein-based molecular motors that are axonemal type. Thus, these dynein molecules are responsible for beating cilia and flagella.

Actin-based Molecular Motors

Actin-based molecular motors make movement of molecules along the actin filaments. These are mainly myosin I–V. However, there are 18 types of myosins. In myosin II, the head binds with actin and pulls actin by bending the neck region. In myosin V, the heads of myosin molecules walk on the microfilaments one after another. Myosin molecules perform various functions like muscle contraction, contraction of intestinal villi, cell migration etc.

The Nucleus

**Structure of Nucleus**

All living cells in humans except red blood cells contain nuclei. Platelets are cellular fragments. Most cells are uninucleated and the nucleus is commonly located at the center of the cell as round, intensely stained structure. However, there are variations in the size, shape and location of the nucleus within the cell, especially in different types of leucocytes and muscle cells. Nucleus is bound by a bilayer nuclear membrane that surrounds the nucleoplasm. The nucleus contains nucleoplasm and nucleolus.

**Nuclear Membrane**

The nuclear membrane is a double-layered membrane (Fig. 4.13). The space between the two membranes is called perinuclear cisterns. In some cells, nuclear membrane is in continuity with the membrane of endoplasmic reticulum. There are circular openings in the nuclear membrane known as nuclear pores that serve as passages for the exchange of materials between the cytoplasm and the nucleoplasm, especially for the transport of mRNA and proteins. There are two special transport proteins in the nuclear membrane, known as importins and exportins.

![Fig. 4.13: Structure of nucleus and nuclear envelope.](image-url)
that regulate transport of molecules across nuclear membrane. Another nuclear membrane protein known as Ran, plays organizing role in these transport mechanism.

**Nucleoplasm**

The nucleoplasm is the nuclear matrix, which is a gel-like ground substance that contains genetic material in the form of DNA. When cell is not dividing the genetic material is present in the form of tangled mass called nuclear chromatin and when cell division begins, the tangled mass unwinds and appears as distinct strands known as chromosomes. The nuclear chromatin is made up of coiled strands of DNA, bound to large quantities of basic proteins (histones).

**Nucleolus**

The nucleus contains nucleolus, a patchwork of granules rich in RNA. There may be multiple nucleoli in a nucleus, especially in developing cells. Nucleoli synthesize ribosomes.

**Functions of Nucleus**

Nucleus regulates cell functions. It contains DNA that is responsible for transmission of hereditary features. It also contains RNA that is essential for protein synthesis. Nucleus is the main regulator of cell divisions. Details of the functions of DNA and RNA are discussed in the next chapter “Physiology of Genetics”.

**INTERCELLULAR JUNCTIONS**

In tissues, junctions formed between the cells are called intercellular junctions or intercellular connections. The cells in tissues are usually held together by the extracellular matrix.

1. In connective tissues such as fibroblasts, cartilage and bones, the extracellular matrix is abundant, and therefore the cells are sparsely distributed within the substance of the matrix.

2. In other connective tissues such as muscle, the cells are held together by cell to cell adhesions.

3. In epithelial tissues that line the free surfaces such as skin, and basement membrane of tubular structures and cavities of the body such as alimentary tract, kidney tubules and urinary bladder, the cells are bound tightly together by specialized intercellular junctions (Fig. 4.14).

4. In epithelial and connective tissues, the normal space between cells (intercellular space) is 20–25 nm. In epithelial tissues, the extracellular matrix is reduced to a thin layer known as the basal lamina.

**Types**

Functionally, intercellular connections or junctions are of two types: junctions that tie cells together and Junctions that allow transfer of ions and small molecules (Table 4.5).

**Tight Junctions**

Tight junctions are commonly found in the epithelium of the GI tract, nephrons, urinary tract, hepatobiliary tract, and choroid plexus. They are also called zonula occludens.

**Special features of tight junctions:**

1. Tight junctions are typically located toward the apical region of the cells (Fig. 4.15).

2. At tight junctions, the outer layer of the cell membrane of neighboring cells fuse with each other that obliterate the intercellular space close to their apical margin.

3. They are made up of ridges, half of which is contributed by both neighboring cells and each half is bound tightly to each other. Therefore, they practically form the barrier for transport of solutes and solvents from the lumen into the interstitial space and between cells.

4. They contain ion and water channels that make them selectively leaky, though the degree of leakiness varies in different epithelia.

5. The membrane proteins that contribute to formation of tight junctions belong to three main families: occludin, claudins and junctional adhesion molecules (JAMs).
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Fig. 4.15: Schematic representation and locations of specialized junctions connecting the cells.

6. Many other membrane proteins from cytosolic side interact with tight junctions.

**Functions of Tight Junctions**
1. In general, they form selective permeability barrier that prevent transport of macromolecules from the luminal fluid into the interstitial space. Thus, macromolecules pass only through the epithelial cell as vesicles (vesicular transport).
2. Due to the presence of leaky channels, small size water-soluble particles are permitted through tight junctions. However, the degree of permeability varies. For example, in the gut, Na⁺ ions pass fairly freely, while in the urinary bladder, the passage for sodium is almost nil. Furthermore, the permeability of a substance through tight junctions can be increased, depending on the osmolality gradient across the epithelium. This forms the paracellular transport of solutes and solvents.
3. In the brain, tight junctions between endothelial cells of cerebral blood vessels contribute to the effectiveness of blood-brain barrier.
4. In the ciliary bodies, they form blood-aqueous barrier between the cells of inner non-pigmented epithelium.

**Anchoring Junctions**

Anchoring junctions are of two types: Cell to cell anchoring junctions and cell to basal lamina anchoring junctions.

**Cell to Cell Anchoring Junctions**

Cell to cell anchoring junctions are desmosome and zonula adherens.

**Desmosomes**
1. Desmosomes are the junctions characterized by focal thickening of two adjacent cell membranes (Fig. 4.15). The thickened area is the presence of dense layer of proteins on the cytoplasmic surface of the membrane.
2. Thickened area of two sides is separated by a gap of 25 nm.
3. Intermediate filaments from cytosol are attached to the thickened areas.
4. The intercellular space between the two membrane thickenings also contains filamentous cell adhesion materials such as desmogleins and cadherins (Fig. 4.16).

**Zonula Adherens**

Zonula adherens is located below the base of tight junctions (Figs. 4.17A and B). It is the major site of attachment for intracellular microfilaments. Cadherins are present in the intercellular space at this junction.

**Cell to Basal Lamina Anchoring Junctions**

Cell to basal lamina anchoring junctions are hemidesmosome and focal adhesion.

**Hemidesmosomes**

In appearance, they look like half of desmosomes, and therefore are called hemidesmosomes. Microfilaments are attached to it intracellularly. In contrast to presence of cadherins and desmogleins in desmosome, hemidesmosomes contain integrins.

**Focal Adhesions**

Focal adhesions (focal spots) connect cell to the basal lamina. Intracellularly, they are associated with actin filaments. Therefore, they assist in cell movement.
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Gap Junctions

Structure

Gap junctions are called nexus between the cells at which the intercellular space is narrowed from its 25 nm diameter to 3 nm (Figs. 4.18A and B).

Special Features

1. Gap junctions are made up of special transmembrane proteins known as connexons. The connexons from the membrane of two adjacent cells are lined up with one another (Fig. 4.19A).
2. Each connexon is formed by six identical protein subunits called connexins. Connexin surrounds an aqueous channel and when connexon of adjacent cells are aligned, the aqueous channels of both cell membranes become a continuous one (Fig. 4.19B). This allows substances of adjacent two cells to pass through the channel without passing through the ECF. The diameter of the channel is about 2 nm.
3. As connexons of one cell membrane are aligned with the connexons of the adjacent cell membrane and they connect the cytoplasm of adjacent cells through their aqueous channels, water, ions, amino acids, sugars and hormones can pass from cell to cell through them.
4. As connexons keep the adjacent cell membranes at a fixed gap, the junction is named as “gap junctions”.
5. There are 20 different connexon genes (Clinical Box 4.6).

Functions

1. Electrical synapses: As the pores of gap junction are larger than ligand-gated or voltage-gated channels, passage of substances is easier through them. Gap junctions easily allow ions to pass through and serve as electrical synapses, as they permit rapid propagation of electrical activity from cell to cell. Therefore, tissues with gap junctions between cells behave as physiological syncytium (Application Box 4.5).
2. Gap junctions also permit passage of organic solutes such as sugars and amino acids with molecular weight up to 1000 from cell to cell.
3. Chemical messengers and hormones are also exchanged between cells through gap junctions.

Regulation of Gap Junction Functions

Though transport of ions through gap junctions is not an active process, they do not just act as mere passive conduits. Their activities are regulated by intracellular calcium concentration and cytosolic pH. Increase in any of these parameters can prevent transport through gap junctions by closing their channels.

Application Box 4.5

Functional syncytium: Gap junctions are present abundantly in heart muscle. As they serve as electrical synapses, electrical impulses pass easily from cell to cell in cardiac muscle. Thus, stimulation of one muscle cell in heart results in activation of all muscle cells. This makes the ventricular and atrial muscle as functional syncytium. However, ventricular and atrial muscles are two separate functional syncytia as ventricles and atria are separated by non-conducting septa. Gap junctions are also present in visceral smooth muscles that conduct electrical impulses from cell to cell. Hence, visceral smooth muscles also have syncytial behavior.

Cell Adhesion Molecules

Cells are attached to each other and to the basal lamina by cell adhesion molecules (CAMs) that are present...
abundantly at intercellular connections (Fig. 4.20). By their property of adhesions between the cells, they provide stability to the tissue.

**Systems of Binding**

They have both extracellular and intracellular bindings.

**Extracellular Binding**

Many of CAMs bind to membrane proteins called **laminins**. Laminins are cross-shaped large membrane molecules that have multiple receptor domains on the extracellular matrix. CAMs bind to these extracellular receptor domains.

**Intracellular Binding**

Cell adhesion molecules (CAMs) pass through the cell membrane to expose into the interior of the cell and attach with the cytoskeleton inside the cell. This intracellular binding of CAMs with cytoskeletal structures enhances strength of cell adhesions.

**Nature of Binding**

Cell adhesion molecules (CAMs) exhibit both homophilic and heterophilic bindings.

**Homophilic Bindings**

In homophilic bindings, CAMs attach with similar molecules present on the other cells.

**Heterophilic Bindings**

In heterophilic bindings, CAMs attach with different molecules of other cells.

**Types of CAMs**

Though there are confusions on classifying CAMs, they can be broadly categorized into four varieties: Integrins, IgG superfamily proteins, cadherins and selectins.

- **Integrins**: Integrins are heterodimeric proteins that bind to various receptors.
- **IgG superfamily proteins**: CAMs belonging to IgG superfamily are immunoglobulins.
- **Cadherins**: These are calcium dependent molecules that mediate homophilic binding.
- **Selectins**: They have carbohydrate binding domains, that resemble lectin-like structure.

**Functions of CAMs**

1. They zip cell to cell. Cell adherence is strengthened by CAMs.
2. For their attachment with cytoskeleton, they play role in cell movement.
3. Cellular signals are transmitted out of the cell or extracellular signals are transmitted into the cell via CAMs.
4. They play significant role in inflammation and wound healing.
5. CAMs prevent apoptosis. Loss of cellular contact from extracellular matrix due to defects in CAMs hastens the process of apoptosis.

**CHAPTER SUMMARY**

The cell is the structural and functional unit of tissues. A cell consists of cell membrane, cytoplasm and nucleus.

1. The cell membrane is the lipid bilayer that forms the boundary in all animal cells and is selectively permeable to the substances.
2. Cells are bound to each other by means of intercellular junctions and cell adhesion molecules. In the upper apical part are the tight junctions and in the base are hemidesmosome and focal adhesion. In the middle of the cells are desmosomes and gap junction. There are many other proteins in the cells like actin and myosin that provide strength and mobility to the cell and also the mechanisms for adhesion to other cells.
3. Gap junctions allow ions to pass from cell to cell much faster. Therefore, they are called electrical synapse. Gap junctions provide the physiological basis for syncytial nature of a tissue like, cardiac tissue.

**Important to Know (Must Read)**

1. **Long Questions** are usually not asked from this chapter.
2. **Cell membrane, Mitochondria, Lysosomes, Cytoskeleton, Molecular motors, Gap junctions, CAMs, Intercellular junctions**, may come as **Short Questions**.
3. In **Viva**, examiner may ask... cell membrane, cell organelles, cytoskeletal proteins, molecular motors, intercellular junctions and gap junctions, and cell adhesion molecules.
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Physiology of Genetics and Apoptosis

CHAPTER 5

PHYSIOLOGY OF GENETICS

Physiology of genomics is a developing branch of medicine. It deals with the understanding of the concept of gene and gene therapy in the treatment of genetic disorders.

Genetics is the science of heredity, dealing with resemblances and differences of related organisms resulting from the interaction of their genes and the environment. Application of knowledge of genetics to understand the heritable basis of the diseases and to improve the management of diseases through gene intervention, is called medical genetics. Mendel’s work in 1886 that hereditary characteristics are transmitted to offspring by separate units laid the foundation of genetics. Later, Johannied, the Danish botanist in 1909 termed these units as genes and, Morgan, the American geneticist established that the hereditary characteristics are transmitted on chromosomes.

Chromosomes

The term chromosome was coined by Waldeyer in 1888. Chromosomes transmit the genetic information from parents to offspring. There are 46 chromosomes in cells of all tissues except gametes that contain 23 chromosomes. Autosomes are present in somatic cells and sex chromosomes in gametes.

Structure of Chromosomes

Each chromosome consists of two chromatids that are connected at the centromeres (or kinetochore). Each chromatid is composed of two chromosomes (Fig. 5.1). Typically, the centromere is not midway between the two ends of chromatids. When chromatid has a short arm and a long arm, the chromosome is called submetacentric. If two arms of the chromatid are of equal length, the chromosome is metacentric, if one arm is too short the chromosome is acrocentric and if centromere lies at one end (each chromatid has only one arm), the chromosome is telocentric (Figs. 5.2A to D). Chromosomes are distinguishable only during mitosis. In the interphase (between successive mitoses), chromosomes elongate and assume the form of a long thread called chromonemata (Application Box 5.1). Though chromosomes are formed mainly by DNA, they also contain RNA, the basic protein histones, complex proteins, organic phosphorous compounds and inorganic salts.
**Application Box 5.1**

**Karyotyping:** Identification of each chromosome and mapping out the chromosomes is called karyotyping. Karyotyping is done for studying the morphology and number of chromosomes. Though karyotyping can be done in any nucleated cell, lymphocytes are preferred. The study is done by arresting the dividing cells in metaphase by colchicine and spreading them on glass slide and staining them with Giemsa stain. In many cases, chromosomal abnormalities can be correlated with specific diseases.

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**The DNA**

Deoxyribonucleic acid (DNA) is found in bacteria, and in nuclei and mitochondria of all eukaryotic cells. DNA is the component of chromosome. Chromosome appears in pairs, except in germ cells. Chromosomes are made up of a mammoth molecule of DNA, which is about 2 m in length. However, DNA is accommodated in the nucleus as most part of it at intervals is wrapped around histone proteins to form **nucleosomes**. About 25 millions of nucleosomes are present in a nucleus. The complex of DNA and the histone protein is called **chromatin**. As cell division begins, acetylation of histone loosens the coiling and pairs of chromosomes become visible.

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**Important Note**

**DNA Content of a Cell:** It has been estimated that total DNA content of a cell (all chromosomes put together) is represented by about $6 \times 10^9$ nucleotide pairs. Of these, $2.5 \times 10^8$ is present in Chromosome 1, which is the largest chromosome. The Y chromosome, which is the smallest chromosome, contains $5 \times 10^7$ nucleotide pairs.

**DNA Nucleotides**

A molecule of DNA is made up of two strands of polynucleotides linked together in the form of a double helix (Fig. 5.3). A nucleotide consists of a nitrogenous base, a sugar molecule (deoxyribose) and a phosphate molecule. In DNA, the nitrogenous bases are **purines** (adenine and guanine) and **pyrimidines** (cytosine and thymine). Nucleotides in DNA from a polymer of polynucleotides through covalent bonds between the sugar molecules. [Nitrogenous bases are often designated by their first letter i.e. A for adenine, T for thymine, G for guanine and C for cytosine]. The purine and pyrimidine bases encode **genetic message** (Application Box 5.2).

**Application Box 5.2**

**The Genetic Message:** The genetic message is encoded by purine and pyrimidine bases in the nucleotide chains of DNA. The amino acid sequence in the protein synthesized in the cell decides the text of the message. The RNA transfers the message form DNA blueprint to the ribosome where protein synthesis occurs. The proteins formed from DNA blueprint include all enzymes that control cell metabolisms.

**Important Note**

**Gene and Proteins:** A **gene** is defined as the amount of information necessary to specify a **single protein** molecule. Proteins determined by a single gene may divide to form different proteins with various physiological actions. Also, different mRNAs may form from a single gene that can guide formation of various proteins.

**Double Helix Structure**

In the double helix, the sugar phosphates form the backbone with all the bases being present inside the helical structure. Each nucleotide consists of a sugar deoxyribose,
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a molecule of phosphate and a base (Fig. 5.4). The bases on one strand pair up by means of hydrogen bonds with bases on the other strand. Linkages in nucleotide form one strand of DNA (Fig. 5.5), and linkages of two chains of nucleotides form the DNA double helix (Fig. 5.6). The paring occurs in such a way that the purine base like guanine in one chain always pairs with the pyrimidine base cytosine in the other chain. Similarly, adenine always pairs with thymine. These base pairs are referred to as complementary base pairs.

Functions of DNA

1. DNA as part of chromosome transmits genetic characteristics (hereditary features) from generations to generations.

2. It possesses information required for the synthesis of RNA and cell proteins (including enzymes).

3. It controls cell division.

The Genome

Each chromosome contains only one type of long-chain DNA molecule. DNA is the component of chromosome that carries the genetic message (blueprint of heritable characteristics) of the cell (Refer, Application Box 5.2).

1. The total genetic information stored in chromosomes of a cell is known as the genome.

2. The human genome contains about 3 billion nucleotide pairs, and in diploid cells they are organized into 23 pairs of chromosomes (all cells of the body, except the gametes).

3. In each pair, one is derived from the mother and the other from the father. However, in males, the X chromosome is inherited from the mother, and the Y chromosome from the father. The X and Y chromosomes are concerned with the determination of sex, and therefore they are called sex chromosomes.

4. The XX are necessary for development of the female and XY pair is necessary for development of the male. As stated in Lyon hypothesis, in female, one of the two X chromosomes (paternal or maternal derived), is
inactivated during embryogenesis. This inactivation is passed to all the somatic cells, while the germ cells in female remain unaffected. That means ovary will have always active X chromosome.

5. The inactive X chromosome in the somatic cells in female lies condensed in the nucleus and is called as sex chromatin. This phenomenon in females helps in nuclear sexing (Clinical Box 5.1).

6. Gametes (sperm and ovum) have half this number of chromosomes, and therefore are said to be haploid cells.

7. During fertilization of an ovum by a sperm, the diploid number is restored, so that each cell carries 23 chromosomes from each parent.

Clinical Box 5.1

Nuclear Sexing: Determination of sex by identification of sex chromatin in the nucleus of somatic cells is called nuclear sexing (Fig. 5.7). This is done for genetic female testing by preparing and staining the smears of squamous cells scraped from oral cavity or by identifying Barr body attached to nuclear lobes in the circulating neutrophil, in females. A minimum of 30% cells positive for sex chromatin indicates the person as female genetically.

Chromosomal Disorders

Chromosomal abnormalities may be either due to the defect in autosomes or in sex chromosomes, and are accompanied by congenital abnormalities.

1. Trisomy 21: The commonest abnormality of autosomal chromosome is the presence of three instead of two chromosomes in the number 21 pair. The condition is known as trisomy 21. The resulting clinical condition is called mongolism, or Down’s syndrome, which is characterized by mental retardation, congenital anomalies and abnormal physical features (Fig. 5.7A).

2. Turner and Klinefelter syndromes: The common sex chromosomal abnormality in female is Turner syndrome in which one X chromosome is absent (i.e. XO), and in male is Klinefelter syndrome in which an extra X chromosomes is added (XXY). In these conditions, the subject is sterile, and has peculiar physical abnormalities. Klinefelter’s syndrome that occurs in males, presents with gynecomastia, osteoporosis, and testicular atrophy (Fig. 5.7B). Turner’s syndrome that occurs in females presents with short stature, webbed neck, small breast size and primary amenorrhea (Fig. 5.7C).

3. X-linked Disorders: An abnormal gene located in an autosome leads to an autosomal trait; whereas location of abnormal gene in a sex chromosome gives rise to sex-linked traits. However, all the known sex-linked genetic disorders are due to the defective genes located on one X chromosomes, and therefore they are called as X-linked disorders. Very few of them are X-dominant, but most are X-recessive. Therefore, many X-linked disorders do not manifest in females who have normal X chromosome allele. However, sex linked genetic disorders at all times manifest in males as they do not have normal neutralizing X allele.

Fig. 5.7A to C: Clinical features of three important chromosomal abnormalities. (A) Down’s syndrome; (B) Klinefelter’s syndrome; (C) Turner’s syndrome.
**Section 1: General Physiology**

Common examples of *X*-recessive disorders are:
- Hemophilia
- G-6-PD deficiency
- Nephrogenic diabetes insipidus.

Rarely do they manifest in females, such as females with Turner’s syndrome.

**The Genes**

The gene is the functional unit of DNA. A gene is defined as the portion of DNA responsible for the production of a single RNA molecule. Each chromosome contains myriad of genes. The genes do not exercise their powers all the time. The inherent control mechanisms are such that only selected genes are switched on at any given time. Though, the DNA of each cell in a multicellular organism has same DNA sequence and same genetic capabilities, the difference between different cell types lies in the differences in genetic expression.

**Gene Expression**

The genetic expression of each cell type is selective, and therefore, the protein synthesis is different in different tissues. Genetic expression occurs in two broad steps: transcription and translation (Flowchart 5.1).

**Transcription**

Transcription is the process in which RNA is synthesized from DNA. In this process:
1. The genetic information stored in DNA is transferred to the RNA.
2. During the process of formation of RNA, the relevant part of the DNA double helix unwinds and exposes the gene unit, which is then copied.
3. The DNA strand that directs the synthesis of mRNA through complementary base pairing is called template strand (also called, coding strand or sense strand) and the other DNA strand is called noncoding strand or antisense strand.
4. The enzyme RNA polymerase carries out transcription by binding to a site on DNA called promoter site.

**Translation**

This is the process by which genetic message transferred to mRNA from DNA is converted into polypeptide chain containing specific sequence of amino acids.

1. Post-transcription: The mRNA is processed from the primary RNA transcript; the process known as maturation in which released introns join with two adjacent exons.
2. Only the exons and introns contain the genetic code.

**Gene Unit**

Each gene unit is organized into three components: regulatory DNA sequence (repressor, promoter and operator), exons and introns (Fig. 5.8). Out of these, introns are removed during post-transcriptional events and adjacent exons join. Regulatory DNA sequence plays major role in transcription.

The regulatory DNA sequences are non-coding sequences that ensure that the gene is transcribed at the right time and in the proper cell. There are three types of regulatory nucleotide sequences per gene.

1. The promoter DNA sequence that contains a sequence of Thymine-Adenine-Thymine-Adenine (T-T-T-A) nucleotides. This acts as the recognition site for the enzyme RNA polymerase to attach with nearby transcription start site before it can move forward to begin transcription of the exons and introns.
2. The promoter separates from the exons and introns by about ten nucleotides known as the operator. The operator should be free of attached molecules for the RNA-polymerase to reach the exons and introns.
3. A repressor nucleotide sequence located ahead of the promoter region is known as 5’ region, which codes for a repressor protein. This protein under certain conditions attaches itself to the operator that prevents the RNA-polymerase from moving towards the structural gene. There is often another regulatory nucleotide sequence at the other end known as 3’ region.

**Exons** are the DNA coding sequences that code for the formation of RNA.

**Introns** are DNA coding sequences inserted at intervals between segments of exons.
During the formation of RNA, both the introns and exons are transcribed, but the later one is translated into sequences of amino acids for the synthesis of a specific polypeptide within the ribosome.

**Steps of Protein Synthesis**

Protein synthesis occurs in three major steps.

1. **Transcription:** The two strands of DNA fiber separate from each other over the area bearing a particular cistron. Thus, the ends of the bases linked to the opposite strand become free. A molecule of mRNA is synthesized as a guide or a template. The code for the sequence in which amino acids are to be linked is passed on from DNA to mRNA (Fig. 5.9). This is called transcription, which occurs under the influence of RNA polymerase. The part of the mRNA strand that bears the code for one amino acid is called codon. The molecule of RNA now separates from the DNA strand and moves from nucleus to cytoplasm, where it attached with ribosome.

2. **Amino acid activation and translation:** On the ribosome, one side of tRNA attaches to amino acid, and on the other side bears a code for three bases (anticodon) that are complementary to the bases coding for its amino acid on mRNA. In the presence of enzyme aminoacyl tRNA synthase, amino acids react with ATP, get activated and attach with specific tRNA. Under the influence of the ribosome, several units of tRNA along with their amino acids become arranged alongside the strand of mRNA. In the sequence determined by the code on mRNA. This is called translation. Translation occurs in three steps:
   - **Initiation:** translation of mRNA with formation of initiation complex.
   - **Elongation:** elongation of polypeptide chain by sequential addition of amino acids to the growing end.
   - **Termination:** termination of polypeptide synthesis evoked by nonsense codon.

3. **Post-translational modification:** Post-translational modifications such as proteolytic degradation, hydroxylation, glycosylation, etc. make the protein more functional.

**Regulation of Gene Expression**

Gene expression is regulated by following mechanisms.

1. **Gene amplification:** Enhancement of gene expression can cause drug resistance. For example, amplification of the gene coding for dihydrofolate reductase causes development of drug resistance by cancer cells to chronic administration of methotrexate.

2. **Gene rearrangement:** This enhances the generation of antigen specific immunoglobulins.

3. **Regulation through transcription factors:** Transcription factor regulate interaction of protein with specific segments of DNA.

**The RNA**

Ribonucleic acid is made up of a single chain of polymers (polymer of ribonucleotides). Unlike DNA, which is double stranded, the RNA has a single strand. RNA is present in the nucleus, cytoplasm, ribosome and to some extent in mitochondria.

RNA differs from a DNA strand in many aspects such as:

1. RNA chain is much shorter in length than the DNA chains.
2. The sugar-phosphate that forms the backbone contains ribose instead of deoxyribose.
3. The base thymine is replaced by uracil.

**Types of RNA**

There are three types of RNA and they have different functions.
1. **Messenger RNA:** Messenger RNA (mRNA) is formed in the nucleus and enters cytoplasm for protein synthesis. It forms the template that directs the synthesis of protein molecules within ribosomes.

2. **Transfer RNA:** Transfer RNA (tRNA) conveys specific amino acids to the site of protein synthesis.

3. **Ribosomal RNA:** Nucleolus is the site of synthesis of ribosomal RNA (rRNA), which is associated with many proteins to form ribosomes, the protein-synthesizing machine.

**Application of Genetics in Medicine**

**Recombinant DNA Technology**

Recombinant DNA technology in genetic engineering refers to the process in which a DNA fragment of interest is transferred from one organism to a self-replicating genetic element such as a bacterial plasmid, which involves cutting, modifying and joining DNA molecules using enzymes such as DNA ligase and others. It is performed in four stages.

**Stage 1:** This is the stage of creation of a copy of gene essential for the purpose. This can be done by making a copy of the gene from its mRNA, by synthesizing the gene artificially or by chopping a part of DNA with restriction enzymes and searching for the part with the required gene.

**Stage 2:** This is the stage of joining the gene to a vector molecule, which is a DNA molecule with which the generated gene is attached for cloning. The usual vectors are plasmids, bacteriophages and cosmids.

**Stage 3:** This is the stage of introduction of vector DNA into the host cell to produce chimeric DNA or recombinant DNA.

**Stage 4:** This is the stage of cloning of chimeric DNA. The chimeric DNA contained in a vector is introduced into bacterial cells by the process called transfection. The host cell allows multiplication of the chimeric DNA of the vector that leads to production of large number of identical target cloned DNA molecules. The cloned DNA is released form the vector by cleavage, which is isolated, characterized and used for different purposes.

---

**Uses of Recombinant DNA Technology**

1. **Synthesis of hormones:** By recombinant DNA technology, hormones like insulin, growth hormone, erythropoietin etc, can be produced in large amount for therapeutic purposes.

2. **Laboratory diagnosis:** Diagnosis of diseases like AIDS has become simple by using recombinant DNA technology.

3. **Gene therapy:** It is useful in therapy for genetic defects.

4. **Use in forensic medicine:** DNA fingerprinting technique is useful in medicolegal cases.

5. **Use in agricultural purposes:** Recombinant DNA technology is used in generating genetically engineered plants to enhance the yield of crops.

**PCR and Blotting Techniques**

**Polymerase Chain Reaction**

Polymerase Chain Reaction (PCR) is a specific and very rapid method of amplifying a target DNA sequence. This involves denaturation of DNA (separation of double stranded target DNA into single strand by heating), cooling the single DNA strand and DNA amplification. The DNA amplification is achieved by formation of new DNA strand in the presence of enzyme DNA polymerase and the substrate deoxyribonucleotide triphosphate. PCR causes million fold amplification of target DNA.

**Scientist contributed**

Kary Mullis (Born 1944, North Carolina, USA)

Kary Mullis, PhD, who conceived PCR as a method to copy DNA and synthesize large amounts of a specific target DNA, was awarded the 1993 Nobel Prize in Chemistry.

**Uses of PCR**

PCR is highly sensitive and can detect the presence of even a single molecule of DNA. PCR is used for quick diagnosis of AIDS, DNA fingerprinting and sex identification.

**Blotting Techniques**

Blotting techniques are methodical techniques used for the identification of a particular DNA, RNA or a protein. There are three techniques: *southern blotting* (for DNA), *northern blotting* (for RNA) and *western blotting* (for protein).
Chapter 5: Physiology of Genetics and Apoptosis

Southern blotting
In southern blotting, DNA is extracted from the cells like leucocytes) and cleaved into fragments by restriction endonucleases.
1. DNA extracted and fragments are separated and denatured.
2. The DNA fragments are transferred into a sheet of nitrocellulose paper from the agarose gel by blotting.
3. Then DNA fragments are fixed to the membrane usually by ultraviolet cross-linking.
4. The labeled DNA is formed by hybrid complexes.
5. Following the hybridization reaction, the membrane is washed and regions of hybridization are identified by autoradiography.

Southern blotting is used for DNA fingerprinting and for detection of mutant gene that causes diseases like cystic fibrosis.

Northern blotting
Northern blotting is similar to southern blotting except that the RNA is used instead of DNA. Northern blotting is used for analysis of gene expression in a particular tissue.

Western blotting
Western blotting is used for identification of specific proteins. First, the SDS polyacrylamide gel electrophoresis is performed for the protein mixture, and then the electrophoresed protein bands are transferred from SDS polyacrylamide gel to a nitrocellulose membrane. Afterward, probing is performed using a labeled antibody probe. Western blotting along with ELISA is a confirmatory test for HIV.

Cloning
Cloning means production of many identical copies of a molecule. Generally, there are four types of cloning: Gene cloning, reproductive cloning, tissue cloning and embryo cloning.

Gene Cloning
Gene cloning, also referred to as DNA cloning or recombinant DNA technology is the process of transfer of a specific DNA fragment of one organism to a self-replicating genetic component of the cloning vector such as bacterial plasmid. Following transfer of the DNA fragment, the molecule is propagated in the host organism. Gene cloning is used widely in genetic engineering for sequencing genomes and in gene therapy.

Reproductive Cloning
Reproductive cloning is used to produce an animal having the same nuclear DNA from the existing animal. The Broad methodology:
1. The technique of reproductive cloning uses the principle of somatic cell nuclear transfer, in which genetic material from the adult donor cell is transferred to an egg from which the genetic material has been removed.
2. The new egg containing the DNA of the donor cell is allowed to divide by chemicals or electric current.
3. After attaining a suitable stage, the cloned embryo is transferred to the uterus of the host female where it grows into a complete fetus.

Reproductive cloning can be used to reproduce animals that are difficult to breed. The Dolly sheep is the first cloned animal (Roslin Institute in Edinburgh, Scotland, 1997).

Embryo Cloning
This is also called therapeutic cloning, which refers to the production of human embryos for research purposes. The aim of this is not to create a cloned human being, rather to yield stem cells to study human evolution and disease treatments. Stem cells are extracted in the blastocyst stage of development, which can practically generate any type of cells in the human body. Stem cells are used to replace degenerating cells as in Alzheimer’s disease, cancer, etc.

Tissue Cloning
This technique is called tissue culture, in which cells are allowed to grow in a suitable medium. The cloned cells are used to study of the action of hormones, antibiotics and pharmaceutical products.

Mutation
Mutation refers to a change in the DNA structure of a gene. Mutation is caused by mutagens, the factors that produce mutation. The known mutagens are X-rays, ultraviolet light, certain chemicals, etc. There are two major types of mutations: Point mutation and frame-shift mutation.

Point Mutation
In point mutation, one base pair of DNA replaces the other. This is of two types: transitions and transversion. Transition is the type of mutation in which one purine or pyrimidine is replaced by another purine or pyrimidine. In transversion type of mutation, a purine is replaced by pyrimidine or pyrimidine is replaced by purine.

Frame-shift Mutations
In this mutation, base pairs are either deleted or inserted into the DNA. Hence, this is also called deletion or inser - tional mutations. The deletion or insertion leads to change in the frame of mRNA. Therefore, mRNA becomes unable to recognize that a new base is added or missing. However, translation continues and proteins formed have many altered amino acid sequence.

Mutation results in diseases such as sickle cell disease, phenylketonuria, cystic fibrosis, etc.
Genetic Screening
Genetic screening is detecting the genetic variations in a human being. It is used for diagnosing diseases at various stages and for various purposes such as prenatal diagnosis, diagnosis of carrier states and prognostic diagnosis.

Prenatal Diagnosis
Prenatal diagnosis aims at identifying the health problems in the fetal stage and therefore has preventive values. This is performed by chorionic villus sampling, amniocentesis and pre-implantation diagnosis.

Diagnosis of Carrier States
A group of people carry and transmit the disease without suffering from it. The identification of such carrier people is important in preventing the spread of disease in the community. The examples of carrier states include sickle cell anemia, cystic fibrosis and phenylketonuria.

Prognostic Diagnosis
Some diseases like type II diabetes mellitus or Huntington’s chorea that are present in childhood are likely to occur in genetically susceptible individuals when they become adult or middle aged. Genetic screening of such people to identify the susceptibility of the disease helps in preventing the disease to occur.

Genetic Basis of Cancer
Some cancers such as cancer of colon and female breast, retinoblastoma, leukemia etc. have hereditary predisposition. Defective DNA and chromosomal abnormalities are among the etiological factors in the genesis of cancers like acute myeloid leukemia and Burkitt’s lymphoma. Ionizing radiations like exposures to ultraviolet rays produce cancer by causing mutagenesis (damaging the genetic constitution) of the individual.

Cancer Genes
There are genes that predispose to cancer and genes that prevent cancers. Cancer producing genes are oncogenes and defective P53 gene. Genes preventing cancer are tumor suppressor genes (or antioncogenes) and RB gene.

Oncogenes
Cancer causing genes are called oncogenes. There are more than 100 oncogenes have been described so far. The proto-oncogenes are normal genes that encode proteins controlling normal cell functions. Many factors stimulate conversion of proto-oncogenes to oncogenes. The factors that promote this conversion are:

1. Chromosomal translocation: In this, a part of chromosome is translocated to other chromosome. For example, an area of chromosome 8 in patients with Burkitt’s lymphoma is translocated to either of the chromosome 2, 14 and 22.
2. Missense mutation: In this, amino acid sequence of proto-oncogene changes that helps the protein to convert into oncogene.
3. Gene amplification: Amplification of some of the genes to become oncogene has been implicated in the genesis of lung, breast, stomach and colon cancer.

Defective P53 Gene
Normally, stimulation of P53 gene results in formation of P53 protein. P53 protein serves as a transcription factor for many physiological functions that prevent malignancy. These are:
1. P53 protein activates genes that promote DNA repair.
2. It activates genes that arrest cell division.
3. It also stimulates genes that help apoptosis.
   About half of malignancies are associated with defects in P53 gene.

Mutator Genes
Normally, damage to DNA is repaired by caretaker genes. Mutator gene is the faulty caretaker gene that has lost normal surveillance function and therefore helps in genesis of cancer.

Telomerase
Telomerase recognizes telomere in cell divisions. Cancer cells express more telomerase that promotes telomere lengthening and this helps in cancerous proliferation.

Genes preventing cancer
RB Gene
Retinoblastoma (RB) gene serves as cancer suppressor. Mutation or deletion of this gene results in retinoblastoma.

Tumor Suppressor Gene
These are also called anti-oncogenes that prevent genesis of cancer. More than ten such genes have been identified. These genes are inactivated by mutation. Thus, mutation causes cancer.

Gene Therapy
Hereditary disorders occur due to transmission of defective genes. Gene therapy aims at providing the correct copy of the gene concerned. This is achieved by:
1. Gene replacement: Replacement of a mutant gene with a normal gene
2. Gene correction: Correction of the specific bases of DNA
3. **Gene augmentation**: Insertion of a foreign DNA into the genome of a cell to rectify the genetic defect

### Somatic Cell Therapy

In humans, somatic (nongametic) cells are used in the gene therapy as changes in these cells cannot be inherited.

The broad steps are:

1. Cells are isolated with the defective gene from the patient and grown in culture.
2. Grown cells are transfected with a remedial gene construct.
3. Transfected cells are then transfused back into the patients.

It is successfully tried in **cystic fibrosis** of the lung and **severe combined immunodeficiency syndrome**. Without ADA (adenosine deaminase), the child develops SCID and dies of infection in the early childhood.

In **cancer**, gene therapy is very useful, in which oncogenic gene is inactivated by introducing a gene like tumor suppressor gene. This selectively kills the cancer cells.

### APOPTOSIS

Apoptosis is the **programmed cell death**. Apoptosis is a Greek word meaning 'falling off' or 'dropping off', first described 1972. It is a distinct process from necrosis. It is a form of coordinated and internally planned cell death, which is of greater significance in a variety of physiologic and pathologic conditions.

Apoptosis is the programmed cell death that has genetic control. **Genes of the own cells** play important role in this programmed cell death. Apoptosis is a natural process, and examples are:

1. Death of neurons in central nervous system during brain development and synapse formation is an example of apoptosis.
2. During fetal development, degeneration of many tissues like web in the fingers is other example.
3. Many blood cells like eosinophils undergo apoptosis.

### Mechanisms of Apoptosis

#### Stimuli

Activation of cysteine proteases in the cell called **caspases** triggers apoptosis. Normally, caspases are present in the inactive form in the cell, and stimulated by external and internal stimuli.

**Internal stimuli**: Mitochondria release cytochrome and a protein called *smac* that causes activation of the *caspase 9*, which induces apoptosis. In mitochondria, apoptosis inducing factor is located in the intermembrane space that migrates to nucleus and destroys DNA.

**External stimuli**: External stimuli are various ligands that bind with cell surface to activate apoptosis. One such factor is tumor necrosis factor that activates the enzyme *caspase 8*. Activation of caspase promotes DNA fragmentation and chromatin condensation.

### Molecular Mechanisms of Apoptosis

#### Initiation of Apoptosis

Stimuli signaling programmed cell death act either on the cell membrane or intracellularly.

(a) Usually it is triggered by absence of stimuli that are normally required for normal cell survival such as absence of certain hormones, growth factors, cytokines, etc.

(b) Activation of receptors like receptors of TNF initiates programmed cell death.

(c) Intracellular stimuli may include heat, radiation, hypoxia, etc.

(d) Genetically programmed events.

### Regulation of Apoptosis

Once apoptosis is initiated, certain intracellular proteins provide signal for the final programmed cell death, which actually determine the outcome. These **regulatory proteins** include the following:

(a) **BCL-2**: BCL-2 is a protein which is equivalent to CED-9, the cell death gene found in nematodes. BCL-2 is located in the outer mitochondrial membrane and control apoptotic process by binding to various cytosolic proteins. Usually it binds to BAX and BAD proteins that are known to promote apoptosis. It may also bind with BCL-XL protein that inhibits apoptosis. Another binding protein present in the cytosol is the pro-apoptotic protease-activating factor (apaf-1), which is a mammalian counterpart of gene CED-4 of nematode. Thus, apoptosis depends on binding of BCL-2 with pro-apoptotic and anti-apoptotic proteins.

(b) **Other apoptotic regulator proteins**: Other regulator proteins of apoptosis are TP53 (p53) protein, caspases, BAX and certain viruses such as adenovirus, papilloma virus, hepatitis B virus, etc.
**Steps of Apoptosis**

Apoptosis occurs in following major steps (Flowchart 5.2).

(a) **FAS receptor activation**: CD 95 or FAS, the surface receptor present on cytotoxic T cells when comes in contact with the target cell, is activated. This leads to activation of caspases and subsequent proteolysis.

(b) **Ceramide generation**: Hydrolysis of sphingomyelin on the cell membrane generates ceramide. Ceramide causes mitochondrial injury.

(c) **DNA damage**: Damage by various agents and activated oxygen species like free oxygen radicals leads to apoptosis. DNA injury influences nuclear protein TP53 that induces the synthesis of cell death promoting protein BAX.

(d) **Cell shrinkage**: Cell reduces in size and cytoplasmic condensation occurs.

(e) **Formation of apoptotic bodies**: Apoptotic bodies are formed as described below.

(f) **Phagocytosis**: The dead apoptotic cells and their fragments are identified and engulfed by phagocytes.

**Physiologic and Pathologic Processes**

Apoptosis is a biologic phenomenon, which has both physiologic and pathologic processes.

**Physiologic Processes**

Physiologic processes occur in three steps (Flowchart 5.3). They are influenced by many factors and examples are:

1. Physiologic involution of cells in hormone-dependent tissues such as endometrial shedding in menstrual cycles, regression of lactating breast after cessation of breast-feeding.
3. Involution of the thymus after childhood.

**Pathologic Process**

Pathologic processes are initiated by following factors.

1. Tumor cell death on exposure to chemotherapeutic agents.
2. Transplant cell death by cytotoxic T cells that cause transplant rejection.
3. Cell death induced by viral infections as occurs in viral hepatitis.
4. Pathologic atrophy following withdrawal of stimuli. For example, prostatic atrophy after orchietomy.
5. Cell death induced by radiation, hypoxia and thermal injury.
6. Degenerative diseases of CNS such as in Alzheimer’s disease, Parkinson’s disease, etc.

**Changes in Apoptosis**

**Pathophysiologic Changes**

Changes in apoptosis confuses with the changes in necrosis. In necrosis, cytoplasm is homogenously eosinophilic, and nuclear changes are pyknosis (condensation and clumping of nucleus), karyolysis (disintegration of the nucleus) and karyorrhexis (nucleus fragments into small bits and disperses into cytoplasm). In apoptosis, there is condensation of nuclear chromatin and cell fragments into apoptotic bodies that are phagocytosed by macrophages (Fig. 5.10).
The characteristic changes in apoptosis include the following:

1. May involve single cells or a cluster of cells. The apoptotic cells become round or oval and reduce in size. The cytoplasm becomes intensely eosinophilic containing condensed or fragmented nuclear chromatin material. Typically, inflammatory response around apoptosis is absent.

2. Though cytoplasm is reduced, organelles remain almost normal.

3. Cell membrane convolutes with formation of membrane-bound spherical structures called apoptotic bodies that contain compacted organelles.

4. Chromatic condensation occurs around the periphery of nucleus.

5. Phagocytosis of apoptotic bodies occurs by macrophages.

Biochemical Changes

Biochemical changes include:

1. Proteolysis of cytoskeletal proteins.
2. Cross-linking of protein molecules.
3. Fragmentation of nuclear chromatin by activation of nuclease.
4. A glycoprotein molecule called thrombospondin and a phosphoprotein called phosphatidylserine appear on the outer surface of apoptotic bodies, which facilitate recognition by macrophages for phagocytosis.

Application

Identification of components of the cell death control and effector mechanisms and the linkage of abnormalities in cell death to human disease, in particular cancers, has unraveled the research for preventing the cell death. Understanding the concept of apoptosis has promising role in future regenerative medicine.

CHAPeR SUMMARy

Physiology of genomics is a developing branch of medicine. It deals with the understanding of the concept of gene and gene therapy in treatment of genetic disorders. Discovery of cancer genes (oncogenes) and genes preventing cancers has changed the concept and modality of treatment of malignancies. Understanding the concept of apoptosis has promising role in regenerative medicine.

Important to Know (Must Read)

1. Long Questions may be asked on ‘Recombinant DNA technology and PCR’ or ‘Mechanism of Apoptosis’ for PGs, but not for UGs.
2. Structure and function of DNA, protein synthesis, blotting techniques, cloning, genetic screening, gene therapy, and oncogenes, may be asked as Short Notes/Questions in exams.
3. In Viva, examiners usually ask on structure and functions of chromosomes, genome, chromosomal disorders, nuclear sexing, gene amplification, uses of recombinant DNA technology and PCR, types of cloning with examples, types of mutation, importance of genetic screening, types of oncogenes, tumor suppressor genes, types of gene therapy, and definition and changes in apoptosis.
Chapter 6

Transport Across the Cell Membrane

Leaming Objectives

On completion of study of this chapter, the student MUST be able to:
1. Name common ion channels in the membrane; and name different types of carrier proteins with examples.
2. Classify transport processes and list the differences between passive and active transport mechanisms.
3. Describe the mechanism, factors affecting and physiological application with example of each transport process, especially of diffusion and osmosis.
4. Explain the primary and secondary active transport processes with examples.
5. Describe the structure, mechanism of action and functions of Na⁺-K⁺ ATPase.
6. Describe the mechanism and importance of endocytosis, exocytosis and transcytosis.

The student MAY also be able to:
1. Describe the gating of ion channels.
2. Describe types of patch clamping.
3. Explain the properties of facilitated diffusion.
4. Define the concept of osmole and application of osmosis in clinical conditions.
5. Describe other types of ATPases.

Fluid and electrolytes on either side of the cell membrane pass through the membrane by various means due to the presence of different channels and carrier molecules. Selective membrane permeability generates the difference in composition between intracellular and extracellular fluids. Transport of specific substances, like drugs, chemicals and hormones also influence cell functions.

Passage of substances through the membrane can be broadly divided into two categories:

1. Direct passage without involvement of carrier molecules: Cell membrane is a lipid bilayer. Hence, lipid-soluble substances, such as gases, fatty acids, alcohol, ketone bodies, aldehydes and many small-uncharged molecules pass through the membrane easily. Water molecules also pass through membrane easily.

2. Passage through channels or carrier proteins: Though water passes easily through the membrane, water-soluble substances, such as electrolytes, glucose and amino acids do not penetrate membrane readily. There are two major means through which these substances are transported across the cell membranes: through water channels formed by integral proteins, and by combination with carrier molecules in the cell membrane.

Protein Channels in the Membrane

Proteins that constitute channels are selectively permeable. They are tubular structures that connect the exterior with the interior of cells. The permeability of channels depends on molecular size, shape and charge.

Types of Protein Channels

Broadly, they are of two types: ion channels and water channels.

Ion Channels

Ion channels are integral proteins that span the entire width of the membrane. Normally, they are formed by several polypeptide subunits.

Mechanism of working of ion channels:
Along the length of the integral protein, an aqueous pore is present, around which the polypeptide subunits are arranged. Ions pass through the aqueous pore from one side of the membrane to the other. Thus, ions cross the membrane without entering through the lipid bilayer of the membrane.

A polypeptide subunit forms a gate at one end of the channel that opens in response to a specific stimulus (Fig. 6.1).

Opening of the gate opens the channel and allows the ions to rapidly move ($10^8$ ions per sec) through the channel.

However, there is a selectivity filter that permits specific ions to pass through the channel. Therefore, ion channels are often selective.

Sodium and potassium channels are extensively studied membrane channels.

**Sodium Channels**

Sodium channels have been studied in detail among all the ion channels. It has subunits surrounding an aqueous pore of about 0.5 nm in diameter. The inner surface of the pore is negatively charged. It is selective for passage of sodium. Sodium channel is blocked by tetrodotoxin.

**Potassium Channels**

There are many types of potassium channels. They are about 0.3 nm in diameter. Potassium channels are blocked by tetraethyl ammonium or 4-aminopyridine.

**Calcium and Chloride Channels**

There are various channels for Ca$^{++}$ and for Cl$^-$.

Ca$^{++}$ channels can be voltage-gated, ligand-gated and stretch-sensitive (for details, refer to chapter 71). Cl$^-$ channels are dimeric (have two pores) and pentameric (e.g. GABA$_A$ and glycine receptors). CFTR protein is a Cl$^-$ channel.

**Water Channels**

Water channels are aquaporins. There are 13 types of aquaporins (for details, refer to ‘Water reabsorption from kidney’ in ‘Renal Physiology’).

**Gating of Ion Channels**

Some of the ion channels remain always open, and, therefore, they are referred to as nongated channels. However, many ion channels open and close by gates. Ion channels are provided with gate on either side of the channel, and opening or closure of the gate regulates the movement of various ions through them.

There are three general mechanisms of gating: voltage-gating, ligand-gating and mechanical-gating.

**Voltage-gating**

Change in the membrane potential beyond a certain threshold value opens or closes the gate of the ion channels. Therefore, they are called voltage-gated channels. For example, ion channels in excitable tissues, such as nerve and muscle are mainly voltage-gated channels like Na$^+$, K$^+$ and Ca$^{++}$ channels. They are also present in pacemaker tissues in the heart and other organs. They are involved in generating and conducting action potentials.

**Mechanism of gating:**

1. The exact mechanism of voltage-gating is not known. It is proposed that alteration in membrane potential induces movement of some charged amino acids in helical segment of the channel protein that causes a conformational change of the channel protein, which in turn opens or closes the gate and allows the ions to pass through the channel rapidly.

2. For Na$^+$ channels, the gate is located on the outer end of the channel (Fig. 6.2A). The gate closes the outer opening of the channel when the cell is at RMP (–70 mV in nerve, –90 mV in muscle and –50 mV in pacemaker tissue). When inside becomes less negative, the gate opens and Na$^+$ influx occurs that leads to first, formation of local response and later, the genesis of action potential.

3. For K$^+$ channels, the gate is located on the inner end of the channel (Fig. 6.2B). The gate closes the cytoplasmic opening of the channel when the cell is at RMP. When interior becomes less negative and approaches towards positivity, more and more gates open and K$^+$ efflux occurs that leads to first, reversal of depolarization potential and later, the repolarization of action potential.

**Important Note**

Speed of opening of ion channel determines the slope of potential: Opening of K$^+$ channels is slower than the opening of Na$^+$ channels. Therefore, in action potential, the phase of repolarization, which is due to K$^+$ permeability, is less steep than the phase of depolarization.
**Ligand Gating**

Interaction of the channel with a ligand (hormone or neurotransmitter) causes opening or closure of the channel. Hence, the channels are called *ligand-gated channels*. As the ligand is a chemical agent, the gating is also called *chemical gating*.

**Mechanism of ligand gating:**
1. The chemical agent binds with the specific receptor protein on the membrane and brings about conformational change in the protein that, in turn, directly or indirectly opens the channel.
2. **Nicotinic cholinergic receptor** channel in postsynaptic neuromuscular junction is a ligand-gated ion channel. Acetylcholine released at the nerve ending binds with these receptors and opens the ion channels that generate postsynaptic potential.
3. Acetylcholine at the parasympathetic endings binds with *muscarinic cholinergic receptors* on the membrane of GI smooth muscle cells and activates G protein, which, in turn, activates the enzyme that causes production of *second messengers* such as cyclic AMP or Ca++. The second messengers activate enzymes like kinase that act on the ion channel by phosphorylation.
4. Other examples are the action of noradrenaline on β-adrenergic receptors on the smooth muscle cell.

**Direct opening of channels:** The receptor for the hormone or neurotransmitter may also be the channel itself, so that the action by the ligand is direct on the channel. For example, acetylcholine released at parasympathetic nerve endings acts on the receptor in the postsynaptic membrane in the cardiac pacemaker cells. This causes direct opening of K⁺ channels in the pacemaker tissue and causes hyperpolarization that decreases the discharge rate of the pacemaker.

**Mechanical Gating**

Some of the channels are *mechanosensitive* and they respond to stretch. Examples are *ion channels in hair cells*.
Types of Patch Clamping

There are three types of patch clamping: cell-attached patch, whole-cell patch and inside-out patch.

Cell-attached patch: In this clamping, the patch of the membrane remains attached with the cell.
Whole-cell patch: In this type of clamping, the patch of the membrane provides access into the interior of the cell.
Inside-out patch: In this method, the patch of the membrane is pulled from inside of the membrane to the outside of the membrane.

However, the patch of the membrane is small enough to contain one or two channels. The channel characteristics can be studied by changing the chemical and electrical gradients across the membrane.

Uses of Patch-clamp Technique

1. The patch clamp technique is an essential tool for scientists studying the activity and behavior of ion channels in cell.
2. This helped to understand how defective regulation of ion channels underlies a host of diseases, including diabetes and cystic fibrosis.
3. Mechanisms of action of drugs that use or block ion channels such as Ca$^{++}$ or K$^+$ channels blockers were studied using this technique.

Scientists contributed

Erwin Neher

Bert Sakmann

The 1991 Nobel Prize in Physiology or Medicine was awarded to two German scientists, Erwin Neher and Bert Sakmann for creating the experimental measuring device that conclusively proved the existence and function of ion channels.

Neher and Sakmann Model

An extremely fine glass pipette with a very small opening is used to make contact with a tiny area, or patch, of the cell's outer membrane that will contain only a single ion channel. Applying a small amount of suction through the pipette forms a seal tight enough such that ions can only flow from the channel into the pipette. By fitting the pipette with a highly sensitive electrode, Neher and Sakmann could record every minute change in current produced as ions flow through the clamped channel into the pipette.

1. With their recording device, they showed how these channels function by opening up and closing in nerve cell membranes to allow certain ions through one at a time.

Types of Carrier Proteins

There are three different types of carrier proteins: Uniport, symport and antiport (Fig. 6.4).

Uniport: Uniport is the carrier protein that transports only one substance. For example, Na$^+$ channels transport Na$^+$ and K$^+$ channels transport K$^+$.

Symport: Symport carriers transport two or more substances from one side of the membrane to the other in the same direction. Transport of Na$^+$ and glucose from the lumen of the intestine or kidney tubule into the epithelial cells through the same carrier protein is an example of symport mechanism. Such substances are said to be co-transported.

Antiport: Antiport carriers transport substances in opposite directions in which one substance is transported to the inside of the cell and other substance from inside the cell to the outside. Substances transported in opposite directions by one carrier are said to be counter-transported. Examples are:

1. A typical antiport carrier is the sodium-potassium pump, which is operated by Na$^+$-K$^+$ ATPase. It actively transports 3 Na$^+$ out of the cell, and 2 K$^+$ into cells for each molecule of ATP hydrolyzed.
2. Na$^+$-H$^+$ exchanger found in the proximal tubular cells of the nephron, for which the inward facilitated diffusion of Na$^+$ is coupled with the outward diffusion of H$^+$. 

Fig. 6.4: Types of carrier proteins in the cell membrane. Uniport transports one substance, symport transports two substances in the same direction and antiport transports two substances in the opposite directions.
Characteristics of Carrier-mediated Transport

Carrier-mediated transport systems exhibit two important properties: Competition for the carrier protein and the saturation of the transport process.

Competition inhibition: Presence of different molecules with similar structure competes for the same carrier protein for their transport, which results in competitive inhibition of the process. For instance, the presence of ketoses in intestinal content decreases the reabsorption of aldoses by the gut.

Saturation kinetics: The number of carrier proteins in a membrane is limited. Therefore, the rate of carrier-mediated transport reaches a maximum which is known as transport maximum or \( T_m \). The \( T_m \) cannot be exceeded by increasing the concentration of the substance. Thus, the carrier-mediated transport exhibits saturation kinetics.

TRANSPORT PROCESSES

Types

Transport mechanisms through cell membrane can broadly be divided into three types: passive transport, active transport, and vesicular transport (Table 6.1). There is another process of transport called transport across epithelia.

Special Features

Passive Transport

There are two common features of passive transport processes (Table 6.2). These are:

a. **Transport is downhill**: In passive transports, the substances pass through the membrane from both sides, however, net movement occurs down the electrochemical gradient;

b. **Transport does not require metabolic energy**: Passive transport does not require utilization of ATP.

Examples are simple diffusion, facilitated diffusion, osmosis, filtration, bulk flow and solvent drag.

Active Transport

Active transport differs from passive transport by its utilization of energy. The common features of active transport are (Table 6.2):

1. **Uphill transport**: Transport occurs against the electrochemical gradient of the substance.
2. **Requires energy**: Energy utilized for transport is derived from the breakdown of ATP. Hence, they are susceptible to metabolic poisons.
3. **Exhibits saturation kinetics**: This is because of limitation in the rate of availability of carriers or supply of energy.

The active transport mechanisms are:

1. Primary active transport.
2. Secondary active transport.

Vesicular Transport

This transport has no relation with the concentration gradient. However, it may utilize metabolic energy.

Vesicular transport mechanisms are:

1. Exocytosis.
2. Endocytosis.

SIMPLE DIFFUSION

Diffusion is the process of passive transport in which molecules move from the area of higher concentration to the area of lower concentration. In simple diffusion, molecules move due to their random movement. The examples of substances that pass through cell membranes by simple diffusions are transport of \( O_2 \), \( CO_2 \), urea, ammonia and ions. Across a membrane, diffusion of a molecule occurs when difference in concentration of the molecule exists on both sides of the membrane. The net movement of molecule ceases when the concentration of molecule on both sides becomes equal and a diffusional equilibrium is achieved.

Table 6.1: Types of transport processes.

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<td>1. Simple diffusion</td>
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<td>2. Facilitated diffusion</td>
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<td>3. Osmosis</td>
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<td>4. Filtration</td>
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<td>5. Bulk flow</td>
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<td>6. Solvent drag</td>
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<th>B. Active transports</th>
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<td>1. Primary active transport</td>
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<td>2. Secondary active transport</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>C. Vesicular transports</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Exocytosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Endocytosis</td>
<td></td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>D. Transport across epithelia</th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>1. Transcellular transport</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Paracellular transport</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 6.2: Differences between active and passive transport processes.

<table>
<thead>
<tr>
<th></th>
<th>Active transport</th>
<th>Passive transport</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Nature of transport</td>
<td>Against electrochemical gradient</td>
<td>Along the gradient</td>
</tr>
<tr>
<td>2. Utilization of energy</td>
<td>ATP hydrolysis occurs</td>
<td>Not needed</td>
</tr>
<tr>
<td>3. Saturation kinetics</td>
<td>Is a feature</td>
<td>May not be present</td>
</tr>
</tbody>
</table>
Factors that Determine Rate of Diffusion

Factors determining the rate of diffusion across the cell membrane can be broadly divided into two categories: (a) properties of the substance and (b) the properties of the membrane.

Properties of the Substance

Properties of the substance that determine diffusion through membrane are mainly, (a) concentration and electrical gradients of the substance, and (b) permeability of the substance through the membrane.

Effect of Concentration and Electrical Gradients

The concentration or the chemical gradients of the substance is primarily responsible for the direction of transport of the substance (Fig. 6.5). The electrical potential difference (electrical gradient) also influences.

1. Normally, the cell membrane is polarized with the negative interior and positive exterior. Therefore, a positively charged molecule that diffuses from the outside to the negative interior is said to be moving along its electrical gradient, and its diffusion from inside to the outside is said to move against its electrical gradient.
2. Usually, substances move along both the electrical and chemical gradients (transfer along the electro-chemical gradients). Electrochemical gradient is mainly used for the ions.
3. For gases, concentration gradient is expressed as partial pressure, and for water as hydraulic pressure.

Thermal energy contributes to random motion: In a liquid or gas, all particles are in constant and random motion due to thermal energy. The motion varies directly with temperature. The motion is absent at absolute zero temperature. This random movement of particle provides the physical basis for diffusion of solutes and gas molecules in body fluids and across the cell membranes.

Unidirectional flux: When two liquids are separated by a semi-permeable membrane, the number of particles in the liquid striking a unit area from each side of the membrane at any given time is proportional to the concentration of the substance on that side. These particles succeed in passing through the membrane along the gradient, which is called unidirectional flux.

1. Unidirectional flux is proportional to the concentration of the substance in the liquid.
2. Similarly, the unidirectional flux of the particles in the opposite side of the membrane is proportional to the concentration of the substance on that side of the liquid.
3. Hence, net diffusion is the difference between the opposing unidirectional fluxes.
4. Diffusion across the membrane ceases when the balance between the fluxes from both sides becomes zero. A distinguishing feature of simple diffusion is the linear relationship between solute concentration and rate of diffusion (Fig. 6.6).

Permeability of the Substance

Permeability refers to the ease at which substances pass through the membrane.

The factors that determine the permeability are:

1. Lipid-solubility: As the membrane is a lipid bilayer, the permeability is proportional to their lipid solubility of the substance.
2. Molecular size: For water-soluble substances like ions that pass through aqueous channels, permeability is inversely proportional to their molecular size. For example, a cation like Na⁺ attracts more water in its hydration shell than K⁺. The atomic weight of Na⁺ is 23, whereas that of K⁺ is 39. However, the effective molecular size of the hydrated Na⁺ is larger than K⁺. Therefore, K⁺ permeability at rest is much more than the permeability of Na⁺.

3. Charge on the molecule: Since membrane is polarized with its negative interior, cations easily enter from outside into the cell and anions in the opposite direction.

4. Temperature: Rate of diffusion is high at higher body temperature as temperature facilitates motion of molecules in the solution.

5. Distribution of channels in the membrane: The number of protein channels for the substance in the membrane determines the permeability of the substance through the membrane.

6. Pressure gradient: In addition to the electrochemical gradients of the substance, the fluid pressure gradient also contributes to the diffusion of the membrane. Diffusion is more from high pressure to low pressure, as the pressure causes greater number of molecule to hit the membrane.

Properties of the Membrane

In addition to the concentration gradient of the substance across the membrane, the cross-sectional area and thickness of the membrane influence diffusion. These factors are governed by Fick’s law of diffusion. The rate of diffusion of a molecule through a membrane is proportional to the surface area (A) available for diffusion, and inversely proportional to the thickness of the membrane (T).

According to Fick’s law, for a substance whose inside and outside concentrations are respectively $C_i$ and $C_o$ mmol/l:

$$-\text{Net Rate of Diffusion (J)} = \frac{-DA}{T} \times (C_i - C_o),$$

where D is the diffusion coefficient.

The minus sign indicates that the direction is down the concentration gradient. As thickness of biological membranes is fairly constant at $10^{-6}$ cm, D/T simplifies to the permeability coefficient ‘P’ of the membrane, and

$$\text{Flux} = -P \times A \times (C_i - C_o)$$

Facilitated Diffusion

When diffusion is facilitated by a carrier protein in the membrane, the process is called facilitated diffusion. This is also called carrier-mediated diffusion as a carrier protein facilitates it. A typical example of facilitated diffusion is the transport of various sugars into red cells, adipose tissue, skeletal and cardiac muscles. Like simple diffusion, facilitated diffusion is also a downhill transport that does not require energy.

Features of Facilitated Diffusion

It differs from simple diffusion by four special features:

1. Faster rate of transport
2. Saturation kinetics
3. Competitive inhibition
4. Specificity.

Faster Rate of Transport

Carrier-mediated (facilitated) diffusion allows the transport of polar or hydrophilic molecules at a much faster rate than the rate expected from their partition coefficient. Therefore, inspite of saturation point for the transfer, the net transport is more than the simple diffusion (Table 6.3).

<table>
<thead>
<tr>
<th>Important Note</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Partition coefficient:</strong> Partition coefficient is the solubility of the substance in oil compared with the solubility in water. Lipophilic substances have high and hydrophilic substances such as ions and sugars have low partition coefficient. Therefore, hydrophilic molecules diffuse at a much faster rate through the membrane.</td>
</tr>
</tbody>
</table>

Saturation Kinetics

In simple diffusion, the rate of diffusion is proportional to the concentration of the substance and there is no saturation point. In facilitated diffusion, the number of carrier proteins available determines the rate of diffusion. When all the available binding sites on the carrier proteins are occupied, the system operates at the maximum capacity. This is called saturation point. However, the rate of diffusion is faster in facilitated diffusion compared to simple diffusion, as there is no involvement of carrier protein in simple diffusion. Thus, the net diffusion in facilitated type is more than in simple type (see Fig. 6.6).

Competitive Inhibition

Many substances share same carrier protein for their transport. When more than one such substance are present on the same side of the membrane, they compete to bind with the carrier protein. Thus, one substance decreases the transport of the other. For example, transport of D-glucose across the epithelial membrane slows down once D-galactose is present along with it as they share same glucose transporter. Also, Na⁺ and Ca⁺⁺ compete for the sodium-calcium cotransporter on the membrane and excess presence of one inhibits the transport of the other. However, it should not be confused with sodium-calcium exchanger and sodium-glucose cotransporter.

1. It should not be confused with sodium-calcium exchanger that promotes the transport of both ions, in which increased concentration of one on one side of the membrane increases the transport of the other from opposite side of the membrane.
2. Also, this should not be confused with carrier-mediated transport mechanisms by co-transporters like sodium-glucose cotransporter that are essentially facilitatory for transport of more than one substance (Application Box 6.1).
Chapter 6: Transport Across the Cell Membrane

Application Box 6.1

Salt is mixed with glucose in ORS: For example, presence of sodium in intestinal fluid increases the absorption of glucose from the gut content by the intestinal epithelial cells, which forms the physiological basis of adding sodium and glucose in equal proportion in the oral rehydration solution (ORS).

Specificity

The carrier proteins are specific for different molecules. For example, the carrier protein for protein does not transport cholesterol or fatty acid. However, specificity is not a rigid phenomenon as, more than one substance share the same carrier protein as for example, sodium-glucose co-transporter. Facilitated diffusion occurs through ion channels as occurs in sodium-glucose cotransport, in which glucose shares the sodium transport mechanism.

Factors Affecting Facilitated Diffusion

Factors that influence simple diffusion also affect facilitated diffusion as described above. However, the major factor is the number of carrier proteins available for the transport. Sometimes, facilitated diffusion is also operated by other factors like hormones. For example, transport of glucose by GLUT-4 into muscles and adipose tissue is insulin-dependent.

OSMOSIS

Definition: Osmosis is the process of movement of solvent from the solution with the lower concentration of solutes to the solution with higher concentration of solute, when both the solutions are separated by a semipermeable membrane.

Concept: Like solute particles, water (solvent) molecules are constantly influenced by thermal disturbance and, therefore, exhibit random movement (called thermodynamic activity of water).

1. They pass through a semipermeable membrane, and their passage is proportional to the solvent molecules on that side.
2. If the membrane that separates two solutions of different solute concentrations is semipermeable, which allows the passage of solvent and not the solute particles, the solution with higher concentration solute will have lower thermodynamic activity of water, and vice versa.
3. Net water movement will, therefore, be from the solution with a higher thermodynamic water activity to the solution with lower thermodynamic water activity, i.e., from the side with lower solute concentration to the side with higher solute concentration (Figs. 6.7A and B).
4. Thus, the net flux of water (or solvent) through a semipermeable membrane from a solution of lower solute concentration to that of higher solute concentration is known as osmosis.

Osmotic Effectiveness of a Substance

A substance to maintain a stable osmotic pressure should be confined to one side of the membrane. Therefore, a

Table 6.3: Differences between simple diffusion and facilitated diffusion

<table>
<thead>
<tr>
<th></th>
<th>Simple diffusion</th>
<th>Facilitated diffusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Mode of diffusion</td>
<td>No carrier molecule involved</td>
<td>Carrier molecule involved</td>
</tr>
<tr>
<td>2. Saturation kinetics</td>
<td>No saturation kinetics, diffusion is linear</td>
<td>Has saturation kinetics. No increase in diffusion once saturation is reached</td>
</tr>
<tr>
<td>3. Competitive inhibition</td>
<td>Absent</td>
<td>Substances that share the same carrier protein compete for transport</td>
</tr>
<tr>
<td>4. Rate of diffusion</td>
<td>May be slow</td>
<td>Faster</td>
</tr>
<tr>
<td>5. Specificity</td>
<td>No specificity</td>
<td>Carrier protein may be specific</td>
</tr>
</tbody>
</table>

Figs. 6.7A and B: The process of osmosis. (A) Compartment ‘a’ containing more number osmotically active solutes is separated by a semipermeable membrane from compartment ‘b’ that contains less number of solutes (solute particles are represented by black dots). Solvent movement from ‘b’ to ‘a’ is prevented by application of osmotic pressure on ‘a’. (B) Solvent (not the solute) moves from ‘b’ to ‘a’ (indicated by arrow mark) till osmotic equilibrium is reached, which results in increase in volume of ‘a’.
substance like urea, which can diffuse readily across cell membrane, cannot impart sustained osmotic effects. Hence, urea is said to be **osmotically ineffective**. Whereas a substance like glucose, which cannot freely diffuse through cell membrane because of its large molecular size, is osmotically active. However, glucose is not permanently osmotically effective as it is metabolized especially in blood, and, therefore, its effect is not sustained.

A better example of **osmotically most effective** substance is **plasma protein** as it is neither transferred from nor metabolized in the compartment. Other examples are various complex polysaccharides such as dextran. Sodium chloride is also osmotically effective even though Na⁺ and Cl⁻ ions can diffuse through the cell membrane (Clinical Box 6.1).

### Clinical Box 6.1

**Normal saline is effective in hypovolemia:** The application of osmotic effectiveness is important in the management of hypovolemia. A solution used to restore circulating blood volume should be the one whose active osmotic constituent remains within the circulation for a longer duration. Therefore, although 5 per cent glucose is isosmotic with plasma, it is usually not used to treat hypovolemia as it is rapidly metabolized, whereas 0.9% NaCl (the normal saline) is the satisfactory replacement for volume loss.

### Osmotic Pressure

Osmotic pressure is the minimum pressure applied to the solution with higher solute concentration to prevent osmosis. When the membrane is impermeable to an osmotically active solute, osmotic flow of water ensues and continues into the side containing the solute until either the membrane bursts (osmotic lysis of cells), or some hydrostatic pressure prevents further osmotic flow. The **hydrostatic pressure necessary to prevent osmotic flow of water** is known as the **osmotic pressure** of the solution.

Osmotic pressure depends on the number of molecules or the ions dissolved in a solution rather than the nature of chemical composition.

1. In case of **nondissociated solutes**, 1 gm mol wt of any substance shall contain similar number of molecules and, therefore, exerts similar degree of osmotic pressure (equal to 22.4 atmospheres).
2. In case of **dissociated solutes**, osmotic pressure depends on the number of molecules resulting from the dissociation.

Osmotic pressure in body fluid is mainly exerted by osmotically active solutes dissolved in the fluid such as **colloidal substances**. Hence, the osmotic pressure is called **colloidal osmotic pressure** (Application Box 6.2).

### Application Box 6.2

**Onctic pressure determines rate of capillary filtration:** In the plasma, the colloidal particles are proteins. The osmotic pressure due to presence of plasma proteins is called **onctic pressure**. The normal onctic pressure is **25 mm Hg**. Ongtonic pressure significantly contributes to filtration across the capillary membrane. Therefore, edema occurs in hypoproteinemia.

### Terms Used in Osmosis

**Mole**

A mole (standard SI unit) is the molecular weight (MW) of a substance in grams, i.e. the gram molecular weight. For example, the MW of glucose is 180, so its mole is 180 gm; and for NaCl, the mole is 58.5 gm.

**Osmole and Milliosmole**

The concentration of osmotically active particles is usually expressed in osmoles (Os). One osmole equals the gram molecular weight (i.e. one mole) of the substance divided by the number of freely moving particles each molecule liberates in solution. The **milliosmole** (mosm) is 1/1000 of 1 Os.

1. If a solute is a **non-ionizing compound** like glucose, one osmole is equal to 1 mole of solute particle. Thus, 1 molar solution of glucose has a concentration of 1 Os (1 osmole per liter).
2. If the solute is an **ionizing compound** like NaCl, each ion is an osmotically active particle. Therefore, in 1 molar solution of NaCl, NaCl would dissociate into Na⁺ and Cl⁻ ions, so that each mole in solution would supply two osmoles of solute per liter of solution. Similarly, one mole of CaCl₂ would dissociate into Ca²⁺, Cl⁻ and Cl⁻, and, thus, supplying 3 osmoles.

### Osmolality and Osmolarity

**Osmolality** of a solution refers to the number of osmoles (number of osmotically active particles) dissolved in a kilogram of water. **Osmolarity** refers to the number of osmoles in one liter of plasma.

1. Unlike osmolality, the value in osmolarity is affected by the volume of other solutes in the solution.
2. Osmolarity is also affected by temperature.
3. Though osmotic pressure is determined by osmolality, the difference between osmolality and osmolarity is negligible.
4. Osmolarity is frequently used in physiology for its easy measurement.

### Important Note

**Osmoles determine osmotic pressure:** Note that the important factor determining the osmotic pressure of a solution is the concentration of the particles released in solution (i.e. the osmoles), not the size, shape or charge of the particles.

### Plasma Osmolality

The total sum of all the particles in plasma determine its osmolality, and over 90 per cent of the osmolality of plasma is due to NaCl. The plasma proteins contribute very little, even though their molecules are large in size. The **normal plasma osmolality is 290 mOsm per kg**, out of which 270 mOsm is due to the effect of NaCl.
Measurement of Osmotic Pressure

By Freezing Point Depression

Osmometers are used to measure osmotic pressure. The molar concentration of a solute in a solution determines the osmotic pressure, and also the vapor pressure and freezing point. A nonvolatile solute will depress both the vapor pressure and the freezing point in a predictable manner, which provides the basis for determining the osmotic pressure of a solution by osmometers. One mol per liter depresses the freezing point of water by 1.86°C. For human plasma, the average freezing point is –0.54°C, which corresponds to an osmolality of 290 mOsm/kg.

Tonicity

Tonicity refers to the osmolality of a solution in relation to plasma (same osmotic pressure or freezing-point depression as plasma).

Isotonic
Solutions, which have osmolality same as that of plasma, like 0.9% NaCl, are said to be isotonic.

Hypotonic
Solutions with lower osmolality are said to be hypotonic.

Hypertonic
Solutions with higher osmolality than that of plasma are said to be hypertonic.

The solution of 0.9% NaCl is isotonic, and, therefore, red cells do not change their shape and size in this solution. In hypotonic solutions, red cells undergo osmotic lysis due to endosmosis and in hypertonic solutions, they shrink due to exosmosis (Clinical Box 6.2).

Clinical Box 6.2

Hypersmolal coma: In diabetes, increased plasma osmolality due to very high plasma glucose concentration causes shrinkage of cells. Especially, dehydration of brain cells leads to coma, which is an acute medical emergency. In chronic renal failure, very high urea and creatinine can cause encephalopathy.

A solution may be isotonic initially, but later becomes hypotonic if the osmotically active particles are transferred into the cell or metabolized. For example, 5% glucose solution is isotonic and remains so temporarily when infused intravenously. However, as glucose is rapidly metabolized, the net effect of infusion is like the infusion of a hypotonic solution. Therefore, infusion of normal saline (0.9% NaCl) is preferred in volume depletion, as described above.

Using Van’t Hoff Equation

The osmotic pressure produced by a concentration difference can be calculated by the Van’t Hoff equation, which is: \( p = C \times R \times T \), where \( p \) is the osmotic pressure (mm Hg), \( C \) is the difference in the concentration of particles between the two solutions (mOsm/l), \( R \) is the natural gas constant (62 mm Hg × 1/mmol × °K), and \( T \) represents absolute temperature (°K).

Measuring Equivalent Hydrostatic Pressure

In experimental set up, osmotic pressure can be measured by measuring the hydrostatic pressure applied to prevent water from entering the solution with higher solute concentration.

OTHER TRANSPORT PHENOMENA

Filtration, Bulk Flow and Solvent Drag

Passage of water and solutes through capillary wall is the example of filtration and bulk flow. Capillary wall separates plasma from interstitial fluid. Water moves out of capillaries when the net hydrostatic pressure exceeds net osmotic pressure and from interstitial space into the capillaries when the net osmotic pressure exceeds the net hydrostatic pressure. This is called filtration.

Filtration
Filtration is defined as the process by which fluid is forced through a membrane mainly because of the difference in hydrostatic and oncotic pressures on two sides.

Bulk Flow
When filtration results in movement of greater quantity of water, the process is called bulk flow.

Solvent Drag
During bulk flow of water, it carries with it, dissolved particles (solute), a phenomenon known as solvent drag.

Donnan Effect

Presence of nondiffusible ion on one side of the membrane affects the distribution of other ions to which membrane is permeable. This results in asymmetrical distribution of ions across the cell membrane.

Gibbs-Donnan Effect

The asymmetrical distribution of ions across the cell membrane at equilibrium has the following effects:

i. There will be an electrical difference across the cell membrane whose magnitude can be determined by Nernst equation.

ii. Because of presence of protein anions (prot- ) in the cells, there are more osmotically active particles in the cells than the interstitial fluid. This can lead to osmotic swelling and rupture, which is prevented by Na+-K+ pump that produces net movement of positive charge out of the cell and keeps the inside and outside of the cell in osmotic equilibrium. Thus normal cell volume and pressure depend on Na+-K+ pump.
**Nonionic Diffusion**

Ions are present either in undisassociated (nonionic) or disassociated (ionic) form. In ionic form they can not be easily transported across the cell membrane as membrane is charged. However, in their undisassociated (nonionic) form they diffuse through the membrane easily. This is called **nonionic diffusion**.

1. Many ions in the body pass the membrane in undisassociated form and then, they dissociate to their ionic form once they reach the other side of the membrane.
2. Nonionic diffusion occurs regularly along the epithelial membrane of kidney and GI tract.

**ACTIVE TRANSPORT**

The transport process that utilizes energy and occurs against the gradient is the active transport. By passive transport processes, the composition of intracellular fluid tends to equalize with that of composition of extracellular fluid. However, this should never happen practically, as it threatens cell volume and intracellular solute concentrations that are not compatible with life. Therefore, nature maintains inequality of fluid composition of intracellular and extracellular compartments by providing special transport mechanisms to the cell membrane that oppose these equilibrating transport processes. These transport processes are active processes. In these processes, substances are transported against their chemical and electrical gradients.

There are three common characteristics of active transport mechanisms:

1. **Uphill transport**: The transport occurs against the electrochemical gradient of the substance transported.
2. **Utilize metabolic energy**: Energy utilized for the active transport is derived from the breakdown of ATP. Therefore, mechanisms that prevent the supply of ATP hinder the active transport process. Especially, the process is susceptible to metabolic poisons.
3. **Exhibit saturation kinetics**: Like any carrier-mediated transport, this has also a saturation point for limitation in the rate of availability of carriers and the supply of energy.

There are two types of active transports:

1. Primary active transport
2. Secondary active transport

**Primary Active Transport**

Primary active transport is the transport mechanism that directly utilizes metabolic energy for the transport process. The mechanism is operated by **ion pumps**. The features are:

1. In this process, the solute is transported against its electrochemical gradient with the help of energy.
2. The energy is derived from ATP.

3. The ion pumps hydrolyze ATP to ADP and use energy in the third phosphate bond for the transport process. As the ion pumps hydrolyze ATP, these are also called **ATPases**.
4. The commonly occurring ATPase is **Na⁺–K⁺ ATPase** or **Na⁺–K⁺ pump**.
5. Other common ATPases are **Calcium ATPases**, **H⁺–K⁺ ATPase** and **H⁺-ATPase**. They are classified into P-type (phosphorylation type), V-type (vacular type), F-type (energy coupling factor type) and ABC transporters (Table 6.4).

**CFTR protein**: Cystic fibrosis transmembrane regulator protein involved in genesis of cystic fibrosis; MDR-1 protein: multidrug resistance 1 protein that pumps atrinancer drug out of cancer cell that causes drug resistance in treatment of cancer.

**Na⁺–K⁺ ATPase**

Na⁺–K⁺ ATPase is present in all eukaryotic cells. This is an antiport that pumps K⁺ into the cell and Na⁺ out of the cell against their concentration gradients. This antiport transport system is primarily responsible for maintaining the high K⁺ and low Na⁺ concentration inside the cells.

**Structure and its Functional Aspects**

The Na⁺–K⁺ ATPase is a heterodimeric protein made up of two subunits: an α and a β subunit.

1. The α subunit is larger catalytic subunit with molecular weight of about 100,000 and β subunit is the smaller one (mol. wt. of about 55,000) of unknown functions.
2. At the cytoplasmic side, the α subunit has ATPase activity and binding sites for 3 Na⁺, ATP and phosphate (Fig. 6.8). At the extracellular side, α subunit has binding sites for 2 K⁺ and ouabain.
3. Binding of three ions of Na⁺ and an ATP molecule with the carrier on its intracytoplasmic surface causes hydrolysis of ATP giving rise to ADP and Pi.
4. The phosphate group is bound to an aspartic acid residue of the α subunit.
5. On activation, Na⁺-K⁺ ATPase pumps three Na⁺ ions out of the cell and two K⁺ into the cell. Na⁺-K⁺ pump
catalyzed the hydrolysis of ATP to ADP, and uses this energy to pump three Na\(^+\) out of the cell and two K\(^+\) into the cell for each mole of ATP hydrolyzed. Therefore, Na\(^+\)-K\(^+\) pump is an **electrogenic pump** as it forces net positive charge out of the cell, and it has the coupling ratio of 3/2 (Application Box 6.3).

**Application Box 6.3**

**Importance of electrogenic pump:** Electrogenic pump creates the electrical potential across the membrane, which is the basic requirement in nerve and muscle fiber for transmitting electrical signal.

**Functions**

The Na\(^+\)-K\(^+\) ATPase is a P-type ATPase, as the carrier protein is phosphorylated during the process. It pumps three Na\(^+\) ions out of the cell and two K\(^+\) into the cell. It serves many important cellular functions:

1. **Cytosolic ion concentration:** Na\(^+\)-K\(^+\) pump opposes Na\(^+\) to accumulate in the cell and K\(^+\) to exit from the cell along their concentration gradient. Thus, Na\(^+\)-K\(^+\) pump maintains high concentration of K\(^+\) and low concentration of Na\(^+\) in the cell.
2. **Cell volume:** By maintaining ion concentration on both sides of the cell, Na\(^+\)-K\(^+\) pump regulates water movement across the cell membrane. This maintains intracellular water content and, therefore, the cell volume. Failure of Na\(^+\)-K\(^+\) pump activity can lead to cell swelling and rupture.
3. **Protein synthesis:** The primary function of Na\(^+\)-K\(^+\) pump is to maintain a high intracellular concentration of K\(^+\), which is essential for protein synthesis.
4. **Resting membrane potential:** Na\(^+\)-K\(^+\) pump also maintains resting membrane potential by maintaining ion gradients across the cell membrane.
5. **Hormone actions:** Na\(^+\)-K\(^+\) pump mediates action of many hormones on the cell. Some important examples are thyroxine, aldosterone and insulin.

**Mechanism of Action**

Being a P type ATPase, phosphorylation and dephosphorylation of the carrier protein lead to transfer of ions across the cell membrane.

1. Binding of three Na\(^+\) and one ATP molecule to their respective sites on a subunit activates ATPases that converts ATP to ADP. This causes phosphorylation of a subunit that changes its configuration and transfers 3 Na\(^+\) to ECF. K\(^+\) binds to K\(^+\) binding site on the extracellular surface that causes dephosphorylation of a subunit and transfers two K\(^+\) from ECF into the cell. Thus, three Na\(^+\) are pumped out for entry of two K\(^+\) into the cell, and one ATP is hydrolyzed.

**Scientist contributed**

The Na\(^+\)-K\(^+\) pump was discovered in 1957 by Danish physiologist Prof Jens Christian Skou of Aarhus University, who received the Nobel Prize for his discovery in 1997, which was the culmination of his four decades of research aimed at explaining the mechanism behind this vital motor of the cells. He received the **Nobel Prize in Chemistry** together with Paul D Boyer and John E Walker for his discovery of Na\(^+\)-K\(^+\)ATPase.

**Jens Christian Skou**

(Born: October 1918, Age 97 years)
4. Dephosphorylation, in turn, causes re-conformational change in the α subunit that transfers two K+ from outside to the inside of the cell.
5. Reconformational change of the carrier protein also returns to its original conformation.
6. Thus, during one cycle of conformational (phosphorylation) and re-conformational (dephosphorylation) change in the α subunit of Na+-K+ ATPase, three Na+ ions are pumped out of the cell and two K+ into the cell, and one ATP is hydrolyzed.

**Regulation of Na+-K+ Pump Activity**

**Activation of Na+-K+ Pump**

Many hormones, chemicals and drugs act by increasing Na+-K+ pump activity such as thyroxine, insulin, aldosterone, G-actin, etc.

**Inhibition of Na+-K+ Pump**

Many hormones and chemicals act by decreasing Na+-K+ pump activity such as dopamine, digitalis, metabolic poisons like DNP (2,4 dinitrophenol), etc. (Clinical Box 6.3). Hypoxia and hypothermia inhibit Na+-K+ pump activity.

**Clinical Box 6.3**

Digitalis inhibits Na+-K+ pump: Cardiac glycosides like ouabain, or digitalis that are routinely prescribed in the management of heart failure inhibit Na+-K+ pump by binding to the external surface of the α-subunit and interfering with the hydrolysis of the aspartic acid-phosphate bond. This accumulates Na+ inside the cell and prevents K+ influx. Intracellular accumulation of Na+ decreases Na+ gradient from outside to inside. Calcium efflux through sodium-calcium exchanger in the membrane utilizes sodium gradient. Hence, decreased sodium gradient decreases calcium efflux causing increase in cytosolic calcium concentration that promotes myocardial contractility.

**Other ATPases**

**Ca++ ATPase**

Other common example of primary active transport is the Ca++ pump, which is present in all cell membranes, membrane of endoplasmic reticulum, and sarcoplasmic reticulum in muscle cell.

Main features and functions Ca++ ATPase are:
1. This is a P-type ATPase.
2. The Ca++ pump present on the cell membrane actively transports calcium out of the cell, and, therefore, maintains a higher concentration of calcium in the ECF (10^-3 molar) compared to inside the cell (10^-7 molar).
3. The Ca++ pump present on the membrane of sarcoplasmic reticulum in muscle cell and endoplasmic reticulum in other cells transports calcium out of the cytoplasm into these organelles, and, therefore, maintains a low cytosolic concentration of calcium.
4. Also, it helps in storage of calcium in these organelles for ready availability of it at the time of need like muscle contraction.

**H+-K+ ATPase**

The H+-K+ pump is present on the luminal membrane of parietal cells of stomach and the intercalated cells of the distal nephrons.
1. In stomach, it pumps proton into the gastric lumen in exchange for K+. This is the primary step in HCl secretion in stomach. Thus, it maintains low pH of gastric content which is essential for gastric digestion and killing of micro-organism in the stomach.
2. In kidney, it secretes H+ into the tubular fluid and reabsorbs K+. Hence, it plays an important role in acidification of urine.

**H+ ATPase or Proton ATPase**

The H+ pump or the proton pump is located on the membrane of lysosome, endoplasmic reticulum and mitochondria.

In lysosome and endoplasmic reticulum: It is a V-type ATPase, named for its first discovery in the vacuoles and vesicles of the cells. It pumps H+ (proton) from cytosol into these organelles. Thus, interior of these organelles become more acidic, which is needed for their physiological activities.

In mitochondria: Proton pump is also located in the inner mitochondrial membrane, but it is F-type ATPase in this organelle. Its main function is to synthesize ATP in mitochondria by utilizing energy stored in the proton gradient created by respiratory chain.

**ABC Transporters**

ATP-binding cassette (ABC) transporters belong to members of a superfamily of membrane transport proteins that bind to ATP. The main features are:
1. They have 12 membrane-binding domains.
2. They use energy derived from ATP for transporting a variety of compounds out of the cells, those include ions, steroids, peptides, bile acids, drugs and xenobiotics.
3. As drugs are transported out of the cells, they are known as MDR 1 protein (multidrug resistance 1 protein). Cells that usually express MDR proteins are liver, kidney and GI tract cells.
4. CFTR protein (cystic fibrosis transmembrane regulator protein), a chloride channel protein involved in gene-expression of cystic fibrosis also belongs to this superfamily of ABC transporters (Application Box 6.4).

**Application Box 6.4**

ABC transporters produce drug resistance in cancer cells: ABC transporters cause expulsion of cytotoxic drugs from cancer cells. This decreases effective concentration of drugs in the cells needed for killing cancer cells. Hence, cancer cells become resistant to anticancer chemotherapy. Examples are MDR 1 protein (multi-drug resistance-associated protein 1) and BRCP (breast cancer resistance protein).
Secondary Active Transport

Many cells have several carrier mechanisms that transfer one solute against its concentration gradient by using the energy generated by gradient of another solute. Usually, Na⁺ is the driver solute for most of these mechanisms. Energy created by Na⁺ gradient is utilized for transport of other solutes. The Na⁺ gradient is generated and maintained by Na⁺-K⁺ pump. Though the transport system by itself does not directly utilize energy, it depends on the function of Na⁺-K⁺ pump. Therefore, when the pump is inhibited by a blocking agent, transport process stops.

Typical example of secondary active transport is reabsorption of glucose from the kidney tubule or intestine.

1. The primary active transport of Na⁺ out of the basal and basolateral membranes of the proximal tubules of the nephron and the small intestine by Na⁺-K⁺ pump leads to decreased concentration of Na⁺ in the cytosol of the epithelial cells.
2. This causes facilitated diffusion of Na⁺ from the lumen into the cells.
3. The carrier protein that transfers Na⁺ from the luminal fluid into the cell also transports glucose in the same direction (Fig. 6.10). Thus, the carrier protein is a symport that transports glucose simultaneously with sodium. The symport also transports amino acids and other solutes.

Fig. 6.10: Mechanism of secondary active transport. Na⁺-K⁺ pump located on basolateral membrane actively pumps sodium out of the cell that decreases cytosolic sodium and creates gradient for transport of sodium from lumen into the cell. The carrier protein (symport) that transports sodium also transports glucose into the cell. Thus, transport of glucose from lumen into the cell is the secondary active transport.

Important Note

The carrier protein may also be an antiport: The example of carrier protein being an antiport in secondary active transport is the counter-transporting hydrogen ion in the nephron out of the cell for bringing in sodium (Na⁺-H⁺ exchanger). Another example is Na⁺-Ca²⁺ exchanger in cardiac cell.

4. Since the transport depends on primary active transport of sodium by the Na⁺-K⁺ pump, it is known as a secondary active transport.
5. As the carrier protein for secondary active transport system is an integral membrane protein it exhibits competitive inhibition and saturation kinetics. However, it differs from the usual carrier transport for its exclusive dependence on primary transport mechanism (the pump) and for transporting against its own electrochemical gradient.

VESICULAR TRANSPORT

The transport process that occurs by either fusion of vesicle or formation of vesicle is called as vesicular transport. Fusion of vesicle with the cell membrane occurs in exocytosis and formation of vesicle from the cell membrane occurs in endocytosis. Special utilities of vesicular transport are:
1. Macromolecules such as large protein molecules cannot be transported by diffusion or active transport process. Therefore, they are transferred across the cell membrane mainly by vesicular transport.
2. Amino acids, sugars, waste products of metabolism, cellular secretions, hormones, neurotransmitters and organisms are transported by this mechanism.

Role of Vesicular Transport Proteins

In vesicular transport, formation and transport of vesicles are facilitated by some vesicular transport proteins. These proteins are: clathrin, coating proteins, dynamin and docking proteins.

Clathrin
Clathrin is a fibrillar protein located in the cell membrane beneath the receptor protein. There are two types of clathrins: AP-1 clathrin and AP-2 clathrin. AP-1 clathrin helps in transportation from Golgi apparatus to lysosome and AP-2 clathrin helps in transportation of endosomes.

Coating Proteins (COP)
There are two types of coating proteins: COP I and COP II. Coating proteins help in transportation of vesicles between endoplasmic apparatus and Golgi apparatus.

Dynamin
Dynamin helps in formation of vesicles from cell membrane, especially in clathrin-mediated endocytosis.
Docking Proteins

Before exocytosis of vesicles, docking of vesicles occurs. Docking proteins facilitate attachment of vesicle with membrane. Examples of docking proteins are \textit{V snare protein} and \textit{T snare protein} (For details, refer to the chapter ‘Synaptic Transmission’).

Types of Vesicular Transports

Vesicular transports are of three types: \textit{endocytosis}, \textit{exocytosis} and \textit{transcytosis}.

Endocytosis

Endocytosis is the process of transport in which a \textit{substance is taken into the cell by means of vesicle formation}.

1. A region of plasma membrane is pinched off to form an endocytic vesicle that causes internalization of the substance to the cell.
2. The vesicle may contain fluid and dissolved solutes or particulate material.
3. The size of the vesicle varies from 0.1 µm to 2 µm.

Mechanisms of Endocytosis

Endocytosis occurs by two mechanisms: constitutive and clathrin-mediated.

Constitutive Endocytosis

Endocytosis by \textit{constitutive pathway} occurs in almost all cells. It is called \textit{constitutive} as the process occurs continuously and does not require any specific stimulus. The steps are as follows:
1. The molecule or the substance makes contact with the cell membrane that invaginates to form an endocytic vesicle.
2. The non-cyttoplasmic side of the membrane then fuses and the vesicle is pinched-off into the cytosol.
Thus, it occurs in \textit{two major steps}: formation of vesicle and separation of vesicle from the membrane (Figs. 6.11A and B).

Clathrin-mediated Endocytosis

Clathrin-mediated endocytosis occurs at the specific site of the cell membrane where clathrin accumulates. \textit{Clathrin} is a fibrillar protein molecule having the shape of a \textit{triskelion} (three legs radiating from the central hub). Clathrin is located in the cell membrane beneath the receptor protein. Clathrin-mediated endocytosis occurs in the following steps:
1. The protein and other macromolecules attach to receptors on the membrane surface. Receptors for these molecules are normally concentrated in small pits called \textit{coated pits}, beneath which are located fibrillar proteins called \textit{clathrin}. Contractile filaments like actin and myosin are also attached to the coated pits.
2. The attachment of the macromolecules to the receptors stimulates the invagination of the coated-pit, enclosing the molecule and a small portion of the ECF in the form of a vesicle. Clathrin molecule forms a geometrical array that surrounds the vesicle (Figs. 6.12A to C). Once the vesicle formation is completed, clathrin molecules detach from the vesicle and recycle to the membrane.
3. The endocytic vesicle detaches from the cell membrane.
4. Fusion of the endocytic vesicle with lysosomes that empty their acid hydrolases into the vesicle leading to the digestion of the substances in the vesicle.

Clathrin-mediated endocytosis internalizes not only various organisms, but also substances like growth factors and lipoproteins. Cell membrane also contains \textit{caveolae}. Caveoli are membrane indentations coated with \textit{caveolin}, the protein that facilitates endocytosis of proteins.

Scientist contributed

\textbf{Discovery of receptor-mediated endocytosis:} Anderson is responsible for two discoveries that changed our view of cell physiology: (1) receptor-mediated endocytosis in coated pits and coated vesicles (Anderson et al., 1976; Anderson et al., 1977 a,b); and (2) identification of caveolin, the protein that lines the surface of caveolae (Rothberg et al., 1992).

\textbf{Richard GW Anderson} (1940–2011)

Types of Endocytosis

Endocytosis is of three types: phagocytosis, receptor-mediated endocytosis and pinocytosis.
**Phagocytosis**

Phagocytosis, otherwise called *cell-eating*, is the process of ingestion of large particles or microorganisms by specialized cells called *phagocytes*. In this process, the phagocytic cells, such as macrophages engulf bacteria, foreign particles and tissue debris and digest them. The **phagocytic vesicles** vary from 1–2 µm in diameter. Phagocytosis occurs in three broad steps:

1. Attachment of the foreign molecule with cell membrane.
2. Engulfment of the foreign molecule in the form of endocytic vesicle.
3. Killing of the organism or degradation of the vesicular content.

Phagocytic cells include neutrophils, monocytes, macrophages and other cells of mononuclear phagocyte system (for details of phagocytosis, refer to Chapter on “White Blood Cells” in hematology section)

**Receptor-mediated Endocytosis**

Receptor-mediated endocytosis is an efficient process of endocytosis in which the extracellular molecule binds with the specific receptor on the cell membrane. Special features in this process are:

1. Receptors are concentrated at a depression on membrane called *coated-pits*.
2. Once the external molecule attaches with receptor, the coated-pit pinches off from the membrane in the form of an **endocytic vesicle** causing rapid internalization of the molecule.
3. Hormones, growth factors, transport-proteins like transferrin, toxins, viruses, etc. enter cells by receptor-mediated endocytosis.

**Pinocytosis**

Pinocytosis is the **fluid-phase endocytosis** or ‘cell drinking’ by which substances in solution in extracellular fluid are internalized to the cell. It is a continuous process that occurs in most cells of the body. The stages in pinocytosis are the same as endocytosis except that the endocytic vesicle contains fluid instead of solid particles.

**Exocytosis**

Exocytosis is the process of export from the cell, which is reverse of endocytosis. By exocytosis, hormones, neurotransmitters, digestive enzymes and undigested foreign particles are released from cells. The steps of exocytosis are:

1. Before exocytosis, the molecules are synthesized in endoplasmic reticulum and packaged in Golgi apparatus to form the **transport vesicles**.
2. The vesicles then move to the cell surface and fuse with the cell membrane.
3. Release the content to ECF (Figs. 6.13A and B), which requires ATP and Ca$^{2+}$.

**Emiocytosis** is the term referring to exocytosis of specific hormones and granules.

**Mechanism of Exocytosis**

Exocytosis occurs in two pathways: **constitutive** and **regulated**.

**Constitutive Exocytosis**

This type of exocytosis is seen in almost all cells. By this mechanism, some proteins are continuously secreted by the cells. For example, secretion of mucus by goblet cells of small intestine.

**Regulated Exocytosis**

In regulatory exocytosis, macromolecules that are stored in the vesicles fuse with cell membrane and release their content in response to specific extracellular stimulus. This is the major mechanism of rapid secretion of hormones, neurotransmitters and digestive enzymes in response to specific stimuli. This is also called **non-constitutive exocytosis**.
Transcytosis
When vesicular transport is limited within the cell, the process is called transcytosis or cytopemisis. Vesicles are formed within the cell and transported in the cytoplasm.

TRANSPORT ACROSS THE EPITHELIUM
Epithelium is a layer of cells present on the basement membrane. Two special features of epithelium make it permeable to various solutes.

1. First, the basolateral membrane contains active transport systems that create gradients for transfer of ions from apical membrane, and various uniports and antiports that regulate exchange of other ions.
2. Second, tight junctions between cells allow selective ions to pass through and prevent large molecules like proteins to be transferred.

Thus, there are two mechanisms for transport across the epithelia:

a. **Transcellular transport** or transport through the cell i.e. from lumen through apical surface into the cytosol and from there through basolateral space or through basal membrane into the interstitial fluid.

b. **Paracellular transport**, which means transport through tight junctions between the cells bypassing the transport through cells (Fig. 6.14).

Reabsorption of sodium and glucose in kidney tubule and intestine is the example of transport across the epithelia.

Ultrafiltration through the glomerular capillary endothelium and Bowman’s capsule epithelium is a special transport mechanism through epithelia. However, in ultrafiltration, transfer of substances occurs mainly due to the difference in pressures across the epithelium.

CHAPTER SUMMARY

**Carrier Proteins**
There are three different types of carrier proteins: uniport, symport and antiport.

- **Uniport**: Uniport is the carrier protein that transports only one substance. For example, Na⁺ channels transport Na⁺ and K⁺ channels transport K⁺.
- **Symport**: Symport carriers transport two or more substances from one side of the membrane to the other in the same direction. Transport of Na⁺ and glucose from the lumen of the intestine or kidney tubule into the epithelial cells through the same carrier protein is the example.
- **Antiport**: Antiport carriers transport substances in opposite directions in which one substance is transported to the inside of the cell and other substance from inside the cell to the outside. Examples of antiport are Na⁺-K⁺ pump and Na⁺-H⁺ exchanger.

**Transport Processes**
Broadly they are of 2 types: Passive transport and active transport

- **Passive Transports**: Have two common features. 1. Transport is downhill (down the electrochemical gradient), and 2. Does not require metabolic energy (ATP is not utilized). Diffusion and osmosis are examples of passive transport.
- **Active Transport**: Active transport utilizes energy. The common features are: 1. Uphill transport (against the electrochemical gradient); 2. Requires energy; and 3. Exhibits saturation kinetics.
The active transport processes are of two types: primary active transport, and secondary active transport. Na⁺-K⁺ pump is the prototype of primary active transport. When a transport system by itself does not directly utilize energy, but depends on the function of a primary active pump (Na⁺-K⁺ pump), the process is called secondary active transport (SAT). Reabsorption of glucose from the kidney tubule or intestine is an example of SAT.

**Vesicular Transport**

The transport process that occurs by either fusion of vesicle or formation of vesicle is called vesicular transport. Fusion of vesicle with the cell membrane occurs in exocytosis and formation of vesicle from the cell membrane occurs in endocytosis. Macromolecules such as large protein molecules that can not be transported by diffusion or active transport process are transported by vesicular transport. Amino acids, sugars, waste products of metabolism, cellular secretions, hormones, neurotransmitters and organisms are transported by this mechanism.

**Important to Know (Must Read)**

In final examination, Long Questions are asked on ‘Passive transport processes (diffusion, facilitated diffusion, osmosis)’ or ‘Active transport mechanisms’.

Carrier proteins, facilitated diffusion, Gibb-Donnan effect, Na⁺-K⁺ pump, secondary active transport, exocytosis, endocytosis, are usually asked as Short Note Question in exams.

In viva, examiners usually ask about different types of ion channels in the membrane, types of gating of ion channels, patch-clamping, examples of uniport, symport and antiport, factors affecting diffusion, examples of facilitated diffusion, osmotic pressure, definitions of osmolality and osmolarity, definitions and examples of filtration, bulk flow and solvent drag, Donnan effect, structure and function of Na⁺-K⁺ ATPase, other ATPases, vesicular transport proteins, types of endocytosis and exocytosis with examples, definition of Transcytosis.
Chapter 7
Membrane Potential

Learning Objectives
On completion of study of this chapter, the student MUST be able to:
1. Understand the concept of resting membrane potential (RMP).
2. Explain the mechanism of genesis and maintenance of RMP.
3. Give the normal values of RMP of different excitable tissues and equilibrium potential of important ions.

The student MAY also be able to:
1. Explain the concept of Gibbs-Donnan Equilibrium.
2. Explain the concept of Nernst Equation.
4. Give the principle of recording of RMP.

An electrical potential difference exists across the membrane of all living cells with the inside being negative in relation to the outside. This potential difference is called membrane potential as ions arrange themselves along the outer and inner surfaces of the cell membrane. At resting state of the cell, the membrane potential is called resting membrane potential (RMP). RMP is also called transmembrane potential. Special features of RMP are:
1. The RMP is different in different tissues (Table 7.1).
2. In a nerve cell, the RMP is −70 mV.
3. When the neuron is stimulated, membrane potential changes and inside of the cell becomes positive due to depolarization. The membrane potential during this state of activation is called action potential.
4. RMP plays an important role in deciding the degree and duration of action potential, as the RMP is the level from where the phase of depolarization starts.
5. In some tissues like visceral smooth muscles, RMP is not stable (fluctuates).

Table 7.1: RMP of important excitable tissues.
<table>
<thead>
<tr>
<th>Tissue/Cell</th>
<th>RMP (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuron</td>
<td>−70</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>−90</td>
</tr>
<tr>
<td>Cardiac muscle</td>
<td>−90</td>
</tr>
</tbody>
</table>

Selective Permeability of the Membrane
The cell membrane is selectively permeable to ions and other solute particles. Some ions are highly permeable, some are less permeable and others are impermeable. The permeability mainly depends on the molecular weight and the radius of ions in their hydrated form (Table 7.2).
1. Though the ions like Na⁺, K⁺, Cl⁻ and HCO₃⁻ are diffusible through the membrane, the permeability is more for K⁺.

Concepts and Physiological Aspects
Membrane potential is mainly due to distribution of ions across the cell membrane, which results mainly from the selective permeability of the cell membrane to various ions at rest. However, this is influenced by various forces acting on the ion distribution. Understanding the following concepts help the students to learn the physiology of RMP:
1. Selective permeability of the cell membrane to various ions
2. Gibbs-Donnan equilibrium
3. Nernst equation
2. Though the particle size of K⁺ is large (atomic weight 39) as compared to Na⁺ (atomic weight 23), the permeability for K⁺ is 500 to 1000 times greater than that for Na⁺ for its effective radius.

3. The membrane is practically impermeable to intracellular proteins and organic phosphates.

**Gibbs-Donnan Membrane Equilibrium**

When two solutions containing ions are separated by a semipermeable membrane, at equilibrium, each solution will be electrically neutral. That means, the total quantity of cations will be equal to total quantity of anions. Also, the product of diffusible ions on one side of the membrane will be equal to product of diffusible ions on the other side of the membrane. This is called Gibbs-Donnan membrane equilibrium.

For example, two solutions A and B containing sodium and chloride ions are separated by a semipermeable membrane. Then, according to Gibbs-Donnan equilibrium, each solution should be electrically neutral (Fig. 7.1), which means:

\[
\text{(cations)}_A = \text{(anions)}_A \quad \text{and} \quad \text{(cations)}_B = \text{(anions)}_B
\]

or

\[
(\text{Na}^+)_{A} = (\text{Cl}^-)_A \quad \text{and} \quad (\text{Na}^+)_{B} = (\text{Cl}^-)_B
\]

The product of diffusible ions on both sides will also be equal, which means,

\[
(\text{diffusible cations})_A \times (\text{diffusible anions})_A = (\text{diffusible cations})_B \times (\text{diffusible anions})_B
\]

or

\[
[(\text{Na}^+)_{A} \times (\text{Cl}^-)_A] = [(\text{Na}^+)_{B} \times (\text{Cl}^-)_B]
\]

Thus, the ratio of diffusible ions will be:

\[
\frac{(\text{Diffusible cations})_{A}}{(\text{Diffusible cations})_{B}} = \frac{(\text{Diffusible anions})_{B}}{(\text{Diffusible anions})_{A}}
\]

or

\[
\frac{[\text{Na}^+]_{A}}{[\text{Na}^+]_{B}} = \frac{[\text{Cl}^-]_{A}}{[\text{Cl}^-]_{B}}
\]

Thus, there will be equal and balanced distribution of ions at equilibrium. However, when a non-diffusible ion 'X' is added on one side (let us say, side A), then as per Gibbs-Donnan equilibrium principle, the distribution of diffusible ions will change to maintain electroneutrality of both sides (Fig. 7.2).

**Nernst Equation**

As described above, in accordance with Gibbs-Donnan equilibrium, asymmetrical distribution of diffusible ions occurs across the cell membrane with more cation (K⁺) present inside. Therefore, K⁺ will try to diffuse into the ECF from ICF, which is opposed by the electrical gradient, created by presence of non-diffusible anions inside the cell. Thus, finally equilibrium is reached between the concentration gradient and the electrical gradient resulting in diffusion potential (equilibrium potential) across the cell membrane.

The degree of this equilibrium potential is determined by Nernst equation:

\[
E_{(m)} = \frac{RT}{2F} \ln \frac{\text{(Conc)}_A}{\text{(Conc)}_B}
\]
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Where,

\( E_{(m)} \): Equilibrium potential (in millivolts) of the ions at which efflux and influx of the ions are equal

\( R \): The natural gas constant (8.316 joules/degree).

\( T \): The absolute temperature

\( F \): The faraday constant (Number of coulomb/mole of charge = 96,500 coulomb/mole)

\( Z \): The valency of the ion

\( \ln \): Natural logarithm

\( \text{(Conc)}_i \): The concentration of the ions in the intracellular fluid

\( \text{(Conc)}_o \): The concentration of the ions in the extracellular fluid

At normal body temperature (37°C), converting from the natural log to the base 10 log and replacing some of the constants with numerical values, the equation can be simplified to:

\[ E_{(m)} = \pm 61 \log \left( \frac{\text{(Conc)}_i}{\text{(Conc)}_o} \right) \]

Thus, Nernst equation helps in calculating the equilibrium potential for each ion individually (Table 7.3).

---

**Goldman-Hodgkin-Katz Equation**

The magnitude of the membrane potential at any given time depends on the distribution of \( Na^+ \), \( K^+ \) and \( Cl^- \) and on the permeability of each of these ions. The role of different ions in the generation of membrane potential is accurately described by Goldman-Hodgkin-Katz (GHK) equation or also called Goldman’s constant field equation.

\[ V = \frac{RT}{F} \ln \left( \frac{P_{K^+}[K^+]_o + P_{Na^+}[Na^+]_o + P_{Cl^-}[Cl^-]_o}{P_{K^+}[K^+]_i + P_{Na^+}[Na^+]_i + P_{Cl^-}[Cl^-]_i} \right) \]

Where,

\( V \): membrane potential,

\( R \): gas constant,

\( T \): absolute temperature,

\( F \): Faraday constant,

\( \ln \): natural logarithm

\( P_{K^+}, P_{Na^+}, P_{Cl^-} \): permeabilities of the membrane to \( K^+, Na^+ \) and \( Cl^- \), and \( i \) and \( o \) refer to inside and outside of the cell respectively.

---

**Scientists contributed**

Sir John Carew Eccles, Alan Lloyd Hodgkin and Andrew Fielding Huxley

The Nobel Prize in Physiology or Medicine 1963 was awarded jointly to **Sir John Carew Eccles, Alan Lloyd Hodgkin and Andrew Fielding Huxley** for their discoveries concerning the ionic mechanisms involved in excitation and inhibition in the peripheral and central portions of the nerve cell membrane.

---

**Importance of Goldman Constant Field Equation**

1. Important ions that generate membrane potentials in nerve and muscle fibers are sodium, potassium and chloride. The voltage of membrane potential is determined by the concentration gradient of each of these ions.

2. The relativity of importance of each ion in determination of the voltage depends upon the membrane permeability of individual ion.

---

**Table 7.3:** Concentrations (mmol/L) of important ions in ECF and ICF and their equilibrium potential (EP) in a mammalian spinal motor neuron.

<table>
<thead>
<tr>
<th>Ions</th>
<th>ECF</th>
<th>ICF</th>
<th>EP (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( Na^+ )</td>
<td>150</td>
<td>15</td>
<td>+60</td>
</tr>
<tr>
<td>( K^+ )</td>
<td>5.5</td>
<td>150</td>
<td>-90</td>
</tr>
<tr>
<td>( Cl^- )</td>
<td>125</td>
<td>9</td>
<td>-70</td>
</tr>
<tr>
<td>( HCO_3^- )</td>
<td>21</td>
<td>5</td>
<td>-25</td>
</tr>
<tr>
<td>Calcium</td>
<td>2.5</td>
<td>10^-4</td>
<td>+130</td>
</tr>
</tbody>
</table>

**Fig. 7.2:** When nondiffusible anion (\( X^- \)) is added to the solution A, more \( Cl^- \) is transferred to B to maintain balance of anion on both sides. Consequently, more \( Na^+ \) is transferred to solution A to maintain electroneutrality of both sides.

---

**Walther H Nernst**

*Born in Briesen, West Prussia, on June 25, 1864, notable for the development of the Nernst equation and winner of 1920 Nobel Prize in chemistry, was a major contributor to the study of membrane potential. He developed the Nernst equation to solve for the equilibrium potential for a specific ion. Goldman, Hodgkin and Katz furthered the study of membrane potential by developing the Goldman-Hodgkin-Katz equation to account for any ion that might permeate the membrane and affect its potential. The study of membrane potential utilizes electrochemistry and physiology to formulate a conclusive idea of how charges are separated across a membrane.*
Chapter 7: Membrane Potential

3. Cation concentration from inside the membrane to outside is responsible for electronegativity inside the membrane. Due to concentration gradient, cations diffuse to outside of the cell leaving the non-diffusible anions inside the cell.

GENESIS OF RMP

Resting membrane potential (RMP) is the membrane potential at rest. In neurons, the RMP is –70 mV.

The RMP is created due to following factors.

1. Permeability of the membrane to K⁺: Normally, K⁺ is more inside and less outside the cell (see Table 7.3). Therefore, a concentration gradient exists for K⁺ from inside to outside that facilitates K⁺ to diffuse out of the cell. At rest, permeability of the membrane to K⁺ is higher than any other ion. Therefore, K⁺ easily diffuses out of the cell (Fig. 7.3), though this is opposed by the electrical gradient. As, K⁺ is the major intracellular cation, its diffusion creates negativity inside the cell. The permeability of the membrane to K⁺ is the major cause of RMP.

2. Permeability of the membrane to Na⁺: At rest, ECF Na⁺ is more than the ICF Na⁺. Therefore, there is a concentration gradient for Na⁺ from outside to inside, for which Na⁺ diffuses into the cell. However, at rest membrane is less permeable to Na⁺ (Fig. 7.3) than to K⁺. Therefore, K⁺ exit is not balanced by Na⁺ entry (more K⁺ goes out). Hence, interior of the cell remains relatively negative.

3. Permeability to anions: Exit of more cations from the cell should be accompanied by proportionate exit of anion to maintain electroneutrality. However, exit of K⁺ (the cation) is not accompanied by exit of same amount of anions as permeability of anions at rest is also not same as that of K⁺. Therefore, more negative ions remain inside.

4. Role of Na⁺-K⁺ pump: The role of Na⁺-K⁺ pump is to maintain RMP rather than to generate it. However, Na⁺-K⁺ pump also contributes to the genesis of RMP, as it is an electrogenic pump. It pumps out three Na⁺ for two K⁺ to come in. Therefore, it pumps more cations out of the cell and less into the cell. Thus, less cations are taken inside, which in other words, a relatively negativity is created inside (Application Box 7.1).

Application Box 7.1

Negativity is only along the membrane: It should be noted that the number of ions that are responsible for membrane potential is a very small fraction of the total number of ions actually present inside the cell. In the cell, the cations and anions are present in equal proportion except along the membrane. Therefore, negativity is created and maintained only close to the membrane.

Maintenance of RMP

K⁺ diffusing out of the cell and Na⁺ diffusing into the cell will come to halt once the concentration gradient ceases to exist for both the ions. However, that does not happen, as Na⁺-K⁺ ATPase helps in building the concentration gradient. It serves to pump back the Na⁺ that diffuses into the cell and K⁺ that diffuses out of the cell. Thus, Na⁺-K⁺ pump plays an important role in maintaining RMP.

Recording of Membrane Potential

The membrane potential is recorded like recording of activity of any excitable tissue. This requires:

1. Microelectrodes
2. Electronic amplifiers
3. Cathode ray oscilloscope (CRO).

Basic Principle

Two microelectrodes are placed on the surface of a nerve fiber and connected to a CRO. When, one of the
Resting membrane potential (RMP) is the membrane potential at rest, which is created mainly by the permeability of the membrane to K⁺, and contribution of other factors are minimal.

1. **Permeability of the membrane to K⁺**: At rest, permeability of the membrane to K⁺ is higher than any other ion. Therefore, K⁺ easily diffuses out of the cell, though this is opposed by the electrical gradient. As, K⁺ is the major intracellular cation, its diffusion creates negativity inside the cell. The permeability of the membrane to K⁺ is the major cause of RMP.

2. **Permeability of the membrane to Na⁺**: At rest, membrane is less permeable to Na⁺. Therefore, K⁺ exit is not balanced by Na⁺ entry (more K⁺ goes out). Hence, interior of the cell remains relatively negative.

3. **Role of Na⁺-K⁺ pump**: The role of Na⁺-K⁺ pump is to maintain RMP rather than to generate it. However, Na⁺-K⁺ pump also contributes to the genesis of RMP, as it is an electrogenic pump. It pumps out three Na⁺ for two K⁺ to come in. Thus, less cations are taken inside, which in other words, a relatively negativity is created inside.

**Important to Know (Must Read)**

1. In examinations, when students are asked about the mechanism of RMP, promptly they answer the role of Na⁺-K⁺ pump, and this is wrong. They should answer that it is the permeability of the membrane to K⁺ that mainly contributes to the genesis of RMP.

2. Resting membrane potential comes usually as Short Note/Question in final exam.

3. In Viva, examiners usually ask about the genesis of RMP, and sometimes about the application of Nernst equation, Gibbs-Donnan equilibrium, and Hodgkin-Katz equation.
Body Fluids

Functions of cells depend on fluids present both outside and inside the cells. Unicellular organisms float in water or air and exchange their nutrients, waste products and gasses through their body surface, i.e., the cell membrane. However, multicellular organisms, especially organisms with complex body systems have no direct access to their immediate environment, and therefore, they contact outer environment through interstitial fluid and transport systems of their body. Therefore, body fluid is compartmentalized into intracellular and extracellular fluids in complex organisms. The body compositions and distribution of fluid in different compartment are as follows:

1. The body composition of a normal adult male on average consists of 15% fat, 18% proteins, 7% minerals and 60% water.
2. The total body water (TBW) as percentage of body weight on average is about 60% in adult males, which is about 10% more than adult females (Table 8.1).
3. In infants and children, though TBW as percentage of body weight is more than in adults, their absolute water content is significantly less.
4. In elderly people in both the sexes, TBW is about 10% less than their adult counterparts.
5. In females, TBW is less than males due to relatively greater amount of adipose tissue (subcutaneous fat) in them.

Of total body water, about two-third is present in intracellular compartment and one-third in extracellular compartment (Table 8.2).

**BODY FLUID COMPARTMENTS**

**Extracellular Fluid Compartment**

Fluid present in the spaces outside the cell constitutes extracellular fluid (ECF) compartment. This includes plasma, interstitial fluid and transcellular fluid.

**Plasma**

Plasma is the fluid part of the blood. It constitutes about 25% of the ECF, which is about 5% of the total body weight (Table 8.3).

Volume of plasma can be calculated from blood volume and PCV (packed cell volume) as:

\[
\text{Plasma volume} = \text{Blood volume} \times \frac{100 \text{ – Hematocrit}}{100}
\]

**Blood Volume and Red Cell Volume**

**Blood volume** is the plasma volume and blood cell volume. It is about 80 ml/kg of body weight or 8% of the total body weight.
### Table 8.1: TBW as % of body weight.

<table>
<thead>
<tr>
<th>Age</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infants</td>
<td>65–70</td>
<td>65–70</td>
</tr>
<tr>
<td>Children (upto 9 years)</td>
<td>60–65</td>
<td>59–62</td>
</tr>
<tr>
<td>10–17 years</td>
<td>58–60</td>
<td>55–58</td>
</tr>
<tr>
<td>18–39 years</td>
<td>58–64</td>
<td>48–55</td>
</tr>
<tr>
<td>40–59 years</td>
<td>52–58</td>
<td>45–50</td>
</tr>
<tr>
<td>60 years and above</td>
<td>50–55</td>
<td>42–48</td>
</tr>
</tbody>
</table>

(BW: Body weight; b.w.: Body water).

### Table 8.2: Distribution of total body water (TBW) in ECF and ICF compartments in a 70 kg adult male.

<table>
<thead>
<tr>
<th>Compartment</th>
<th>% of BW</th>
<th>% of b.w.</th>
<th>Volume</th>
<th>% of b.w.</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBW</td>
<td>60</td>
<td>100</td>
<td>42 L</td>
<td>8</td>
</tr>
<tr>
<td>ECF</td>
<td>20</td>
<td>33</td>
<td>14 L</td>
<td>25</td>
</tr>
<tr>
<td>ICF</td>
<td>40</td>
<td>67</td>
<td>28 L</td>
<td></td>
</tr>
</tbody>
</table>

(BW: Body weight; b.w.: Body water).

### Table 8.3: Distribution of ECF in a 70 kg adult male.

<table>
<thead>
<tr>
<th>Compartment</th>
<th>% of ECF</th>
<th>% of BW</th>
<th>Volume</th>
<th>% of b.w.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>25</td>
<td>4–5</td>
<td>3.5 L</td>
<td>8</td>
</tr>
<tr>
<td>Interstitial and transcellular fluid</td>
<td>10.5</td>
<td>15</td>
<td>10.5 L</td>
<td>25</td>
</tr>
</tbody>
</table>

(BW: Body weight; b.w.: Body water).

### Clinical Box 8.1

**Dehydration is common and rapid in children:** Ratio of ECF volume to ICF volume is larger in infants and children than in adults. However, the total volume of ECF is much smaller in children than in adults. In addition, the regulatory mechanisms for maintaining ECF volume are not well developed in infants and children. Therefore, dehydration is more common and occurs rapidly in these age groups.

### Interstitial Fluid

It is ECF volume present in the space between the cells (ECF outside the vascular system). Interstitial fluid surrounds all cells except blood cells and includes lymph (lymph constitutes 2–3% of the total body weight). It is in constant motion throughout the body and is exchanged rapidly with the circulating blood.
1. Interstitial fluid volume is about 15% of body weight.
2. It can not be measured directly as substances used for it rapidly equilibrate with plasma.
3. It is calculated by subtracting plasma volume from ECF volume.

### Transcellular Fluid

Transcellular fluid represents fluid in the lumen of structures lined by epithelium.
1. It includes fluid in the secretion of exocrine digestive glands, cerebrospinal fluid (CSF), pleural, peritoneal, synovial and pericardial fluids, intraocular (aqueous and vitreous humors) fluids, bile, luminal fluids of the gut and fluid in the cochlea and kidney tubules.

### Intracellular Fluid Compartment

Intracellular fluid (ICF) is the fluid contained within the cells of the body. It constitutes about two-thirds of total fluid of the body. It can not be measured directly. It is measured by subtracting ECF volume from total fluid volume.

### MEASUREMENT OF BODY FLUID VOLUMES

**General Principle**

The volume of fluid compartment is usually measured by the *indicator dilution principle*, which is based on the following relationship:

\[ C = \frac{A}{V} \quad \text{i.e.,} \quad V = \frac{A}{C} \]

Where, A is the amount of a substance injected intravenously, V is the volume in which the substance is distributed and C is the final concentration attained.

**Characteristics of an Indicator**

1. Should be relatively easy to measure.
2. Should remain in the compartment being measured.
3. Should not change the fluid distribution in the compartment being measured.
4. Should be non-toxic.
5. Must mix evenly throughout the compartment being measured.
6. Should remain unchanged by the body during the mixing period or the amount changed must be known.

**Principle of Measurement**

Size of fluid compartment is measured by injecting a substance and then calculating the volume of fluid in which the test substance is distributed. This is called the *volume distribution* of the injected substance, which is equal to the amount injected divided by concentration of the substance in the sample.

If indicator *leaves the compartment by excretion or metabolism* during the time allowed for mixing, then calculation is done as follows:
Volume distribution = \frac{\text{Amount injected} - \text{Amount removed}}{\text{Final concentration of the substance}}

**Measurement of ECF Volume**

The ECF volume is difficult to measure, as the limit of this space is ill-defined, and few substances mix rapidly in all parts of the space while remaining exclusively extracellular.

**Methods of Measurement**

ECF volume is measured by volume distribution principle using inulin, a polysaccharide having molecular weight of 5200.

1. **Radioactive inulin** is prepared by substituting $^{14}$C for one of the carbon atoms of the molecule. Radioactive inulin levels are easily determined by counting the samples with suitable radiation detectors.

2. Also $^{36}$Cl, $^{38}$Cl, $^{82}$Br, mannitol and sucrose are used for measurement of ECF volume.

3. Cl$^-$ is largely extracellular. Therefore, radioactive isotopes of Cl$^-$ ($^{36}$Cl and $^{38}$Cl) are used for the purpose. However, ECF volume determined by using Cl$^-$ is greater than actual volume as some Cl$^-$ is also present in intracellular fluid.

4. $^{82}$Br, sulphate, thiosulphate, thiocyanate and ferrocyanide are also used for measuring ECF volume. As these ions interchange with Cl$^-$ in the body, they determine greater values for ECF.

5. Mannitol and sucrose have also been used to measure ECF volume.

**Measurement of Plasma Volume**

Plasma volume is measured by two dilution methods.

**First Method:** In the first method, the substance used neither leaves the vascular system nor penetrates red cells. Examples are:

i. **Evans Blue Dye** (T-1824) that remains bound to plasma proteins.

ii. **Radio-iodinated human serum albumin** (RISA) i.e. serum albumin labeled with radioactive iodine. It slowly escapes from circulation into the interstitial fluid. Suitable sample of injected solution and plasma samples obtained after injection are counted in a scintillation counter.

iii. **Radio-iodinated gamma globulin and fibrinogen.** These substances generally do not leak out of the blood stream.

**Second Method:** In the second method, **radio-isotopes of phosphorus ($^{32}$P), iron ($^{55,59}$Fe) and chromium ($^{51}$Cr)** are used that penetrate and bind to red cells. Therefore, the red cells volume, i.e. volume occupied by all the circulating RBCs in the body can be measured by injecting tagged red cells intravenously. After thorough mixing has occurred, fraction of the RBCs that is tagged is measured.

The red cell volume, i.e. volume occupied by all the red cells (RBCs), is measured by injecting tagged red cells intravenously. After thorough mixing has occurred, fraction of the RBCs that is tagged is measured.

Commonly used tag is $^{51}$Cr, which is attached to the red cells by incubating in a suitable ‘Cr’ solution. Then, plasma volume is calculated by using the formula:

\[
\text{Plasma volume} = \frac{100 - \text{PCV}}{100} \times \text{Blood volume}
\]

(PCV is packed cell volume, which is practically the red cell volume).

**Measurement of Interstitial Fluid Volume**

Interstitial fluid volume cannot be measured directly as it is difficult to sample and no substance penetrates exclusively in this compartment. Substance that equilibrates in interstitial fluid also equilibrates in plasma. Therefore, the interstitial fluid volume is estimated as the difference between ECF volume and plasma volume.

**Measurement of ICF Volume**

Intracellular fluid volume (ICFV) cannot be measured directly by dilution principle as no substance remains confined only to this compartment. It is determined indirectly by subtracting ECF volume from total body water (TBW). Thus, first TBW is measured.

**Measurement of TBW**

Measurement of TBW is performed by indicator dilution principle:

1. **Deuterium oxide** ($^{2}$H$_2$O, heavy water) is usually used as it has properties that are slightly different from H$_2$O. However, in equilibration experiments for measuring TBW, it gives accurate results.

2. **Tritium oxide** and aminopyrine can also be used for measuring TBW.

Measurement of red cell volume, plasma volume effective blood volume is described in next chapter.

**IONIC COMPOSITION OF BODY FLUIDS**

**Basic Features**

1. The distribution of electrolytes varies in body compartments.

2. Sum of the concentrations of the cations equals the sum of the concentrations of the anions in respective compartments. This makes the fluid in each compartment electrically neutral.

3. Na$^+$, Ca$^{2+}$, Cl$^-$ and HCO$_3^-$ are largely extracellular (Table 8.4) and K$^+$, Mg$^{2+}$, organic phosphates (PO$_4^{3-}$) and proteins (prot$^-$) are mainly intracellular.

4. Essentially, almost all the K$^+$ in the body is in the exchangeable pool, whereas only 65%–70% of the body Na$^+$ is exchangeable. Solutes that are exchangeable are osmotically active.

5. Almost all of the body Ca$^{2+}$ (in bone) and most of the body Mg$^{2+}$ (in bone and cells) are nonexchangeable.
CHAPTER SUMMARY

Key Concepts

1. Total body water (TBW) as percentage of body weight on average is about 60% in adult males, which is about 10% more than in females.
2. In infants and children, though TBW as percentage of body weight is more than in adults, their absolute water content is significantly less. Therefore, dehydration occurs faster and often may be fatal, if not treated early and adequately.
3. Measurement of body fluid volume is based on ‘indicator dilution principle’.

Table 8.4: Concentration (mmol/L of H₂O) of major ions in ECF (plasma) and ICF.

<table>
<thead>
<tr>
<th>Ions</th>
<th>ECF</th>
<th>ICF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺</td>
<td>145</td>
<td>12</td>
</tr>
<tr>
<td>K⁺</td>
<td>5</td>
<td>140</td>
</tr>
<tr>
<td>Ca²⁺ (ionized)</td>
<td>1.2</td>
<td>0.1 μmol/L</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>1.5</td>
<td>20</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>110</td>
<td>5</td>
</tr>
<tr>
<td>HCO₃⁻</td>
<td>27</td>
<td>10</td>
</tr>
<tr>
<td>PO₄³⁻</td>
<td>2</td>
<td>60</td>
</tr>
</tbody>
</table>

Equivalents

The equivalent is the standard unit for expressing the solutes in the body which are in the form of charged particles. One equivalent (Eq) is 1 mole of an ionized substance divided by its valency. One mole of KCl dissociates into 1 Eq of K⁺ and 1 Eq of Cl⁻. One Eq of K⁺ = 39 gm/1 = 39 gm; whereas 1 Eq Ca²⁺ = 40 gm/2 = 20 gm. The milliequivalent (mEq) is 1/1000 of 1 Eq.

The normality (N) of a solution is the number of gram equivalents in 1 liter. Therefore, 1 N solution of hydrochloric acid (HCl) contains 1 + 35.5 gm/L = 36.5 gm/L.

Osmolarity and Osmolality

The number of osmoles per liter of solution is called osmolarity, whereas the number of osmoles per kilogram of solvent is osmolality. Osmotically active substances in the body are dissolved in water. As the density of water is 1, osmolar concentration is expressed in osmoles per liter (Osm/L) of water. Details of osmole, milliosmole, osmolarity and osmolality are described in Chapter 6, under ‘Osmosis’. Regulation of composition, osmolality and volume of body fluid and the related applied physiology are described in in chapter “Regulation of volume, osmolality and composition of body fluid compartments”, in Volume II, Section 13 (Integrative Physiology).

pH of Body Fluids

The hydrogen ion concentration of body fluids is expressed as H⁺ or pH (negative logarithm of H⁺).

pH = –log₁₀[H⁺].

1. When pK (K is the ionization or dissociation constant) of a buffer system is known, it is possible to determine the effective pH range of the buffer. Hence, pK = negative log of K (–log K) and is equal to the pH at which half of the acid molecules are dissociated and half are undisassociated.
2. Blood pH is 7.35–7.45, at the range of H⁺ conc. 20–126 mEq/L.
3. Acidosis refers to blood < 7.35 and alkalosis refers to blood > 4.45.

Concept and mechanism of pH-buffer system and acid-base balance are described in detail last section of the book “Integrative Physiology” in chapter ‘Basic principle of acid-base homeostasis’.

Units for Measuring Solutes

The number of molecules, electrical charges, or particles of a substance per unit volume of a particular body fluid is frequently expressed in moles, equivalents or osmoles.

Moles

The mole is the standard unit for expressing the amount of substances in the SI unit system. A mole is the gram-molecular weight of a substance, i.e. the molecular weight of a substance, in grams. Each mole (mol) consists of approx. 6 × 10²³ molecules. Thus, 1 mole of KCl = 39 + 35.5 gm = 74.5 gm (i.e. sum of atomic masses of all the atoms in the molecule).

The millimole (mmol) is 1/1000 of a mole. Thus, 1 mmol of KCl = 74.5 mg.

The concepts are:

1. The concentrations of two different substances on the basis of number of grams per liter of solution do not indicate how many molecules of each compound are present. Therefore, concentrations in units of grams per liter are often used when the chemical structure of the solute is unknown.
2. When the structure of a molecule is known, concentrations are expressed as moles per liter. This provides a unit of concentration based upon the number of molecules of the solute in solution. Thus a solution containing 74.5 gm of KCl in 1 liter of solution is said to be one-molar (1M or 1 mol/L) solution of KCl.
3. Since 1 mole of any molecule will have the same number of molecules (6 × 10²³), 1M solution of KCl contains the same number of solute molecules per liter as 1M solution of glucose or any other substance.
4. K\(^+\) is more in ICF (140 mmol/L of water) and less in ECF (5 mmol/L of water), whereas Na\(^+\) is less in ICF (12 mmol/L of water) and more in ECF (145 mmol/L of water).

**Points to be Noted (Must Read)**

1. Measurements of plasma volume, blood volume and ECF volume are usually asked in written examinations.
2. In *Viva*, questions from the following topics/concepts are asked:
   - % of total body water in males and females in different age groups.
   - Principle of measurement of fluid volumes (indicator dilution principle).
   - Characteristics of an indicator.
   - Basic method of measurement of ECF and ICF.
   - Different isotopes/dyes used for measuring different fluid volumes.
   - Why is dehydration common in children?
   - Concept of moles, osmoles, equivalents, osmolality and osmolarity.
SECTION–2

Blood and Immunity

9. Composition and Functions of Blood and Plasma Proteins
10. Bone Marrow and Hemopoiesis
11. Red Blood Cells
12. Erythropoiesis
13. Destruction of Red Blood Cells
14. Hemoglobin and Blood Indices
15. Pathophysiology of Anemia and Polycythemia
16. Blood Groups and Physiological Basis of Blood Transfusion
17. White Blood Cells
18. Thymus, Lymphoid Tissues and Lymph
19. Physiology of Immunity
20. Platelets and Their Role in Hemostasis
“The spirit shall look out through Matter’s gaze
And Matter shall reveal the Spirit’s face
Then man and superman shall be at one
And all the earth become a single life.”

Sri Aurobindo (in ‘SAVITRI’)
Hematological investigations are commonly performed laboratory tests in medical practice because many bodily dysfunctions reflect through alteration in blood even in their early stage and hematological changes are reliable indices of the intensity and progress of the diseases. Moreover, blood is an easily accessible and readily available tissue sample for investigations, and many blood tests can be performed in physician’s office. Therefore, examination of blood is common and an essential component in patient management. Therefore, a student of medicine should have adequate knowledge in physiology of blood.

**Hematology** is the study of physiology of blood and blood disorders. Study of blood physiology includes the study of different components of blood, their formation, their role in different body functions and dysfunctions, and study of blood disorders includes the pathophysiology of diseases of blood and their management, and pathological alterations in blood in other (non-hematological) diseases.

Blood examination reveals two fundamental aspects of hematology:

1. If the bone marrow is producing adequate number of mature cells of hematopoietic lineages
2. If development of each hematopoietic lineage is qualitatively normal.

Though investigations on peripheral blood by different blood counts and smear examination help to answer these two major questions, study of bone marrow provides a better picture of the disease and adds to hematological diagnosis.

**BLOOD**

Blood is defined as liquid connective tissue that fills the heart and blood vessels. The normal blood volume in an average adult is 5–6 liters, which accounts for about 8% of the body weight.

**Composition of Blood**

Blood consists of two components: cells and fluid. The cellular component comprises of different formed elements, and the fluid component is the plasma.

1. The **formed elements** are red blood cells (erythrocytes), white blood cells (leucocytes), and platelets (thrombocytes).
2. The **plasma** consists of about 55% of the total blood volume which is made up of water and solid particles. When blood is collected in an anticoagulated tube and allowed to settle by centrifugation, **three layers** are
distinctly visible. The upper plasma layer is separated from lower red cell mass by a thin buffy coat (Fig. 9.1).

**Buffy Coat**

The thin middle layer that separates the upper plasma and lower red cell mass after centrifugation of blood is the buffy coat.

1. The buffy coat contains leucocytes and platelets.
2. In diseases, abnormal cells are found in the buffy coat like LE cells in systemic lupus erythematosus and atypical or primitive blood cells (atypical mononuclear cells, promyelocytes, metamyelocytes, blast cells, megakaryocytes etc.) in malignant or premalignant conditions (Clinical Box 9.1).

**Clinical Box 9.1**

**Buffy coat preparation:** The blood film may be prepared from buffy coat for the detection of the abnormal cells in blood. Also, buffy coat preparation is very useful for the detection of bacteria, fungi or parasites within neutrophils, monocytes or circulating macrophages.

**Functions of Blood**

1. **Respiratory functions:** Blood transports oxygen from lungs to tissues, and carbon dioxide from tissues to the lungs. Thus, by transporting gases blood serves an important function of respiration.
2. **Transport medium:** Blood acts as the transport medium for various hormones, chemical substances, nutrients, vitamins, etc. Hormones secreted from various endocrine tissues, and nutrients absorbed from GI tract circulate in blood and distribute to all tissues of the body. Thus, blood regulates growth and metabolism of the tissues.
3. **Temperature regulation:** Blood plays an important role in temperature regulation as it conducts heat from the interior of body to the surface through blood vessels. Temperature is continuously produced in the body. Blood absorbs this heat and transfers to the body surface. Therefore, vasoconstriction preserves body temperature and vasodilation facilitates heat dissipation from the body.
4. **Excretory function:** Blood helps in excretion of waste materials by transporting them from different parts of the body to the kidney.
5. **Water homeostasis:** Fifty-five percent of blood contains plasma and 92% of plasma contains water. Loss of water from body as occurs in diarrhea, vomiting, excessive sweating etc. results in decreased blood volume (hypovolemia). Persistent hypovolemia results in cellular dehydration. In response to hypovolemia, mechanisms are activated to increase the water content of blood that aims at maintaining cellular hydration. Thus, by maintaining blood volume, water homeostasis of the body is maintained.
6. **Acid-base balance:** Blood contains plasma proteins and hemoglobins. They (protein and Hb buffers) play important role in maintaining acid base balance of the body. Also, bicarbonate in blood buffers the acids.
7. **Immunology:** Blood forms a critical component of body immunity. It contains cells that play primary role in cellular immunity and also contains antibodies that play major role in humoral immunity. Blood also contains cells and chemicals that take part in nonspecific defenses of the body.
8. **Storage:** Blood is the storage site of electrolytes, nutrients, chemicals, hormones, etc.
9. **Body color:** Blood provides natural color to the body. In anemia and hypovolemia, body becomes pale.
10. **Oncotic pressure:** The albumin present in blood exerts osmotic pressure, known as oncotic pressure that controls capillary filtration and prevents edema formation (discussed more in ‘functions of plasma proteins’).

**Blood Volume**

The total blood volume can be roughly calculated as 70–80 ml/kg of body weight. In adult males, the total volume of blood is about 5 to 6 liters and in females about 4.5 to 5.5 liters. In children, though the absolute volume of blood present is significantly less than adults, the volume expressed per kg of body weight is more (80–90 ml/kg-BW). Blood volume is also expressed per square meter of body surface area, which is normally 2.8 lit/m² of the body.

**Determination of Blood Volume**

Blood volume is determined by determining the plasma volume and cell volume separately. The normal ratio of plasma volume to cell volume is 55:45.
Measurement of Cell Volume

Cell volume is measured by measuring the volume of red cells. The volume of leucocytes and platelets is ignored as they constitute a minor fraction of the total cell volume. Red cell volume is measured by radioactive isotope study.

Red cell volume

Red cell volume is the volume occupied by all circulating red cells.
1. It is usually calculated by subtracting plasma volume from total blood volume.
2. It is also directly calculated by injecting tagged red cells. The radioactive isotope of chromium is usually used as a tag, though isotopes of iron ($^{59}$Fe) and phosphorus ($^{32}$P) are also used.
3. The commonly used chromium isotope is $^{51}$Cr, which is attached to red cells through incubation of cells in the chromium solution.

Determination of Plasma Volume

Plasma volume is usually measured by using dyes. The dye used is Evans blue (T-1824). Plasma volume is also measured by injection of serum albumin labeled with radioactive iodine. An average plasma volume is about 3.5 L (5% of the body weight of a 70 kg man).

Total blood volume is calculated by multiplying plasma volume with 100/(100 – hematocrit). (Hematocrit is to be determined). If hematocrit is 40 and plasma volume is 3.5 L, then blood volume will be:

$$3.5 \times \frac{100}{100 - 40} = 5.8 \text{ L}$$

Effective Blood Volume

Effective blood volume = the total blood volume – the volume that is sequestered.

This means the volume which is present actually in the circulation that helps in perfusion of tissue. However, it is difficult to estimate the sequestered volume of blood in the visceral organs.

Specific Gravity and Viscosity of Blood

Specific gravity: Specific gravity or density of whole blood is approximately 1.050.

**PLASMA**

Plasma is the fluid component of blood which constitutes about 55% of the total blood volume. The remaining 45% is constituted by cells (formed elements). If the whole plasma is to be used for investigations, blood is collected with anticoagulants (anticoagulated blood). Plasma can be stored for years for future investigations (Application Box 9.1).

**Composition of Plasma**

Plasma constitutes water and solids (Table 9.1). Water content of plasma is about 92% of the total plasma. The major solute (solid) content is the plasma proteins, which constitutes about 7% of the plasma and other solutes make up the rest 1% (Fig. 9.2).

---

**Table 9.1: Composition of plasma.**

<table>
<thead>
<tr>
<th>1. Water (92%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. Solids or Solutes (8%): plasma proteins make 90% of solids</td>
</tr>
<tr>
<td>a. Inorganic:</td>
</tr>
<tr>
<td>i. Anions: Chloride, bicarbonate, phosphates, sulphates, etc.</td>
</tr>
<tr>
<td>ii. Cations: Na⁺, K⁺, Ca²⁺, Mg²⁺, etc.</td>
</tr>
<tr>
<td>b. Organic:</td>
</tr>
<tr>
<td>i. Colloids: Plasma proteins</td>
</tr>
<tr>
<td>ii. Crystalloids: Glucose, lipid, urea, uric acid, etc.</td>
</tr>
</tbody>
</table>
Serum
During blood coagulation, a soluble plasma protein called fibrinogen is removed from plasma as it is utilized in the formation of fibrin (the blood clot). Plasma without clotting factors is called serum.
1. Many biochemical tests are performed by separating serum from blood by allowing the blood to clot (coagulated blood) (Application Box 9.2).
2. Serotonin content of serum is high as platelets release serotonin during clotting.
3. Serum is not only used for biochemical investigations, but also as a supplement to cell culture media.
4. Serum ensures a better environment for growth of cells in vitro as it contains all nutrients, sugar, proteins, hormones and other factors.
5. Fetal calf serum is used often for growth of human cells.

Application Box 9.2
Separating serum: Plasma is separated by centrifuging anticoagulated blood. The serum is separated from blood by allowing the blood to coagulate (serum = blood – fibrinogen and other clotting factors).

PLASMA PROTEINS
Plasma proteins are the major solute constituents of plasma. The normal plasma protein concentration is 6–8 g/100 ml of plasma.

Types of Plasma Proteins
Plasma proteins are of three types: albumin (4–5.5 g%), globulin (1.5–3 g%), and fibrinogen (0.3 g%). Plasma proteins are formed mainly in the liver.

Albumin
This is the major constituent of plasma proteins.
1. Albumin is formed in the liver.
2. It has half life of about 20 days and its molecular weight is 66000.
3. Being smallest in diameter among the plasma proteins, in kidney diseases with glomerular injury it appears early in urine (albuminuria).
4. In kidney diseases, albuminuria decreases plasma protein concentration that leads to hypoalbuminemia. Hypoalbuminemia also occurs in in liver diseases due to decreased formation of albumin. In such conditions, decreased colloidal osmotic pressure (oncotic pressure) of plasma results in edema formation (see below).

Globulin
This is formed in the liver, cells of reticuloendothelial system and plasma cells.
1. Globulins are divided into three categories: α (α₁, α₂), β (β₁, β₂) and γ.
2. The molecular weight of globulins is 90000–156000.
3. Globulins include different transport proteins like transferrin, ceruloplasmin, hemopexin, etc.
4. They form different lipoproteins in combination with lipids in plasma.
5. Antibodies (immunoglobulins) are γ-globulins that are formed by plasma cells.
6. The normal albumin-globulin ratio (AGR) is 1.5 to 2.5:1. In many diseases, AGR is altered. Detection of AGR helps in diagnosing and assessing the prognosis of some diseases.
7. Albumin-globulin ratio (AGR) is important (Clinical Box 9.2)

Fibrinogen
The molecular weight is 340000. It is produced in the liver. It plays an important role in blood coagulation. It also contributes to the viscosity of plasma and determination of ESR.

Functions of Plasma Proteins
1. Osmotic pressure: Plasma proteins are osmotically active molecules, and the osmotic pressure of plasma due to plasma proteins is called oncotic pressure. The normal oncotic pressure is 25 mm Hg. This pressure helps in maintaining volume of the vascular compartment. Oncotic pressure retains fluid in the vascular compartment and, therefore, prevents loss of fluid from capillaries into the interstitial tissue space (functionally, it opposes the action of hydrostatic pressure). Therefore, when oncotic pressure decreases due to hypoproteinemia as occurs in liver and kidney diseases, edema manifests due to escape of water into the interstitial tissue space. The oncotic pressure is due to the presence of albumin in the plasma.
2. Viscosity: Plasma protein contributes to about 50% of the viscosity of blood (red cells account for rest of the viscosity). The viscosity depends on the molecular shape of the plasma protein. This is why fibrinogen molecules that are elongated and fibrillar in shape contribute more to blood viscosity than albumin molecules that are ellipsoid in structure.
3. Immunity: Antibodies are plasma proteins (gamma globulins). Antibodies mediate humoral immunity that protects the body from infections, especially extracellular pathogens and from the effect of toxic substances.
4. **Coagulation**: Blood clotting depends on concentration of fibrinogen that forms fibrin thread, the final step in blood coagulation. Also, other clotting factors like prothrombin are plasma proteins.

5. **Transport**: Plasma proteins serve as carrier molecules for transport of various substances like hormones, drugs, metals, etc.

6. **Buffering**: Plasma proteins form an important buffering system of the body called protein buffers. This helps in acid-base balance of the body.

7. **Protein store**: Plasma proteins serve as mobile protein reserve of the body, which can be utilized for tissue growth, especially in situations of protein depletion.

8. **Synthetic function**: Plasma proteins provide substrate for the synthesis of protein hormones like erythropoietin, etc. and various enzymes.

9. **Determination of ESR**: ESR mostly depends on the concentration of fibrinogen in the plasma. Fibrinogen facilitates rouleaux formation, which in turn increases the rate of sedimentation of red cells. Therefore, conditions in which fibrinogen concentration is more like acute inflammations, ESR becomes more (Details of ESR are described in chapter “The Red Blood Cells”).

**Separation of Plasma Proteins**

Plasma proteins can be separated by methods like salt separation, paper electrophoresis, Cohn’s fractionation, ultracentrifugation, immunochemical analysis, etc. Determination of concentration of various fractions is useful for estimation of albumin-globulin ratio (AGR) and for diagnosis and prognosis of different diseases.
Bone Marrow and Hemopoiesis

**Learning Objectives**

On completion of study of this chapter, the student **MUST** be able to:

1. Name the types of bone marrow and give their functions.
2. Comprehend the basic cellular architecture of bone marrow.
3. Appreciate the importance of myeloid-erythroid ratio in bone marrow.
4. Define hemopoiesis and name the sites of hemopoiesis in different stages.
5. Understand the difference between medullary and extramedullary hemopoiesis.
6. Name the sites of hemopoiesis in different age groups.
7. Name the types of stem cells and give their properties.
8. Outline the steps of different series (erythropoiesis, leucopoiesis and thrombopoiesis) of hemopoiesis.

The student **MAY** also be able to:

1. List the indications of bone marrow biopsy.
2. Understand the need for bone marrow aspiration.
3. Draw and describe the cells in hemopoietic series.
4. Describe the cellular patterns in bone marrow biopsy.

**Bone Marrow**

Bone marrow is the site of normal hemopoiesis after birth. Hemopoiesis (or hematopoiesis) describes the formation of blood cells, which is an active process that must maintain normal number of blood cells in peripheral blood and also should be able to respond to the increased demands in situations like hemorrhage or infections. During fetal life, hemopoiesis mainly occurs in the spleen and liver, and subsequently in red bone marrow present in the medullary cavity of all bones. From childhood, red marrow is progressively replaced by fat tissues (yellow marrow). Therefore, normal hemopoiesis in adults is restricted to vertebrae, sternum, ribs, clavicles, pelvic bones, skull and ends of humerus and femurs.

Bone marrow contains a range of hemopoietic precursor cells and a storage pool of mature cells for their release into peripheral circulation. All blood cells are derived from the pluripotent stem cells, referred to as hemopoietic stem cells (HSCs). HSCs sustain life long production of all blood lineages. HSCs provide homeostasis of blood cells through their ability to generate the hundreds of millions of red cells, white cells and platelets needed every day.

**Types of Bone Marrow**

There are three types of bone marrow: red marrow, yellow marrow and white marrow.

**Red Marrow**

The red marrow is the active marrow and is red in colour. It consists of many blood vessels that contain sinusoidal capillaries (vascular sinuses), and the marrow stroma (Fig. 10.1). **Marrow stroma:** The marrow stroma consists principally of a network of sinuses that originate at the endosteum from cortical capillaries and terminate in the collecting vessels that enter the systemic circulation. Hematopoiesis takes place in the **intersinus spaces.** The trilaminar sinus wall contains three components:

1. Endothelial cells
2. Thin basement membrane
3. Adventitial reticular cells.
The bone forming cells are present between the sinusoidal capillaries. The adventitial reticular cells are fibroblasts capable of transforming into adipocytes (fat cells). Endothelium and reticular cells are the sources of cytokines that profoundly influence hemopoiesis. All types of precursor cells (blast cells, progenitor cells, megakaryocytes) are present in the bone marrow trabeculae. Red cells are produced from the erythroid ‘nests’.

Red bone marrow is present in almost all bones of the body at birth. However, with advancement of age red marrow is replaced by fatty tissue and slowly becomes inactive. At the age of 20–30 years, red marrow of long bones cease to produce cells, and active marrow is limited to the membranous bones like skull bones, vertebrae, ribs, sternum and pelvic and pectoral girdles (Fig. 10.2).

Yellow Marrow

The yellow marrow contains less blood vessels and more fat cells and fibro-fatty tissue. The yellow marrow does not participate in hemopoiesis. Fat cells replace hematopoietic cells in the bones of hands, feet, legs and arm at about age of 20 years. Fat occupies 50% of the space of red marrow in adult and further fatty degeneration continues slowly with aging. Yellow marrow can revert back to hemopoietically active red marrow when prolonged demand persists as occurs in chronic hemolytic anemia. Differences between red and yellow marrow are listed in Table 10.1.

**White Marrow**

In very old individuals, a gelatinous transformation of fat to a mucoid material occurs in bone marrow. This type of marrow in elderly persons is called white marrow.

**Bone Marrow Examination**

Bone marrow examination is indicated in complex hematological disorders and malignant hematological conditions that require detailed analysis of cellularity and activity of the marrow. The bone marrow examination is done for the following types of assessments:

1. Cellularity of the marrow (normocellular, hypercellular or hypocellular)
2. Myeloid-Erythroid ratio
3. State of erythropoiesis, myelopoiesis and megakaryopoiesis
4. Number of lymphocytes (normal, increased or abnormal)
5. Plasma cells (normal or increased)
6. Other cells : Metastatic tumor cells, parasites (malaria, Leishmania Donovani, etc.), fungus or any other cells.
7. Iron stores evaluated by Persian blue reaction (normal, increased or decreased)

**Indications for Bone Marrow Examination**

Usual indications for bone marrow examination are:

1. Anemias: Aplastic anemia and refractory anemias
2. Aleukemic leukemia
3. Differentiation of leukemias
4. Myelofibrosis and myelosclerosis
5. Multiple myeloma
6. Agranulocytosis
7. Megaloblastic anemia
8. Thrombocytopenic purpura
9. Assessment of iron stores
10. Therapeutic (Bone marrow transplant)

**Procedure**

Bone marrow examination is usually done by needle aspiration and bone marrow trephine biopsy. Aspiration is a safe and easy procedure. Under local anesthesia, aspiration or biopsy needle is introduced into the center of the marrow and about 1 ml of marrow content is aspirated or the tissue is obtained, which is examined after preparing a stained smear. Staining is usually done by Romanowsky dyes or May–Grünwald–Giemsa stain. The usual sites of aspirations are sternum (sternal puncture), iliac crest and spinous process of vertebra.

**Bone Marrow Needle Aspiration**

The usual sites of aspirations are sternum, iliac crest and spinous process of vertebra (Clinical Box 10.1).

**Clinical Box 10.1**

Sites of marrow aspiration: Bone marrow aspiration is relatively an easy procedure. Commonly used needle is Salah Needle that has the provision of adjusting the depth of penetration into the marrow. The common sites of bone marrow aspiration are: sternum, posterior-superior iliac spine, iliac crest, anterior-superior iliac spine, and spinous process of lumbar vertebra. In infants, upper end of tibia is the ideal site for marrow aspiration.

**Sternal Puncture**

This is the commonest procedure for bone marrow aspiration, especially in adults. The needle is introduced into the marrow cavity of mid-manubrium sterni.

**Iliac Crest Puncture**

The iliac crest, particularly the region of the posterior spine is the preferred site for bone marrow aspiration, especially in children. It is also done in adults.

**Vertebral Puncture**

Spinous process of vertebrae of the lumbar segments is preferred when sternal puncture fails to reveal proper cytology or when sternum is otherwise contraindicated for the process.

**Bone Marrow Biopsy**

Bone marrow biopsy is performed by biopsy needle. This is called *trephine biopsy*. The sites of biopsy are same as for needle aspiration. Biopsy is more tedious and risky than aspiration.

**Features of Marrow Smear**

The examination of stained marrow smear is performed for assessing cellularity of the marrow, effectiveness of hemopoiesis (erythropoiesis, leucopoiesis and thrombopoiesis), myeloid-erythroid ratio, and presence of tumor cells, plasma cells, LD bodies (Leishman Donovan bodies as seen in Leishmaniasis), and malaria parasites.

**Cells in Bone Marrow**

In bone marrow, hemopoietic cells are observed in different stages. These include stem cells, CFUs, blast cells and other progenitor cells (Fig. 10.1). The stem cells normally constitute about 0.5% of marrow cells. In red bone marrow, cells are mostly of myeloid series that produce leucocytes (granulocytes and agranulocytes), and constitute about 70–75% of the cell mass. The erythroid series constitutes about 25% of the marrow cell mass. Thus, the myeloid-erythroid ratio (MER) in the bone marrow is 3:1 (Application Box 10.1). However, in the peripheral blood, red cells are much more than the white cells, *leucocyte-erythrocyte ratio* being 1:700.

**Application Box 10.1**

MER in bone marrow preserves leucocyte population in peripheral blood: The average life span of red cell is 120 days, whereas white cells stay in peripheral blood for few hours to few days. Therefore, to preserve white cell population in peripheral blood nature has provided more white cell precursors in bone marrow. This (more myeloid cells in the marrow) helps to replace adequate leucocytes in the blood as leucocytes have shorter life span than erythrocytes in circulating blood.

**Bone Marrow Transplantation**

Bone marrow transplantation (BMT) is the process of collection and infusion of hematopoietic stem cells obtained from the bone marrow or peripheral blood, of either of the other individuals or of the own hematopoietic stem cells (HSC).

**Important Note**

Sources of hematopoietic stem cells:

1. **Bone Marrow**: Bone marrow is the richest source of HSC.
2. **Peripheral Blood**: Few HSC are present in peripheral blood. They are mobilized into peripheral blood from bone marrow by administration of G-CSF or GM-CSF.
3. **Umbilical Cord Blood**: Collected following delivery. This is a rich source.

**Scientist contributed**

Dr E Donnall Thomas received Nobel Prize in Physiology and Medicine in 1990, for Bone Marrow Transplantation. Dr. Thomas and his colleagues pioneered the successful use of bone marrow transplantation. This procedure replaces cancerous cells and stem cells damaged by chemotherapy and radiation with donated healthy cells that engraft within a patient’s bone marrow. The discovery was a cure for leukemia and other blood cancers, and earned Thomas the 1990 Nobel Prize in physiology or medicine.
hematopoiesis. Blood cells are continuously destroyed in the body. Therefore, replacement of blood cells is also a continuous phenomenon and is an essential part of homeostasis of blood cells.

**Scientist contributed**

A Maximow is the first scientist to study the process of hematopoiesis. He studied the contribution of endothelium to blood regeneration (hematopoiesis). He especially studied the relation hematopoiesis with connective tissue and vascular endothelium.


**Alexander Maximow (1874–1928)**

**Sites and Stages of Hematopoiesis**

Location of hematopoiesis depends on the stage of hematopoiesis. During intrauterine life, hematopoiesis occurs first in the yolk sac, and later in the liver and spleen. After birth, hematopoiesis is confined to bone marrow. The environment in these organs (yolk sac, liver, spleen and bone marrow) where hematopoiesis takes place is called hematopoietic microenvironment. Hematopoiesis occurs in three stages: mesoblastic, hepatic and medullary.

**Mesoblastic Stage**

Mesoblastic stage is the stage of development of blood cells in the yolk sac and non-yolk sac regions during embryonic stage of intrauterine life. Yolk sac contains cells with multilineage differentiating capabilities that start functioning as early as second week of gestation. There are also few non-yolk sac regions that participate in hematopoiesis. Important among them are paraaortic splanchnopleura (PSP) and aorta-gonad mesonephros (AGM) regions. PSP region gives rise to B-cell progenitors and AGM region contains pleuripotent stem cells. Early lymphoid precursors have also been identified in the day-8 yolk sac. Also, lymphohematopoietic stem cells have been detected in vivo in the 9th day yolk sac.

**Hepatic Stage**

During second trimester of pregnancy, hematopoiesis takes place in the liver and spleen. This is called hepatic stage. In liver, hematopoiesis *starts as early as 5th week of gestation*, which reaches its peak in 5th month and ceases at birth (Fig. 10.3). In spleen, hematopoiesis *occurs mainly in the second trimester* and the degree of hematopoiesis is quite less than that in the liver. Normally, hematopoiesis *does not occur in liver and spleen during post-natal life* (after birth). However, when the demand for blood cells is more than the rate of production, hematopoiesis does occur in liver and spleen.

**Medullary Stage**

Formation of blood cells in the bone marrow is called medullary hematopoiesis. Marrow cavities in the bone appear as early as *5th month of fetal life*, and soon they become hematopoietic. In initial phase, bone marrow is the exclusive site for granulocytic and megakaryocytic proliferation, during which erythropoietic activity is confined to liver. Erythropoiesis in bone marrow occurs effectively towards the end of third trimester of pregnancy and then continues throughout life.

After birth, hematopoiesis stops in liver and gets confined only to the bone marrow. When hematopoiesis occurs in liver and spleen in postnatal life, it is called extramedullary hematopoiesis. Extramedullary hematopoiesis after birth is always non-physiological (Application Box 10.2).

**Application Box 10.2**

Extramedullary hematopoiesis after birth is abnormal: Hematopoiesis in liver and spleen is physiological during intrauterine life. However, hematopoiesis in these organs or in any other organ (extramedullary hematopoiesis) after birth is considered abnormal.

**Rate of Medullary Hematopoiesis**

Bone marrow is one of the largest organs in the human body. It produces about 6 billion cells (2.5 billion red cells, 2.5 billion platelets and 1 billion granulocytes) per kg of body weight per day in adolescents and adults. However, the rate of production is adjusted to the needs of day-to-day life. Different cell population and their rate of productions are listed in Table 10.2.

**Sites of Medullary Hematopoiesis**

Bone marrow is the sole site of erythropoiesis in human beings. In long bones, active marrow regresses rapidly after first decade of life and ceases to produce cells between 20–30 years of life (Fig. 10.2). Hematopoiesis continues to occur in active marrow of vertebrae, pectoral and
pelvic girdles, ribs, sternum and skull. However, with age the degree of hemopoiesis also decreases in these bones.

**Steps of Hemopoiesis**

Hemopoiesis occurs in the bone marrow from hematopoietic stem cells (Flowchart 10.1). The mother hematopoietic stem cell is the pluripotent stem cells (PPSC), also called as hemocytoblast. PPSC is the *multilineage stem cell* capable of producing two important groups of stem cells. These are myeloid stem cells and lymphoid cells. Lymphoid stem cells are unipotent stem cells (UPSC) that produce only lymphocytes, whereas myeloid stem cells are pluripotent in nature that from a group of different progenitor cells that are meant to produce red cells, granulocytes, monocytes and platelets. Thus, bone marrow contains two types of cells: stem cells and progenitor cells. In fact, stem cells develop into progenitor cells. The differences between these cells are listed in Table 10.3.

**Stem Cells**

**Properties**

Stem cells in the bone marrow have two important properties: self renewal and differentiation.

**Self Renewal**

This is the property of duplicating themselves. That means they renew themselves and therefore physiologically they do not die and at the same time they proliferate into different lineage of cells. Due to self renewal, the bone marrow never goes out of stock for stem cells. Stem cell reserve in bone marrow remains always adequate.

**Differentiation**

This is the property of developing into specific lineage of cells. Due to the property of differentiation, stem cells differentiate into progenitor cells of various cell lines. Thus, different cell lineages are formed from stem cells that give rise to specific blood cells.

**Scientist contributed**

Stem-cell scientists led by Dr John Edgar Dick was the first to study the details of properties and nature of stem cells. He has transformed the study of human hematopoiesis and leukemogenesis, with his development of methodologies for transplanting human bone marrow into immune-deficient mice, with resultant multilineage repopulation of murine bone marrow and other hematopoietic tissues. Using this approach, he has identified long-term repopulating human hematopoietic stem cells and generated mouse models of leukemia.

**Types**

There are two different stem cells: myeloid stem cells and lymphoid stem cells (Fig. 10.4).

**Myeloid Stem Cells**

Myeloid stem cells are pluripotent (tri-lineage) in nature that give rise to three types of different progenitor cells. These are erythroid progenitors that form erythroid series, the granulocytic progenitors that form granulocytes (neutrophil, eosinophil, and basophil), monocyctic progenitors form monocytes, and megakaryocytic progenitors that form platelets.

**Lymphoid Stem Cells**

Lymphoid stem cells are unipotent stem cells (UPSC) that produce only cells of lymphocyte series.

**Progenitor Cells**

Progenitor cells develop from stem cells. Progenitor cells form colony forming units (CFUs) that give rise to different types of blast cells. *Myeloid stem cells* differentiate into CFU-GEMM, i.e. CFU for granulocyte, erythrocyte, megakaryocyte and monocyte. CFU-GEMM develops into three major CFUs (Fig. 10.4).
Fig. 10.4: Types and stages of hemopoiesis and major cytokines influencing them. (SCF: Stem cell factor, BFU: Burst-forming unit, CFU: Colony-forming unit, CSF: Colony-stimulating factor, GM: Granulocyte-monocyte, IL: Interleukins, EPO: Erythropoietin, TPO: Thrombopoietin).

1. **CFU-E**: CFU-E is the CFU for erythroid series that form proerythroblast, which in turn develops into precursor cells of erythroid series.

2. **CFU-GM**: CFU-GM is the multilineage CFU that in turn forms CFU for granulocytes (CFU-G) and CFU for monocytes (CFU-M). CFU-G develops into myeloblast that differentiates into three lineage of granulocytes (neutrophil, eosinophil, and basophil). CFU-M develops into monoblast that differentiates into monocyte.

3. **CFU-Mega**: CFU-Mega differentiates into megakaryoblast that in turn forms platelets.

**Types of Hemopoiesis**

Hemopoiesis is broadly divided into three types: erythropoiesis, leukopoiesis and thrombopoiesis. Erythropoiesis is the development of red cells, leukopoiesis is the development of leucocytes and thrombopoiesis is the development of thrombocytes (platelets).

The PPSC differentiates into five major blast cells (CFUs are excluded here) that give rise to five different cell lines (Fig. 10.4).

1. The proerythroblast that forms erythroid series for final development of red cells.
2. The myeloblast that differentiates into three lineages of granulocytes series (neutrophil, eosinophil, and basophil).
3. The monoblast that differentiates into monocytes.
4. The lymphoblast that develops into lymphocytes.
5. The megakaryoblast that finally forms platelets.

Details of development of each cell line have been described in their respective sections.
Regulation of Hemopoiesis

Hemopoiesis is regulated by various cytokines. Cytokines that influence development of blood cells are called **hemopoietic growth factors** (HGFs). As many of them stimulate development of CFU, they are popularly called colony stimulating factors (CSFs). Many of them are nonspecific, influencing more than one cell line (Table 10.4), whereas few are very specific in their actions (for details of cytokines, refer the chapter “Immunity”). The major HGFs are:

1. **Erythropoietin**: It is secreted from kidney and stimulates development of erythrocyte precursors (for details, refer “regulation of erythropoiesis”).
2. **Interleukins (IL)**: There are many interleukins, IL_1_ to IL_7_. IL_1_ controls eosinophil development, IL_2_ and IL_6_ control lymphocyte development, IL_3_ and IL_7_ control monocyte-macrophage development, etc.
3. **Colony stimulating factors**: There are three major groups of CSFs: M-CSF, G-CSF and GM-CSF. M-CSF controls development of monocyte precursors, G-CSF controls growth of granulocyte precursors and GM-CSF promotes proliferation of both granulocytes and monocytes.
4. **Other growth factors**: Colony stimulating factor 1 (CSF 1) that influences macrophage development, leukemia inhibitory factor (LIF) that controls growth of megakaryocyte and stroma cell derived cytokine (SCDC) that influences B cell differentiation.

### Table 10.4: Target cells of hemopoietic growth factors (HGFs).

<table>
<thead>
<tr>
<th>Cell lineage</th>
<th>Cytokines (HGFs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Erythropoietin-BFU</td>
<td>Erythropoietin, IL_3_, IL_4_ and GM-CSF</td>
</tr>
<tr>
<td>2. Erythropoietin-CFU</td>
<td>Erythropoietin</td>
</tr>
<tr>
<td>3. Neutrophil-Macrophage</td>
<td>GM-CSF</td>
</tr>
<tr>
<td>4. Macrophage</td>
<td>CSF-1, IL_3_, IL_4_ and IL_6_</td>
</tr>
<tr>
<td>5. Neutrophil</td>
<td>G-CSF</td>
</tr>
<tr>
<td>6. Eosinophil</td>
<td>IL_3_ and IL_4_</td>
</tr>
<tr>
<td>7. Megakaryocyte</td>
<td>IL_3_, IL_4_, IL_6_, IL_7_, GM-CSF and LIF</td>
</tr>
<tr>
<td>8. Basophil-Mast cell</td>
<td>IL_7_ and GM-CSF</td>
</tr>
<tr>
<td>9. B lymphocytes</td>
<td>IL_2_, IL_3_, IL_4_ and SCDC</td>
</tr>
<tr>
<td>10. T lymphocyte</td>
<td>IL_2_, erythropoietin and GM-CSF</td>
</tr>
</tbody>
</table>

### CHAPTER SUMMARY

**Bone Marrow**

There are three types of bone marrow: red marrow, yellow marrow and white marrow.

- **Red Marrow**: The red marrow is the active site of hemopoiesis and is present in almost all bones of the body at birth. With advancement of age, red marrow is replaced by fatty tissue and slowly becomes inactive. At the age of 20-30 years, red marrow of long bones cease to produce cells, and active marrow is limited to the membranous bones like skull bones, vertebrae, ribs, sternum and pelvic and pectoral girdles.
- **Yellow Marrow**: The yellow does not participate in hemopoiesis. Fat cells replace hematopoietic cells in the bones of hands, feet, legs and arm at about age of 20 years.

**Bone Marrow Examination**

Bone marrow examination is indicated for detailed analysis of cellularity and activity of the marrow, which is done mainly for the following types of assessments:

1. **Cellularity of the marrow** (normocellular, hypercellular or hypocellular)
2. **Myeloid-Erythroid ratio** (Normal M/E ratio is 3:1)
3. **State of erythropoiesis, myelopoiesis and megakaryopoiesis**

**Hemopoiesis**

Hemopoiesis occurs in three stages: mesoblastic, hepatic and medullary.

- **Mesoblastic Stage**: Mesoblastic stage is the stage of development of blood cells in the yolk sac and non-yolk sac regions during embryonic stage of intrauterine life.
- **Hepatic Stage**: During second trimester of pregnancy, hemopoiesis takes place in the liver and spleen. This is called hepatic stage. In liver, hemopoiesis starts as early as 5th week of gestation, which reaches its peak in 5th month and ceases at birth. In spleen, hemopoiesis occurs mainly in the second trimester and the degree of hemopoiesis is quite less than that in the liver.
- **Medullary Stage**: Formation of blood cells in the bone marrow is called medullary hemopoiesis. Marrow cavities in the bone appear as early as 5th month of fetal life, and soon they become hemopoietic. Erythropoiesis in bone marrow occurs effectively towards the end of third trimester of pregnancy and then continues throughout life.

**Steps of Hemopoiesis**

Hemopoiesis occurs in the bone marrow from hematopoietic stem cells that are pluripotent stem cells (PPSC). PPSC is the multilineage stem cell capable of producing two important groups of stem cells: myeloid stem cells and lymphoid cells. Lymphoid stem cells are unipotent stem cells (UPSC) that produce only lymphocytes, whereas myeloid stem cells are pluripotent in nature that from a group of different progenitor cells that are meant to produce red cells, granulocytes, monocytes and platelets.

**Important to Know (Must Read)**

1. Stages, types and regulation of hemopoiesis, may come as a Long Questions.
2. Types of bone marrow, Stages of bone marrow, Stem cells, Regulation of hemopoiesis, Hemopoietic growth factor, may come as Short Questions.
3. In Viva, examiner may ask... types of bone marrow, structure of bone marrow, when hemopoiesis starts in different stages, medullary and extramedullar hemopoiesis, properties of stem cells, HGFs for different types of hemopoiesis.
CHAPTER 11

Red Blood Cells

Learning Objectives

On completion of study of this chapter, the student MUST be able to:
1. Give the dimensions of red cell and normal red cell count in different age groups in males and females.
2. List the functions of red cells.
3. Give the list of abnormal forms of red cells and the common condition in which these abnormalities are observed.
4. Appreciate the importance of specialties of red cell membrane.
5. Understand the meaning of red cell fragility and give the causes of increased and decreased fragility of red cells.
6. Give the values of hematocrit in males and females and common conditions of variations in hematocrit.
7. Explain the mechanism of erythrocyte sedimentation rate (ESR), list the factors affecting ESR, give the values of ESR in males and females, and list the physiological and pathological variations in ESR.

The student MAY also be able to:
1. Understand the importance of special type of metabolism in red cell.
2. Learn the types of abnormal red cells in common conditions.

The red blood cells (or red cells) are named as erythrocytes as they appear red (erythros means red) in a stained smear of peripheral blood.

1. The red color of erythrocytes is due to the presence of hemoglobin that accounts for approximately 90% of weight of red cells.
2. In a stained smear, the central part of red cells is pale that gives the appearance of a halo and peripheral part is more red, as in these cells the center is thin and periphery is thick.
3. Red cells are the major cellular elements of blood and perform transport of oxygen from lungs to tissues, and carbon dioxide from tissues to the lungs.

Structure and Functions

Structure

The normal human red cell is a circular, non-nucleated and biconcave disc (Fig. 11.1). The surface area of the red cell is much greater than that of a sphere of the same size. Therefore, exchange of oxygen and carbon dioxide is maximal with the biconcave configuration of red cells.

Red Cell Dimensions

<table>
<thead>
<tr>
<th>Shape</th>
<th>Biconcave</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size</td>
<td>7.5 (7 to 8) µm</td>
</tr>
</tbody>
</table>

Fig. 11.1: Structure of a red cell. Note the biconcave dimension of red cell. The center is thin (minimum 0.8 µm), and periphery is thick (2 µm).
Thinness: 2 µm at the periphery and 1 µm at the center
Surface area: 140 µm²
Volume: 87 µm³ (78 to 94 µm³)

The advantages of biconcavity of red cells:
1. When exposed to hypotonic environment, red cells withstand lysis to a greater degree as they swell to a larger extent to attain spherical shape before bursting. Thus, they do not easily lyse when blood becomes hypotonic.
2. It increases the surface area for exchange of gases (oxygen and carbon dioxide).
3. It helps in easy folding of red cells and therefore, facilitates their movement through the narrow capillaries.

Red Cell Composition
Red cells are composed of water 62.5%, hemoglobin 35%, and other substances 2.5% that constitute glucose, lipids (cephalin, cholesterol and lecithin), proteins (glutathiones and albumin like insoluble proteins) and enzymes (glycolytic enzymes, carbonic anhydrase and catalase).

Red Cell Count
In adult males: 4.5–6 (average 5.2) millions per cu mm of blood
In adult females: 4–5.5 (average 4.7) millions per cu mm of blood
In newborns: 6–8 millions per cu mm of blood
In children: 3–5 millions per cu mm of blood

The count is more in infants mainly due to hemoconcentration. The count rapidly decreases to its lowest level at about 2–4 months. Then, count slowly increases from one year of life to reach about 5 millions/mm³ at about 10 years.

The decrease in red cell count is called anemia and increase in count is called polycythemia (for details, refer the chapter “Pathophysiology of Anemia and Polycythemia”).

Life Span of Red Cells
Red cells usually live for 120 days. Aged red cells are destroyed by tissue macrophage system. Life span of red cells can be measured by injecting radioactive iron or RBCs tagged with radioactive chromium (⁵¹Cr). The tagged red cells lose radioactivity as they are destroyed. Complete disappearance of radioactivity gives the life span of red cells.

Abnormal Red Cells

Based on Size
Normocyte, Microcyte and Macrocyte
1. Normocyte: A normal red cell is called a normocyte, the volume of which is about 87 µm³ (78–94 µm³). Red
cells are non-nucleated biconcave discs. The cell diameter is about 7.5 µm. The normal red cell is also called discocyte, as it is a smooth, biconcave disc (Figs. 11.2 and 11.3A).
2. Microcyte: When the volume of red cell is less than 80 µm³, is called a microcyte (Figs. 11.2 and 11.3B). Microcytosis occurs in conditions of decreased cell size like iron deficiency anemia.
3. Macrocyte: When the volume of red cell is more than 94 µm³, is called a macrocyte (Figs. 11.2 and 11.3C). Macrocytosis occurs in conditions of increased cell size like megaloblastic anemia.

Anisocytes
This means the red cells are of different sizes. Anisocytosis is seen in different types of anemias (Fig. 11.3E).

Hypochromic Cells
Hypochromic red cells (hypochromia) are cells with pale appearance. Hypochromia occurs either due to decreased hemoglobin concentration as seen in iron deficiency or due to abnormal thinning of red cells. Usually the cells are microcytic.

Based on Shape
Poikilocytes
This means red cells are of different shapes. Poikilocytosis is usually seen when older cells are present in circulation (Fig. 11.3D).

Spherocytes
When red cells assume spherical shape, are called spherocytes (Fig. 11.3F). This is seen in conditions like hereditary spherocytosis.

Elliptocyte
When red cells assume oval shape with varying degrees of elliptical aberration, are called elliptocytes (Fig. 11.3G). Elliptocytosis occurs in hereditary elliptocytosis, thalassemia and iron deficiency anemia.
Chapter 11: Red Blood Cells

Acanthocyte
When a red cell has irregular shape with 2 to 10 hemispherically tipped spicules of variable length and diameter is called acanthocyte. Acanthocytosis is seen in abetalipoproteinemia, alcoholic liver disease and malabsorptive states (Fig. 11.3H).

Drepanocyte (Sickle Red Cells)
Drepanocyte is the red cell having sickle shape (Fig. 11.3I). Drepanocytosis is seen in sickle cell anemia.

Echinocyte (Burr Cells)
A “burr cell” or crenated red cell is an echinocyte (Fig. 11.3J). Echinocytosis occurs in uremia and liver disease.

Dacryocyte (Tear Drop)
When the red cell assumes the appearance of a tear drop, is called dacryocyte (Fig. 11.3K). Dacryocytosis occurs in myelofibrosis with myeloid metaplasia, thalassemia and myelophthisic anemia.

Keratocytes
Keratocytes are red cells having a pair of spicule. Keratocytosis usually occurs by mechanical damage or by removal of a Hinz body by pitting action of the spleen. ‘Hamlet cells’ and ‘bite cells’ are also used to describe keratocytes (Fig. 11.3L).

Schistocytes
Schistocytes are fragments of red cells (see Fig. 11.3M). They are smaller than red cells and they have sharp angles and spurs. Schistocytosis (red cell fragmentation) occurs in thalassemia, mechanical stress (microangiopathic hemolytic anemia, cardiac hemolytic anemia, etc.), and thermal injury (severe burns).

Target Cells
When a red cell has a central deeply stained round area in addition to its normal feature, the cell is called target cell (see Fig. 11.3N). Target cells are seen in chronic liver disease, obstructive jaundice, iron deficiency anemia and thalassemia.
**Stomatocyte**
This is a red cell having a slit-like area at the center of the cell in a dried film. In wet film, cell appears like cup. *Stomatocytosis* occurs in hemolytic anemia, liver disease and alcoholism (Fig. 11.3O).

**Basophilic Stippling**
This means presence of numerous basophilic granules (coarse and dark-blue granules) in red cells (see Fig. 11.3P). This condition is called punctate basophilia. It is typically seen in lead and other heavy metal poisoning. It also occurs in thalassemia, megaloblastic anemia, infections and liver disease.

Sometimes in lead poisoning, Cabot’s ring (ring shape or figure of 8) at the periphery of red cells or Howell Jolly bodies (small nuclear fragments appear in cytoplasm) are found in erythrocytes. Nucleated red cells are seen in severe hemolytic anemia.

**Cell Membrane and Metabolism**

**Red Cell Membrane**
For its easy accessibility, red cell membrane is the most thoroughly studied biological membrane. Red cell membrane is made up of three major structural elements: lipid bilayer, integral proteins and membrane skeleton.

**Lipid Bilayer**
The lipid bilayer is primarily composed of phospholipid and cholesterol:
1. The lipid bilayer provides an impermeable barrier between cytoplasm and external environment.
2. It also helps to maintain a slippery exterior, so that the red cells do not stick to the vascular endothelium.

**Integral Proteins**
Integral proteins are embedded in the lipid bilayer. The important membrane proteins are band-3 protein (anion exchanger-1), the glycophorins, Rh D protein and various ion channels (Refer to Fig. 4.5; Chapter 4).
1. These proteins allow red cells to participate in wide range of functions including antigenic determination and cellular metabolism.
2. Band-3 is the major anion-exchanger (chloride-bicarbonate exchange) and also regulates metabolic pathways by sequestering key enzymes like enzymes of glycolytic pathways.
3. Glycophorins constitute more than 60% of negative surface charge of red cells; they modulate interaction between red cells and interaction of red cells to endothelium.
4. There are five different types of glycophorins: A, B, C, D and E. *Glycophorin-C* provides stability and shape to the red cell membrane, and its deficiency leads to elliptocytosis.

**Membrane Skeleton**
A membrane skeleton is present on the internal side of the red cell membrane. Two important proteins are part of membrane skeleton proteins: *ankyrin* and *spectrin*.

**Spectrin:** Spectrin is the most abundant skeletal protein and constitutes 75% of the mass of membrane skeleton.
1. Spectrin is composed of two subunits: α and β. The αβ heterodimers align and intertwine with each other in antiparallel fashion to form flexible rod like structure.
2. These dimers further self-associate to form tetramers that are composed of multiple repeats and provide a strong elastic filament to the overlying cell membrane via formation of lattice-like meshwork linked to integral membrane proteins. Thus, spectrin molecules maintain cellular shape, provide structural support to membrane lipid bilayer and regulate lateral mobility of integral membrane proteins.
3. Defect in association of αβ heterodimers results in hereditary elliptocytosis and poikilocytosis.

**Ankyrin:** Ankyrin is an asymmetric polar protein that provides primary linkage between membrane skeleton and lipid bilayer. Disruption of this linkage decreases membrane stability. Abnormalities of ankyrin are the most common causes of hereditary spherocytosis (Application Box 11.1).

**Application Box 11.1**

**Common membrane defects:** Presence of ankyrin and spectrin in the membrane provides biconcave shape, deformability, membrane stability and durability to the red cells. Therefore, deficiency of ankyrin and spectrin results in membrane defects like *spherocytosis, elliptocytosis* and *poikilocytosis*, etc. These defects also make the cells rigid so that the cells are destroyed prematurely.

**Deformability of Red Cells**
An important determinant of red cell survival is its deformability. Deformability is ascribable to the intrinsic deformability of the red cell membrane. When red cells become rigid, the viscosity of blood increases and cells are lysed while passing through the splenic pulp. The primary cause of decreased deformability is due to spherocytosis of the cell rather than stiffening of their membrane.

**Metabolism of Red Cells**
Red cells have no nuclei, mitochondria and ribosomes. Therefore, adequate synthesis of proteins and lipids does not occur in red cells.
1. Glucose is the primary fuel for red cells. Though enzymes for glycolysis are present, enzymes for TCA cycle are absent. ATP is formed by *Embden-Mayerhoff pathway* (EM pathway). The *HMP shunt* provides NADPH.
2. Glucose entry into the red cells occurs easily by facilitated diffusion, which is independent of insulin action. Red cells depend mostly on glucose metabolism for their energy supply.
3. 90% of glucose is oxidized by EM pathway and 10% by HMP shunt.

**EM Pathway**
Red cells metabolize glucose, usually by anaerobic glycolysis using EM pathway:
1. Two ATP molecules are generated by glycolysis through EM pathway.
2. 2, 3-DPG is produced in red cells. 2, 3-DPG influences oxygen affinity of hemoglobin and therefore, plays an important role in red cell function.
3. Hypoxia inhibits Kreb cycle and therefore, stimulates 2, 3-DPG formation, whereas, acidosis inhibits glycolysis and therefore, decreases 2, 3-DPG formation.

**HMP Shunt**
The enzyme in the red cell, glucose-6-phosphate dehydrogenase (G-6-PD) is the main enzyme for HMP shunt:
1. HMP shunt generates NADPH, keeps glutathione in reduced state, which is a strong reducing agent and prevents damage to the red cell.
2. Therefore, G-6-PD deficiency interferes with red cell functions (Clinical Box 11.1).

<table>
<thead>
<tr>
<th>Clinical Box 11.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-6-PD deficiency causes hemolysis: In the absence of G-6-PD, an <em>inborn error of metabolism</em>, red cells are susceptible to the damages due to oxidation. Therefore, hemolytic anemia is common in G-6-PD deficiency. Especially, hemolysis is facilitated on exposure to anti-malarial drugs, though these individuals are generally malaria resistant.</td>
</tr>
</tbody>
</table>

**Functions of Red Cells**
1. The most important function of red cell is to transport oxygen from lungs to the tissue. This is due to the presence of hemoglobin in the red cell which has high affinity for oxygen.
2. Hemoglobins also participate in carbon dioxide transport from tissues to lungs and maintenance of acid base balance.
3. Red cells contribute to 50% of viscosity of blood.
4. Antigen on red cell membrane helps in blood group classification.

**APPLIED ASPECTS**

**Red Cell Fragility**
The tendency of the cells to hemolyze is called fragility of the cells. There are 2 types of fragility: mechanical and osmotic.

**Mechanical Fragility**
Lysis of red cells due to mechanical stress and strain is called *mechanical fragility*. Red cell diameter is slightly less than the diameter of average capillaries. Therefore, when red cells pass through capillaries and splenic pulp, their membrane undergoes mechanical stress:
1. On average, a red cell passes about three lakh times though capillaries during its life span, which makes the cell more fragile.
2. Also, when red cells become older, the membrane becomes rigid. Increased membrane stiffness and mechanical stress make cell vulnerable to rupture.
3. Red cell membrane defects increase mechanical fragility.

**Osmotic Fragility**
Lysis of red cells on exposure to different osmotic solutions is called osmotic fragility. Osmotic fragility is defined as the ease with which the red cells are ruptured when they are exposed to hypotonic solutions. It assesses the integrity of red cell membrane. The *osmotic fragility test* helps in the diagnosis of anemia in which the physical properties of the red cells are altered. This test detects whether or not the red cells can easily be hemolyzed. The red cell membrane allows water to pass through while restricting solutes. This is called osmosis.
1. In an *isotonic solution*, the solution of equal concentration as that of red cell content, the red cells remain intact. Such a solution has same tonicity with that of plasma. Examples are 0.9% NaCl, 5% glucose, 10% mannitol and 20% urea.
2. When suspended in *hypertonic solution*, a solution with more tonicity (> 0.9% NaCl), red cells shrink due to loss of water from them by exosmosis.
3. Red cells absorb water by *endosmosis*, when kept in hypotonic solutions, a solution with less tonicity (< 0.9% NaCl). Endosmosis results in hemolysis due to swelling and rupture of the cells (Clinical Box 11.2).

**Clinical Box 11.2**
| Shape of red cell determine hemolysis: Increased osmotic fragility denotes decreased resistance of cells to rupture. As the resistance of the red cell membrane to rupture is related to its geometric configuration, red cells that are spherical demonstrate increased hemolysis, whereas red cells that are flat like sickle cells demonstrate decreased hemolysis. |

**Normal Value and Variations**
Normally, osmotic fragility begins at 0.45 to 0.50 and completes at 0.30 to 0.33.

**Conditions of Diminished Fragility**
- Iron deficiency anemia
- Thalassemia
- Sickle cell anemia
- Obstructive jaundice
- Post-splenectomy
Section 2: Blood and Immunity

Conditions of Increased Fragility
- Hereditary spherocytosis
- Congenital hemolytic anemia
- Other conditions in which spherocytes are found in the blood.

Packed Cell Volume (Hematocrit)
Hematocrit is the fractional volume of blood that erythrocytes occupy. Hematocrit or packed cell volume (PCV) is the amount of packed red blood cells following centrifugation. When blood is centrifuged in a tube the red cells are packed together at the bottom of the tube by centrifugal force, as cells are heavier than the plasma. The hematocrit is a macroscopic observation by which the percentage volume of the packed red blood cells is measured. Hematocrit is therefore known as PCV.

1. PCV is a reliable index of the red cell population. It provides useful information about the red cell mass which is correlated with red cell count and their hemoglobin content.
2. These measurements (PCV, red cell count and Hb content) are essential for determination of red cell indices that help in detecting and classifying the various types of anemias.

Normal Value and Variations
- Adult male: 46% (40–50%)
- Adult female: 42% (37–47%)

Hematocrit decreases in conditions of decreased red cell count and increases in conditions of increased red cell count. When cells are deformed as occurs in spherocytosis or sickle cell disease, more plasma is trapped between the packed cells, which gives a false high result.

Erythrocyte Sedimentation Rate
Red cells have the property of rouleaux (piling one on the other) formation (Fig. 11.4). The piled red cells are heavier than the individual red cells. Therefore, when whole blood is allowed to settle, sedimentation of erythrocytes is facilitated due to the presence of rouleaux. The rate at which the red cells fall (sediment), is known as the erythrocyte sedimentation rate (ESR).

Factors Affecting ESR
ESR depends on 3 major factors: (1) The shape and number of red cells, (2) Size of rouleaux, and (3) Plasma factors. Other factors also contribute.

Shape and Number of Red Cells
Shape of red cells: The most important factor determining ESR is the shape of red cells:
1. The biconcave shape favors rouleaux formation.
2. The alteration of shape of erythrocyte decreases rouleaux formation.
3. Therefore, ESR is less in hereditary spherocytosis and sickle cell anemia.

Red cell mass: Red cell mass contributed to blood viscosity.
1. Increase in cell mass increases viscosity of blood. Increase in viscosity decreases ESR.
2. Thus, ESR decreases in polycythemia and increases in anemia (except sickle cell anemia).

Size of Rouleaux
ESR depends on the mass of the falling particles, i.e. the size of rouleaux (the red cell aggregates). The larger the size of rouleaux, the faster is the fall. Therefore, the factors that increase the size of rouleaux facilitate ESR. Increase in red cell size without change in their shape increases the size of rouleaux.

Plasma Factors
The size and number of rouleaux mainly depends on the fibrinogen concentration of plasma. Globulin content of plasma also contributes:
1. In normal blood, red cells remain separate as they have negatively charged surface that tend to repel one another. When fibrinogen concentration increases...
CHAPTER SUMMARY

**Key Concepts**

1. Red cells are anuclear biconcave discs. The cells are highly deformable due to presence of ankyrin and spectrin in the membrane skeleton.
2. Deformability of the red cells allows them to pass through capillaries and splenic pulp.
3. Change in shape and rigidity of the membrane make the red cells susceptible to hemolysis.
4. Also, biconcave shape and membrane plasticity help red cells to resist osmotic lysis (less osmotically fragile).
5. PCV (hematocrit) is a reliable index of red cell mass.
6. Negative charges on cell surface inhibit bigger rouleaux formation. In infection, inflammation, malignancy and collagen diseases, the inflammatory or abnormal proteins produced by the disease neutralize the negative charge and facilitate rouleaux formation. This increases ESR in these conditions, which is a marker of intensity of the disease.

**Important to Know (Must Read)**

1. In examinations, usually Long Questions do not come from this chapter.
2. Osmotic fragility, ESR, and hematocrit may be asked as Short Notes in exams.
3. Many questions are asked in Viva from this chapter. Examiners invariably ask the dimensions of RBC, why is RBC biconcave and what are the benefits of it, normal red cell count, and the conditions of increase and decrease in count, common abnormalities of red cells, basics of red cell metabolism, types of fragility and factors affecting them, importance of PCV, factors increasing and decreasing ESR, common conditions of increase and decrease in ESR.
4. When a student fails to answer the size and shape of normal red cells, basics of red cells, and normal red cell count, it becomes difficult for examiner to give the pass marks.
The process of production of red cells is called erythropoiesis. Red cells have a finite lifespan of about 120 days. The new cells are formed at a pace that replaces the cells destroyed. Bone marrow precisely replaces the cells lost by senescence, hemorrhage or destruction. Red cell mass in a normal man is 26–32 ml/kg and in women 23–29 ml/kg of body weight. The volume of red cells (red cell mass) in the body is maintained and regulated by the bone marrow.

**SITES AND STAGES OF ERYTHROPOIESIS**

*Definition:* Erythropoiesis is defined as the process of formation of red cells. This is an important component of hemopoiesis.

**Stages of Erythropoiesis**

There are three stages of erythropoiesis: mesoblastic, hepatic and medullary.

**Mesoblastic Stage**

During intrauterine life, erythropoiesis first takes place in the mesoderm of yolk sac and mesoderm of the body. This is called mesoblastic stage. During this stage, erythropoiesis is intravascular.

**Hepatic Stage**

From the 5th week of gestation, erythropoiesis takes place in the liver and spleen (hepatic stage).

**Medullary Stage**

From the 5th month of intrauterine life, the bone marrow starts forming red cells (medullary stage). However, erythropoiesis in bone marrow is very slow in second trimester.

1. **Medullary erythropoiesis** becomes more effective towards the end of third trimester (Refer to Fig. 10.3; Chapter 10). After birth, bone marrow becomes the sole site of erythropoiesis.
2. *Extramedullary erythropoiesis in postnatal life is always considered abnormal.*
3. Till adolescent period, marrow cavities of all bones are involved in erythropoiesis, after which erythropoiesis regresses in the limb bones.
4. After the age of 20–30 years, erythropoiesis is mostly limited to sternum, ribs, vertebrae, skull, pelvic and pectoral girdles (Refer to Fig. 10.2; Chapter 10).

**Important Note**

*Extramedullary erythropoiesis after birth is abnormal:* After birth, erythropoiesis occurs only in bone marrow. Extramedullary erythropoiesis in postnatal life is always considered abnormal.
Chapter 12: Erythropoiesis

Steps of Erythropoiesis

There are four major cell-stages or steps of erythropoiesis: stem cells, progenitor cells, precursor cells and mature cells. Each stage has sub-stages. Red cells are formed from pluripotent hemopoietic stem cells, which give rise to committed stem cells. Committed stem cells form subsequent progenitor and precursor cells that finally produce reticulocyte and mature red cells (Fig. 12.1).

In general, during erythropoiesis, following cellular changes take place:

2. Size of nucleus and number of nucleoli decrease, chromatin material condenses and finally nucleus disappears.
3. Staining reaction of cytoplasm changes from deep basophilic to polychromatophilic (acidophilic plus basophilic) and finally to acidophilic type. This occurs mainly due to gradual reduction in quantity of RNA material.
Stem Cells

Pluripotent Stem Cells

Pluripotent stem cells are the mother stem cells that form stem cells for different cell lines. Stem cells (including committed stem cells) have two unique properties: 1. self renewal, and 2. differentiation. They have the capacity to renew themselves. Therefore, at no time, bone marrow is depleted of the stem cells. Also, they have the capacity to develop (differentiate) into the subsequent progenitor cells.

Committed Stem Cell

These cells develop from pluripotent stem cells. There are specific two categories of stem cells: stem cells for myeloid series and lymphoid series. Myeloid stem cells form erythroid series, megakaryoid series, monocytic series and granulocytic series (Fig. 10.4; Chapter 10). Erythroid stem cells give rise to progenitor cells for erythroid cell lines. There are two progenitor cells: BFU-E and CFU-E.

Progenitor Cells

There are two types of progenitor cells: 1) BFU-E, and 2) CFU-E. The BFU-E and CFU-E develop from a common progenitor CFU-Mg/E.

BFUs-E are burst forming units-erythroid progenitor cells that give rise to large number of CFU-erythroid cells. CFUs-E are colony-forming units-erythroid progenitor cells that give rise to moderate number of blast cells of erythroid series. Thus, BFU-E forms CFU-E, which, in turn, forms blast cells (erythroblasts).

Precursor Cells

As blast cells are first to be morphologically identifiable cells in the bone marrow in erythropoiesis, they are the true precursors for erythrocytic series. Hence, precursors for erythrocytes are called erythroblasts, also called normoblasts. Normoblasts develop from pronormoblasts. There are three successive forms of normoblasts: early, intermediate and late (Fig. 12.1).

Pronormoblast

Pronormoblasts (proerythroblasts) are the first blast cells to appear in bone marrow and the first identifiable cells of erythrocyte series. Following are the characteristics of pronormoblast.

1. This is a large cell, having diameter of about 15–20 µm. Cell is irregularly rounded or slightly oval.
2. The cytoplasm is less in amount, occupies about 20% of the cell. Presence of high concentration of polyribosomes makes the cytoplasm intensely basophilic. Cytoplasm has high content of RNA, which indicates vigorous protein synthesis in the cell.
3. The nucleus is large and occupies 80% of the cell. It contains multiple nucleoli.
4. Mitosis is present.
5. Hemoglobin is not yet formed in the cell.

Early Normoblast (Basophilic Erythroblast)

Early normoblast or early erythroblast is the first erythroblast to appear in bone marrow. The features are:

1. Diameter of these cells is 12–18 µm.
2. Cells exhibit active mitosis.
3. Cytoplasm is scanty, deep blue and basophilic. The basophilic cytoplasm is due to continuation of many polyribosomes in it. Therefore, early normoblast is also called basophilic erythroblast.
4. Nucleus is large and occupies three-fourth of the cell area. Nucleus is composed of dark violet heterochromatin clumps interspersed with pink clumps of euchromatin. The chromatin is connected by linear strands. This often gives nucleus the appearance of wheel spokes.
5. Hemoglobin appears first time in these cells in erythropoiesis.

Intermediate Normoblast (Polychromatic Erythroblast)

This is also called intermediate erythroblast. This appears following mitotic division of early erythroblast. Following are features of intermediate erythroblast.

1. The cell is smaller than the early normoblast, having the diameter of 10–15 µm. 
2. The cytoplasm changes from blue to pink as hemoglobin dilutes the polyribosome content. 
3. Nucleus is small and occupies about half of the cell area. The distribution of heterochromatin clumps in nucleus gives the appearance of checkerboard pattern. There are no nucleoli. 
4. Hemoglobin synthesis increases, which makes the cell acidophilic. The presence of RNA material makes the cytoplasm eosinophilic. Thus, mixture of acidophilic hemoglobin and eosinophilic RNA in cytoplasm makes it polychromatic. Therefore, intermediate normoblast is also called polychromatic erythroblast. 
5. Mitosis is sluggish in intermediate normoblast.

Late Normoblast (Orthochromatic Erythroblast)

This is also called late erythroblast. This is produced by final mitosis in the erythropoietic series. The features are:

1. This is smallest in the erythroblastic series (7–12 µm in diameter) and mitosis is absent.
2. Cytoplasm is deeply eosinophilic giving the appearance of an orthochromatic cell. Hence, this is also called orthochromatic erythroblast.
3. Nucleus is small and pyknotic at the beginning. Sometimes, dark nuclear chromatin materials are arranged in a typical pattern to give the appearance of a cart-wheel. Finally, the pyknotic nucleus disintegrates.
4. Hemoglobin synthesis increases and almost completes in this stage.

**Mature Cells**

**Reticulocytes**

Reticulocytes are the immediate precursors of red cells. Therefore, they are also called juvenile red cells.

1. They are mature cells with full complement of hemoglobin.
2. They are slightly larger than red cells.
3. They have a network of reticular nuclear material. The reticular network is nothing but the remnants of disintegrated organelles, and especially of the nuclear fragments. Due to the presence of reticular network, the cells are called reticulocytes.
4. The reticulum is stained by supravital stains.
5. Hemoglobin synthesis continues to some extent in some reticulocytes. The reticulocyte count is 0–1 per cent of red cells in adults (details of reticulocyte are given below).

**Erythrocytes**

Erythrocytes are the final cells in erythropoiesis. They are biconcave discs. They have the diameter of about 7.5 µm (for details of red cells, refer to the previous chapter).

**Duration of Erythropoiesis**

The total period for erythropoiesis occurs in 7 to 9 days. It takes 5 to 7 days for progenitor cells to become reticulocytes and another 2 days for reticulocytes to become red cells.

### REGULATION OF ERYTHROPOIESIS

There are many factors that effectively control erythropoiesis. In physiological conditions, total cell mass is maintained at its optimal size by the suitable adjustments in the rate of erythropoiesis.

**Feedback Controls**

In animals, spleen is the reservoir of red cells, but not in humans. Therefore, there is no specific origin of signals from a particular reservoir for the production of red cells and consequently, there is no anatomical tissue feedback for erythropoiesis. Hence, mechanisms controlling erythropoiesis can broadly be divided into two categories: the functional feedback and the end-product feedback.

**Functional Feedback**

The functional feedback is the feedback that originates from the tissues signaling the rate of their requirement which is normally served by red cells. Red cells supply oxygen to the tissues, and, therefore, oxygen requirement of the tissue is the major functional feedback for red cell production. Erythropoietin is the chief mediator of this functional feedback.

**End-product Feedback**

The end-product feedback is due to the products of red cell destruction. It is believed that the products released from red cell destruction (hemolysis) influence red cell production. This is supported by the observation that hemolytic anemia is associated with more erythroid hyperplasia and reticulocytosis than the hemorrhagic anemia of equal severity. However, the precise mediator of end-product feedback is not clearly identified, though in vitro studies have demonstrated the stimulatory effects of hemin on erythropoiesis.

**Factors Controlling Erythropoiesis**

The factors controlling erythropoiesis can broadly be divided into three categories: hormonal, dietary and others (Table 12.1).

**Hormonal Factors**

**Erythropoietin**

**History**

In 1906, French Professor Dr Paul Carnot and his associates suggested that hypoxia generates humoral factor capable of stimulating red cell production. In 1950, Kurt Ressmann provided strong support for existence of a hormonal mechanism, and few years later, it was named as erythropoietin. In 1957, Jacobson and coworkers found that the erythropoietin is produced by kidney.

| Table 12.1: Factors controlling erythropoiesis. |
| A. Hormonal factors |
| 1. Erythropoietin |
| 2. Androgens |
| 3. Estrogen |
| 4. Thyroxine |
| 5. Anterior pituitary hormones |
| – Growth hormone |
| – TSH, ACTH, LH, FSH, Prolactin |
| 6. Corticosteroid |
| 7. Interleukins |
| B. Dietary factors |
| 1. Vitamins (vitamin B₁₂, folic acid, vitamin C) |
| 2. Proteins |
| 3. Minerals (iron, copper, cobalt and nickel) |
| C. Other factors |
| 1. Intrinsic factor |
| 2. Chemical factor |
| 3. Environmental factor (hypoxia) |
| 4. Drugs |
Source
Erythropoietin is produced mainly by the interstitial cells in peritubular capillary bed of kidney. To some extent, it is also produced by juxtaglomerular cells and extraglomerular mesangial cells of kidney. Kidney secretes about 85% of erythropoietin (Application Box 12.1). The rest 15% comes from liver. The nonparenchymal cells (Kupffer cells) of liver and perivenous hepatocytes produce erythropoietin. There are also evidences that erythropoietin is produced in brain, uterus and oviducts.

Application Box 12.1
Renal disease causes anemia: As kidney is the major source of erythropoietin, chronic renal diseases that reduce renal mass significantly produce anemia. Anemia is also produced following nephrectomy. In such conditions, erythropoietin produced by liver fails to meet the normal demand of erythropoiesis as the amount secreted from liver is not adequate.

Structure
Erythropoietin is a glycoprotein containing 165 amino acids. There are four oligosaccharide chains attached with polypeptide chain. These oligosaccharide chains are necessary for the physiological activity of erythropoietin. Erythropoietin has molecular weight of 34,000 dalton.

Mechanism of Action
Erythropoietin acts on erythropoietin receptors that belong to cytokine receptor superfamily. The receptor has tyrosine kinase activity. Binding of erythropoietin with its receptor initiates activation of a cascade of serine and threonine kinases that finally leads to activation of JAK-2 protein. Formation of JAK and other signaling proteins brings about changes in cell functions in a similar way to that of growth hormone.

Functions
Erythropoietin stimulates erythropoiesis in several ways:
1. It acts mainly on the progenitor cells and early precursor cells. It especially stimulates BFU-E and CFU-E to form pronormoblasts.
2. It decreases cell cycle length of precursor cells, and therefore, enhances mitosis.
3. It facilitates maturation of normoblasts.
4. It increases hemoglobin synthesis in normoblasts.
5. It acts on the stem cells to promote their transformation towards erythroid series.
6. It stimulates early release of immature erythrocytes (reticulocytes) into circulation.

Regulation of Erythropoietin (Ep) Production
Factors regulating Ep production can be divided into factors increasing and factors decreasing the production.

Factors that increase Ep production: Hypoxia, low blood volume, anemia, lung diseases and hormones like epinephrine, norepinephrine, androgen, thyroxine, prolactin, ACTH, etc. facilitate Ep production. Hypoxia stimulates erythropoiesis by increasing erythropoietin production. This is the mechanism of polycythemia that occurs at high altitude.

Factors that decrease Ep production: Estrogen inhibits erythropoiesis. Adenosine antagonist such as theophylline decreases erythropoietin secretion.

Metabolism
Ep is inactivated mainly in liver. The usual half-life of Ep in circulation is about 5 hours. However, if carbohydrate component, especially sialic acid residue of erythropoietin molecule is damaged (even partly), the half-life is shortened to 5 minutes.

Interleukins and GM-CSF
Interleukin 1, 3 and 5 that are produced mostly from T-cells act mainly on the stem cells to convert them to the progenitor cells. GM-CSF (granulocyte-macrophage colony-stimulating factor), which is produced from T-cells, endothelial cells, and fibroblasts stimulates production of committed stem cells.

Androgens
Androgens stimulate erythropoiesis. This is the major cause of higher red cell count in males than in females. Red cell count is same in both genders till puberty. Androgen stimulates erythropoietin production. Also, it directly stimulates erythropoiesis.

Estrogens
Estrogen inhibits erythropoiesis by inhibiting erythropoietin production and also by decreasing the response of stem cells to erythropoietin. Estrogen also decreases hepatic synthesis of globulin.

Thyroxine, Cortisol and Growth Hormone
1. Thyroxine stimulates erythropoiesis. Though the exact mechanism of thyroxine increasing red cell count is not known, it stimulates erythropoietin production.
2. Growth hormone increases the mitosis and maturation of erythroid precursors.
3. Cortisol produces mild erythrocytosis.

Dietary Factors
Iron
Iron is the raw material for synthesis of heme component of hemoglobin. Therefore, iron deficiency results in hypochromic microcytic anemia.

Vitamin B12 and Folic Acid
These two vitamins are necessary for maturation of red cell precursors as they promote DNA synthesis.
1. For synthesis of DNA, thymine is required and tetrahydrofolate is necessary for thymine synthesis, which is formed from folic acid.
2. Folate, after its absorption from intestine, becomes methyl-tetrahydrofolate in blood, which in the tissue
becomes 5,10-methylene tetrahydrofolate, the active form of tetrahydrofolate. This promotes formation of deoxythymidylate from deoxyuridylate, which is required for synthesis of DNA.

3. Therefore, folate deficiency leads to arrest of mitosis and chromosome division in the absence of DNA synthesis.

4. Vitamin B₁₂ promotes conversion of methyl-tetrahydrofolate to its active tetrahydrofolate (THF) form. Methyl-THF accumulates in the cell, which is known as methyl-THF trap. Therefore, vitamin B₁₂ deficiency also causes arrest of chromosomal division.

5. In fact, folic acid and vitamin B₁₂ interact with each other for synthesis of DNA (Fig. 12.2). Thus, in deficiencies of these two vitamins, megaloblasts are produced in the bone marrow instead of normoblasts (Fig. 12.3B). Therefore, the resulting anemia is called megaloblastic anemia (Clinical Box 12.1). Megaloblasts produce macrocytes that manifest as macrocytic anemia (Fig. 12.3A).
Megaloblastic anemia: Folate and vitamin B$_12$ deficiencies cause megaloblastic anemia. Patient usually presents with glossitis and neurologic manifestation (subacute combined degeneration of spinal cord and peripheral neuropathy). Macrocyes are seen in peripheral blood smear and megaloblasts are seen in bone marrow smear (Figs. 12.3A and B).

Protein
Protein is essential for synthesis of globin component of hemoglobin. Therefore, protein deficiency is invariably associated with hypochromic anemia.

Other Nutritional Factors
1. Vitamin C helps in absorption of iron. Therefore, vitamin C deficiency causes anemia.
2. Minerals like copper and cobalt influence hemoglobin formation. Thus, deficiency of these minerals leads to anemia.

Other Factors
Intrinsic factor
Intrinsic factor is secreted from oxyntic cells of stomach along with hydrochloric acid. It helps in absorption of vitamin B$_{12}$ (extrinsic factor of Castle) from terminal part of ileum. Therefore, intrinsic factor deficiency produces megaloblastic anemia (pernicious anemia).

Environmental Factors
Hypoxia due to any cause increases erythropoiesis by increasing erythropoietin production. Hypoxia occurs at high altitude, and is commonly seen in cardiac and respiratory diseases.

Drugs and Chemicals
Vasoconstrictor agents like catecholamines, nucleotides like cAMP, NAD, NADP, products of red cell destruction (hemolysates), cobalt salts and thyroxine stimulate erythropoiesis.

Effective vs Ineffective Erythropoiesis
Under normal conditions, most of the red cells produced in the marrow are actively alive or have the potential to live a normal life span. This is called effective erythropoiesis. However, a fraction of red cell production is ineffective with destruction of nonviable red cells either within the marrow or shortly after they are released into circulation. When percentage of production of these nonviable red cells is increased, the erythropoiesis is called ineffective erythropoiesis. Ineffective erythropoiesis is suspected when reticulocyte count is normal despite erythroid hyperplasia of marrow.

Reticulocytes
Structure
Reticulocytes are juvenile red cells in the bone marrow. They develop into mature red cells and few of them pass into circulation along with red cells.

Development
Reticulocytes are produced in the bone marrow from late normoblast (orthochromatic erythroblast).
1. The nucleus is removed from the late normoblast to become reticulocyte.
2. Reticulocytes lose their mitochondria and ribosomes, and basophilic tint to become mature red cells.
3. About 1% of circulating red cells is replaced everyday by newly formed red cells. 
4. With the release of young red cells into the circulation, a few reticulocytes are also released. When the bone marrow sends out red cells at an increased rate, more reticulocytes are released. Thus, the number of reticulocytes in the peripheral blood is an index of erythropoiesis, especially in anemia (Clinical Box 12.3).

Clinical Box 12.3

Reticulocytes indicate bone marrow activity: Reticulocytes are the immediate precursors of red cells. Therefore, when the demand for red cells in the body is increased, reticulocyte formation and release are also increased. Thus, reticulocyte count increases along with red cell count. Sometimes, the demand may be so high that nucleated red cells are also released from bone marrow.

Table 12.2: Differences between reticulocytes and red cells.

<table>
<thead>
<tr>
<th></th>
<th>Reticulocytes</th>
<th>Red cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size</td>
<td>8 μm</td>
<td>7.5 μm</td>
</tr>
<tr>
<td>Nuclear material</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>Count</td>
<td>Very few (1% of red cells)</td>
<td>Plenty (5 million/mm³)</td>
</tr>
<tr>
<td>MCHC*</td>
<td>Less</td>
<td>More</td>
</tr>
</tbody>
</table>

* MCH is same in both the cells

Reticulocyte Count

The normal reticulocyte count in adult is 0–1% of the red cells. In newborns, the count is 2–6%. The number falls during the first year of life to less than 1% and then the level is maintained throughout life.

Reticulocyte Response

The reticulocyte count is performed to assess the therapeutic response of anemias to treatment in which the patient is deficient in one of the substrates essential for the synthesis of red cells. When therapy begins, new red cells are formed and released rapidly into the circulation before cells are fully matured. Therefore, many reticulocytes are also released with the release of young red cells. This results in increase in reticulocyte, which is called reticulocytosis. Increase in reticulocyte count is called reticulocytosis. This is seen in various physiological and pathological conditions.

Physiological: Newborns and infants have higher count than adults. Count is more at high altitude.

Pathological: Hemolytic anemia, acute hemorrhage, during treatment for deficiency anemias (reticulocyte response), and any condition that stimulates bone marrow to produce red cells.

Reticulocytopenia

There is no physiological reticulocytopenia. Reticulocytopenia always indicates diseased state. It is seen in aplastic anemia, myxedema, hypopituitarism and leucoerythroblastic anemia.

Leucoerythroblastic Reaction

The leucoerythroblastic reaction is used to describe the presence of immature myeloid cells and nucleated red cells in the peripheral blood, which occurs often as a consequence of disturbance of the bone marrow architecture by abnormal tissues. This is seen in secondary carcinoma of bone, myelofibrosis, postsplenectomy states, thalassemia major and multiple myeloma.

Reticulocyte Count in Ineffective Erythropoiesis

Reticulocyte remains almost normal in ineffective erythropoiesis. In fact, ineffective erythropoiesis is suspected when reticulocyte count is normal despite erythroid hyperplasia of marrow.

Reticulocyte Index

Effective erythropoiesis is estimated by reticulocyte count, which is normally expressed as the % of red cells. However, it can also be expressed as the total number of circulating reticulocytes per unit of blood, i.e.:

Absolute reticulocytes = reticulocyte percentage × red cell count

To use reticulocyte % as a measure of the rate of red cell production, the % may be corrected for the hematocrit. This is called reticulocyte index or corrected reticulocyte count.

Reticulocyte index = Reticulocyte % × ActualHct / NormalHct

Alteration in Reticulocyte Count

Reticulocytosis
**CHAPTER SUMMARY**

**KEY CONCEPTS**

1. Erythropoiesis occurs in 3 stages in fetal life: mesoblastic (in yolk sac), hepatic (in liver and spleen) and medullary (in red bone marrow). After birth, erythropoiesis is completely medullary. Extramedullary erythropoiesis after birth is always considered abnormal.

2. In erythropoiesis, hemopoietic stem cells become progenitor cells that, in turn, form precursor cells. Hb appears in early normoblast and completes in late normoblast. Nucleus becomes pyknotic and extruded in late normoblast.

3. Erythropoietin secreted mainly from kidney is the primary regulator of erythropoiesis. Hypoxia, low blood volume, anemia, lung diseases and hormones, like epinephrine, norepinephrine, androgen, thyroxine, prolactin, ACTH, etc. facilitate Ep production, and, therefore, these factors/conditions stimulate erythropoiesis.

4. Reticulocyte has no nucleus, but contains RNA materials that make the reticular appearance in cytoplasm in supravital stain. Reticulocyte count is about 1% in adults.

5. Reticulocyte count increases when vitamin B\( \text{subscript} 12 \) is administered as part of the treatment for pernicious anemia. The response is called reticulocyte response. It is also seen when iron therapy is given in iron-deficiency anemia.

6. Reticulocyte count is an index of effective erythropoiesis, especially in anemia.

**Important to Know (Must Read)**

1. In examinations, ‘steps of erythropoiesis and its regulation’ usually comes as a Long Question. This is a common question, very often asked in final exam. A student must not miss it.

2. Erythropoietin, regulation of erythropoiesis, reticulocyte, and reticulocyte response, are usually asked as Short Questions in exams.

3. In Viva, examiners usually ask stages of erythropoiesis, medullary and extramedullary erythropoiesis, factors affecting erythropoiesis, regulation of erythropoiesis, source and functions of erythropoietin, role of folic acid and vitamin B12 in erythropoiesis, differences between red cells and reticulocyte, reticulocyte response, and causes of reticulocytosis and reticulocytopenia.
CHAPTER 13

Destruction of Red Blood Cells

LEARNING OBJECTIVES

On completion of study of this chapter, the student MUST be able to:
1. Say the lifespan of normal red cell.
2. Give the site and mechanism of red cell destruction.
3. Describe the fate of destroyed red cells.
4. List the common causes of hemolytic jaundice.
5. Explain why hemolytic jaundice is common in newborns.

The student MAY also be able to:
1. Give the physiological basis of hemolytic jaundice.

The average lifespan of normal red cell is 120 days. After red cells live their normal life, they become less active and more rigid, and get trapped by cells of reticuloendothelial system, especially by the macrophages in the liver and spleen. These macrophages engulf red cells by phagocytosis and release hemoglobin. Also, the rigid cells get ruptured while passing through narrow capillaries.

MECHANISMS OF DESTRUCTION

There are two mechanisms of red cell destruction: intravascular and extravascular (Fig. 13.1).

Intravascular Destruction

Red cells are destroyed in the circulation, when their cell membrane is breached. Normally, intravascular demise of red cell is less frequent. The causes are:
1. The membrane of older red cells becomes rigid and becomes susceptible to rupture during circulation inside the blood vessel.
2. If there is a defect in the membrane, hemolysis in circulation is facilitated.
   For example, in paroxysmal nocturnal hemoglobinuria, complement complexes create holes in the red cell membrane that promotes intravascular hemolysis.
3. Intravascular destruction is also common in cardiac valve hemolysis and microangiopathic hemolytic anemia.

Extravascular Destruction

Macrophages differentiate young and senescent red cells. They ingest older red cells.

Two important changes occur in senescent red cells that make them vulnerable to mechanical destruction or phagocytosis by macrophages:
1. Decreased deformability
2. Alteration in surface properties.

Decreased Deformability

Deformability of red cells helps them to easily pass through the narrow capillaries and splenic pulps. The biconcave shape and to some extent the viscosity of hemoglobin solution in the cell facilitate the ability of red cells to deform. Therefore, decreased deformability due to change in shape as occurs in spherocytosis or elliptocytosis, and increased internal viscosity as occurs in sickle cell or Hb-C disease, make the red cells vulnerable to lysis while passing through the splenic pulp.

Alteration in Surface Properties

Binding of antibodies or complement components to the surface of red cells, changes the chemical composition of the membrane. Especially, binding of IgG or C3 causes oxidation of membrane components. This is detected by macrophages that ingest red cells.
Section 2: Blood and Immunity

FATE OF DESTROYED RED CELLS

Destruction of red cells causes release of Hb, which is then converted to heme and globin. Heme is converted to bile pigments. Fate of destroyed red cells depends on whether the cells were destroyed intravascularly or extravascularly.

Fate in Intravascular Destruction

Destruction of red cells in circulation releases Hb into plasma. In plasma, Hb binds with haptoglobin (Application Box 13.1). The Hb-haptoglobin complex is transported to liver, where the heme of Hb is converted to iron...
and biliverdin by heme oxygenase. CO is released during cleavage of heme by heme oxygenase. Biliverdin is further degraded to bilirubin (Fig. 13.1). Heme in the plasma binds with hemopexin, a plasma glycoprotein. When the capacity of hemopexin to bind with heme is saturated, heme binds with albumin to form methemalbumin.

### Application Box 13.1
**Plasma haptoglobin indicates hemolysis:** In intravascular hemolysis, Hb-haptoglobin complex clears haptoglobin from plasma. Therefore, haptoglobin content of the plasma is decreased in hemolysis and the degree of decrease is apparently proportionate to the rate of hemolysis. Therefore, estimation of plasma haptoglobin level is an index of hemolysis.

### Fate in Extravascular Destruction
In the phagocytic cells, red cells are degraded by lysosomes into lipids, proteins and heme. Heme is catabolized to bilirubin, and protein and lipids are reprocessed in their catabolic pathways.

1. Bilirubin formed from destruction of red cells regardless of their site of destruction is finally excreted through bile into the GI tract. In the intestine, bilirubin is converted to urobilinogen by bacterial reduction. Urobilinogen is further converted to stercobilinogen and stercobilin that are excreted in stool (Fig. 13.1).
2. A small fraction of urobilinogen is absorbed from intestine and excreted through urine as urinary urobilinogen.

### Important Note
**Assessment of hemolysis:** Estimation of fecal stercobilinogen and urinary urobilinogen content indicates the intensity of hemolysis as they are good markers of the rate of hemolysis.

### HEMOLYTIC JAUNDICE
Hemolytic jaundice occurs from increased destruction of red cells or their precursors in the marrow, causing increased bilirubin production (Table 13.1). Jaundice due to hemolysis is usually mild because the healthy liver can excrete a bilirubin load of six times of its normal concentration. However, this does not happen in newborn. Hence, hemolytic jaundice is common in newborn (Clinical Box 13.1).

1. Heme is converted to biliverdin by heme oxidase, and biliverdin is converted to bilirubin by biliverdin reductase (Fig. 13.2).
2. Unconjugated bilirubin bound to albumin enters liver and gets conjugated by the action of glucuronosyltransferase, to form bilirubin diglucuronide.
3. Following enterohepatic circulation, bilirubin is excreted through bile into intestine, where it is converted to urobilinogen by bacterial reduction.
4. A fraction of it is absorbed into blood from intestine and excreted in urine. Urobilinogen content of stool and urine indicates rate of hemolysis. As bilirubin is unconjugated, which is mostly bound to protein, there is no excretion of bilirubin in urine. Hence, hemolytic jaundice is called acholic jaundice.

### Table 13.1: Causes of hemolytic jaundice.

<table>
<thead>
<tr>
<th>A. Inherited disorders</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Spherocytosis, elliptocytosis</td>
</tr>
<tr>
<td>2. Glucose 6-PD deficiency</td>
</tr>
<tr>
<td>3. Pyruvate kinase deficiency</td>
</tr>
<tr>
<td>4. Sickle cell anemia</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B. Acquired disorders</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Microangiopathic hemolytic anemia</td>
</tr>
<tr>
<td>2. Paroxysmal nocturnal hemoglobinuria</td>
</tr>
<tr>
<td>3. Spur cell anemia</td>
</tr>
<tr>
<td>4. Immune hemolysis</td>
</tr>
<tr>
<td>– Warm and cold antibodies</td>
</tr>
<tr>
<td>– Hemolytic diseases of newborn</td>
</tr>
<tr>
<td>– Incompatible blood transfusion</td>
</tr>
<tr>
<td>5. Drug-induced (rifampicin, probenecid)</td>
</tr>
<tr>
<td>6. Burn</td>
</tr>
</tbody>
</table>

![Fig. 13.2: Metabolism of bilirubin (hepatic phase and intestinal phase) and pathophysiology of hemolytic jaundice.](image-url)
5. Increased fecal stercobilinogen can cause **dark-brown colored stool**.
6. The plasma bilirubin is usually less than 6 mg% and liver function tests are usually normal. For details, refer Chapter 41 “Functions of liver and pathophysiology of jaundice” in GI System.

**Clinical Box 13.1**

**Hemolytic jaundice is common in newborn:** Normally, healthy liver can excrete a bilirubin load of six times of its normal concentration. However, in newborns as hepatic enzyme systems are not well developed, the bilirubin metabolism is not effective. Hence, hemolytic jaundice is common in them, especially in premature deliveries.

**CHAPTER SUMMARY**

**Key Concepts**

1. Red cells after their life span of 120 days (senescent red cells) are destroyed intravascularly or picked up and phagocytosed by the cells of reticuloendothelial cells (extravascular destruction).
2. Bilirubin released from macrophages enters liver for metabolism, and from there it enters intestine, where it is converted to stercobilinogen and excreted in feces.
3. About 20% of bilirubin enters kidney and excreted as urobilinogen.
4. Levels of stercobilinogen and urobilinogen indicate the rate of hemolysis.
5. Excess of hemolysis causes bilirubin accumulation, leading to hemolytic jaundice.

**Important to Know (Must Read)**

1. Usually, **Long Questions** are not asked from this chapter.
2. Mechanism of red cell destruction and hemolytic jaundice are usually asked as **Short Questions**.
3. In **Viva**, examiners usually ask the life span of red cells, fate of red cells, mechanism of hemolysis, bilirubin metabolism, causes of hemolytic jaundice, physiological basis of hemolytic jaundice, laboratory tests to detect hemolysis, and why hemolytic jaundice is common in newborns.

**5.** Increased fecal stercobilinogen can cause **dark-brown colored stool**.

**6.** The plasma bilirubin is usually less than 6 mg% and liver function tests are usually normal. For details, refer Chapter 41 “Functions of liver and pathophysiology of jaundice” in GI System.

**Clinical Box 13.1**

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Hemoglobin (Hb) is a conjugated protein present in red blood cells and it constitutes more than 90% of the dry weight of these cells. It transports oxygen from the lungs to the tissues, and carbon dioxide from tissues to the lungs. Hb also serves to destroy the nitric oxide molecule, which is physiologically important.

1. Oxygen affinity of Hb is an important property of Hb.
2. This affinity increases with oxygenation that makes the oxy-Hb dissociation curve sigmoid-shaped.
3. The oxy-Hb releases proton and deoxy-Hb binds proton.
4. The concentration of Hb within red cells of human beings is very high (34 g/dl), which increases its ability to carry adequate oxygen.

**Structure and Synthesis of Hb**

Hb is synthesized in the precursors of red cells during their development in the bone marrow. It appears in the early normoblast stage and attains maximum concentration in the late normoblast stage.

**Structure**

Hb is made up of heme and globin.

**Heme**

Heme is a complex molecule made up of a series of tetapyrrole rings, terminating in protoporphyrin, with a central iron atom. Following destruction of red cells, the components of hemoglobin undergo metabolic degradation (Flowchart 14.1).
1. The iron part of heme is recycled and used up again in the hemoglobin synthesis.
2. The only component of Hb that cannot be recycled is protoporphyrin, which forms bilirubin.

**Globin**

This is a protein substance that consists of two pairs of polypeptide chains. Each amino acid chain is attached to a heme moiety to form a single hemoglobin molecule. After the degradation of hemoglobin, the globin component breaks down into its amino acid constituents that are recycled for hemoglobin synthesis.
Synthesis of Hb

Formation of Hb requires heme and globin. Heme is formed in mitochondria and globin in ribosomes.

1. **Succinyl-CoA**, an intermediary product of TCA cycle combines with glycine to form α-amino-β-ketoadipic acid with the help of pyridoxal phosphate, which in turn forms α-amino-δ-levulinic acid (ALA) in the presence of ALA synthase (Flowchart 14.2).

2. ALA is converted to porphobilinogen, which forms protoporphyrin-IX.

3. Ferrous iron is introduced into protoporphyrin-IX and in the presence of heme synthase, heme is formed.

4. Globin, the protein component formed by ribosome combines with heme to form hemoglobin.

Types of Hemoglobin

Hemoglobins can be broadly divided into normal and abnormal types (Table 14.1).

**Normal Hemoglobins**

**Adult Hemoglobins**

Adult Hb are of two types: Hb A and Hb A₂.

**Hemoglobin A (Hb A)**

Hb A is the major Hb and comprises about 97% of hemoglobin in adult red cells. It consists of two α and two β chains with the structural formula α₂β₂. Hb A is detected in small amount in the fetus as early as the eighth week of intrauterine life. During the first few months of post-natal life, Hb A replaces Hb F and the adult pattern is fully established in 6 months.

**Fetal Hemoglobin (Hb F)**

Hb F is the major hemoglobin in intrauterine life. It has the structural formula of α₂γ₂. Hb F accounts for 70 to 90% of hemoglobin at term. It then falls rapidly to 25% in one month, and 5% in six months. The adult level of 1% is not reached in some children until puberty. Hb F concentration in adults increases in some types of anemia, hemoglobinopathies, and sometimes in leukemia.

Hemoglobin Bart’s (Hb Bart’s)

This is the minor hemoglobin present in fetal life. It consists of 4 gamma (γ) chains, γ₄. Hb Bart’s concentration increases in fetal life in thalassemia.
Chapter 14: Hemoglobin and Blood Indices

Embyronic Hemoglobins
These hemoglobins are confined to the very early stages (embryonic stage) of development. There are 3 embryonic hemoglobins:
1. **Gower Hb 1**: It consists of 2 zeta and 2 epsilon chains (ζ2ε2).
2. **Hb Gower 2**: It consists of 2 alpha and 2 epsilon chains (α2ε2).
3. **Hb Portland**: It consists of 2 zeta and 2 gamma chains (ζ2γ2).

Abnormal Hemoglobins
There are four clinically important abnormal hemoglobins: Hb S, Hb C, Hb D, and Hb E. These are present in different hereditary hemoglobinopathies.
1. The most commonly encountered hemoglobin is Hb S which consists of α2β2, but in the beta chain, valine is substituted for glutamic acid at the sixth position.
2. Hb S is present in sickle cell anemia.

Unstable hemoglobins: Unstable hemoglobins are hemoglobin variants that undergo denaturation, and precipitate in the red cells as Heinz bodies. Unstable hemoglobins are present in some form of congenital non-spherocytic hemolytic anemia.

Normal Values, Functions and Variations

Normal Values
**Adult males**: 14 to 18 (16 ± 2) g/dL of blood  
**Adult females**: 12 to 16 (14 ± 2) g/dL of blood

In newborns, hemoglobin concentration is normally 16 to 22 g/dL, which occurs mainly due to hemoconcentration. It decreases to 9 to 14 g/dL at about two to four months of age. By ten years of age, the normal hemoglobin concentration will be 12 to 14 g/dL. There may be a slight decrease in hemoglobin level after 50 years of age.

Functions of Hb
Hemoglobin serves three important functions.
1. It transports oxygen from lungs to the tissues by forming oxyhemoglobin and carbon dioxide from tissues to lungs by forming carboxyhemoglobin. When fully saturated, 1g of hemoglobin carries 1.34 ml of oxygen. Thus, **oxygen carrying capacity** of blood can be calculated easily if the value of blood hemoglobin concentration is known. For example, if Hb is 15 g%, 100 ml of blood will carry 15 × 1.34 = 20.7 ml of oxygen.
   - Iron component of Hb is essential for the primary function of the hemoglobin; i.e., the transport of oxygen.
   - When reduced hemoglobin is exposed to oxygen at increased pressure, oxygen is taken up at the iron atom until each molecule of hemoglobin has bound four oxygen molecules, one molecule at each iron atom. This is not a true oxidation-reduction reaction, and therefore, combination of hemoglobin with oxygen is known as **oxygination**.
     - The oxygen molecules combine with hemoglobin to form oxyhemoglobin.
     - Hemoglobin returning with carbon dioxide from tissues is called reduced hemoglobin.
2. Hemoglobin acts as a buffer in maintaining blood pH.
3. Hb serves to destroy physiologically important nitric oxide molecule.
4. It imparts red color to the blood. Erythrocytes look red due to the presence of hemoglobin in them, which is a red pigment.

Variations in Hb Concentration

Conditions that Decrease Hb Concentration

**Physiological**
Children have values lower than that in adults and women have values lower than that in males. Hb is less during pregnancy due to hemodilution.

**Pathological**
Hb is less in different types of anemia (for details, see next chapter). However, relative decrease in Hb concentration occurs in different pathological conditions that produce hemodilution like excess ADH secretion as occurs in pituitary tumors.

Conditions that Increase Hb Concentration

**Physiological**
Hb level is high at high altitude that occurs due to hypoxia. Hb is more in newborns. In excessive sweating, relative increase in Hb occurs due to hemoconcentration.

**Pathological**
Hb concentration increases in conditions that produce hemoconcentration like severe diarrhea, vomiting etc; and conditions that produce hypoxia like congenital heart disease, emphysema, etc. It may also be due to polycythemia vera (for details, see next chapter).

Hemoglobin Ligands (Complexes)

Besides its binding with oxygen to form oxyhemoglobin (and release of oxygen from hemoglobin to form reduced or deoxygenated Hb), Hb combines with other chemicals and gases to form Hb-complexes, some normally and some abnormally. Some commonly encountered complexes are nitrosohemoglobin, carboxyhemoglobin, methemoglobin, sulfhemoglobin, cyanmethemoglobin and glycosylated and glycated hemoglobins.

Nitrosohemoglobin
Nitric oxide (NO) is a physiologic ligand of Hb.  
1. NO stimulates a cytosolic heme protein, guanylate cyclase to from cyclic GMP that mediates many physiological
activities including vasodilation, inhibition of platelet aggregation and macrophage cytotoxicity.
2. Hb and myoglobin rapidly react with NO and prevent guanylate cyclase activation.
3. Binding of Hb with NO destroys NO and forms nitrosohemoglobin by formation of nitrosoheme.
4. Especially, stroma-free Hb inactivates NO. Therefore, stroma-free Hb as occurs in hemoglobinemia causes vasoconstriction.

**Carboxyhemoglobin**
Carbon dioxide (CO) is a physiologic ligand of Hb.
1. Binding of CO2 with Hb leads to formation of carboxyhemoglobin.
2. CO2 combines with globin, not with heme.
3. It helps in transport of CO2 from tissues to lungs.

**Carboxyhemoglobin**
Carboxyhemoglobin, also called carbmonoxyhemoglobin, is formed when heme iron of Hb combines with carbon monoxide (CO).
1. Hemoglobin has a much greater affinity for CO than for oxygen. Hb binds with CO about 200 times more strongly than it binds oxygen.
2. Therefore, it readily combines with CO even when CO is present in low concentration.
3. However, formation of carboxyhemoglobin is reversible, which means, once CO is removed from the blood, the hemoglobin combines back with oxygen.
4. Carboxyhemoglobin is found in very low concentrations in normal persons.
5. In smokers, its concentration is high, ranging from 1 to 10 g/dL. Therefore, tissue oxygenation is impaired in smokers.

**Methemoglobin**
Methemoglobin is Hb in which iron has been oxidized from ferrous to ferric state.
1. Methemoglobin is not capable of reversibly binding with oxygen. Therefore, in methemoglobinemia oxygen carrying capacity is grossly reduced.
2. Normally, its concentration is very low.
3. But, its formation increases in the presence of certain chemicals or drugs.
4. However, the formation of methemoglobin can be reversible.

**Cyanmethemoglobin**
Cyanmethemoglobin (hemiglobincyanide) is formed by the action of cyanide anion (CN−), for example potassium cyanide (KCN). However, the combination is reversible.
1. Hemoglobin is the hemoglobin in which the iron has been oxidized by cyanide to the ferric state.
2. Hemiglobincyanide is the methemoglobin bound to cyanide ions.

**Sulfhemoglobin**
Sulfhemoglobin is formed on exposure to some toxic agents, usually sulfur-containing drugs and chemicals.
1. In sulfhemoglobin, the iron is in ferrous state, but oxygen affinity is about 100 times lower than the normal Hb.
2. Sulfur is not attached with iron, rather is bound to porphyrin ring.
3. Once sulfhemoglobin is formed, it is irreversible and remains throughout the life of the carrier RBC.

**Glycosylated or Glycated Hemoglobin**
In our body, glucose gets attached to protein by enzymatic and nonenzymatic processes and hemoglobin being the major protein in circulation undergoes the same too. The resulting structure is called glycosylated hemoglobin. This is a natural physiological process of body.
1. The fraction of HbA, known as HbA1c is of actual clinical interest.
2. In HbA1c, glucose is attached to terminal valine in β chain.
3. In normal individuals, glycosylated Hb (HbA1c) is present in very less concentration, less than 4% of total Hb. The normal HbA1c is less than 4%.
4. As per WHO criteria, if HbA1c is more than 6.5%, it is considered abnormal (Clinical Box 14.1).
5. Its concentration increases in conditions in which blood glucose is chronically elevated like diabetes mellitus, Cushing’s syndrome, hyperthyroidism, etc.
6. As per ADA (American Diabetic Association) criteria:

<table>
<thead>
<tr>
<th>Clinical Box 14.1</th>
</tr>
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<tbody>
<tr>
<td>Glycated Hb (HbA1c) indicates persistent hyperglycemia: When blood glucose is chronically elevated for more than three months, glycated Hb is formed. Therefore, instead of fasting blood glucose, emphasis is given on estimation of glycated Hb. Thus, glycated Hb is an index of chronic hyperglycemia in diabetic subjects.</td>
</tr>
</tbody>
</table>

**In Oxidative Stress**
Currently, the term glycosylation is not used. The process is called glycation of proteins. The resulting hemoglobin is called glycated hemoglobin.
1. This nomenclature has been used recently to differentiate the nonenzymatic glycation from enzymatic glycosylation of proteins that occurs normally in the ribosome-Golgi complex.
2. Glycation requires two factors, increased level of sugars and increased level of free radicals that promote this type of addition in order to gain neutrality.
3. Hence, glycated hemoglobin has recently been considered to reflect the degree of oxidative stress (Clinical Box 14.2).

<table>
<thead>
<tr>
<th>Clinical Box 14.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Importance of Glycated Hb: As noted above, glycated Hb is measured to detect the degree of hyperglycemia that is harmful to the body and it is also a measure of degree of oxidative stress. Oxidative stress is a potent inducer of aging.</td>
</tr>
</tbody>
</table>
**Applied Physiology**

**Hb Destruction**

Hb destruction occurs with destruction of red cells. When red blood cells are destroyed in the tissue macrophage system, or in the circulation, hemoglobin is degraded into heme and globin. Globin returns to the body’s metabolic pool where its amino acids are subsequently reutilized (Flowchart 14.1). The porphyrin ring of heme is cleaved by the microsomal enzyme, heme oxidase, yielding biliverdin. The biliverdin is further reduced to form bilirubin by biliverdin reductase (Refer to Fig. 13.2, Chapter 13). Therefore, excess hemolysis leads to jaundice (hemolytic jaundice) and hemoglobinuria (Application Box 14.1).

**Application Box 14.1**

**Tissue Hypoxia and ARF:** The primary function of hemoglobin is to carry oxygen from lungs to the tissues. Therefore, anemia-related hypoxia causes tissue hypoxia. The major effects of anemia are due to tissue hypoxia. In hemolysis, hemoglobin is released into blood (hemoglobinemia) and is filtered in the renal tubules and appear in the urine (hemoglobinuria). In the tubules, hemoglobin forms casts that block the renal tubules and cause acute tubular necrosis (acute renal failure). Hemoglobinemia also exerts osmotic effect and increases blood viscosity that affects cardiac output and alters dynamics of blood flow.

**Hemoglobin Estimation**

Hemoglobin estimation is the most frequently ordered laboratory test in clinical practice. It is performed as a routine investigation in outpatient departments and also as a bedside test in hospital patients. It is mandatory to assess the hemoglobin status of a patient prior to any surgical intervention. As it is more convenient, estimation of hemoglobin is preferred to the total RBC count to detect anemia. **Anemia** is said to be present when the hemoglobin level in blood is below the lower limit of the normal range for the age and gender of the individual (for details of anemia, refer to the next chapter).

**Blood Indices**

The values of hemoglobin, PCV and total RBC count are used to calculate red cell volume and red cell hemoglobin content and concentration. These are called **red blood cell indices**. The commonly estimated blood indices are:

1. Mean cell volume (MCV)
2. Mean cell hemoglobin (MCH)
3. Mean cell hemoglobin concentration (MCHC)

   The MCV defines the volume or size of the average RBC, the MCH defines the weight of hemoglobin in the average RBC, and the MCHC defines the hemoglobin concentration or color of the average RBC.

   Other direct quantitative measurements of red cell indices are the **mean corpuscular diameter** (MCD) and **red cell distribution width** (RCDW). Determination of these indices is of considerable clinical importance and is widely used in the classification of anemia.

**Mean Corpuscular Volume**

Mean corpuscular volume (MCV) is the average volume of an RBC expressed in femtoliters (1 fl = 10^{-15} L). If the PCV and the number of red cells per liter are known, MCV is calculated by dividing the volume of red cells per liter by the number of red cells per liter.

\[
MCV \text{ (fl)} = \frac{\text{Hematocrit (%) } \times 10}{\text{RBC count in millions/mm}^3}
\]

Where the factor 10 is introduced to convert the hematocrit reading (in %) from volume of packed red cells per 100 ml to volume per liter. For example, if the hematocrit reading is 40%, and the red cell count is 5 millions, then MCV is calculated as:

\[
MCV = \frac{40 \times 10}{5} = 80 \text{ fl.}
\]

**Normal value:** The MCV in normal adults is between 78 and 96 fl.

**Applied aspect:** The MCV is the **index of the size of red cells**. It depicts whether the red cells are microcytic, normocytic or macrocytic. If the MCV is less than 78 fl, the red cells are considered **microcytic** and if greater than 96 fl, they are considered **macrocytic**.

**Mean Corpuscular Hemoglobin**

The mean corpuscular hemoglobin (MCH) is the average weight of hemoglobin content in an RBC expressed in picograms (1 pg = 10^{-12} g). It is calculated by dividing the hemoglobin content of 1 liter of blood (in g/L) by the number of RBCs in 1 liter.

\[
MCH \text{ (pg)} = \frac{\text{Hb (g/dL) } \times 10}{\text{RBC count in millions/mm}^3}
\]

For example, if the hemoglobin content is 14 g/dL, and the RBC count is 5 millions:

\[
MCH = \frac{14 \times 10}{5} = 28 \text{ pg.}
\]

**Normal value:** The normal range for MCH is 27 to 33 pg. It may be as high as 50 pg in macrocytic anaemia, or as low as 20 pg or less in hypochromic microcytic anaemia.

**Mean Corpuscular Hemoglobin Concentration (MCHC)**

The mean corpuscular hemoglobin concentration (MCHC) is the average hemoglobin concentration per unit volume of packed red cells. It is expressed as g/dL, or percent.

\[
MCHC \text{ (%) } = \frac{\text{Hb (g/100 ml) } \times 100}{\text{PVC/100 ml}}
\]

For example, if the hemoglobin concentration is 15 g/dL and hematocrit is 45%:

\[
MCHC = \frac{15}{45} \times 100 = 33.3\%.
\]
Section 2: Blood and Immunity

CHAPTER SUMMARY

**Key Concepts**

1. The normal hemoglobin is HbA and HbA₂. Hb A constitutes 97% of total Hb, it has the structure of two α and two β chains with the structural formula α₂β₂.
2. Main function of Hb is the transport of oxygen from lungs to tissues and CO₂ from tissues to lungs.
3. Hb in males is 14 to 18 g%, and in females is 12 to 16 g%.
4. Persistent elevation in blood glucose manifests in increased HBA1c. If HBA1c is more than 6.5%, it is considered abnormal.
5. Blood indices (MCV, MCH and MCHC) are useful in classification of anemia.

**Important to Know (Must Read)**

1. In examinations, usually Long Questions are not asked from this chapter.
2. Structure of Hb, functions of Hb, abnormal Hb, all types of hemoglobin complexes, and all blood indices are usually asked as Short Questions in exams.
3. In Viva, examiners usually ask types of Hb, structure of Hb, normal values of Hb in males and females, conditions of increased and decreased Hb, broad steps of Hb synthesis, major steps of Hb degradation, Hb complexes and their clinical significance, importance of HbA1c, normal values and significance of all blood indices. These questions are very common in oral exam and students are expected to answer these questions related to Hb.

**Color Index (CI)**

This is the ratio of hemoglobin percent and RBC percent.

\[
CI = \frac{\text{Hb percent}}{\text{RBC percent}}
\]

100% of Hb = 14.8 g/100 ml.
100% of RBC = 5 millions/mm³.

**Normal value:** The normal range of CI is between 0.85 and 1.10.
CI less than 0.85 indicates hypochromic anemia.

### Normal value

The normal range of MCHC is between 33 and 37 g/dL (or %).

**Applied aspect:** MCHC above 40% indicates malfunctioning of the instrument or error in the calculation of the manual measurements used, as an MCHC of 37% is near the upper limits for hemoglobin solubility, thus limiting the physiologic upper limit of the MCHC. In hypochromic anemias, the hemoglobin concentration is reduced and values as low as 20% to 25% are not uncommon.

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CI less than 0.85 indicates hypochromic anemia.
CHAPTER 15
Pathophysiology of Anemia and Polycythemia

Learning Objectives
On completion of study of this chapter, the student MUST be able to:
1. Define anemia and polycythemia.
2. Classify anemia, and give the common causes of each category of anemia.
3. Give the salient blood picture of common types of anemia.
4. List the types and causes of polycythemia.

The student MAY also be able to:
1. Explain the causes and salient blood picture of iron-deficiency anemia, spherocytosis, sickle-cell anemia and thalassemia.
2. Briefly describe the blood picture and bone marrow picture of polycythemia vera.

Alterations in red cell count are grouped into two major types, anemia (erythrocytopenia) and polycythemia (erythrocytosis). Anemia is the decreased red cell mass and polycythemia is the increased red cell mass.

1. As anemia decreases oxygen-carrying capacity of blood, it is usually expressed in terms of hemoglobin concentration of blood, and the clinical features of anemia are primarily due to tissue hypoxia and hypoxia-induced compensatory mechanisms.

2. Polycythemia is better expressed in terms of hematocrit percentage because the clinical features of it are primarily due to the size of red cell mass and the degree of viscosity.

3. Though, defining strictly, polycythemia is the increase in all formed elements of blood, it usually refers to increased red cell mass as red cells are the major constituents of blood.

Anemia is defined as decreased red cell count or hemoglobin content of blood.

1. Detection of anemia is usually performed by estimating hemoglobin content of blood.
2. It can also be performed by performing either total RBC count of blood or by estimating packed cell volume (hematocrit).

3. Clinically, anemia is detected by assessing the degree of paleness usually by looking at the lower palpebral conjunctiva or nail beds.

4. Detection of morphological type of anemia depends on various blood indices (as described in the previous chapter).

Classification

Anemias can be classified either morphologically or etiologically. Common causes of anemia are:
1. Inadequate supply of nutrients resulting in deficiency anemias (deficiency of iron, vitamins and proteins)
2. Aplasia of bone marrow
3. Anemia associated with chronic diseases
4. Anemia associated with renal failure
5. Anemia due to inherited diseases (e.g. thalassemia)
6. Anemia due to blood loss
   However, they are best classified etiologically into two broad groups as decreased red cell production and increased red cell destruction (Table 15.1).

Morphological Classification

Morphologically, anemia are classified into hypochromic microcytic, normochromic, microcytic and macrocytic normochromic types (Refer to Fig. 11.3, Chapter 11).
Section 2: Blood and Immunity

Hypochromic Microcytic Anemia

MCV, MCH, and MCHC are below normal. Such subnormal red cell indices correspond to microcytosis and hypochromia of red cells in the blood film (Figs. 15.1A and B). Micro-normoblasts are seen in bone marrow examination. This occurs due to the result of a defect in red cell formation in which hemoglobin synthesis is impaired to a great extent. The common examples are:

- **Iron-deficiency anemia** in which there is inadequate iron for the formation of the heme component of the hemoglobin, and
- **Thalassemia** in which the formation of the globin component of hemoglobin is defective.

Normochromic Normocytic Anemia

MCV, MCH, and MCHC are within the normal range. Size and hemoglobin concentration of the red cells are normal in the blood film. It usually occurs in following conditions:

- Substantial blood loss (*blood loss anemia*),
- Hemolysis (*hemolytic anemia*) and
- Impairment of red cell production by bone marrow failure or chronic renal failure (*aplastic anemia*).

Macrocytic Normochromic Anemia

The MCV is above the upper limit of the normal. It corresponds to macrocytosis of red cells in the blood film. The red cells are usually normochromic, though they are macrocytic. Megaloblasts are seen in bone marrow examination (Fig. 15.2). Howell Jolly body, hypersegmentation of neutrophils, basophilic stippling are also seen (Refer to Fig. 12.3, Chapter 12). The typical example of this type of anemia is *megaloblastic anemia* that occurs due to deficiency of vitamin B_{12} or folic acid.

**Etiological Classification**

**Blood Loss Anemia**

Anemia due to blood loss mainly occurs due to acute hemorrhage or chronic hemorrhage.

*Acute hemorrhage*: Anemia due to acute hemorrhage depends on the extent of blood loss and the time that has lapsed since bleeding.

---

**Table 15.1: Classification of anemia.**

<table>
<thead>
<tr>
<th>A. Decreased red cell production</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Stem cell failure</td>
</tr>
<tr>
<td>- Aplastic anemia</td>
</tr>
<tr>
<td>- Anemia of leukemia</td>
</tr>
<tr>
<td>2. Progenitor cell failure</td>
</tr>
<tr>
<td>- Pure red cell aplasia</td>
</tr>
<tr>
<td>- Chronic renal failure</td>
</tr>
<tr>
<td>- Chronic diseases</td>
</tr>
<tr>
<td>3. Precursor cell failure</td>
</tr>
<tr>
<td>- Megaloblastic anemia</td>
</tr>
<tr>
<td>- Iron-deficiency anemia</td>
</tr>
<tr>
<td>- Thalassemia</td>
</tr>
<tr>
<td>- Hemoglobinopathies</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B. Increased red cell destruction or loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Acquired causes</td>
</tr>
<tr>
<td>- Acute blood loss</td>
</tr>
<tr>
<td>- Hypersplenism</td>
</tr>
<tr>
<td>- Micro- and macroangiopathic</td>
</tr>
<tr>
<td>- Antibody-mediated</td>
</tr>
<tr>
<td>2. Hereditary causes</td>
</tr>
<tr>
<td>- Membrane defects</td>
</tr>
<tr>
<td>- Enzyme defects</td>
</tr>
<tr>
<td>- Globin defects</td>
</tr>
</tbody>
</table>

Figs. 15.1A and B: Microcytic hypochromic anemia. (A) Peripheral blood smear (note microcytosis); (B) Schematic picture of the smear (note presence of microcytes with hypochromia).

Chapter 15: Pathophysiology of Anemia and Polycythemia

1. In acute blood loss, usually there is a reduction in the total blood volume. Therefore, hemoglobin in the residual blood is normal.

2. However, when the compensatory mechanisms set in to expand the blood volume, hemodilution decreases hemoglobin content.

3. Therefore, estimation of hemoglobin after few hours of acute blood loss does not assess the actual degree of anemia.

Chronic hemorrhage: Chronic hemorrhage occurs mainly in gastrointestinal, genitourinary and respiratory tract diseases.

1. Gastrointestinal blood loss: Peptic ulcer, hemorrhoids, hiatus hernia, carcinoma of the stomach and colon, esophageal varices, chronic aspirin ingestion, ulcerative colitis, hookworm infestation, etc.

2. Respiratory diseases: Respiratory diseases that produce epistaxis, hemoptysis as occurs in pulmonary tuberculosis or bronchogenic carcinoma produce anemia.

3. Genitourinary diseases: Diseases that cause hematuria and hemoglobinuria produce anemia.

4. Diseases of genital tract: In females, loss of blood from genital tract like menstrual disorders (menorrhagia, metrorrhagia) and uterine pathologies produce anemia.

Aplastic Anemia

Aplastic anemia is the anemia due to impaired red cell production. Marrow examination shows a near absence of hematopoietic precursor cells. The common causes of aplastic anemia are listed in Table 15.2.

Hemolytic Anemia

Hemolytic anemia results from increase in the rate of red cell destruction. Defects causing premature red cell destruction may be divided into two broad categories: intracorpuscular, and extracorpuscular (Table 15.3).

Common Anemias

Iron-deficiency Anemia (IDA)

This is the commonest form of anemia in the developing countries. It usually occurs due to deficiency of iron in the diet. There are three major factors in the pathogenesis of IDA.

1. Increased physiological demand for iron as occurs in pregnancy, lactation, growing children. Therefore, in these groups, extra iron should be available in the diet; otherwise IDA occurs.
2. **Inadequate iron intake**, as occurs due to deficiency in the diet.

3. **Pathological blood loss** like bleeding peptic ulcer, piles, worm infestations, epistaxis, hemoptysis etc.

   Anemia of IDA is **microcytic hypochromic** type. Laboratory findings are decreased all blood indices (MCV, MCH and MCHC), microcytic hypochromic cells in blood smear (Fig. 15.1) and micronormoblasts in bone marrow, and decreased marrow iron store (Fig. 15.3).

### Table 15.3: Causes of hemolytic anemia.

<table>
<thead>
<tr>
<th><strong>A. Intracorpuscular defects</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Membrane defects: hereditary spherocytosis, hereditary elliptocytosis</td>
</tr>
<tr>
<td>2. Hemoglobin defects:</td>
</tr>
<tr>
<td>i. Hemoglobinopathies: Sickle cell anemia, abnormal hemoglobins like, Hb-C, Hb-E, Hb-D, etc., unstable hemoglobin disease.</td>
</tr>
<tr>
<td>ii. Thalassemia: Thalassemia major and minor</td>
</tr>
<tr>
<td>3. Enzyme defects: Deficiency of pyruvate kinase and glucose-6-phosphate dehydrogenase.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>B. Extracorpuscular defects</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Acquired:</td>
</tr>
<tr>
<td>i. Immune mechanisms: Warm and cold antibodies, hemolytic diseases of newborn, incompatible blood transfusion, drug induced anemia.</td>
</tr>
<tr>
<td>ii. Non-immune mechanisms: Cardiac hemolytic anemia, March hemoglobinuria</td>
</tr>
<tr>
<td>iii. Drug-induced</td>
</tr>
<tr>
<td>iv. Infections</td>
</tr>
<tr>
<td>v. Burn etc.</td>
</tr>
<tr>
<td>2. Hereditary</td>
</tr>
</tbody>
</table>

### Hereditary Spherocytosis

This is a hemolytic anemia in which the fundamental abnormality is the increased defect of red cell membrane (due to decreased quantity of spectrin) that results in spherocytic shape of the cell. Spherocytes have a decreased surface area to volume ratio and the cells are more rigid (less deformable). Therefore, when cells pass through the splenic pulp, they are destroyed.

1. This is an **autosomal dominant disorder** which affects males and females equally.
2. The usual features are anemia, jaundice, enlarged spleen, and may present with gall stone.
3. The blood picture typically shows anemia with spherocytosis (Figs. 15.4A and B), increased osmotic fragility, hyperbilirubinemia, and reticulocytosis.
4. Usually, anemia is **normocytic and normochromic**.
5. Splenectomy helps in improving the condition.

### Sickle-cell Anemia

This is a hereditary disorder in which red cells contain an abnormal hemoglobin called Hb S. Hb S is the hemoglobin in which glutamic acid is replaced by valine at the 6th position of beta chain. In the deoxygenated state, conformational changes induced by Hb S makes the cell more rigid and deformed to take the shape of a sickle (Fig. 15.5). Therefore, cells undergo **intravascular hemolysis**.

1. Diagnosis is usually made by **sickle test** (demonstrating sickling of red cells when the blood is mixed with freshly prepared solution of a reducing agent like sodium metabisulphite) (Figs. 15.6), **hemoglobin solubility test** (relative insolubility of reduced Hb S in phosphate buffer), and **hemoglobin electrophoresis**.

### Table: LABORATORY FINDINGS

<table>
<thead>
<tr>
<th><strong>LABORATORY FINDINGS</strong></th>
<th><strong>NORMAL</strong></th>
<th><strong>IRON-DEFICIENCY ANAEMIA</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BLOOD</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>RED CELL MORPHOLOGY</strong></td>
<td>Normal red cell</td>
<td>Microcytic hypochromic red cell</td>
</tr>
<tr>
<td><strong>RED CELL INDICES</strong></td>
<td>MCV, MCH, MCHC all normal</td>
<td>MCV ↓, MCH ↓, MCHC ↓</td>
</tr>
<tr>
<td><strong>MARROW ERYTHROPOIESIS</strong></td>
<td>Normoblastic</td>
<td>Micronormoblastic</td>
</tr>
<tr>
<td><strong>MARROW IRON Stores</strong></td>
<td>Normal</td>
<td>Deficient</td>
</tr>
</tbody>
</table>

**Fig. 15.3:** Iron deficiency anemia. Note microcytic hypochromic red cells in peripheral blood smear and micronormoblasts in bone marrow with decreased iron store.
2. Hyposplenism is usually associated, due to microinfarctions in spleen. Autosplenectomy may occur.
3. The anemia is usually normochromic and normocytic.

**Thalassemia**

Thalassemia is a genetically determined heterogenous group of commonest monogenic disorder in which the rate of synthesis of one or more types of hemoglobin polypeptide chain is decreased. Thus, there are two major classes of thalassemia: α thalassemia and β thalassemia, in which α and β globin genes are involved respectively. This causes decrease in the respective polypeptide chain of hemoglobin (Fig. 15.7).

**β thalassemia**

In β thalassemia (failure to synthesize β chain), which is more common, there is excess α chain production that damages red cell precursor and red cells.
1. There are many homozygous and heterozygous, and major and minor forms of the disease.
2. In β thalassemia major, anemia develops in first few months of life and becomes progressively severe.
3. Splenomegaly, hepatomegaly and skeletal deformities are common.
4. Though anemia is usually microcytic and hypochromic, all forms and combinations are not uncommon (Fig. 15.8).

**α-thalassemia**

Anemia of α-thalassemia (failure to synthesize α chain) is more hemolytic than dyserythropoietic.

The thalassemias are diagnosed by:

1. Hemoglobin electrophoresis
2. Demonstration of Hb-H inclusions (in the absence of sufficient alpha chains, excess of beta or gamma chains aggregate to form Hb-H)

3. Study of rate of globin chain synthesis
4. Alkali denaturation test
5. Acid elution test

**POLYCYTHEMIA**

Though polycythemia strictly denotes increase in all cell types of blood, traditionally it represents increase in number of red cells. It exists in two main forms:

1. The primary form, also called polycythemia vera, is a clonal neoplastic disorder of hematopoietic stem cells.
2. The secondary forms are conditions of increased red cell production that usually occurs due to appropriate or inappropriate increase in secretion of erythropoietin.

In primary form, the cause of the disease is the abnormality of hemopoietic stem cells characterized by uncontrolled proliferation of cells of erythroid, granulocytic and megakaryocytic series, resulting in increase of all forms of formed elements of blood. Thus, primary polycythemia is appropriate for the primary form.

In secondary forms, the cause of the disease is excess erythropoietin secretion that results in increase in red cell production (mostly without increase in granulocytes and platelets). Thus, secondary erythrocytosis is more appropriate than secondary polycythemia for the secondary forms.

**Types of Polycythemia**

Polycythemia is broadly divided into three forms (Table 15.4). The primary form is the polycythemia vera. The secondary
**CHAPTER SUMMARY**

**Key Concepts**
1. Anemia can be classified of morphologically and etiologically. Morphologically, anemias are classified into hypochromic microcytic, normochromic microcytic, and macrocytic normochromic types.
2. The commonest cause of anemia in the Indian subcontinent is nutritional deficiency anemia, especially the iron-deficiency anemia (IDA).
3. IDA manifests with microcytosis and hypochromic red cells in peripheral smear.
4. The next common anemia is megaloblastic anemia that occurs due to vitamin B<sub>12</sub> deficiency. In this, macrocytosis is seen in peripheral smear, and megaloblasts in bone marrow smear.
5. Polycythemia vera occurs due to hemopoietic stem cells producing more erythroid lineage, resulting in excessive production of red cells.

**Important to Know (Must Read)**
1. In examinations, classification of anemia with causes and blood picture may come as a Long Question.
2. Types of anemia, megaloblastic anemia, iron-deficiency anemia, thalassemia and polycythemia are usually asked as Short Questions in exams.
3. In Viva, examiners usually ask about types of anemia, causes of anemia, peripheral blood picture in common anemias, like IDA and megaloblastic anemia, and definition and types of polycythemia.
4. As anemia is common in India, questions are frequently asked from this chapter, and the student is expected to answer these questions.
CHAPTER 16
Blood Groups and Physiological Basis of Blood Transfusion

Learning Objectives
On completion of study of this chapter, the student MUST be able to:
1. List the uses of blood groups, classify blood groups and give the physiological basis of blood grouping.
2. Describe the agglutinogens, agglutinins in ABO system, give % distribution of ABO blood groups in population and understand the mechanism of inheritance of ABO blood groups.
3. Define Landsteiner’s law and give its physiological basis.
4. Describe the antigens and antibodies in Rh system, and the mechanism of Rh incompatibility.
5. Give the etiology, features, physiological basis of treatment and prevention of erythroblastosis fetalis.
6. Understand the concept of universal donor, universal recipient, and major and minor cross matching.
7. List the usual hazards of blood transfusion.

The student MAY also be able to:
1. Explain the role of H antigen in ABO typing.
2. Appreciate the importance of Bombay blood group.
3. Name the anticoagulants used for blood collection and list the changes in red cells following storage of blood.
4. Name the common diseases associated with various blood groups.
5. List the indications of blood transfusion and learn the procedure of transfusion.

BLOOD GROUPS

Human red cells contain numerous surface structures that are recognized as antigens by the immune system of the individual who does not possess them. These surface structures are called blood group antigens. Based on the presence of antigens on red cell membrane, usually corresponding antibodies are absent in the plasma of that individual. This forms the physiological basis of blood groups and principle of blood transfusion:
1. The blood group antigens are called agglutinogens and the antibodies against them are called agglutinins as the reaction between them results in clumping or agglutination of red cells.
2. There are more than 30 known blood group systems in our blood containing about 400 antigens (Table 16.1).
3. Many of them have cold antibodies that do not react at body temperature. Therefore, only few of them are immunologically active.
4. The blood group systems that are most important for blood transfusion are ABO and Rh systems.
5. Other major blood group systems that have medical and medicolegal importance are MNS, Lewis, Duffy, Kell, P and Lutheran systems.

Uses of Blood Groups

Red cells have traditionally been considered as less active cells that contain mainly hemoglobins. However, they play important role in many physiological processes of the body due to the presence of antigens on their surface:
1. Characterization of red cell antigen and antibodies forms the basis of compatibility testing for blood transfusion. When the blood group of an individual is known, in emergency situations, blood can be transfused immediately with the suitable blood. Therefore, ABO and Rh blood groups are usually displayed in the identity cards of individuals.
2. The blood group antigens have provided the scientific basis for understanding hemolytic diseases of the newborn and autoimmune hemolytic anemias.
3. Blood group antigens play a critical role in susceptibility to infections by malaria parasites, bacteria and viruses. Also, few blood groups provide resistance to some diseases (see above).

4. Red cell antigens are also associated with clinical disorders. Absence of certain red cell antigens is associated with specific dysfunctions, and certain inherited and acquired diseases are associated with alteration of red cell antigen expression. These associations have led to understanding of pathophysiology of these pathologic processes.

5. Medicolegal experts take the help of characterization of red cell antigen and antibodies in establishing the identity of the father in cases of disputed paternities (and also to identify the mother in cases of disputed maternity).

6. Specific blood groups have been reported to be associated with human behaviors. Therefore, blood groups are believed to contribute to the personality of the individual.

### ABO System

ABO system was the first blood group system to be known (described in 1901):

1. This is the most important system in transfusion medicine, as ABO agglutinogens are highly antigenic.
2. Physiologically, it is most significant of all blood group systems, because of natural occurrence of A and B antibodies in the plasma of individuals who lack corresponding antigen on their red cells.
3. In addition, transfusion of incompatible ABO blood groups instantly leads to serious consequences.

### ABO Agglutinogens

In ABO system, in essence there are two antigens, the antigen A and the antigen B. A antigen has two sub-types, A and A. The genes for antigens for ABO system are ABO genes located on chromosome 9.

### Occurrence

These antigens are present on the membrane of red cells:

1. They are also found in other tissues like salivary gland, testes, lungs, kidney and pancreas. Therefore, they are secreted in minute quantity in saliva, pancreatic secretion and seminal fluid.

2. Some individuals, especially blood group O, secrete blood group H antigens in saliva and other body fluids; they are called H secretors (see below, role of H antigen).

### Time of Appearance

ABO antigens are present since fetal life. They first appear at sixth week of intrauterine life. At birth, the concentration is about one-fifth of concentration in adults. Concentration rises slowly to reach a significant level at puberty and reaches maximum at 15–17 years of life (Application Box 16.1). Therefore, for collection of blood for blood transfusion, the age of the donor should be minimum of 15 years.

### Chemical Nature

Blood group antigen A and B are complex oligosaccharides. There is difference in their terminal sugars. For agglutinogen A, terminal sugar is N-acetylgalactosamine, and for agglutinogen B, terminal sugar is galactose.

### Blood Groups Based on Antigens

Based on the presence or absence of A and B antigens on the red cell membrane individually or in group, four blood groups have been described.
Section 2: Blood and Immunity

Group A : Antigen A is present
Group B : Antigen B is present
Group AB : Both A and B antigens are present
Group O : Neither A nor B antigen is present

Subtypes
As A antigen has two sub-types, A and A, the group A is subdivided into two groups: the group A containing A and A antigens, and group A containing A antigen alone. Similarly, the AB blood group is subdivided into A,B and A,B blood groups.

Distribution
In the Indian population, the approximate distribution of the ABO blood group is as follows:

A : 27%
B : 31%
AB : 8%
O : 34%

In the A blood groups, A is 75% and A is 25%.

Role of H Gene and H Antigens
The expression of A and B antigens are dependent on the presence of H genes. Most of the individuals are homozygous for the H gene. The sequence of events in the formation of A and B red cell antigens is:

1. A and B red cell antigens are glycoproteins and their formation starts with basic precursor substances (Fig. 16.1).
2. Basic precursor substance is first converted to H substance (by transferase) under the influence of H gene.
3. H substance is partially converted under the influence of A and B genes (and specific transferase) into A and B antigens. Some of the H substance remains unconverted.
4. Since O group individuals do not have A and B genes, neither A nor B antigen is formed, and these have only H substance (H remain unchanged). A, B and H antigens may be detected in the saliva and other body fluids also. Such individuals are called secretors, while the remaining without it are non-secretors.

Important Note
Secretors and Nonsecretors: The individuals who secrete A, B and H antigen substances in the saliva and other body fluids are called secretors and those who do not are called nonsecretors.

Bombay Blood Group
In 1952, Bhende, Bhatia and Deshpande discovered a new blood group called as Bombay blood group (Phenotype Oh). These individuals lack the H gene, and therefore the basic precursor substance can not be converted into H substance. This in turn results in failure to form A or B antigen. When their blood sample is tested for routine AB grouping, they will be labeled as blood group O. However, their serum contains anti A and anti B and anti H antibodies. These individuals therefore, should be transfused with only Bombay blood group.

ABO Agglutinins
In ABO system, agglutinins are of two types: anti-A (α) and anti-B (β). The α agglutinin has two subtypes, α proper and α.

Occurrence
These antibodies are found in plasma. Usually, specific antibodies are formed against a particular antigen, when they are exposed to that antigen. But, blood group antibodies are naturally occurring antibodies as they are formed without prior exposure to the antigens and are
present in the blood of individuals in whom the respective antigens are absent. However, the mechanism of development of blood group antibodies (as naturally occurring) is not known.

**Time and Mechanism of Appearance**

Anti-A and anti-B agglutinins are not formed during fetal life and are absent at birth. They appear in the second week of neonatal life and increase very slowly in concentration to attain the peak level at about 10 years of life:

1. The exact mechanism of formation of agglutinins in the absence of their corresponding agglutinogen is not clearly known. It has been proposed that intestinal bacteria and food contain antigens similar to that of blood group agglutinogens. Therefore, when the baby starts eating and food gets absorbed from intestine, antigens are absorbed into the blood.
2. As these antigens are recognized as non-self antigens by the body’s immune system, they stimulate antibody production.
3. However, these antigens are present in very low concentration and antibodies against them are produced very slowly; therefore, antigen-antibody reaction does not occur (Application Box 16.2).
4. Moreover, antigens are removed by phagocytic cells.

**Chemical Nature**

The α and β agglutinins are globulins and belong to Ig M category. Therefore, they do not cross the placenta. They function efficiently at low temperature, i.e. between 5° to 20°C. Therefore, they are called cold antibodies.

**Agglutinins Against Blood Groups**

Antibodies are directed against the antigens:

1. The individuals with blood group A antigen on the red cell membrane do not have anti-A antibody, rather possess anti-B antibody in their plasma.
2. Persons with blood group B do not contain anti-B, rather have anti-A antibody in their plasma (Table 16.2).
3. Individuals with blood group AB do not have any antibody.
4. Individuals with blood group O have both the anti-A and anti-B antibodies in their plasma.

These facts were first noted and described by Karl Landsteiner in 1900, and his theory is popularly known as Landsteiner’s law.

**Landsteiner’s Law**

This law states that if a particular agglutinogen is present on the red cell membrane of an individual, the corresponding agglutinin must be absent in his plasma. Conversely, if the agglutinogen is absent in the red cells, the corresponding agglutinin must be present in the plasma. This law holds good for ABO system. However, the second part of the law does not apply for Rh and many other blood group systems as there are no naturally occurring agglutinins in these systems.

**Antigen and Antibodies for ABO Blood Groups**

Thus, in ABO system, blood group A has antigen A on the red cell membrane and anti-B antibody in the plasma, blood group B has antigen B on the red cell membrane and anti-A antibody in the plasma, blood group AB has both antigens A and B on the red cell membrane and no antibodies in the plasma, and blood group O has no antigens on the red cell membrane and possesses both agglutinins anti-A and anti-B in the plasma (Table 16.2).

**Blood Grouping**

Blood grouping is performed by suspending the red cells of the person’s blood group to be checked in the anti-A and anti-B antisera that are commercially available. If the agglutination (clumping of red cells, as checked under the microscope) is present, red cells contain that particular antigen (Fig. 16.2)

**Inheritance of ABO Blood Groups**

Blood groups are genetically determined. Normally, presence of a blood group antigen is a dominant characteristic. Therefore, the antigen is present in the phenotype irrespective of the genotype (Table 16.3).
There are three types of genes: O, A and B. Therefore, a person can have any of the six possible genotypes: OO, OA, OB, AA, BB, and AB. Gene O is genetically not important. Therefore, the respective phenotype may be A or B or AB or O (Clinical Box 16.1). For example, if blood group of one parent is A and other parent is B, then there is possibility of child having any of the four blood group of ABO system (Fig. 16.3).

Clinical Box 16.1

Disputed paternity: The knowledge of inheritance of blood group is useful to solve the cases of disputed paternity. When the blood group of a child and the mother is known, it can be told whether the man in suspicion could have been the father of the child or not. However, blood group can never prove that a particular man is the father of a particular child. This is because the blood group of the man can be one out of several other possible men. Therefore, determination of blood group gene only helps to identify, not confirms the identity. MNS system is more useful in this regard.

Rh System

The Rh (Rhesus) system is the second most important blood group system in transfusion medicine. This was first described in Rhesus monkeys in 1940 by Landsteiner and Weiner; hence it is called Rh system (for Rhesus). First, it was discovered that injection of red cells of Rhesus monkey into rabbit results in development of antibody against rhesus red cells, later it was found that rabbit serum containing anti-Rhesus antibody agglutinate human red cells. This led to the discovery of Rh system in humans.

Rh Antigens

In Rh system, there are six antigens, but there are no naturally occurring antibodies. The antigens are C, D, E, c, d and e. Out of these six antigens, immunologically most active is the D antigen:
1. Thus, in Rh system, there are two blood groups: Rh positive (D antigen present) and Rh negative (D antigen absent).
2. The gene for Rh antigen is the RHD and RHCE genes that are located on chromosome 1.
3. The antigens of Rh system participate in cation transport and membrane integrity of red cells.
4. Rh antigens are membrane proteins found in red cell membrane, not in any other tissues.

Inheritance

Rh antigen is inherited as dominant gene:
1. In Indian population, 95 to 98% are Rh +ve and 2 to 5% are Rh –ve.
2. Rh +ve individual may have homozygous (DD) or heterozygous (Dd) genotypes. Usually, 60% of Rh +ve individuals have Dd genotype and 40% have DD genotype. The genotype of Rh –ve person is dd.
3. If one of the parents is homozygous positive and the other is homozygous negative, all offsprings will be heterozygous positives (Fig. 16.4).
4. Similarly, if the father and mother are homozygous negatives, all offsprings will be homozygous negatives, and when one of the parents is heterozygous positive and the other homozygous negative, 50% of offsprings will be heterozygous positive and 50% will be homozygous negatives.

**Rh Antibody**

The antibody in the system is called *anti-D antibody*, which is produced only when an Rh negative individual receives the Rh positive blood:

1. These antibodies develop very slowly in the first encounter, but form rapidly following subsequent encounters. Rh antibody is of *Ig G type*, which can cross placental barrier.
2. Hence, if antibodies are present in mother’s blood, can be transferred to the fetus.
3. Rh antibody is best reactive at body temperature, and therefore, designated as *warm antibody*.

**Rh Incompatibility**

Rh incompatibility occurs when an Rh negative individual receives Rh positive blood:

1. Normally, when an Rh negative person receives Rh positive blood, there will be no immediate reactions as Rh negative individual does not normally has anti-Rh antibody.
2. However, the donor’s red cells induce an immune response in the recipient to synthesize anti-Rh antibodies, which takes about two to four months to reach a significant titer. However, by that time the donor’s red cells die their natural death within 120 days. The anti-Rh antibody cannot produce any harm to the recipient’s red cells because the Rh negative recipient’s red cells contain no Rh antigens.
3. But, if the same Rh negative person who has already received a Rh positive blood before, receives a second Rh positive transfusion later, the anti-Rh antibodies are synthesized in large amount immediately by the memory cells. This antibody reacts against the donor cells and causes reactions of mismatch transfusion.
4. Thus, the Rh negative individual can safely receive Rh positive blood once in life time.

The similar Rh incompatibility occurs in pregnancies when Rh negative mother bears Rh positive fetus, which leads to *erythroblastosis fetalis*. The first child does not suffer. However, subsequent pregnancies carry risk for the fetus. The disorder is called erythroblastosis fetalis.

**Erythroblastosis Fetalis**

**Etiopathogenesis**

This is a *hemolytic disease of the newborn* which occurs due to Rh incompatibility when an Rh negative mother carries Rh positive fetus during pregnancy:

1. Usually, no reaction occurs in the first pregnancy. However, at the time of delivery during placental separation, a small amount of fetal blood leaks into the maternal circulation. This induces formation of anti-Rh agglutinins in the mother.
2. Therefore, in subsequent pregnancies, the anti-Rh agglutinin from mother, which is predominantly IgG type crosses placenta to enter into fetal circulation and causes hemolysis.
3. In third and subsequent pregnancies, the degree of hemolysis becomes severe.

**Clinical Features**

The features are mainly due to hemolysis. Hemolysis leads to anemia, extramedullary hemopoiesis and neonatal hyperbilirubinemia. If the hemolysis is severe, the fetus may die *in utero* or if the fetus is born alive, he may have the following features:

1. **Anemia**: Anemia is proportionate to the degree of hemolysis.
2. **Hemolytic jaundice**: Occurs due to hemolysis. Serum bilirubin level may be more than 25 mg% in severe cases.
3. **Generalized edema**: Edema occurs in the whole body due to anemia and hypoproteinemia. This is called *hydrops fetalis*.
4. **Kernicterus**: This is a neurologic syndrome with major motor deficits that occurs due to the *deposition of bilirubin in the basal ganglia*. Hyperbilirubinemia occurs due to hemolysis. Basal ganglia have more affinity for bilirubin. However, bile pigments cannot cross the blood-brain barrier (BBB) in adults. Therefore, *hemolysis in adults does not produce kernicterus*. As BBB is not fully developed in fetuses, infants and children, in them bilirubin enters brain and gets deposited in the basal ganglia. Therefore, *hemolysis in infancy and early childhood causes kernicterus*. The dysfunctions mainly manifests in the form of motor dysfunctions.
5. **Extramedullary hemopoiesis**: Due to severe anemia, extramedullary hemopoiesis occurs, for which *erythroblasts* (nucleated red cells) are released into the blood. Erythroblasts are seen in plenty in peripheral smear. Hence, the disease is called erythroblastosis fetalis.
Treatment
About 50% of the fetuses and newborns with erythroblastosis fetalis have mild hemolysis and do not require treatment. In severe cases, the major modalities of treatment are intrauterine fetal transfusion, exchange transfusion and phototherapy:
1. **Intrauterine fetal transfusion:** If the disease is diagnosed in fetus and found to be severe, the treatment is intrauterine fetal transfusion. Presently, the fetal transfusion is carried out by intraperitoneal route, which has replaced the direct intravascular fetal transfusion.
2. **Exchange transfusion:** The treatment of newborns with severe anemia, jaundice and hydrops is exchange transfusion soon after birth. Exchange transfusion removes sensitized red cells, bilirubin and maternal antibody from the plasma. A double-volume exchange transfusion (2 x 80 ml/kg) replaces 90% of the infant’s blood volume with antigen negative red cells. Blood chosen for exchange should be ABO negative, Rh negative and cross-matched against mother’s blood.
3. **Phototherapy:** Intensive phototherapy is very effective in reducing serum bilirubin level.

Clinical Box 16.2

**Phototherapy for neonatal jaundice:** Bilirubin in excess is toxic to the body, especially it produces neurotoxicity. However, on exposure to light bilirubin becomes nontoxic, which is the physiological basis of phototherapy. Bilirubin, on exposure to light undergoes structural and configurational isomerization and photo-oxidation. These changes in bilirubin make it less toxic and less lipophilic products that are excreted efficiently without hepatic conjugation. Therefore, phototherapy is the primary treatment for unconjugated hyperbilirubinemia in newborns. Neonatal jaundice is mainly hemolytic and unconjugated.

Prevention
Erythroblastosis fetalis is prevented by administering a single dose of anti-Rh antibodies in the form of Rh immunoglobulin during the postpartum period following the first delivery. The disease can also be prevented by passive immunization of the mother with a small dose of Rh immunoglobulins during pregnancy.

Other Blood Group Systems

**MNS System**
The MNS system of blood group was first described in 1927 (MN in 1927; S in 1947). This system has three antigens: M, N and S. The antigens are expressed in red cells and endothelial cells of renal capillaries. The MNS antigens are major contributor for negative charges on the red cell membrane. The common phenotypes are M, N, MN and S. This system is helpful for solving the paternity dispute. MNS groups are also useful for anthropological and genetic studies.

**H System**
In the H system, the common antigen is the H antigen. The expression of H antigen depends on the expression of ABO groups, especially O. The blood group O having H antigen in plasma or saliva is called OH group or Oh (if H is not dominant). The gene for H antigen is the FUT1 (H), located on the chromosome 19. The H antigen is present in plasma, most body secretions like saliva, sweat and semen, tissues and epithelial cells. Accordingly they are called secretors or nonsecretors.

**Lewis System**
The Lewis system was described in 1946. This system has two antigens: Le^a^ and Le^b^. These are not truly red cell antigens as they are produced in the plasma and then are absorbed into the red cells. Antigens are also present in body secretions and lymph. The gene for antigen Le^a^ is FUT3(Le) gene located in chromosome 19. The antigens of Lewis system serve as receptor for Helicobacter Pylori. The antibodies are of the IgM type. They do not cross the placental barrier and therefore, do not cause hemolytic disease of the newborn.

**Ii System**
Ii system was first described in 1956. There are two antigens in the Ii system, the ‘i’ and the ‘i’ antigens. The antigens are present in plasma, red cells, lymph and secretions. The Ii system differs from other blood groups in several ways:
1. At birth the I antigen is poorly developed, but red cells of fetus and neonate are rich in the i antigens.
2. There is gradual changeover from i to I antigen in the first two years of life.
3. In conditions like hemoglobinopathies, red cells show increased i antigens without any decrease in I antigens.

**PI System**
The PI system or also called P system was first described in 1927. There are three antigens in this system: P, P and P^k^.

**F Duffy System**
This system was described in 1950. There are three blood group antigens in this system: the Fya, Fyb, and Fyb. A close relationship between Duffy blood group and malaria has been well established. The Fyb blood groups is resistant to Plasmodium Vivax, whereas the Fya and Fyb are susceptible to parasitic infections.
vivax malaria. This is because, Fya and Fyb antigens serve as receptors for plasmodium if present separately on the red cell membrane, and thus increase their entry into the red cells.

Kidd System
This system was described in 1951. There are three antigens in this system: the Jk\(^a\), Jk\(^b\) and Jk\(^3\). The genes for these antigens are Jk (HUT11) genes that are located on chromosome 18. The antigens are specifically present on the red cell membrane (and in no other tissues). They are responsible for delayed hemolytic transfusion reactions.

Kell System
The Kell system was described in 1946. In this system, the common antigens are: K, k, Kp\(^a\) and Kp\(^b\). There are two sets of genes for these antigens: KEL and XK. The KEL genes are located on chromosome 7 and are expressed in red cells, bone marrow and fetal liver. The XK genes are located on X chromosome and are expressed in red cells, skeletal and cardiac muscles and neural tissues. People having K+ve blood group (containing K antigen on the red cell membrane) are susceptible to chronic granulomatous diseases.

Lutheran System
The Lutheran system was described in 1951. There are three blood group antigens in this system: the Lu\(^a\), Lu\(^b\) and Lu\(^3\). The genes for these antigens are LU genes that are located on chromosome 19. The antigens are distributed in red cells, brain, heart, kidney, lung, pancreas, placenta and skeletal muscles. In addition to antibody recognition, they play possible role in cell adhesion, receptor function and intercellular signaling.

Blood Group-associated Diseases
There are diseases that are prevalent in different blood groups. There are diseases that occur in the absence or alteration of blood groups (Table 16.4).

<table>
<thead>
<tr>
<th>Table 16.4: Blood group-associated diseases.</th>
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<tbody>
<tr>
<td><strong>A. Diseases due to occurrence blood groups</strong></td>
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<tr>
<td>Group A</td>
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<td>B</td>
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<tr>
<td>O</td>
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<tr>
<td>Le</td>
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<td>Fya/b</td>
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<td>K</td>
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<tr>
<td>Rh–ve</td>
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<td>ii</td>
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<tr>
<td><strong>B. Blood group associated with disease resistance</strong></td>
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<tr>
<td>P/<strong>P</strong></td>
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<tr>
<td>Fya<strong>b</strong></td>
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<tr>
<td><strong>C. Absence of antigen associated with diseases</strong></td>
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<tr>
<td>Rh<strong>null</strong></td>
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<tr>
<td>Kh</td>
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<tr>
<td><strong>D. Diseases associated with alteration of antigens</strong></td>
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<td>Weak AB</td>
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<td>Weak Rh, K</td>
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<tr>
<td>Weak MN</td>
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<td>Acquired A</td>
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<td>Acquired B</td>
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<td>Acquired K</td>
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<td>Acquired Jk(^b)</td>
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**PHYSIOLOGICAL BASIS OF BLOOD TRANSFUSION**
Transfusion of whole blood or a component of blood is common in medical practice. The common is the transfusion of whole blood or red cell concentrates that are required for the treatment of acute hemorrhage or severe anemia. However, one should be careful for the possibility of transmissions of infectious diseases like HIV and hepatitis, and other hazards of transfusions, when transfusion is given frequently.

**Indications for Transfusion**
1. **Acute blood loss:**
   - Whole blood is preferred.
2. **Chronic anemia:**
   - Packed red cells are preferred to whole blood.
3. **Bone marrow failure:**
   - Leukemia
   - Aplastic anemia
   - Bone marrow infiltration by neoplastic cells.
4. **Purpura:**
   - Platelet transfusion is preferred.
5. **Clotting factor deficiencies:**
   - Fresh frozen plasma is preferred. In hemophilia, cryoprecipitate (rich in factor VIII and fibrinogen) is given.
6. **Preparation for surgery and during surgery:**
   - If the patient is anemic, blood is transfused prior to surgery. During major surgeries, blood transfusion is routinely administered. Loss of 500 ml of blood is usually tolerated during surgery in a patient with normal Hb prior to surgery. If blood loss is expected to be more than this, transfusion is always planned.
7. **Burns:**
   - Plasma rich in protein is lost from the burn surface. This is corrected with plasma and colloids. If anemia is severe, packed red cells are transfused.
8. **Anemia:**
   - Usually, blood transfusion is not required in chronic stable anemia. In hemoglobinopathies like thalassemia and sickle cell anemia, blood transfusion is
the cornerstone of the treatment. Repeated and multiple transfusion in thalassemia and sickle cell disease not only aim to improve Hb content, but also to inhibit erythropoiesis so that defective cells are not produced. However, transfusional hemochromatosis limits usefulness of this treatment.

Procedure of Blood Transfusion

The first step in any transfusion is the identification of a healthy and appropriate donor. Blood grouping for Rh and ABO types of both recipient and the donor, and cross-matching of blood of both recipient and the donor are the next important steps in all blood transfusions to ensure safety and compatibility. In mismatch transfusion, agglutination (clumping) of red cells occurs in recipient’s blood leading to acute hemolysis, which may be life threatening. Therefore, a suitable transfusion should always include:

1. Selection of appropriate donor
2. ABO and Rh typing
3. Cross matching
4. Antibody screening of the patient (to detect the presence of clinically significant antibodies).

Selection of Appropriate Donor

Healthy donors free from transfusion-transmissible diseases should be identified for blood transfusion:

1. Ideally, the age of the donor should be 18 years or above and should not be above 60 years. Donor should be voluntarily willing to donate blood.
2. The hemoglobin status of the donor should be estimated. There should be minimum of 12 g% Hb in female, and 13 g% Hb in male donors.
3. Donor must be screened for AIDS, hepatitis, lymphomas, and protozoal organisms like malaria, filaria, etc.

ABO and Rh Typing

The two major blood group systems for transfusion are ABO and Rh systems. ABO incompatibility causes immediate reactions and may even kill the persons. The Rh incompatibility causes delayed reactions. Therefore, ABO and Rh compatibility must be ensured before any transfusion. The concept of universal donor and recipient helps in this process.

Universal Donor

The blood group O negative (O group and Rh –Ve) persons are considered to be the universal donor because their red cells contain no antigens. Therefore, their blood cells on transfusion to any individual do not cause agglutination reaction. Hence, blood group O –Ve can be given to any individual (Clinical Box 16.3).

Universal Recipient

Individuals having blood group AB positive (AB group and Rh +Ve) are considered to be the universal recipient because their plasma contains no antibodies. Therefore, they can safely receive blood from any one as they do not cause agglutination reaction against any blood cells.

Clinical Box 16.3

Cross-matching is a must before transfusion: Though technically the concept of universal recipient is true, transfusion of blood based on this concept alone may not provide full assurance of compatibility always as there are many other minor blood group systems. Therefore, prior to blood transfusion cross-matching should always be done to eliminate the possibilities of any form of mismatching. However, in an emergency condition, this concept may be used in selecting the donor, if the blood groups of donor and recipient are known without waiting for the report of cross-matching.

Cross-matching

There are two types of cross-matching: major and minor.

Major Cross-matching

In major cross-matching, the cells of the donor are directly matched against the plasma of the recipient:

1. As it is important to ensure that antibodies present in the recipient’s plasma do not harm the donor’s red cells, this cross-matching is called major cross-matching.
2. The ABO agglutinogens are highly antigenic and produce agglutination even in low concentration. Therefore, though donor’s red cells are less in quantity than the recipient, they produce agglutination reactions.
3. ABO agglutinins do not react highly, as they are cold antibodies. Moreover, donor’s agglutinin is diluted in large volume of recipient’s plasma; hence not capable of inducing agglutination reaction.
4. Therefore, it is important to match donor’s agglutinin (red cells) against recipient’s agglutinin (plasma).

Minor Cross-matching

In minor cross-matching, the donor’s plasma is checked against the red cells of the recipient:

1. It is called minor cross-matching, because it is not very important to assess the reactivity of donor’s agglutinin against recipient’s cells.
2. This is because; small volume of the donor’s plasma is diluted in a large volume of the recipient’s plasma.
3. Therefore, the agglutinin titer of donor’s plasma falls to such a low level after transfusion that they are unlikely to damage the red cells of the recipient.
4. However, for full compatibility, minor cross-matching should also be performed.

Antibody Screening of the Patient

The patient who receives transfusion, his serum should be screened for various antibodies like autoantibodies that
may react against the donor’s cells and cause hemolysis. Therefore, it is ideal to do the antibody screening of the patient.

**Blood Collection and Storage**

**Collection of Blood**

Venipuncture is done to collect blood:

1. Ideally, not more than 350 ml of blood is collected from a single donor at a time.
2. From antecubital vein, blood flows freely into a collecting bottle or bag that contains anticoagulant.
3. The usual collection time is 7–10 min.
4. The blood mixes with 50 to 70 ml of anticoagulant consisting of citrate, phosphate and dextrose (CPD). *Disodium hydrogen citrate* is used instead of trisodium citrate as anticoagulant, because this favors fall of pH which is required for survival of red cells.
5. BP and heart rate are monitored after collection, especially before they get up from the supine posture.
6. Normally, some degree of diaphoresis, bradycardia, hypertension and dizziness accompany this bleeding.
7. Donors are advised not to go for work for rest of the day and should also avoid exercise.
8. They are also advised not to donate blood for at least next three months.

**Autologous Donor Blood**

When the donor is the recipient himself, the blood collected is called autologous donor blood and the transfusion is called autologous transfusion:

1. Autologous transfusion was not practiced before. However, AIDS epidemic has increased the necessity and importance of autologous transfusion in transfusion medicine.
2. This can be done prior to a planned surgery.
3. The Hb of the person should be more than 12.5 g%.
4. Blood is collected every 72 hours up to 72 hours prior to surgery or till the Hb level has not gone below 11 g%.
5. Usually 2 to 4 units of blood of 250 ml each can be collected. Then, the same blood (recipient’s own blood) is used during surgery.

**Storage of Blood**

Blood is stored in the blood bank at 4°C. Stored blood for transfusion should ideally be used within 2 weeks of storage. However, blood should not be used if it is stored for more than 3 weeks, because gross hemolysis occurs after this period.

**Red Cell Changes during Storage**

Red cells undergo rapid changes during storage in simple citrate solutions even at 4° C. During cold storage, the changes that occur are mainly due to reduction of metabolism of cells. The changes are:

1. Increase in sodium and decrease in potassium concentration in the red cells. This occurs due to decreased active transport of ions across the cell membrane. At low temperature, Na⁺-K⁺ pump activity is grossly decreased. This results in net increase in the total base and water of the cell. The inhibition of Na⁺-K⁺ pump increases intracellular sodium concentration that causes water to enter the cell by endosmosis.
2. Cells swell and become more spherocytic. This results in spontaneous hemolysis. Leucocytes and platelets almost totally disappear within 24 to 48 hours of storage.
3. The ATP content in the cell decreases and inorganic phosphate concentration increases. This is due to imbalance between phosphorylation and dephosphorylation processes in the cell.

**Changes in Stored Blood after Transfusion**

1. Within 24 hours of transfusion, the cell metabolism greatly increases. Consequently, the sodium is extruded from the cells and the potassium is drawn back into the cells.
2. The volume, shape, and fragility of the red cells come back to normal within 24 to 48 hours.
3. Red cells show 80% of survival 24 hours after transfusion if the transfusion is given within 14 days of storage of the blood. But the survival rate greatly decreases if the blood is stored for more than 2 weeks.
4. Therefore, it is advisable to use blood ideally within 14 days of storage, though blood can be used up to 21 days.
5. Blood should never be used after 30 days of storage.

**Hazards of Blood Transfusion**

Blood transfusion, even in ideal condition carries risks of adverse reactions. Mostly, transfusion reactions occur due to human error:

1. **Due to Mismatched Transfusion:** When an incompatible blood is transfused, the mismatched transfusion reaction occurs immediately. The reaction is primarily due to agglutination of donor’s red cells followed by their hemolysis. This is called **acute hemolytic transfusion reactions.** Usually it occurs due to ABO incompatibility. The severity of the reaction depends on the degree of hemolysis. The complications of mismatched transfusion are:
   i. Shivering and fever (febrile reactions) occur usually
   2. Hemoglobinemia and *hemoglobinuria*
   3. Hemolytic jaundice
   4. **Acute renal failure.**

Renal failure occurs due to:

i. hemoglobin casts blocking the renal tubules and damaging the tubules,
ii. release of toxic substances from the lysed red cells cause renal vasoconstriction, and
iii. circulatory shock.
5. **Hyperkalemia** (due to release of potassium ions from red cells). This may cause cardiac arrest in diastole.

II. **Due to Faulty Techniques of Giving Blood**: Due to wrong method of transfusion, following complications may arise:

1. **Thrombophlebitis**: This is a common complication in those who receive repeated transfusions.
2. **Air Embolism**: Air enters the venous circulation and gets lodged at the outlet of the right ventricle and blocks the flow of blood to the lungs. Death may occur in severe cases. Use of plastic bags has reduced this complication.

III. **Due to Massive Transfusion**: This occurs when more than 10 units of blood are given within 24 hours or when the total blood volume is exchanged within 24 hours. This leads to **circulatory overload**. Cardiac arrhythmias and even sometimes cardiac arrest occur due to high potassium level in the stored blood.

IV. **Febrile Reaction**: The patient feels cold and may get rigor due to rise in body temperature. This occurs mainly due to presence of pyrogens in the transfusion apparatus.

V. **Allergic Reactions**: This is less frequent and is characterized by itching, erythema, nausea, vomiting, and in severe cases may cause anaphylactic reactions.

VI. **Transmission of Diseases**:

1. Hepatitis
2. Malaria
3. AIDS
4. Syphilis.

### CHAPTER SUMMARY

#### Key Concepts

1. Blood group of an individual depends on presence or absence of a specific blood group antigen on the surface of red cell (RBC membrane).
2. If a blood group antigen is present on the surface of red cell, the corresponding antibody must be absent in his plasma. However, if a blood group antigen is absent on the surface of red cell, the corresponding antibody may (or may not be) present in his plasma. This is called Landsteiner’s law.
3. In ABO system, the agglutinogens are highly antigenic. Therefore, for compatible blood transfusion, blood grouping and cross-matching must be done before the transfusion.
4. In major cross-matching, cells of the donor are matched against the plasma of the recipient.
5. Blood group AB positive is the universal recipient, and O negative is the universal donor.
6. For blood collection, disodium hydrogen citrate is the preferred anticoagulant, as it ensures low pH, which facilitates red cell survival.
7. Erythroblastosis fetalis occurs in Rh incompatibility when a mother with Rh negative blood group bears the fetus with positive blood group during pregnancy. It is treated by exchange transfusion and prevented by passive immunization with anti-D antibody.

#### Important to Know (Must Read)

1. In examinations, ‘Physiological basis of blood grouping of ABO system’ comes as a Long Question.
2. Landsteiner’s law, ABO agglutinogens, ABO agglutinins, inheritance of blood group, Rh incompatibility, erythroblastosis fetalis, cross matching, universal donor and recipient, changes in red cells following storage, and hazards of blood transfusion are usually asked as Short Questions in exams.
3. In Viva, examiners usually ask about types of blood groups, uses of blood groups, Landsteiner’s law, ABO agglutinogens, ABO agglutinins, H antigens, Bombay blood group, inheritance of blood group, Rh incompatibility, causes, features, treatment and prevention of erythroblastosis fetalis, types and importance cross matching, universal donor and recipient, method of blood collection, how to store blood in blood bank, changes in red cells following storage, and hazards of blood transfusion, and common diseases prevalent in various blood groups.
4. As blood groups have general and special interest in medicine, a student is expected to answer these questions.
White Blood Cells

**Learning Objectives**

On completion of study of this chapter, the student **MUST** be able to:

1. Classify leucocytes, and give the percentage of different leucocytes in blood.
2. Give the steps of leucopoiesis.
3. Give the general life history of leucocytes.
4. Describe the structure and functions of leucocytes, and list the common causes of decrease and increase of each type of leucocyte.
5. Explain the mechanism of phagocytosis and killing of organisms by neutrophil.
6. List the components of mononuclear-phagocyte system (MPS).
7. Understand the general role of leucocytes in defence mechanisms of the body.

The student **MAY** also be able to:

1. Describe the role of eosinophil in allergy.
2. Explain the role of MPS in defense of the body.
3. Briefly describe different types of leukemia.

**General Introduction**

Leucocytes or white blood cells perform the defense functions of the body. There are two series of leucocytes: **myeloid series** and **lymphoid series**. Each cell line has separate pathway of development from distinct primitive cells (Fig. 17.1):

1. The **myeloid series matures into granulocytes and monocytes**. The **granulocytes** are neutrophils, eosinophils and basophils.
2. The **lymphoid series matures into lymphocytes**.

Though monocytes and lymphocytes have some granules, they are not usually visible with commonly used stains. Therefore, monocytes and lymphocytes are traditionally classified under **agranulocytes** (Table 17.1). The leucocytes of granulocytic series develop from myeloblasts that are virtually agranular cells. During the process of development, the precursor cells synthesize proteins and store them in cytoplasmic granules. The enzymes of granules take part in killing organisms:

1. Granulocytes exhibit active motility and migrate to the site of inflammation by amoeboid movement to kill organisms by phagocytosis.
2. Neutrophils are highly amoeboid and phagocytic, and kill a variety of microorganisms, especially bacteria. Therefore, neutrophils are considered as **first line of defense** against acute bacterial infections.
3. Monocytes are also actively phagocytic and are considered as **second line of defense** against infections, especially in chronic and parasitic infections.
4. The eosinophils and basophils are specialized to participate in **allergic inflammatory responses**.
5. Lymphocytes are involved in **immunological responses**: **Life of leucocytes**: In general, leucocytes have **three phases** in their life: marrow phase, circulatory phase and tissue phase.

1. In the **marrow phase**, they develop from progenitor cells where they proliferate and grow into mature leucocytes and get released into circulation.
2. In the **circulatory phase**, they circulate for few hours before entering into tissues where they live a longer period of their life.
3. In the **tissue phase**, they enter the tissues. In the tissue, monocytes become macrophages that take part in nonspecific defenses of the body as a component of mononuclear phagocyte system.
Section 2: Blood and Immunity

LEUKOPOIESIS

Leukopoiesis is the development of leucocytes.

Stages of Leukopoiesis

The pluripotent stem cells in the bone marrow give rise to committed stem cells. The committed stem cells in the bone marrow are broadly of two types: The myeloid stem cells and lymphoid stem cells. The stem cells for lymphoid series form lymphocytes.

The stem cells for myeloid series are trilineage stem cells that form stem cells for three series of blood cells:

1. Erythroid series for red cells,
2. Megakaryocytic series for platelets and

Table 17.1: Types of leucocytes and their distribution in peripheral blood as DLC and absolute count.

<table>
<thead>
<tr>
<th>Leucocytes</th>
<th>Percentage (DLC)</th>
<th>Absolute count (per cu mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Granulocytes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Neutrophils</td>
<td>50–70</td>
<td>2000–2750</td>
</tr>
<tr>
<td>2. Eosinophils</td>
<td>1–4</td>
<td>40–440</td>
</tr>
<tr>
<td>3. Basophils</td>
<td>0–1</td>
<td>20–80</td>
</tr>
<tr>
<td><strong>B. Agranulocytes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Monocytes</td>
<td>2–8</td>
<td>1500–4000</td>
</tr>
<tr>
<td>2. Lymphocytes</td>
<td>20–40</td>
<td>500–800</td>
</tr>
</tbody>
</table>

Usually, life span is 6 to 10 days in the marrow phase, 6 to 8 hours in the circulatory phase and 4 to 5 days or more in tissue phase (Flowchart 17.1)
The stem cells for granulocyte-monocyte of myeloid series give rise to three categories of progenitor cells (colony forming units or CFUs): CFU-GM, CFU-Eo and CFU-Bas (Flowchart 17.2):

1. **CFU-GM** is the phagocytic series progenitor cell that on further differentiation from CFU-G (for neutrophils) and CFU-M (for monocytes).
2. The CFUs form blast cells.
3. CFU-G forms myeloblasts, which on further development form neutrophils.
4. CFU-M forms monoblast that develops into monocytes.
5. CFU-Eo forms eosinophil-myeloblast that develops into eosinophils.
6. CFU-Bas forms basophil-myeloblast that develops into basophils.

**Steps of Development**

The **myeloblasts** form promyelocytes. Promyelocytes form myelocyte, metamyelocytes that in turn develop into mature leucocytes (Fig. 17.2).

**Colony-forming Units**

Colony forming units (CFUs) are progenitor cells that develop from committed stem cells. There are different
CFUs for different cell lines (as described above). However, they possess the properties of stem cells and can not be distinguished morphologically.

**Blast Cells**

These are earliest precursor cells of leucocyte development that are **morphologically distinguishable**:
1. The blast cells for neutrophils are **myeloblasts**, for eosinophil are eosinophil-myeloblasts, and basophils are basophil-myeloblasts, for monocytes are **monoblasts** and for lymphocytes are **lymphoblasts**.
2. The blast cells are large cells (16–20 μm) with large nucleus containing multiple nucleoli.
3. The nucleoli are prominent features of blast cells and are the sites of assembly of ribosomal proteins and r-RNA.
4. The cytoplasm is scanty.
5. Cells are actively mitotic.

**Myeloblasts**: The earliest precursor cells for neutrophils are myeloblasts. They have scanty blue cytoplasm with 5–10 nucleoli in a large nucleus. There are separate myeloblasts for eosinophils (eosinophil-myeloblasts) and basophils (basophil-myeloblasts).

**Monoblasts**: These are the blast cells for monocytes. As they resemble myeloblasts morphologically, they are also called myelomonoblasts.

**Lymphoblasts**: These are blast cells for lymphocytes. They have fewer nucleoli. Nuclear chromatins are clumped.

**Promyelocytes and Promonocytes**

The promyelocytes and promonocytes develop from myeloblasts and monoblasts respectively:
1. The cells are also large and cytoplasm is granular.
2. Nucleus is round and condensed and contains less nucleoli.
3. Cells are mitotic.
4. **Promonocytes** are larger cells (larger than myeloblasts) that on further development form monocytes, the **largest cells** in peripheral blood.
5. The promyelocytes are also larger cells and contain large population of **peroxidase-positive granules**. The granules are large in size having diameter of about 500 nm.

**Neutrophilic Myelocytes**

Myelocytes have diameter of about 12–20 μm:
1. They contain round concentric nucleus. Nucleoli are absent.
2. Cells show some degree of mitosis. About three cell divisions occur in this stage of maturation.
3. The characteristic feature of this stage is appearance of **peroxidase negative specific granules**.
4. The granules are usually spherical (about 200 nm) or rod shaped.
5. The peroxidase positive granules are less than peroxidase negative granules. This is because, with each mitotic division, peroxidase positive granules are reduced whereas peroxidase negative granules continue to be formed.

**Metamyelocytes**

The diameter of metamyelocyte is 12–18 μm. The cells are nondividing (no mitosis) and have mixed granule populations.

**Neutrophils**

Neutrophils developed from metamyelocytes are **juvenile neutrophils** (band forms) that further grow into **matured neutrophils** (segmented neutrophils) (Fig. 17.2).

**Regulation of Leukopoiesis**

Leucopoiesis is mainly regulated by cytokines (for details of cytokines, refer ‘Immunity’), especially, colony stimulating factors (CSFs), interleukins and tumor necrosis factors (TNFs). T lymphocytes, monocytes, fibroblasts, endothelial cells, liver and kidney are the major sources of hemopoietic growth factors (HGFs) (Fig. 17.3). Types, sources and functions of HGFs are listed in Table 17.2.

**Interleukins**

IL-1, IL-6 and IL-3 promote maturation of stem cells. IL-5 helps in development of eosinophil, and therefore, IL-5 is also called eosinophilic growth factor. IL-3 and IL-4 facilitate development of basophils. IL-2 inhibits myelopoiesis.

**Colonystimulating Factors**

There are different colony stimulating factors (CSFs). The important CSFs that influence leucopoiesis are GM-CSF, G-CSF and M-CSF.

**GM-CSF**: This is the granulocyte-monocyte colony stimulating factor (GM-CSF), secreted by fibroblasts, vascular endothelial cells, monocytes and T lymphocytes. GM-CSF stimulates differentiation and proliferation of pluripotent stem cells into committed cells. GM-CSF also helps in differentiation of CFU into granulocyte and monocyte precursors and their further development into mature cells. It synergizes with IL4 to produce dendritic cells.

**G-CSF**: This is the granulocyte colony stimulating factor. It is produced by monocytes, endothelial cells and fibroblast. It helps in development and functions of granulocytes.

**M-CSF**: This is the monocyte colony stimulating factor. This is also called CSF-1. It is produced by fibroblasts, endothelial cells and macrophages. It promotes proliferation of monocytes and macrophages, and also stimulates functions of monocytes.

**Tumor Necrosis Factor**

Tumor necrosis factors (TNFs) help in proliferation and differentiation of stem cells.
Table 17.2: Major hematopoietic growth factors their sources and actions.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Growth factor</th>
<th>Sources</th>
<th>Actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Interleukin-1 (IL-1)</td>
<td>Activated macrophages</td>
<td>Mediates synthesis and release of acute phase proteins by liver cells, synthesis of other cytokines</td>
</tr>
<tr>
<td>2.</td>
<td>Interleukin-2 (IL-2)</td>
<td>T lymphocytes</td>
<td>Growth factor for activated T cells</td>
</tr>
<tr>
<td>3.</td>
<td>Interleukin-3 (IL-3)</td>
<td>T lymphocytes</td>
<td>Growth factor for hematopoietic stem cells</td>
</tr>
<tr>
<td>4.</td>
<td>Interleukin-6 (IL-6)</td>
<td>T lymphocytes/macrophages</td>
<td>Growth factor for B and T lymphocytes mediates acute phase response</td>
</tr>
<tr>
<td>5.</td>
<td>C kit ligand (CSF)</td>
<td></td>
<td>Acts with other growth factors to stimulate pluripotent stem cells</td>
</tr>
<tr>
<td>6.</td>
<td>GM-CSF</td>
<td>T cells, fibroblasts, endothelial cells</td>
<td>Multilineage growth factor for factor for neutrophils, monocytes/ macrophages,</td>
</tr>
<tr>
<td>7.</td>
<td>G-CSF</td>
<td>Monocytes/macrophages, fibroblasts</td>
<td>Lineage restricted growth factor for neutrophils</td>
</tr>
<tr>
<td>8.</td>
<td>M-CSF</td>
<td>Monocytes/macrophages, fibroblasts, endothelial cells</td>
<td>Lineage restricted growth factor for monocytes and macrophages</td>
</tr>
<tr>
<td>9.</td>
<td>Erythropoietin</td>
<td>Kidneys and liver</td>
<td>Lineage restricted growth factor for erythrocytes</td>
</tr>
<tr>
<td>10.</td>
<td>Thrombopoietin</td>
<td>Kidneys and liver</td>
<td>Lineage restricted growth factor for platelets</td>
</tr>
</tbody>
</table>

Life History of Leukocytes

Leukocytes have mainly three phases in their life: The marrow phase, the circulation phase and the tissue phase (Fig. 17.4).

Marrow Phase

This is the phase of development in bone marrow, hence also called development phase. Marrow phase has two pools: mitotic pool and maturation pool.

Mitotic Pool

The development from myeloblasts to myelocytes is the phase of mitotic pool, as cells undergo mitosis in these stages.

Maturation Pool

This is the phase of maturation of metamyelocytes into mature cells. Cells do not undergo mitosis in these stages.

Duration of Leucopoiesis: From myeloblast to matured leukocytes, the process of development usually takes 10 days. About 5 days are spent in the mitotic pool (development up to myelocytes) and another five days are utilized in the maturation pool (development from metamyelocyte to the mature cells).

Circulation Phase

The matured cells are released into circulation and remain in circulation for few hours before they enter the tissues (circulation pool):
Chapter 17: White Blood Cells

1. At rest, many leucocytes especially neutrophils, adhere to the endothelial lining of blood vessels, which is called **margination pool** of leucocytes (Application Box 17.1).

2. In addition, leucocytes actually circulate in the blood (the **active circulation pool**).

**Application Box 17.1**

*Disruption of margination causes leucocytosis:* Leucocytes adhere to inner lining of blood vessel, called margination. In exercise and other conditions of increased hemodynamics, the leucocytosis occurs mainly due to disruption of margination of leucocytes.

**Tissue Phase**

After their activities in circulation, leucocytes enter the tissues. This is called **tissue pool** of leucocytes. Granulocytes live for few days in the tissues (Application Box 17.2), whereas monocytes remain for a longer period. In the tissues, monocytes are transformed into **tissue macrophages** that remain for many years.

**Application Box 17.2**

*Apoptosis of leucocytes:* Apoptosis is the programmed cell death. This is a physiologic phenomenon of elimination of mature cells from the body. Biochemically, apoptosis occurs due to internucleosomal DNA fragmentation, and morphologically, by nuclear and cytoplasmic condensation. The apoptotic cells are phagocytosed by macrophages. Senescent neutrophils and eosinophils undergo apoptosis.

---

**NEUTROPHILS**

Neutrophils are most prevalent leucocytes in blood. They provide the **major defense against acute pyogenic infections**. Neutrophils exhibit ameboid movements. In infections, they migrate to the site of microbial invasion in response to chemical factors. They ingest organisms and kill them. Thus, in neutropenia, body is vulnerable to bacterial infections.

**Structure**

Neutrophils are common granulocytes. The major identifying features of neutrophils are:

1. The average size of neutrophils varies between 10–14 μm.
2. The cytoplasm of neutrophils contains **fine pink colored granules**.
3. The nucleus is usually **multilobed** and the lobes are connected by thin strand (Fig. 17.5). Nucleus may contain **Barr body** (Application Box 17.3).

It is not known why the segmentation of nucleus of neutrophil occurs. The nucleus of **juvenile neutrophil** is single lobed (**band form**), whereas in mature neutrophils the number of lobes increases with age.

**Arneth count:** The oldest neutrophils may have 6 or 7 lobes in their nucleus. Based on the nuclear lobes
neutrophils are classified as N₁ to N₆/N₇. This is called Arneth count (Figs. 17.6A to F).

1. Normally, N₂ and N₃ constitute most of the neutrophils in peripheral blood.
2. Presence of younger neutrophils (mostly N₁ and N₂) indicates stimulation of bone marrow (shift to left).
3. Presence of older cells (mostly N₄ and N₅) indicates suppression of bone marrow (shift to right).
4. Hypersegmented nucleus of neutrophils is typically seen in megaloblastic anemia that occurs due to folate and vitamin B₁₂ deficiency (Figs. 17.7A and B).

**Granules of Neutrophils**

Neutrophils have four types of granules: primary or azurophilic granules, secondary or specific granules, tertiary granules and secretory granules. Primary granules are formed during granulopoiesis, whereas other granules are formed at later stages. The granular contents are determined by the time of their appearance in the leucopoiesis.

**Primary Granules**

These are peroxidase positive granules. They are also called azurophilic granules:

1. In addition to myeloperoxidase, they contain lysosomal enzymes and elastase, proteinase and α-1 antitrypsin.
2. They also contain antimicrobial proteins like cathepsin-G, defensins and bactericidal-permeability increasing proteins.
3. Contents of azurophil granules also cause tissue destruction during inflammation.

**Secondary Granules**

By definition, secondary granules do not contain peroxidase (peroxidase negative):

1. They are also called specific granules.
2. The secondary granules contain lactoferrin, gelatinase, lysozyme, vitamin B₁₂ binding protein and other proteins.
3. Lysozyme is microbicidal and lactoferrin is antibacterial.
4. About 16% granules contain only lactoferrin, 24% contain only gelatinase and 60% contain both.
5. In inflammatory responses, gelatinase containing granules are more readily released than the other granules.
6. The number of specific granules may be decreased in different conditions. The granules may disappear and nuclear lobes may become big giving spectacular appearance in Pelger-Huet anomaly. Morphologically, abnormalities of specific granules are Alder-Reilly anomaly and May-Hegglin anomaly (Fig. 17.8).

**Tertiary Granules**

Tertiary granules contain gelatinase, alkaline phosphatase and cytchrome-b. The alkaline phosphatase is located on the luminal side of the granule membrane. The low leucocyte alkaline phosphatase score is associated with chronic myeloid leukemia.

**Secretory Granules**

These are secretory vesicles and different from azurophilic and specific granules. They contain CD3, phospholipase and tyrosine kinase. Toxic granulations occur in severe infections (Clinical Box 17.2).
Figs. 17.7A and B: Hypersegmented neutrophil in megaloblastic anemia (in the inset), as shown in original peripheral blood smear (A) and drawn picture of the smear (B).


<table>
<thead>
<tr>
<th></th>
<th>Mode of inheritance</th>
<th>Characteristic morphology</th>
<th>Appearance of leukocyte</th>
<th>Functional abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pelger-Hütet</td>
<td>Autosomal dominant</td>
<td>Lack of neutrophil nuclear segmentation beyond 2 lobes</td>
<td>Nil</td>
<td></td>
</tr>
<tr>
<td>anomaly</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alder-Reilly</td>
<td>Autosomal recessive</td>
<td>Large lilac inclusions in cytoplasm of all leukocytes.</td>
<td>Nil</td>
<td></td>
</tr>
<tr>
<td>anomaly</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>May-Hegglin</td>
<td>Autosomal dominant</td>
<td>Large basophilic inclusions in all leukocytes. Giant platelets and thrombocytopenia</td>
<td>Abnormal bleeding due to thrombocytopenia</td>
<td></td>
</tr>
<tr>
<td>anomaly</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chediak-Higashi</td>
<td>Autosomal recessive</td>
<td>Large gray blue granules in cytoplasm of monocytes and granulocytes. Defective lysosomal granules</td>
<td>Poor chemotaxis. Increased susceptibility to pyogenic infections. Bleeding tendency</td>
<td></td>
</tr>
<tr>
<td>anomaly</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic granulomatous disease of childhood (CGD)</td>
<td>X-linked Autosomal recessive</td>
<td>Normal appearance but defective function</td>
<td>Deficient NADPH oxidase, with absent H₂O₂ production. Phagocytosis and killing of organisms is impaired</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 17.8: Qualitative dysfunctions of neutrophils.

Courtesy: Figure 19.3, Essentials in Hematology and Clinical Pathology by Ramadas Nayak et al., 1st edition, 2012; Jaypee Brothers Medical Publishers (P) Ltd.
Toxic granulations of neutrophils: In severe infections, toxic granulations and Döhle bodies are seen in neutrophils in addition to nuclear pyknosis.

**Life History**

Neutrophils like other leucocytes have four stages in their life: marrow pool, circulation pool, margination pool and tissue pool.

**Marrow Pool**

This is the developmental stage of neutrophil. As soon as neutrophils are developed from metamyelocytes, they are released into circulation. However, large number of juvenile neutrophils are present in bone marrow, which constitutes marrow pool of the cell. This serves as the reservoir for peripheral neutrophils.

**Circulation Pool**

In peripheral blood, about 50% of neutrophils are present in circulation pool that actually circulate in the blood. Rest, 50% remains in the margination pool.

**Margination Pool**

About 50% of the neutrophils in the blood remain adhered (marginated) to endothelial lining of the blood vessels. This is called margination of leucocytes. Neutrophils present in the margination pool serve as the immediate source for circulation pool. In fact, any factor that causes disruption of margination increases neutrophil count and causes acute leucocytosis.

**Tissue Pool**

After their usual life in circulation for about 6–8 hours, neutrophils enter tissues where they live for about 4 days.

**Neutrophil Count**

Normally, neutrophils constitute 50–70% of the total leucocytes in the peripheral blood:

1. The neutrophil count in the blood is maintained due to the balance between neutrophilopoiesis that releases neutrophils into circulation and the shift of neutrophils into marginated and tissue pools.
2. In circulation, many neutrophils adhere to endothelium called margination.
3. Margination is due to firm attachment of neutrophils to endothelial lining, which is mediated by selectins, polypeptides that contain sugar binding site.
4. Temporary release of neutrophils from marginated pool also accounts for increase in count of neutrophils.
5. Neutrophil count alters in various conditions (Table 17.3).

**Table 17.3: Conditions that alter neutrophil count.**

<table>
<thead>
<tr>
<th>Neutrophilia</th>
<th>Neutropenia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Acute neutrophilia</strong></td>
<td><strong>A. Decreased neutrophil production</strong></td>
</tr>
<tr>
<td>1. Physical stimuli</td>
<td>1. Congenital, e. g. Kostmann syndrome</td>
</tr>
<tr>
<td>Exercise, cold, pain, labor, surgery</td>
<td>2. Infections: Typhoid and paratyphoid fevers</td>
</tr>
<tr>
<td>2. Emotional stimuli</td>
<td>3. Drugs: Chloramphenicol, phenylbutazone, phenytoin</td>
</tr>
<tr>
<td>Panic, severe stress, depression</td>
<td>4. Aplastic anemia</td>
</tr>
<tr>
<td>3. Infections</td>
<td><strong>B. Chronic neutrophilia</strong></td>
</tr>
<tr>
<td>Acute bacterial, mycotic and rickettsial infections</td>
<td>1. Inflammation Pancreatitis, myositis, colitis, rheumatoid arthritis</td>
</tr>
<tr>
<td>4. Inflammation or tissue necrosis</td>
<td>2. Endocrine disorder ACTH excess, thyroid storm</td>
</tr>
<tr>
<td>Burn, infarction, trauma, electric shock, gout</td>
<td>3. Tumors Gastric, renal, bronchial and hepatic tumors</td>
</tr>
<tr>
<td>5. Drugs Epinephrine, glucocorticoids, vaccines</td>
<td>4. Blood diseases Chronic hemolysis, meyloproliferative diseases</td>
</tr>
</tbody>
</table>

**Functions**

Neutrophil is actively phagocytic. They contain many antimicrobial and bactericidal chemicals in their granules (see above). Therefore, neutrophils are capable of ingesting and killing microbial organisms. During inflammation due to acute bacterial infections, neutrophils soon migrate to the site of infection and kill the organisms. Hence, neutrophils are considered as the first line of defense against acute bacterial infections. Neutrophils provide major non-specific defense against invasion of pyogenic organisms. Consequently, neutropenia predisposes body to pyogenic infection.

**Neutrophil Phagocytosis**

Phagocytosis is the process of ingestion and killing of microbes or a foreign substance by a phagocyte. Actively phagocytic cells are neutrophils, monocytes and macrophages. Steps of phagocytosis include chemotaxis, diape-desis, adherence, ingestion and killing (Fig. 17.9).
Chemotaxis

Chemotaxis is the process of migration of neutrophils to the site of infection. The bacterial invasion triggers acute inflammatory response:

1. The chemical substances are released from the site of inflammation or infection by the infecting organisms or inflammatory cells.
2. These chemical factors attract neutrophils to the site of infection. Consequently, they are called chemotaxins (taxin means movement; chemical that produces movement is a chemotaxin) or chemoattractants or chemotactic factors.

3. Chemotaxins are usually the microbial products or chemicals secreted from leucocytes or chemicals released from damaged tissue.
4. Complement proteins, especially C5a and C3 also act as chemotaxins.
5. During chemotaxis, neutrophils change their shape and become highly ameboid.
6. Also, the bone marrow is stimulated in response to plasma factors and more neutrophils are produced.

Diapedesis

The neutrophils must enter the tissue from their circulation pool to kill organisms at the site of inflammation in the tissue. The process, by which neutrophils pass through the capillary endothelial cells to reach the invader in the tissue, is called diapedesis. The activated neutrophils first marginate (margination and pavementing) adhere tightly to endothelial lining (rolling and adhesion) with the help of L-selectins and then by their ameboid movement they squeeze through the space between endothelial cells (emigration and diapedesis) (Figs. 17.10A to D).

Opsonization and Adherence

The process by which the bacteria are made tasty to the phagocyte is called opsonization. In this process, antigen is coated by opsonins. The chemicals that facilitate the process of opsonization are called opsonins:

1. IgG antibody and complement proteins (C5a, C3b) are known high-quality opsonins.
2. Bacteria coated by opsonins bind to the receptor on the neutrophil membrane. The attachment of membrane of phagocyte to the membrane of microbe is called adherence.
3. Opsonization facilitates the process of adherence (Fig. 17.11A).

**Ingestion (Endocytosis)**
Adherence facilitates motor activity of neutrophils. The membrane of phagocyte extends projections from both the sides to encroach on to the microbe (Fig. 17.11B). These extensions are called pseudopodia. Pseudopodia finally surround the microbe and form phagocytic vesicle (Fig. 17.11C). The phagocytic vesicle fuses with the lysosome to form **phagolysosome** (Fig. 17.11D).

**Killing**
The bactericidal (killing of bacteria) mechanisms can broadly be divided into two types: nonoxidative and oxidative.

**Nonoxidative Mechanisms**
Neutrophil granules contain a wide variety of **antibacterial chemicals** such as degradative enzymes, proteases, defensins and cationic proteins:
1. Lysozyme that hydrolyzes the cell wall of bacteria and lactoferrin that sequesters iron (iron is required for bacterial growth) are nonoxidative components of bacterial killing.
2. Defensins (α and β defensins) released from azurophil granules have unusual cyclic structure and kill bacteria by disrupting their outer membrane and breaking single-strand DNA structure.

**Oxidative Mechanisms**
Activated neutrophils produce a number of oxygen metabolites that are antimicrobial.
1. The metabolites are superoxide anion (O$_2^-$), H$_2$O$_2$, free hydroxyl radicals (OH·), hypochlorous acid (HOCl) and singlet oxygen (¹O$_2$). These reactive metabolites are generated by a nicotinamide adenine dinucleotide phosphate (NADPH)-dependent oxidase that reduces molecular oxygen to O$_2^-$. The oxidase is quiescent in resting neutrophils and is stimulated following neutrophil activation and promotes O$_2^-$ formation. O$_2^-$ is the most effective oxidant metabolite.
2. Activation of NADPH oxidase is associated with increased oxygen intake of neutrophil (Clinical Box 17.3). This is called **respiratory burst**. This leads to generation of O$_2^-$ by following mechanism:

   \[ \text{NADPH} + \text{H}^+ + 2\text{O}_2 \rightarrow \text{NADP}^+ + 2\text{H}^+ + 2\text{O}_2^- \]

   Two superoxide anions (O$_2^-$) react with two H$^+$ to form H$_2$O$_2$ by the action of **superoxide dismutase** (SOD) (Application Box 17.4). Both O$_2^-$ and H$_2$O$_2$ are active oxidants and are effective bactericidal agents. H$_2$O$_2$ is converted to H$_2$O and O$_2$ by the enzyme **catalase**. Hence, catalase is considered as an **antioxidant**.
3. Myeloperoxidase, the enzyme of primary granules facilitate conversion of Cl$^-$ to HOCl, which is also a potent oxidant.

**Clinical Box 17.3**
CGD occurs due to defective NADPH-oxidase: In chronic granulomatous disease (CGD), a genetic disorder, neutrophils fail to generate O$_2^-$ and related metabolites. Neutrophils and monocytes ingest catalase positive microorganisms but can not kill them due to lack of adequate active oxidants that result from decreased NADPH-oxidase activity. This leads to formation of chronic granulomas, the abnormal inflammatory tissue reactions.

**Application Box 17.4**
ALS occurs due to defective dismutase: Amyotrophic lateral sclerosis (ALS) is a motor system disease in which progressive degeneration of spinal motor neurons results in atrophy of skeletal muscles (amyotrophy). O$_2^-$ reacts with H$^+$ to form H$_2$O$_2$ with the help of cytoplasmic dismutase and H$_2$O$_2$ is converted to H$_2$O and O$_2$ by the enzymes catalase. Defective dismutase results in accumulation of O$_2^-$ in the motor neurons that damages the neurons. In ALS, genetic mutation of dismutase results in oxidative damage to the motor neurons in the spinal cord, which is progressively fatal.

**Interaction of Oxidative and Nonoxidative Mechanisms**
Nonoxidative mechanism facilitates the activity of oxidative mechanism for bacterial killing. The protease enzymes...
of primary granules and collagenase enzymes of secondary granules produce a killing zone around the activated neutrophil by locally damaging the inflammatory tissue, which helps the oxidant metabolites to promote their bactericidal properties. However, in diseases like rheumatoid arthritis, this becomes detrimental due to destruction of larger quantity of host tissue.

**Applied Aspects**

Chemotaxis and phagocytosis require active movements of neutrophil. When neutrophil is activated, its cytosolic calcium concentration increases that promotes contraction of microfilaments, microtubules and myosin-1 filaments. This increases the ameboid movement and activity of neutrophils. Neutrophil hypomotility, a primary phagocytic dysfunction of neutrophil, decreases phagocytic activity. There are about 15 primary disorders of neutrophil function.

**Eosinophils**

Eosinophils are known for their protective function against allergy including asthma, and helminthic parasite infections. Like neutrophils, eosinophils produce proinflammatory mediators:

1. Eosinophil-specific granule proteins are toxic for many mammalian cells and parasitic larvae.
2. In addition, cytokines produced by eosinophil such as leukotrienes and PAF, aid to their defense functions.
3. Eosinophils have short life span in circulation, whereas they live longer in tissue. They are primarily tissue dwelling cells. There are 100 eosinophils in tissue to 1 eosinophil in peripheral blood.
4. Eosinophils are present in the epithelia of respiratory, gastrointestinal and genitourinary tract.

**Table 17.4: Eosinophil-derived chemicals.**

<table>
<thead>
<tr>
<th>A. Granule derived chemicals</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Major basic protein</td>
<td></td>
</tr>
<tr>
<td>2. Eosinophil cationic protein</td>
<td></td>
</tr>
<tr>
<td>3. Eosinophil peroxidase</td>
<td></td>
</tr>
<tr>
<td>4. Eosinophil-derived neurotoxin</td>
<td></td>
</tr>
<tr>
<td>5. Lysophospholipase</td>
<td></td>
</tr>
<tr>
<td>6. Phospholipase D</td>
<td></td>
</tr>
<tr>
<td>7. Arylsulphatase</td>
<td></td>
</tr>
<tr>
<td>8. Acid phosphatase</td>
<td></td>
</tr>
<tr>
<td>9. Catalase</td>
<td></td>
</tr>
<tr>
<td>10. Histaminase</td>
<td></td>
</tr>
<tr>
<td>11. Hexoseaminidase</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B. Cytokines (may or may not be stored in granules)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. GM-CSF</td>
<td></td>
</tr>
<tr>
<td>2. TGF-α and TGF-β</td>
<td></td>
</tr>
<tr>
<td>3. Macrophage inhibition factor (MIF)</td>
<td></td>
</tr>
<tr>
<td>4. IL-1α, IL-1β and IL-12</td>
<td></td>
</tr>
<tr>
<td>5. Tumor necrosis factor (TNF-α)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>C. Lipid-derived chemicals</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Leukotriene C</td>
<td></td>
</tr>
<tr>
<td>2. Platelet activating factor</td>
<td></td>
</tr>
<tr>
<td>3. PGE₁ and PGE₂</td>
<td></td>
</tr>
<tr>
<td>4. Thromboxane B₂</td>
<td></td>
</tr>
</tbody>
</table>

5. Their production and function are mainly influenced by IL-5. IL-5 also stimulates production and function of basophils that are closely related to eosinophils.

**Structure**

Eosinophils are granular leucocytes having the size same as that of neutrophils. However, the granules are coarse and brick red in color in blood smear stained by Leishman stain. Moreover, the nucleus is usually bilobed and the lobes are separated by a thick strand. This gives eosinophil nucleus an appearance of a pair of spectacles (Fig. 17.12). The granules are plenty, and sometimes encroach upon the nucleus.

**Chemicals Secreted by Eosinophils**

Eosinophil secretes various chemicals that mediate many physiological activities. These chemicals may be divided into three categories: chemicals secreted from granules, cytokines, and lipid-derived chemicals (Table 17.4). The last two groups of chemicals are secreted from many other cells, whereas chemicals secreted from granules are eosinophil specific.

**Eosinophil Granular Contents**

The granules of eosinophil contain following major chemicals:
1. Major basic protein
2. Eosinophil cationic protein
3. Eosinophil-derived peroxidase
4. Eosinophil-derived neurotoxin
5. Cytokines
6. Other chemicals

**Major Basic Protein**

Major basic protein (MBP) is major toxic product of eosinophil. It is toxic against many intestinal parasites and their larvae such as:
1. Schistosomula (a larva of *S. mansoni*, which is an intestinal parasite, blood fluke) and the larva of *Ancylostoma duodenale* (hookworm)
2. *Ascaris lumbricoid* (roundworm)
3. *Toxocara canis*
4. *Wuchereria bancrofti* (filarial larva)
5. *Trichinella spiralis*

Eosinophil adheres with the IgG coated larva and this adherence results in secretion of MBP from eosinophil into the integument of the larva that causes damage to the larval tissue.
- MBP is also toxic to pneumocytes and epithelial cells of respiratory tract.
- MBP also makes the bronchial tree hyper-responsive to bronchoconstrictors.
- Therefore, these worm infestations are usually associated with respiratory symptoms like bronchial asthma (Clinical Box 17.4).
- Eosinophil peroxidase (EP) and MBP activate platelets, basophils and mast cells.
- Basophils also contain MBP, but the concentration is only 2% of eosinophils.

**Clinical Box 17.4**

**Asthma like symptoms occurs in worm infestations:** MBP released from eosinophil granules to kill worms, is also toxic to lungs. MBP makes the bronchial tree hyper-reactive to bronchoconstrictors. Therefore, these worm infestations are usually associated with respiratory symptoms like bronchial asthma.

**Eosinophil Cationic Protein**

Eosinophil cationic protein (ECP) is rich in arginine.
- Though ECP has 66% structural homology with eosinophil-derived neurotoxin (EDN) and 31% homology with pancreatic ribonuclease, its ribonuclease activity is less compared to the ribonuclease activity of EDN.
- ECP is toxic to helminthic parasites and tracheal epithelium.
- ECP along with EDN produces neurotoxicity.

**Eosinophil Peroxidase**

Eosinophil peroxidase (EP) is a heme containing protein.
- It has 68% homology with neutrophil myeloperoxidase and other peroxidases.
- EP is toxic to the adult parasites and pulmonary tissue.
- When combined with H₂O₂, its toxicity increases.

**Eosinophil-derived Neurotoxin**

Eosinophil-derived neurotoxin (EDN) is a glycosylated protein having striking ribonuclease activity. EDN is also secreted by mononuclear cells. It is toxic to the neural tissue. It is not toxic against nonneural tissue of parasites.

**Cytokines**

Cytokines are synthesized outside the granules but stored in granules. The important cytokines secreted from eosinophils are:
1. Interleukins (IL1-6, IL8 and IL12)
2. GM-CSF
3. Macrophage inhibition factor (MIF)
4. Transforming growth factors (TGF α and β)
5. Tumor necrosis factor-α.

TGF-α mediates the important role of eosinophil in wound healing. Many of the cytokines like interleukins such as IL5, IL3 and GM-CSF act in autocrine fashion as eosinophil growth factor. MIF plays a role in the genesis of adult respiratory distress syndrome.

**Other Chemicals**

Lysophospholipase constitutes 10% of eosinophil proteins. The enzymes secreted from eosinophils like phospholipase D, arylsulphatase-B, acid phosphatase, catalase and histaminase are other granular proteins that take part in eosinophil-mediated inflammatory reactions and killing of parasites.

**Functions**

Like neutrophils, eosinophils migrate into the tissues. There are selective chemoattractants for eosinophils such as eosinophil chemotactic factor of anaphylaxis (ECF-A). ECF-A in humans is a tetrapeptide that facilitates tissue accumulation of eosinophils. Eosinophils exhibit endothelial cell adhesion and chemotaxis to migrate into the tissues. Eosinophils participate in two important defense mechanisms of the body:
1. Against helminthic infections
2. Against allergy.

**In Helminthic Infections**

Eosinophils attack intestinal parasites especially the larva of *S. mansoni*, *Trichinella spiralis*, *Toxocara canis*, *N. brasiliensis*, *Fasciola hepatica*, roundworms and hook worms:
1. They attack the larva opsonized by IgG, IgE and complements. First, eosinophils attach themselves to the larva, which activates the eosinophils.
2. Within about 3 hours of this adherence, the activated eosinophils secrete proteins from their granules onto surface of the larva.
3. Once, the tegument is breached, eosinophils crawl under it and secrete toxic mediators that destroy the tissues of larva and phagocytose the larva or larval fragments.

4. In this process, degranulation of eosinophils occurs.

In Allergy

In allergic condition like bronchial asthma, eosinophils play a major role in pathogenesis:

1. Bronchial hyper-reactivity is correlated with eosinophilia and airway eosinophil content.
2. Inhibition of airway eosinophilia by glucocorticoid has been reported to cause improvement in bronchial hyper-responsiveness and asthmatic symptoms.

3. Though, glucocorticoid causes eosinopenia by eosinophil apoptosis, the main mechanism is the inhibition of production of IL5 and IL3 from type 2 helper cells (T4 lymphocytes).

4. It is not clear whether eosinophils prevent allergy and therefore eosinophilia occurs in allergy or eosinophilia in allergy aggravates the situation.

5. However, recruitment of eosinophil at the site of allergic inflammation is accompanied by increase in number of activated T cells and monocytes.

6. It has been proposed that asthma is an eosinophil-mediated disease driven by type-2 helper lymphocytes that secrete IL5 and IL3.

Alterations in Eosinophil Count

The normal eosinophil count is 2–4% in differential count (by examining blood smear), or 40–440 per μl of blood in absolute count (by hemocytometry). When eosinophil count is more than normal, called eosinophilia and less than normal, called eosinopenia (Table 17.5).

**Table 17.5: Causes of eosinophilia and eosinopenia.**

<table>
<thead>
<tr>
<th><strong>A. Eosinophilia</strong></th>
<th><strong>B. Eosinopenia</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. Helminthic infections</strong></td>
<td><strong>1. Glucocorticoid therapy</strong></td>
</tr>
<tr>
<td>- Ascariasis (roundworm)</td>
<td><strong>2. Cushing’s syndrome</strong></td>
</tr>
<tr>
<td>- Filariasis</td>
<td><strong>3. Aplastic anemia</strong></td>
</tr>
<tr>
<td>- Toxocariasis</td>
<td><strong>4. Drug-induced agranulocytosis</strong></td>
</tr>
<tr>
<td>- Ancylostomiasis (hookworm)</td>
<td></td>
</tr>
<tr>
<td>- Strongyloidiasis</td>
<td></td>
</tr>
<tr>
<td>- Trichinosis</td>
<td></td>
</tr>
<tr>
<td>- Schistosomiasis</td>
<td></td>
</tr>
<tr>
<td>- Fascioliasis</td>
<td></td>
</tr>
<tr>
<td>- Echinococcosis</td>
<td><strong>2. Allergic diseases</strong></td>
</tr>
<tr>
<td></td>
<td>- Bronchial asthma</td>
</tr>
<tr>
<td></td>
<td>- Allergic rhinitis</td>
</tr>
<tr>
<td></td>
<td>- Atopic dermatitis</td>
</tr>
<tr>
<td></td>
<td>- Urticaria</td>
</tr>
<tr>
<td></td>
<td>- Food allergy</td>
</tr>
<tr>
<td></td>
<td>- Hay fever</td>
</tr>
</tbody>
</table>

| **C. Drug reactions (drug allergy)** | **D. Eosinophilic leukemia** |
| **2. Allergic diseases** | **5. Tropical pulmonary eosinophilia** |
| | **6. Addison’s disease** |
| | **7. Eosinophilia-myalgia syndrome** |

<table>
<thead>
<tr>
<th><strong>B. Eosinopenia</strong></th>
<th><strong>B. Eosinopenia</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. Glucocorticoid therapy</strong></td>
<td><strong>2. Cushing’s syndrome</strong></td>
</tr>
<tr>
<td><strong>3. Aplastic anemia</strong></td>
<td><strong>4. Drug-induced agranulocytosis</strong></td>
</tr>
</tbody>
</table>

**Table 17.6: Differences between basophils and mast cells.**

<table>
<thead>
<tr>
<th><strong>Basophils</strong></th>
<th><strong>Mast cells</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Origin</strong></td>
<td>Bone marrow</td>
</tr>
<tr>
<td><strong>Cells in blood</strong></td>
<td>Present</td>
</tr>
<tr>
<td><strong>Normal residence in connective tissue</strong></td>
<td>No</td>
</tr>
<tr>
<td><strong>Life span</strong></td>
<td>Few days</td>
</tr>
<tr>
<td><strong>Growth factor</strong></td>
<td>IL-3</td>
</tr>
<tr>
<td><strong>Major secretion</strong></td>
<td>Histamine, IL-4, heparin</td>
</tr>
<tr>
<td><strong>Receptors for</strong></td>
<td>IgE, IgG</td>
</tr>
</tbody>
</table>

**Basophils and Mast Cells**

Basophils are least frequent granulocytes. They account for less than 0.5% of leucocytes in blood. Though they recruit into tissues in response to immunological and inflammatory reactions, unlike eosinophils they ordinarily do not reside in the tissue. Though basophils and mast cells resemble functionally, they are not identical (Table 17.6):

1. Mast cells are derived from blood precursors, but they mainly reside in the connective tissues, particularly beneath the epithelial surfaces and around the blood vessels, where they live a longer period.
2. Basophils and mast cells have high-affinity receptors for IgE on their surface.
3. As IgE is a reagin antibody, these cells mediate many allergic responses of the body.
4. Basophils and mast cells also contribute to protect host responses associated with IgE production.

**Structure**

**Basophils**

Identifying features of basophils are (Fig. 17.13):

1. They have the same diameter as of neutrophils (10–14 μm).
2. The nucleus is usually less segmented and often appear ‘S’ shaped.
3. Nuclear chromatin shows marked condensation.
4. The granules are large in size and oval or round in shape, and more in number. As cell is heavily studded with granules, nucleus is often not visible. Granules are surrounded by membranes and contain dense particles called Charcot-Lyden crystals.

5. Cytoplasm contains glycogen deposits, mitochondria, free-ribosomes and few lipid bodies. Basophil granules secrete histamine, chondroitin sulphate, tryptase, carboxypeptidase A, cathepsin G, leukotrienes, eosinophil chemotactic factor of anaphylaxis (ECF-A), neural protease and MBP.

Mast Cells

Mast cells that remain in the tissues are round or elongated cells with nonsegmented nucleus. Granules are numerous, but smaller in size. There are many cytoplasmic filaments, numerous lipid bodies and no glycogen deposits. Mast cell granules secrete histamine, heparin, chondroitin sulphate, carboxypeptidase, cathepsin, ECF-A and neural protease. There are two types of mast cells: mucosal mast cells (mast cell present in the mucosa) and connective tissue mast cell.

Differences between Basophils and Mast Cells

Though there are functional homology between basophils and mast cells, there are many differences between them (Table 17.6).

Functions

Basophils and mast cells are mainly involved in allergic reactions. During allergy, these cells release the content of their granules. Mediators such as histamine released by degranulation produce antimicrobial and anti-host effects. The usual stimulus for basophil and mast cell degranulation is an allergen, which should ideally cross-link IgE molecule bound to the surface of basophils or mast cells via its high affinity Fc receptor for IgE.

Role in Acute Allergic Reactions

Basophils and mast cells have receptors for Fc region of IgE:

1. IgE binds to membrane of these cells and initiate degranulation. This leads to release of histamine. Acute allergies such as acute rhinitis, urticaria, food allergy etc are mediated by this mechanism.

2. However, massive release of histamine produces immediate hypersensitivity reactions, also known as anaphylaxis. Anaphylaxis is acute systemic allergic reaction that occurs in conditions like injections of penicillin or xylocaine anesthesia in sensitive individuals.

3. In anaphylaxis, histamine released from basophils and mast cells cause vasodilation and inhibition of cardiac output that lead to acute hypotension.

Role in Chronic Allergic Reactions

Basophils and mast cells also contribute to late-phase reactions. In chronic allergic conditions such as bronchial asthma, basophils and mast cells are recruited to the site of allergy. Especially, mast cells secrete cytokines that attract leucocytes, eosinophils and basophils, which in turn mediate chronic allergy. This mechanism of pathogenesis of late-phase reaction is termed as mast cell-leucocyte cascade.

Role in T-cell Dependent Responses

Activation of mast cells in the affected tissues along with infiltration of basophils occurs in a variety of T-cell dependent immunological responses. T-cells secrete growth factors for basophils and mast cells.

Role in Host Defense

Basophils and mast cells play critical role in host resistance to some viral, bacterial and parasitic infections. These cells increase in conditions like chicken pox, small pox and tuberculosis.

Normal Count

Normal basophil count is 0–1%, and absolute count is 20–80 per μl of blood. Mast cells are normally not found in blood. Increase in basophil count is called basophilia and decrease in count is called basopenia (Table 17.7).

Mastocytosis

Secondary increase in mast cell count usually occurs in allergic conditions like asthma, and connective tissue disorders like rheumatoid arthritis. However, primary increase in mast cell number occurs in a group of systemic disorders called systemic mastocytosis. Depending on the degree of mastocytosis, the condition has been classified into 4 categories:

- Category I: Indolent mastocytosis as seen in urticaria pigmentosa.
- Category II: Mastocytosis with myeloproliferative disorder.
- Category III: Aggressive mastocytosis i.e., lymphadenopathic mastocytosis with eosinophilia.
- Category IV: Mast cell leukemia.
MONOCYTES AND MACROPHAGES

Monocyte is the largest leucocyte in the peripheral blood. After spending life in blood, monocytes enter the tissues, where they are transformed into macrophages:
1. Monocytes and macrophages are mononuclear phagocytes.
2. In the tissues, they play an important role in nonspecific defense against microbial invasion.
3. The tissue macrophage system was previously called as reticuloendothelial system (the term has become obsolete). Presently, this is known as mononuclear phagocyte system.
4. Monocyte is the second line of defense against microbial infections.

Monocytes

Morphology

Monocytes are the largest blood cells. The identifying features are:
1. The diameter of monocytes varies between 12–25 μm.
2. The nucleus occupies half of the cell and remains eccentrically. Cytoplasmic-nuclear ratio is 50:50.
3. Often the nucleus is reniform (kidney shaped), but may be horse-shoe shaped, round or irregular (Fig. 17.14).
4. There are characteristic fine nuclear chromatin net connecting small chromatin clumps. This gives the nucleus a non-homogenous or stringy appearance.
5. Cytoplasm is abundant and ground glass in appearance.
6. Though monocyte is traditionally classified as agranular leucocytes, fine pink-purple granules are present in about 40% of cells. Sometimes, large azurophil granules are also seen in monocytes. The monocyte granules contain hydrolytic enzymes such as acid phosphatase, lysozymes, etc. However, alkaline phosphatase is absent.

Monocytes have a number of receptors on its surface such as Fc receptors for IgG, IgA and IgE, receptors for complements, cytokines, and hormones like insulin, glucocorticoid and angiotensin. Monocytes and macrophages express HLA class II and their receptors on their surface that help in antigen presentation.

Functions

1. Phagocytosis and microbial killing: Monocyte is an active phagocyte:
   - It exhibits motility and chemotaxis.
   - The presence of various surface receptors enhances their phagocytic activity by facilitating the recognition of various host-derived factors including immunoglobulins, complements, cytokines, and hormones like insulin, glucocorticoid and angiotensin.
   - The receptors also identify various sugar units on microbial membranes.
   - The organisms once phagocytosed, are destroyed by oxidants produced by NADPH oxidase, nitric oxide synthase, and intracellular hydrolytic enzymes.
   - Monocyte also kills intracellular pathogens like viruses, and parasites.
   - Monocyte is the second line of defense against infections.

2. Antigen presentation: Monocyte is an important antigen presenting cell (APC):

### Table 17.7: Alteration in basophil count.

<table>
<thead>
<tr>
<th>A. Basophilia</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Allergic and inflammatory conditions</td>
</tr>
<tr>
<td>- Ulcerative colitis</td>
</tr>
<tr>
<td>- Erythroderma</td>
</tr>
<tr>
<td>- Urticaria</td>
</tr>
<tr>
<td>- Drug and food hypersensitivity</td>
</tr>
<tr>
<td>2. Infections</td>
</tr>
<tr>
<td>- Chicken pox</td>
</tr>
<tr>
<td>- Small pox</td>
</tr>
<tr>
<td>- Influenza</td>
</tr>
<tr>
<td>- Tuberculosis</td>
</tr>
<tr>
<td>3. Endocrinal disorders</td>
</tr>
<tr>
<td>- Myxedema (hypothyroidism)</td>
</tr>
<tr>
<td>- Diabetes mellitus</td>
</tr>
<tr>
<td>4. Iron deficiency</td>
</tr>
<tr>
<td>5. Basophilic leukemia</td>
</tr>
<tr>
<td>6. Polycytemia</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B. Basopenia</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Cortisol therapy</td>
</tr>
<tr>
<td>2. Cushing's syndrome</td>
</tr>
<tr>
<td>3. Hyperthyroidism</td>
</tr>
<tr>
<td>4. Ovulation</td>
</tr>
<tr>
<td>5. Hypersensitivity reactions</td>
</tr>
</tbody>
</table>

**Fig. 17.14:** Structure of monocyte.
Partially digested product of the antigen (Ag) combines with the MHC II molecules produced by APC, and MHC + Ag complex expresses on the surface of APC.

Lymphocytes, especially T cells are activated when they come in contact with MHC + Ag present on the APC surface.

This is the first step in the activation of cellular immunity.

3. Release of cytokines: Monocyte secretes various chemokines that carry out different physiological functions:
- Monocyte produces IL-1 and IL-6 that are essential for coactivation of immunological responses.
- Monocyte secretes TNF-α and interferons that facilitate killing of viruses and other microbial organisms.
- Monocyte also secretes various growth factors like GM-CSF, M-CSF and erythroid colony potentiating factor that promote leucopoiesis and erythropoiesis, and transforming growth factors (TGF), PDGF and fibroblast growth factors (FGF).
- Monocytes by secreting complement factors augment the local tissue defense responses.
- Monocyte releases various enzymes like collagenase, elastase, plasminogen activator, etc. that participate in wound healing and tissue remodeling.

Table 17.8: Causes of alteration in monocyte count.

<table>
<thead>
<tr>
<th>A. Monocytopenia</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Aplastic anemia</td>
</tr>
<tr>
<td>2. Hairy cell leukemia</td>
</tr>
<tr>
<td>3. Septicemia</td>
</tr>
<tr>
<td>B. Monocytosis</td>
</tr>
<tr>
<td>1. Acute monocytic leukemia</td>
</tr>
<tr>
<td>2. Chronic myelomonocytic leukemia</td>
</tr>
<tr>
<td>3. Hodgkin's disease</td>
</tr>
<tr>
<td>4. Polycythemia vera</td>
</tr>
<tr>
<td>5. Hemolytic anemia</td>
</tr>
<tr>
<td>6. Postsplenectomy state</td>
</tr>
<tr>
<td>7. Cytomegalovirus infection</td>
</tr>
<tr>
<td>8. Collagen diseases</td>
</tr>
<tr>
<td>9. Malaria</td>
</tr>
<tr>
<td>10. Kala azar</td>
</tr>
<tr>
<td>11. Glucocorticoid therapy</td>
</tr>
<tr>
<td>12. Chronic idiopathic monocytosis</td>
</tr>
</tbody>
</table>

Table 17.9: Distribution of cells of mononuclear phagocyte system.

<table>
<thead>
<tr>
<th>A. In blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>– Monocytes</td>
</tr>
<tr>
<td>B. In bone marrow</td>
</tr>
<tr>
<td>– Monoblasts</td>
</tr>
<tr>
<td>– Promonocytes</td>
</tr>
<tr>
<td>C. In tissues</td>
</tr>
<tr>
<td>– Kupffer cell in liver</td>
</tr>
<tr>
<td>– Osteoclasts in bone marrow</td>
</tr>
<tr>
<td>– Alveolar macrophages in lungs</td>
</tr>
<tr>
<td>– Histiocytes in connective tissue</td>
</tr>
<tr>
<td>– Microglia in brain</td>
</tr>
<tr>
<td>– Red pulp macrophages in spleen</td>
</tr>
<tr>
<td>– Macrophages in lymph nodes and thymus</td>
</tr>
<tr>
<td>– Messangial cells in kidney</td>
</tr>
<tr>
<td>– Dendritic cells/histiocytes in skin</td>
</tr>
<tr>
<td>– Type A cells in synovium</td>
</tr>
<tr>
<td>D. In body cavities</td>
</tr>
<tr>
<td>– Pleural macrophages</td>
</tr>
<tr>
<td>– Peritoneal macrophages</td>
</tr>
<tr>
<td>E. In inflammatory tissues</td>
</tr>
<tr>
<td>– Epitheloid cells</td>
</tr>
<tr>
<td>– Multinucleate giant cells</td>
</tr>
</tbody>
</table>

Life Span

Monocytes in circulation have a wide range of half life between 10–72 hours. Then, they enter the tissues where they live few weeks to months. The average life in tissues is three months. In tissue, they are transformed into tissue macrophages. If there is tissue infection or inflammation, within few hours monocytes migrate to the site of injury. However, initially the monocyte numbers are less than neutrophils. If inflammation persists for more than 12 hours, monocytes predominate over neutrophils.

Normal Count

Normal monocyte count is 2–8%, and the average absolute count is 400/μl of blood. Increase in count is called monocytosis and decrease in count is called monocytopenia (Table 17.8). The monocyte count is more in neonates and infants, which is about 1000/μl of blood. Men tend to have slightly higher monocyte count than women. However, in adults count more than 800/μl of blood is considered as monocytosis.

Macrophages

Monocytes after their life span in blood enter tissues and transform into tissue macrophages. They form mononuclear phagocyte system in various tissues (Table 17.9) (Also, refer Fig. 19.2, Chapter 19):

1. Macrophages are capable of cell division and resident or noninflammatory macrophages in the tissue are self-sustaining.
2. The exact mechanism of differentiation of monocyte to macrophages is not known. However, on becoming macrophage there is increase in cell size, number of cytoplasmic granules and vacuoles, and increase in heterogeneity of the cell shape.
3. The average diameter of macrophages varies from 25 to 50 μm.
4. The nucleus is fusiform or reniform and is eccentrically placed with one or two nucleoli in it.
5. Cytoplasm contains multiple large azurophil granules.
6. They contain all the surface receptors that are present in monocytes.
7. In chronic tissue inflammations, macrophages are converted into multinucleated giant cells that are highly phagocytic and microbicidal.

**Dendritic Cells**

Few monocytes are transformed into highly specialized mononuclear cells called dendritic cells:
1. They play important role in antigen processing and presentation to the T cells.
2. They are specialized in antigen capture, rather than in phagocytosis. Thus, they are specific antigen presenting cells.
3. However, unlike macrophages, dendritic cells lack receptors for immunoglobulins, complements and colony-stimulating factors, and specific granules in cytoplasm. Therefore, they are weakly phagocytic.
4. They are present in blood and bone marrow, where they account for about 0.1 to 1% of total mononuclear cells.
5. They are also present as Langerhans cells in skin, interdigitating cells in thymic medulla, and interstitial cells in the lung and heart.

**LYMPHOCYTES**

Lymphocytes are a heterogeneous group of cells with characteristic morphology. Lymphocyte is the only leucocyte that has different varieties of cells both morphologically and functionally. Structurally, lymphocytes are divided into two groups: small and large lymphocytes.

**Structure**

**Small Lymphocytes**

Small lymphocytes are same in size to that of red cells. They constitute 35% (20–50%) of total lymphocytes. The identifying features are:
1. The cells are 6–9 μm in diameter.
2. They have ovoid or kidney shaped nucleus with densely packed nuclear chromatin. Nucleus is usually eccentrically placed and occupies about 90% of the cell area (Fig. 17.15A).
3. There is a thin rim of bluish cytoplasm that does not contain granules.

**Large Lymphocytes**

Large lymphocytes constitute 65% (50–80%) of total lymphocytes. The identifying features are:
1. The cells are 10–15 μm in diameter (Fig. 17.15B).
2. The nucleus is homogenous and compact with dense nuclear chromatins. Nucleus is usually oval or kidney shaped and eccentrically placed.
3. The cytoplasm is navy blue in colour and usually does not contain granules. However, about 3% of large lymphocytes contain coarse pink granules (5 to 15 granules per cell). These granular lymphocytes are usually NK or T cells.

**Normal Count and Functions**

Normal lymphocyte count is 20–40% of total leucocytes. The absolute count is 500–8000 per cu mm of blood. Increase in count is called lymphocytosis and decrease in count is called lymphocytopenia (Table 17.10).

Functionally, lymphocytes are divided into three categories: B cells, T cells and NK cells. B cells on stimulation are transformed into plasma cells that secrete antibodies. B cells mediate humoral or antibody-mediated immunity. T cells mediate cellular or cell-mediated immunity. NK cells mediate natural and nonspecific immunity. Details of lymphocyte functions are discussed in the chapter “Immunity”.

**Summary of Blood Cells**

To summarize, blood cells are formed elements of blood. Erythrocytes are anuclear cells having diameter of 7 to 8 μm that are primarily meant for transport of oxygen from lungs to the tissues. Leucocytes are of various sizes and they mainly play role in defence mechanisms. Platelets are anuclear and smaller cells (cellular fragments) that principally participate in homeostasis (Table 17.11).
**Table 17.10:** Causes of lymphocytosis and lymphocytopenia.

<table>
<thead>
<tr>
<th>A. Lymphocytosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Primary lymphocytosis</td>
</tr>
<tr>
<td>– Acute lymphocytic leukemia</td>
</tr>
<tr>
<td>– Chronic lymphocytic leukemia</td>
</tr>
<tr>
<td>– Adult T-cell leukemia</td>
</tr>
<tr>
<td>– NK-cell leukemia</td>
</tr>
<tr>
<td>– Monoclonal B-cell lymphocytosis</td>
</tr>
<tr>
<td>2. Reactive lymphocytosis</td>
</tr>
<tr>
<td>– Infectious mononucleosis</td>
</tr>
<tr>
<td>– Bordetella pertussis</td>
</tr>
<tr>
<td>– Tuberculosis</td>
</tr>
<tr>
<td>– Postplenectomy</td>
</tr>
<tr>
<td>– Cigarette smoking</td>
</tr>
<tr>
<td>– Septic shock</td>
</tr>
<tr>
<td>– Drugs</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B. Lymphocytopenia</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Acquired lymphocytopenia</td>
</tr>
<tr>
<td>– Aplastic anemia</td>
</tr>
<tr>
<td>– AIDS</td>
</tr>
<tr>
<td>– Hepatitis</td>
</tr>
<tr>
<td>– Glucocorticoid therapy</td>
</tr>
<tr>
<td>– Typhoid fever</td>
</tr>
<tr>
<td>– Systemic lupus erythematosus</td>
</tr>
<tr>
<td>2. Inherited lymphocytopenia</td>
</tr>
<tr>
<td>– Severe combined immunodeficiency states</td>
</tr>
<tr>
<td>– Wiskott-Aldrich syndrome</td>
</tr>
<tr>
<td>– Immunodeficiency with thymoma</td>
</tr>
<tr>
<td>– Cellular immunodeficiency with immunoglobulins</td>
</tr>
</tbody>
</table>

**LEUKEMIA**

**Definition and Concept**

Leukemia is defined as a malignant neoplasia of hemopoietic cells in which there is abnormal proliferation of leucocytes and their precursors resulting in appearance of abnormal and immature cells in the peripheral blood associated with very high leucocytosis, and infiltration of tissues by leukemic cells.

1. There is increased infiltration of bone marrow by the proliferating leukemic cells.
2. The total leucocyte count is usually very high, except in subleukemic or aleukemic form of leukemia.
3. Usually, the proliferation involves leucocytic series.
4. Occasionally, erythroid precursors or megakaryocytes may also be involved in the disease process.

**Types**

Leukemia is classified into two main categories: myeloid (myelocytic) and lymphocytic leukemia. These two varieties are subclassified into acute and chronic types.

**Acute Leukemias**

**Acute Lymphoblastic Leukemia**

Acute lymphoblastic leukemia (ALL) is primarily a disease of children and young adults:

1. This constitutes 80% of childhood acute leukemias. It rarely occurs in adults.

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**Table 17.11:** Summary of the formed elements in blood.

<table>
<thead>
<tr>
<th>Name</th>
<th>Count</th>
<th>Features</th>
<th>Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Red blood cells</strong> (erythrocytes)</td>
<td>5.4 million/mm³ in males; 4.8 million/mm³ in females.</td>
<td>7–8 μm diameter, biconcave discs, without a nucleus, life span about 120 days.</td>
<td>Transport oxygen and carbon dioxide.</td>
</tr>
<tr>
<td><strong>B. White blood cells</strong> (leukocytes)</td>
<td>4000–11,000/mm³.</td>
<td>Live for a few hours to few days.</td>
<td>Kill pathogens (body defence).</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>50–70% of all WBCs.</td>
<td>10–14 μm diameter; nucleus is multilobed, connected by thin strands of chromatin; cytoplasm has fine, pink granules.</td>
<td>Phagocytosis of organisms (first line of defence).</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>1–4% of all WBCs.</td>
<td>10–14 μm diameter; nucleus is bilobed; coarse brick-red granules in cytoplasm.</td>
<td>Combat the effects of histamine in allergic reactions, kill parasitic worms.</td>
</tr>
<tr>
<td>Basophils</td>
<td>0–1% of all WBCs.</td>
<td>10–14 μm diameter; nucleus is bilobed or irregular in shape; Large cytoplasmic granules are deep blue-purple.</td>
<td>Release heparin, histamine, and serotonin in allergic reactions that promote overall inflammatory response.</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>20–40% of all WBCs.</td>
<td>Small lymphocytes are 6–9 μm in diameter; large lymphocytes are 10–14 μm in diameter; nucleus is round or slightly indented; cytoplasm forms a clear rim around the nucleus.</td>
<td>Mediate immune responses.</td>
</tr>
<tr>
<td>Monocytes</td>
<td>2–8% of all WBCs.</td>
<td>12–25 μm diameter; nucleus is oval or kidney-shaped or horseshoe-shaped; cytoplasm turbid in appearance.</td>
<td>Phagocytosis (after transforming into tissue macrophages).</td>
</tr>
<tr>
<td><strong>C. Platelets</strong> (thrombocytes)</td>
<td>150,000–400,000/mm³</td>
<td>2–4 μm diameter, cell fragments, no nucleus.</td>
<td>Form platelet plug in hemostasis (temporary hemostatic plug).</td>
</tr>
</tbody>
</table>
2. The most common mode of presentation is with symptoms of anemia or hemorrhage, infective lesions of the mouth and pharynx, fever, prostration, headache and malaise.
3. Generalized **lymphadenopathy, splenomegaly and hepatomegaly** are common and occur due to infiltration of organs by leukemic cells.
4. The typical blood picture is of **anemia and thrombocytopenia**, with a moderate or marked increase in white cells, the majority of which are blast cells ‘lymphoblasts’ (Figs. 17.16A and B).

**Acute Myeloblastic Leukemia**

Acute myeloblastic leukemia (AML) primarily affects **adults between the ages of 15 and 40 years**. It constitutes only 20% of childhood leukemias:

1. The presentation is like that of ALL, but lymphadenopathy and hepatosplenomegaly is not common.
2. Blood picture presents anemia, thrombocytopenia, and moderate to high leucocytosis.
3. More than 60% of leucocytes in the peripheral blood are blast (myeloblast) cells (Figs. 17.17A and B).

**Chronic Leukemias**

**Chronic Myeloid Leukemia**

Chronic myeloid leukemia (CML) accounts for about 20% of all cases of leukemia. It is primarily a disease of **adults between the ages of 30 to 60 years** with the peak incidence in the 4th and 5th decades of life.

1. Onset is usually slow with nonspecific features like anemia, weight loss, weakness, and easy fatigability.
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2. Splenomegaly is the outstanding physical sign. Hepatomegaly may be present, but lymph node enlargement is rare.
3. Markedly elevated total leucocyte count usually more than one lakh cells per cubic mm of blood is seen commonly.
4. Neutrophils, myelocytes and metamyelocytes constitute most of the circulating cells (Figs. 17.18A and B).
5. Blasts cells are present rarely except in the blastic crisis.


Chronic Lymphocytic Leukemia
Chronic lymphocytic leukemia (CLL) is the most indolent of all leukemias. It occurs typically in persons over 50 years:
1. Males are affected twice as frequently as females.
2. Patients present with nonspecific symptoms.
3. Lymphadenopathy is the outstanding physical sign.
4. Hepatosplenomegaly may be present.
5. Mild to severe increase in leucocyte count is seen.
6. More than 90% of leucocytes are mature lymphocytes (Figs. 17.19A and B).

CHAPTER SUMMARY

Key Concepts
1. In leucopoiesis, granulocytes and monocytes develop from myeloid stem cells and lymphocytes develop from lymphoid stem cells. Major hemopoietic growth factors are GM-SCF and interleukins.
2. Development of leucocytes in bone marrow occurs in 6 to 10 days (marrow phase), they spend 6 to 8 hours in peripheral blood (circulation phase), and then enter tissues (tissue phase).
3. Neutrophils are highly phagocytic cells, and are the first line of defense against acute pyogenic infection.
4. Eosinophils and basophils defend the body against allergy.
5. Monocytes are phagocytic, and defend the body against chronic infections. Monocytes enter the tissue to become tissue macrophages (Mononuclear phagocyte system).
6. Lymphocytes participate in immunity.
7. Leukemia is the malignancy of leucocyte precursor cells. ALL occurs in children, AML in young adults, CML in adults and CLL in elderly.

**Important to Know (Must Read)**

1. In examinations, ‘Steps and regulation of leucopoiesis’ and ‘Development, life history, structure and functions of neutrophils’ come as **Long Questions**.
2. Regulation of leucopoiesis, Life history of leucocytes, Types and fund functions of neutrophil granules, Phagocytosis by neutrophil, Oxidative mechanism of killing by neutrophils, Role of eosinophil in allergy, Functions of monocytes, and Leukemias, are usually asked as **Short Questions** in exams.
3. In **Viva**, examiners usually ask… the steps of leucopoiesis, hemopoietic growth factors controlling leucopoiesis, structure and function of each leucocyte, conditions that increase and decrease each leucocyte, role of neutrophil in acute defence, steps of phagocytosis, types of killing by neutrophil, definition and types of leukemia, and which leukemia is common in which age.
4. As TLC and DLC are routine and very useful hematologic investigations, questions on WBCs are very common in all exams (practical, theory and viva). Students are supposed to answer these questions well.
CHAPTER 18

Thymus, Lymphoid Tissues and Lymph

LEARNING OBJECTIVES

On completion of study of this chapter, the student **MUST** be able to:

1. Name the lymphatic tissues of the body.
2. Give the structure and functions of thymus.
3. Give the structure and functions of lymph node.
4. Give the structure and functions of spleen.
5. Give structure and functions of other lymphoid structures.
6. Composition, circulation and function of lymph.

The student **MAY** also be able to:

1. Describe the role of various lymphatic tissues in defence of the body.
2. Explain the development of lymphocytes in thymus.
3. Describe the lymphatic circulation.

THYMUS

The thymus is the organ usually not seen by most students and teachers as it is rudimentary in adults. The thymus is located in the superior mediastinum (Fig. 18.1). At birth, the thymus weighs 10–15 grams, which increases to 30–40 grams at puberty. Subsequently, much of the organ is replaced by fat and is atrophied in adults. The thymus is important as it produces T lymphocytes throughout life.

Structure

The thymus consists of right and left lobes that are joined together by fibrous tissue. Each lobe has a connective tissue capsule, which has septa passing inwards from the capsule that subdivide the lobe into a larger number of **lobules**.

1. Each lobule is about 2 mm in diameter. It has an outer **cortex** and an inner **medulla** (Figs. 18.2A to C).
2. Both the cortex and medulla contain cells of two distinct lineages. The medulla of adjoining lobule is continuous.
3. The thymus has a rich blood supply. It does not receive any lymph vessels, but gives off efferent vessels.

Epithelial Cells

Epithelial cells are called **epitheliocytes**. Seven types of epitheliocytes are recognized.

1. **Type 1** epitheliocytes line the inner aspect of the capsule, the septa, and blood vessels. These are the cells forming the partial haemotyphic barrier mentioned above.
2. **Type 2 and type 3** cells are present in the outer and inner parts of the cortex respectively.
3. **Type 4** cells lie in the deepest parts of the cortex, and also in the medulla. They form a network containing spaces that are occupied by lymphocytes.

4. **Type 5** cells are present around corpuscles of Hassall.

5. Cortical epitheliocytes are also described as **thymic nurse cells**. They destroy lymphocytes that react against self-antigens.

### Lymphocytes of the Thymus

Lymphocytes of thymus are called **thymocytes**.

1. In the medulla of each lobule also contains lymphocytes (medullary thymocytes), but these are less densely packed than the cortex (cortical thymocytes). Consequently, the epithelial reticulum is more obvious in the medulla than in the cortex.

2. As thymocytes divide they pass deeper into the medulla.

3. Thymocytes leave the thymus by passing into blood vessels and lymphatics.

### Macrophages

Apart from epithelial cells and lymphocytes the thymus contains a fair number of macrophages that are part of mononuclear phagocyte system (MPS). The **subcapsular macrophages** are highly phagocytic. Macrophages present more deeply are **dendritic cells**.

### Corpuscular Hassall

These are small rounded structures present in the medulla of the thymus.

1. Each corpuscle has a central core formed by epithelial cells that have undergone degeneration. These cells ultimately form a pink staining **hyaline mass**.

2. Around this mass there is a wall formed by concentrically arranged epithelial cells.

3. The central mass of the corpuscle may also contain degenerating macrophages.

### Functions of the Thymus

T cell develops from pre-T cell. The thymus is the site for T cell development. The **epithelial cells** play an important role in the development of bone marrow-derived pre-thymic cells into mature T cells (discussed below). Though thymus is reduced to a very small structure in adults, and in the atrophic adult thymus, cortex is mainly replaced by adipose tissue, the less cellular medulla remains apparently normal (Clinical Box 18.1).

#### Clinical Box 18.1

**Effects of thymectomy:** Thymectomy in young animals leads to lymphocytopenia, increased susceptibility to infections, atrophy of lymphatic tissues, failure to reject transplanted organs and suppression of delayed hypersensitivity reactions. Thymectomy in young animals leads to depletion of T cells and immunodeficiency within few months. These findings depict the importance of thymus in development of T cells in childhood and maintenance of T cells in adult life.

### Role in Lymphopoiesis

The role of the thymus in lymphopoiesis is significant.

1. Stem cells from bone marrow that reach the superficial part of the cortex divide repeatedly to form smaller lymphocytes.
2. It has been proposed that during these mitoses, the DNA of the lymphocytes undergoes numerous random mutations, as a result of which different lymphocytes acquire the ability to recognize a very large number of different proteins and to react to them.

3. As it is not desirable for lymphocytes to react against the body's own proteins, all lymphocytes that would react against them are destroyed. It is for this reason that 90% of lymphocytes formed in the thymus are destroyed within three to four days.

4. The remaining lymphocytes that react only against proteins foreign to the body are thrown into the circulation as circulating, immunologically competent T lymphocytes. They lodge themselves in secondary lymph organs like lymph nodes, spleen, etc. where they multiply to form further T lymphocytes of their own type when exposed to the appropriate antigen.

**Thymus as a Primary Lymphoid Organ**
Thymus is regarded as a primary lymphoid organ along with bone marrow.

1. It has been observed that, within the thymus, lymphocytes are not allowed to come into contact with foreign antigens, because of the presence of the **blood-thymic barrier**. It has also been stated that because of this thymocytes do not develop into large lymphocytes or into plasma cells, and do not form lymphatic nodules.

2. Recently, it has been postulated that the medulla of the thymus (or part of it) is a separate “compartment”. After thymocytes move into this compartment they probably come into contact with antigens presented to them through dendritic macrophages. Such contact may be necessary step in making T lymphocytes competent to distinguish between foreign antigens and proteins of the body itself.

3. The proliferation of T lymphocytes and their conversion into cells capable of reacting to antigens, probably takes place under the influence of hormones produced by epithelial cells of the thymus. T lymphocytes are also influenced by direct cell contact with epitheliocytes. **Hormones** produced by the thymus may also influence lymphopoiesis in peripheral lymphoid organs. This influence appears to be specially important in early life, as lymphoid tissues do not develop normally if the thymus is removed.

4. Thymectomy has much less influence after puberty as the lymphoid tissues have fully developed by then.

**Thymic Hormones**
A number of hormones produced by the thymus have now been identified as follows:

1. **Thymulin**: Thymulin enhances the function of various types of T cell, specially that of suppressor cells.

2. **Thymopoietin**: Thymopoietin stimulates the production of cytotoxic T cells. The combined action of thymulin and thymopoietin allows precise balance of the activity of cytotoxic and suppressor cells.

3. **Thymosin**: Thymosin α-1 stimulates lymphocyte production and also the production of antibodies. Thymosin β-4 is produced by mononuclear phagocytes.

4. **Thymic humoral factor** controls the multiplication of helper and suppressor T cells.

**Clinical Correlation**
Enlargement of thymus is often associated with a disease called **myasthenia gravis**. In this condition there is a great weakness of skeletal muscles. In many such cases the thymus is enlarged and there may be a tumor in it. **Removal of the thymus** results in considerable improvement in some cases. Thus, myasthenia gravis is now considered to be a disturbance of the immune system, in which, antibodies are produced against these proteins rendering them ineffective (autoimmune disease).

**LYMPHATIC TISSUES**
Lymphatic system consists of lymphatic vessels and lymphatic tissues.

**Lymphatic Vessels**
When circulating blood reaches the capillaries, part of its fluid content passes into the surrounding tissues as tissue fluid. Most of this fluid reenters the capillaries at their venous ends. Some of it is, however, returns to the circulation through a separate system of lymphatic vessels (usually called lymphatics). The fluid passing through the lymphatic vessel is called **lymph**.

1. The smallest lymphatic (or lymph) vessels are lymphatic capillaries that join together to form larger lymphatic vessels.

2. The largest lymphatic vessel in the body is the thoracic duct, which drains lymph from the greater part of the body.

3. The thoracic duct ends by joining the left subclavian vein at its junction with the internal jugular vein (Fig. 18.3).

**Fig. 18.3**: Origin, course and termination of thoracic duct.
4. On the right side there is the right lymphatic duct that has a similar termination.

**Lymphoid Tissues**

Lymphoid tissue may be broadly classified as: Diffused lymphoid tissue and dense lymphoid tissue.

**Diffused Lymphoid Tissue**

Diffused lymphoid tissue consists of diffusely arranged lymphocytes and plasma cells in the mucosa of large intestine, trachea, bronchi and urinary tract.

**Dense Lymphoid Tissue**

It consists of an aggregation of lymphocytes arranged in the form of nodules. These nodules are found either as discrete encapsulated organs or in close association to the lining epithelium of the gut. Dense lymphoid tissue can therefore be further divide as:

1. **Discrete lymphoid organs**: these include thymus, lymph nodes, spleen, and tonsils.
2. **Mucosa associated lymphoid tissue (MALT)**: small numbers of lymphocytes may be present almost anywhere in the body, but significant aggregations are seen in relation to the mucosa of the respiratory, alimentary and urogenital tracts. These aggregations are referred to as MALT.

**Mucosa associated lymphoid tissue in the respiratory system**: in the respiratory system the aggregations are relatively small and are present in the walls of the trachea and large bronchi. The term bronchial associated lymphoid tissue (BALT) is applied to these aggregations.

**Mucosa associated lymphoid tissue in the alimentary system**: This is also called gut associated lymphoid tissue (GALT) and includes Payer’s patches of ileum, adenoids (located in the roof of pharynx), lingual tonsils in posterior 1/3rd of tongue, palatine tonsils and lymphoid nodules in vermiform appendix.

*Note*: Thymus and bone marrow are primary lymphoid organ while others are secondary lymphoid organ.

**Lymph Node**

Lymph nodes are small encapsulated organs present in the pathway of lymphatics. They are found usually in groups, especially in neck, submandibular region, axilla and inguinal regions.

1. They have afferent and efferent lymphatics. **Afferent lymphatics** penetrate the capsule and join the sinus inside.
2. **Trabeculae** extend from capsule into the stroma in the node.
3. **Stroma** is made up of reticular lymphoid tissue arranged in outer cortex and inner medulla (Fig. 18.2A and B).
4. **Cortex** has lymphatic nodules or follicles with definite germinal centers.
5. **Each follicle has central pale area that represent germinal center and surrounding little darker area representing B cell area of the node.**
6. The **T cell area** is present in the paracortex, the junction between cortex and medulla (Fig. 18.4).
7. **Medulla** has lymphoid cells arranged in strands forming medullary cords.
8. Lymph flows from afferent lymphatics to subcapsular sinus, and then along trabeculae to the cortical and medullary sinuses, and exits via efferent lymphatics at the hilus.
9. Thus, lymph nodes filter the lymph that flows through it (Clinical Box 18.2). Lymph nodes also assist in processing of lymphocytes that takes place in the germinal centers.
Clinical Box 18.2

Lymphadenopathy indicates infection in the area: Lymph nodes draining a particular area get enlarged in response to infection or inflammation in the area. For example, axillary lymphadenopathy indicates infection, tumor or malignancy in the mammary gland, especially in females, and submandibular lymphadenopathy in throat infections. Thus, clinically it helps in detecting the area of pathology.

Spleen

Spleen is the largest lymphoid organ in the body.
1. It is surrounded by a capsule made up of elastic and smooth muscle fibers.
2. A number of trabeculae extend from capsule into the organ.
3. Stroma contains network of reticular fibers and cells and many venous sinuses.
4. Parenchyma contains splenic pulps made up of white pulp and red pulp. Red pulps are present around the white pulps (Figs. 18.5A and B).
5. The white pulp appears as circular patches. It consists of lymphoid nodule containing B cells, central artery and germinal center. The periarteriolar lymphocytes are mainly T cells. However, B cells slightly predominate T cells in spleen.
6. The red pulp is made up of diffuse lymphoid tissue organized as splenic cords separated by venous sinuses. Red pulp contains red cells, lymphocyte, other white cells and macrophages.
7. The cords contain fine network of reticular fibers and cells. This fine reticular network in the splenic red pulp filters the blood cells that pass through it. The arrangement of splenic circulation (open and closed circulation) in the red pulp and location of the sinusoids help in filtering senescent and abnormal cells (Fig. 18.6).
8. The aged and defective cells are easily detected and destroyed in the pulp. Otherwise also blood cells are trapped in the splenic pulp (Clinical Box 18.3).

Figs. 18.5A and B: Histological picture of spleen, in low magnification (A), and in photomicrograph (B). (1: Red pulp; 2: White pulp; 3: Germinal center; 4: Arteriole; Ca: Capsule; T: Trabeculus).

Fig. 18.6: The splenic circulation. Note the location of sinusoids that filter cells.
Functions of Spleen

1. Spleen plays important role in immunity (Clinical Box 18.4). Macrophages in spleen help in phagocytosis of pathogens or antigens. Spleen is the site for production of B and T cells. Germinal centers in the spleen helps in development of lymphocytes. Spleen also helps in production of antibodies.
2. Spleen removes old and abnormal red cells, white cells and platelets. Cells of MPS detect these senescent and abnormal cells and remove them from blood.
3. Spleen helps in hepatic stage of hemopoiesis during intrauterine life. Extramedullary hemopoiesis takes place in spleen in postnatal life in pathological conditions.
4. Spleen acts as an important reservoir of blood in mammals.
5. Macrophages in spleen recycle iron, which has been released from destroyed red cells. This iron is reutilized for synthesis of hemoglobin.

Mucosa-associated Lymphoid Tissue

As discussed significant aggregation of lymphocytes seen in relation to the mucosa of the respiratory, alimentary and urogenital tracts are refereed as mucosa-associated lymphoid tissue (MALT). The total volume of MALT is more or less equal to that of the lymphoid tissue present in lymph nodes and spleen. Mucosa associated aggregations of lymphoid tissue have some features in common as follows:

- These aggregations are in the form of one or more lymphatic follicles (nodules) having a structure similar to nodules of lymph nodes. Germinal centers may be present. Diffuse lymphoid tissue (termed the parafollicular zone) is present in the intervals between the nodules. The significance of the nodules and of the diffuse aggregations of lymphocytes is the same as already described in the case of lymph nodes. The nodules consist predominantly of B, while the diffuse area consists of T lymphocytes.
- These masses of lymphoid tissue are present in very close relationship to the lining epithelium of the mucosa in the region concerned, and lie in the submucosa. Larger aggregations extend into the submucosa. Individual lymphocytes may infiltrate the epithelium and may pass through it into the mucosa.
- The aggregations are not surrounded by a capsule, nor do they have connective tissue septa. A supporting network of reticular fibers is present.
- As a rule these masses of lymphoid tissue do not receive afferent lymph vessels, and have no lymph sinuses. They do not therefore, serve as filters of lymph. However, they are centers of lymphocyte production. Lymphocytes produced here pass into lymph nodes of the region through efferent lymphatic vessels. Some lymphocytes pass through the overlying epithelium into the lumen.

Mucosa-associated Lymphoid Tissue in Respiratory System

In the respiratory system the aggregations are relatively small and are present in the walls of the trachea and large bronchi. The term bronchial associated lymphoid tissue (BALT) is applied to these aggregations.

Mucosa-associated Lymphoid Tissue in Alimentary System

This is also called gut associated lymphoid tissue (GALT). In the alimentary system examples of aggregations of lymphoid tissue are tonsils, Peyer’s patches and lymphoid nodules in vermiform appendix.

LYMPH AND LYMPHATIC CIRCULATION

Lymph is a transudate from blood and contains the same proteins, but in smaller amounts and in different proportions. Suspended in lymph there are cells that are chiefly lymphocytes. Most of these lymphocytes are added to lymph as it passes through lymph nodes, but some are derived from tissues drained by the nodes. Large molecules of fat (chylomicrons) that are absorbed from the intestines enter lymph vessels. After a fatty meal these fat globules may be so numerous that lymph become milky (is called chyle). Under these conditions the lymph vessels can be seen easily as they pass through mesentery.

Functional Anatomy

Lymphatics drain lymph from different tissues of the body and empty the same into the subclavian veins at their junctions with internal jugular veins.

1. Lymphatic vessels typically begin as blind tubular bulbs (lymphatic bulbs) that drain into meshwork of interconnected lymph vessels. For example, in the intestine, villous lacteals are lymphatic bulbs.
Section 2: Blood and Immunity

2. **Blood and Immunity**

![Diagram: The lymphatic drainage system. Note, there is no valve in initial lymphatics, but the valves are present in collecting lymphatics. Black arrows indicate the direction of flow of fluid movement into lymphatic bulb.](image)

**Fig. 18.7:** The lymphatic drainage system. Note, there is no valve in initial lymphatics, but the valves are present in collecting lymphatics. Black arrows indicate the direction of flow of fluid movement into lymphatic bulb.

2. Tissue fluid enters into the bulbs that in turn drain to interconnected lymph vessels and then from these vessels into tubular lymphatic vessels and the larger lymphatic vessels (Fig. 18.7).

3. Lymphatic vessels coalesce into increasingly larger vessels.

4. Larger vessels are surrounded by contractile cells similar to that of smooth muscles of blood vessels.

5. Lymph nodes are interposed in the path of lymphatics.

**Important Note**

Organs with no lymphatics: Lymphatics are absent in bone, teeth, cartilage, placenta and CNS.

**Types of Lymphatics**

Lymphatics are of two types: Initial lymphatics and collecting lymphatics.

**Initial Lymphatics**

Initial lymphatics are lymphatic bulbs and interconnected vessels (lymphatic capillaries).

Lymphatic capillaries differ from vascular capillaries in several ways:

1. No basal lamina (or scanty basal lamia) under the endothelium.

2. Junctions between endothelial cells are open.

3. No fenestrations in endothelium (non-fenestrated)

4. Tight junctions (tight intercellular connections) are absent

Initial lymphatics lack valves and smooth muscles in their walls. Tissue fluid enters into them through gaps or loose junctions between the endothelial cells present in their linings. Movement of fluid in them is facilitated by contraction of organs in which they are present and the contraction of arterioles and venules with which they are often associated. They drain into collecting lymphatics.

**Collecting Lymphatics**

Collecting lymphatics are tubular and larger lymphatics.

1. They have valves and smooth muscles in their walls.

2. They exhibit peristaltic contraction that propels lymph in forward direction.

3. Lymph flow is further facilitated by skeletal muscle contraction, negative intrathoracic pressure (during inspiration), and suction effect created by higher velocity of flow in veins into which the lymphatics drain.

4. Unidirectional flow of lymph is maintained by presence of valves in the larger lymphatic ducts.

5. Substances that increase lymph flow are called lymphagogues. Usually, agents that increase capillary permeability also act as lymphagogues.

**Formation and Composition of Lymph**

Lymph is formed from tissue fluid. Therefore, essentially it is a modified tissue fluid (Table 18.1). It is formed by transcapillary exchange and exchange between tissue fluid and lymphatic ducts. Lymph flow is much slower (1 mL/min in thoracic duct) than blood. Its colloidal osmotic pressure is less than that of plasma. Presence of more lipid gives milky color to the lymph, hence called chyle.

**Functions of Lymphatic Circulation**

1. Extra amount of fluid left in the tissue space by capillary filtration is taken up by lymphatics and returned back to circulation. Thus, lymphatics prevent accumulation of excess free fluid in the interstitial space (prevent edema formation), and at the same time contribute to water content of plasma to some extent.

2. In liver and intestine, a significant quantity of protein enters into interstitial space. This protein is returned to circulation via lymphatics. This accounts for 25 to 50% of circulating protein in the blood. Thus, lymphatics maintain protein content of plasma.

<table>
<thead>
<tr>
<th>Table 18.1: Composition of lymph.</th>
</tr>
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<tbody>
<tr>
<td>1. Proteins: 2–6 mg% (more in liver)</td>
</tr>
<tr>
<td>2. Lipids: Intestinal lacteals contain more lipid</td>
</tr>
<tr>
<td>3. Carbohydrate: Less than plasma</td>
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<tr>
<td>4. Clotting factors: Lymph draining from liver mainly contains clotting factors.</td>
</tr>
<tr>
<td>5. Cells: Mainly lymphocytes and few monocytes</td>
</tr>
<tr>
<td>6. Ions: Sodium, potassium, calcium, chloride, sulfates, phosphate</td>
</tr>
<tr>
<td>7. Water</td>
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</tbody>
</table>
3. Long-chain fatty acids, cholesterol and fat-soluble vitamins absorbed from intestine are transported to circulation via lymphatics.
4. Mononuclear phagocytes in lymph nodes remove bacteria and pathogenic organisms from lymph draining from the organ. Thus, they play protective function (Clinical Box 18.5).

Clinical Box 18.5

Lymphadenopathy: Specific lymph nodes draining from the site of infection and inflammation are usually enlarged during the active phase of the disease process. Enlarged lymph nodes (lymphadenopathy) in a particular part of the body provide clinical clue to the physician to locate the site of infection. For example, enlarged submandibular lymph glands indicate infection in the throat.

CHAPTER SUMMARY

Key Concepts
1. Thymus is a primary lymphoid organ involved in T cell development.
2. Lymph node, spleen and associated lymphatic tissue are secondary lymphoid organs where the lymphocytes reside and mature.
3. Spleen is the site of destruction of old and abnormal blood cells.
4. Splenomegaly and lymphadenopathy indicates infection or proliferation of abnormal cells in general.

Important to Know (Must Read)
1. In examinations, Long Questions do not come from this chapter.
2. Functions of thymus, functions of spleen, composition and functions of lymph, & lymphatic circulation may come as Short Questions in exams.
3. In Viva, examiners may ask… hormones secreted from thymus, how T cells are developed in thymus, functions of thymus, spleen and lymph node, composition of lymph, and design of lymphatic circulation.
The process of defense of the body against harmful elements that threaten our normal health is the immunity. Our environment is rich in varieties of infectious microbes, such as bacteria, viruses, fungi, protozoa and multicellular parasites. When these organisms enter the body they multiply, and if their growth and multiplication are unchecked, they produce disease in the host and eventually they may even kill the host. Therefore, the living beings require quick and continuous mechanisms to kill and remove the pathogens from their body.

1. Adequacy of the defense systems mainly depends on the activities of immune mechanisms.
2. Mammalians are endowed with various defense mechanisms that can broadly be divided into specific and non-specific defenses.
3. The specific defense mechanisms of the body are collectively known as immunity.
4. The immune responses greatly depend on the site of infection and the type of infecting organism (pathogen).

**CLASSIFICATION OF IMMUNITY**

The ability of the body to defend against invading agents is called immunity. The invader may be a living organism or a nonliving substance.

Immune responses broadly involve two steps:

1. Recognition of pathogen or the foreign material,
2. Reactions or responses to eliminate it. The responses are called immune responses (Table 19.1).
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Largely, immune responses are of two types:
1. Innate or nonadaptive response (Innate immunity).
2. Acquired or adaptive response (Acquired immunity).

Scientist contributed

Paul Ehrlich a German physician and scientist worked in the fields of immunology, and antimicrobial chemotherapy. He invented the precursor technique to Gram staining bacteria. The methods he developed for staining tissue made it possible to distinguish between different types of blood cells, which led to the capability to diagnose numerous blood diseases. He was awarded Nobel Prize in Physiology or Medicine in 1908 for his pioneering work on humoral immunity.

Paul Ehrlich (1854–1915)

Innate immunity: Innate immunity is mostly nonspecific:
1. It include phagocytosis, inflammation, release of cytokine and antibacterial peptides from phagocytes and inflammatory cells, activation of complement system, etc.

Scientists contributed

Bruce A Beutler Jules A Hoffmann Ralph M Steinman

The Nobel Prize in Physiology or Medicine 2011 was divided, with one half jointly to Bruce A Beutler and Jules A Hoffmann for their discoveries concerning the activation of innate immunity, and the other half of the prize was given to Ralph M Steinman, for his discovery of the dendritic cell and its role in adaptive immunity.

2. Though, highly developed in mammals, this is also the major mechanism of immunity in invertebrates and plants.

Acquired immunity: Vertebrates, especially mammals are gifted with acquired immunity in which specific set of lymphocytes are activated with specific antigens and eliminate antigens by specific mechanisms (specific immunity).

NONSPECIFIC DEFENSE SYSTEM
(Innate Immunity)

Non-specific defense systems include the skin and mucous membrane (mechanical factors and chemical factors), antimicrobial mechanisms (interferons and complements), natural killer cells, phagocytosis, inflammation and fever. Thus, nonspecific defence mechanisms are:
1. Mechanical defense
2. Chemical defense
3. Defense by NK cells
4. Defense by phagocytic cells
5. Defense by inflammation and fever

Mechanical Defense

Skin and epidermis: Skin and epidermis are the most important and natural defense barriers of the body. They form the partition between the body and the external environment that immediately checks the entry of organisms into the body. Therefore, loss of skin as occurs in burns leads to infection and septicemia.

Mucous membranes: Mucous membrane also forms the physical barrier for the organisms and prevents their entry into the body. Wherever there is no skin on the body surface, mucous membrane covers the body, especially the surface of body cavities such as oral cavity, nasal cavity etc. Mucous membrane mechanically traps the organisms and secretes chemicals that inhibit the organisms.

Mucus: Mucus is secreted from glands located in the mucosal epithelium. Mucus, by virtue of its physical property traps microorganisms, especially in the respiratory, gastrointestinal and genitourinary tracts.

Hairs: Almost all openings (entry points) of the body such as nostrils, ears, eyes, anus, urethra and vagina (in females) are guarded by hairs. They filter microorganisms and prevent their entry into the body.

Cilia: Cilia also filter microorganisms. They are present in mucous membranes. They remove dust and microbes from upper respiratory tract. Along with mucus they also trap microbes.

Tear: Tear is secreted from lacrimal apparatus. It washes the microbes and dilutes the chemical substances produced by microbes.

Saliva: Saliva washes microbes from the oral cavity and keeps the mouth and teeth clean. It also contains antimicrobial chemicals.
Urine: During micturition, urine comes out of the urinary tract forcefully. Therefore, urine flow washes microbes from urinary tract.

Defecation: During the act of defecation, fecal matter is forcefully evacuated from the rectum and anal canal. Thus, along with fecal matters microbes are washed from lower GI tract.

Chemical Defense

Acidic pH of Skin: Acidic pH of skin inhibits growth of micro-organisms.

Bactericidal Substance of Sebum: Sebum is the secretion of the glands in the skin. It contains bactericidal substances like unsaturated fatty acids that kill microbes.

Chemicals in Body Secretions: Antimicrobial substances like lysozyme secreted in saliva, tear, sweat and other body secretions inhibit growth of micro-organisms.

Acidic pH of Stomach and Vagina: Strongly acidic pH of gastric secretion kills many micro-organisms and also destroys toxins in the stomach. Low pH of vaginal secretion also prevents entry of microbes into the body.

Complement Proteins: There are different complement proteins in plasma that are activated in response to entry of microorganisms into the body. They help in destroying the organisms by facilitating opsonization and phagocytosis. They also participate in immunity (refer 'complement system').

Interferons: These are chemicals secreted from activated lymphocytes, etc., These are usually antiviral substances that kill viruses.

Defense by NK Cells

NK Cells or natural killer cells are third category of lymphocytes that are neither B nor T cells:

1. They have the ability to kill a wide variety of micro-organisms and tumor cells.
2. They do not mature in thymus.
3. Unlike B and T cells, they lack surface antigen receptors.
4. They carry surface molecules CD2, CD16, and CD56, but negative for T cell marker CD3.
5. These are large granular lymphocytes (Fig. 19.1).
6. They constitute about 15% of total lymphocytes in the body.
7. They are present in the spleen, lymph nodes, bone marrow and blood.
8. They take part in natural or innate immunity.
9. They recognize antibody coated target cells and kill them by antibody-dependent cell-mediated cytotoxicity (ADCC), which works more effective against viruses and tumour cells.

Mechanism of Killing

NK cells kill microbes by following mechanisms:

1. Osmotic lysis by incorporating perforins into the surface of the microbes.
2. They release interferons that activate phagocytosis and immunity.
3. They possess Fc receptors that allow them to kill antibody coated viruses.
4. Kill by antibody-dependent cell-mediated cytotoxicity (ADCC), especially the viruses and tumour cells.

Special Features: NK cells differ from other lymphocytes by following characteristics:

1. They do not require prior sensitization to kill microbes.
2. They do not involve major histocompatibility complex (MHC) antigen for killing micro-organisms.
3. They are the first line of defense against viral infections.
4. They are also active against tumor cells, especially malignant cells.
5. They usually attack cells that do not display proper markers.

Defense by Phagocytic Cells

Role of Granulocytes and Mononuclear Cells

Granulocytes (neutrophils, eosinophil and basophil), monocytes and macrophages kill micro-organisms by phagocytosis:

1. Phagocytosis is the process of ingestion of microbes or foreign cells or solid materials by a phagocyte. Phagocytes are neutrophils, monocytes and macrophages (scavenger cells). Monocytes transform themselves into macrophages in the tissue.
2. There are two types of macrophages: the wandering macrophages and fixed macrophages. The macrophages that are mobile in the tissues are called wandering macrophages.
3. The fixed macrophages are present in specific tissue sites in the body. These macrophages are mononuclear cells, and therefore this system of phagocytes is called as mononuclear phagocyte system (MPS). Previously, this system of cells was known as reticuloendothelial system, but they neither are reticular in appearance nor have endothelial origin. Therefore,
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the term reticuloendothelial system is obsolete. The important examples of MPS include the following (Fig. 19.2):

a. Kupffer cells of the liver
b. Alveolar macrophages in the lungs
c. Microglia of brain
d. Tissue macrophages in the spleen and lymph nodes
e. Osteoclasts in bone
f. Mesangial cells in kidney
g. Histiocytes in connective tissues
h. Langerhans cells in the skin

Scientist contributed

Élie Metchnikoff was a Russian zoologist best known for his pioneering research in immunology. In particular, he is credited with the discovery of phagocytes (macrophages) in 1882. This discovery turned out to be the major defence mechanism in innate immunity. He and Paul Ehrlich were jointly awarded the 1908 Nobel Prize in Physiology or Medicine “in recognition of their work on immunity”. He established the concept of cell-mediated immunity, while Ehrlich described humoral immunity. Their works are regarded as the foundation of the science of immunology. In immunology, Élie Metchnikoff is given an epithet the “Father of natural immunity.”

Inflammation

In the process of inflammation, microorganisms are killed by the chemicals released from the inflammatory cells, and also by phagocytes.

Defense by Inflammation and Fever

In the process of inflammation, microorganisms are killed by the chemicals released from the inflammatory cells, and also by phagocytes.

Inflammation

The response of the tissue to injury is known as inflammation. This is a defensive response of the body against the tissue injury. It is characterized by appearance of four features in sequence at the site of injury:

1. Rubor (redness)
2. Dolor (pain)
3. Calor (increased temperature)
4. Tumor (swelling).

The fifth component of inflammation is the functiolesia, the loss of function of the injured part of the body. Inflammation helps in killing the organisms and disposing them off. It also destroys toxins released by microbes, and helps in tissue repair. Thus, inflammation restores tissue homeostasis.

Stages of Inflammation

There are three stages of inflammation: vasodilation and increased permeability, phagocyte migration and phagocytosis, and tissue repair (Fig. 19.3).
Vasodilation and Increased Permeability
Vasodilation occurs immediately following injury, which allows more blood to flow to the site of injury. This causes redness of the area. This is followed by increased permeability which allows fluid to escape into the tissue that causes local swelling (wheal), and also permits antibodies and phagocytes to enter the injured area (Figs. 19.4A and B):

1. Vasodilation and increased capillary permeability are produced by histamine released from mast cells, basophils, and platelets, bradykinin formed in the blood and in tissues from plasma kininogen and tissue kininogen respectively, prostaglandins released from damaged cells, and complement proteins formed by activation of complement system.
2. Contraction of vascular endothelial cells at the site of injury increases the gap between the cells that facilitates vascular permeability (Fig. 19.5).
3. The arteriolar dilation increases further blood flow that causes increased local temperature (calor) and flare i.e., spread of redness to the surrounding area, the phenomenon called triple response (Figs. 19.4A and B).

Phagocyte Migration and Phagocytosis
Within minutes to hours of inflammation, phagocytes appear at the site of injury. The first to appear is neutrophil, followed by monocyte and macrophage (Application Box 19.1):

1. Phagocytes reach the site of injury by diapedesis and chemotaxis (for details, refer Figs. 17.12 and 17.13, Chapter 17).
2. As the inflammatory response continues, phagocytes die in the process of killing the microbes.
3. Within few days, the dead phagocytes and damaged tissue cells form viscous fluid called pus. Usually, over a period of days pus is absorbed.
4. The pus that cannot be drained out from the region of inflammation slowly forms abscess (localized accumulation of pus).

**Application Box 19.1**

Alteration in cell count indicates nature of inflammation: As neutrophils first participate in inflammation, neutrophilia indicates acute inflammation or infection. As inflammation continues, monocytes and macrophages take part in phagocytosis. Therefore, monocytosis is the feature of chronic inflammation.

**Tissue Repair**

Tissue repair is the healing stage in inflammation. This is facilitated by migration of fibroblasts, macrophage and epithelial cells to the site of injury that repair and restore the epithelium by secreting growth factors. Tissue plasmin promotes migration of keratinocytes that aid to the healing process. Proliferation of collagen produces scar.

**Role of NF-κB**

Recent evidences suggest that a transcription factor, called nuclear factor kappa B (NF-κB), plays an important role in inflammation:

1. Normally, NF-κB is bound to another cytoplasmic protein called IκBα, and this binding keeps NF-κB in an inactive state.
2. Viruses and cytokines that induce inflammation separate NF-κB from IκBα. NF-κB then migrates to nucleus and attaches with the DNA.
3. This induces the transcription of genes for formation of various chemicals that participate in inflammation.
4. Thus, NF-κB stimulates synthesis and secretion of mediators of inflammation (Application Box 19.2).

**Systemic Response to Inflammation**

Cytokines produced during inflammation induce systemic responses. The major change is the increase in acute phase reactants (APRs) in plasma. APRs are plasma proteins whose concentration increases at least by 25% in response to acute inflammations.

These acute phase proteins are mainly synthesized by liver. The important APRs are:

1. C-reactive protein (CRP)
2. Orosomucoid protein
3. Fibrinogen
4. Haptoglobin
5. Serum amyloid protein A
6. C3 complement protein

The first to appear in acute inflammation is CRP. In many acute conditions, the plasma level of high-sensitive CRP (hsCRP) is an important marker of inflammation (Clinical Box 19.1). However, CRP concentration decreases rapidly within first week of the onset of the disease. The fibrinogen concentration increases slowly and remains elevated for 2 to 3 weeks. Increase in ESR in acute inflammation is due to increased level of fibrinogen that neutralizes the negative charges on the red cell surface.

**Clinical Box 19.1**

hsCRP is a prognostic marker: Estimation of ultrasensitive-CRP (us-CRP) has recently been used for diagnosis, and assessing the prognosis of inflammatory diseases and coronary artery disease.

**Inflammatory Cells and Cytokines**

Cells for acute inflammation: Neutrophils, eosinophils and basophils.

Cells for chronic inflammation: Monocytes, macrophages, lymphocytes and plasma cells.

These cells kill organisms by phagocytosis and also by secreting cytokines. Many of the mediators of inflammation are secreted by the inflammatory cells.

**Fever**

Fever is one of the protective phenomena of the body against infections:

1. Increased body temperature as occurs in acute infections and inflammations, prevents growth of microorganisms.
2. Fever also facilitates the actions of interferon and different body enzymes that inhibit the growth of many microorganisms.
3. Therefore, unless very high and deleterious, fever should not be brought down immediately by antipyretics.

**Contribution of Toll-like Receptors**

Innate immunity in Drosophila is mainly due to the presence of a receptor protein called ‘Toll’. The toll binds with fungal antigens that activates of genes coding for antifungal proteins. A large number of toll like receptors (TLRs) are found in mammals including human beings. TLR4, one of these proteins, binds to bacterial polysaccharides and to CD14. It is proposed that this binding initiates intracellular events that activate transcription of genes for a variety of proteins required for innate immune responses (Application Box 19.3).
Pattern Recognition Receptors: TLRs are referred to as pattern recognition receptors (PRRs) as they organize and respond to the molecular patterns expressed by pathogens. Few PRRs such as nucleotide-binding oligomerization protein domain (NOD proteins) may be intracellular. NOD 2 has recently been identified as the product of a candidate gene involved in inflammatory bowel disease and Croh'n disease.

**SPECIFIC DEFENSE MECHANISMS**

(ACQUIRED IMMUNITY)

Acquired immunity is broadly divided into two categories: the cellular (cell mediated) immunity, and humoral (antibody mediated) immunity.

**Cellular Immunity:** Cell mediated immunity is due to the presence of **cytotoxic T cells** (killer cells) in the body:
1. These cells develop and proliferate in response to a particular antigen (or a specific organism) and kill that organism (or destroy that antigen).
2. This immunity is particularly effective against **intra-cellular organisms** like viruses, parasites and fungi, cancer cells, tumor cells, and transplanted tissues.

**Humoral Immunity:** Humoral immunity is due to the presence of **antibodies** in the body:
1. Antibodies are formed by plasma cells. Plasma cells are developed from activated B cells in response to antigen.
2. This immunity works mainly against **extracellular organisms** such as bacteria and the antigens dissolved in the body fluids.

Though pathogens stimulate activation of a particular immune mechanism predominantly, often they provoke both cellular and humoral immunity simultaneously.

**Development of Immunity**

Development of immunity is the development of lymphocytes. Immunity develops naturally in the body, but is activated in response to an antigen, which is generally an infective organism or a nonliving substance:
1. A specific antigen stimulates the development of a specific set of lymphocyte.
2. Functionally, lymphocytes are of three types: **B cell**, **T cell**, and **NK cell**.
3. NK cell (described earlier) is mostly involved in relatively nonspecific defenses of the body.
4. The major sites of lymphocyte development are the **primary lymphoid organs**. In these organs, lymphocytes differentiate from their precursors that originate from the bone marrow, and proliferate and mature into functionally potent cells. In mammals, **T cells mature in the thymus**, and **B cells mature in the bone marrow and bursal equivalent**. Thus, **thymus, bone marrow and bursal equivalent** are the primary lymphoid organs.
5. After their maturity, they circulate in blood and migrate to **secondary lymphoid organ** where they reside.

Flowchart 19.1: General outline of the development of immunity. T4 cells induce development of both cellular and humoral immunity.

Secondary lymphoid organs are **lymph nodes**, **spleen** and **gut associated lymphoid organs**.

T cells develop in thymus, and B cells in bursal equivalents. The general outlines of development of immunity are summarized in Flowchart 19.1. For details of lymphoid tissues (thymus, lymph node, spleen, and other lymphoid tissues), and their functions, especially their role in immunity, refer the previous chapter. Distribution of T and B cells in different lymphoid tissues is depicted in Table 19.2.

**Development of Bursa:** B cells develop in bursa. In the birds, lymphoid tissue is present in bursa of Fabricius, which is located near the cloaca:
1. This bursa helps in development and processing of **B lymphocytes**, where pre-B cells develop into B cells. Therefore, these cells are called bursa-dependant cells or B cells.
2. However, in mammals there is no such bursa, but they have **bursa equivalents**. The bursal equivalent issues are **bone marrow and fetal liver**.

**Development of Gut-Associated Lymphoid Organs:**

Lymphatic tissues are present at many places in GI tract starting from oral cavity to colon:
1. **Tonsils** (palatine, lingual and nasopharyngeal) that are present in the oral cavity and pharynx contain lymphatic follicles.
2. Lymphoid tissues are present extensively in the form of **Payer's patches** along the entire wall of intestine in the submucosal layer.
3. Vermiform appendix is rich in lymphatic tissue.
4. Lymphatic tissues are also present in colon.
5. These lymphatic structures in GI tract provide immunity to body by killing organisms, parasites and larvae that try to enter blood from intestinal lumen.

**Development of T Cells**

**Mechanism of Development**

Development of T cells takes place in thymus. Hence, they are called thymus-dependent or T cells:

1. The stem cells or precursor cells that migrate to thymus before they mature into competent lymphocytes in the thymic environment are called pre-T cells. The lymphocytes that develop from pre-T cells are called T cells.
2. It is not clearly known whether the migration of prethymic (pre-T) cells is selective or random, and whether they are multipotent or destined to from only T cells.
3. Experimental evidences suggest that migration of stem cells into thymus is not a random process, but results from chemotactic signals that are periodically emitted from the thymus. β2-microglobulin, a component of MHC-I molecule is putative chemoattractant for pre-T cells.
4. Though experimental evidences suggest that stem cells destined for thymus express CD7, a marker of T cells, they are multipotent in nature. However, in the thymic environment they develop only into T cells.
5. In the thymus, epithelial cells, macrophages and interdigitating cells are rich in MHC class II antigens. These cells are important in the differentiation of pre-T cells into T cells.
6. The most important among them is the epithelial cell. Specialized epithelial cells in the peripheral areas of cortex are called thymic nurse cells. The thymic nurse cells contain pockets of thymocytes (cortical and medullary) that secrete IL-7, which supports differentiation and proliferation of pre-T cells.

**Steps of Development**

Stem cells (pre-T cells) first colonize in the subcapsular cortical region of the thymus. Then, they develop into large actively proliferating lymphoblasts that possess the property of self-renewal:

1. Lymphoblasts differentiate and proliferate into pre-T cells.
2. Pre-T cells develop into T cells.
3. Pre T cells become Naïve T cells, that further proliferate to form T immunoblasts (Fig. 19.6).
4. There are 2 types of T immunoblasts. One set expresses CD8+ on the surface that develops into CD8+ cells (Tcells, or cytotoxic T cell) and the other set expresses CD4+ on the surface that develops into CD4+ cells (T helper T cell).
5. As cells mature, they migrate to the medulla, and mature T cells leave thymus via postcapillary venules located at the corticomedullary junction, and also via lymphatic vessels.
6. After processing and development in the thymus, the mature T cells migrate into the lymph nodes, spleen, bone marrow, other tissues and blood.
7. T cells participate in cell-mediated or cellular immunity.

**Changes during Development**

During their processing (differentiation and proliferation) in the thymus, they undergo two morphological and chemical changes that confer on them the ability to recognize and kill the antigens. These changes are formation of specific receptors and synthesis of chemokines:

1. **Formation of specific receptors on T cells**
   - The receptors to recognize the particular antigen are formed on T cell surface (Fig. 19.7), and are called T cell receptors (TCR). There are four types of polypeptides for TCR: α, β, γ and δ. However, only two sets are present in TCR. The combinations are either αβ or γδ. Accordingly, TCR are either αβ TCR or γδ TCR. The T cells with αβ TCR are called αβ T cells, and T cells with γδ TCR are called γδ T cells. 95% of circulating T cells are αβ T cells and only 5% are γδ T cells.

2. **Synthesis of chemokines to kill antigens**
   - The cells acquire capacities to from various chemokines that are capable of killing invading organisms. Important chemokines are lymphotxin and interferons.

**Types of T Cells**

There are three types of T cells: the helper T cells (T4 cells), cytotoxic T cells (T8 cells), and memory T cells.

**Helper T Cells (T4 Cells)**

The helper T cells are called T4 cells as they contain CD4 protein on their cell surface. They are known as helper or inducer cells as they assist in induction of both cellular and humoral immunity. There are two sets of helper T cells: **Type 1 helper cells** (TH1 cells) that assist in cellular immunity and **type 2 helper cells** (TH2 cells) that assist in humoral immunity.
immunity. Activated T_{H1} cells secrete IL-2 and γ-interferon, and T_{H2} cells secrete IL-4, IL-5, IL-6 and IL-10 (Fig. 19.7).

**Cytotoxic T Cells**

These are called T_{8} cells as they contain CD8 protein on their cell membrane. They are called cytotoxic or killer cells as they kill invading organisms by producing cytotoxicity. They are sometimes also referred to as suppressor T cells (T_{S}).

**Memory T Cells**

A small subset of T cells remains in the tissue as memory T cells. These cells remember the initial immunologic insult,
and on subsequent exposure to same challenges they are activated immediately without presentation of antigen by antigen presenting cells (see below) and proliferate to millions of T cells that instantaneously fight to eliminate the antigens. Memory T cells live a very long life, may be the entire life of the individual. Therefore, similar antigenic exposure at any time during the life of the person induces prompt and focused cell-mediated immune response.

**T Cell Receptor**

The T cell receptor (TCR) complex consists of seven polypeptide chains:
1. In the majority of (95%) of T cells, δ and β chains form the antigen-binding site of TCR (δβ TCR).
2. Each of these chains has a variable and a constant region similar to immunoglobulins.
3. The δ and β chains are linked together by a disulfide bond to form δ-β complex which is composed of five polypeptide chains.
4. The variable regions of δ and β chains bind antigen while CD3 converts this antigen recognition into intracellular activating signals.
5. In a minority of T cells, γ and δ polypeptide chains are present instead of δ and β chains (γδ TCR).

**TCR Gene Arrangement**

The genetic structure of TCR bears resemblance to that of immunoglobulin. The TCR β chain gene is located on chromosome 7 and TCR α chain gene is on chromosome 14:
1. Although all somatic cells contain T cell receptor gene in germ line configuration, rearrangement occurs only in T cells.
2. The TCR β gene consists of variable (V), diversity (D), joining (J) and constant (C) regions.
3. One segment each from V, D and J regions join together with deletion of intervening sequences. The rearranged gene is transcribed into mRNA. Rearrangement of other peptide chain occurs similarly.
4. As there are a number of V, D and J segments which code for amino acid sequences in variable region, it is possible to generate T cell receptor with different antigen specificities by various combinations during rearrangements.
5. Rearrangement of TCR β gene precedes the rearrangement of TCR α gene.

**T Cell Ontogeny**

Progenitor T cells from the bone marrow are transported to thymus where they undergo maturation. During maturation,
there is rearrangement of TCR genes, expression of some cell surface proteins, and acquisition of ability to distinguish self antigen from foreign antigens:

1. Initially immature cortical thymocytes express CD7, TdT and cytoplasmic CD3 (cCD3).
2. Those T cells which subsequently are going to form δ and β polypeptides (δβ TCR), first rearrange TCR β gene followed by TCR α gene.
3. Expression of δβ TCR occurs in association with expression of CD3 on surface of cells.
4. Initially both CD4 and CD8 antigens are acquired, but with further maturation cell retains either CD4 or CD8 antigen.
5. CD4⁺ cells are called as helper or inducer T cells whereas CD8⁺ cells are called cytotoxic T cells.
6. The mature T cells are released from thymus, circulate in peripheral blood and are transported to peripheral lymphoid organs.

Development of B Cells

The lymphocyte precursors that enter the bursa equivalents like fetal liver and bone marrow in mammals form B cells. They are called B cells as they develop in the bursa equivalent tissues (hence, bursa-dependent cells or B cells):

1. In the birds, a lymphoid tissue is present near the cloaca (the bursa of Fabricius) help in development and processing of B lymphocytes, where pre-B cells develop into B cells. However, in mammals there is no such bursa, but they have bursa equivalents.
2. During their development in bursa equivalent structures, B cells acquire characteristic surface molecules. They acquire receptors to recognize antigen and receptors for various cytokines, and most importantly the genes for immunoglobulin synthesis. First, the gene rearrangement occurs for heavy chain and then the gene rearrangement occurs for light chain of immunoglobulins.
3. Pre B cells become Naive B cell, that transform into Mantle Cell, Centroblast and Centrocyte. Centrcytes mature into plasma cell or transform to memory B cells (see Fig. 19.6)
4. Each B cell lineage is committed for making only one specific antibody against a specific antigen. This is the central theme of clonal selection theory of antibody production.
5. Pre B cells once mature in bursa equivalents migrate to lymph nodes, bone marrow, blood and other tissues like lymph nodes and lymphoid follicles.
6. In these structures (bone marrow, lymph nodes and lymphoid follicles), they further process to become B immunoblasts or centroblasts, those under on specific immunologic stimulation undergo further transformation to form plasma cells (Fig. 19.8).
7. Plasma cells produce large quantities of antibodies. Plasma cells are not normally found in blood. Antibodies formed by plasma cells kill or neutralize antigens.
8. A small set of B cells form memory B cells that on subsequent exposure to an antigen get readily converted into effective B cells (plasma cells) to carry out immuno-

![Fig. 19.8: Development of B cells. Note pre B cells migrate to lymph node or lymphoid follicles to become B immunoblasts or centroblasts, which further transform to form plasma cell.](image-url)
Antigens

Definition
Antigens are living organisms or substances that on entry into the body induce specific immunological reactions. Antigens have two important properties: immunogenicity and reactivity:

1. **Immunogenicity** is the ability to provoke an immune response i.e., to stimulate the production of specific antibody or proliferation of specific T cells or both.
2. **Reactivity** is the ability of the antigen to react specifically with an antibody or a cell or both.
3. An antigen that possesses both the properties is defined as a complete antigen.
4. An antigen that has the reactivity but lack immunogenicity is called partial antigen or a hapten.

Nature of Antigen
Antigens may be the entire micro-organism like a bacterium or a virus, or a part of the organism like capsule of the virus, flagella of bacteria or the cell wall of the organism. The antigen may also be a nonmicrobial substance, such as pollen, egg white, transplanted tissue or incompatible blood cells.

Chemical Nature: Antigens are large complex molecules. Usually, they are protein in nature. However, nucleoproteins, glycoproteins, or large polysaccharides also behave as antigens. Usually T cells respond to protein antigens, whereas B cells respond to proteins and non-protein antigens.

Antigenic Determinant
Specific portion of an antigen that triggers the immunological reaction is called antigenic determinant or epitope. Usually an antigen possesses many antigenic determinants, each of which induces production of a specific antibody or proliferation of a specific set of T lymphocytes.

MHC Antigens
Major histocompatibility complex or MHC antigens are self-antigens that help in identifying and rejecting the foreign antigens:

1. They are also called HLA antigens (human leucocyte associated antigens), as they were first identified on the membrane of leucocytes. However, afterward they were found to be present on the surface of all the body cells except in red cells (remember, red cells contain blood group antigens).
2. Like blood group antigens they are chemically glycoproteins. They are made up of α and β subunits.
3. Though MHC antigens are responsible for rejection of transplanted tissues, their normal function is to identify the foreign antigens and present them to the T cell for induction of cellular immunity.
4. The recognition of an antigen is the first and foremost step in the process of activation of immunological responses, especially for initiation of cellular immunity.

Types of MHC Antigens
There are two types of MHC antigens: type 1 or MHC I, and type 2 or MHC II antigens.

- **MHC I antigen**: MHC I antigens are present on the cell membranes of all body cells except red cells. MHC I is made up of three α and one β subunits.
- **MHC II antigen**: MHC II antigens are present on the surface of antigen presenting cells (APCs), thymus cells and activated T cells. MHC II is made up of two α and two β subunits.

Mechanism of Action of MHC Antigens
The proteins in the cells are continuously broken down to their peptide fragments. MHC I molecules pick up the peptide fragments containing 8–10 amino acids, whereas MHC II molecules pick up peptides containing 13–17 amino acids:

1. When a peptide fragment of a self protein is picked up by the MHC antigen and expressed on the surface of the APC along with MHC proteins, T cells ignore it.
2. However, when the peptide fragment is of a foreign protein, T cells recognize it and get activated that induce cell-mediated immunological responses.

Significance of HLA Antigens
1. They are important as histocompatibility antigens in organ transplantation.
2. HLA antigens play a major role in recognition of foreign antigens and in immunity.
3. In transfusion medicine, they are responsible for autoimmunization against platelet antigens and refractoriness to platelet transfusion, febrile transfusion reactions and graft versus host disease.
4. A relationship exists between presence of some HLA antigen and susceptibility to certain diseases.
5. HLA antigen typing can also be used for paternity testing.

Recognition of MHC-Ag Complex by TCR
The MHC protein-Antigen complex on the surface of the APCs binds to appropriate receptor on T cells. Therefore, receptors on the T cell should recognize a wide variety of protein complexes (Figs. 19.9A and B). As discussed above, TCR is made up of α and β subunits that form heterodimers to recognize the MHC proteins and the antigen fragments with which they are combined. These cells are called αβ T cells. Details of TCR recognition of antigen presented by APCs are discussed below. About 10% of
circulating T cells are αδ T cells. These T cells are prominent in the mucosa of the gastrointestinal tract. The αδ T cells form a link between the innate and acquired immune systems, and help in secretion of cytokines.

**CELLULAR IMMUNITY**

Cell mediated immunity is mediated by T cells. Cellular immunity is particularly effective against *intra-cellular organisms* like viruses, parasites and fungi, cancer cells, tumor cells, and transplanted tissues. This immunity is induced following entry of an antigen into the body. However, antigen directly does not induce cellular immunity unless presented appropriately to the T cells. The antigen is recognized and processed by the antigen presenting cells (APCs) and then presented to the immunocompetent lymphocytes that are activated and proliferated to destroy the antigen.

**Steps of Cellular Immunity**

Mechanism of cellular immunity includes following steps:
1. Antigen recognition, processing and presentation
2. Activation and proliferation of T cells
3. Elimination of the invader

**Antigen Recognition, Processing and Presentation**

There are different varieties of antigens but human body has the ability to recognize each of them. This recognition ability is innate, which develops without prior exposure to the antigen. The precursor stem cells of lymphocytes differentiate into millions of different T and B cells. Each T or B cell type has the ability to respond to a particular antigen. On entry into the body, antigens bind with an appropriate receptor present on the B cell surface and activate the B cells:
1. However, for activation of T cell to occur the antigen should be processed and presented by the APCs to the appropriate receptors on the T cell.
2. The cells that process and present antigens to the T cells are called antigen presenting cells (APCs).
3. APCs include macrophages, dendritic cells and B cells.
4. APCs are present in more numbers specifically at locations that are the usual sites for antigen entry into the body like skin, and mucous membrane of respiratory, gastrointestinal and genitourinary tracts.

**Steps of Antigen Presentation**

There are five major steps in antigen presentation: ingestion of antigens, digestion of antigen, fusion of vesicles, binding of peptide fragments to MHC molecule, and incorporation of antigen-MHC complex into the cell membrane of APC (Fig.19.10):

1. **Ingestion of the antigen:** Antigens are *ingested by APCs* by the process of endocytosis.
2. **Digestion of the antigen and formation of vesicles:** Following phagocytosis, antigen is digested partially by the lysosomal enzymes and form *phagosome* within the cytoplasm of APCs. The phagosomal vesicles contain peptide fragments of antigens. Simultaneously, MHC II molecules are formed in the cell (*MHC vesicles*).
3. **Fusion of vesicles:** The phagosomal vesicles containing peptide fragments of antigen combine with the vesicles containing MHC II molecules. These two vesicles merge to form single vesicle.
4. **Binding of peptide fragments with MHC II molecules:** Following fusion of vesicles, antigen fragments bind with MHC II molecules. The fused vesicles containing MHC II and peptide fragments (MHC-Ag complex) undergo exocytosis.

5. **Incorporation of antigen-MHC complex into the cell membrane of APC:** During the process of exocytosis, the peptide fragments (antigens)–MHC-II complex get incorporated into the cell membrane of APC:
   - An APC containing the MHC-antigen complex migrate into the lymphatic tissue or circulate in the blood.
   - When APC containing this MHC-Ag complex comes in contact with a particular T cell, the complex attaches with T-cell receptor (Fig. 19.11).
   - Consequently, the T cell gets activated that triggers cell mediated immunity.

### Immunologic Synapse

For antigen recognition, antigen presenting cell (APC) presents the antigen to T cell. T cell receptors are surrounded by adhesion molecules. Proteins bind to complementary proteins in the APC when the two cells transiently join. The junction between T cell and APC is called immunologic synapse. The activity at this synapse permits the T cell activation to occur. It is now generally accepted that two signals are necessary to produce activation. One is produced by the binding of the digested antigen to the T cell receptor. The other is produced by the joining of the surrounding proteins in the synapse. If the first signal occurs but the second does not, the T cell is inactivated and becomes unresponsive.

### Activation and Proliferation of T Cells

The cell-mediated immunity depends on the activation of T cells by a particular antigen. An activated T cell undergoes differentiation and proliferation into the **effector T cells**, which eliminates the antigen. Activation of cellular immunity involves **two steps**: activation of T cells and proliferation and differentiation of T cells (Fig. 19.12).
Activation of T Cells
There are receptors on the T cell surface, known as TCR. When TCRs come in contact with MHC-antigen complex located on the surface of the APCs, T cells are activated (see Fig. 19.11). This is the first step in induction of cellular immunity.

Proliferation and Differentiation of T Cells
The activated T cells with proper costimulation by inducer cells (type 1 Helper Cells), undergo proliferation and differentiation into two sets of T cells: the cytotoxic T cells, and the memory T cells:
1. **The cytotoxic T cells** are also called T₈ cells, as they display CD8 proteins on their cell membrane. They are also known as **killer T cells** as they kill the microbes.
2. The **memory T cells** are programmed to recognize the original invading antigen in future, when the body is reexposed to the similar antigens. Therefore, when the same antigen invades the body at a later date, thousands of T cells are formed immediately and the invader is killed promptly.

Elimination of the Invader
The cytotoxic T cells kill the invading microbes by following three major mechanisms:
1. **Cytolysis**: Killer cells synthesize and secrete **perforins**, and incorporate them into the membranes of the invading organisms. Perforins are water channels that freely allow water to enter the microorganism along the osmotic gradient. The invading cell swells and finally undergoes **osmotic lysis**.
2. **Lymphotoxin**: Activated T cells secrete **lymphotoxins** that kill the microbes. Tumor necrosis factor (TNF)-β is an important known lymphotoxin.
3. **Interferons**: Cytotoxic T cells secrete gamma-interferons that are mainly **antiviral**. They also increase the phagocytic activity of neutrophils and macrophages by promoting opsonization.

Cellular immunity is activated mainly against intracellular pathogens and tumor cells, as in:
1. Viral infections
2. Fungal infections
3. Tumor cells, especially cancer cells
4. Transplanted cells
5. Chronic bacterial infections like tuberculosis, brucellosis, etc.
6. Parasitic infections

Humoral Immunity
Humoral immunity is the immunity mediated by antibodies. Antibodies are produced by plasma cells. Plasma cells are normally not present in the blood, but are formed from B cells on specific antigenic stimulation:
1. In response to an antigen, the B cells are activated and are differentiated into plasma cells.
2. Normally, B cell activation does not depend on presentation of antibody by APCs.
3. Antigens can directly stimulate B cells. However, antigens on the surface of APCs also activate B cells.

Steps of Humoral Immunity
Activation of humoral immunity involves **six steps** (Fig. 19.13):
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1. Presentation of antigen
2. Activation of B cells
3. Differentiation of B cells into plasma cells
4. Proliferation of plasma cells and antibody production
5. Killing of the invaders by antibodies that include activation of complement system
6. Formation of memory B cells and subsequent immunological responses

**Presentation of Antigen**
Antigen presenting cells in humoral immunity include B cells themselves. Therefore, antigen presentation through APCs is **not a must** for induction of humoral immunity. However, antigen combined with MHC II molecules on the surface of APC (Ag-MHC complex) may also be presented to B cells.

**Activation of B Cells**
When an antigen binds directly to the receptors present on the surface of the B cells, the B cells are activated. B cells are also activated when they come in contact with MHC-antigen complex presented by the APCs:
1. This process of activation is accentuated by the **costimulation from type 2 helper cells**.
2. T_{H,2} cells stimulate B cell by secreting IL-2, IL-4, and IL-5.
3. Activated B cells form plasma cells and memory B cells.

**Differentiation of B Cells into Plasma Cells**
The activated B cell enlarges in size that undergoes complete transformation to become plasma cell. This is not a simple differentiation as occurs for T cells in cellular immunity:
1. The cell is transformed completely in structure and function.
2. Plasma cell has larger cytoplasm containing numerous rough endoplasmic reticulums.
3. The enlarged B cell appears almost like a **lymphoblast**, called **B immunoblasts** (see Figs. 19.6 and 19.8). Therefore, sometimes this differentiation is called as **blast transformation**. Co-stimulation by T helper cells (T_{H,2} cells) facilitates the process of transformation.

**Proliferation of Plasma Cells and Antibody Production**
Activated B cells are differentiated into plasma cells. Plasma cells then undergo proliferation to form millions of plasma cells. Plasma cells secrete a large quantity of antibodies:
1. A specific antigen stimulates activation of specific B cells that undergo proliferation of a selective clone of plasma cell.
2. This is called **clonal selection** of plasma cell.
3. Specific clone of plasma cells produce specific antibodies.

**Killing of the Invaders**
Antibodies attack antigens and kill organisms (destroy antigens) by following mechanisms:
1. **Neutralizing antigen**: Antibodies neutralize the antigens. For example, antigens detoxify the bacterial toxins and prevent them to induce immunological reactions.
2. **Immobilization of microbes**: Antibodies prevent the motility of bacteria thereby inhibit their spread to the surrounding tissue.
3. **Activation of complement system**: Antibodies (antigen-antibody complex) activate the classical pathway of complement system by activating C1 (for details, see below) and complements kill organisms.
4. **Precipitation of antigens**: Antibodies cause agglutination and precipitation of antigens. By this mechanism, soluble antigens become insoluble. Insoluble antigens are easily phagocytosed by phagocytic cells.
5. **Facilitation of phagocytosis**: Antibodies enhance phagocytosis by opsonizing the antigen. Opsonization is the process by which microbes or antigens are made tasty to phagocytes for ingestion by coating antigen with opsonins like antibody, complement proteins, etc.
6. **Providing immunity to newborns**: IgG antibody can easily cross placenta and enter fetal circulation from the maternal blood. Thus, it provides immunity in newborns. IgA antibody from the maternal milk also provides immunity to the newborn.

**Formation of Memory B Cells and Subsequent Immunological Responses**
A small subset of activated B cells differentiates into memory B cells. However, this differentiation is not like blast transformation. Memory B cells normally remain inactive, unless stimulated by the similar antigens. They respond quickly and vigorously to the same antigens on subsequent exposures. The immunological responses on second and subsequent exposures to antigen are much more in intensity and duration than on first exposure. This is called **secondary immunological response** (see below). These memory B cells live a longer life, may be the life of the individual.

**Types of Humoral Immune Responses**
There are two types of immune responses: the primary response, and the secondary response.

**Primary Response**
When antigen enters into the body for the first time, the immune response induced is called primary response:
1. The antibodies are formed slowly with a latent period of about four days to four weeks.
2. The concentration of antibodies rises gradually and attains a smaller peak (Fig. 19.14).
Fig. 19.14: Primary and secondary immunological responses.

3. Though IgM and IgG antibodies contribute to the primary response, the response is **mainly due to IgM**.
4. Also, antibody titer in primary response returns back to normal within few days to weeks.

**Secondary Response**

When the same antigen enters the body for the second time, the immune response triggered is the secondary response:
1. It occurs speedily and intensely, which is due to the **immunological memory**.
2. Persistence of memory B and T cells help in carrying out the secondary response.
3. Antibodies are formed rapidly. Therefore, concentration of antibody rises fast (fast rising slope) and reaches a greater peak (see Fig. 19.14).
4. Antibody titer falls very slowly and never returns to normal, rather remains elevated for a longer duration. The antibodies in the secondary response are primarily of IgG type.

**Complement System**

There is a group of plasma proteins designated as complement proteins as they complement the effects of antibodies in destroying antigen. These proteins constitute the complement system:
1. Though there are more than 30 complement proteins, eleven are categorized in this system and are designated as C1–C9. C1 is further divided into C1q, C1r, and C1s (thus, total is 11).
2. These are a system of plasma enzymes that are normally present in the inactive form.
3. Once activated, they exert their immunological and inflammatory actions and boost the humoral immune mechanism.

**Mechanisms of Activation of Complement System**

There are **three mechanisms** of complement activation: classical pathway, alternative pathway and mannose-binding lectin pathway.

**Classical Pathway**

In this process, C1 binds with immunoglobulin attached to an antigen. This binding triggers a sequence of events that activates other complement proteins:
1. The mechanism of activation of complements is similar with the activation of intrinsic pathway of blood coagulation (the enzyme cascade theory), in which one activated protein activates the other protein (see Fig. 19.15).
2. Once, antigen-antibody complex activates C1, activated C1 (C1a) activates C2 and C4. Activated C2 and C4 (C4b, and C2a) activates C5, which (C5a) in turn activates C6, C7, C8, and C9.
3. These activated complement proteins kill microorganisms and facilitate inflammation by **following mechanisms:**
   - C3a, C4a, and C5a: Promote **phagocytosis** by facilitating chemotaxis, releasing histamine from mast cells, and causing arteriolar dilation.
   - C3b: Promote **opsonization** (makes the bacteria tasty to the phagocytes).
   - C5b, C6, C7, C8, and C9: Cause **cytolysis** of the microbes.

   The activated complements incorporate perforins (pore-forming molecules) into the membranes of microbes. This results in transport of ions and water into the microbes through the pores, which finally result in **osmotic lysis** of microbes.

**Alternative Pathway**

This is also called **properdin pathway** as the key protein in the plasma for this pathway is the **properdin**. In this system, a circulating protein called **factor-1**, recognizes the polysaccharide unit present on the surface of microorganisms (but not in the normal mammalian cells). The interaction of factor-1 with the polysaccharide on microbes triggers a reaction that activates C3 and C5. Once C3 is activated, it activates other complement proteins (see Fig. 19.15).
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Mannose-binding Lectin Pathway

The plasma protein lectin binds with mannose group on the surface of bacteria, which activates complement proteins.

The complement system activates B cell, and thus serves as the link between innate and acquired immunity. Following apoptosis, complement proteins help in disposing the debris.

Antibodies

Antibodies are immunoglobulins (Ig). Ig binds specifically with the antigenic determinant of the antigen. This binding triggers the production of further antibodies. Binding of antigen with Ig is like the key fitting into the lock. There are specific antigen receptors on B cells. Binding of specific antigen with the specific receptor on B cell, results in production of specific antibody.

**Types of Antibodies**

There are five types of antibodies: IgG, IgA, IgM, IgD, and IgE (Table 19.3). They are produced in response to specific stimuli (specific antigens). A specific clone of B cells that are activated by specific antigenic stimulus produce specific antibody, i.e. monoclonal antibody (details, given below). The concentration of IgG in plasma is maximum (1000 mg%), followed by IgA (200 mg%), IgM (120 mg%), IgD (3 mg%), and IgE (0.05 mg%).
Structure of Antibodies

Most antibodies have four polypeptide-chains. The two of them are heavy (H) chains and the other two are light (L) chains (Fig. 19.16):

1. The two H chains are identical to each other and contain about 450 amino acids, which may be either of α, γ, µ, δ and ε chains. The two L chains consist of about 220 amino acids. The polypeptides in L chain are κ and λ chains that are same in all antibodies. L chains are also identical to each other.
2. There may be some additional polypeptide chains in certain antibodies.
3. Antibodies are glycoproteins and their carbohydrate residue is attached to the heavy chains.
4. Disulfide bonds bridge the L and H chains. H chains are attached to each other at the middle by disulfide bonds. At this point, H chains display flexibility. Therefore, this region of H chain is called hinge region.
5. The tip of H and L chains is called variable region, as this region is different for each type of antibody. The variable region contains antigen binding site. This part of antibody recognizes and specifically attaches to a particular antigen.
6. The flexibility at hinge region allows the antibody to bind to two antigenic determinants (of antigens).
7. The rest of the H and L chain is called constant region, as this part of antibody is same in all types of antibodies.

Functions of Antibodies

Different antibodies play different role in immunological responses:

- **IgG**: Complement fixation by classical pathway
- **IgA**: Localized protection in body secretions like tear, salivary and intestinal secretions, etc.; complement fixation by alternate pathway
- **IgM**: Complement fixation by classical pathway
- **IgD**: Antigen recognition by B cells
- **IgE**: Histamine releases from mast cells and basophils; also has reagin activity

MECHANISMS OF SELF-RECOGNITION (Immunological Tolerance)

Self recognition is the process by which own MHC proteins do not recognize self antigens (identify only foreign antigens). This is due to the mechanism of immunological tolerance (Application Box 19.4). Immunological tolerance is defined as unresponsiveness of the individual to an antigen. Immunological tolerance to self antigen provides the physiological basis for self recognition. Development of immunological tolerance is based on the process of negative and positive selections.

**Scientists contributed**

The Nobel Prize in Physiology or Medicine for the year 1960 was awarded to Australian scientist Sir Frank Macfarlane Burnet and Brazilian scientist Sir Peter Brian Medawar for their discovery of the mechanism of acquired immunological tolerance.

**Negative Selection**

During the process of development of T cells in thymus, cells with TCR that recognize peptide fragments of self proteins are eliminated while cells with TCR that do not recognize peptide fragments of self proteins are retained. This occurs either due to deletion (the self-reactive T cells undergo apoptosis) or anergy (the self-reactive T cells remain unresponsive to immunologic stimulation).

**Clonal Deletion**

During embryonic life, the clone of T cell and B cells containing receptors that react against self antigens are selectively removed from the body during their thymic and bursal development, so that they are not available in post-natal life to react against self antigens. These self reactive lymphocytes undergo apoptosis.

**Clonal Anergy**

The self-reactive clone of T and B cells those remain alive during post-natal life loose the capacity to be activated in
response to self antigens. This is because they lose the activity of their receptors and lymphokine systems to react against self antigens, which may be due to the immune suppression of these cells by body’s self regulating systems. This state of idleness is called clonal anergy.

Positive Selection

Immunological tolerance is also achieved by positive selection in which the T cells that are capable of recognizing self-MHC molecules during their thymic development survive and those that do not recognize undergo apoptosis. The T cells recognize MHC - self antigen complex as the self antigens and do not react against them.

Application Box 19.4

Fetal tolerance: Though fetus that grows in the mother’s uterus is genetically a different organ (fetal graft) and foreign tissue immunologically, is not rejected during pregnancy like rejection of a transplanted organ. This is an example of immunological tolerance, called as fetal tolerance. Fetal tolerance is due to four mechanisms:

1. During the formation of placenta, trophoblast cells that separate the mother and fetus do not express MHC I and II antigens, and therefore, lose their immunogenicity. Instead, placenta expresses, HLA-G a nonpolymorphic antigen. Therefore, antibodies against fetal tissue do not develop.
2. Placenta has Fas ligand on its surface which attaches to the T cells. This attachment causes apoptosis of T cells that are supposed to react against fetal tissue.
3. Alpha fetoprotein (AFP) that is formed during fetal development causes suppression of T and B cells that react against fetal graft.
4. High level of progesterone during pregnancy is believed to suppress immunity against fetal tissue.

APPLIED PHYSIOLOGY

Organ Transplantation

Tissue or organ transplantation involves replacement of an injured or diseased tissue or organ by a new tissue or organ. Sometimes, it is required for the treatment of debilitating diseases like renal failure, liver failure, etc. Commonly transplanted organs are skin, kidney, bone and liver. However, the transplanted tissue is rejected as the recipient develops an immune response against the transplanted organ, which is a foreign tissue to him:

Scientists contributed

The Nobel Prize in Physiology or Medicine for the year 1990 was awarded to American transplant surgeon Joseph E Murray and E Donnall Thomas for their discoveries concerning organ and cell transplantation in the treatment of human disease.

1. Transplant rejection is due to the activation of cellular immunity.
2. The immune system recognizes the transplanted tissue as a foreign organ and initiates a series of immunological reactions that finally rejects the tissue.
3. The rapidity at which the transplanted tissue is rejected depends on degree of matching of MHC antigen (HLA antigen) of donor with the HLA antigen of the recipients.

Types of Transplants

1. Autograft: Transplantation of a tissue from one part of the body to another part of the same individual is called autograft. The best example is the skin graft on a burnt or scarred surface of the patient by taking a flap of skin from other part of his body. Autografts are never rejected.
2. Isograft: Transplantation of organs between the genetically identical individuals. The best example is the transplantation of tissues from the identical twin. Isograft is also not rejected.
3. Allograft: This is the transplantation of organs between individuals of the same species, but with different genetic background. The example is transplantation from an individual other than identical twin. Allograft is rejected. If the graft is taken from the very close relations, rejection occurs slowly.
4. Xenograft: This is a transplant from or between different species. For example, transplantation of organ between animal and man. Commonly used xenograft is the physiological dressing using animal skin over severe burn surfaces. Xenograft is rejected fast.

Prevention of Transplant Rejection

A number of treatments are available to prevent rejection of a transplanted organ. These are:

1. Immunosuppressive drugs: The commonly used drug is azathioprine. This is a purine antimetabolite that kills T lymphocytes. However, the individual on azathioprine is susceptible to severe infection because of immunosuppression.
2. Glucocorticoid therapy: Glucocorticoids have strong immunosuppressive activity. They inhibit proliferation of cytotoxic T cells by inhibiting the production of interleukin 2 (IL-2) from T cells.
3. Antilymphocyte globulins: These are monoclonal antibodies produced against T lymphocytes. They inhibit T cell activity.
4. Antibiotics: Antibiotics usually used for the prevention of transplant rejection are cyclosporine, tacrolimus and rapamycin. Cyclosporine is a fungal extract and tacrolimus is an antibiotic of fungal origin. They prevent T cells from transcribing the IL-2 gene by preventing dephosphorylation of NF-AT, a transcription factor.
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*Rapamycin* prevents activation of IL-2 receptor from producing T cell differentiation and proliferation.

5. **Monoclonal antibodies**: They prevent activation and proliferation of T cells.

**Monoclonal Antibodies**

Monoclonal antibodies are antibodies prepared from a single clone of B or plasma cells. They are used for immunotherapy of different diseases or to prevent rejection of transplanted tissue. They are obtained by fusing a B cell with a tumor cell that produces antibodies in large quantity.

**Method of Production**

An animal is immunized by injecting a particular antigen. The animal is then sacrificed and the antibody producing **cells** are extracted from the spleen of the animal:

1. The antibody producing cells are fused to the myeloma cells that are obtained from B lymphocyte tumor in a patient suffering from multiple myeloma.
2. Fusion of myeloma cell with antibody producing cell results in formation of a hybridoma which grows to become an antibody producing tumor.
3. The fused cells (hybridoma cells) are separated and every single cell is allowed to form a clone of cells.
4. The single clone of hybridoma cells produce specific antibody consisting of heavy or light chains of spleen cell or of myeloma cells. These are called monoclonal antibodies.

**Uses of Monoclonal Antibody**

Monoclonal antibodies are used in a wide variety clinical spectrum:

1. For measurement of drug level in patient’s blood.
2. Diagnosis of allergic diseases, hepatitis, sexually transmitted diseases, etc.
3. Detection of cancer in the early stage.
4. Preparation of vaccines.
6. Treatment of autoimmune diseases.

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**Immunotherapy**

Immunotherapy is the treatment that aims at induction of the immune system of an individual. This is called **immunostimulation** or **immune enhancement**. This is especially helpful for the treatment of different malignant diseases. There are different types of immunotherapies: cellular immunotherapy, cytokine therapy, antibody therapy and adjuvant therapy.

**Cellular Immunotherapy**

Cells that have anti-tumor activity are injected into the blood of the cancer patient. These cells destroy the malignant tumor cells:

1. Patient’s own inactive cytotoxic T cells or NK cells are used for this purpose.
2. In practice, these cells are removed from the blood of the patient and cultured with IL-2, which activates them.
3. These activated cells are called lymphokine-activated killer cells (**LAK cells**). LAK cells are then transfused into the patient’s blood.

**Cytokine Therapy**

Different cytokines (see below) can be used in the treatment of different diseases. The best example is **interferon**. Interferons are especially used for the treatment of viral infections or malignancies. **Interleukin-2** is also used for the treatment of cancer.

**Antibody Therapy**

Monoclonal antibodies are extensively used for the treatment of malignancies or for preventing transplant rejection.

**Adjuvant Therapy**

Adjuvant is a compound, which is introduced with an antigen to enhance immune responses nonspecifically against the antigen. This was first discovered by Freund, hence called **Freund’s adjuvant**:

1. Adjuvants may be complete adjuvants like tubercular bacilli and gram negative bacilli or incomplete adjuvants like aluminum hydroxide, aluminum phosphate, mineral oil, etc.
2. Adjuvants nonspecifically stimulate lymphocytes and macrophages.
3. However, adjuvants may cause delayed hypersensitivity reactions.

**Immunological Disorders**

**Allergy**

This is a hyper-reactive response of the body to an antigen which is usually tolerated by others. There are two types of allergies: the **local allergy** and the **systemic allergy** (anaphylaxis).
Local Allergy
When allergic reactions are limited to an area of the body, are called localized allergy. Examples are swelling of the lips, eczema, hives, urticaria, etc.

Systemic Allergy
When allergic reaction is generalized (affects systemic functions), is called systemic allergy or anaphylaxis. For example, anaphylactic reaction that occurs due to injection of penicillin or xylocaine in sensitive individuals. Anaphylaxis is an acute medical emergency, which may results in shock, called anaphylactic shock.

Hypersensitivity Reactions
There are four types of hypersensitivity reactions:

Type I Reaction or Anaphylaxis
In this reaction, the individual over-reacts to a sensitized allergen. Usually it occurs on re-exposure. But in highly sensitive individual, it occurs on first exposure:
1. The antigen-antibody complexes release histamine and slow-releasing substance A from mast cells and basophils.
2. Histamine produces bradycardia and rapid vasodilation leading to acute hypotension.
3. Myocardial contractility is also inhibited resulting in reduced cardiac output. It is an acute medical emergency, which requires prompt medical support.

Type II Reaction or Cytotoxic Reaction
This reaction is caused by IgG or IgM antibody, which is directed against antigen present on person’s red cells. Incompatible blood transfusions, hemolytic diseases of newborn and autoimmune hemolytic anemias are examples. This causes acute hemolysis and requires early medical intervention.

Type III Reaction or Immune Complex Disease
In this reaction, the antigen and antibodies form complexes that escape phagocytosis. These immune complexes are deposited on the basement membrane of the blood vessels. They activate complements and induce inflammation. Examples are glomerulonephritis, systemic lupus erythematosus (SLE) and rheumatoid arthritis.

Type IV Reaction or Cell Mediated Reaction or Delayed Hypersensitivity Reactions
These reactions are mediated by macrophages that are activated by T cells.
1. Allergens (antigens) are taken up by APCs and presented to T cell.
2. This causes proliferation of T cells, some of which migrate to the location of allergen entry into the body. There, they secrete cytokines that activate macrophages and induce inflammatory reactions.
3. The appropriate example is the skin test for tuberculosis.

Autoimmune Diseases
Normally, the immune system does not react against self proteins. However, in some conditions, immune system fails to neglect self antigens and triggers immune reaction against body’s own antigens. This results in autoimmune diseases:
1. This may be due to the persistence of T cells or B cells that are active against self antigens. Especially, failure of the process that removes antibodies against self antigens leads to formation of autoantibodies (Clinical Box 19.2). Thus, B cells produce antibodies against self antigens.
2. The common examples are SLE, rheumatoid arthritis, insulin-dependent diabetes mellitus, myasthenia gravis, Grave’s disease, etc.
3. Autoantibodies may eliminate the receptors or activate the receptors. For example, in myasthenia gravis, antibodies are formed against nicotinic cholinergic receptors that destroy the receptors, whereas in Grave’s disease, antibodies formed against TSH receptors activate the receptors.

Immunodeficiency States
Immunodeficiency diseases may be broadly classified into two categories: congenital or primary and acquired or secondary.

Congenital Immunodeficiency Syndromes
There are list of diseases in which severe combined immunodeficiency (SCID) occurs due to defects in development in either cellular or humoral immunity:
1. **Autosomal recessive SCID:** In this syndrome, stem cells (pre-T cells) in thymus fail to develop into mature T cells. Therefore, severe deficiency occurs in the cellular immunity.

2. **X-linked SCID:** In this disease, pluripotent stem cells fail to develop into progenitor cells of lymphoid series. Therefore, severe deficiency occurs in the development of both humoral and cellular immunity.

3. **X-linked agammaglobulinemia:** In this defect, pre-B cells fail to develop into B cells. Therefore, severe deficiency of humoral system of immunity ensues.

4. **MHC class II deficiency:** Deficiency of MHC II antigen leads to deficiency of CD4 cells. Thus, both cellular and humoral immunities fail to develop.

**Acquired Immunodeficiency Syndrome (AIDS)**

AIDS occurs due to infection by human immunodeficiency virus (HIV). HIV is a retrovirus that binds to CD4 protein on the surface of helper cells and decreases number of circulating T4 cells:

1. As helper cells are required for induction of both cellular and humoral immunity, HIV infection causes severe immunodeficiency state that predisposes the individual to all type of serious infections and malignancies. Consequently, the disease becomes a dangerous as cancer.

2. The usual clinical features are fever, lymphadenopathy, sore throat, diarrhea and weight loss.

3. Oral candidiasis, anogenital herpes simplex infection, Pneumocystis carinii pneumonia, cryptococcus meningitis and cytomegalovirus infections are common.

4. Finally, the patient dies due to secondary infection or cancer.

5. The commonest malignancy is Kaposi’s sarcoma.

6. The disease is transmitted by blood, semen, or vaginal secretions or through breast feeding. The usual mode of transmission is sexual contact.

7. Though there is no definite treatment, interferon, azathioprine and protease inhibitors are used to reduce the severity. Efforts have been made recently to develop vaccines to prevent AIDS.

**Cytokines**

Cytokines are a group of hormone like substances that usually act in a paracrine fashion to control immunological responses. They are secreted from activated lymphocytes, macrophages, endothelial cells, glial cells, etc. They are classified into: interleukins, tumor necrosis factors (TNF), interferons, transforming growth factors (TGF) and GM-CSF (Table 19.4).

**Interleukins**

There are different types of interleukins. However, physiologically important interleukins are **IL-1 to IL-13:**

1. They are produced by macrophages, activated T cells, APCs, NK cells, mast cells and other somatic cells.

2. They assist in proliferation of B and T cells, activation of leucocytes, lymphopoiesis, erythropoiesis, thrombopoiesis and leucopoiesis.

3. The specific interleukins have specific functions. For example IL-1 produces fever, induces anorexia, increases neutrophil count and decreases lymphocyte count. Though it increases capillary permeability, it produces hypertension.

**Tumor Necrosis Factors (TNFs)**

There are two types of TNFs, TNF-α, and TNF-β. TNF-α is produced by activated macrophages. It acts like interleukin-1. It produces vascular thrombosis and tumor necrosis. TNF-β is produced by activated type 1 helper cells. The physiological effects are same as TNF-α.

**Interferons (IFN)**

There are three types of IFNs: IFN-α, IFN-β, and IFN-γ.

**IFN-α:** This is produced by macrophages, neutrophils, and other somatic cells. It prevents growth of viruses (antiviral). It also activates macrophages and NK cells, and induces the expression of type I MHC on all somatic cells.

**IFN-β:** Same as IFN-α.

**IFN-γ:** Secreted by activated type 1 helper cells and NK cells. It induces expression of MHC I on all somatic cells and MHC II on APCs. It activates macrophages, neutrophils and NK cells. It stimulates cellular immunity. It is also antiviral.

**GM-CSF**

Functions of GM-CSF have been discussed in detail in ‘leucopoiesis’.

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>Source</th>
<th>Cell target</th>
<th>Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL1</td>
<td>Macrophage and monocyte Fibroblasts, endothelial cells Some epithelial cells</td>
<td>All cells</td>
<td>Activation of cellular immunity, activation of inflammation, and hemopoiesis, expression of adhesion molecules, Emigration of neutrophils and macrophages, role in fever and shock, hepatic production of acute phase protein</td>
</tr>
</tbody>
</table>

Contd...
### Chapter 19: Physiology of Immunity

#### T/Cytotoxic Factors

<table>
<thead>
<tr>
<th>IL2</th>
<th>T&lt;sub&gt;h&lt;/sub&gt;1 cells</th>
<th>T cells</th>
<th>Stimulates cellular immunity, NK cells and macrophages</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL3</td>
<td>T cell</td>
<td>Progenitor cells</td>
<td>Activates hemopoiesis</td>
</tr>
<tr>
<td>IL4</td>
<td>T&lt;sub&gt;h&lt;/sub&gt;2 cells, mast cells, basophils and eosinophils</td>
<td>B cells</td>
<td>Stimulates humoral immunity, activates monocytes, IgE class switching</td>
</tr>
<tr>
<td>IL5</td>
<td>T&lt;sub&gt;h&lt;/sub&gt;2 cells, mast cells and eosinophils</td>
<td>Eosinophil</td>
<td>Stimulates eosinophil development</td>
</tr>
<tr>
<td>IL6</td>
<td>T&lt;sub&gt;h&lt;/sub&gt;2 cells, fibroblast Monocytes and macrophages, Same as IL1</td>
<td>T and B cells epithelial cells hepatocytes, Monocyte/ Macrophage</td>
<td>Differentiation and growth of T and B cell, Hepatic production of acute phase proteins (ARP), activation of lymphocytes</td>
</tr>
<tr>
<td>IL7</td>
<td>Monocyte/ Macrophages</td>
<td>Nutrophil, Basophil T cells Monocyte Macrophages Endothelial cells</td>
<td>Chemotaxix of neutrophils and basophils, induces migration of Nutrophil, Macrophages and T cells Stimulates release of histamine from basophils stimulates angiogenesis</td>
</tr>
<tr>
<td>IL11</td>
<td>Osteoblasts, fibroblasts</td>
<td></td>
<td>Stimulates hemopoiesis and production of ARP</td>
</tr>
<tr>
<td>IL12</td>
<td>Macrophage and B cell Dendritic cells and nutrophils</td>
<td>T cell NK cells</td>
<td>Stimulates synthesis of IFNγ; activates T&lt;sub&gt;h&lt;/sub&gt;1 cell induces formation of T helper cells and killer cells Promotes CTL cytolytic activity, increases IFNγ production, decreases production of IL17</td>
</tr>
<tr>
<td>IL17</td>
<td>CD4&lt;sup&gt;+&lt;/sup&gt; T cells</td>
<td>Fibroblast Endothelial cells</td>
<td>Increases secretion of other cytokines migration of nutrophils and monocytes</td>
</tr>
<tr>
<td>TNFα</td>
<td>T cell, B cells, NK cell Monocyte/ Macrophages Mast cells, basophils, eosinophils</td>
<td>All cells except RBC</td>
<td>Hepatic production of acute phase proteins, Systemic features (fever, shock), anoxia, expression of endothelial adhesion molecule Enhanced leukocyte toxicity, Induction of proinflammatory cytokines</td>
</tr>
<tr>
<td>TNFβ</td>
<td>B cells and T&lt;sub&gt;h&lt;/sub&gt;1 cells</td>
<td></td>
<td>Promotes inflammation</td>
</tr>
<tr>
<td>IFNα and β</td>
<td>Virally infected cells</td>
<td></td>
<td>Kill viruses</td>
</tr>
<tr>
<td>IFNγ</td>
<td>T&lt;sub&gt;h&lt;/sub&gt;1 cells, NK cells</td>
<td>All cells</td>
<td>Activation of macrophage and NK cells, inhibition of TH2 cells Stimulates secretion of Igs by B cells, Differentiation of T helper cells</td>
</tr>
<tr>
<td>TGFβ</td>
<td>T cell, B cells, and Mast cells</td>
<td></td>
<td>Suppresses immunity</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>T cell, B cells, NK cell and Macrophages</td>
<td></td>
<td>Stimulates granulopoiesis and monocyte production</td>
</tr>
<tr>
<td>Mcp-1</td>
<td>Fibroblasts, smooth muscle cells, Blood mononuclear cells</td>
<td>Monocytes, macrophages, NK cells, T cells</td>
<td>Chemoattractant for monocytes, T cells, NK cells Stimulates release of histamine from basophils</td>
</tr>
<tr>
<td>Eotaxin</td>
<td>Alveolar cells, Myocardium</td>
<td>Eosinophil, basophil</td>
<td>Chemoattractant for eosinophil, basophil Induces allergic pulmonary diseases</td>
</tr>
<tr>
<td>PF-4</td>
<td>Platelet Megakaryocytes</td>
<td>Fibroblasts endothelial cells</td>
<td>Chemoattractant for fibroblasts inhibitory to haematopoietic precursors and endothelial cell proliferation</td>
</tr>
</tbody>
</table>

(TH1 cells: Type 1 helper cell; TH2 cells: Type 2 helper cell; TNF: Tumor necrosis factor; IFN: Interferon; TGF: Transforming growth factor)

#### Chapter Summary

**Key Concepts**

1. Nonspecific immune mechanism (innate immunity) is mainly due to NK cells, phagocytosis by mononuclear phagocyte system, inflammatory cells, and chemicals present in skin and mucous membrane.
2. T cell (T<sub>8</sub> cell or cytotoxic T cell) is for cellular immunity, which is mainly targeted against intracellular pathogens like viruses, parasites and fungi, cancer cells, tumor cells, and transplanted tissues.
3. B cell is for humoral immunity mediated by antibodies, which is mainly targeted against extracellular pathogens like bacteria.
4. Type 1 helper cell induces cellular immunity and type 2 helper cell induces humoral immunity.
5. Complement system activates B cells, and also helps in nonspecific immunity.
6. Monoclonal antibodies help in transplant rejection and used in immunotherapy.
Important to Know (Must Read)


2. NK cells, Inflammation, Phagocytosis, Mononuclear phagocyte system, Development of T cell, Development of B cell, Types and functions of T cells, T cell receptor, MHC antigens, Immunoglobulins, Cellular immunity, Humoral immunity, Immune responses, Antigen presenting cells, Mechanism of antigen presentation by APCs, Complement system, Monoclonal antibodies, Immunological tolerance, Immunotherapy, Prevention of transplant rejection, Immunodeficiency states, AIDS, Autoimmunity, and Cytokines are usual Short Questions in exams.

3. In Viva, examiners may ask any questions from the topics listed as short notes. In addition, classification of immunity, types of antibodies and their functions, and all immunological disorders, may be asked.
Platelets and Their Role in Hemostasis

**Learning Objectives**

On completion of study of this chapter, the student **MUST** be able to:
1. Define hemostasis and list the major steps of hemostasis.
2. Give the steps of thrombopoiesis and briefly describe its regulation.
3. Describe the structure (cytoskeletal systems and granules) of platelet and correlate the structure with platelet functions.
4. Appreciate the properties of platelet, especially platelet adhesion, aggregation and release reaction.
5. Explain the role of platelet in hemostasis, with special reference to the role in temporary hemostatic plug formation.
6. List the causes of thrombocytosis and thrombocytopenia.
7. List the platelet function tests.
8. Understand common platelet dysfunctions.

Student **MAY** also be able to:
1. Describe the role of platelets in hemostasis.
2. Describe platelet function test.
3. Describe the physiological basis of common platelet dysfunctions, especially ITP.

**Definition and Concept**

Hemostasis is defined as the process of **arrest of bleeding** while maintaining blood in a fluid state within the vascular system.

Hemostasis is the mechanism by which loss of blood from vascular system is prevented by a complex interaction of vessel wall, platelets and plasma proteins. This happens broadly in two stages: primary and secondary.

**Primary hemostasis:** This is the initial stage during which vascular wall and platelet interact to limit the blood loss from damaged vessel. This is also called temporary hemostasis, as platelet plug alone cannot stop bleeding for a longer duration.

**Secondary hemostasis:** A stable fibrin clot is formed from clotting factors by enzymatic reactions. This is also called definitive hemostasis, as formation of clot in the vicinity of platelet plug finally stops bleeding.

Although formation of blood clot is needed to arrest blood loss, ultimately blood clot needs to be dissolved to resume the normal blood flow, and the process of dissolution of clot is called fibrinolysis.

Bleeding occurs when blood vessels are damaged. The vascular injury initiates a series of events in sequence that finally forms clot at the site of injury and prevents loss of blood from the body. Thus, hemostasis is a life-saving process that maintains homeostasis of blood volume.

**Steps of Hemostasis**

Hemostasis occurs in **three stages**: vasoconstriction, temporary hemostatic plug formation, and blood coagulation (Flowchart 20.1).

**Vasoconstriction**

Immediate response of blood vessel to injury is vasoconstriction.
1. This occurs due to the contraction of vascular smooth muscle in response to injury.
2. It instantaneously decreases loss of blood and also helps in platelet plug formation (see below).
3. Contraction of vascular smooth muscle to injury is initially a mechanical response (**stretch-induced contraction**).
4. However, later vasoconstriction is maintained and potentiated by secretion of vasoconstrictor substances like serotonin from the activated platelets.

**Temporary Hemostatic Plug Formation**

Formation of platelet plug at the site of injury is called temporary hemostatic plug. This occurs due to three properties of platelets: adhesion, aggregation, and release reaction (secretion) (Fig. 20.1).

1. The initial response of platelet to vascular injury is the change in shape of platelets and its increased surface adhesiveness to the injured vascular endothelium. This is called adhesion.
2. Platelets also stick to each other (aggregate) at the site of the injury. This is called aggregation.
3. Simultaneously, platelets are also activated to release a number of chemicals (release reaction or secretion) that further facilitate vasoconstriction, adhesion and aggregation.

The whole process finally results in formation of a platelet plug that arrests bleeding temporarily. As platelet plug is not a stable one, the plug is called temporary hemostatic plug (or primary hemostatic plug), and the process is called temporary hemostasis. This is also called primary hemostasis as this is the first step in hemostasis that occurs very quickly and stops bleeding instantaneously.

**Blood Coagulation**

Immediately following injury, the clotting mechanism of blood is initiated. This results in formation of blood clot at the site of injury that stops bleeding.

1. The formation of blood clot occurs in and around the platelet plug and the plug formed is a stable one. Therefore, this plug is called a definitive hemostatic plug (or secondary hemostatic plug).
2. This process is also called secondary hemostasis as this step follows the primary hemostasis.
3. Blood coagulation is initiated by the vessel injury that releases tissue factors and exposes collagen of the vessel wall (for details, see the next chapter).

**THROMBOPOIESIS**

The development of platelets is called thrombopoiesis or thrombocytopenia. Platelets are the smallest formed elements of blood. They are anuclear fragments of mega-karyocytes. Megakaryocytes are the giant cells in the bone marrow: The major steps of thrombopoiesis are summarized in Flowchart 20.2.

### Stages of Development

Thrombopoiesis occurs in three broad steps (Figs. 20.2):

1. **Formation of megakaryoblast** from hemopoietic stem cells (Pluripotent stem cells, Committed stem cells and CFU-Mega).
2. **Megakaryopoiesis**: This is formation of megakaryocyte (Promegakaryocyte, basophil megakaryocyte, granular megakaryocyte and mature megakaryocyte) from megakaryoblast.
3. **Formation of platelets** from megakaryocytes

### Stem Cells

Platelets develop from myeloid stem cells that form CFU-Mega, which in turn develop into megakaryoblast (for details, refer ‘Hemopoiesis’).

### Megakaryoblasts

Megakaryoblasts are the first identifiable cells of the thrombopoietic series in the bone marrow. Their size is more than 15 µm. The cytoplasm is basophilic and nucleus is multilobed.

### Megakaryocytes

Megakaryocytes grow in three stages: promegakaryocyte, granular megakaryocyte, and mature megakaryocyte.

#### Promegakaryocytes

The cells are bigger than megakaryoblast (size more than 20 µm).

1. Cytoplasm is basophilic and contains few azurophilic granules around the centrosome.
2. The nucleus is horse-shoe shaped.
3. This is also called basophil megakaryocyte.

#### Granular Megakaryocytes

The cells are very big having the size between 25 and 50 µm.

1. Cytoplasm is acidophilic and contains numerous azurophilic granules around the centrosome.
2. The nucleus is irregular or horse-shoe shaped.

#### Mature Megakaryocytes

These are the largest cells in bone marrow. The characteristic features are:

1. These are polyploid bone marrow cells (ploidy means DNA content; diploidy means double amount of DNA; polyploidy means increased amount of DNA in the nucleus). Megakaryocyte has large nucleus, which is polyploid.
2. The cells are still bigger in size (more than 25 to 50 µm).
3. Cytoplasm contains numerous granules of different kinds. Four types of granules have been described in megakaryocyte:  
   - α-granules, dense granules, lysosomal granules, and myeloperoxidase granules.
4. Most of the proteins and granules of megakaryocyte determine the characteristic of platelets. When the need for platelet increases, the megakaryocytes increase in size, number and ploidy, and the opposite effects occur when the demand decreases.
5. Granules are present around the centrosome.
6. The nucleus is reduced in size.

### Platelets

Megakaryocytes form platelets by pinching off bits of cytoplasm and extruding them into the circulation (Figs. 20.2). On average, each megakaryocyte produces about 1,000 to 3,000 platelets. Normally, platelet production per day is about 35,000 to 45,000 per microliter of blood.
Regulation of Thrombopoiesis

The production and release of platelets from bone marrow remains normally constant. The production of platelets is enhanced by removal of platelets from blood (thrombocytopenia) and decreased by transfusion of platelets. This indicates that a feedback regulatory mechanism exists for platelet production. Thrombopoietin, interleukins, and GM-CSF control thrombopoiesis.

Thrombopoietin

The endogenous thrombopoietin is a short-lived polypeptide, produced mainly from liver parenchymal cells. To some extent, it is also formed in kidney. However, the naturally occurring thrombopoietin for medical uses is very less. Therefore, commercial thrombopoietin have been synthesized by recombinant technology for use in thrombocytopenic conditions. Recombinant thrombopoietins have half-life of 20–40 hours. They are produced by E. coli. Thrombopoietin stimulates differentiation and proliferation of megakaryocytes.

Interleukins

Interleukin-1, interleukin-3, interleukin-6, and interleukin-11 stimulate platelet production. They do not stimulate thrombopoietin release.

GM-CSF

GM-CSF is produced by monocytes, T lymphocytes, fibroblasts, and endothelial cells. It stimulates erythropoiesis, granulopoiesis, and thrombopoiesis.

Life History

Platelets have the half-life of about 4 days. They survive in circulation for about 8–12 days. The aged platelets are removed from circulation by reticuloendothelial systems. Spleen plays an important role in destruction of platelets. Therefore, platelet count increases after splenectomy and decreases in splenomegaly.

Structure and Functions

Structure

Platelets are small, anucleate cell fragments adapted to participate in hemostasis. By their membrane properties they adhere to damaged blood vessels and aggregate with each other. Though the cells are small, platelets have developed cellular structures (Fig. 20.3)

1. They have highly developed cytoskeletal system suited for their functions.
2. They have specialized canalicular system.
3. They have developed membrane systems. When activated, they change their shape due to change in activity of membrane structure and cytoskeleton. This helps in release of chemicals from its granules.

4. They contain extensive microtubules and microfilaments and numerous granules. Frequently, they change their shape with very long and thin processes extending out from their body called as **filopodia**.

**Dimensions**

Platelets are small, granulated, spherical, or oval bodies having diameter ranging from 1.5 to 3.0 µm. The size of platelets is approximately one-third to one-fourth of red cells. However, there is considerable variation in size of platelets even in the same individual. Few platelets may have diameter more than half the diameter of red cells (> 4 µm).

**Cell Membrane**

Cell membrane is covered by an **exterior glyocalix coat** that consists of **glycoproteins** including glycoprotein receptors, glycolipids, and mucopolysaccharides.

1. Platelets have negative charge on their surface due to presence of sialic acid residues attached to the exterior coat. This **prevents the resting platelets to attach to each other** or to the negatively charged endothelial cells.

2. The cell membrane is a **trilaminar unit** consisting of a bilayer of phospholipids in which cholesterol, glycolipids, and glycoproteins are embedded.

3. **Glycoproteins** aid to prevent adherence of platelets to the normal vascular endothelium.

4. However, **glycoprotein receptors** help in adhesion and aggregation of platelets.

5. Platelets have **receptors** on their cell membrane for collagen, ADP, von Willebrand factor (vWF), and fibrinogen.

**Membrane Glycoproteins**

The cell membrane contains integral membrane glycoproteins (Gp) (Fig. 20.4), which play an important role in hemostasis. Important platelet membrane glycoproteins and their functions are as follows:

- **Gp Ib-IX-V**: This is a constitutively active receptor that mediates vWF dependent adhesion of platelets to subendothelial collagen.

- **Gp IIb/IIIa**: On activation, serves to bind fibrinogen and thus mediates aggregation. Also, this is the receptor for vWF, fibronectin, and thrombospondin.

- **Gp Ia-IIa**: Constitutively active receptor for collagen and mediates platelet adhesion independent of vWF.

**Cytoskeletal Systems**

Cytoskeletal system of platelets can be broadly divided into membrane and cytoplasmic cytoskeletal systems.

**Membrane Cytoskeleton**

An elongated tetramer of **spectrin molecule** is present in the cell membrane, which is connected to the ends of actin filaments that are situated close to the cell membrane and membrane of open canalicular system.

1. **Filamin 1** (actin binding protein) **interacts with membrane glycoprotein and actin** present below the membrane. Filamin 1 also connects these proteins (membrane glycoprotein and submembrane actin filaments) to the transmembrane spectrin network, and this **provides stability** to the membrane and discoid shape of the platelets.

2. Other membrane proteins include **talin, vinculin, and vimentin**. Membrane cytoskeleton plays important role in **platelet spreading** following their adhesion to the vascular endothelium.

**Cytoplasmic Cytoskeleton**

Cytoplasm contains a well-developed contractile system, which consists of an **extensive network of microtubules** and **microfilaments**.

**Microtubules**

There is a sub-membrane microtubular system consisting of many microtubules that are arranged beneath the membrane.

1. Microtubules are made up of **α and β tubulins**.

2. Cytosolic motors like **dynein and kinesin filaments** are also associated with microtubules.

3. Microtubules **provide stability to the membrane and support the discoid shape** of the cell.
### Microfilaments

Platelet microfilaments consist mainly of actin molecules that polymerize into microfilamentous bundles. There are also few myosin filaments attached to actin filaments.

1. Actin and myosin filaments constitute contractile system in platelets.
2. The microfilament system is not prominent in the resting cells. However, when platelets are activated and they change their shape, microfilament bundles become more extensive.
3. They form the major contractile system of the cell that helps to change the shape of platelets and discharge their granular content to the exterior through canaliculi (cell release).

### Cellular Systems

There are two developed cellular systems in platelets that help in platelet functions: open canalicular system and dense tubular systems (see Fig. 20.3).

#### Open Canalicular System

There are many canaliculi in the platelets that open to the exterior.

1. The open canaliculi are extensive invaginations of cell membrane deep into the cytoplasm that form the conduits for discharge of contents of granules.
2. Presence of numerous canaliculi in platelet eliminates the dependence of the cell for its chemical release solely on the migration of granules to the periphery for their fusion with cell membrane for exocytosis, as the granule-movement toward the interior of the cell and fusion of the granules with the canalicular membrane facilitates the process of release of chemicals from platelet granules.
3. Thus, open canalicular system enhances the degree and rate of platelet release (see below).

#### Dense Tubular System

This a closed system of channels formed by residual endoplasmic reticulum that are less extensive than canalicular system.

1. This system is found close to the open canalicular system.
2. They sequester the ionized calcium.
3. The membrane of tubular system contains peroxidase activity, and the interior contains calreticulin, a calcium binding protein that helps in calcium accumulation.
4. Release of calcium from dense tubular system facilitates platelet release.

### Cytoplasmic Organelles

Cytoplasmic matrix contains microfilaments, structured filaments, and microtubules. Interspersed in the matrix are various types of granules and electron dense bodies. Organelles are mainly mitochondria. Sometimes Golgi apparatus is found.

### Platelet Granules

Platelet contains numerous peroxisomes, mitochondria, lysosomes, and granules. Activation of platelet leads to discharge of content of granules that facilitate the process of hemostasis. The granules of platelets are of two types: the alpha granules and dense granules (Fig. 20.4).

#### Alpha granules:

The alpha granules are abundant in platelets. About 50–80 granules are present per platelet. They are about 200 nm in diameter. They contain various chemicals (Table 20.1).

#### Dense granules:

There are about 3–8 dense granules per platelet. They are also called dense bodies. They have diameter of about 20–30 nm. They contain chemicals like serotonin, ADP, calcium, ATP, etc.
Chapter 20: Platelets and Their Role in Hemostasis

Properties of Platelets

Platelets have three unique properties: adhesion, aggregation, and activation and release.

Adhesion

Platelets easily adhere to the damaged vascular endothelium. This is called platelet adhesion. Platelets have the tendency to stick to the exposed collagen of the injured vessel wall. Adhesion is facilitated by von Willebrand factor. However, platelets do not adhere to the normal vessel wall.

Role of membrane proteins: This means binding of platelets to non-endothelial surfaces, particularly subendothelium which is uncovered following vascular injury. von Willebrand factor mediates adhesion of platelets to subendothelium via GpIb on the surface of platelets (Clinical Box 20.1).

Clinical Box 20.1

Deficiency of membrane proteins causes bleeding disorder: Congenital absence of glycoprotein receptor GpIb (Bernard-Soulier syndrome) or of von Willebrand factor in plasma (von Willebrand’s disease) causes defective platelet adhesion and bleeding disorder.

Aggregation

Platelets not only stick to the injured vessel wall but also to each other. The property of platelets to stick to each other is called platelet aggregation. Fibrinogen and GpIb-IIIa mainly promote aggregation of platelet to each other (Fig. 20.5). Also, thrombin, ADP and PAF promote platelet aggregation.

Activation and Release (Secretion)

Platelets are activated when they bind to collagens or to each other.

1. The activation is facilitated by thrombin and ADP.
2. Platelets normally circulate as round to oval disc like structures. With the activation platelets undergo shape change, i.e. they become more spherical and form pseudopodia. This shape change is due to recognition of microtubules and contraction of actomyosin of microfilaments.
3. The activated platelets change their shape and discharge their granular contents (Fig. 20.6). The discharge of the granular content to the exterior through open canalicular system is called platelet release (see below).
4. The release reaction (secretion) is facilitated by chemicals released from platelet granules.

Functions of Platelets

In addition to their important role in hemostasis (described below), platelets serve many other functions of the body. Important functions of platelets are as follows:

1. Temporary hemostasis: Platelets prevent bleeding by forming plugs at the site of injury (temporary hemostatic plug). Platelets also promote vasoconstriction by producing serotonin that helps in hemostasis.
2. Blood coagulation: Platelets contribute to blood coagulation by releasing platelet factor 4, and synthesizing clotting factors V and XI.
3. Clot retraction: Platelets promote clot retraction, which is essential for stabilization of clot.
Section 2: Blood and Immunity

5. **Phagocytosis**: Platelets phagocyte smaller molecules like immune complexes and viruses.

6. **Storage and transport**: Platelets synthesize, secrete, and transport many chemical substances (as listed in Table 20.1).

7. **Vascular growth**: Platelets help in growth of vascular endothelium by secreting platelet-derived growth factor (PDGF). PDGF is also produced by macrophages and endothelial cells. PDGF is synthesized in three forms: PDGF-AA, PDGF-BB, and PDGF-AB (containing either of the two polypeptide chains A or B).

### Normal Count and Variations

**Normal count of platelet** is 1.5–4 lakhs/mm$^3$ of blood.

**Thrombocytopenia**

Thrombocytopenia is defined as the platelet count less than 1.5 lakhs/mm$^3$ of blood.

1. However, significant bleeding occurs when platelet count decreases below 50,000/mm$^3$ of blood. Therefore, platelet count below 50,000/mm$^3$ of blood is called **critical count**.

2. Thrombocytopenia occurs in idiopathic thrombocytopenic purpura (ITP), aplastic anemia, hypersplenism, acute leukemia, cytotoxic chemotherapy and radiation (Table 20.2).

3. About 70% of platelets released from bone marrow are present in circulation and 30% are sequestered in spleen.

4. Therefore, splenectomy usually causes thrombocytosis and hypersplenism invariably causes thrombocytopenia.

**Thrombocytosis**

Thrombocytosis commonly occurs in polycythemia vera, chronic myeloid leukemia, iron deficiency anemia, splenectomy, chronic infection, surgery, and following acute hemorrhage.

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**Table 20.2**: Variations in platelet count.

**A. Thrombocytosis**

1. Essential thrombocythemia

2. Other myeloproliferative diseases like polycythemia rubra vera, CML, etc.

3. Familial thrombocytosis

4. Acute blood loss

5. Iron deficiency

6. After splenectomy

7. Malignancies

8. Drugs: vincristine, epinephrine

9. After surgery

10. Chronic infection

**B. Thrombocytopenia**

1. Hereditary thrombocytopenias like Fanconi anemia, Alport syndrome, May-Hegglin anomaly, etc.

2. Hypersplenism

3. Idiopathic thrombocytopenic purpura (ITP)

4. HIV infection

5. Aplastic anemia

6. Massive transfusion of stored blood

7. Drugs: Heparin, quinidine, quinine, rifampicin, etc.

8. Acute leukemia

9. Cytotoxic chemotherapy

10. Irradiation of bone marrow

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**ROLE OF PLATELET IN HEMOSTASIS**

Hemostasis is the **arrest of bleeding**. Hemostasis occurs in two major steps: temporary hemostasis and definitive hemostasis. Following injury to blood vessels, platelets aggregate at the site of injury and seal the opening in the vascular tree. This is the first and important step in prevention of bleeding, which is called **temporary hemostasis**. Simultaneously, mechanisms of coagulation of blood are also activated that form blood clot, the processes combine known as **definitive hemostasis** (refer Flowchart 20.1).

Though platelet is essential for temporary hemostasis, it also contributes to **blood coagulation and clot retraction**. Platelet also controls **thrombolysis**.

**Role in Temporary Hemostasis**

Immediate hemostasis following vascular injury is achieved by formation of platelet plug (also called, **platelet thrombus**) at the site of injury. As platelet thrombus can be washed away, unless supported by a clot, this is called **temporary hemostatic plug**. Temporary hemostatic plug formation depends on adhesion, platelet aggregation, and release reaction.
Platelet Adhesion

The damage to blood vessel exposes underlying portion of the vessel wall that are normally concealed from the circulatory platelets by intact lining of the endothelium. Platelets have high affinity to adhere to the exposed vascular wall, which is called platelet adhesion (Fig. 20.7A). Especially, exposed collagen and von Willebrand factor in the injured vessel wall attract platelets for them on their cell membrane. However, platelet adhesion is also controlled by following factors:

1. **Depth and degree of injury:** Deeper and extensive the injury more is the platelet aggregation. This occurs due to release of more quantity of platelet activating factors from the tissue.
2. **Site of injury:** Injury in mucocutaneous vascular bed depends more on platelet for hemostasis, whereas injury of vascular beds in muscles and joints rely more on coagulation mechanism. Platelet aggregation is more in mucocutaneous tissue.
3. **Age of the individual:** As composition of vessel wall changes with age, platelet aggregation alters with age of the individual. In elderly individuals, the aggregation is less.

Platelet Aggregation

Platelet adhesion is immediately followed by platelet aggregation at the site of injury (Fig. 20.7B). Fibrinogen, GpIIb-IIIa, and thrombin promote platelet aggregation. Simultaneously, platelets are activated, and activated platelets release chemicals from their granules (refer Fig. 20.5). These chemicals, especially ADP and platelet activating factor (PAF) further facilitate platelet aggregation (Clinical Box 20.2). Thus, accumulation of more platelets at the site of vascular injury facilitates the process of temporary hemostatic plug (platelet thrombus) formation. Then, formation of clot is initiated (Fig. 20.7C).

Clinical Box 20.2

**Use of ADP inhibitors:** ADP facilitates platelet thrombus formation. There are ADP receptors on the surface of platelets. In human beings, three types of ADP receptors have been identified on platelet membrane: P2Y1, P2Y2, and P2X1. ADP released from dense granules during platelet activation binds with ADP receptors on platelet membrane and facilitates platelet aggregation that promotes platelet thrombus formation. Therefore, inhibitors of ADP (ADP receptor antagonists that prevent platelet aggregation) have been clinically tried in the prevention of myocardial infarction and stroke that occur due to platelet thrombus obstructing blood flow to the concerned tissues.

Important Note

**Use of antiplatelet drugs such as aspirin:** Aspirin inhibits platelet aggregation by inhibiting the membrane enzyme cyclooxygenase (Flowchart 20.3). Cyclooxygenase facilitates thromboxane A2 formation that facilitates platelet aggregation and causes vasoconstriction. Therefore, dispirin (soluble aspirin) is used in treatment and prevention of myocardial infarction and stroke. Steroids inhibit phospholipase.

Platelet Activation and Release

Adhesion of platelets to damaged vascular endothelium activates the platelets. Polymerization of microfibrillar actin occurs in activated platelets that results in pseudopodia or spicules formation. Platelets are also activated by tissue factors and chemicals released from granules of the platelets, especially PAF. Thrombin and ADP also promote activation.
The activated platelets release various chemicals from their granules to the exterior via canalicular system; the process is called release reaction or platelet release (secretion). Following are the possible mechanisms of platelet release.

**Through Platelet Activating Factor**
Platelet adhesion activates platelets. Activation of platelet is facilitated by PAF, a cytokine secreted from neutrophils, monocytes and platelets.
- PAF is a phospholipid produced from membrane lipids. PAF acts via G-proteins and activates phospholipase-C and diacylglycerol (DAG).
- DAG increases cytoplasmic calcium by promoting calcium release from dense tubular systems that are present close to open canaluli.
- Calcium causes contraction of microfilaments that helps in movement of granules to the open canalicular system.
- Membranes of granules fuse with the membranes of canaluli and the contents of granules are released into the canalici by exocytosis.
- From canalici, chemicals are transferred to the exterior of the cell. During this process, platelets change their shape and exhibit movement.

**Through Thromboxane and Serotonin**
Formation of DAG (as described above) activates the enzyme phospholipase A_2, which converts membrane phospholipid into arachidonic acid.

- Arachidonic acid is converted to endoperoxides by cyclooxygenase. Endoperoxides in turn form thromboxane A_2 (TX-A_2).
- TX-A_2 is a potent vasoconstrictor and also promotes platelet aggregation (Flowchart 20.3).
- Serotonin released from platelet produces vasoconstriction and promotes hemostasis.

**Through Thrombospondin and Thrombonectin**
Thrombospondin and thrombomectin released from platelet granules facilitate the activity of contractile system and further promote exocytosis of granules and release of their content to the exterior.

Normally, platelet thrombus formation is prevented due to the balance between the factors that promote and inhibit platelet adhesion, aggregation and activation (Table 20.3). Alteration in this balance, even without vascular injury leads to intravascular platelet thrombus formation resulting in tissue infarction.

**Other Hemostatic Functions**

**Role in Blood Coagulation**
Activation of platelet leads to increased platelet coagulant activity.
1. Platelet factor 4 (PF4) acts as a cofactor for blood coagulation (see next chapter).
2. Platelet synthesizes coagulation factors V and XI.
3. Platelet phospholipids present on the platelet surface accelerate the formation of Va, VIIIa, and Xa. Va is essential for conversion of prothrombin to thrombin.
Chapter 20: Platelets and Their Role in Hemostasis

Flowchart 20.4: Procoagulant activity of platelet. Platelets provides surface for activation of few clotting factors.

Fig. 20.8: Role of blood vessel in hemostasis. Note the factors produced by endothelial cells that promote and inhibit hemostasis.

4. They also facilitate the conversion of X to Xa and prothrombin to thrombin (Flowchart 20.4). Thus, platelets accelerate thrombin formation.

Role in Clot Retraction

If platelets are present in the clot in a test tube, within minutes to hours, the clot contracts, extruding a very large fraction of serum. This is called clot retraction.

1. The platelet filopodia extends into fibrin clot and fibrin strands tug with filopodia. Thus, shrinkage of platelet with contraction of filopodia causes internalization of fibrin that causes clot retraction.
2. In vivo, the phenomenon akin to clot retraction is the consolidation of the thrombus, which not only firmly seals the opening in the injured vessel, but also facilitates wound healing.
3. Clot retraction also prevents the process of thrombolysis.
4. Platelet deficiency prolongs the clot retraction time.

Role in Thrombolysis

Role of platelet in fibrinolysis is complex. Both profibrinolytic and antifibrinolytic actions of platelets have been demonstrated.

1. Thrombospondin, tPA, and plasmin that are profibrinolytic are synthesized and released by platelets.
2. However, antifibrinolytics like plasminogen activator inhibitor-1 and a2-antiplasmin are also secreted from platelets.
3. Platelets by causing clot retraction decrease the efficiency of thrombolysis.
4. It appears that antifibrinolytic effects of platelets predominate over their profibrinolytic effects in vivo as platelet rich thrombus is known to resist thrombolysis.

Role of Vascular Wall in Hemostasis

Vascular endothelium plays an important role in hemostasis. Endothelial cells of blood vessel synthesize certain substances that are inhibitory and substances that have facilitatory influence on hemostasis (Fig. 20.8).

Inhibitors of Hemostasis

Endothelial cells synthesise certain substances which have inhibitory influence on hemostasis. These include;

- Thrombomodulin
- Protein S
- Heparin-related substances
- Prostacyclin (PGI2)
- Tissue plasminogen activator (tPA).

Thrombomodulin: Binding of thrombomodulin to thrombin causes activation of protein C. Protein C inactivates factors V and VIII and is a potent inhibitor of coagulation.

Protein S: Protein S is a cofactor for protein C. deficiency of protein C or protein S is associated with tendency toward thrombosis.

Heparin: Heparin-like substances on the surface of endothelial cells potentiates the action of antithrombin.

Prostacyclin: Prostacyclin, a prostaglandin synthesized by endothelial cells, induces vasodilation and also inhibits platelet aggregation. Endothelial cells also synthesize tissue plasminogen activator, which converts plasminogen to plasm, and activates fibrinolytic system.

Promoters of Hemostasis

Certain factors synthesized by endothelial cells promote hemostasis. These are tissue factor, von Willebrand factor and platelet activating factor.

1. Tissue factor or thromboplastin activates extrinsic system of coagulation.
2. von Willebrand factor mediates adhesion of platelets to subendothelium.
3. Platelet activating factor induces aggregation of platelets.
4. Another vascular factor promoting hemostasis is vasoconstriction of small vessels following injury.
5. Subendothelial collagen promotes platelet adhesion and also activates factor XIII (intrinsic pathway).

PLATELET FUNCTION TESTS

1. Platelet count: Decreased platelet count is associated with prolongation of bleeding time. Though, normal count of platelet is 1.5–4 lakhs/mm$^3$ of blood, significant prolongation of bleeding time occurs when platelet count is less than 50,000/mm$^3$ of blood (critical count). Platelet count is not routinely ordered in clinical practice. However, for assessing integrity of hemostatic mechanisms, especially in the diagnosis of a bleeding disorder, platelet count is a must.
2. Bleeding time: Bleeding time assesses integrity of platelets. Bleeding time is routinely assessed before any surgery or dental procedures. It is prolonged in thrombocytopenia and thrombasthenia. Thrombosthenia is a condition in which platelet count is normal but functionally platelets are abnormal.
3. Platelet aggregation tests: Ability of platelets to aggregate is assessed with the help of aggregometer. In this test, an aggregating agent is added to a suspension of platelets in plasma and the response is measured turbidometrically as a change in transmission of light through the suspension.
4. Platelet adhesiveness test: Platelet’s ability to adhere to glass surface is measured in this test. Anticoagulated blood is allowed to pass at a constant rate through a plastic tube containing glass beads, during which platelets stick to the beads. Platelet adherence is measured by estimating the platelet retention in the tube.
5. Clot retraction time: When blood is collected without anticoagulant, clotted blood retracts and 50% retraction occurs by the end of one hour. Clot retraction completes at the end of 18 to 24 hours. When retraction is less than 50% in one hour, thrombocytopenia or platelet malfunction is reported.

COMMON PLATELET DYSFUNCTIONS

Idiopathic Thrombocytopenic Purpura

Idiopathic thrombocytopenic purpura (ITP) is a primary autoimmune purpura characterized by thrombocytopenia which usually occurs due to formation of antibody against platelets. Antiplatelet antibodies get attached to platelet membrane glycoproteins, and these platelets are phagocytosed in spleen resulting in thrombocytopenia (Fig. 20.9).

It occurs in two forms: childhood ITP and adult ITP.

Childhood ITP: Childhood ITP is common but less severe and occurs equally in both genders that usually resolves spontaneously in six months.

Adult ITP: Adult ITP is less common and occurs predominantly in females.

1. The common feature of the disease is bleeding that usually occurs spontaneously. Skin is the commonest site of hemorrhage, exhibiting petechiae or ecchymoses. Bleeding occurs from mucus membranes in the form of epistaxis (bleeding from the nose), or bleeding-gums.
2. Diagnosis is made by demonstrating thrombocytopenia, anemia, and anti-platelet antibodies in the patient.

Fig. 20.9: Pathogenesis of ITP. Note that platelets having antiplatelet antibodies on their surface are destroyed in spleen. Courtesy: Figure 35.1, Essentials in Hematology and Clinical Pathology by Ramadas Nayak et al., 1st edition, 2012; Jaypee Brothers Medical Publishers (P) Ltd.
Increased numbers of megakaryocytes are seen in bone marrow smear (Fig. 20.10).

The treatment consists of administration of corticosteroids, and splenectomy.

Thrombocytopenia in Pregnancy

Thrombocytopenia is common during pregnancy and usually asymptomatic.

1. A form, called as gestational thrombocytopenia that occurs during late gestation (count may be less than 70,000/cu mm of blood in last trimester) and resolves following delivery, but causes excessive bleeding during parturition.

2. Another form is associated with preeclampsia.

3. Pregnancy-induced hypertension (PIH) and thrombocytopenia (platelet count less than 50,000/cu mm of blood) have lower prognostic value.

Neonatal Autoimmune Thrombocytopenia

Sometimes, neonatal autoimmune thrombocytopenia (NATP) occurs in newborns and platelet count is less than 50,000/cu mm of blood in this condition. It may be associated with autoimmune hemolytic anemia. Antibodies are demonstrated against platelet specific alloantigens. It is more severe when the mother has ITP. NATP is treated by platelet transfusion, steroid and immunoglobulins.

CHAPTER SUMMARY

Key Concepts

1. Platelets are subcellular elements produced by pinching off from megakaryocytes.

2. Though platelets are small in size, they have highly developed membrane and cellular cytoskeletal system, open canalicular system, alpha and dense granules, and membrane glycoproteins. All these cellular specializations help in platelet functions: adhesion, aggregation, activation, and secretion.

3. Primary hemostasis (temporary hemostatic plug formation) is the first and important step in hemostasis, which mainly depends on platelets. Therefore, platelet deficiency leads to bleeding.

4. Platelet also play role in clotting by activating few clotting factors and promoting clot retraction.

5. Splenomegaly causes thrombocytopenia as platelets are destroyed in spleen.

6. Platelet count less than 50,000 per cu mm of blood is called critical count, as bleeding is significant below this count.

Important to Know (Must Read)

1. In theory examination, “Role of platelets in hemostasis”, “Steps and regulation of thrombopoiesis”, and “Structure, properties and functions of platelets” may come as Long Questions.

2. Temporary hemostatic plug, properties of platelets, thrombopoiesis, platelet granules, cytoskeletal and canalicular systems of platelets, functions of platelets, and platelet function tests may come as Short Questions in exams.

3. In Viva, examiners may ask… platelet count, functions of platelets, causes of thrombocytopenia and thrombocytosis, mechanism of temporary hemostatic plug formation, specialities of platelet structure, properties of platelets, steps of thrombopoiesis, regulation of thrombopoiesis, chemicals secreted from platelet granules, mechanism of platelet adhesion, mechanism of aggregation, mechanism of platelet release, causes and features of ITP, and platelet function tests.
Blood Coagulation

Chapter 21

Learning Objectives

On completion of study of this chapter, the student MUST be able to:
1. Name the clotting factors.
2. Describe the mechanisms (intrinsic and extrinsic) of blood coagulation.
3. Understand the importance of clot retraction.
4. Explain the anticoagulant mechanism (process of fibrinolysis).
5. Appreciate the physiological basis of use of fibrinolytic agents in coronary artery disease and stroke.
6. List the anticoagulants.
7. List the investigations for detection of defects in temporary and definitive hemostatic plug formation.
8. Enumerate the common abnormalities of coagulation.

The student MAY also be able to:
1. Describe various clotting factors.
2. Describe the mechanism of action and uses of anticoagulants.
3. Understand the pathophysiology of common abnormalities of coagulation, such as DIC, hemophilia and von Willebrand disease.
4. Describe the physiological basis of investigations of bleeding disorders.

Introduction

Coagulation of blood is a vital physiological process, as hemorrhage is detrimental to life. Blood is maintained in a fluid state in the vascular compartment, yet swiftly coagulates to seal a vascular injury. Failure to form clot results in hemorrhage or thromboembolic phenomenon. Immediately following vascular damage, platelet plug (temporary hemostatic plug) formation occurs at the site of injury that immediately stops bleeding. However, unless associated with definitive hemostatic plug, hemorrhage continues as platelet thrombus is not a stable plug. The definitive hemostasis is the coagulation of blood, the process which is simultaneously activated along with the activation of platelets. Coagulation of blood occurs due to activation of clotting factors (coagulation proteins) that are normally present in their inactive form in plasma.

Clotting Factors

Coagulation of blood depends on a series of chemical reactions involving clotting factors. There are known 12 clotting factors (Table 21.1) that were depicted earlier as factors I to XIII (factor VI absent). Later, few more were added to the list.

Scientist contributed

In 1905, Paul Morawitz was the first scientist who systematically assembled coagulation factors into the scheme of coagulation and demonstrated that, in the presence of calcium and thromboplastin, prothrombin (II) was converted to thrombin, which, in turn, converted fibrinogen (I) into a fibrin clot. This theory persisted for 40 years until the discovery of factor V.

Factor I

Factor I is the fibrinogen.
1. It is a dimeric glycoprotein with molecular weight of 340,000. Each of the two subunits consists of three chains: Aα, Bβ and γ. The A and B fibrinopeptides are separated by action of thrombin.
2. The genes for three chains of fibrinogen are found in the chromosome 4 at q23–q32.
3. Fibrinogen is present in plasma and α-granules of platelet.
4. Its plasma concentration is \(2000-4000\ \mu\text{g/ml}\).
5. Its half-life in plasma is 3–5 days.
6. **Fibrinogen is converted to fibrin** by IIa (thrombin) and is stabilized by physical contact with XIIIa (activated fibrin-stabilizing factor).
7. Fibrin forms the *structural meshwork that transforms the loose platelet plug into a solid hemostatic plug*.
8. Fibrinogen is an **acute phase reactant** and is synthesized by liver.
9. Its concentration is greatly increased in **acute inflammations**.
10. IL-6 stimulates fibrinogen synthesis.

### Factor II

Factor II is **prothrombin**, a vitamin K-dependent coagulation protein primarily synthesized in liver.
1. It is a single chain zymogen with molecular weight 72,000 and plasma half-life of about 60 h.
2. Its plasma concentration is \(100–150\ \mu\text{g/ml}\).
3. The gene for human prothrombin is located on **chromosome 11** close to the centromere.
4. **Prothrombin is activated to thrombin** by Xa (activated Stuart-Prower factor).
   
   In clotting mechanism, **thrombin has many functions**, such as:
   1. It converts fibrinogen to fibrin monomers by removing fibrinopeptides A and B from fibrinogen. Fibrin monomers then form fibrin polymers (clot).
   2. It is a potent activator of platelets.
   3. It activates procarboxypeptidase B that inhibits plasmin-mediated fibrinolysis.
   4. It has also anticoagulation activity. It binds to cofactor thrombomodulin on endothelial cells that allows protein C to be activated.
   5. It has growth factor and cytokine like activities that play a role in inflammation, wound healing and atherosclerosis.

   **Inhibitors of thrombin**: The primary inhibitor of thrombin in plasma is **antithrombin III**. Heparin cofactor II also inhibits thrombin.

#### Factor III

Factor III is a tissue factor, known as **tissue thromboplastin** (TPL).
1. It is a membrane protein containing 263 amino acids.
2. The gene for factor III is located on **chromosome 1**.
3. Though TPL is not expressed normally in the cells in close contact with flowing blood, it is expressed in many extravascular tissues and in the adventitia of blood vessels.
4. It is an important physiological **initiator of extrinsic pathway for blood coagulation**.
5. When blood comes in contact with TPL following extravasation of blood from the injured vessel, circulating factor VII binds with TPL and gets rapidly converted to VIIa, an active protease that converts X to Xa. This initiates the process of coagulation.

#### Factor IV

Factor IV is **calcium**. Calcium acts as a cofactor in many steps of intrinsic and extrinsic pathways of blood coagulation, especially for **conversion of X to Xa** and **prothrombin to thrombin**.

#### Factor V

Factor V is **proaccelerin**.
1. This is a large glycoprotein with molecular weight 333,000 and plasma half-life of about 12h.
2. The gene for factor V is located in chromosome 1 at q21–q25.
3. The primary function of factor V is to **activate prothrombin to thrombin**, though it also participates in activation of Xa.
4. The acidic regions of factor V contain high aspartic and glutamic acid residues that are important in activating factor II. Sulfation of factor V potentiates its activity.

#### Table 21.1: Clotting factors.

| Factor I | Fibrinogen |
| Factor II | Prothrombin (α2-globulin) |
| Factor III | Tissue thromboplastin |
| Factor IV | Calcium ion |
| Factor V | Preaccelerin |
| Factor VI | Factor VI does not exist |
| Factor VII | Proconvertin |
| Factor VIII | Antihemophilic factor (antihemophilic globulin (AHG) or antihemophilic factor A) |
| Factor IX | Christmas factor (antihemophilic factor B) |
| Factor X | Stuart-Prower factor |
| Factor XI | Plasma thromboplastin antecedent (antihemophilic factor C) |
| Factor XII | Hageman factor, contact factor |
| Factor XIII | Fibrin stabilizing factor (Laki-Lorand factor) |
| HMW-K | High-molecular weight kininogen (Fitzgerald factor) |
| Pre-K | Prekallikrein (Fletcher factor) |
| Ka | Kallikrein |
| PL | Platelet phospholipid |

**Inhibitors**

| Protein C |
| Protein Z |
| Thrombomodulin |
| Antithrombin III |
| Tissue factor pathway inhibitor |
| Protein Z-dependent protease inhibitor |
**Factor VII**
Factor VII is also called proconvertin. It is among the vitamin-K-dependent clotting factors.
1. It consists of single chain polypeptide.
2. It has the half-life of about 3.5 h, which is shortest amongst procoagulant factors.
3. The gene for factor VII is located on chromosome 13.
4. It is activated by the tissue factor and inhibited by tissue factor pathway inhibitor (TFPI).
5. The primary function is to activate X to Xa. It also activates IX to IXa.

**Important Note**

Naming of Stuart-Prower Factor: Factor X deficiency was described in 1957 in a woman named Prower and a man Stuart, in whose blood the defects in blood clotting were observed. Later the clotting factor was identified and named after the patients.

**Factor VIII**
Factor VIII is antihemophilic factor.
1. The gene for factor VIII is located on the X chromosome at q28. It is synthesized in liver.
2. Its half-life is 8 to 12 h when it is associated with vWF. The half-life is reduced when concentration of vWF is reduced in plasma. Therefore, deficiency of vWF also reduces the level of VIII.
3. Factor VIII is activated by thrombin or Xa and the activation results in its release from vWF.
4. The main function of VIIIa is to activate X to Xa.

**Factor IX**
Factor IX is also called Christmas factor or anti-hemophilic factor B.
1. It is synthesized in liver dependent on vitamin K.
2. It has the half-life of 18–24 h.
3. The gene for factor IX is located on the tip of long arm of X chromosome. Therefore, hemophilia B is a sex-linked disorder.
4. Factor IX is activated by Xla or VIIa-TF complex.
5. Along with cofactor VIIIa, it activates factor X. This activity is physiologically expressed on the surface of platelets and evidences suggest that platelets have receptors for IXa.

**Factor X**
Factor X is known as Stuart-Prower factor.
1. It is a vitamin K-dependent polypeptide synthesized by liver.
2. It has molecular weight of 59,000 and plasma half-life of 34 to 40 hours.
3. The gene for factor X is located on chromosome 13 in close proximity to factor VII gene.
4. Activation of factor X is the target of both intrinsic and extrinsic pathways of coagulation.
5. It is activated by IXa of intrinsic pathway and VIIa of extrinsic pathway.
6. The primary function of factor Xa is the conversion of prothrombin to thrombin. It has also mitogenic activity for smooth muscle cells. It possesses receptor mediated proinflammatory activity.
7. Factor Xa is inhibited by antithrombin III (AT-III), which is accelerated by heparin. TFPI also inhibits Xa.

**Scientists contributed**

Oscar D Ratnoff and Joan E Colopy worked in the field of blood coagulation. In 1955, Ratnoff and Colopy identified a patient named John Hageman with a Factor XII deficiency that died from a thrombotic event and not a bleeding disorder. The factor was named as Hageman’s factor.

**Factor XI**
Factor XI is known as Hageman’s factor.
1. This is also called glass factor or contact factor.
2. The gene for factor XII was located on chromosome 5.
3. Factor XII is activated when it comes in contact with negatively charged surface. This is called contact activation. HMW kininogen and prekallikrein facilitate the process.
4. Xla promotes the conversion XI to Xla.

**Factor XII**
Factor XII is known as Hageman’s factor.
1. It is a glycoprotein consisting of A and B subunits.
2. It has molecular weight of 320,000 and half-life of about 10 days.
3. The B chain is not active enzymatically. The A chain (XIII-A) is a member of transglutaminase family, which contains 731 amino acids with molecular weight of 83,000.
4. In addition to its presence in plasma, it is also found in platelets, monocytes and macrophages.
5. The plasma XIII contains both A and B chains, and the cellular XIII contains only A chain.
6. The gene for A chain is located on chromosome 6 and the gene for B chain is located on chromosome 1.
7. Thrombin causes cleavage of A and B subunits of XIII that causes exposure of active site on A chain. This activates XIII to XIIIa. The XIIIa cross-links α and β chains of fibrin to stabilize the fibrin plug. In XIIIa deficiency, though clot formation occurs, hemostasis becomes inadequate.
8. It also promotes wound healing and tissue repair. It helps in maintenance of pregnancy.

**HMW Kininogen**

High molecular weight kininogen is also called *Fitzgerald factor*.
1. It is a nonenzymatic cofactor that circulates in complex with factor XI and prekallikrein. It is a contact factor for coagulation.
2. The gene for HMW kininogen is located on chromosome 3.
3. Its plasma half-life is 150 h.
4. It is synthesized by liver.
5. In addition to its nonenzymatic function in contact activation, it acts as a thiol protease inhibitor.
6. It is cleaved by *prekallikrein to bradykinin*, a potent vasodilator.

**Prekallikrein**

Prekallikrein is also called *Fletcher factor*. It is a contact factor for coagulation.
1. The gene for prekallikrein is located on chromosome 4.
2. It has half-life of 35 h in plasma. It is synthesized by liver.
3. It circulates as a complex with factor XI and HMW kininogen.
4. It causes contact activation of blood coagulation.
5. Along with HMW kininogen and factor XII, it participates in inflammatory response.

**Protein C**

Protein C is a vitamin K-dependent protein. However, unlike other vitamin K-dependent proteins (factors II, VII, IX and X), it is *not a procoagulant*.
1. It regulates clotting by inactivating Va and VIIIa.
2. The gene for protein C is located on chromosome 2.
3. It has molecular weight of 59,000 and half-life of about 6 h.
4. It is activated by thrombomodulin.

**Protein S**

Protein S is a single-chain glycoprotein cofactor synthesized primarily in liver. It is also synthesized by endothelial cells, megakaryocytes, Leydig cells and osteoblasts.
1. It has molecular weight of 75,000 and half-life of about 42 hours.
2. The gene for protein S is located on chromosome 3.
3. Protein S acts as a cofactor for protein C. It forms a complex with protein C that inactivates Va and VIIIa. Thus, it is an important component of anticoagulation mechanisms. However, it does not require proteolytic activation for its activity.
4. It has also its independent anticoagulant activity by virtue of its ability to compete with factor Xa for binding with factor Va.

**Protein Z**

Protein Z is synthesized in liver. It has half-life of about 60 hours. The gene for protein Z is located on chromosome 3. Function of protein Z is similar to that of protein S.

**Tissue Factor Pathway Inhibitor (TFPI)**

TFPI is a single chain polypeptide with molecular weight of about 35,000.
1. It is mainly synthesized by endothelial cells of blood vessels.
2. The gene for TFPI is located on chromosome 2.
3. It has three protease inhibitor domains. It inhibits factors Xa and VIIa.
Antithrombin III (AT-III)

AT-III is member of a large family of serine protease inhibitors, known as serpins.
1. The gene for AT-III is located on chromosome 1.
2. It inhibits thrombin, Xa and IXa, which is facilitated by heparin.
3. Factor VIIa is normally resistant to the action of AT-III. However, VIIa complexed with tissue factor is inhibited by AT-III in the presence of heparin.

MECHANISMS OF BLOOD COAGULATION

Blood coagulation occurs in three major stages (Fig. 21.1):

**Stage 1:** Activation of Stuart-Prower factor (formation of prothrombin activator)

**Stage 2:** Formation of thrombin from prothrombin

**Stage 3:** Formation of fibrin from fibrinogen

**Activation of Stuart-Prower Factor (Factor X)**

Activation of Stuart-Prower factor or factor X is the key to blood coagulation. Factor Xa is called prothrombin activator as it activates prothrombin to form thrombin. Therefore, this process is also called prothrombin activation.

This is achieved by two pathways: the intrinsic pathway and the extrinsic pathway (Fig. 21.1).

**Intrinsic Pathway**

Intrinsic mechanism of prothrombin activation occurs in four steps:

**Step 1 (Activation of XII)**

Activation of intrinsic pathway starts with contact of Hageman factor with a negatively charged surface or exposed collagen of the injured vessel wall.
1. High molecular weight (HMW) kininogen and kallikrein act as cofactors to facilitate the activation of factor XII.
2. Exposed collagen stimulates platelet adhesion and aggregation before initiating blood coagulation.

**Step 2 (Activation of XI)**

Activated XII (XIIa) converts XI to its active form (XIIa). This step is accelerated in the presence of HMW kininogen.

**Step 3 (Activation of IX)**

XIIa then converts IX to IXa, which is accentuated by VIIa. Calcium accelerates this process.
Step 4 (Activation of X)

Final step in activation of prothrombin activator is activation of X. IXa causes activation of X to Xa, the activated Stuart-Prower factor. The membrane phospholipid, calcium and activated factor VIII act as cofactors for the activation of Stuart-Prower factor.

Formation of VIIIa is crucial in Stage 1

Formation of VIIIa is the key to the process of activation of X. Factor VIII circulates as a complex with vWF.
1. Separation of VIII from vWF activates VIII.
2. Activation of VIII is facilitated by Xa and thrombin, the factors that are the consequences of formation of VIIIa.
3. Thus, activation of factor VIII and further activation of clotting mechanism become a vicious cycle.

Enzyme Cascade Hypothesis

In the intrinsic system of blood coagulation, activation of one clotting factor acts as an enzyme for the activation of next factor that leads to sequential activation of subsequent factors in a series of steps. Therefore, the intrinsic process of blood coagulation is called enzyme cascade hypothesis.

Extrinsic Pathway

Extrinsic pathway of blood coagulation occurs in three steps.

Step 1 (Release of TPL)

Key to the clotting mechanism is the release of tissue thromboplastin (TPL) from the injured tissue. As TPL is the tissue factor which is viewed as extrinsic to circulating blood, this system of blood coagulation is called extrinsic system of clotting.

Step 2 (Activation of VII)

TPL converts factor VII to its active form (VIIa). This is the key step in extrinsic mechanism. VIIa directly activates not only X (Stuart-Prower factor), but also IX (Christmas factor). Thus, it also influences intrinsic mechanism of activation of factor X.

Step 3 (Activation of X)

VIIa converts X to Xa. This process is accelerated in the presence of calcium, platelet phospholipid and TPL.

Note: Though there are intrinsic and extrinsic systems of blood coagulation, recently it is believed that these two systems do not operate independently, and all the clotting factors are interrelated for homeostasis.

Formation of Thrombin from Prothrombin

This is the second stage of blood coagulation in which activated Stuart-Prower factor (Xa) converts prothrombin to thrombin, in the presence of platelet phospholipid, calcium and activated factor V (Va). Factor Va acts as cofactor for acceleration of this process. Therefore, it is also called proaccelerin or accelerator globulin.

Important Note

Thrombin formation occurs rapidly: Factor II is prothrombin, a vitamin K-dependent protein formed in liver. Prothrombin forms thrombin, which is basically a proteolytic enzyme having molecular weight of 34,000 dalton. It is formed rapidly and in large amount as both intrinsic and extrinsic mechanisms stimulate its formation.

Functions of Thrombin in Blood Coagulation

1. It converts fibrinogen to fibrin monomers by removing fibrinopeptides A and B from fibrinogen. Fibrin monomers then form fibrin polymers (clot).
2. It activates formation of prothrombin activator by stimulating the activation of VIII, V, and XIII. Thus, it provides positive feedback to the coagulation process.
3. It is a potent activator of platelets.
4. It activates procarboxypeptidase B that inhibits plasmin-mediated fibrinolysis.
5. It has also an anticlotting activity. It binds to cofactor thrombomodulin on endothelial cells that allows thrombin to activate protein C to be activated. Thus, thrombin balances the coagulation and anticoagulation processes in the body.
6. It helps in repair of vessel wall. It has growth factor and cytokine-like activities that play role in inflammation, wound healing and atherosclerosis.

Inhibitors of thrombin: The primary inhibitor of thrombin in plasma is antithrombin III. Heparin cofactor II also inhibits thrombin.

Formation of Fibrin from Fibrinogen

This is the final stage of blood coagulation in which thrombin acts as an enzyme to convert fibrinogen to fibrin. In this process, first, the fibrin monomers are formed and afterward, they are polymerized to fibrin thread (blood clot). This occurs in three steps:
1. Proteolysis of soluble fibrinogen
2. Polymerization of fibrin monomers
3. Stabilization of fibrin polymer

Proteolysis of Soluble Fibrinogen

Fibrinogen has three domains: two peripheral (D) domains and one central (E) domain.
1. Thrombin binds with central domain and proteolytically releases two fibrinopeptides A and B from aminoterminals of Aα and Bβ chains of each fibrinogen molecules.
2. Release of fibrinopeptides leads to the formation of fibrin monomer (Flowchart 21.1).
Polymerization of Fibrin Monomers

Fibrin monomers join to form **protofibrils**.
1. About 15 to 20 protofibrils aggregate to form **thick fibers of fibrin**.
2. Protofibrils also branch out to form a meshwork of interconnected thick fibrin fibers. This is called **polymerization of fibrin monomers**.
3. Thrombin initiates the process of polymerization and simultaneously activates XIII.
4. XIIIa completes the process of polymerization.

Stabilization of Fibrin Polymer

Fibrin stabilizing factor (XIIIa) stabilizes the fibrin polymers by cross-linking them.
1. XIII is converted to XIIIa by thrombin.
2. **Calcium acts as a cofactor** for this conversion.
3. **Covalent cross-linking** of fibrin polymers provides adequate strength to the fibrin thread and to the fibrin meshwork.
4. The stabilized fibrin meshwork is the blood clot.
5. Red cells and platelets are trapped inside the fibrin meshwork to give the volume to the clot.

Clot Retraction

The clotted blood consists of fibrin meshwork containing red cells and platelets trapped within the clot. **Fibrin threads spread in all directions** and adhere to the endothelial wall. When blood is allowed to clot in a test tube, the **fibrin mesh spreads all around** trapping all the serum within it. However, within minutes to hours, clot shrinks expressing serum out of it. This phenomenon is called **clot retraction**.
1. The process of clot retraction is believed to occur in vivo that causes **consolidation of thrombus** (intravascular clot).
2. Clot retraction is the **function of platelets**.
3. For effective clot retraction to occur, normally functioning **platelets must be present in adequate number**.
4. Retracted clot decreases the efficiency of thrombolysis (fibrinolysis). Therefore, a **thrombus rich in platelets is resistant to fibrinolytic agents**.

**Mechanism of Clot Retraction**

The platelets form spicules (filopodia) that extend along the fibrin threads.
1. Fibrin strands tug with the filopodia. Also, protofibrils of thick fibrin strand get embedded within the filopodia by the action of membrane cytoskeleton.
2. Later, platelets shrink with contraction of their filopodia. This causes **internalization of fibrin within the contracted platelets**. Thus, retraction of clot occurs.
3. **Thrombin and calcium** accelerate clot retraction.
4. **Glycoprotein IIb/IIIa** receptors on platelets facilitate clot retraction.

**Functions of Clot Retraction**

A retracted clot is a **consolidated and stable thrombus**, which not only firmly seals the opening in the injured vessel, but also has other functions. The functions of retracted clot are as follows:
1. Retracted clot (thrombus) strongly seals injured vessel.
2. It facilitates wound healing.
3. It prevents thrombolysis.

**Prolongation of Clot Retraction Time**

Normally, clot retraction begins within thirty seconds after clot formation. However, the process of retraction is very slow. About 50% retraction occurs at the end of one hour and completes in 18 to 24 hours.
1. Clot retraction time is said to be prolonged when retraction is less than 50% at the end of one hour.
2. In **Glanzmann thrombasthenia**, deficiency of IIb/IIIa receptors results in inhibition of clot retraction that causes prolongation of clot retraction time.
3. **Platelet deficiency** also prolongs the clot retraction time.

**ANTICLOTTING MECHANISM (Fibrinolysis)**

Tendency of blood to clot in vivo is prevented by naturally occurring anticlotting mechanisms. Thus, there is a balance between clotting and anticlotting mechanisms.
1. In our body, normally, there is a low level of activation of coagulation factors. This results in a **basal coagulation** of blood. The basal coagulation may be due to minor injuries to blood vessels that occur during normal daily activities (**normal vascular stress**). However, coagulation process proceeds only when enough thrombin is generated in response to a significant vascular injury.
2. Moreover, the basal coagulation is balanced by the activity of basal anticoagulation, which is evidenced by the presence of low levels of protein C and tissue plasminogen activator activity in normal individuals. Anticlotting mechanisms include three naturally occurring systems:

1. Presence of naturally occurring anticoagulants in the blood
2. Interaction between thromboxane $A_2$ and prostacyclin
3. An important role played by vascular endothelium.

**Mechanism of Fibrinolysis**

The process of blood coagulation automatically initiates the anticlotting mechanisms so that intravascular clot does not spread beyond the site of injury. Fibrinolysis occurs in three steps:

1. Activation of protein C
2. Activation of plasmin
3. Fibrinolysis

**Activation of Protein C**

Thrombin which is produced by clotting mechanism acts as an enzyme to activate protein C to its active form. The anticlotting mechanism activated by thrombin can also be initiated by thrombomodulin, a hormone secreted from endothelial cells of blood vessel. Thrombomodulin activates protein C (Fig. 21.2).

**Activation of Plasmin**

Activated protein C inactivates the inhibitors of plasmin activator. This, in turn, activates plasminogen activator, which promotes formation of plasmin from plasminogen. Activated protein C along with its cofactor protein S also inactivates Va and Villa into their respective inactive forms. This aids in prevention of coagulation.

**Fibrinolysis**

Plasmin acts as an enzyme to cause fibrinolysis (lysis of clot).

1. This process is facilitated by cofactors thrombin, tissue-type plasminogen activator and urokinase-type plasminogen activator.
2. Fibrin is degraded by plasmin to fibrin degradation products (FDP) (Clinical Box 21.1). The details of fibrinolysis by plasmin are given below.

**Clinical Box 21.1**

FDP level indicates the rate of fibrinolysis: Activation of clotting process as occurs in DIC activates the anticlotting mechanism (fibrinolysis). Fibrinolysis causes accumulation of FDP in plasma, which is subsequently excreted in urine. Hence, measurement of FDP in urine is an index of rate of fibrinolysis as well as intravascular coagulation.

**Functions of Plasmin**

Functions of plasmin can be broadly divided into two categories: Fibrinolytic actions and nonfibrinolytic actions.

**Fibrinolytic Actions**

Plasmin cleaves both fibrinogen and fibrin.

1. Plasmin initially cleaves $\alpha$ and $\beta$ chains within the D domain of fibrinogen that releases $A\alpha$ and $B\beta$ fragments.
2. Subsequently, plasmin cleaves polypeptide chains connecting D and E domains that forms, D, E and Y fragments.
3. Degradation of fibrin by plasmin results in production of fibrin degradation products (FDP), such as D-dimers.

**Nonfibrinolytic Actions**

Plasmin has many functions other than fibrinolysis:
1. Plasmin is involved in tissue remodeling.
2. Plays a role in inflammation and tumor cell invasion.
3. Controls ovulation and embryogenesis.
4. Regulates development of neurons.
5. Plasmin plays a role in activation of growth factors.

**Plasminogen**

Plasminogen is a polypeptide consisting of 791 amino acids. Its molecular weight is 92,000 and plasma half-life is approximately two days. It has two chains: a heavy chain containing 560 amino acids and a light chain of 231 amino acids. Plasminogen is synthesized mainly in liver.
1. The gene for plasminogen formation is located on chromosome 6.
2. Activation of plasminogen occurs from cleavage by plasminogen activators of the bond between arginine and valine at 560 and 561 positions respectively.
3. Thus, plasminogen is converted into plasmin, an active protease.

**Plasminogen Activators**

There are two major endogenous plasminogen activators: the tissue plasminogen activator and urokinase.

**Tissue Plasminogen Activator (t-PA)**

t-PA is a polypeptide having 527 amino acids and molecular weight 72,000. The gene for t-PA is located on chromosome 8. It is synthesized primarily by endothelial cells. The half-life of t-PA is very short, about 5 minutes.
1. Thrombin, histamine, bradykinin, epinephrine, ADH, acetylcholine and gonadotropins control the secretion of t-PA.
2. Though, normally t-PA is a poor plasminogen activator by itself, in the presence of fibrin, its activity increases enormously.
3. Fibrin facilitates t-PA activity by increasing affinity of t-PA for plasminogen (Clinical Box 21.2).

**Urokinase and Streptokinase**

Urokinase plasminogen activator (u-PA) is a glycoprotein consisting of 411 amino acids. The gene for u-PA is located on chromosome 10. It is formed by endothelial cells, macrophages and renal epithelial cells.
1. It exists in **two forms**: high molecular weight u-PA (molecular weight of 54,000) and low molecular weight u-PA (molecular weight of 33,000).
2. Though both the forms have ability to activate plasminogen, only high molecular weight u-PA binds to u-PA receptor.
3. Physiologically, **u-PA is a more effective activator of plasminogen** than t-PA even in the presence of fibrin. u-PA has lower affinity for fibrin than t-PA.
4. **Streptokinase** is a variant of urokinase.

**Clinical Box 21.2**

| t-PA and urokinase are used in treatment of AMI: | t-PA and urokinase for their fibrinolytic activity are used for lysis of intracoronary clot in acute myocardial infarction (AMI). t-PA is very effective for this purpose as it causes lysis of clot at the site of thrombosis. |

**Inhibitors of Fibrinolysis**

Inhibitors of fibrinolysis can be divided into two categories: plasmin inhibitors and plasminogen-activator inhibitor.

**Plasmin Inhibitors**

Plasmin is inhibited by serpins, a family of serine protease inhibitors. They form an irreversible complex with the active site serine of the target protease.
1. α₂-antiplasmin is a serpin peptide containing 452 amino acids. Though it is synthesized by liver and kidney, it is a constituent of α₂-granules of platelet.
2. α₂-macroglobulin is a nonserpin plasmin inhibitor, formed by endothelial cells and macrophages.

**Plasminogen-Activator Inhibitors (PAI)**

There are two PAIs: PAI-1 and PAI-2.
1. **PAI-1** is single chain polypeptide containing 402 amino acids, secreted by liver, monocytes, endothelial cells and adipocytes. This is the most important and rapidly acting physiologic inhibitors of t-PA and u-PA.
2. **PAI-2** is secreted by placenta, monocytes and tumor cells. It is less effective than PAI-1 in inhibiting plasminogen activators.

**Plasmin Generation Defects**

**Fibrinolytic Deficiency**

Fibrinolytic deficiency is usually due to plasminogen deficiency. There are **two types** of congenital plasminogen deficiencies: **type 1** and **type 2**.

- **In type 1 plasminogen deficiency**, both concentration and function of plasminogen are reduced.
- **In type 2 plasminogen deficiency**, though the concentration of plasminogen is normal its function is reduced.

The patient with plasminogen deficiency exhibits repeated episodes of thrombophlebitis, intracranial and mesenteric venous thrombosis, and pulmonary embolism (Application Box 17.1). Mutation in plasminogen activators also results in decreased fibrinolysis.

**Enhanced Fibrinolysis**

This usually occurs due to congenital or acquired loss of fibrinolytic inhibitor activity.
1. **Deficiency of \( \alpha_2 \)-antiplasmin** is a common cause. The patient presents with bleeding diathesis. In promyelocytic leukemia, bleeding occurs due to high level of plasmin generation and deficiency of \( \alpha_2 \)-antiplasmin.

2. **Acquired \( \alpha_2 \)-antiplasmin deficiency** may be seen in liver disease, DIC, nephritic syndrome and thrombotic therapy.

### Application Box 17.1

**Pregnancy is a hypofibrinolytic state:** In pregnancy, overall fibrinolytic activity is reduced. This leads to increased fibrin deposition, as suggested by increased D-dimer levels. The PAI levels also increase in pregnancy. The hemostatic and fibrinolytic imbalance is increased in preeclampsia. This predisposes to intravascular thrombosis.

### Regulation of Blood Coagulation

Blood coagulation at the site of injury is a life saving process as it prevents loss of blood volume. However, once the clot (fibrin-platelet plug) is formed, the process of clotting must be terminated to avoid thrombotic occlusion in the adjacent normal areas of blood vessel. If clotting remains unchecked, coagulation of blood can spread to the entire vascular tree with modest procoagulant stimulus.

Coagulation in a normal blood vessel or circulation is highly dangerous. Fluidity of blood and absence of thrombosis (intravascular clot) are important physiological states that are essential for normal circulation and blood supply to tissues of the body.

The balance between coagulation and anticoagulation is due to play of various regulatory mechanisms, as listed below:

1. **Dynamism of blood flow:** Flow of blood is a dynamic process. When the rate of blood flow decreases, as occurs in vascular stasis, intravascular coagulation is facilitated. Thus, continuous and dynamic flow of blood should be maintained to prevent intravascular coagulation.

2. **Role of vascular endothelium:** Vascular endothelium plays an important role in restricting coagulation process to the site of injury.
   - Damage to vascular endothelium initiates the clotting mechanism by exposing its collagen and negatively charged particles.
   - Once adequate hemostasis is achieved, thrombomodulin, a hormone secreted by endothelial cells of blood vessel prevents further blood coagulation by activating protein-C-protein S complex.
   - Also, protease inhibitors AT-III and tissue factor pathway inhibitor are expressed on the surface of the endothelial cells.
   - Endothelial cells also inhibit platelet activation by releasing prostacyclin and EDRF that inhibit platelets (Refer to Fig. 20.11, Chapter 20).

3. **Heparin-antithrombin-III system:** Heparin is a naturally occurring anti-coagulant, secreted from mast cells and basophils. Heparin acts by activating the antithrombin III activity. Heparin-antithrombin III complex prevents activation of clotting factor IX, X, XI and XII.

4. **Negative feedback by thrombin:** Thrombin, which is a major mediator of blood coagulation, initiates the process of anticlotting mechanism (fibrinolysis). Thus, coagulation is an autoregulatory process.

5. **Role of liver:** Many coagulation factors are plasma proteins, formed mainly in the liver. Therefore, in liver diseases, clotting time is prolonged. Also, liver plays an important role in preventing intravascular coagulation by removing activated clotting factors from blood. This hepatic clearance of activated clotting factors is accelerated when clotting is spontaneously activated.

6. **Role of platelets:** In addition to its role in primary hemostasis, platelets contribute to coagulation in various ways.
   - Following platelet activation, platelet phospholipids (platelet factor 3 and 4) released to their surface act as cofactors to accelerate the formation of Va, VIIia and Xla. Cofactors Va and VIIia are rapidly localized to the membrane of activated platelet.
   - Also, factor IXa formed by VIIia-tissue factor complex binds to the platelet surface. This leads to the formation of IXa-VIIia complex on the platelet surface, following which factor X is recruited from the plasma to the platelets and is activated to Xa on platelet membrane. Thus, **platelet plays an important role in activation of factor X**, which is a crucial step in blood coagulation. Thus, major clotting reactions occur in close interaction with platelets (Refer to Fig. 20.8, Chapter 20).
   - As platelets adhesion and aggregation are localized at the injured vessel wall, platelets help in restricting clotting reactions to the site of injury.

### ANTICOAGULANTS

Anticoagulants prevent blood from clotting. They are mainly used:

1. For collection of blood sample for laboratory investigations
2. For preserving blood for transfusion
3. For anticoagulation therapy

#### Anticoagulants for Blood Collection

Anticoagulants are added to the blood sample especially during collection of blood by venipuncture, when whole anticoagulated blood is required for laboratory investigations. Though several anticoagulants are available for the purpose, commonly used are EDTA, trisodium citrate, double oxalate, sodium fluoride, and heparin.

1. While EDTA is used frequently for hematologic tests, citrated blood is used for coagulation studies and for preserving blood in the blood bank.
2. Use of heparin and fluoride (oxalated) is limited for the determination of blood gases and pH, and plasma glucose respectively.

**EDTA (Ethylene-diamine Tetra-acetic Acid)**
The sodium and potassium salts of EDTA are the commonly used and most powerful anticoagulants.
1. It acts by its **chelating effect on the blood calcium**. It inactivates calcium by forming insoluble complex with it. As calcium is an important cofactor for blood coagulation, chelation of calcium prevents blood to clot.
2. The chelating effect of EDTA is achieved at a concentration of 1.2 mg of the anhydrous salt per ml of blood.
3. Excess EDTA irrespective of its salts, affects both red cell and leucocyte morphology by causing shrinkage and degenerative changes. Therefore, EDTA in excess of 2 mg/ml of blood results in **decrease in PCV and increase in MCHC**. Platelets also swell and disintegrate causing an **artificially high platelet count**, as the platelet fragments are large enough to be counted as normal platelets.

**Sodium Citrate**
Trisodium citrate is the commonly used anticoagulant in coagulation studies. It is prepared as 0.106 M solution of trisodium citrate in distilled water and then sterilized.
1. It **prevents coagulation by inactivating calcium ions**.
2. For coagulation studies like determination of prothrombin time or partial thromboplastin time, 9 volumes of blood are added to 1 volume of sodium citrate solution (9 : 1).
3. It is also used in **collection of blood for estimation of ESR by Westergren method**, where 4 volumes of venous blood are added to 1 volume of the sodium citrate solution (4 : 1).

**Double Oxalate**
As this anticoagulant contains ammonium and potassium oxalates, it is called **double oxalate**. Potassium oxalate alone causes shrinkage of red cells whereas ammonium oxalate increases their volume. Hence, double oxalate is also called **balanced oxalate**.
1. It is prepared as the solution containing 1.2% ammonium oxalate and 0.8% potassium oxalate. Usually, 0.25 or 0.5 ml of the double oxalate solution is taken in a penicillin bottle and evaporated in an oven or in an incubator and then kept for collecting blood.
2. The oxalates form an **insoluble complex with the calcium** in the blood, and thereby prevent coagulation.

**Sodium Fluoride**
Sodium fluoride is used mainly for collecting blood specimen for **plasma glucose estimation**. Fluoride is an **inhibitor of glycolytic enzymes** and thus prevents loss of glucose. However, as fluoride is not a strong anticoagulant, it is mixed with the oxalate.

**Oxalates**
Oxalates of sodium, potassium, ammonium or lithium act as anticoagulant. They form **insoluble complexes with calcium**.

**Heparin**
Heparin is a natural constituent of blood. Sodium, lithium, potassium and ammonium salts of heparin are commercially available.
1. It prevents coagulation for approximately 24 hours by neutralizing thrombin. It **facilitates action of antithrombin III**. Thus, it **prevents formation of fibrin from fibrinogen**.
2. Usually, it is used at a concentration of 10–20 IU per ml of blood.
3. It is commonly used for osmotic fragility test, blood gas determination and pH assays.
4. It is also commonly used in anticoagulation therapy and in preventing blood coagulation during animal experiments.

**Anticoagulants for Treatment (Anticoagulation Therapy)**
Anticoagulants are used for **treatment or prevention of thrombosis** (intravascular clots). They are usually required for:
1. Management of thrombotic diseases like venous thromboembolism
2. Placement of mechanical heart valves as heart valve prosthesis is associated with risk of thromboembolism
3. Prevention of thromboembolism in chronic nonrheumatic atrial fibrillation
4. Prophylaxis after attacks of cerebral ischemia
5. Management of ischemic heart disease
6. Peripheral vascular diseases
7. Placement of central venous catheters.

**Oral Anticoagulants**
Routinely used oral anticoagulants are vitamin K antagonists, glycosaminoglycans, inhibitors of factor Xa and inhibitors of thrombin.

**Vitamin K Antagonists**
The commonly used vitamin K antagonist is warfarin. This is a coumarin derivative.
1. Warfarin is an acronym for WARF, i.e. Wisconsin Alumni Research Foundation that first synthesized it in 1942 (Coumarin of WARF, named as warfarin).
2. The four vitamin K-dependent coagulation factors are II, VII, IX and X. They undergo post-translational \( \gamma \)-carboxylation of approximately 10 glutamic acid residues in the N-terminal Gla-domain (the domain where \( \gamma \)-carboxylation of clotting proteins takes place is called the Gla-domain).

3. The \( \gamma \)-carboxylation of these cofactors is necessary to enable them to bind calcium and to localize enzymatic processes to a phospholipid surface like membrane of activated platelet.

4. When \( \gamma \)-carboxylation is reduced by 1 to 6 glutamic acid residues, coagulation activity of factors II, VII, IX and X is impaired significantly.

**Mechanism of action of warfarin**: Warfarin inhibits \( \gamma \)-carboxylation of about 3 to 10 glutamic acid residues, especially of factor II (prothrombin). Warfarin achieves this by inhibiting the reductase enzymes that cause formation of vitamin KH\(_2\) (Fig. 21.3).

- Vitamin KH\(_2\) is required for \( \gamma \)-carboxylation of vitamin K-dependent coagulation factors.
- During the process of \( \gamma \)-carboxylation, vitamin KH\(_2\) is converted to vitamin K-epoxide by vitamin K epoxide reductase, vitamin K-epoxide is converted back to vitamin KH\(_2\) by vitamin K reductase.
- Both these enzymes are inhibited by coumarin derivatives such as warfarin.

**Protein C and protein S** (the inhibitors of blood coagulation) also undergo \( \gamma \)-carboxylation and vitamin K antagonists prevent their activity by inhibiting the process.

*Dicoumarol* is another commonly used vitamin K antagonist.

**Glycosaminoglycans**

Oral heparin sulfate and iduronyl-glycosaminoglycan are used as anticoagulants.

**Inhibitors of Factor Xa**

Formation of Xa is the key step in blood coagulation. Therefore, selective inhibitor of Xa is a potent antithrombotic agent without affecting bleeding time as demonstrated in animal models. However, its use in human is under trial.

**Inhibitors of Thrombin**

Many low-molecular weight selective inhibitors of thrombin have been recently identified and are under clinical trials.

**Intravenous or Subcutaneous Anticoagulants**

Most commonly used intravenous anticoagulants are heparin and hirudin.

**Heparin**

Heparin is a glycosaminoglycan consisting of chains of alternating residues of D-glucosamine and iduronic acid. Due to its variation in chain lengths, heparin is available as LMW-heparin and HMW-heparin, molecular weight ranging between 5,000 to 30,000. Its anticoagulant activity is due to its facilitation of the effects of antithrombin III (AT-III).

1. The pentasaccharide component of heparin has high affinity binding to AT-III.
2. AT-III inactivates thrombin, Xa and IXa. However, heparin inhibition of thrombin is different from inhibition of Xa and IXa.

**HMW heparin**: HMW heparin molecules inhibit thrombin by forming a ternary complex in which it binds with both thrombin and AT-III, and facilitates the thrombin inhibition by AT-III. But for inhibition of Xa, AT-III/heparin complex does not require heparin to bind directly to factor Xa or IXa in a ternary complex.

**LMW heparin**: LMW heparin molecules are unable to bind thrombin and AT-III simultaneously. Therefore, they can not augment the inhibition of thrombin by AT-III. However, they retain the ability to inhibit factor Xa and IXa. LMW heparin is as effective as unfractionated heparin for the treatment of venous thromboembolism or acute coronary syndrome. It is given subcutaneously and is cost-effective in comparison to IV heparin.

**Hirudin**

Hirudin is a polypeptide containing 65 amino acids. It is produced by the salivary glands of *Hirudo medicinalis*, a medicinal leech.

1. It is a naturally occurring most specific inhibitor of thrombin.
2. It is also produced by recombinant DNA technology. It directly inactivates thrombin by binding to the active site and main fibrinopeptide binding region of thrombin.
3. It has been tried in the treatment of acute coronary syndrome, patients undergoing coronary angioplasty and deep vein thrombosis.
4. It is mainly used as anticoagulant in patients who develop heparin-induced thrombocytopenia.
Laboratory investigations of bleeding disorders are carried out for patients who have a history of spontaneous bleeding or excessive bleeding after injury or surgery. Bleeding disorders are primarily due to the defects in formation of either in temporary hemostatic plug or in definitive hemostatic plug. Therefore, initial investigations aim at differentiating these two primary defects. If it is a coagulation disorder, tests are performed to detect the deficiency of clotting factor that has caused the disease and also to assess whether the defect is in intrinsic or extrinsic system.

Assessment of Defects in Temporary Hemostatic Plug

1. Bleeding time
2. Capillary fragility test
3. Platelet aggregation test
4. Platelet adhesiveness test

Bleeding Time (BT)
The normal BT by Duke method is 1 to 5 minutes and by Ivy method is 5 to 11 minutes. BT assesses platelet number and functions.

Capillary Fragility Test
This test measures the ability of the capillaries to withstand increased stress. Petechiae appear in the fore arm of the subject when the blood pressure cuff in the arm is inflated to a maximum pressure of 100 mm Hg for about 5 minutes.
1. Normally, zero to 10 petechiae appears.
2. More than 10 petechiae indicate capillary weakness, thrombocytopenia or both.

Platelet Aggregation Test
An aggregating agent is added to a suspension of platelets in plasma and the response is measured turbidometrically as a change in the transmission of light by the instruments called aggregometers. Measurement of platelet aggregation is an essential part of the investigation of any patient with suspected platelet dysfunction in a modern laboratory.

Platelet Adhesiveness Test
This test measures the ability of platelets to adhere to glass surface. When anticoagulated blood is passed through a plastic tube containing glass beads at a constant rate, some platelets will adhere to the glass beads.
1. The percentage difference of the platelet count done prior to and after passage through the glass bead column is calculated.
2. The normal range is 75 % to 95 % of platelet retention.
3. The platelet adhesiveness test is nonspecific. It is abnormal in several platelet functional disorders.

Assessment of Defects in Definitive Hemostatic Plug

1. Clotting time
2. Prothrombin time
3. Prothrombin consumption test
4. Partial thromboplastin time
5. Activated partial thromboplastin time
6. Thrombin time
7. Plasma recalcification time
8. Clot retraction time

Clotting Time (CT)
Determination of CT is routinely performed before any surgical procedure. It is usually performed by two methods: capillary tube method, and Lee-White (venipuncture) method.
1. The capillary method is used routinely in clinical laboratories. The normal range of CT by capillary glass tube method ranges from 2 to 8 minutes. CT is prolonged in diseases in which there is deficiency of clotting factors.
2. If the CT is prolonged more than 10 minutes, the patient should be subjected to more detailed investigations for the identification of the missing coagulation factors.
3. The normal range of CT by Lee-White method ranges from 5 to 12 minutes.

Prothrombin Consumption Test
This test is performed to determine the quantity of prothrombin remaining in the serum after clot is formed. Normally, prothrombin is used if it is converted to thrombin. Increased serum prothrombin results from a quantitative or qualitative platelet deficiency.

Prothrombin Time (PT)
PT detects the integrity of stage 2 clotting process. In this, preparation of rabbit brain emulsion (which contains tissue thromboplastin) is added to plasma in the presence of calcium. This, in the presence of factor VII, triggers stage 2 of the coagulation mechanism, and the clotting time is recorded after the addition of calcified thromboplastin to the plasma.
1. Normal PT is 12 to 16 seconds. Prolonged PT suggests the possibility of deficiency of factor II, V, VII, and X.
2. In stage 2, prothrombin is converted to thrombin which triggers the transformation of fibrinogen to fibrin. Thus, abnormal prothrombin time suggests defect in stage 2.
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Partial Thromboplastin Time (PTT)
PTT detects the integrity of stage 1 clotting process. The platelet substitute in the form of partial thromboplastin is prepared from rabbit brain as chloroform extract. The extract is mixed with test plasma containing excess of calcium, which leads to clot formation.
1. Normal PTT is 60 to 80 seconds. PTT is prolonged when there is a deficiency of one or more clotting factors XII, XI, IX, VIII, X, V, II, and I.
2. Abnormal PTT indicates stage 1 defect and missing of one or more of these intrinsic factors.

Activated Partial Thromboplastin Time (APTT)
APTT is a more reliable test than PTT in evaluating the coagulation disorders. Its use in conjunction with PT provides a simple method for differentiating between stage 1 intrinsic defects and deficiency of other factors. The platelet substitute, in the form of partial thromboplastin, is prepared from rabbit brain. This is incubated with a contacting agent (kaolin) to provide optimal activation of the intrinsic coagulation factors. The clotting time is determined after the addition of an excess of calcium.
1. Normal APTT is 35 to 40 seconds.
2. APTT is prolonged in deficiencies of factor XII, XI, X, IX, and VIII.
3. APTT is mainly estimated in hemophilias that involve deficiencies of factor VIII, IX or XI.

Thrombin Time (TT)
TT assesses the integrity of stage 3 of blood coagulation. Thrombin (commercially available) is added to the plasma along with calcium and clotting time is determined.
1. Normal value of TT is 15 to 20 seconds.
2. Thrombin time detects the effectiveness of the final stage of coagulation in which fibrinogen is converted to fibrin.
3. A prolonged TT is considered to be due to either a decrease in fibrinogen concentration or the presence of dysfunctional fibrinogen.

Plasma Recalcification Time (PRT)
PRT assesses efficiency of intrinsic system of coagulation. When excess of calcium is added to the citrated plasma, clotting occurs. As platelet factor 3 acts as a cofactor for coagulation, clotting occurs in less time in platelet rich plasma than in platelet poor plasma.
1. Normal value of PRT of platelet rich plasma is 100 to 150 seconds and of platelet poor plasma is 135 to 240 seconds.
2. PRT is a screening test to detect the deficiencies of the factors of the intrinsic pathway, especially XII, XI, IX, VIII, X, V, and II (all coagulation factors except VII and XIII).

Clot Retraction Time
It assesses the clot stability and platelet functions (for details of clot retraction time, refer to previous chapter).

ABNORMALITIES OF COAGULATION

Hemorrhagic disorders are broadly classified into inherited and acquired defects.
1. Acquired defects are more common than inherited defects and platelet defects are more common than the coagulation defects.
2. Deficiencies of factor VIII (hemophilia) and factor IX (Christmas disease) are more common inherited coagulation defects.
3. The common acquired defects are thrombocytopenia, vitamin K deficiency, disseminated intravascular coagulation and liver failure resulting in clotting defects.

Hemophilia A

Etiology
Hemophilia A, also known as classic hemophilia, is a bleeding disorder that occurs due to deficiency of factor VIII. It is an X-linked recessive hereditary disease. Though it is less common than von Willebrand disease, it is more common than other inherited defects of coagulation. Women are carriers and generally do not suffer from the disease as they are protected by the second X-chromosome which is usually normal.

Clinical Features
The disease manifests with the bleeding tendency which appears in infancy, but in mild cases, may appear in adult life.
1. Soft tissue hematomas and hemarthroses (bleeding into joints) leading to severe crippling hemarthropathy are highly characteristic of the disease.
2. In severe cases, spontaneous hemarthropathy from infancy is the common feature.
3. In mild to moderate cases, continuation of hemorrhage secondary to trauma or surgery is the feature.
4. Bleeding usually persists from days to weeks inspite of formation of clots.
5. Bleeding may also occur spontaneously into tissues, and cavities of the body.

Diagnosis
Patients have prolonged activated partial thromboplastin time (APTT). Prothrombin time and bleeding time are normal. Assay of factor VIII in plasma is diagnostic. Functional factor VIII coagulant activity can also be measured.

Treatment
The treatment consists of transfusion of fresh blood (as on storage factor VIII is rapidly lost), or transfusion of factor
VIII-concentrate. Many plasma products are available for raising factor VIII to hemostatic level. Fresh-frozen plasma and cryoprecipitate both contain factor VIII. Attempt should be made to avoid aspirin, nonsteroidal anti-inflammatory drugs and other drugs that interfere with platelet aggregation.

Christmas Disease (Hemophilia B)

Etiology
Christmas disease or hemophilia-B occurs due to deficiency of factor-IX (antihemophilic factor-B or Christmas factor). This is a sex-linked recessive hemorrhagic disease.

Features
The disease is clinically indistinguishable from hemophilia A. Bleeding episodes are clinically identical to those in hemophilia A. Therefore, this is also called hemophilia-B. Hematoma, hemarthroses and crippling hemarthropathy occur.

Diagnosis
In most cases, PT is normal and partial thromboplastin time (PTT) is prolonged. Specific assay of factor IX coagulant activity confirms diagnosis.

Treatment
The specific treatment of hemophilia B is the replacement of factor IX.

Scientist contributed
In 1947, Dr Alfredo Pavlovsky, a doctor in Buenos Aires, Argentina, distinguished two types of hemophilia in his lab—A and B. He reported that the blood from some hemophilic patients corrected the abnormal clotting time in others. In 1952 this was called Christmas disease, after the family in which it was discovered.

von Willebrand Disease

Etiology
von Willebrand disease (vWD) is the most common inherited bleeding disorder in humans that occurs due to deficiency of von Willebrand factor (vWF). In 1926, Eric von Willebrand described this bleeding disorder in both genders of 24 persons of a 66 members of family from Åland Island.
1. vWF plays a central role in hemostasis as it serves as a carrier for factor VIII and is essential for adhesion of platelets to the injured vessel wall.
2. Therefore, deficiency of vWF leads to defects in the formation of both temporary and definitive hemostatic plugs.

Variants
There are different variants of vWD: Type 1, type 2A, type 2B, type 2M, type 2N and type 3.
1. Type 1 is the most common variant, which occurs due to decrease in 20–50% in quantity of vWF.
2. Type 2 variants are mainly qualitative abnormalities of vWF.
3. Type 3 is the most severe form in which vWF is grossly decreased.
   Factor VIII activity is decreased in type 1, decreased or normal in type 2, and markedly decreased in type 3.

Clinical Features
Mucocutaneous bleeding is the most commonest presentation in type 1. Epistaxis, easy bruising, hematoma, menorrhagia and GI bleeding are common. In type 3, patients suffer from severe bleeding and present with hemarthroses and muscle hematomas like hemophilia A. Type 2 presents with moderate symptoms.

Diagnosis
As vWF is primarily responsible for platelet adhesion, prolongation of bleeding time is a standard screening test for vWD. However, clotting time is prolonged or normal depending on the degree of deficiency of factor VIII. Quantitative and functional assays of plasma vWF are diagnostic.

Treatment
Desmopressin (DDAVP), an analogue of ADH increases factor VIII activity, vWF-Ag concentration and ristocetin cofactor activity. Therefore, DDAVP is very useful in type 1 and 2 diseases. However, vWF replacement therapy is required in type 3 disease.

Disseminated Intravascular Coagulation (DIC)

Definition and Etiology
DIC is a clinicopathological syndrome in which there is widespread intravascular coagulation that occurs due to procoagulants that are introduced into or produced by blood circulation.
1. The procoagulant activity overcomes the natural anticoagulant mechanisms. This is also called consumption coagulopathy or defibrination syndrome.
2. This is a hemorrhagic disorder in which diffused intravascular coagulation results in defects of hemostasis.
3. In this disease, coagulation factors and platelets are overutilized. This results in bleeding.
4. The most common procoagulant stimulus is the tissue factor (tissue thromboplastin) exposure to the blood, that activates extrinsic pathway of coagulation (Fig. 21.4).
5. Activation of plasmin causes excess fibrinolysis, resulting in increased levels of fibrin degradation product (FDP)

Common causes of DIC are:
1. Septic abortions
2. Amniotic fluid embolism
3. Gram-negative septicemia
4. Fungemia
5. Severe trauma
6. Severe eclampsia
7. Burns (burn sepsis)
8. Snake bite (Russell viper)
9. Sometimes in hemolytic transfusion reactions.

Manifestations
The clinical manifestations of DIC include two important features:
1. Multiorgan dysfunctions caused by widespread microembolism
2. Bleeding caused by consumption of platelets, fibrinogen, factor V and factor VIII.

Therefore, this is also called consumption coagulopathy. There is also activation of secondary fibrinolysis (hence, called defibrination syndrome).

Diagnosis
Laboratory features include thrombocytopenia, hypofibrinogenemia, increased d-dimer and fibrin degradation product (FDP) and prolonged PT, PT and thrombin time (TT). Diagnosis is usually done by demonstrating FDP in urine and decreased concentration of coagulation factors and fibrin monomers in blood.

Treatment
Treatment is based on early diagnosis, elimination of the precipitating factors, and replacing coagulation factors and platelets. Blood component therapy is needed for those who bleed excessively.

Thrombosis
Thrombus is an intravascular clot. Normally, a balance is maintained between the processes of coagulation and anticoagulation and therefore thrombus is not formed. However, in pathological situations, intravascular clotting occurs. Three factors (Virchow’s triad) predispose to the formation of thrombosis. These are:
1. Endothelial injury: Injury to vascular endothelium occurs in chronic and sustained hypertension, ulcerated atherosclerosis, arterial diseases etc. Injured site becomes the site for platelet adhesion and aggregation and intravascular clot formation.
2. Sluggishness of blood flow: Stasis of blood promotes thrombosis (as described above).
3. Hypercoagulability of blood: Increased activity of procoagulants such as fibrinogen, prothrombin and other coagulants leads to thrombosis.

Platelet aggregation at the site of injury initiates the process of thrombosis. Venous thrombosis is more common than arterial thrombosis.
1. One common example is thrombosis of lower limb veins in varicosities.
2. Deep vein thrombosis is not uncommon.
3. Thrombosis also occurs in cardiac chambers and valve leaflets.

   The major complication of thrombosis is thromboembolism. Emboli are dislodged from thrombus and circulate to be lodged in microcirculation in visceral organs, such as brain (cerebral embolism), lungs (pulmonary embolism), heart (coronary embolism) and intestine (intestinal embolism). Coronary and cerebral thrombosis leads to ischemic tissue death (infarction), which causes heart attack and stroke respectively. Prophylactic anticoagulation and antiplatelet therapy is the mainstay of prevention of complications of thrombosis.

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### CHAPTER SUMMARY

### Key Concepts

1. Blood coagulation is initiated when tissue factor (tissue thromboplastin) is released into blood following tissue injury that activates VII, which is called extrinsic pathway of clotting mechanism, or, exposed collagen activates Factor XII through the intrinsic system. Finally, factor X is converted to Xa. This is the first stage in clotting.
2. In the second stage, prothrombin is converted to thrombin, and thrombin converts fibrinogen to fibrin in the third stage.
3. Clot retraction, which is the function of platelets, makes the clot stable.
4. Fibrinolysis is initiated by plasmin that checks the spread of clot beyond the site of injury.
5. Increased FDP level in blood and urine, the marker of excess fibrinolysis, is diagnostic of DIC. DIC is a state of consumptive coagulopathy.
6. In pregnancy, fibrinolytic activity is less.

### Important to Know (Must Read)

1. In examinations, ‘Mechanism of blood coagulation’ usually comes as a Long Question.
3. In Viva, examiners may ask… name the clotting factors, stages of blood clotting, steps of intrinsic and extrinsic pathways of clotting, clot retraction and its importance, mechanism of fibrinolysis, mechanism of action of Vitamin K antagonist, anticoagulants and their mechanisms, tests to detect defects in extrinsic and intrinsic mechanisms of clotting, and causes, features and treatment of common coagulation disorders.
4. Anticoagulants are frequently asked for as they are commonly used in clinical as well as laboratory practices.
SECTION–3

Nerve and Muscle

**Part A: Nerve**
22. Structure and Functions of Neurons  
23. Nerve Potentials  
24. Properties, Classification and Applied Aspects of Nerve Fibers

**Part B: Neuromuscular Junction**
25. Neuromuscular Transmission

**Part C: Muscles**
26. Structure of Skeletal Muscle: Physiological Aspects  
27. Mechanism of Skeletal Muscle Contraction  
29. Smooth Muscle and Cardiac Muscle
“The great are strongest when they stand alone.  
A God-given might of being in their force,  
A ray from self’s solitude of light the guide;  
The soul that can live alone with itself meets God.”

Sri Aurobindo (in ‘SAVITRI’)
A neuron is the structural and functional unit of the nervous system. Its primary function is to receive the various stimuli and transmit the signals to other neurons and tissues. The neuron is an excitable cell where message transmission occurs in the form of action potentials. There are about $10^{11}$ neurons present in the human central nervous system (CNS). There are also supporting cells called glial cells, which are 10 to 30 times more in number than the neural cells.

**Structure of a Neuron**

A neuron has three parts: a cell body, dendrites, and an axon that ends at axon terminal (Fig. 22.1).
Cell Body

Cell body, also known as soma or perikaryon, is the center of the neuron. It contains the nucleus and cytoplasm containing cell organelles. The plasma membrane of the soma is a bilayered lipid protein membrane, called plasmalemma.

Cell Organelles

Cell organelles are mainly numerous Nissl granules, many mitochondria, Golgi apparatus and lysosomes. Cytoplasm also contains cytoskeletal proteins like neurofilaments, microtubules and actin microfilaments (Fig. 22.2).

1. **Nissl granules**: Also called Nissl bodies, are stacks of rough endoplasmic reticulum with their membranes studded with ribosomes and polysomes. Nissl granules extend into the dendrites, but not into the axon.

2. **Neurofilaments** (Neurofibrils): Another distinctive feature of neurons is the presence of networks of fibrils permeating the cytoplasm. These neurofibrils consist of microfilaments and microtubules. In Alzheimer’s disease, the neurofilaments form a characteristic neurofibrillary tangle.

Nucleus

The nucleus usually contains one nucleolus; sometimes there may be two nucleoli, but centrioles are absent.

Functions of Soma

1. Soma contains the genetic information and is capable of protein synthesis. Though neuron is an active cell, it has lost the capacity to regenerate as indicated by the absence of centriole.

2. The soma at one end gives rise to small branching processes called dendrites and at the other end a long process called axon.

**Table 22.1: Differences between axons and dendrites.**

<table>
<thead>
<tr>
<th>Axons</th>
<th>Dendrites</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Axon is a single long thin process of nerve cell which terminates away from the nerve cell body</td>
<td>Dendrites are multiple, short, thick and tapering processes of the nerve cell which terminate near the nerve cell</td>
</tr>
<tr>
<td>2. Axon rarely branches at the right angle (axon collaterals) but ends by dividing into many fine processes called axon terminals</td>
<td>Dendrites are highly branched. Their branching pattern forms a dendritic tree</td>
</tr>
<tr>
<td>3. Free of Nissl granules</td>
<td>Nissl granules are present in dendrites.</td>
</tr>
<tr>
<td>4. The nerve impulse travel away from the cell body</td>
<td>Nerve impulse travels toward the cell body</td>
</tr>
</tbody>
</table>

Dendrites

The numerous short extensions from the cell body are called dendrites.

1. They increase the cell surface area many folds. Dendrites have dendritic spines, that help in increasing the surface area for receiving information.

2. In neurons of cerebral and cerebellar cortex, small knobby projections called dendritic spines further increase the surface area.

3. Dendrites receive the incoming signals from other cells and transmit it to the cell body.

4. At some areas of the brain, they can cause protein synthesis, also generate and conduct the action potentials.

5. On an average, there are about 10,000 dendrites per neuron present in the CNS.

Axon

Axon or the axis cylinder of the neuron forms the nerve fiber. It is a long tubular process that extends away from the cell body to transmit output signals to target organs. Dendrites transmit impulses toward cell body, whereas axons carry impulses away from cell body (Table 22.1).

1. The cytoplasm is known as axoplasm that contains mitochondria, Golgi apparatus, and cytoskeletal proteins.

2. The axon arises from a thickened, tapered area of the cell body called the axon hillock.

3. The initial portion of the axon is known as the initial segment.

4. The axon hillock continues as initial segment and this part is known as axon hillock—initial segment portion of the neuron.

5. The action potential is generated at the initial segment in motor neurons and at the first node of Ranvier in sensory neurons.

**Axon terminal**: The terminal part of axon loses myelin and divides into several branches, called telodendria. The endings of telodendria form bulb like enlargements.
called terminal buttons or synaptic knobs. The buttons contain neurotransmitter vesicles.

Types of Axon
Axons are of two types: myelinated and unmyelinated.

Myelinated Axons
In the peripheral nervous system (PNS), myelinated axons have a sheath around, called myelin sheath (Figs. 22.3A and B).
1. Myelin sheath in PNS formed by the Schwann cells. The large diameter somatic nerve fibers as well as the preganglionic fibers of the autonomic nervous system are myelinated.
2. In the CNS of mammals, myelination is carried out by oligodendrocytes. Each oligodendrocyte projects several processes that wrap around many axons.

Role of Schwann Cell in Myelination
The double layers of the membrane of a single Schwann cell wrap several times (about 100 times) over 1 mm length of the axon forming a covering of 8–10 µm thickness (Figs. 22.3A and B).
1. The adjacent layers stick to each other tightly with the help of a protein called protein zero ($P_0$) present in the Schwann cell membrane.
2. The extracellular portion of $P_0$ in one layer locks to the extracellular portion of $P_0$ in the apposing layer resulting in compaction of myelin.
3. Mutation of $P_0$ cause defective myelination and decreased conduction as occurs in various peripheral neuropathies.
4. The nucleus of the Schwann cell lies beneath the plasma membrane in the outermost layer (Fig. 22.3A). Thus, the myelin sheath extending along the length of an axon is formed by many Schwann cells, which are present at regular intervals along the course of the axon.
5. The gaps between the Schwann cells are called the nodes of Ranvier (Fig. 22.3B), where the plasma membrane is exposed to the ECF. Each node is 0.5–1.0 µm in length and the internodal distance is 1–2 mm.
6. In multiple sclerosis, an autoimmune disease, patchy destruction of myelin occurs causing decreased conduction velocity in both motor and sensory neurons.

Myelinogenesis
Myelin sheath when present is seen outside the axolemma. The cells providing this sheath for axons in the peripheral nervous system are called Schwann cells and in CNS are oligodendrocytes. The nature of this sheath is best understood by considering the mode of its formation.
1. An axon lying near a Schwann cell invaginates into the cytoplasm of Schwann cell. In this process the axon comes to be suspended by a fold of the cell membrane of the Schwann cell. This fold is called mesaxon (Fig. 22.4).
2. In some situations the mesaxon becomes greatly elongated and comes to be spirally wound around the axon, which is thus surrounded by several layers of cell membrane. Lipids are deposited between adjacent layers of the membrane. These layers of the mesaxon, along with the lipids, form the myelin sheath.
3. Outside the myelin sheath a thin layer of Schwann cell cytoplasm persists to form an additional sheath that is called neurilemma (also called the neurilemma sheath or Schwann cell sheath).
4. An axon is related to a large number of Schwann cells over its length. Each Schwann cell provides the myelin sheath for a short segment of the axon. At the junction of any two such segments there is a short gap in the myelin sheath. These gaps are called the nodes of Ranvier (Fig. 22.5).
5. The nodes of Ranvier have great physiological importance. When an impulse travels down a nerve fiber it does not proceed uniformly along the length of the axis cylinder, but jumps from one node to the next. This is called saltatory conduction (in unmyelinated neurons the impulse travels along the axolemma). Such conduction is much slower than saltatory conduction and consumes more energy.
6. The segment of myelin sheath between two nodes of Ranvier is called internode.
Composition of Myelin Sheath

Myelin contains protein, lipids, and water. The main lipids present include cholesterol, phospholipid, and glycosphingolipids. Other lipids are present in smaller amount.

Important Note

Myelin sheath defects: Myelin sheath can be seriously impaired and there can be abnormal collections of lipids, in disorder of lipid metabolism. Various proteins have been identified in myelin sheath and abnormality in them can be the basis of some neuropathy.

Objectives of Myelination

Myelination serves following four purposes.
1. It increases the speed of conduction.
2. It reduces energy expenditure by the cell.
3. It provides a protective covering to the axon.
4. It is responsible for the color of the white matter of the brain and spinal cord.

Timing of Myelination during Development

Myelination of different types of nerve fibers takes place at different times.

1. The sensory fibers of the dorsal column system first get myelinated, which occurs at 4th–5th month of intrauterine life.
2. The corticospinal tract fibers start myelinating at two months of age and the process gets completed at about 2nd year of life, when the child has learned to walk (Application Box 22.1).

Application Box 22.1

Axonal Growth: During development of a neuron, axonal growth depends on the interaction between the growing axon and the surrounding tissue environment. At the growing end is present a growth cone, which is motile and contains actin. The direction of axonal growth is determined by cell adhesion molecules, influenced by trophic factors secreted from target cells and guided by glial cells.

Unmyelinated Axons

Unmyelinated axons do not have myelin sheath.
1. The Schwann cells are present near these axons, but their mesaxons do not completely spiral around them (Fig. 22.6).
2. Another difference is that several such axons may invaginate into the cytoplasm of a single Schwann cell.
3. Somatic nerve fibers of very small diameter, postganglionic sympathetic neurons of the autonomic nervous system, dorsal root fibers and most of the fibers in invertebrates are unmyelinated.
4. The speed of conduction of impulse is slower in unmyelinated nerve fibers (Table 22.2).

Scientist contributed

Theodor Schwann advanced the concept of cellular organization of living beings, described the structure of neurilemma (covering of the nerve) and showed the necessity of air for embryonal growth. His intense study on digestion was important, in which he discovered pepsin and its action, and highlighted the importance of bile in digestion. His discovery of yeast and its role in purification and fermentation was an important revelation. He is popularly remembered for his discovery of myelin sheath and myelogenesis (Schwann cells that form the covering of myelinated neurons) of axons; hence these cells are known as Schwann cells.

Axoplasmic Transport

Transfer of substances between cell body and axon terminal is called axoplasmic transport.

1. Various proteins, organelles and other cellular substances required for the development, growth, and maintenance of the neuron are transported mainly along the length of the axon.

2. Axoplasmic transport can be abolished by application of colchicine, dinitrophenol, azide, cyanide, and prolonged anoxia.

3. Colchicine disrupts the movement of microtubules; others block the process of oxidative phosphorylation.

Types of Axoplasmic Transport

In the axoplasm, transport process can occur in both directions by different transport mechanisms. Accordingly, they are called anterograde, retrograde, and transneuronal transports.

Anterograde Transport

The transport of materials from the cell body toward the axon terminals is known as anterograde transport. For example, various neurotransmitters synthesized in the cell body are packaged in vesicles and get secreted at the nerve endings through axoplasmic microtubules. Anterograde transport process is mapped by $[^{3}H]$-leucin. The rate of transport process may be fast or slow.

Fast axoplasmic transport: Fast axoplasmic transport occurs at the speed of about 400 mm/day, which is accomplished by kinesin, a microtubule associated protein that transports many organelles, vesicles and membrane glycoproteins.

Slow axoplasmic transport: Slow axoplasmic transport occurs at the rate of about 0.5–2 mm/day. Various structural proteins like actin, neurofilaments and microtubules get transported by slow transport. It has an important role in supplying the required materials for the regeneration of axons following nerve injury.

Retrograde Transport

Transport of substances from the axon terminals to the cell body is known as retrograde transport. It occurs at a speed of about 200 mm/day, brought about by dynein, another microtubule associated protein. This mechanism keeps the soma informed about the synaptic environment. Retrograde transport is mapped by horse-radish peroxidase.

The examples of retrograde transport are:

1. Transport of viruses: The chickenpox virus, known as varicella zoster that causes herpes simplex reaches cell body from nerve terminals in the skin by retrograde transport. The virus may remain in a dormant state in nerve root for many years before causing herpes zoster afterwards.

2. Transport of toxins: Tetanus toxin at motor neuron ending is transported to the cell body by this retrograde process.

3. Transfer of nerve growth factor: Nerve growth factor is taken up by presynaptic terminal and transferred to soma by retrograde transport.

4. Reuptake of synaptic transmitters: Neurotransmitters like norepinephrine (NE) released at the nerve terminals are rapidly removed from the synaptic cleft by reuptake.
into the presynaptic neuron. In the presynaptic terminal, NE is repackaged into vesicles or deaminated by mitochondrial monoamine oxidase. This reuptake is an active retrograde transport process. Some of the vesicles may be transported back to the cell body and this may provide a feedback signal to the cell body for further synthesis of transmitters. Choline is also taken up by the axon terminal and reused for new Ach synthesis.

**Transneuronal Transport**

Trophic substances like nerve growth factors are transported across the synapse to the presynaptic membrane of another neuron. This is called transneuronal transport. This helps in maintenance of the synaptic contacts.

**FUNCTIONS OF NEURONS**

The cell body and dendrites serve as the receptor zone to receive the information, axon hillock and initial segment for generation of action potential, axon for transmission of nerve impulse, axon terminal for discharge of neurotransmitters (Fig. 22.7).

**Cell body:** It maintains the functional and anatomical integrity of the axon. The proteins associated with synaptic transmitters are synthesized in Nissl granules of the cell body and are transported to axon terminal by axoplasmic flow.

**Dendrites:** They form the receptor zone of the neuron, i.e. they receive impulses and transmit the impulses toward the cell body. In this region, non-conducted local potential changes generated by synaptic connections are integrated.

**Axon:** The initial segment is the site where propagated action potentials are generated. The axonal process transmits propagated impulses from the cell body to the axon terminal.

**Synaptic knobs:** This is the nerve ending where arrival of action potentials results in the release of synaptic transmitter.

**Important Note**

**Concentration of voltage gated Na**⁺ **channels:** Na⁺ channels are highly concentrated in the nodes of Ranvier and the initial segment in myelinated nerve fibers.

**METABOLISM AND GROWTH OF NEURONS**

**Metabolism**

Neurons are metabolically active cells as mitochondria are present in adequate numbers. Neurons are always active as the membrane potentials and neuronal cytosolic activities are continuous phenomena. About 70% of total energy required is used to maintain polarization of the membrane by the action of Na⁺-K⁺ pump. During the peak activity, the metabolic rate of nerve doubles compared to skeletal muscle cell metabolism.

**Special features of neuronal metabolism are:**
1. The excitability, conductivity and recovery process from the activities can happen in a nerve for a considerable period in the absence of oxygen.
2. Chemical changes in the nerve are similar to that in muscles, i.e. pyruvic acid is formed and if O2 supply is insufficient, lactic acid accumulates.
3. Energy requirement of the resting nerve to maintain polarization of the membrane is supplied primarily by combustion of sugar and phospholipids.
4. During activity, hydrolysis of ATP and creatine phosphate supply energy for the propagation of the nerve impulse.
5. The nerve cells are rich in K⁺ and vitamin B₁ that further assist in metabolism. Vitamin B₁ is essential for oxidation of pyruvic and lactic acids in the neurons.

**Growth of Neurons**

Various factors affecting neuronal development, growth and survival have been isolated and studied. These can be broadly arranged into two groups:

1. **Neurotrophins**
2. **Other growth factors:** Other factors affecting neural development such as ciliary neurotrophic factor (CNTF), glial cell line-derived neurotrophic factor (GDNF), leukemia inhibitory factor (LIF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), transforming growth factor (TGF), insulin like growth factor (IGF-I), etc.

**Neurotrophins**

Neurotrophins are trophic proteins to the neurons, as they promote nerve growth and survival. They are produced by the nerves, muscles, glands, and astrocytes.
Chapter 22: Structure and Functions of Neurons

Known neurotrophins are:
1. Nerve growth factor (NGF),
2. Brain-derived neurotrophic factor (BDNF),
3. Neurotrophin-3 (NT-3), and
4. Neurotrophin-4/5

**Nerve Growth Factors**
Nerve growth factor (NGF) is the first neurotrophin to be identified. It promotes the growth of sympathetic nerves and some sensory nerves. NGF is made up of 2α, 2β, and 2γ subunits.
1. The α subunits have trypsin-like activity.
2. The β subunits are similar to insulin and have nerve growth-promoting activity.
3. The γ subunits are serine proteases.
NGF decreases apoptosis of neurons by acting through tyrosine kinase A receptor.

**Brain-derived Neurotrophic Factor (BDNF)**
BDNF promotes growth of peripheral sensory nerves. It acts through tyrosine kinase A.

**Neurotrophin 3**
The neurotrophin-3 (NT-3) promotes growth of cutaneous mechanoreceptors. It acts through tyrosine kinase A, B, and C.

**Neurotrophins 4 and 5**
They act through tyrosine kinase B. Exact function is not known.

**TYPES OF NEURONS**

Neurons are classified according to the number of processes, length of axon, functions of neurons, and patterns of dendrites.

**According to the Arrangement of Axon**
According to the arrangement and number of processes, the neurons are classified into unipolar, pseudounipolar, bipolar, and multipolar neurons (Figs. 22.8A to D).

**Unipolar Neurons**
They have a single process (Fig. 22.8A). They are usually found in invertebrates. In vertebrates, they are found in ANS.

**Pseudounipolar Neurons**
In pseudounipolar neurons, axon after originating from soma splits into central and peripheral processes (Fig. 22.8B). The example is dorsal root ganglion cell (primary sensory neurons with cell bodies in dorsal root ganglion).

**Bipolar Neurons**
In these neurons, two processes arise from the cell body (Fig. 22.8C). The example is the bipolar cell of retina.

**Multipolar Neurons**
In multipolar neurons, many processes arise from the soma (Fig. 22.8D). The example is a spinal motor neuron.

**According to the Length of Axon**
According to the length of the axon, neurons are classified into two categories: Golgi type 1 and type 2.

**Golgi Type 1**
These are the neurons with short axons. Dendrites of these neurons terminate near the soma. The example is cortical inhibitory neurons.
Golgi Type 2
Axons of Golgi type 2 neurons are long. Cortical motor neurons (neurons that give rise to corticospinal tract) are the examples.

According to Function
According to functions, neurons can be divided into sensory and motor neurons.

Sensory Neurons
These are the neurons that carry impulses from the receptors to the central nervous systems. These are called afferent neurons (afferent fibers).

Motor Neurons
These are the neurons that carry impulses from the central nervous system to the target organs. These are called efferent neurons (efferent fibers).

According to Dendritic Pattern
According to dendritic pattern, two types of neurons are present: pyramidal cells and stellate cells.

Pyramidal Cells
Dendrites of these cells spread like pyramids. The example is hippocampal pyramidal neurons.

Stellate Cells
Radial shaped spread of dendrites occurs in these cells. The examples are cortical stellate cells.

ARRANGEMENT OF NEURONS AND NEUROGLIA

Arrangement of Neurons in Nerve Fibers
The neuronal structures are present in endoneurium, perineurium, and epineurium (Figs. 22.9A and B).

Endoneurium
In the peripheral nerves each nerve fiber with its Schwann cell and basal lamina is surrounded by a layer of connective tissue called endoneurium.
1. The endoneurium contains collagen, fibroblast, Schwann cells, endothelial cells and macrophages.
2. Many nerve fibers together form bundles or fasciculi.
3. Endoneurium holds adjoining nerve fibers together and facilitates their aggregation to form fasciculi.

Perineurium
Each fasciculus is surrounded by thicker layer of connective tissue called perineurium.
1. The perineurium is made up of layers of flattened cells separated by layers of collagen fibers.
2. The perineurium probably controls diffusion of substances in and out of axons.
3. A very thin nerve may consist of single fasciculus, but usually a nerve is made of several fasciculi.

Epineurium
The fasciuli are held together by a fairly dense layer of connective tissue that surrounds the entire nerve and is called the epineurium.
Flowchart 22.1: Types of neuroglia in the nervous system (PNS and CNS).

(PNS: Peripheral neurons system; CNS: Central neurons system).

Important Note

Clinical Correlation of Neuronal Structures:

- The epineurium contains nerve fibers. Loss of this fat in bedridden patients can lead to pressure on nerve fibers and paralysis.
- Blood vessels to a nerve travel through the connective tissue that surrounds it. Severe reduction in blood supply can lead to ischemic neuritis and pain.

Neuroglia

In addition to neurons, the nervous system contains several types of supporting cells called neuroglia (Flowchart 22.1). As neuroglia are present mostly in CNS, their structure and functions are discussed in first chapter of Neurophysiology, in Section XI.

CHAPTER SUMMARY

Key Concepts

1. Neuron is the structural and functional unit of the nervous system.
2. Neurons are divided into myelinated and unmyelinated, based on the presence or absence of myelin sheath. Myelination improves the speed of conduction.
3. Myelination of axon in PNS occurs by Schwann cells and in CNS by oligodendroglia.
4. The cell body and dendrites serve as the receptor zone to receive the information from other neurons. Axon hillock and initial segment are the sites of generation of action potential. Axon is meant for transmission of nerve impulse. Axon terminal is for discharge of neurotransmitters into the synapse.
5. Neurotrophins are the main nerve growth factors.

Important to Know (Must Read)

1. In examinations, usually Long Questions are not asked from this chapter.
2. Structure of neuron, Mechanism of myelination, Axoplasmic transport, Types of neurons, and Neurotrophins are usually Short Questions in exams.
3. In Viva, examiners may ask…… different parts of neurons and their functions, how the myelination occurs and what are the functions of myelination, functions of Schwann cells, types of axoplasmic transport, types of neurons, special features of metabolism of neurons, axoplasmic transport, types of neurons with examples for each, and different neurotrophins.
Learning Objectives

On completion of study of this chapter, the student MUST be able to:
1. Appreciate the distribution and working of ion channels on the neuronal membrane.
2. Understand the importance of electrotonic potentials.
3. Define rheobase, chronaxie and utilization time and draw the strength-duration curve.
4. Draw a labeled schematic diagram of nerve action potential (AP) and describe the ionic basis of each phase of AP.
5. Define refractory periods (ARP and RRP) of nerve AP and explain their importance.
6. Explain the mechanism of propagation of action potential along the axon and understand the importance of saltatory conduction.
7. List the differences between graded potential and action potential.

The student MAY also be able to:
1. Describe the mechanism and genesis of electrotonic potentials.
2. Describe the mechanism of strength-duration curve.
3. Explain the application of nerve potential in various aspect of neuromuscular physiology.

Electrophysiological Aspects

For nerve impulses to be transmitted from neuron to neuron, the action potential must be generated and propagated along the nerve cell membrane. All these events depend on the activities of ion channels present on the membrane of the neurons.

Neuronal Ion Channels

Like any other cell membrane, neuronal membrane possesses numerous ion channels like Na⁺, K⁺, Ca²⁺, Cl⁻, etc. They are broadly categorized into three types:

i. Nongated or leaky channels

ii. Gated (voltage-gated, ligand-gated and mechanical-gated) channels.

iii. ATP-driven pumps:

1. The nongated or leaky channels of Na⁺, K⁺, Cl⁻ are present throughout the neuronal membrane.
2. The voltage-gated Na⁺ channels are concentrated at the nodes of Ranvier.
3. The voltage-gated Ca²⁺ channels are mainly present at the axon terminals, where they play important role in the secretion of neurotransmitters.

4. The mechanical-gated Na⁺ channels are involved in the genesis of receptor potential in the somatic sensory nerve endings.

5. Ligand-gated ion channels are present predominantly on dendritic spines, dendrites and cell body of the neuron. They are important for receiving information from other neurons at synaptic sites, in the form of released neurotransmitters.

Distribution of Na⁺ Channels

In myelinated neurons, the number of Na⁺ channels per square micrometer of membrane in different segments of the neuron is as follows:

1. At cell body: 50–75
2. At initial segment: 350–500
3. On the surface of the myelin: 25
4. At the nodes of Ranvier: 2000–12,000
5. At the axon terminals: 20–75

Thus, the channels are concentrated in areas where the action potential is first initiated (initial segment) and in regions where it is regenerated (nodes of Ranvier) during its propagation.
In unmyelinated neurons, about 110 Na⁺ channels are present per square micrometre of the axonal membrane. Abnormalities of channels are called channelopathies (Clinical Box 23.1).

**Clinical Box 23.1**

Channelopathies: The diseases caused due to the structural or functional changes in the ion channels are known as channelopathies. Those affecting neurons include episodic and spinocerebellar ataxias, some forms of epilepsy and familial hemiplegic migraine.

**Recording of Nerve Responses**

To study the various activities and properties of a nerve, intracellular and extracellular recording methods are adopted. For both recordings, electrical stimulus is usually used as it is convenient to apply the stimulus and accurately measure the strength of the stimulus.

**Intracellular Recording**

Usually, an isolated single giant axon taken from invertebrates such as cuttlefish (sepia) or squid (Loligo) is used for experimental set-up. As their fiber diameter is large (about 500 µm) it allows vertical penetration of microelectrode (diameter of 100 µm). The tip of the recording electrode is placed inside the axon, and the reference electrode is kept on the surface of the axon. A steady negative potential of −70 mV is recorded by the cathode ray oscilloscope (CRO) in the resting set-up. Then on application of a stimulus, the monophasic response obtained, is observed.

**Extracellular Recording**

In this type of recording, both the recording and reference electrodes are placed on the external surface of the isolated nerve fiber or on the skin surface of the intact animals or human beings. When the set-up is arranged, at rest, no potential is recorded by the CRO. Since both the electrodes are placed in same environment, both pick up the same voltage and there is no potential difference. On stimulation, a biphasic response is observed.

**Terms Used for Membrane Potentials**

Certain terms are used to explain the change in membrane potential relative to the resting membrane potential (RMP):

1. When there is a voltage difference between the inside and outside of the membrane, the membrane is said to be polarized.
2. When a stimulus allows influx of positive charges or efflux of negative charges, it decreases the membrane potential (i.e. the membrane potential approaches towards zero) and the stimulus is called a depolarizing stimulus. Thus, the membrane is said to be depolarized when the membrane potential becomes positive or less negative in relation to RMP.
3. After the depolarization phase, return of the potential towards the resting value is known as repolarization.
4. Similarly, as the interior of the cell becomes more negative in relation to RMP, due to influx of negative charges or removal of positive charges, the membrane is said to be hyperpolarized. In this state, the membrane potential is more negative in relation to RMP.

**GENESIS OF NERVE POTENTIALS**

The ability of the cells to generate action potential in their membrane is known as excitability. Nerve is a highly excitable tissue, which can be stimulated by electrical, chemical and mechanical forms of energy. When a stimulus is applied, it induces ions to flow across the membrane and alters the ionic balance on both sides of the membrane, producing a voltage change. With application of a stronger stimulus, much larger disturbance in ionic balance occurs. However, the ionic balance is promptly restored by 2 factors (repolarizing forces):

1. Diffusion of ions across the cell membrane
2. Increased activity of Na⁺-K⁺ ATPase.

The voltage changes across the membrane generate electrical signals, which on recording show a wave like pattern. The transient and small voltage changes spread along the length of the nerve fiber and die out after some time. When the stimulus is strong enough, the response does not die out fast, rather, it travels along the whole length of the axon, being regenerated at regular intervals. This phenomenon is possible because the neuronal membrane is a biological membrane studded with different ion channels, whose activation time is modifiable with change in external environment.
The message transmission occurs by means of generation and propagation of the electrical signals in the axon from one end to the other. These generated signals can be of two types:
1. Electrotonic or graded potentials
2. Action potentials.

Graded potentials spread the signal over short distances, whereas action potentials transmit the message throughout the length of the plasma membrane. Another type of response is seen in neuronal membrane that is called local response.

Electrotonic or Graded Potentials

Definition
Electrotonic potentials are local, nonpropagated potentials of small magnitude, in response to a depolarizing or hyperpolarizing stimulus of lesser strength.

Types
Electrotonic potentials are two types: catelectrotonic and anelectrotonic.

Catelectrotonic potential: When a membrane is electrically stimulated, the cathodal end of the stimulator evokes a depolarizing response called catelectrotonic potential.

Anelectrotonic potential: The hyperpolarizing potential produced due to stimulation at the anodal end is known as anelectrotonic potential (Fig. 23.1).

Concept
In the resting state, negatively charged ions are lined along the interior of the membrane and positively charged ions are lined along the exterior of the membrane:

1. With the application of a cathodal stimulus of smaller strength to a small area of the membrane, few Na+ ions enter through the leaky sodium channels into the cell.
2. At that instant, at the site of stimulus, the inside of the membrane becomes positive compared to the previous resting state.
3. With application of greater strength of stimulus, more positive charges enter into the cell and the voltage change is larger.
4. The repolarizing forces try to neutralize the disturbance in RMP, created by the Na+ entry. K+ tends to come out of the cell and Cl- enters through the leaky channels to maintain the electrical neutrality. Also, Na+ moves away by diffusion from the site of stimulus. Moreover, activity of the Na⁺-K⁺ ATPase is increased pumping 3 Na⁺ out and 2 K⁺ in. All these lead to the gradual return of the membrane potential towards the resting value.

In the neuron, graded potentials are recorded from the membranes of dendrites and cell body.

Properties of Graded Potential

1. Graded in nature: The term graded potential comes from the fact that the potential change increases in a stepwise manner with application of increasing strength of stimulus, i.e. the magnitude of potential change is proportionate to the stimulus strength.
2. Decremental conduction: Graded potentials decay progressively with time and distance, which is known as decremental conduction:
   i. When recorded near the site of stimulus, the amplitude of the potential is larger and recorded at a farther place, it is smaller. The potentials die out within a distance of 3 mm from the site of stimulus.
   ii. If recorded immediately after the application of the stimulus, the amplitude is larger and with delay in recording, the amplitude is smaller.
3. Depolarizing or hyperpolarizing nature: If the change in potential is plotted in the y-axis and time in the x-axis, the graph will be like a wave (Fig. 23.1), which primarily depends on the type and strength of the stimuli:
   i. With application of increasing strength of cathodal stimulus, more positive charges enter the cell and the graph displays gradually rising upward waveforms.
   ii. Similarly, when the membrane is stimulated with anodal end, due to entry of negative charges or removal of positive charges, the membrane potential becomes more negative; the graph displays a downward waveform.

Therefore, according to the type of stimulus, graded potentials can occur in either a depolarizing or a hyperpolarizing direction.

Fig. 23.1: Electrotonic potential and local response. (RMP: Resting membrane potential).
4. **Summation**: if a second stimulus is applied before the potential produced by the first stimulus has disappeared, both the potential changes are added together producing a larger and/or prolonged wave in the recording. This happens due to the arrival of more Na\(^+\) ions at the site of stimulus before neutralization of all Na\(^+\) influx caused by the first stimulus. Similarly the anelectrotonic potentials exhibit the property of summation.

**Forms of Graded Potentials**

Wherever a cell responds to a stimulus, graded potentials are produced along its membrane:
1. According to the type and location of the membrane from where they are recorded, they are described as **end-plate potential**, recorded from skeletal muscle membrane at neuromuscular junctions.
2. **Receptor potential**, recorded from sensory nerve endings.
3. **Synaptic potential**, recorded from membrane of postsynaptic neurons at neuro-neuronal junctions.
4. **Pacemaker potential**, recorded from pacemaker cells in the heart, intestine, etc. and so on.

**Local Response**

As the axon is stimulated with slowly increasing strength of stimuli, the amplitude of the electrotonic potential gradually rises. When the membrane potential is decreased by 7 mV, the pattern of graded potential is altered. The response becomes greater than what is expected for that strength of stimulus. This enhanced response is known as **local response**:
1. Similar to the graded potential, the local response gradually dies out with increasing distance.
2. Local response is seen only with a depolarizing stimulus of lower strength, and not with a hyperpolarizing one.

**Ionic Basis of Local Response**

As the applied cathodal stimulus is progressively raised from zero the **influx of Na\(^+\) ions through the leaky sodium channels** increases proportionately. Consequently, the membrane potential gradually **decreases from –70 to –63 mV**. At –63 mV, the few voltage-gated sodium channels start **opening**, which allows entry of some more Na\(^+\) ions. The **extra Na\(^+\) ions** entering into the cytoplasm are added to the existing Na\(^+\) influx, resulting in a heightened response called local response. This is observed within a potential change from –63 to –55 mV.

**Types of Stimuli**

**Threshold, Subthreshold and Suprathreshold Stimuli**

At membrane potential of –55 mV, the neuron starts generating action potentials:
1. This is termed as **firing or discharge** of the neuron and the membrane potential at –55 mV is known as the **firing level** or **threshold**.
2. The stimulus that brings the membrane potential to –55 mV is known as threshold stimulus. **Threshold stimulus** is defined as the lowest strength of stimulus that elicits an action potential.
3. The stimuli less in strength than the threshold are known as **subthreshold stimuli** and the stimuli higher in strength than the threshold are known as **suprathreshold stimuli**.

**Strength-Duration Relationship**

Strength and duration are two important aspects of a stimulus. Both of them have a complimentary role in determining the excitability of a tissue. These two parameters are inversely related to each other. The relationship between the strength and duration of stimuli depicted in graph form is known as **strength-duration curve** (Fig. 23.2):
1. To excite a tissue, the lowest amplitude of current required is termed as **rheobase**.
2. The minimum time for which the rheobase must be applied to elicit an action potential is known as **utilization time**. A stimulus weaker than rheobase does not excite the tissue and a stimulus stronger than rheobase requires less time (less than utilization time) to elicit a response.
3. **Chronaxie** is the time required for a stimulus of double the rheobase strength to produce an action potential.
4. Usually chronaxie gives us a better idea about the excitability of a tissue. The lesser the chronaxie, the greater is the excitability.
5. Nerves have a shorter chronaxie compared to muscles.

**Fig. 23.2**: Strength-duration curve.
Accommodation

If the intensity of a stimulus is slowly raised to the threshold value, the tissue fails to produce an action potential because it adapts to the stimulus. This phenomenon is known as accommodation:
1. During the slow depolarization, some of the voltage-gated Na⁺ channels start opening at a membrane potential of −63 mV. But, they soon get inactivated before another set of channel opens; because, the membrane takes longer time to arrive at the next level of potential.
2. Thus, the sufficient number of activated sodium channels required to fire an action potential is never accomplished. Like this, the membrane may attain the normal threshold level or even surpass that level, but action potential is not produced.
3. Besides, the potassium channels that open in response to the depolarization drain off the positive charges.

Action Potential

Definition

Action potential is defined as a transient change in membrane potential of about 100 mV, which is conducted along the axon in an all-or-none fashion.

It has following features:
1. It is characterized by a gradual depolarization to threshold, and a rapid ascent in the membrane potential followed by a phase of repolarization.
2. It travels along the axon with the same shape and amplitude being regenerated at regular intervals.
3. It is also known as an impulse or spike potential.

Duration and Amplitude

The duration of a single nerve action potential is about 1 msec, during which the membrane potential sharply rises from −70 to +35 mV, and then returns to its resting value.

Latent Period

Action potential is always preceded by a latent period, which is the interval between the application of a stimulus and the onset of action potential:
1. Duration of latent period depends on the distance between stimulating and recording electrodes and the type and diameter of nerve fiber.
   The action potentials never summate and a definite interval (refractory period) exists before the second action potential is fired.

Phases of an Action Potential

It has two phases, a phase of depolarization and a phase of repolarization:

i. The phase of depolarization is recorded as a sharp upward wave during which the membrane potential approaches zero and then attains a positive value. It consists of slow depolarization to threshold, rapid rising phase, overshoot and peak. During overshoot, the membrane potential crosses the zero or isopotential level and then at peak, it reaches a maximum potential of +35 mV.

ii. The phase of repolarization is recorded as down-stroke during which the membrane potential returns to the resting level. It includes a rapid falling phase and slower terminal part called after-depolarization.
   The phase of repolarization is followed by an after-hyperpolarization phase during which the membrane potential undershoots (becomes more negative) and then returns back to the resting level (Fig. 23.3).

Ionic Bases of Action Potential

The depolarization and repolarization phase of the action potential are due to sequential changes in membrane permeability to sodium and potassium leading to large fluxes of these ions across the membrane, along their gradients.

Depolarization is due to influx of sodium and repolarization is due to efflux of potassium. The voltage-gated Na⁺ and K⁺ channels contribute to the different phases of the action potential.

Depolarization

Depolarization is due to opening of voltage-gated Na⁺ channels, causing massive influx of sodium ions:
1. When a threshold or suprathreshold stimulus is applied, the influx of Na⁺ through leaky channels and later through opening of few voltage-gated Na⁺ channels decreases the membrane potential from −70 mV to −55 mV (threshold level).
2. At this threshold potential, there occurs simultaneous opening of a large number of the voltage-gated Na⁺ channels, increasing the membrane permeability to sodium ions several hundredfold. This leads to massive influx of sodium ions producing a swift, large and steep depolarization, changing the membrane potential to +35 mV (a change in membrane potential by 105 mV starting from the resting value of –70 mV to +35 mV).

The initial change in membrane potential by +15 mV (–70 mV to –55 mV) is essential for instantaneous activation of a large number of voltage-gated Na⁺ channels. At threshold level, the number of Na⁺ channels that have already opened, cause concomitant opening of almost all the Na⁺ channels in the stimulated part of the membrane (positive feedback control) (Application Box 23.1). The activation gate of Na⁺ channels opens that brings them to the activated state (for details, see below). Therefore, this process of simultaneous activation of huge number of Na⁺ channels is called auto-activation, which occurs very rapidly.

**Application Box 23.1**

**Hodgkin’s cycle:** The opening of few Na⁺ channels leading to further opening of other Na⁺ channels is called Hodgkin’s cycle. This is an example of positive feedback control in which a stimulus triggering an event further facilitates the process (Refer to Fig. 3.3, Chapter 3).

The concentration gradient as well as electrical gradient favors the entry of sodium ions across the membrane. In fact, there occurs reversal of membrane potential with the inside becoming positive than outside as the membrane potential crosses the isopotential value of 0 mV and finally attains a peak potential of +35 mV.

**Membrane Potential Remains Below Na⁺ Equilibrium Potential**

During depolarization, the membrane potential approaches but does not reach the equilibrium potential for sodium, which is +60 mV as derived from Nernst equation, because of the following three factors.

At the peak of the action potential:
1. Sodium influx abruptly ceases due to the closure of the inactivation gates of the Na⁺ channels. The Na⁺ channels open very fast, remain open for a very brief period, and they close very fast. The process of speedy closure is called rapid autodeactivation.
2. The voltage-gated potassium channels being fully open, allow the exit of positively charged K⁺ ions.
3. The electrical gradient for Na⁺ is reversed subsequent to the overshoot, i.e. after crossing RMP of 0 mV, inside of the cell membrane becomes positive and it hinders the positively charged Na⁺ to enter the positive interior, slowing down further sodium entry.

During an action potential, approximately 20,000 Na⁺ ions enter into the cell. The repolarizing forces try to restore the resting membrane potential, but the depolarization is large enough to overcome the opposing forces and produce an action potential.

**Repolarization**

Repolarization is due to opening of voltage-gated K⁺ channels, causing efflux of K⁺. Actually, these K⁺ channels are sensitive to the same depolarization that opens the voltage-gated Na⁺ channels but they open more slowly:

1. At the peak of the action potential, the voltage-gated Na⁺ channels enter a closed state whereas the voltage-gated K⁺ channels are fully open.
2. The membrane permeability to potassium ions increases several times causing increased potassium efflux. The K⁺ concentration is much higher inside the cell and at the peak of the action potential, outside of the membrane is negative in comparison to inside, which is positive (+35 mV).
3. These two factors favor the electrochemical gradient for potassium efflux.
4. Thus, the rapid falling phase of repolarization is brought about by decline in sodium influx together with increase in potassium efflux.
5. The termination of action potential due to activation of voltage-gated potassium channels is a negative feedback process.

Following the phase of rapid fall, once the membrane potential drops close to the isopotential level and moves towards RMP, the inside of the membrane becomes negative that limits the efflux of potassium. Thus, after-depolarization phase is due to the slower exit of potassium ions that considerably decreases the rate of repolarization and makes the repolarization curve oblique (less steep).

**Voltage-gated Na⁺ and K⁺ Channels**

The Na⁺ channel has two gates, an activation gate and an inactivation gate:

1. When the membrane is at rest, the inactivation gate is open and the activation gate is closed. This is the resting state of the channel in which Na⁺ influx cannot occur. The K⁺ channel has only one gate that remains closed during the resting state (Fig. 23.4A).
2. As the membrane is depolarized to the firing level, the activation gate of Na⁺ channel opens. This is the activated state of the Na⁺ channels in which, both the gates are open permitting massive influx of Na⁺ that brings the membrane potential to +35 mV (Fig. 23.4B).
3. The gate of the K⁺ channel start opening at the same time as the activation gate of Na⁺ channel, but K⁺ gates open slowly.
4. At the peak of the action potential, the inactivation gate of Na⁺ channel closes. This is the inactivated state of the Na⁺ channels, in which Na⁺ influx stops. Also, the activation gate of Na⁺ channel is about to close (Fig. 23.4C).
5. At this time, the gates of K⁺ channel are fully open allowing K⁺ efflux and causing rapid repolarization (Fig. 23.4D).
6. In the later part of repolarization, the activation gate of Na⁺ channel is closed and the inactivation gate starts opening slowly. This is the closed state of the Na⁺ channels (Fig. 23.4E).
7. By now, the K⁺ channel gates have started to close but they take a longer time to shut down completely (Fig. 23.4F).
8. Then the channels proceed to the resting state, where, the inactivation gate of Na⁺ channel is fully open; the activation gates of Na⁺ channels and the K⁺ channel gates are fully closed causing no ion movement across the channels.

**After-Hyperpolarization**

1. Following after-depolarization, the membrane reaches the resting potential (RMP). At this level, though most

of the voltage-gated K⁺ channels are closed, some of them still remain open allowing continued efflux of K⁺.
2. As a result, the membrane potential becomes more negative than the RMP, giving rise to the prolonged and slow undershoot, which is called as phase of after-hyperpolarization.
3. Finally, the K⁺ channels completely close, restoring the membrane potential back to the resting level.

** Ionic Conductance during Action Potential **

Conductance of an ion means the ease with which the ion passes through the plasma membrane. It indicates the permeability of the membrane to the ion. Conductance is reciprocal of resistance, offered by the membrane as well as the potential gradient across the membrane hindering the passage of the ion through the membrane:

1. The Na⁺ conductance rises gradually from the RMP to the firing level, and then it fast increases reaching a peak. At the peak of the action potential, it declines rapidly and comes to the base line.
2. The K⁺ conductance rises after the rise in Na⁺ conductance. Initially, the conductance increases at a much slower rate during the phase of depolarization. Just after the peak of the action potential, the conductance increases to its maximum during the rapid falling phase of repolarization. After that, the K⁺ conductance decreases but takes a long time to reach the base line (Fig. 23.5).

**Ionic Activity after Action Potential**

At the end of an action potential, the ionic composition on both sides of the membrane is altered. This leaves more sodium and less potassium inside the cell:
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1. The ionic composition is restored by **increased activity of the Na⁺-K⁺ ATPase**.
2. The number of ions that take part in generation of a single action potential is very little compared to the total number of ions in the cell (1 of every one million Na⁺ go into the cell and about the same number of K⁺ come out of the cell during an action potential). This produces a negligible change in the intracellular ionic concentration.
3. However, if this imbalance was not taken care of, in the long run, following repeated generation of action potentials, it would be difficult to generate action potentials further, because slowly the concentration gradients of sodium and potassium across the membrane will cease to exist.

**Effects of Extracellular Ionic Changes**

1. When the **extracellular Na⁺ concentration is decreased**, the amplitude of the action potentials becomes smaller than usual because the concentration gradient for Na⁺ that drives sodium into the cell is reduced.
2. When the **extracellular Na⁺ concentration is increased**, the amplitude of the action potentials may increase.
3. When the **extracellular K⁺ concentration is decreased**, the membrane potential becomes more negative as the resting K⁺ efflux is increased favoured by the increased concentration gradient across the membrane.
4. When the **extracellular K⁺ concentration is increased**, the membrane potential come closer to the firing level and the membrane becomes more excitable.
5. When the **extracellular Ca²⁺ concentration is decreased**, the electrical potential difference across the membrane is decreased, as the inside becomes less negative compared to outside. The RMP come closer to the firing level, so that, the magnitude of depolarization needed to reach the firing level is less. Hence, decrease in extracellular Ca²⁺ concentration increases the excitability of the tissue, as observed in hypocalcemic tetany, occurring in hypoparathyroidism.
6. If the **extracellular Ca²⁺ concentration is increased**, the RMP goes away from the firing level due to increased potential difference across the membrane. Consequently, the magnitude of depolarization needed to reach the firing level is more, decreasing the tissue excitability.

**Ion Channel Blockers**

**Na⁺ Channel Blockers**

The drugs like lidocaine, procaine ‘etc., and neurotoxins like tetrodotoxin, saxitoxin’ etc., block the voltage-gated Na⁺ channels and hinder the generation of action potentials.

**Local Anesthetics**

Lidocaine and procaine are used clinically as local anesthetics during various surgical procedures. When injected into the affected tissue or sprayed over an area, the ionizable hydrophobic drug molecules diffuse readily through the plasma membrane and block the Na⁺ channels and decrease the transmission of pain and other sensations. Axons with a smaller diameter are more sensitive to the local anesthetics than the large diameter fibers.

**Membrane Toxins**

The neurotoxins like tetrodotoxin (TTX) is found in ovaries of puffer fish and in tissues of salamanders, and saxitoxin (STX) is accumulated in the tissues of shellfish. Both are lethal paralytic toxins and eating a small quantity of tissue containing the toxins can lead to death. These animals have survived by developing resistance against the toxins’ i.e. they have TTX-resistant or STX-resistant sodium channels.

**K⁺ Channel Blockers**

The voltage-gated K⁺ channel blockers are tetraethylammonium (TEA) and 4-aminopyridine (4-AP). These chemicals along with TTX are used extensively by scientists in electrophysiological research, like study of voltage-clamp techniques.

**Na⁺–K⁺ ATPase Blocker**

Drugs like digitalis and dinitrophenol block the Na⁺–K⁺ ATPase pump.

**All-or-None Law**

**Definition**

All or none states that the action potential occurs with a constant amplitude and shape irrespective of magnitude of the stimulus. A **subthreshold stimulus** fails to excite the tissue. Only a stimulus of **threshold magnitude** elicits an action potential. If **suprathreshold stimuli** are applied, the action potentials resulting from them have the same amplitude, duration and form as those produced by threshold stimuli, provided the experimental conditions like electrical potentials on both sides of the membrane, concentration of ions in ICF and ECF, ‘temperature’ etc. remain same (Fig. 23.6).

**Mechanism**

The activation gates of voltage-gated Na⁺ channels open as soon as 15 mV of depolarization is achieved. Following that, any extra degree of depolarization is of no further use, as the membrane automatically achieves another +90 mV of depolarization (−55 to +35 mV):

1. Thus, **after the threshold level is achieved**, the amount of sodium influx becomes independent of the stimulus factor.
2. The number of voltage-gated Na⁺ channels over the axonal membrane of unmyelinated axons as well as at the nodes of Ranvier in myelinated axons remain fairly constant.
3. Once the action potential is formed, it appears with its maximum size and shape, otherwise it does not form at all.
4. Therefore, the action potential follows the all-or-none law; i.e. the action potential occurs with a constant amplitude and shape whether the stimulus is of threshold or suprathreshold magnitude.

**Refractory Periods**

During the action potential, the stimulated area of the membrane happens to be unresponsive to a second stimulus in most part, and later it requires a stronger stimulus to get excited again. The length of time during which the membrane is unresponsive to a second stimulus no matter how strong is the stimulus, is known as refractory period. The periods of total and relative refractoriness are known as absolute and relative refractory periods respectively.

**Absolute Refractory Period**

**Definition and Duration**

Absolute refractory period (ARP) is defined as the period in the action potential during which, application of a second stimulus of any strength and duration does not produce another action potential. The ARP corresponds to the period from the time the firing level is reached until repolarization is about one-third complete (Fig. 23.7).

**Mechanism**

At the peak of the action potential, the inactivation gates of the voltage-gated sodium channels close and they remain in that inactivated state for some time before returning to the resting state. These sodium channels can reopen in response to a second stimulus, only after attaining the resting state. Hence, even if a stronger stimulus is applied during this interval, it will not produce a second action potential, and the membrane is said to be in its absolute refractory period.

**Relative Refractory Period**

**Definition and Duration**

Relative refractory period (RRP) is defined as the period following ARP during which, application of a suprathreshold stimulus can elicit a second action potential. The RRP starts from the end of ARP to the start of after-depolarization.

**Mechanism**

The following factors contribute to RRP:
1. All the sodium channels present at the site of stimulus do not achieve the open state or inactivated state or resting state, exactly at the same time. Few of them open when the membrane potential is ~63 mV, causing local response. By the time of relative refractory period, some of the channels have returned to their initial resting state. These channels in resting state can open their activation gate and allow the influx of Na⁺.
2. A suprathreshold stimulus can spread to larger area over the membrane and open extra voltage-gated sodium channel.

**Physiological Importance**

The absolute refractory period limits the number of action potentials that the nerve can fire in a given period of time. ARP serves two important functions:
1. ARP determines the rate of discharge of nerve fiber. In our body, nerves fire at a rate of 10 to 1000 impulses per second. Generally, large diameter nerve fibers have an ARP of about 0.4 msec, with a firing rate of 2500 impulses per second, whereas small diameter fibers have an ARP of about 4 msec, with a firing rate of 250 impulses per second.
2. The ARP is also responsible for the one-way conduction of action potentials (this will be discussed under the propagation of action potential).
Thus, only a suprathreshold stimulus and not any threshold stimulus can open up sufficient number of sodium channels to elicit an action potential during the RRP.

The excitability of the membrane keeps on changing in different phases of the action potential. Usually, the time the membrane potential is closer to the firing level, the membrane is more excitable; and the excitability of the membrane decreases, when its potential is away from the firing level. During the ARP, it is least excitable.

**Initiation and Propagation of Action Potential**

**Initiation of Action Potential**

The production of action potentials requires the presence of large number of voltage-gated ion channels that are present mostly on the axons. Therefore, it is the axon, not the cell body or the dendrites that generate and conduct the action potentials:

1. The action potential is first initiated in the specialized areas in the axon called the first node of Ranvier in sensory neurons and initial segment-axon hillock in motor neurons.
2. These areas are known as trigger zones that have a very high concentration of voltage-gated sodium and potassium channels.
3. The synaptic potential generated at the dendrites and, or the cell body is integrated by the cell body and transmitted to the axon hillock.
4. If this potential is sufficient to depolarize the membrane of the axon hillock to firing level, the membrane easily fires an action potential.

**Propagation of Action Potential**

Once formed, the action potential is regenerated at regular intervals to be transmitted from the initial segment of the axon to the axon terminal. This is known as the propagation of action potential. In myelinated axon, the speed and mode of propagation of action potential is different from that in unmyelinated axon.

The speed of conduction of the impulse depends on two factors:

1. **Myelination**: Conduction velocity is more in myelinated axon and is proportionate to the degree of myelination.
2. **Diameter of the axon**: Conduction velocity is proportionate to the diameter of the fiber. Fibers with larger diameter have faster rate of conduction. The large diameter fibers have less cytoplasmic resistance. So, the flow of ions across the membrane is easier.

**In Unmyelinated Axon**

At the site of genesis of an action potential, large influx of positive charges into the membrane occurs, which is known as current sink. The positive charges diffuse away from the site of accumulation. The adjacent membrane, which is in its resting state, has a potential of –70 mV:

1. This potential difference allows the positive charges to flow toward the adjacent negative area. Consequently, the potential of the adjacent membrane decreases and reaches the threshold value, as the fraction of Na⁺ ions that move to the nearby negative area are sufficient enough to bring the adjacent membrane to the firing level. This results in opening of the voltage gated Na⁺ channels present in that area, firing an action potential.
2. Similarly, from the site of second action potential, positive charges flow to the adjacent resting membrane and decrease its potential to the threshold level. This activates the voltage gated Na⁺ channels present in that part of the membrane resulting in another action potential.
3. In this manner, each point of the membrane gets depolarized to the firing level and produces an action potential.
4. As the depolarization and repolarization phases of the ensuing action potentials go on, there is a sequential opening and closing of sodium and potassium channels along the axonal membrane (Fig. 23.8).
5. The action potential does not move by itself but helps to generate a new action potential in the membrane ahead of it. As the number of voltage gated Na⁺ and K⁺ channels are distributed uniformly along the axon, the action potential arriving at the end of the axon is almost identical in appearance to the initial one. Thus, due to the local current flow produced following an action potential, there occurs serial depolarization of the adjacent membrane to the firing level and action potential travels, being successively regenerated along the membrane in an all-or-none manner.
6. At the same time, the exterior of the membrane which becomes negative due to current sink attracts flow of positive charges from the adjacent regions toward the site of application of stimulus. Thus, on both sides of the site of action potential, a circular pattern of current flow occurs across the membrane; i.e., inside the membrane, positive charges flow away from the site of action potential, whereas outside the membrane, positive charges flow toward the site of action potential. This circular pattern of current flow tries to restore the resting potential of the membrane where the action potential was previously generated.
In Myelinated Axon (Saltatory Conduction)
There are few voltage gated Na⁺ channels on the surface of the myelin:

1. **Myelin acts as an insulator** and does not allow free flow of ions across the membrane. Therefore, as the positive charges flow from the site of action potential to the adjacent area, large Na⁺ influx (as occurs during an action potential) does not occur in the myelinated portion of the membrane, though it may attend the threshold potential of –55 mV. Also, in the myelin sheath, the concentration of positive charges does not decrease fast because of less ‘leakage’. This helps the charges to spread farther along the axon.

2. The local current (the positive charges) travels like a graded potential and dies away 37% of its maximal strength over a distance of about 3 mm.

3. As the internodal distance is 1–2 mm, the local current definitely arrives at the adjacent node of Ranvier and decreases its membrane potential. Most importantly, the **voltage gated Na⁺ channels are present in large numbers at the nodes of Ranvier**. Therefore, as soon as the nodal membrane gets depolarized to threshold level, an action potential is quickly fired.

4. Thus, in the myelinated axon, the action potential is **generated at each node of Ranvier** (Fig. 23.9).

5. Because the action potential rapidly proceeds from one node to the next and pause at each node to get regenerated, the mode of propagation of action potential in myelinated axon is known as **saltatory conduction** (Latin word ‘saltare’ means to jump).

**Advantages in Myelinated Axon**
In myelinated axon, the velocity of conduction is faster. Besides, myelination also helps to conserve energy. Since the ionic flux occurs only at the nodes of Ranvier, the total membrane area across which ionic balance has to be restored is much less compared to the unmyelinated axon. Therefore, in unmyelinated axons, voltage-gated channels open throughout the axonal length causing activation of a larger number of Na⁺-K⁺ ATPase and higher expenditure of energy.

**Direction of Propagation of Action Potential**
In the motor neuron, the action potential is conducted from axon hillock toward axon terminal. In the sensory neuron, it propagates from the first node of Ranvier toward CNS. This is called **anterograde conduction** of impulse:

1. The axon contains a large number of voltage gated Na⁺ channels that promotes in quick generation of an action potential in the axonal membrane next to the trigger zone.

2. The action potential **does not travel from the axon back toward the trigger zone**. This is because; following depolarization, the area on the membrane where action potential was produced becomes refractory.

3. Therefore, though local currents from the site of next action potential tend to bring the membrane toward threshold value, the membrane does not fire an action potential, as the sodium channels remain inactivated.

4. Hence, the action potential can be conducted only in the direction away from the site of previous action potential.

5. The action potential can spread from the point of stimulus in both directions along the axon, if it is initiated between trigger zone and axon terminal.

**Differences between Graded Potential and Action Potential**
From the above discussions, we note that many differences exist between graded potentials and action potentials. The major differences are listed in Table 23.1.

---

**Table 23.1:** Differences between graded potential and action potential.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Graded potential</th>
<th>Action potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Amplitude</td>
<td>Amplitude is proportional to the strength of the stimulus; small amplitude</td>
<td>Once threshold potential is reached, amplitude remains same irrespective of the strength of the stimulus; large amplitude</td>
</tr>
<tr>
<td>2. Conduction</td>
<td>Travels in a decremental fashion; amplitude gradually decreases with time and distance</td>
<td>Conducted in an all-or-none manner; appears with the same amplitude and shape all along the axon</td>
</tr>
<tr>
<td>3. Summation</td>
<td>Can be summated</td>
<td>Cannot be summated</td>
</tr>
<tr>
<td>4. Nature</td>
<td>Can be a depolarizing or hyperpolarizing potential</td>
<td>Always a large depolarizing potential</td>
</tr>
<tr>
<td>5. Mechanism</td>
<td>Due to opening of ligand-gated or leaky ion channels</td>
<td>Due to opening of voltage-gated ion channels</td>
</tr>
<tr>
<td>6. Properties</td>
<td>Does not have a threshold or refractory period</td>
<td>Have a threshold and refractory period</td>
</tr>
</tbody>
</table>
# CHAPTER SUMMARY

## Key Concepts

1. Electrotonic potentials are local, non-propagated potentials of small magnitude, in response to a depolarizing or hyperpolarizing stimulus of lesser strength.

2. **Threshold stimulus** is the lowest strength of stimulus that elicits an action potential. This brings the membrane potential to –55 mV. The stimuli less in strength than the threshold are known as **subthreshold stimuli** and the stimuli higher in strength than the threshold are known as **suprathreshold stimuli**.

3. **Chronaxie** is the time required for a stimulus of double the rheobase strength to produce an action potential. Chronaxie indicates excitability of a tissue. Lesser the chronaxie, greater is the excitability.

4. In an action potential, **depolarization is due to influx of sodium** and **repolarization is due to efflux of potassium**.

5. Absolute refractory period (ARP) is defined as the period in the action potential during which, application of a second stimulus of any strength and duration does not produce another action potential. ARP is the period from the firing level to the one-third of repolarization.

6. The speed of conduction of impulse in myelinated neuron is much faster due to presence of large number of Na$^+$ channels at nodes of Ranvier that makes the conduction saltatory in nature.

## Important to Know (Must Read)

1. In examinations, usually **Long Questions** are not asked from this chapter.

2. Electrotonic potentials, Local response, Strength-duration curve, Labeled diagram and ionic basis of action potential, All or none law, Refractory period, Propagation of action potential along the axon, and Saltatory conduction, are usual **Short Questions** in exams.

3. In **Viva**, examiners may ask........ definition and importance of electrotonic potentials, meaning and ionic basis of local response, definition and importance of rheobase, chronaxie and utilization time, different phases and ionic basis of action potential, latent period, definition of firing level, threshold, subthreshold and suprathreshold stimuli, All or none law, definition of absolute and relative refractory period, their mechanisms and importance, how is action potential propagated in unmyelinated and myelinated axons, saltatory conduction, and differences between graded potential and action potential.
Learning Objectives

On completion of study of this chapter, the student MUST be able to:
1. List the properties of nerve fibers.
2. Classify nerve fibers and mention their functions.
3. Describe the degenerative (Wallerian degeneration) and regenerative changes following nerve injury.

The student MAY also be able to:
1. Describe the properties of nerve fibers.
2. Classify nerve fibers and mention their functions.
3. Explain the changes in nerve fibers following injury.

Properties of Nerve Fibers

Important properties of the nerve fibers are as follows:
1. Excitability
2. Conductivity
3. Unfatigability
4. Refractory period
5. All-or-none law
6. Summation
7. Accommodation

Refractory period and ‘All-or-none law’ of nerve fibers have already been described in the previous chapter.

Factors Affecting Excitability

1. Strength and duration of the stimulus
2. Effect of extracellular Ca^{++}
   i. Decrease in ECF Ca^{++} increases excitability of neuron by decreasing the amount of depolarization necessary to initiate the changes in the Na^{+} and K^{+} permeability that produces the action potential.
   ii. Increase in ECF Ca^{++} stabilizes the membrane by decreasing excitability. Ca^{++} entry contributes to depolarization.

Conductivity

On stimulation, action potential is generated in the nerve fiber, which is propagated along its entire length to the axon terminal (described in detail in the previous chapter).

Orthodromic and Antidromic Conduction

An axon can conduct in either direction. If the stimulus is applied in the middle junction of axon, the action potential initiated in the middle of it can travel in both directions, due to set-up of electronic depolarization on either side of the initial current sink.

Excitability

Excitability is the property by virtue of which cells or tissues respond to changes in the external or internal environments. It is due to the disturbances in the ionic equilibrium across the receptive zone of cell membrane. The nerve fibers are highly excitable tissues. They respond to various forms of stimuli—mechanical, thermal, chemical or electrical. In experiment set-up, ‘electrical’ stimulus is usually employed, because its strength and frequency can be accurately controlled, nerves respond well to chemical and thermal stimuli. The production of a wave of depolarization, and (excitation or activation) impulse demonstrates that a nerve has been excited.
1. Impulses normally pass from synaptic junction to the axon terminal, which is called **orthodromic conduction**.

2. Conduction in the opposite direction is called **antidromic conduction**, seen in sensory nerve supplying the blood vessels. **Axon reflex** is an example of antidromic conduction.

**Summation**

Application of a subthreshold stimulus does not evoke an action potential. However, if subthreshold stimuli are applied in rapid succession, they are summated and they produce an action potential. This property is called **summation**.

**Accommodation**

Application of continuous stimuli may decrease the excitability of the nerve fiber, a phenomenon called **accommodation**. More than nerve fiber, it is nerve endings that adapts. This decreases the transmission of impulse across the neurons.

1. If a nerve is submitted to the passage of constant strength of current, the site of stimulation shows decrease in excitability. The accommodation consists of a rise in threshold of the membrane during stimulation

2. A similar feature observed at at nerve endings is called **adaptation**.

3. Thus, nerve fiber accommodates while the nerve endings adapt.

**Unfatigability**

Nerve fibers cannot be fatigued, even when they are stimulated continuously. This is because the nerve fibers primarily conduct impulses (propagation of action potential) that do not involve expenditure of energy (ATP).

**CLASSIFICATION OF NERVE FIBERS**

Based on function, nerves are classified as motor, sensory and secretomotor, and based on myelination, they are classified as myelinated and unmyelinated. Likewise, though there are many classifications of nerve fibers, the most appreciated and popular classification is of **Erlanger and Gasser**, which is based mainly on their diameter and conduction velocity.

### Table 24.1: Classification of nerve fibers.

<table>
<thead>
<tr>
<th>Fiber types</th>
<th>Fiber diameter (µm)</th>
<th>Conduction velocity (m/s)</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aα</td>
<td>12–20</td>
<td>70–120</td>
<td>Somatic motor and proprioception</td>
</tr>
<tr>
<td>Aβ</td>
<td>5–12</td>
<td>30–70</td>
<td>Touch-pressure</td>
</tr>
<tr>
<td>Aγ</td>
<td>3–6</td>
<td>15–30</td>
<td>Motor to muscle spindle</td>
</tr>
<tr>
<td>Aδ</td>
<td>2–5</td>
<td>12–30</td>
<td>Pain, cold and touch</td>
</tr>
<tr>
<td>B</td>
<td>&lt; 3</td>
<td>3–15</td>
<td>Autonomic preganglionic fibers</td>
</tr>
<tr>
<td>C-dorsal root fiber</td>
<td>0.4–1.2</td>
<td>0.5–2</td>
<td>Somatic sensations</td>
</tr>
<tr>
<td>C-sympathetic fiber</td>
<td>0.3–1.3</td>
<td>0.7–2.3</td>
<td>Postganglionic sympathetic fibers</td>
</tr>
</tbody>
</table>

**Scientists contributed**

Joseph Erlanger (1874–1965)  
Herbert S. Gasser (1888–1963)

The **Nobel Prize in Physiology or Medicine for the year 1944** was awarded jointly to two neurophysiologists from USA, Joseph Erlanger and Herbert Spencer Gasser, “for their discoveries relating to the highly differentiated functions of single nerve fibers”. They worked extensively on nerve fiber types and the classification of nerve fibers is named after them ‘Erlanger-Gasser classification’.

**Erlanger-Gasser Classification**

This is the most popular classification of nerve fibers, based on their diameter and conduction velocities. Broadly fibers are classified into three categories: A, B and C.

**Type A Nerve Fibers**

These are the fastest conducting fibers with conduction velocity ranging from 70 to 120 m/sec. The fiber diameter varies from 12 to 20 µm. Type A fibers are further subdivided into α, β, γ and δ fibers (Table 24.1).

1. **Aα fibers** supply extrafusal fibers in skeletal muscles and also carry proprioception.
2. **Aβ fibers** carry touch pressure sensation and act as motor fibers.
3. **Aγ fibers** supply intrafusal fibers in muscle spindles.
4. **Aδ fibers** are mainly fast conducting nociceptive fibers.

**Type B Nerve Fibers**

These are preganglionic autonomic afferent and efferent fibers. They have a diameter of less than 3 µm and conduction velocity ranging from 3 to 15 m/sec.

**Type C Nerve Fibers**

These are unmyelinated fibers. They are subdivided into two broad categories: **dorsal root fibers** that carry various...
sensations and have diameter of 0.4 to 1.2 µm and their conduction velocity varies from 0.5 to 2 m/sec, and post-ganglionic sympathetic fibers with diameter 0.3 to 1.3 µm and conduction velocity of 0.7 to 2.3 m/s (Table 24.1).

**Numerical Classification**

Sensory nerve fibers are typed into Ia, Ib, II, III and IV. A comparison of the numerical classification and the later classification is shown in Table 24.2.

**Classification by Susceptibility to Various Agents (Hypoxia, Pressure and Local Anesthetics)**

**Hypoxia:** Type B fibers are most susceptible to hypoxia. Type C is the least susceptible.

**Pressure:** Type A fibers are most susceptible to pressure and type C is the least. This is because of fiber diameters.

**Local anesthetics:** Type C fibers are most susceptible to local anesthetics and type A is least susceptible.

### APPLIED ASPECTS

#### Demyelinating Diseases

**Multiple Sclerosis**

In multiple sclerosis, patchy loss of axonal myelin occurs at several areas in the nervous system, resulting in decreased conduction velocity along the fiber.

1. The pathways commonly affected are pyramidal tracts, cerebellar tracts, medial longitudinal fasciculus, optic nerve and posterior columns.
2. The symptoms vary depending on the site of affection. The usual symptoms are sensory abnormalities (decreased or abnormal sensations or pain), muscle weakness, fatigue, spasticity, optic neuritis, diplopia and ataxia (poor coordination).

**Demyelinating Form of GBS**

In demyelinating form of Guillain-Barre syndrome, loss of myelin causes abnormal conduction.

### Nerve Injury

When a nerve is sectioned, the distal part of the nerve separated from the cell body degenerates. This is called **Wallerian degeneration**. The proximal portion of the axon still attached to the cell body and then develops a growth cone and grows distally toward the distal portion; the changes associated are known as **regenerative changes**. The soma gets informed about the injury by absence of substances transported retrogradely from the axon terminals.

#### Grading of Nerve Injury

Based on severity, nerve injury is typed into five degrees:

1. **First-degree injury** is transient loss of function that occurs due to a mild pressure on the nerve. The temporary loss of function is mainly caused by local ischemia following obstruction to the blood flow. Nerve fibers completely recover within few hours to few weeks.
2. **Second-degree injury** includes nerve damage with intact endoneural tube. This occurs due to prolonged pressure on the nerve. The regeneration is delayed but complete as endoneural tube is intact.
3. **Third-degree injury** is the severe damage to the nerve fiber that interrupts endoneural tube.
4. **Fourth-degree injury** refers to a severe damage to the nerve associated with disorganization of nerve fasciculi.
5. **Fifth-degree injury** is the complete transection of the nerve. The degenerative changes occur early.

#### Degenerative Changes (Wallerian Degeneration)

The degenerative changes occurring in the distal segment of the axon are called **Wallerian degeneration**, named after its discoverer August Waller. The changes are as follows (Figs. 24.1A to D):

1. **Transmission of Impulse:** Shortly after the injury, synaptic transmission occurring at the axon terminal stops within hours.
2. **Axonal changes:** The axon swells and the terminals retract from the postsynaptic target. Gradually, over a period of several weeks, the axon and all its terminals degenerate. If the axon is myelinated, round...
fatty enlargements form all over the myelin sheath that looks like a series of beads (Fig. 24.1B). The myelin sheath breaks down but the myelinating cells remain viable (Schwann cell in the PNS and oligodendrocyte in the CNS). The debris created by the disintegration of the axon and its myelin is devoured by macrophages and Schwann cells in the PNS and microglia in the CNS. The phagocytic process is rapid in the PNS than in the CNS.

3. **Effect of stimulation:** On stimulation, the distal axon can conduct an action potential up to 3 days; this ability is impaired from 3rd to 5th day, and action potential cannot be initiated after the 5th day.

4. **Changes in soma and the stump:** Following injury, degenerative changes are seen in the soma and, to some extent in the proximal stump. These changes are known as **retrograde degeneration.** The changes are:
   i. Cell body swells and becomes rounded.
   ii. The nucleus also swells and moves to an eccentric position (Fig. 24.1B).
   iii. Endoplasmic reticulum instead of being closer to the nucleus reassembles around the periphery of the cell body.
   iv. The ribosomes appear disorganized.
   v. The Nissl granules gradually disintegrate and are stained weakly with basic dyes; this is known as **chromatolysis** (Fig. 24.1C).

**Regenerative Changes**

The soma tries to repair the axon by synthesizing new structural proteins that fills up and distends the cisterns of the rough endoplasmic reticulum. This is known as **axonal reaction.** Chromatolysis is reversible, if the neuron survives and re-establishes its contact with the appropriate target.

**Axonal Changes**

The proximal end of the axon develops **many sprouts** (Fig. 24.1C).

1. The sprouts elongate by formation of a **growth cone** at their growing ends (Fig. 24.1D).
2. The Schwann cells that had survived the degeneration **multiply** and **form rows** along the pathway previously taken by the disintegrated distal axon.
3. Out of the many sprouting branches, one branch finds the way through the Schwann cells and finally **reinnervates the original target structure.**
4. The regeneration rate of the growing axon is about 1–4 mm/day.
5. The Schwann cells then lay down their bilayered membrane to form the myelin sheath around the newly formed axon.

**Somatic Changes**

The soma regains its size. The number of Nissl granules slowly reappears. Other organelles also reappear. The cell loses excess fluid and regains its normal size. The nucleus occupies the central position (Fig. 24.1E).

**Changes in Target Structure**

Following degeneration of the nerve, the neurotransmitter release at axonal terminal decreases.

1. Decreased neurochemical at the synaptic cleft leads to **upregulation of the receptors** in the target structure.
2. Therefore, when a neurotransmitter is released following regeneration, the response of the target tissue to the neurotransmitter is increased. This is called denervation hypersensitivity (for details, refer to next chapter).

Factors Influencing Regeneration
Successful axonal and somatic regeneration depends mainly on four factors: severity of the injury, degree of damage to the cell body, site of injury and secretion of neurotrophins.

Severity of Injury
If the gap between the proximal and distal parts is more than 3 mm, the multiple outgrowths intermesh and form a tumor-like swelling called neuroma.

1. If neuroma is formed, successful regeneration can never occur.
2. If the neuroma involves sensory fibers, pain is felt at the site when touched.

Condition of Soma
If the axonal damage is close to the cell body, a larger part of the neuronal membrane and cytoplasm is lost. In such situations, the neuron often dies instead of regenerating.

Location of Injury
In the CNS, a single oligodendroglia sends out many processes to myelinate several (around 15) axons. Therefore, even though the axonal sprouting occurs, the oligodendroglia cannot form a path along which the sprouts can grow. Besides, glial scars formed by astrocytes pose obstruction in the pathway of the growth cone. Hence, axons in the CNS regenerate less successfully than axons in the PNS.

Neurotrophins
When specific neurotrophins are administered to the site of injury, the growth of the regenerating sprout is enhanced and enmeshing of the branches does not occur.

CHAPTER SUMMARY

Key Concepts
1. Nerve fibers are highly excitable tissues, strictly follow all-or-none law, and they do not exhibit fatigability.
2. Nerve fibers are classified based on their fiber diameter and conduction velocity. A fiber has maximum diameter (12–20 µm) and maximum conduction velocity (70–120 m/s). Type C fibers are most susceptible to local anesthetics.
3. The degenerative changes in the distal segment following nerve injury are called Wallerian degeneration.
4. The target organ (muscle) responds more to stimuli due to upregulation of receptors. This phenomenon is called denervation hypersensitivity.
5. Neurotrophins facilitate regeneration.

Important to Know (Must Read)
1. In the examinations, ‘degenerative and regenerative changes following nerve injury’ may sometimes come as a Long Question.
2. ‘Properties of nerve fibers, Erlanger-Gasser classification of nerve fibers, Wallerian degeneration, Regenerative changes following nerve injury’ are usual Short Questions in exams.
3. In Viva, examiners may ask about the properties of nerve fibers, definition of refractory period, All-or-none law, types of classification of nerve fibers, Erlanger-Gasser classification of nerve fibers, types of nerve fibers, features of Wallerian degeneration, regenerative changes following nerve injury.
Neuromuscular Transmission

Learning Objectives

On completion of study of this chapter, the student **MUST** be able to:
1. Draw the schematic diagram of neuromuscular junction (NMJ).
2. Describe the events of neuromuscular transmission at presynaptic, synaptic and postsynaptic parts of NMJ.
3. Classify neuromuscular blockers and give their mechanism of action.
4. Name the common disorders of NMJ and give their physiological basis.
5. Understand the mechanism of denervation hypersensitivity.

The student **MAY** also be able to:
1. Explain the physiological basis of etiology, features and treatment of myasthenia gravis and Lambert-Eaton syndrome.

The junction between a motor neuron and a muscle fiber is known as the neuromuscular junction (NMJ). This is also called **myoneural junction** or **motor end plate**, through which action potential from the neuron is transmitted to the muscle fiber. At this junction, the neuronal membrane and the sarcolemma remain apposed to each other, but do not touch.

1. The nerve endings release vesicles containing **acetylcholine** (ACh) that diffuse across the gap and initiates action potential in the muscle fiber.
2. The NMJ is the most extensively studied and best understood synaptic connection in the nervous system where **chemical synaptic transmission** was first studied.
3. It is readily visible under the light microscope, has a relatively simple mechanism and easily accessible to experimentation. Therefore, it is an ideal site for **studying the basic features of chemical transmission**.

**Presynaptic Portion (Axon Terminal)**

The neurons innervating skeletal muscle fibers are known as **motor neurons** that have their cell bodies in anterior horn of the spinal cord or in the brainstem. Their axons are myelinated and are the **largest-diameter axons in the body**.

The **features of presynaptic portion** of NMJ are:
1. As the axon of motor neuron approaches the muscle fiber, it **loses its myelin sheath** and divides extensively into **several fine branches** of about 2 µm in diameter, called **axon terminals**.
2. The terminals are covered by Schwann cells, known as **teloglia** (glial cells at terminals).
3. Each terminal forms a junction with a single skeletal muscle fiber, midway along its length. Thus, each muscle fiber is supplied by one motor neuron terminal. The motor neuron, including its axon and axon terminals and the muscle fibers supplied by it are called a **motor unit**.
4. Each terminal is expanded at its end to form a knobby structure, called **synaptic knob (terminal button)**, which contains plenty of mitochondria and neurotransmitter vesicles (Fig. 25.1). The terminal button lies in the groove (**synaptic trough**) in the surface of the muscle fiber, but outside the muscle cell membrane. Presence of large number of mitochondria indicates higher metabolic activity at terminal region.

**STRUCTURE OF NEUROMUSCULAR JUNCTION**

The anatomy of neuromuscular junction may be broadly divided into three parts: Presynaptic, synaptic and postsynaptic portions.
Section 3: Nerve and Muscle

5. The vesicles are clustered around specific points called **active zones**. The membrane at the active zones is modified to form **dense bar** that contains numerous **voltage-gated Ca\(^{++}\) channels**. These channels mediate ACh release.

### Synaptic Cleft

This is the gap between the terminal button and the muscle fiber, which is about 40–100 nm wide.

1. The muscle fiber is covered by a layer of amorphous connective tissue called the **basement membrane** or **basal lamina**, consisting of collagen, glycoproteins and other extracellular matrix proteins.
2. The basement membrane in the cleft contains the enzyme **acetylcholine-esterase** (AChE), which is anchored to the collagen fibrils and is secreted into the basement membrane by the presynaptic terminal and the muscle fiber.
3. AChE rapidly hydrolyzes ACh into acetate and choline. The basal lamina helps organize the presynaptic buttons with the postsynaptic junctional folds.

### Postsynaptic Portion (End Plate Membrane)

The postsynaptic portion of the neuromuscular junction is known as the end-plate membrane (**motor end plate**). This is the part of the sarcolemma that lies directly under the terminal button (Fig. 25.1).

1. The area of the end plate membrane increases many times as it is thrown into several folds called **junctional folds**.
2. The endplate membrane contains numerous **ACh receptors** (AchR), which are concentrated at the crests of the junctional folds. It also contains voltage-gated Na\(^{+}\) channels.

### ACh Receptors at NMJ

ACh receptors (AChR) at NMJ are **nicotinic type**. An average end plate contains about 15–40 million AChR, which accounts for about **10,000 AchR per square micrometer** of end-plate membrane.

1. The AChR protein is a **chemically gated ion channel** that undergoes a configurational change and opens when two molecules of ACh bind to it.
2. The channel is **blocked by α-bungarotoxin**.
3. Because of its larger pore diameter (0.8 nm), AChR is permeable to both Na\(^{+}\) and K\(^{+}\). But, as the electrochemical gradient is favorable for Na\(^{+}\) entry, more sodium moves in than potassium coming out resulting in **net Na\(^{+}\) influx**.
4. AChR allows the **passage of only cations** because the anions are repelled by the fixed negative charges present in it.

### MECHANISMS OF NEUROMUSCULAR TRANSMISSION

Neuromuscular transmission (NMT) is the transmission of impulse from the motor neuron to the skeletal muscle fibers supplied by the neuron. Mechanisms involved in NMT are broadly divided into **three parts**: presynaptic events, synaptic-cleft events and postsynaptic events.

### Presynaptic Events

The purpose of presynaptic mechanism is to release acetylcholine into the synaptic cleft. This occurs in following steps:

1. Arrival of an action potential at the axon terminal **depolarizes the membrane** of the terminal buttons.
2. This causes activation and **opening of the voltage-gated calcium channels**, which leads to **calcium influx**.
3. Increased cytoplasmic calcium concentration increases movement of microtubules and microfilaments that leads to **migration of neurotransmitter vesicles** to the inner surface of the presynaptic membrane.
4. The vesicles **fuse with the membrane** and discharge their contents into the synaptic cleft by **exocytosis**. Various membrane proteins present in the vesicular membrane as well as in the neuronal membrane are involved in the fusion process. Effective interaction between **synaptobrevin**, a vesicular membrane protein with **syntaxin**, a neuronal membrane protein occurs in the presence of SNAP-25, α/γ SNAP, NSF and many other associated proteins.

**Quantal Release**

Depolarization of the terminal button causes synchronous releases of about 60–200 vesicles from different parts of the presynaptic membrane. All the vesicles are roughly of the same size. Each vesicle contains around 4000–10,000 AcCh molecules.

1. The vesicle or the multimolecular **packet of ACh** is known as **quantas**, and, the process of release is known as **quantal release** of neurotransmitter.

2. With each action potential, the presynaptic terminal discharges about the same amount of neurotransmitter, collectively known as a “quantum of neurotransmitter” or “**quantal content**”, which is synonymous with “number of vesicles released”.

**Scientists contributed**


Ulf von Euler (1905–1983)

Julius Axelrod (1912–2004)

The Nobel Prize in Physiology or Medicine 1970 was awarded jointly to Sir Bernard Katz, Ulf von Euler and Julius Axelrod “for their discoveries concerning the humoral transmitters in the nerve terminals and the mechanism for their storage, release and inactivation”. They contributed to the understanding of synaptic transmission, especially they explained the mechanism of presynaptic events.

**Events at the Synaptic Cleft**

The released ACh molecules diffuse across the synaptic cleft and **bind to the receptors present at the post-synaptic membrane**.

1. On their way to the end plate membrane, some ACh molecules are **hydrolyzed by AChE**, and some **diffuse out of the synaptic cleft**.

2. However, the amounts that arrive at the end plate region and get bound to AChR, is **sufficient enough** to produce an action potential in the muscle fiber.

**Events at the End Plate**

The major purpose of postsynaptic mechanism is to **generate action potential in sarcolemma** adjacent to end plate. This occurs in following steps (Flowchart 25.1):

1. The binding of ACh to AChR in the end-plate membrane allows Na⁺ influx, producing a rapid local depolarization of the motor end plate known as **end plate potential (EPP)**.

2. EPP decreases the membrane potential to the firing level and opens the voltage-gated Na⁺ channels resulting in an **action potential** at the membrane adjacent to the end plate region. The voltage-gated Na⁺ channels are present all over the sarcolemma.

3. The action potential propagates along the sarcolemma and T-tubule network, activating the contractile machinery. As the motor end-plate is present at the middle of a muscle fiber, action potential **propagates in both directions**.

4. The binding of ACh to AChR is **loose and reversible**. Soon ACh diffuses away and gets **rapidly hydrolyzed by AChE to acetate and choline**.

5. The action of ACh is terminated leading to closure of ion channels, cessation of end plate potential and consequently action potential. The membrane potential of the end plate **returns to the resting state**.

6. The acetate diffuses away into the extracellular fluid and choline is taken up by the presynaptic terminal to be reused for the synthesis of new ACh.

All these events at the neuromuscular junction occur in a **few milliseconds** and can be repeated several times per second without fatigue.
End Plate Potential (EPP)

The EPP is like a graded potential, the amplitude of which is proportionate to the amount of neurotransmitters released.
1. EPP decays slowly with time and distance from the end plate region, which is known as decremental conduction. As AChE rapidly hydrolyzes ACh, the amplitude of EPP decreases with time.
2. Because the ACh-gated ion channels (AChR) are localized to the end plate membrane, EPP is generated at and confined to the end plate region only and therefore, the amplitude of EPP declines progressively with increasing distance from the end plate region.

EPP vs EPSP

In the CNS, most presynaptic neurons generate excitatory postsynaptic potential (EPSP) less than 1 mV, so that several EPSPs are required to attain the threshold and generate an action potential in the postsynaptic neuron. Whereas, in NMJ, the amplitude of an EPP is much larger compared to an EPSP in CNS.
1. Stimulation of a single motor neuron produces an EPP of about 70 mV.
2. EPP of this magnitude is much bigger than that required (15 mV) to bring the membrane potential to firing level.
3. Therefore, stimulation of a motor nerve always produces action potential in each muscle fiber supplied by it; i.e. all the muscle fibers in a motor unit contract in response to discharge of its motor neuron. In skeletal muscle, the EPP is always a depolarizing potential.
4. The EPP rises quickly resulting in an action potential as soon as the end plate membrane attains threshold level. During intracellular voltage recording, the magnitude of EPP gets flanked by the rising phase of action potential (Application Box 25.1).
The EPP was first recorded in 1950 by Paul Fatt and Bernard Katz by application of curare to the end plate membrane that blocked the Ach receptors, thereby decreased the amplitude of EPP below threshold level.

Application Box 25.1

Safety factor of EPP: A stimulated motor neuron produces an EPP of about 70 mV. Under normal circumstances, the amount of neurotransmitters released is enough to activate about 10 times the number of receptors required to produce an EPP that can bring the membrane potential to firing level. Due to this 10-fold safety factor, stimulation of a motor neuron never fails to excite an action potential in the muscle fibers of its motor unit. This reserve helps to elicit muscle contraction even in different pathological situations like (i) decrease in number or function of ACh receptors, (ii) decline in the quantity of ACh released, and (iii) abnormal voltage-gated Na+ channels in the end plate region.

Miniature End Plate Potential

When the motor nerve is at rest, spontaneously occurring potentials of minute amplitude (0.1 to 4 mV) are recorded from the postsynaptic membrane. These potentials are known as miniature end plate potential (MEPP).
1. They occur several times a second and have all the properties of an EPP.
2. MEPP occurs due to release of the contents of a single Ach vesicle by exocytosis (a quantal event) that opens around 2000 Ach channels in a very small area of the end plate membrane.

BLOCKADE OF NEUROMUSCULAR TRANSMISSION

The neuromuscular transmission is disrupted at different steps by drugs, chemicals, toxins and trauma. Failure to generate required EPP leads to paralysis of the skeletal muscles. The paralysis of the respiratory muscles endangers the life of the patient and has to be taken care of immediately. The drugs causing muscle relaxation are used during surgery and in some hyperactive states. The blockade can occur at presynaptic or postsynaptic level.

Presynaptic Blockade

Interruption of events taking place at the presynaptic axon terminal leads to impaired calcium influx causing decreased vesicle release.

Botulinum Toxins

The bacterium Clostridium botulinum releases a toxin that causes a paralytic disease called botulism.
1. The botulinum toxin is one of the most potent natural toxins, a minute amount of which causes serious illness.
2. The lethal dose for an adult human is around 2 to 3 µg.
3. Botulinum toxin B, D, F and G inactivate synaptobrevin, a vesicle membrane protein that is required for the binding and fusion of ACh vesicles with the plasma membrane of the axon terminal.
5. Thus, the botulinum toxin inhibits the release of acetylcholine from the axon terminals resulting in cessation of muscle contraction (flaccid paralysis) (Clinical Box 25.1).

Clinical Box 25.1

Clinical Uses of Botulinum Toxin: The paralytic effect of botulinum toxin is utilized for therapeutic and cosmetic purposes. Local injections of highly diluted toxin (botox treatment) produce muscle relaxation at the desired site. It is injected into the lower esophageal sphincter to treat achalasia cardia; into extraocular muscles to decrease their overactivity, which is used in the management of strabismus and blepharospasm; into facial muscles to reduce aging-related skin wrinkle and is used in cases of cervical dystonia. However, it can also be a lethal bioterrorism agent by dispersing it as an aerosol or by contaminating the food supply.
Hemicholinium
The drug inhibits choline uptake by the presynaptic terminal resulting in depletion of ACh. Consequently, the EPP decreases and action potential cannot be formed.

Postsynaptic Blockade
A variety of agents can act on the postsynaptic membrane interfering with generation of EPP by different mechanisms. They may be classified into competitive blockers and depolarizing blockers.

Competitive Blockers
Competitive blockers are blockers that block the receptors on postsynaptic membrane. Curare and gallamine are examples of competitive blockers of AChR. These substances diffuse to the end plate membrane and compete with ACh for AChR. Their attachment with AChR does not lead to opening of ion channels, as they do not have the biological activity of ACh. Moreover, they do not get hydrolyzed by AChE. So, the ACh binding sites in the receptors remain occupied permanently. In these conditions, motor nerve releases ACh, but ACh fails to bind to its receptor resulting in absence of EPP and lack of muscle contraction.

Curare
Curare is a plant product used as a South American arrowhead poison for hunting. The animal hit by such arrow suffers from paralysis in all skeletal muscles including the respiratory muscles. Consequently, the animal dies of asphyxiation.

Gallamine
Gallamine is given before surgery to relax the skeletal muscles.
1. It reduces the required dose of general anesthetic as well as bleeding and other complications.
2. As the respiratory muscles are also relaxed, patients are supported with artificial ventilation.

Depolarizing Blockers
Drugs like succinylcholine and carbamylcholine have the biological activity of ACh, but they are not hydrolyzed by AChE. Therefore, their action is long lasting. When they bind to AChR, the ion channels remain open in the end plate. The maintained depolarization keeps the voltage-gated Na⁺ channels in an inactivated state and blocks subsequent action potentials. There are other drugs that produce the blockade by inactivating AChE reversibly or irreversibly.

Reversible AChE Inhibitors
These agents compete with ACh to bind to AChE, thereby, prevent the hydrolysis of ACh by AChE. The accumulated ACh at the synaptic cleft binds to AchR, leading to a depolarizing block.
1. This is of use in diseases like myasthenia gravis, where excess ACh is a requirement in the face of decreased AChR.
2. Neostigmine and physostigmine (eserine) are examples of reversible AChE inhibitors. Their attachment with AChE decreases, when the concentration of the drug falls and so the block is removed after some time.
3. This block can be overcome by application of curare that attaches to AChR and reduces the effect of excess ACh on the end plate membrane.

Irreversible AChE Inhibitors
The organophosphorous compounds (pesticides like parathion, malathion and baygon) and nerve gases used in chemical warfare like disopropylfluoro-phosphate (DFP) and sarin, bind to AChE very tightly. This binding is irreversible.
1. Lack of hydrolysis of ACh produces sustained depolarization of the end plate resulting in no further contractions in response to subsequent nerve stimulation.
2. Poisoning with these agents lead to skeletal muscle paralysis and death from asphyxiation.
3. Nerve gases also cause accumulation of ACh at the cardiac pacemaker cells. Therefore, in cases of nerve gas exposure, atropine (a muscarinic receptor antagonist) is given as antidote.

NEUROMUSCULAR DYSFUNCTIONS

Myasthenia Gravis
It is a neuromuscular disorder of autoimmune nature, characterized by weakness and fatigability of skeletal muscles that gradually worsens as the muscles are used.

Etiology
Myasthenia gravis results from a decrease in the number of AChR present on the motor end plate due to production of circulating autoantibodies against these receptors (Figs. 25.2A and B). Moreover, the postsynaptic folds are flattened, or simplified. Due to these changes, though ACh is released normally, the magnitude of the end plate potential falls below the threshold for initiating a muscle action potential.

The anti-AChR antibodies have following three major functions:
1. They compete with ACh to bind to AChR, producing receptor blockade.
2. They induce endocytosis of AChR.
3. They damage the postsynaptic membrane.
Section 3: Nerve and Muscle

Features

1. **Fatigue**: Normally, on repeated stimulation of the motor nerve, the amount of ACh released per action potential gradually decreases (presynaptic rundown). But the amount is enough to generate an EPP well above the threshold level. In myasthenic patients, due to the postsynaptic changes along with the presynaptic rundown, after a few motor nerve impulses, the successive contractile responses become feeble, causing fatigue. So there is decremental response to repetitive nerve stimulation on EMG recording from the affected muscle.

2. **Women are affected more** than men in a ratio of 3:2.

3. The muscle weakness increases during prolonged use of the muscle and improves after rest or sleep. Thus, the patient feels better in the morning, but symptoms aggravate toward evening or after exertion.

4. **The extraocular muscles and eyelids are often involved** early in the course of the diseases. Therefore, diplopia and ptosis (drooping of eyelids) are early symptoms (Fig. 25.3).

5. **Physiological Basis of Treatment**

   Current modalities of treatment are as follows.

   1. **Administration of AChE inhibitors**: Examples of these drugs are pyridostigmine and neostigmine. They increase the concentration of ACh at the neuromuscular junction.

   2. **Thymectomy**: Recently it has been observed that immune responses mediated by T cells are part of the pathophysiological mechanisms. Thymectomy is therefore helpful. It blunts down the immune response and improves the condition.

   3. **Immunosuppression**: Immunosuppressive drugs glucocorticoids and azathioprine are useful. They inhibit the immunological mechanisms.

   4. **Plasmapheresis**: Plasmapheresis removes AChR antibodies from plasma.

Lambert-Eaton Myasthenic Syndrome

It is a presynaptic disorder of the neuromuscular junction due to production of autoantibodies against voltage-gated Ca\(^{++}\) channels. The decreased Ca\(^{++}\) influx in the presynaptic axon terminals results in impaired ACh release from the nerve endings. The muscular weakness is primarily seen in the limb muscles. Patients show incremental response to repetitive nerve stimulation as Ca\(^{++}\) level raises with each action potential. Thus, with prolonged contractions, muscle strength increases.
NERVE ENDINGS IN SMOOTH AND CARDIAC MUSCLES

The smooth and cardiac muscle cells are innervated by autonomic nervous system. There are no well-defined end plate regions or precise synaptic terminals.

1. Especially in smooth muscles, the neurotransmitter vesicles are present in dilated areas of the axon all along its length. These dilated portions (swellings) are known as varicosities (Fig. 25.4).

2. On stimulation of the neuron, the neurotransmitters are released and then diffused to the adjacent muscle fibers, initiating action potentials in many fibers simultaneously.

3. In a noradrenergic nerve, there can be as many as 20,000 varicosities per neuron. This type of synaptic arrangement is called synapse en passant.

4. The depolarizing potentials produced in the smooth muscle cells are known as excitatory junction potentials (EJPs).

5. EJPs are like graded potentials and can be summated. The hyperpolarizing potentials produced in the smooth muscle cells are known as inhibitory junction potentials (IJPs).

DENERVATION HYPERSENSITIVITY

When a neuron is cut and the distal axon degenerates, the target tissue innervated by its synaptic terminals becomes more reactive to the neurotransmitter secreted at the nerve endings. This phenomenon of increased responsiveness is known as denervation hypersensitivity. This is seen in both skeletal and smooth muscles. Due to degeneration of the nerve endings, the chemical is no more released from the terminals. The tissue reacts to the neurotransmitter present in the circulation, where concentration of the chemical is lower than at the synaptic endings.

The hypersensitivity occurs due to three reasons:

1. Increased number of receptors at the postsynaptic membrane (up-regulation). Both number and distribution of receptors increase. For example, after degeneration of the motor nerve, the AChR appears all over the sarcolemma (Fig. 25.5B), whereas normally it is confined to the end plate region only (Fig. 25.5A).

2. Increased responsiveness (sensitivity) of receptors to the chemical.

3. In case of noradrenergic neurons, the reuptake of neurotransmitter molecules by the presynaptic terminals is decreased resulting in higher plasma noradrenaline concentration.
### Key Concepts

1. Arrival of an action potential at the axon terminal causes opening of the voltage-gated calcium channels, and causes calcium influx. Calcium mediated exocytosis of neurotransmitter vesicles is facilitated by membrane proteins synaptobrevin and syntaxin.
2. Binding of Ach to AChR causes Na\(^+\) influx that leads to genesis of end plate potential (EPP).
3. EPP at threshold causes activation of voltage-gated Na\(^+\) channels causing influx that causes genesis of muscle fiber action potential.
4. Botulinum toxin causes presynaptic blockade and curare causes postsynaptic blockade (competitive blockade of AChR) of neuromuscular transmission.
5. Myasthenia gravis results from a decrease in the number of AChR present on the motor end plate due to production of circulating autoantibodies against these receptors.
6. When the distal axon degenerates, the target tissue innervated by its synaptic terminals becomes more reactive to the neurotransmitter secreted at the nerve endings, which is known as denervation hypersensitivity. This is due to increased number of receptors as well as sensitivity of receptors to chemicals.

### Important to Know (Must Read)

1. In examinations, ‘Structure of NMJ and Neuromuscular transmission’ usually come as a **Long Question**.
2. Mechanism of neuromuscular transmission, end-plate potential, Neuromuscular blockers, Denervation hypersensitivity, are usual **Short Questions** in exams.
3. In **Viva**, examiners may ask…… Structure of NMJ, Steps of neuromuscular transmission, Meaning and importance of Quantal release, role of syntaxin and synaptobrevin, Features of end-plate potential, and how it differs from action potential, Name and mechanism of presynaptic blocker, Name and mechanism of post-synaptic blocker, Neuromuscular blockers, Cause, features and treatment of myasthenia gravis, Special feature of autonomic innervation in smooth muscle, and Definition and mechanism of denervation hypersensitivity.
Structure of Skeletal Muscle: Physiological Aspects

**CHAPTER 26**

**Learning Objectives**

On completion of study of this chapter, the student **MUST** be able to:
1. Understand the organization of muscle fibers and fibrils in skeletal muscle.
2. Name the contractile proteins in the skeletal muscle and give their functions.
3. Draw the labeled diagram of sarcomere.
4. Draw the picture of sarcotubular system (STS) and give function of each component of STS.

The student **MAY** also be able to:
1. Describe the details muscle proteins.
2. Describe the role of STS in muscle contraction and relaxation.

For muscle contraction, muscles use chemical energy to generate force. All muscles transduce a chemical or electrical command into a mechanical response. The trigger for muscle contraction is the rise in free cytosolic Ca\(^{2+}\) concentration. However, on the basis of structure, metabolism, control mechanisms and contractile properties like rate and duration of contraction, fatigability and the ability to regulate contractile strength, muscles can be classified into three types:
1. Skeletal muscle
2. Smooth muscle
3. Cardiac muscle.

Skeletal muscle, as the name implies, is attached to the skeleton (bones). Its contraction supports and moves the skeleton (through joint movements). The contraction in skeletal muscle is mostly under voluntary control and is initiated by discharge of the motor neuron supplying it. Skeletal muscle is capable of rapid force development and shortening. It can maintain contractile force for relatively longer periods.

**Structure of Skeletal Muscle**

A typical skeletal muscle contains many muscle bundles or fascicles. Each fascicle consists of large number of muscle fibers arranged parallel to each other (Figs. 26.1A and B). Connective tissue layers in the muscle bundles are epimysium, perimysium and endomysium (Fig. 26.2).

![Figs. 26.1A and B: Structure of skeletal muscles. (A): Muscle bundle; (B) Muscle fiber.](image)

1. The connective tissue layer around the muscle is called **epimysium**.
2. The layer covering each fascicle is known as **perimysium**.
3. The layer surrounding each muscle fiber is termed as **endomysium**.

The blood vessels and nerves supplying the muscle fibers are present within the perimysium. All the three layers are made up of collagen and elastin that helps to transmit the force from the muscles to the bones. Muscles are usually attached to bones by collagen fibers known as tendons.
Myocyte

The structural unit of muscle is muscle fiber that is a single skeletal muscle cell (myocyte). The cell membrane of myocyte is known as sarcolemma and the cytoplasm as sarcoplasm (Fig. 26.1B).

1. The muscle fibers are long, cylindrical, multinucleated cells, extending the entire length of the muscle, and attach to tendons at both ends.
2. They are 10–100 µm in diameter and may extend up to 20 cm in length.
3. In embryonic fibers, the nuclei are centrally placed; but following differentiation after birth, they lie beneath the sarcolemma, hence peripherally placed (Fig. 26.3A).

The important functions of sarcolemma are:
1. It transmits the wave of depolarization originating at the motor end plate over the entire cell surface to initiate contraction.
2. Besides shielding the muscle fiber, the sarcolemma contributes to the parallel elastic resistance, i.e. the resistance to stretch offered by all the connective tissue layers.
3. It has no gap junctions or tight junctions, thereby promotes electrical separation between fibers.

Myofibrils and Organelles

Characteristically, the sarcoplasm is filled with myofibrils that extend along the axis of the cell and are connected to the tendons at both ends of the muscle fiber.

1. Each myofibril is 1 µm in diameter and consists of cylindrical bundles of two types of myofilaments that are referred to as thick and thin filaments.
2. The sarcoplasm contains the usual cytoplasmic organelles including mitochondria (sarcosomes), sarcoplasmic reticulum, and Golgi apparatus.

Development of Myocytes

The muscle fibers are formed during fetal development by the fusion of a number of undifferentiated, mono-nucleated cells, known as myoblasts.

1. At the time of birth, the differentiation of skeletal muscle fibers is complete. These differentiated fibers continue to increase in size during growth from infancy to adulthood, but no new fibers are formed from myoblasts.
2. After birth, following destruction of skeletal muscle due to any cause, the existing muscle fibers do not divide to replace the damaged fibers. Instead, new fibers are formed by differentiation of satellite cells that are located in the adjoining myocytes.

Fig. 26.2: Connective tissue layers in the muscle bundles (epimysium, perimysium, and endomysium).

Figs. 26.3A and B: Longitudinal section through skeletal muscle showing multinucleate myocytes (A) and photomicrograph showing transverse striations (B). 1. Peripherally placed nuclei; 2. Transverse striations.
3. Though the satellite cells are capable of forming a large number of new muscle fibers, that may not be adequate enough to restore a severely damaged muscle to its full strength. In such situations, hypertrophy (increase in cell size) of the remaining muscle fibers usually compensates for the loss.

4. Recently, it has been demonstrated that a transcription factor called myogenin stimulates fibroblasts to become muscle cells.

**Striations:** The most striking feature of skeletal muscle is the presence of striations due to alternate light and dark bands throughout the length of the fiber as seen through a light microscope (Fig. 26.3B).

1. The dark band is also called **A band** because it is anisotropic to polarized light.
2. The light band is known as **I band** because it is isotropic to polarized light (refer to Fig. 26.1B).

Each myofibril is made up of units called **sarcomere** that contains different muscle proteins (see below).

**Muscle Proteins**

There are three types of proteins in skeletal muscle:
1. **Contractile proteins:** myosin and actin
2. **Regulatory proteins:** troponin and tropomyosin
3. **Attachment proteins:** titin, nebulin, alpha actinin, desmin, myomesin, and dystrophin

**Contractile Proteins**

There are two contractile proteins in skeletal muscle: myosin and actin.

**Myosin**

There are thick and thin filaments in muscle. The thick filaments are polymers of myosin II (MW 480,000). Myosin filament is a bundle of myosin molecules. The head of myosin molecules projects from the filaments that are arranged in a helical manner (Fig. 26.4). The filament is composed of following units:

1. Two intertwined heavy chains,
2. Two regulatory light chains, and
3. Two alkali (essential) light chains.

The myosin II has three regions: two globular heads, neck (hinge region) and a long tail (Fig. 26.5).

**Myosin Head**

Each head is made up of amino terminal portions of one heavy chain forming a complex with two light chains, one regulatory and one alkali.

1. The myosin head contains two binding sites, one for actin and one for ATP (Fig. 26.6).
2. The ATP binding site functions as an ATPase (hydrolyzes ATP).
3. The carboxy terminals of the heavy chains coil around each other in an alpha-helical configuration forming a long rod-like tail.
4. The alkali light chain stabilizes the myosin head and the regulatory light chain regulates its ATPase activity.

**Myosin Tail**

The tail of each myosin molecule lies along the axis of the thick filament, and the two globular heads extend out to the sides, forming the cross-bridges.

**Myosin Hinge**

In the hinge region, the tail joins the globular head.

**Arrangement of myosin molecules:** The myosin molecules aggregate with a definite directional arrangement, such that their tail-ends are directed toward the center of the thick filaments creating a bare region in the middle consisting of myosin tails only, while the globular heads point away from both sides of the tail.
Section 3: Nerve and Muscle

1. The site of the reversal of polarity of myosin molecules is the M line where slender cross connections preserve the organization and alignment of the thick filaments in the sarcomere (See below).
2. Besides titin, proteins like myomesin and C-protein contribute to the bipolar organization and packing of the thick filaments.

Actin

The thin filaments are made up of actins. The actin filament (F-actin) is made up of globular molecules of G-actin (Fig. 26.7).
1. G-actin is a globular protein with a diameter of 4–5 nm and MW 42,000.
2. G-actin molecules (monomers) are joined from front to back into long chains that wind about each other forming a double stranded alpha helical filament known as F-actin (or filamentous actin) that forms the backbone of the thin filament.
3. The cytoskeletal protein nebulin extends along the length of the F-actin and plays a role in the regulation of the length of the thin filament.
4. Each actin monomer contains binding sites for myosin, tropomyosin, troponin I, and other actin monomers.
5. As the F-actin undergoes a half-turn every seven G-actin monomers, a groove is formed down the length of the helix where lies the long, filamentous protein tropomyosin.

Regulatory Proteins

Regulatory proteins are tropomyosin and troponin.

Tropomyosin

Tropomyosin is a rod-shaped molecule (MW 70,000) composed of two strands of polypeptides intertwined in an alpha helical configuration with a length approximately equal to seven actin monomers. It is located in the groove between two chains of actin (Fig. 26.8).
1. The tropomyosin molecules are connected to one another serially that extends over the entire actin filament covering myosin-binding sites on the actin monomers.
2. The function of tropomyosin is to interfere with the binding of myosin head to actin.

Troponin

Troponin is a complex of three proteins: Troponin T, Troponin I, and Troponin C (Fig. 26.8)

Troponin T

Troponin T has molecular weight 30,000. It binds the troponin complex to tropomyosin.

Troponin I

Troponin I (MW 22,000) binds the troponin complex to actin. It is called I, because it inhibits the binding of actin to myosin by blocking the myosin binding site on actin.

Troponin C

Troponin C (MW 18,000) binds to calcium.

Note:

Interaction of troponin with tropomyosin: Each troponin complex interacts with one tropomyosin molecule, which in turn interacts with seven actin monomers. The troponin complex also interacts directly with the actin filaments. Troponin is known as the regulatory protein in the contraction of skeletal muscle.

Attachment Proteins

Titin

It is a large, elastic, filamentous, cytoskeletal protein, which extends from the Z line to the M line. It provides framework for the thick filaments by connecting the Z line to the M line. It prevents overextension of the thick filaments and maintains the central location of the A band.

Nebulin

This is a large, filamentous protein that extends along the length of the thin filaments. It is believed to play a role in stabilizing the length of the actin filaments during muscle development.

Alpha Actinin

Alpha actinin anchors the thin filaments to the Z line.
Desmin is present in the intermediate filament of cytoskeleton of myocyte. It connects the Z line to the sarcolemma. It also connects myofibrils to each other.

**Dystrophin**

It is a large rod like protein that anchors the thin filaments to the membrane spanning protein beta-dystroglycan, which in turn is connected to the extracellular matrix protein laminin through alpha-dystroglycan.
1. Dystrophin helps in transfer of force from the contractile system to extracellular regions through the transmembrane proteins.
2. These proteins are disrupted in the group of genetic diseases called muscular dystrophy.
3. Absence of these proteins or their mutations leads to muscle degeneration, weakness and even death.

**Myomesin**

It is present in the region of M disc. It binds the tail end of myosin filament to the disc.

**Sarcomere**

Sarcomere (in Greek; sarco means muscle, and mer means part) is the structural and functional unit of myofibril. A single myofibril may be composed of hundreds or thousands of sarcomeres that are joined end-to-end. In each myofibril, thick and thin filaments are arranged in a repeating pattern in the form of sarcomere (Fig. 26.9).
1. Sarcomere is defined as the portion of the muscle fibril between two successive Z lines.
2. The average length of a sarcomere is 2 µm.

**Structure**

Electron micrographic studies reveal the detailed structure of sarcomere (Fig. 26.10).

**Z Line**

The Z line or Z disc is a network of interconnecting proteins. It is oriented perpendicular to the axis of the muscle fiber. The thin filaments are anchored to the two sides of each Z line by alpha actinin.

**Thin Filament**

Each thin filament is 1 µm long and 7 nm in diameter.
1. It is composed of the contractile proteins actin, tropomyosin, and troponin in a ratio of 7:1:1.
2. From the Z line, the thin filaments project in parallel arrays toward the center of the sarcomere where they overlap a portion of the thick filaments.
3. Thus, each sarcomere contains two sets of thin filaments, one at each end. The Z disc not only binds the thin filaments of a single myofibril together, but connections between the Z discs also anchor each myofibril to its neighbors.

**Thick Filament and A Band**

The parallel array of thick filaments located in the middle of each sarcomere produces A band.
1. The thick filament is 1.6 µm long and 10 nm in diameter.
2. It consists of the protein myosin (see above).

**I Band**

I band lies between the ends of A bands of two adjacent sarcomeres and includes those portions of the thin filaments that do not overlap the thick filaments. The Z line bisects I band.
1. During muscle contraction, the length of the sarcomere (the distance between two Z lines) decreases. This results in shortening of I band but, length of A band remains unchanged (Fig. 26.11).
2. Thus, sarcomeric length is decided by the length of I band.

**H Band**

H band (H zone) is a narrow, light band in the center of A band. This is the portion of A band showing no overlap (represented by thick filaments only) as can be seen in a relaxed muscle.

**M Line**

A transverse M line runs through the middle of H band. The M line contains proteins that bind the parallel array of thick filaments.
1. Besides, the cytoskeletal protein, titin binds the thick filaments to the Z lines.
2. In fact, **titin filaments** extend from Z line to M line and thus, are linked to M-line proteins, thick filaments and Z line proteins.
3. Therefore, it plays a key role in organizing and maintaining the regular array of thick filaments in the middle of each sarcomere.

**Hexagonal and triangular arrangement:** A cross section through the overlap region of A band shows that each thick filament is surrounded by a hexagonal array of six thin filaments, and each thin filament is surrounded by a triangular arrangement of three thick filaments (Fig. 26.12).

**Cross-Bridges**

The globular heads of myosin II project from the surface of the thick filaments toward the thin filaments and bridge the gap between them.
1. These projections are known as **cross-bridges** (Fig. 26.13).
2. During muscle contraction, they attach to the thin filaments and pull them.

**Sarcotubular System**

Inside a muscle fiber, fibrils are surrounded by membranous structures known as sarcotubular system. The relationship of sarcotubular system with the I and A bands of sarcomere in a myofibrils is shown in Figure 26.14.

**Structure**

It consists of sarcoplasmic reticulum and T (transverse) tubules (Fig. 26.15).

**T-tubules**

The T-tubules, also called **sarcotubules** are tubular extensions of the sarcolemma, about 0.03 µm in diameter.
1. They penetrate to the center of the muscle fiber and surround the individual myofibrils at the junctions of A and I band.
2. The lumen of the T tubule contains extracellular fluid, which is a continuation of the ECF surrounding the muscle fibers.

3. Once initiated, an action potential is rapidly conducted along the sarcolemma over the surface of the muscle fiber as well as into the interior of the muscle cell by the way of the T-tubules so that deep lying myofibrils are fast activated.

**Sarcoplasmic Reticulum**

The sarcoplasmic reticulum (SR) in skeletal muscle corresponds to the endoplasmic reticulum found in other cells.

1. It forms an elaborate membranous network having extensive anastomosis around each myofibril and runs parallel to the myofilaments.

2. The slender tubules, about 0.04 µm in diameter, have an elongated portion in the middle and dilated region at both ends known as lateral sacs or terminal cisternae.

3. The cisternae lie in close contact with the T-tubules at the A-I junction.

4. The combination of the T-tubule membrane and its two neighboring cisternae is called a triad, which plays a vital role in linking membrane action potentials to muscle contraction (details see below). In cardiac muscle, it forms a diad due to the presence of one cistern.

**Receptors and Channel Proteins in STS**

The T-tubules are separated from the terminal cisternae by a narrow space of about 12–14 nm that contains areas of densities known as feet. The junctional feet involve two integral membrane proteins, one in the T-tubule membrane, and the other in the membrane of the terminal cistern.

1. The T-tubule protein is a modified voltage-sensitive calcium channel known as the dihydropyridine (DHP) receptor. It is so named because it is blocked by the drug dihydropyridine.

2. The DHP receptors are L-type calcium channels and they are clustered in groups of four called tetrads.

3. Primarily the receptor acts as a voltage sensor rather than a calcium channel.

4. The portion of the terminal cisternae membrane that faces the T-tubules contains proteins known as the ryanodine receptors. They are so called because they bind to the plant alkaloid ryanodine. They mainly act as calcium release channels.

5. There is also calcium ATPases present on the membrane of SR that pumps calcium back into the SR.

**Functions**

**Role of STS in Muscle Contraction and Relaxation**

Sarcotubular system (STS) plays a vital role in muscle contraction as it links T-tubular action potentials (excitation) with calcium release from the SR that activates the contractile machinery (contraction). When calcium is pumped back into the SR, decline in cytoplasmic calcium level leads to muscle relaxation. Thus, STS is crucial in both contraction and relaxation process by mediating calcium release and calcium uptake.
Mechanism of Calcium Release

Following depolarization at the motor end plate, action potentials propagate along the sarcolemma and down the T-tubule membrane.

1. Depolarization of the triad region of the T-tubules induces conformational changes in each of the four DHP receptor proteins, which leads to a conformational change in the ryanodine receptor resulting in opening of the ryanodine receptor channel.
2. Calcium is thus released from the terminal cisternae of the SR into the cytoplasm, activating cross-bridge cycling.
3. The entire process, starting from depolarization of the T-tubule membrane to the initiation of the cross-bridge cycling is termed excitation-contraction coupling.
4. Further, conformational change in DHP receptor allows calcium entry from the ECF into the cytoplasm through the DHP receptor channel and this increase in calcium level inside the cell can as well activate the ryanodine receptors resulting in calcium release. This mechanism is known as calcium-induced calcium release (CICR). However, this pathway has a much lesser role in contraction of skeletal muscle.
5. CICR plays an important role in cardiac muscle contraction.
6. Increase in Ca$^{2+}$ concentration near the sarcoplasmic reticulum is known as calcium spark.

Mechanism of Calcium Uptake

The membrane of the SR contains a protein called SERCA (Sarcoplasmic Endoplasmic Reticulum Calcium ATPase). This is so named as it is present in all cells in connection with the endoplasmic reticulum.

1. The protein is a Ca$^{2+}$ ATPase that pumps free calcium ions from the sarcoplasm to the SR, from where the calcium ions are moved to their storage sites in the terminal cisternae.
2. The pump relocates two molecules of Ca$^{2+}$ into the SR for each molecule of ATP hydrolyzed. It becomes active as soon as the Ca$^{2+}$ concentration in cytoplasm becomes high.
3. In the terminal cisternae, a Ca$^{2+}$ binding protein called calsequestrin, favors storage of calcium at high concentration.
4. With decrease in cytoplasmic calcium concentration, contractile activity ceases and relaxation process begins (Flowchart 26.1).

Functions of STS

1. It transfers action potential from the surface of the muscle fiber to the interior, closer to the myofibrils.
2. It raises cytoplasmic calcium concentration by calcium release from SR.
3. It ensures muscle relaxation by calcium reuptake by SR.
4. The cisternae of SR act as storage sites for calcium.
CHAPTER SUMMARY

Key Concepts
1. Sarcomere, the functional unit of muscle fibril, is the distance between two Z lines.
2. During muscle contraction, the length of the sarcomere decreases, due to shortening of I band, but the length of A band remains unchanged.
3. The globular heads of myosin project from the thick filaments toward the thin filaments and bridge the gap between them. Hence, this head region is called cross-bridges. During muscle contraction, cross bridge attach to the thin filaments and pull them.
4. T tubule of sarcotubular system that invaginates into the myofibril transmits action potential to the contractile machinery. Release of calcium from cistern causes muscle contraction and active pumping of calcium back into the cistern causes muscle relaxation.

Important to Know (Must Read)
1. In examinations, usually Long Questions are not asked from this chapter.
2. Muscle proteins, Sarcomere, Sarcotubular system, Role of sarcotubular system in muscle contraction, are usual Short Questions in exams.
3. In Viva, examiners usually ask… Type of muscle proteins, Structure and function of each muscle protein, what is cross bridge, What are the regulatory proteins and their functions, What are the attachment proteins and their functions, Definition and structure of sarcomere, What are the I and A bands and what are their significance, Components of sarcotubular system and function of each component, Name the receptors and channel proteins in sarcotubular system, and what are their functions, and, How sarcotubular system participates in muscle contraction and relaxation.
4. Types of muscle proteins, sarcomere and sarcotubular system are usually asked.
Learning Objectives

On completion of study of this chapter, the student **MUST** be able to:
1. Describe the mechanism of excitation-contraction coupling in skeletal.
2. Understand the process of cross-bridge cycle.
3. Appreciate the relationship between the action potential and contractile response.
4. List the types of muscle contraction and the differences between them.

The student **MAY** also be able to:
1. Describe the detailed mechanism of cross-bridge cycle.

The action potential from sarclemma spreads to the interior of muscle fiber through T tubules and results in calcium release from cisternae. This increases sarcoplasmic calcium concentration. The muscle response to the action potential (through increased calcium) is the **excitation**, and the mechanical response is the **contraction** of the muscle. Calcium diffuses to troponin C and induces muscle contraction through a series of well controlled steps. Therefore, this phenomenon of pairing of electrical event with the mechanical event is called **excitation-contraction coupling**.

**Excitation-Contraction Coupling**

Increase in sarcoplasmic calcium concentration in response to arrival of action potential from sarcolemma leads to muscle contraction. The process is called excitation-contraction coupling.

1. The term contraction means attachment of myosin heads to actin and activation of the cross bridge cycles so that force is generated within to shorten the muscle fibers.

2. However, it may not always lead to shortening as occurs in isometric contraction. While lifting a heavy object, shortening occurs, but holding the object at a constant position, activates the cross bridge cycles without apparent shortening.

**Sliding-Filament Theory of Muscle Contraction:** In 1957, AF Huxley proposed the sliding filament theory of muscle contraction based on the changes observed in the lengths and widths of various bands and zones of the sarcomere. Shortening of a muscle fiber occurs due to **sliding of thin filaments over thick filaments** toward the center of the sarcomere (described below in “molecular mechanisms of muscle contraction”). It was observed that the length of A band does not change during contraction, but the H zone and I band decrease in width and the Z lines move closer together.

**Scientists contributed**

Sir Andrew Fielding Huxley was a Nobel Prize-winning English physiologist and biophysicist. He discovered the basis for propagation of nerve impulses (called an action potential) earned the **Nobel Prize in Physiology or Medicine in 1963**. He developed **interference microscopy** suitable for studying muscle fibers. In 1952, German physiologist Rolf Niedergerke joined him, and in 1954 together they discovered the mechanism of muscle contraction, popularly called the “sliding filament theory”, which established the foundation of understanding of muscle mechanics. He contributed enormously to the understanding of nerve impulses and muscle contraction. He was conferred a Knight Bachelor by Queen Elizabeth II in 1974, and was appointed to the Order of Merit in 1983. He was a fellow of Trinity College, Cambridge, until his death.
Molecular Mechanisms of Muscle Contraction

The molecular mechanism of muscle contraction is based on the principle that the sliding motion of the filaments occurs as a result of a cyclic interaction between the myosin cross bridges and the actin filaments, in the presence of calcium and ATP.

1. This is known as the cross bridge theory or the ratchet theory as the strokes are similar to the action of a ratchet.
2. The force generation and shortening are produced at the cross bridge sites where the chemical energy stored in the muscle is converted into mechanical energy.

Role of Troponin, Tropomyosin and Calcium

Calcium ions provide the most important chemical link in the regulation of muscle protein interactions during the course of excitation-contraction coupling.

1. In resting skeletal muscle, cytoplasmic calcium ion concentration is low, which is about $10^{-7}$ M in the region of the myofilaments. Troponin I is attached to myosin binding site on actin and partially covers the myosin binding site. Rest of myosin binding site is covered by tropomyosin filament.
2. Troponin I and tropomyosin inhibits myosin-actin interaction.
3. Troponin T is attached to tropomyosin.
4. Each tropomyosin molecule is held in this blocking position by troponin that is bound to both tropomyosin and actin.

5. Thus, the troponin-tropomyosin complex behaves as a relaxing protein that prevents undesirable contraction.

Troponin–Tropomyosin Interaction

Following propagation of action potential along the sarcolemma and down the T tubules, calcium is released from the SR and calcium concentration rises to $10^{-4}$ M in the sarcoplasm, which accounts for about 1,000-fold rise of the ion.

1. Calcium binds to troponin C and induces a conformational change in the troponin molecule.
2. This result in shifting of troponin I from the actin filament, allowing tropomyosin to move deeper into the actin groove, unmasking the myosin binding sites (Figs. 27.1A and B). Moreover, the conformational change alters the position of troponin T that drags tropomyosin away from the myosin-binding sites.
3. The exposure of the binding sites allows myosin heads to interact with actin and engage in cross-bridge cycling.
4. Thus, the troponin-tropomyosin complex regulates the contraction of skeletal muscle.
5. As the regulation of the contractile process by calcium is implemented through the actin filaments, it is also called actin-linked regulation.

Role of Calcium

An increase in cytoplasmic Ca$^{2+}$ concentration triggers the contractile process by removing the inhibition on cross bridge cycling. On the contrary, decrease in cytoplasmic Ca$^{2+}$ concentration leads to detachment of calcium from troponin that reverses the process and turns off contractile activity.

Figs. 27.1A and B: Role of troponin-tropomyosin interaction in muscle contraction. Binding of calcium with troponin C induces conformational change in the troponin molecule, which shifts troponin I from the actin filament, and simultaneously troponin T that drags tropomyosin away from the myosin-binding site on actin. Exposure of this binding site allows myosin heads to interact with actin and engage in cross-bridge cycling. Note, ATP is converted to ADP during this interaction.
1. In skeletal muscle, increase in cytosolic calcium in response to a single action potential is usually sufficient to saturate all troponin-binding sites on actin filaments.
   - At a calcium concentration of $10^{-4}$ M, there is full force development and the contractile system is fully saturated.
   - With further increase in concentration, there is only small increase in the force.
2. Whereas, in cardiac and smooth muscle, the amount of calcium regulates the degree of actin-myosin interaction as the contractile system is only partially saturated under normal conditions.

**Cross-Bridge Cycle**

The sequence of events that occur during the interaction between the myosin cross-bridges and the actin molecules is termed as a cross-bridge cycle.

1. During each cycle, cross bridge (myosin head) attaches to thin filament causing displacement of thick filament over thin filament followed by detachment of myosin head in a repetitive fashion.
2. ATP is required during the cycle; for the movement of the cross bridge as well as for its detachment.
3. However, all the cross-bridges do not go through the same phase at any instant during a cycle. This helps in a smooth and sustained contraction.

**Events in the Resting Muscle**

When the muscle is at rest, the cytoplasmic ATP gets attached to its specific binding site in the head of the myosin molecule and the globular head is not attached to actin.

1. The intrinsic ATPase activity of the myosin head breaks down ATP to ADP and Pi (Fig. 27.2A).
2. This results in rotation of the myosin head around the hinge in such a way that the globular head is positioned at 90° angle in relation to the thick filament, pointing toward, but not attached to the thin filament.
3. This rotation moves the myosin head about 10 nm along the thin filament, so that, the head is positioned against a new actin molecule. This resting conformation is known as the energized state or the 'cocked position' of the myosin head' in which, energy is stored in the form of Pi, similar to the potential energy stored in a coiled spring.
4. When most of the cross-bridges in a muscle are in this energized state, it keeps the muscle in a relaxed state. ATP hydrolysis in the resting muscle keeps the muscle prepared for future contraction.

**Events in the Stimulated Muscle**

When action potential arrives at the T-tubules, calcium is released from the SR and the myosin-binding sites on actin are exposed.

1. The myosin-ADP-Pi complex has higher affinity for actin, which is called as the charged or energized myosin head. The charged myosin head binds to actin forming the actomyosin complex, also called cross-bridge formation (Fig. 27.2B).
2. The binding of myosin head to actin has two effects:
   - First, Pi is released from myosin head producing the power stroke, i.e. a conformational change in which the myosin head bends on the hinge at an angle of about 45 degrees pulling the actin filament about 11 nm toward the center of the sarcomere (Fig. 27.2C).
   - Second, the binding causes the dissociation of ADP from the myosin head leaving the actomyosin complex in a rigid state. That means the myosin head stay attached to the actin molecule in the same position and at a 45° angle with respect to the thick and thin filaments.
3. For detachment to occur, a new molecule of ATP must bind to the myosin head and initiate next cycle. Usually, the sarcoplasmic ATP concentration in normal physiological conditions is always adequate to produce the necessary detachment of cross-bridges. So, the ADP-free actomyosin complex (attached state) quickly binds to ATP.
4. This decreases the affinity of myosin for actin resulting in detachment of cross-bridges from the thin filaments, called as detached state (Fig. 27.2D). Soon after, ATP hydrolysis occurs and the myosin head attains the energized state.

If the stimulus for contraction persists, i.e. the sarcoplasmic Ca$^{2+}$ remains elevated, then myosin-binding sites on actin get exposed and the next cross, bridge cycle ensues. The cycle can go on as long as the sarcoplasmic Ca$^{2+}$ remains high, ATP is available, and the physiological limit to shortening has not been reached.

**Effects of Cross-Bridge Formation**

During contraction, being attached to an actin molecule, each myosin cross-bridge pivots around its hinge. Each thick filament has 500–600 myosin heads, and each one can pivot about five times per second during a rapid contraction.

1. One stroke of a cross-bridge produces 11 nm displacement of the thin filament over the thick filament.
2. As long as the muscle is stimulated, these swiveling motions of many cross-bridges with each cross-bridge repeating its cycle many times, drag the thin filaments attached to successive Z lines toward the center of the sarcomere, producing large displacements of the thin filaments.
3. This leads to decrease in the sarcomeric length and ultimately muscle shortening.
4. When shortening does not occur as in isometric contraction, the repetitive binding and pull on the actin generate tension.
Figs. 27.2A to D: Cross-bridge cycle. (A) Resting state (cross-bridge is energized by braking down of ATP to ADP and Pi that provides energy; (B) Myosin head-ADP-Pi complex develops more affinity for actin and bind with actin to form actomyosin complex; (C) Myosin Head pulls actin filament that moves Z line toward center of sarcomere (power-stroke), and actomyosin complex is free of ADP and Pi; (D) ATP binds with myosin head and detaches it from actin filament (detached state).

Role of ATP in Cross-Bridge Formation
ATP is required for the detachment of myosin head from the thin filaments.
1. If most of the cross-bridges in a muscle remain in the detached state, it would produce relaxation in the muscle.
2. Not all the cross bridges remain in the detached state even in a completely relaxed muscle; some of them bind to actin and generate tension that contributes to the tone of the muscle.
3. If cellular ATP stores are depleted, as happens after death, the cross-bridges cannot detach and it is called a rigor cross bridge, the cycle stops there and the filaments remain in the attached state. This produces stiffening in the muscle, known as rigor mortis (Clinical Box 27.1).
Clinical Box 27.1
Rigor mortis: Stiffening of the muscle after death is called rigor mortis. Within 3 to 6 h of death, rigor mortis sets in as the ATP concentration decreases in cells, including muscle cells. The depletion of ATP fails to detach the cross-bridge. Therefore, muscle remains in a state of contraction or rigidity. The rigidity of rigor mortis completes in about 12 h and disappears about 40 to 60 h after death due to disintegration and putrefaction of muscle fibers. Due to rigor mortis, body remains in same position for a longer time. Thus, rigor mortis not only speaks about the time of death, but also the nature of death, which helps in medicolegal investigations in case of mysterious deaths.

The process of cross-bridge cycle can be summarized in four steps (Figs. 27.2A to D, as depicted above):
1. ATP hydrolysis in the myosin head leading to energized state of the myosin.
2. Cross-bridge formation in the presence of increased calcium.
3. Release of Pi and ADP from myosin head producing power stroke.
4. Binding of ATP to myosin head causing detachment of myosin from actin.

Note, during muscle contraction, Z lines move toward M line that decreases sarcomeric length (muscle shortening), and during muscle relaxation, Z lines remain wide apart (Figs. 27.3A and B). In this process of contraction and relaxation, A band remains constant and changes occur in I band and H zone. In extreme contracted state, I band and H zone almost obliterate.

Summary of Skeletal Muscle Contraction
Molecular basis of skeletal muscle contraction as discussed above is an elaborate process. For students to quickly revise, understand and memorize quickly, it is summarized in Flowchart 27.1.

Mechanism of Muscle Relaxation
Like muscle contraction, relaxation is also an active process.
1. The Ca$^{2+}$ ATPase present on the membrane of SR pumps Ca$^{2+}$ from the sarcoplasm into the SR (for details read the mechanism of calcium uptake by Sarco-Tubular System in previous chapter).
2. If no further action potential arrives at the myoneural junction, the activity of Ca$^{2+}$ ATPase decreases calcium concentration in the vicinity of the myofilaments to a very low level.
3. Consequently, very few calcium ions are available to bind with troponin.
4. This leads to **cessation of interaction** between actin and myosin.
5. The contractile activity stops and relaxation process begins (Flowchart 27.2).

Thus, contraction as well as relaxation in skeletal muscle requires energy in the form of ATP.

**Action Potential and Contractile Response**

**Action Potential**

The **resting membrane potential** of skeletal muscle is \(-90 \text{ mV}\). The action potential is generated at the end plate region and spreads at a speed of 5 m/sec along the sarcolemma and down the T tubules activating the myofilaments.

1. The **duration** of the action potential is 2–4 ms and **absolute refractory period** is 1–3 ms.
2. The **depolarization phase** of the action potential is due to **sodium influx** and **repolarization** is a manifestation of **potassium efflux**.
3. The action potential does not directly activate the contractile proteins but instead produces a state of increased cytosolic calcium concentration, which activates the contractile apparatus long after the electrical activity in the membrane has ceased (Fig. 27.4A).

**Contractile Response**

In response to the motor nerve discharge, the thick and thin filaments slide past each other producing shortening of the activated fibers. This contractile response (twitch contraction) can be recorded in a graphical form (Fig. 27.4B).

1. A **twitch** is defined as the mechanical response of single muscle fiber to an action potential consisting of a phase of contraction followed by a relaxation phase.
2. When the action potential and the twitch contraction are plotted on the same time scale, the contractile response begins about 2 ms after the onset of the action potential. This delay is known as the **latent period**, during which the excitation-contraction coupling takes place.

In nerve-muscle preparation, where a motor nerve is stimulated to obtain a contraction, the latent period occurs due to the time taken for:

1. Conduction of impulse along the axon from the point of stimulus.
2. Secretion of Ach, its diffusion through the synaptic cleft and generation of end plate potential.
3. Origin and spread of action potential along the sarcolemma and T tubules.
4. Activation of the contractile mechanism.
5. Overcoming the viscous resistance of the muscle.
6. Overcoming the inertia of the writing lever (if the twitch is being recorded).

Calcium released during a twitch can fully activate the contractile machinery, but the relaxation process starts soon after. So, the magnitude of peak tension developed is relatively low. The duration from the start of the contractile response to the attainment of peak tension is the **contraction time** and from the point of peak tension to
the end of contractile response is the relaxation time. The duration of the twitch is about 7.5 ms in the fast muscle fiber and may be up to 100 ms in slow muscle fiber. Normally, twitches occur occasionally in a healthy person. In the clinics, twitches are usually elicited while testing the tendon jerks (myotatic reflexes). In lower motor neuron diseases, presence of twitches that occur spontaneously helps to establish the diagnosis.

### Types of Contraction

**Tension** is the force of the contracting muscle acting on an object. **Load** is the force exerted on the muscle by an object, i.e. weight of the object against which muscle tension acts. Thus, muscle tension and load act against each other. When an object is to be lifted, muscle tension has to be more than the opposing load. Contractions are of two types:
1. **Isometric contraction**
2. **Isotonic contraction**

#### Isometric Contraction

When muscle contraction is associated with no apparent change in muscle length, the phenomenon is called isometric contraction. The muscle develops tension but does not shorten or lengthen; for example, when somebody is trying to lift a heavy object.

1. During this type of contraction, the cross bridges bind with the actin molecules and attempt to pull them but cannot drag the thin filaments because the load is greater than the tension exerted by the muscle fiber. This exerts a force on the thin filament, the isometric tension.
2. If isometric contraction is continued, cycling cross bridges again and again bind to the same actin molecule.
3. According to the law of physics, if displacement is zero (position of the object does not change), the work done (force × displacement) is also nil, though force is generated and energy is spent. Thus, in isometric contraction **no external work is done**.
4. Isometric tension can be recorded with the help of an isometric lever when the muscle is stimulated to contract with its two ends held at fixed points.

#### Isotonic Contraction

When muscle contraction is associated with no apparent change in muscle tone, the phenomenon is called isotonic contraction.

1. The shortening of the muscle fiber occurs due to sliding of thin over thick filaments. Examples of such muscle contractions are walking, running, lifting an object, etc.
2. Isotonic contraction is also known as **concentric contraction**.

| Table 27.1: Differences between isometric and isotonic contractions. |
|-----------------------------|-----------------------------|
|                            | Isometric | Isotonic |
| 1. Length of muscle         | Remains same | Shortening occurs |
| 2. Tension                  | Tension increases | No change |
| 3. External work            | No work done | Work is done |

The major difference from isometric contraction is that in isotonic contraction, the external work is done (Table 27.1).

### Positive and Negative Works

The muscle does **positive work** when an object is lifted from the ground. **Negative work** is done when a heavy object is lowered onto the ground.

1. In negative work, the muscle actively opposes the descent of the object by contracting, but, as the load exerted on the muscle is greater than the tension generated due to actomyosin interaction, the load pulls the muscle to a longer length. Such lengthening of the muscle is called **lengthening contraction** or **eccentric contraction**, for example, the extensors of the knee lengthen when somebody sits on the ground.
2. This is not an active process produced by cross bridge activation, rather a passive phenomenon, where external load stretches the muscle.

When stimulated, a muscle fiber always tends to shorten, unless an external lengthening force is present. While recording for isotonic contraction, one end of the muscle is fixed and the other kept free, so that on stimulation, the muscle shortens by contraction producing a constant force.

#### Processes in Isometric and Isotonic Contractions

When an object is to be lifted, initially tension increases in the muscle till it becomes equal to the downward pulling force (due to the weight of the object) without any change in muscle length, i.e. the fibers undergo isometric contraction.

1. Once the tension in the muscle is greater than the opposing load, shortening contraction of the muscle lifts the object and brings it to the new position; here the fibers undergo isotonic contraction.
2. Now, if the object is to be held in space in the new position, the fibers undergo isometric contraction, the tension generated being just equal and opposite to the load of the object.
3. Pushing against a wall is an example of isometric contraction if the elbow does not bend during the act, i.e. the distance between shoulder and hand remains unchanged.


CHAPTER SUMMARY

**KEY CONCEPTS**

1. Muscle contraction is an active process in which ATP is utilized, and muscle relaxation is also an active process.
2. Sliding filament theory of muscle contraction is based on cross-bridge formation, in which myosin head pulls the thin filament on thick filament.
3. During each cross bridge cycle, cross bridge (myosin head) attaches to thin filament causing displacement of thick filament over thin filament followed by detachment of myosin head in a repetitive fashion.
4. ATP is required during the cycle for the movement of the cross bridge as well as for its detachment.
5. In skeletal muscle, the contractile response (that lasts for about 15 ms) begins almost toward the end of electrical response (that lasts for about 4 ms). Therefore, skeletal muscle can be tetanized.

**Important to Know (Must Read)**

1. In examinations, “Describe the molecular basis of muscle contraction” is a very common Long Question. Cross bridge cycle may sometime come as long question.
2. Excitation-contraction coupling, Molecular basis of muscle contraction, Cross bridge cycle, Relationship between electrical and mechanical response in skeletal muscle, Types of muscle contraction, are usual Short Questions in exams.
3. In Viva, examiners usually ask… What is the meaning of Excitation-contraction coupling, Who described Sliding-Filament Theory, What is the meaning of Sliding-Filament Theory, Steps in molecular basis of muscle contraction, Mechanism of calcium release from cisterns, Mechanism of cross-bridge cycle, Role of troponins and tropomyosin, Steps of muscle relaxation, Relationship between electrical and mechanical response in skeletal muscle and its importance, and Types of muscle contraction.
4. Mechanism of skeletal muscle contraction is invariably asked in both theory and oral exams.
Learning Objectives

On completion of study of this chapter, the student **MUST** be able to:
1. List the properties of skeletal muscle.
2. Understand the link of muscle properties to function.
3. Name the energy sources in skeletal muscle.
4. Explain the mechanism of oxygen debt.
5. Define the motor unit and understand the concept of size principle.
6. Define different types of muscle heat and understand their importance.
7. Name the type of muscle fibers and basic differences between them.
8. Correlate the application of knowledge of function with dysfunctions.

The student **MAY** also be able to:
1. Describe each property of skeletal muscle.
2. Understand the mechanism of muscle fatigue and rigor mortis.

**Properties of Skeletal Muscle**

**Important Properties**

Important properties of skeletal muscles (in addition to the properties, like contractility, excitability, fatigability, etc.) are:
1. Summation of contraction
2. Staircase phenomenon
3. Tetanization
4. Post-tetanic potentiation
5. Length-tension relationship
6. Load-velocity relationship

**Summation of Contraction**

It states that isometric tension developed in a single fiber or a muscle depends on the frequency of the stimulus applied to it.

1. The action potential of the skeletal muscle is brief (1–4 ms) compared to its contraction time. The absolute refractory period (which includes the depolarization phase and part of the repolarization phase) of the action potential is over by the time contraction starts.
2. Thus, during the contraction period, if the motor neuron is stimulated repeatedly, it will generate several action potentials in the muscle fiber. The **contractile elements have no refractory period**; they do not follow-all-or none law and contraction can continue till Ca	extsuperscript{2+} and ATP are available.

If a second twitch is produced when the contraction phase of the first twitch is still continuing, the phase of contraction of the second twitch is added to the phase of contraction of the first twitch, resulting in a **summated contractile response** (Figs. 28.1A to C).

1. The Ca	extsuperscript{2+} released due to the second action potential is added to the Ca	extsuperscript{2+} released due to the first action potential, producing a higher sarcoplasmic Ca	extsuperscript{2+} concentration that results in a **bigger contractile response**. Therefore, the tension generated during summated response is greater than that of a single twitch. The tension increases further as the stimulus interval shortens (Fig. 28.1C).
2. Thus, repeated stimulation of the muscle before relaxation has occurred produces a phenomenon known as **summation of contractions**.

**Staircase Phenomenon**

If a skeletal muscle is stimulated rapidly (but below the tetanizing frequency) with a maximal stimulus, there is a progressive increase in the force of contraction for the
first few contractions, until a maximum uniform tension per contraction is reached. This is the staircase phenomenon or treppe (in German). Treppe is also seen in cardiac muscle. In 1871, Bowditch first described Treppe in the frog’s heart.

1. During repeated and rapid stimulation, if a second contraction occurs during the relaxation phase of the first one, the amplitude of the second twitch happens to be higher than the first one (Figs. 28.2A to D).

2. This is because when the muscle has not completely relaxed, the cytoplasmic calcium concentration remains elevated as all the calcium released due to the first stimulus has not been pumped back into the sarcoplasmic reticulum.

3. This leftover calcium is added to the calcium released during the second contraction and results in greater amplitude. Also, the heat produced during preceding contraction decreases sarcoplasmic viscosity and facilitates the enzymatic activity that increases the height of succeeding contractions.

4. In this manner, if subsequent contractions occur before the relaxation phase of the previous one is over, first few twitches gradually increase in amplitude.

5. This is known as staircase phenomenon as the graph is in an ascending order, like a staircase. The amplitude does not increase after a few contractions as the calcium level becomes saturated.

**Tetanization**

If the muscle is stimulated repeatedly at a very high frequency, continuous activation of the contractile mechanism occurs without any relaxation, resulting in a sustained contraction known as tetanus (tetanic contraction).

1. In complete/fused tetanus, there is no relaxation between the contraction phases.

2. In clonus or incomplete or unfused tetanus, there are periods of incomplete relaxation between the maintained contractions (Figs. 28.2C and D).

3. While the frequency of the action potentials progressively increases to tetanizing frequency, the tension generated in the muscle gradually rises and summed until a maximal tetanic tension is reached. Beyond this, tension does not increase further with increase in frequency of the action potentials.

The critical frequency (CF) at which summation of contractions occur differs from muscle to muscle since it depends on the twitch duration of a fiber.

\[
\text{CF} = \frac{1}{\text{Twitch duration (in sec)}}
\]

For example, a fiber has twitch duration of 10 ms or 0.01s. The CF for this fiber will be 1/0.01s = 100/s.

When a muscle is stimulated with a frequency more than CF, summation of contractions occurs. Stimulation of the muscle with less than CF produces distinct contractions with intermittent relaxations. A fast muscle needs a larger CF and a slow muscle needs a smaller CF to summate the contractions. The tension generated during a complete tetanus is usually about four times that of a single twitch.

1. Following a single action potential, enough Ca^{2+} is released to expose all the myosin-binding sites on actin molecules. But, the attachment of activated myosin heads to these sites takes time. Also, the Ca^{2+} concentration starts decreasing as the pumping back of Ca^{2+} into the sarcoplasmic reticulum commences immediately.
2. Therefore, all the cross-bridges are not engaged and the tension-generating mechanism is not saturated.
3. In a tetanic contraction, more and more Ca\(^{++}\) is released with each successive action potential, exhausting the pumping-back mechanism.
4. Thus, the cytosolic calcium remains at a constantly high level so that all the myosin-binding sites on the thin filaments are available.
5. Therefore, the tension generated during tetanic contraction is very high. Such contraction occurs in a disease called tetanus caused by the clostridium tetani.

**Post-Tetanic Potentiation**

When a single stimulus is applied to a muscle immediately after the tetanic contraction is over, the amplitude of contraction is higher than that of a single twitch. This phenomenon is known as post-tetanic potentiation.

1. Following tetanic contraction, the released Ca\(^{++}\) takes some time to be pumped back into the sarcoplasmic reticulum.
2. This left-over Ca\(^{++}\) is added to the Ca\(^{++}\) released by the next stimulus resulting in a higher cytosolic Ca\(^{++}\) level and, therefore, a bigger contraction.

**Length-Tension Relationship**

Isometric tension developed in a muscle depends on the initial length of the muscle. For the isometric tension to be recorded in an experimental set-up, the ends of the muscle are attached to two fixing points so that changing the distance between the fixing points can alter the length of the muscle.

1. When the muscle fiber is stretched, the tension registered by it is known as the passive tension, which is due to the elongation of the elastic titin filaments, not due to activation of the contractile elements.
2. If this stretched muscle is stimulated, the tension developed by it is known as the total tension.
3. At any length, the amount of tension actually generated by the cross-bridge movements is known as the active tension, which is the difference between the two values, that is the total tension minus passive tension.

The length-tension relationship graph (Fig. 28.3) shows that within physiological limit, the tension in the muscle increases, as the length is gradually increased.
1. The length of the muscle prior to contraction is called the **initial length**.

2. The length of the muscle at which it develops maximal isometric active tension is known as the **optimal length** or **resting length**, beyond which the tension generated on stimulation decreases until the muscle fibers are torn by the stretch.

3. It has been found that when the skeletal muscles in the body are relaxed, if the tendon is cut from its bony attachment, the muscle shortens about 20%. This indicates that the skeletal muscles are under a certain degree of stretch in the resting state.

4. Experiments have shown that this degree of stretch of resting muscles generates maximum tension on stimulation. Therefore, the term ‘resting’ length is used to describe the muscle length at which maximum response is produced when it is stimulated.

**Length-Tension Relationship in a Sarcomere:**

The length-tension relationship in skeletal muscle has been explained by the sliding filament mechanism of muscle contraction described earlier. According to this mechanism, during contraction, the amount of tension generated depends on the total number of cross-linkages formed due to interaction between actin and myosin molecules.

1. **At resting length**, there is utmost overlap between actin and myosin filaments that can lead to maximum tension development.

2. As the muscle is stretched beyond this length, overlap between thick and thin filaments decreases progressively and the active tension developed declines proportionately, such that, when there is no overlap, the tension generated becomes nil (Fig. 28.4).

3. On the contrary, when the length becomes less than the resting length, overlap decreases and the tension declines.

4. With extreme reduction in length, the thin filaments overlap each other in the center of the sarcomere so that the binding sites on actin are in the bare area of the thick filaments, producing minimal tension. The optimal sarcomeric length in frog muscle is 2.0–2.2 µm.

**Load-Velocity Relationship**

When a muscle contracts against a load (isotonic contraction), the velocity of fiber-shortening is inversely proportional to the degree of load. When there is no load, the muscle contracts with maximum velocity and the velocity decreases with increasing load on the muscle (Fig. 28.5).

1. When the load becomes equal to the maximal isometric tension the muscle can develop, the shortening velocity is zero. If the load increases further, the muscle lengthens (**lengthening contraction**).

2. This load-velocity relationship is a common experience in our day-to-day life. As we know, light objects can be lifted faster than heavy objects.

3. The rate of cross-bridge cycling in a muscle, which depends on its ATPase activity, determines the shortening velocity of that muscle. In general, a light load offers less resistance to the sliding of the filaments so that thin filaments move quickly over thick filaments allowing few cross-bridges to form at a time, producing less tension and faster contraction.

4. On the contrary, increasing the load on a cross-bridge slows its forward movement during a power stroke and reduces the ATP hydrolysis; the slower contraction allows more time for actomyosin interaction and greater tension development.

Now, if we correlate both the length-tension and load-velocity relationships in a skeletal muscle, we can derive that for any given load, the shortening velocity is maximal when the muscle is at its resting length. When a muscle is stretched by a load prior to start of contraction, the condition is known as free-loaded (pre-loaded); and when the load is applied to the muscle after it starts contracting, the condition is called after-loaded.
Energy Sources in Skeletal Muscle

Adenosine Tri-phosphate
Adenosine Tri-phosphate (ATP) is the immediate source of energy for contraction in skeletal muscles. During ATP hydrolysis, the terminal high-energy phosphate bond of ATP splits to form ADP and Pi, with release of 7.3 kcal for each mole of ATP hydrolyzed.

ATP + Pi + H2O → ADP + Pi + 7.3 kcal

The functions of ATP are as follows:
1. It supplies the energy required for cross-bridge movement during muscle contraction.
2. ATP binding to myosin breaks the actin-myosin interaction and allows the cross-bridge cycle to continue.
3. It provides energy to the Ca2+-Mg2+ ATPase pump that transports Ca2+ back into the SR and initiates relaxation.
4. The Na+/K+ pump in the sarcolemma utilizes the energy derived from ATP hydrolysis to maintain the membrane excitability (RMP).

In resting muscle, less ATP molecules are present in the sarcoplasm. Once contraction is initiated, ATP stores can sustain contraction for a fraction of a second. For contractions to continue, muscle requires an enormous supply of ATP, which has to be generated immediately. ATP is regenerated by the addition of one phosphate group to ADP by three pathways:
1. Phosphorylation of ADP by creatine phosphate
2. Oxidative phosphorylation of ADP in the mitochondria
3. Phosphorylation of ADP by the breakdown of glucose to CO2 and H2O.

Creatine Phosphate
Creatine phosphate (CP) is also called phosphorylcreatine. Phosphorylation of ADP by creatine phosphate is a very rapid, nonoxidative process, which provides ATP by transfer of a phosphate group from CP to ADP, catalyzed by the enzyme creatine kinase (CK).

CP + ADP ⇌ CK C + ATP

The concentration of CP is about five times that of ATP in the resting muscle. The above reaction is stimulated by a fall in ATP and a rise in ADP concentration. The amount of ATP that can be formed is limited by the initial concentration of CP in the sarcoplasm. The ATP supplied by this mechanism sustains contractions for a few seconds. During recovery period, the concentration of CP is restored back to the normal level by addition of ATP formed by glycolysis to creatine (C). Also some mitochondrial ATP transfers its phosphate group to creatine.

C + ATP ⇌ CK CP + ADP

As the levels of ATP and CP fall, other mechanisms are activated to provide ATP to the contracting muscle. At rest and during light exercise, muscle utilizes free fatty acids as its energy source. During moderate to heavy exercise, metabolism of glucose and glycogen has the primary role to generate ATP. In the presence of O2, aerobic glycolysis takes place, the end-products being CO2, H2O, and 38 molecules of ATP. If the O2 supply is inadequate, anaerobic glycolysis delivers lactic acid and 2 ATP per molecule of glucose.

Oxygen Debt
During rapid strenuous activity, even with the high respiratory rate and maximum possible dilation of the blood vessels, ATP generated by aerobic glycolysis is not rapid to meet with body’s energy need. In that case, anaerobic glycolysis occurs to fulfill the ATP demand. For example, when a person runs for an hour, around 95% of the energy spent comes from aerobic glycolysis and only 5% from anaerobic glycolysis whereas in a 100 meter dash completed in 10 seconds, 85% of the energy is derived from anaerobic glycolysis.

After the exercise is over, body has to accomplish the following functions:
1. Convert the surplus lactate into pyruvate
2. Replenish the ATP and CP stores in the muscle
3. To replaced the O2 that came from myoglobin.
4. For all the above functions, O2 is required. Therefore, even after the termination of the exercise, the respiratory rate remains high for some time according to the severity of the exercise. The amount of extra oxygen taken up by the body above the basal O2 consumption following exercise is known as oxygen debt (Application Box 28.1).

Application Box 28.1
Effects of training: Trained athletes can increase muscle oxygen consumption greatly than the nonathletes. They can also effectively utilize free fatty acids. Thus, they sustain exercise without loss of glycogen and accumulation of lactate. Hence, oxygen debt in them is much smaller even for severe exercise. They also practice more carbohydrate meal several days before the final competition to increase their muscle glycogen store.

Muscle Fatigue
When a skeletal muscle is repeatedly stimulated, the tension developed by the muscle gradually decreases, even though the stimulation continues. The decrease in muscle tension as a result of previous contractile activity is known as muscle fatigue. There is decline in the amplitude of contraction as well as the shortening velocity, and the relaxation period is prolonged. Fatigue can be of early or late onset and the rate of its progress can be fast or slow. In general, the factors affecting skeletal muscle fatigue are:
1. The type of fibers present in the muscle
2. The intensity and duration of the exercise
3. Availability of nutrients like oxygen and glucose
4. Accumulation of metabolites like lactic acid, H+, K+ and substance P
5. Previous regular training of the similar type of activity
6. CNS factor
Out of all these, the CNS factor plays an important role in delaying the muscle fatigue in human beings. As we know, motivation and encouragement significantly prolong the duration of exercise. They lead to secretion of certain neurotransmitters by an unknown mechanism that reduces the sensory inputs from the exercising muscle as well as the whole body, reaching the level of consciousness. One probable mechanism is that they activate the endorphin system. The degree and source of encouragement also affect fatigue significantly. A muscle that fatigues early also recovers early and the one that takes longer time also recovers late.

**Rigor Mortis**

During the cross-bridge cycle, binding of ATP to the myosin head breaks the actin-myosin interaction and brings in relaxation of the muscle. If cellular energy stores are depleted, as happens after death, cross-bridge detachment cannot occur due to lack of ATP. Therefore, the myosin heads remain in the attached state. Following death, the cytoplasmic calcium concentration remains elevated because of the following reasons:

1. Calcium is not pumped back into the sarcoplasmic reticulum in the absence of ATP.
2. Ca\(^{2+}\) diffuses from ECF into the cytoplasm. This is because after death, the muscle membrane becomes inactive, which cannot maintain the high gradient of Ca\(^{2+}\) between ECF and ICF, as occurs in life.
3. The inactive membrane of the SR cannot hold back Ca\(^{2+}\), which diffuses out of SR to the sarcoplasm.

The raised cytoplasmic calcium concentration initiates contraction by exposing the myosin heads on actin and permitting formation of cross-bridges that do not detach due to lack of ATP. This leads to stiffness in the muscle, which is known as rigor mortis. It starts about 3 to 4 h after death and gets completed in about 12 h after death. The stiffness disappears 48 to 60 h after death due to disintegration of muscle proteins.

**Heat Production in Muscles**

During muscle contraction, most of the energy is spent in the form of heat, part of the energy is used to do the work and a small portion is used to build up the ATP stores. The mechanical efficiency of a muscle is expressed as the percentage of total energy spent for the work done. In isometric contraction, work done is zero; so the mechanical efficiency is 0%, whereas in isotonic contraction, the efficiency lies between 20 and 25%, and can be as much as 50%. The amount of heat generated can be measured accurately with the help of sensitive thermocouples.

The heat generated is of the following types:

a. **Resting heat**: When the muscle is at rest, the heat generated due to basal metabolism is known as resting heat.

b. **Initial heat**: Initial heat is the heat liberated during muscle contraction in excess of resting heat. It can be further divided into two parts:

i. **Activation heat**, which is produced after the muscle is stimulated and before the contraction starts.

ii. **Shortening heat**, which is released during contraction leading to shortening and is proportionate to the degree of shortening.

iii. **Relaxation heat**: When a previously shortened muscle returns to its original length, the heat generated is known as relaxation heat.

iv. **Recovery heat**: This is the heat produced by the metabolic processes to restore the muscle to its resting state, in excess of resting heat. The recovery heat is liberated for a prolonged period and is equal in magnitude to the initial heat.

### Fiber Types in Skeletal Muscle

Based on the speed of contraction and the process of deriving energy from the body metabolism, skeletal muscle fibers can be classified into two categories: type I and type II (Table 28.1).

<table>
<thead>
<tr>
<th>Table 28.1: Differences between type I and II muscle fibers.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type</strong></td>
</tr>
<tr>
<td>1. Other names</td>
</tr>
<tr>
<td>2. Contraction velocity</td>
</tr>
<tr>
<td>3. Myosin ATPase</td>
</tr>
<tr>
<td>4. Ca(^{2+})-pumping capacity of SR</td>
</tr>
<tr>
<td>5. Rate of fatigue</td>
</tr>
<tr>
<td>6. Fiber diameter</td>
</tr>
<tr>
<td>7. Size of motor unit</td>
</tr>
<tr>
<td>8. Glycogen content</td>
</tr>
<tr>
<td>9. Glycolytic capacity</td>
</tr>
<tr>
<td>10. Oxidative capacity</td>
</tr>
<tr>
<td>11. Mitochondria</td>
</tr>
<tr>
<td>12. Myoglobin content</td>
</tr>
<tr>
<td>13. Capillaries</td>
</tr>
<tr>
<td>14. Primary source of ATP production</td>
</tr>
</tbody>
</table>
Type I Muscle Fibers
Muscles consisting mainly of type I fibers have many capillaries and high myoglobin content that imparts them the red color. This ensures an increased blood flow to supply more oxygen.

1. As there are plenty of mitochondria and the oxygen binding protein myoglobin, they synthesize ATP by oxidative phosphorylation and are named as oxidative fibers.
2. Due to their low myosin ATPase activity, they break down ATP slowly; thus, the rate of cross-bridge cycling is less causing a decreased contraction velocity and so they are known as the slow fibers.
3. They are more resistant to fatigue; that means they can remain contracted for longer time, thereby help maintain posture.
4. Therefore, type I fibers in back and proximal limb muscles are known as postural muscles.

Type II Muscle Fibers
Muscles containing mainly type II fibers have a greater contraction velocity and are mainly white muscles. They carry out fine, precise movements. The examples are intrinsic muscles of the hand and extraocular muscles of the eye.

Motor Units

Definition
A motor unit consists of a single motor neuron, its axonal branches and all the muscle fibers supplied by them (Fig. 28.6).

Size of a Motor Unit
The cell bodies of motor neurons are present in the anterior horn of the spinal cord. A single motor neuron branches out and innervates many muscle fibers. When a motor neuron discharges, contraction is produced in all the muscle fibers supplied by it.

1. The size of a motor unit can be large or small depending on the number of muscle fibers innervated by it.
2. In the intrinsic muscles of the hand, one motor neuron innervates less than ten muscle fibers so, stimulation of a motor unit produces a small rise in tension.
3. The back muscles have hundreds or even thousands of muscle fibers per motor unit, where activation of a single motor unit causes a large increase in tension.

Recruitment of Motor Units
At the resting state of the muscle, hardly any motor unit is activated. When a single motor neuron discharges, there is some rise in tension in the muscles of the concerned limb. If a greater total tension has to be attained, additional motor neurons fire and there is increase in the number of active motor units. The process of activating more number of motor units is called recruitment of motor units.

1. In hand muscles, gradual activation of motor units produces a stepwise increase in muscle tension because, with activation of each new unit, a small amount of tension is added. As the tension goes up in a graded manner, it can precisely be regulated. Therefore, the muscles carrying out skilled movements have smaller motor units, for which finer regulation of muscle tension is of primary importance.
2. In contrast, in back muscles, with recruitment of additional motor units, each time a huge amount of tension is added. Therefore, the back muscles are useful in maintenance of posture, as there is need to develop a greater tension to resist the downward pull of the gravity (Application Box 28.2).

Asynchronous discharge of motor units prevents fatigue: The motor units discharge asynchronously. When some of the motor units are active, other units are silent. Afterward, the active units go to rest and the silent units become active. At any instant of time, the inputs from different units are summed and this results in a smooth contraction of the muscle. This also helps the muscle to work for longer time without being fatigued.

Size Principle
All the muscle fibers in a motor unit are of the same type, i.e. the muscle fibers innervated by a spinal motor neuron are either oxidative or glycolytic in nature. Based on this,
the motor units can be designated as oxidative or slow and glycolytic or fast motor units. Usually the large diameter, fast-conducting motor neurons innervate the muscle fibers of the fast motor units and the small diameter, slow-conducting motor neurons innervate muscle fibers of the slow motor units. This is called the size principle. As the strength of contraction gradually increases, the small motor units are recruited initially, followed by the recruitment of large motor units. Thus, during the activity of mild to moderate intensity, the less fatigable, oxidative fibers mostly take part in the contractile activity, whereas the glycolytic fibers are recruited during more intense contraction. In general, a muscle has both types of motor units (Application Box 28.3).

**Whole-Muscle Contraction**

During any voluntary activity, muscles contract or relax around a joint to facilitate or inhibit movement in a particular direction. A whole muscle (for e.g. biceps or triceps) consists of many muscle fibers that are organized into several motor units. The central nervous system exerts a precise control over the voluntary movements by matching the force required by the act with the tension developed in the active muscles. Depending on the type and degree of activity, tension develops in the muscles in a smooth and graded fashion. The CNS achieves this by recruitment of more motor units and increasing the frequency of discharge of the motor units. The motor units discharge asynchronously and this results in a smooth and sustained contraction preventing early fatigue of the muscle.

The total tension developed in a muscle depends on the number of active fibers at any instant during contraction, which is decided by the following factors:

1. The total number of active motor units
2. The number of fibers present in each motor unit
3. The type of fibers present in each motor unit (based on the initial length of the fiber, its diameter and fatigability).

**Muscle Strength**

The strength of a muscle is measured by the maximum load it is capable of lifting. There are muscles of different lengths and shapes. Therefore, in order to compare the relative strengths of muscles, the maximum load it can lift in kilograms is divided by the cross-sectional area in square centimeters. The strength of human skeletal muscles is about 3–4 kg/cm², a value typical of all mammals. In the same age group, males have a higher value than females, the ratio being 1.0:0.65. The male hormone testosterone contributes to better development and growth of skeletal muscles. So, the differences are apparent in the postpubertal life. Genetic build plays a role. Also, regular and systematic training enhances muscle power.

**Applied Aspects**

**Muscular Dystrophy**

Muscular dystrophy (MD) includes a variety of degenerative muscle diseases that are due to mutations in the genes coding for the various components of the dystrophin-glycoprotein complex.

**Duchenne Muscular Dystrophy**

The commonest of muscular dystrophy is Duchenne muscular dystrophy (DMD), also called pseudohypertrophic MD, an X-linked recessive disease that affects mostly male children.

1. DMD is characterized by progressive muscular weakness that becomes apparent by age 4, and enlargement of affected muscles (muscle hypertrophy), especially of the calf muscles, and pelvifemoral muscles. Cardiomegaly is common.
2. Enlargement occurs due to gradual degeneration and necrosis of muscle fibers that are replaced by more fibrous and fatty tissue.
3. Typically, the child uses his hands to climb up, while getting up from the floor. By age 12, most sufferers are no longer ambulatory and death occurs by age 30.
4. The pathology is mainly due to absence of dystrophin in the muscles, caused by mutations of the dystrophin gene.
5. Dystrophin gene is a large gene, located in the p21 region of the X chromosome and has a high mutation rate.

**Baker’s Muscular Dystrophy**

Baker’s muscular dystrophy (BMD) is a less severe and rare form MD. It is similar in presentation to DMD, but patients often survive into adulthood.

1. In BMD patients, dystrophin is reduced in amount or present in an abnormal form. Research is underway to identify and stimulate the production of dystrophin-related proteins that may help to correct the symptoms of BMD.
2. A gene expressing a truncated form of dystrophin, called utrophin, has been inserted into mice using transgenic methods and has improved the myopathy.
3. In several forms of MD, laminin 2, a protein associated with the basal lamina of muscle cells and concerned
with mechanical connections between the exterior of muscle cells and the extracellular matrix, is absent along with dystrophin.

**Myopathies**
Mutations in the gene coding for the protein desmin cause skeletal and cardiac myopathies.

**Metabolic Myopathies**
Metabolic myopathies occur due to mutations in the genes coding for various enzymes involved in the metabolism of carbohydrates, proteins and fats. In these patients, muscle breakdown occurs due to accumulation of toxic metabolites.

**Inflammatory Myopathy**
Poliomyelitis is an inflammatory myopathy in which weakness of proximal limb muscles is an early feature. It is due to destruction of motor neurons present in the anterior horn of spinal cord by the poliovirus, resulting in paralysis of skeletal muscles. Death may occur due to respiratory failure.

**Myotonia**
These are conditions where muscle relaxation is prolonged after voluntary contraction. They are due to abnormal genes on chromosomes 7, 17 or 19 that lead to malfunction of Na⁺ or Cl⁻ channels.

**Focal Dystonias**
Dystonia means faulty contraction. Usually the abnormal contraction is limited to a small and specific region of muscles, so it is called focal dystonia.
1. These are neuromuscular disorders characterized by involuntary and repetitive or sustained skeletal muscle contractions that cause twisting, turning or squeezing movements in a body part.
2. They often result in abnormal postures, considerable pain and also physical impairment.
3. The common dystonias are spasmodic torticollis and cervical dystonia that usually affect neck and shoulder muscles, blepharospasm that affects eyelid muscles, strabismus and nystagmus that affect extraocular muscles, writer’s cramp that affect finger muscles, spasmodic dysphonia that affect muscles of speech apparatus including vocal cord and hemifacial spasm that affect facial muscles.
4. Injection of botulinum toxin into the affected muscles has shown promising results in the treatment of these disorders.

**Muscle Sprain**
Muscle sprain often occurs during sports activity or physical labor due to overstretching or forced extension of an active muscle.
1. Very often the myotendinous junction is injured or, at times, there is separation of the fibers.
2. Ultrastructural damage to the contractile elements especially at the Z lines may occur. Pain, soreness, weakness, and swelling are the usual symptoms.
3. Treatment includes ice packs, rest and immobility. Drugs to relieve pain may be prescribed and surgery is performed to correct the damage if needed.

**Muscle Cramp**
This is a painful condition due to involuntary tetanic contraction of skeletal muscle.
1. It is caused by generation of nerve action potentials at a very high rate.
2. This abnormal activity of nerve occurs in conditions like electrolyte imbalances in the extracellular fluid surrounding both the muscle and nerve fibers due to overexercise or persistent dehydration.

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**CHAPTER SUMMARY**

**Key Concepts**
1. Skeletal muscle exhibits properties, such as summation of contraction, treppe, tetanization and typical length-tension and load-velocity relationship that are suited for its functions.
2. Major energy sources in skeletal muscles are ATP, and creatine phosphate.
3. After the termination of the exercise, the respiratory rate remains high for some time to meet the amount of extra oxygen taken up by the body above the basal O₂ consumption during exercise. It is known as oxygen debt. Oxygen debt is much less in athletes compared to nonathletes, even in severe exercise.
4. A motor unit consists of a single motor neuron, its axonal branches and all the muscle fibers supplied by them. At rest, hardly any motor unit is activated. For more muscle tension to be attained, additional motor neurons should fire and there should be increase in the number of active motor units. The process of activating more number of motor units is called recruitment of motor units.
### Important to Know (Must Read)

1. In examinations, ‘properties of skeletal muscle’ may be asked as a **Long Question**.
2. Treppe, Length-tension relationship, Load-velocity relationship, Oxygen debt, and Heat produced in muscle come as usual in **Short Questions** in exams.
3. In **Viva**, examiners usually ask: Name the properties of skeletal muscle, What is staircase phenomenon, Define treppe and speak about its mechanism and importance, What is the difference between tetanization and tetany, What are the critical frequency for tetanization, What is post-tetanic potentiation, Define initial length and resting length, What is passive tension, active tension and total tension, What is Length-tension relationship and Load-velocity relationship, Define oxygen debt, what is its mechanism and its importance, What are the types of heat produced in a muscle, How muscle fatigue can be delayed, What is rigor mortis and what is its mechanism, What are the types of muscle fibers and what are the differences between them, Define motor unit, What is motor unit recruitment, What is size principle, Name common muscle dystrophies and myopathies, What is the defect in Duchenne muscle dystrophy and what are the features in this problem.
4. Oxygen debt, length-tension relationship and motor units are invariably asked in both theory and oral exams.
On completion of study of this chapter, the student **MUST** be able to:

1. Classify smooth muscle, and mention the differences between them.
2. List the properties of smooth muscle.
3. Describe the mechanism of smooth muscle contraction.
4. List the special features of cardiac muscle.
5. List the differences between skeletal, smooth, and cardiac muscle.

The student **MAY** also be able to:

1. Describe the properties of smooth muscle and cardiac muscle.
2. Explain the differences between skeletal, cardiac, and smooth muscles.

**SMOOTH MUSCLES**

Smooth muscles are mainly present in the visceral organs and blood vessels. The distinctive features in smooth muscles and major differences from skeletal and cardiac muscles are:

1. They are characterized by absence of regular pattern of cross-striations as found in skeletal and cardiac muscles, hence the name *smooth muscles*.
2. The nerve supply to smooth muscles is through the branches of autonomic nervous system.
3. Another striking difference is that the length-tension relationship is not linear as in skeletal and cardiac muscle.
4. Smooth muscles can remain contracted for relatively longer periods without expenditure of much energy, a property that endows them with a better metabolic economy.

**Types of Smooth Muscles**

In some organs, groups of smooth muscle fibers behave as *syncytium*, like that of cardiac muscles and in few other organs they follow the functional properties of skeletal muscles. Based on this, they are classified into two types: single-unit smooth muscles, and multiunit smooth muscles.

**Single-unit Smooth Muscle**

The muscle fibers in single-unit smooth muscles are connected to each other by *gap junctions*.

1. The action potential generated at one place spreads rapidly to all the fibers of that unit. Therefore, contraction occurs simultaneously in all the fibers. Due to this *synchronous electrical and mechanical activity*, the fibers behave as a *syncytium* or a single unit.
2. Single-unit smooth muscles are far more abundant. They form the *walls of hollow viscera* such as the gastrointestinal tract, from the esophagus to the rectum, including the gallbladder and ducts of digestive glands; the ureters and urinary bladder; the uterus and small diameter blood vessels.
3. As they are present in many visceral organs, they are also called *visceral smooth muscles*.

**Multiunit Smooth Muscle**

Multiunit smooth muscles *do not have gap junctions*; therefore, they do not act as a syncytium.

1. They *resemble skeletal muscles functionally* as they are largely under neural control, though not under voluntary control.
2. They are abundantly innervated by the fibers of the autonomic nervous system.
3. The intrinsic muscles of the eye (ciliary body and iris), muscle in the large airways to the lungs, precapillary sphincters, and piloerector muscles are examples of multiunit smooth muscles.

**Functional Organization**

Though the structure of smooth muscles remains apparently same in various body parts, they accomplish different tasks at different body locations. For example, they regulate movement of fluids in the blood vessels, cause propulsive movements at different parts of GIT, expel the contents of the hollow organs like uterus, etc. To suit these varieties of functional needs, arrangements of smooth muscles differ in body parts.

Chiefly, arrangement of fibers is of three types:

1. **Circular**: Circular pattern of arrangement is seen in the blood vessels and in the airways of the lungs where contraction of the muscles narrows the diameter of the passage and reduces the flow. In sphincters, the contraction can totally block the passage and stop the flow.

2. **Circular and longitudinal**: Circular and longitudinal organization is found in GI tract, where an inner thick circular muscle layer and an outer thin longitudinal muscle layer regulate the many types of movements of intestine and promote the mixing and forward movement of the digested food.

3. **Circular, longitudinal and oblique**: Circular, longitudinal and oblique arrangement is typically found in the uterus and urinary bladder. When the increased contents of these viscera stretch these muscles, they contract and decrease the volume by expelling the contents.

**Structure**

Unlike skeletal muscle fibers that cannot multiply once differentiation is complete around birth, the smooth muscles are capable of dividing throughout the life of the individual. The special features of smooth muscles are as follows:

1. The cells are quite small in size compared to the whole tissue. The small size of cells is an advantage for smooth muscle for precisely controlling visceral functions.

2. Each smooth muscle fiber is a spindle-shaped cell, 100–300 µm long and 5–10 µm in diameter at the middle (Fig. 29.1).

3. Smooth muscle cells contain a single elongated nucleus at the center and few mitochondria. The cell derives energy mainly from glycolytic pathway.

4. The sarcoplasmic reticulum is well developed only in some types of smooth muscles; in others it is rudimentary.

5. Myofilaments are organized differently.

6. There are no sarcomeres and striations are not visible (Figs. 29.2A and B).

7. There is lack of an organized T tubular system.

8. The cell membrane shows invaginations called caveolae that increase the surface area.

**Myofilaments**

The cytoplasm contains three types of myofilaments: thick, thin, and intermediate. In addition to the filaments, the cytoplasm contains a calcium-binding protein called calmodulin, which is structurally related to troponin.

**Thick Filaments**

The thick filaments are about 2.2 µm long and composed of myosin molecules that are not arranged in a regular pattern around the axis of the filament as in the skeletal muscle. They are interspersed among the thin filaments (Fig. 29.3) and are much fewer than in skeletal or cardiac muscle.

**Intermediate Filaments and Dense Bodies**

The diameter of intermediate filaments is about 10 nm, which is present between the thick and thin filaments. Intermediate filaments provide cytoskeletal support and have negligible roles in contractile activity.
Section 3: Nerve and Muscle

1. Small, electron-dense, dark areas called dense bodies are present throughout the cytoplasm, as well as attached to the cell membrane.

2. Those associated with the cell membrane are often called membrane-associated dense bodies or patches, or focal adhesions. Dense bodies are functionally analogous to the Z lines in skeletal muscle fibers.

Thin Filaments
In smooth muscles, thin filaments are composed of actin and tropomyosin molecules but troponin protein complex is absent.

Organization of Filaments
The relationship between the thick and thin filaments is poor and they are less well organized.

1. In cross sections through the overlap region of the filaments, the geometric pattern as found in striated muscles are not seen.

2. Because of the absence of a highly organized arrangement of contractile apparatus, the length tension relationship in the smooth muscles is very much flexible.

3. The thick and thin filaments are obliquely oriented to the long axis of the cell. The actin filaments anchor to the dense bodies by means of the protein \( \alpha\)-actinin.

4. During the sliding-filament mechanism, the gap between the actin filaments reduces and the shortening force is transmitted through the dense bodies to the plasma membrane producing contraction of the muscle fiber.

Organization of Muscle Fibers
The smooth muscle fibers are organized in sheets and the cells are connected to adjacent cells by short strands of connective tissue. In addition, collagen and elastin fibers are embedded in a reticular connective tissue that traverses through the whole organ, connecting and holding the cells together.

1. Most importantly, the cells are linked to each other by gap junctions, by which many cells are activated at a time by single action potential.

2. With the help of the stroma and the gap junctions, the mechanical as well as electrical activities of the cells are coupled, so that contraction occurs in an integrated and coordinated fashion.

Innervations of Smooth Muscles
Smooth muscles exhibit a spontaneous, slow wave rhythm. Neural stimulation only modulates (increases or decreases) this basic pattern. Branches of the autonomic nervous system innervate the smooth muscles, most of which are supplied by sympathetic as well as parasympathetic fibers:

1. In response to a nerve action potential, neurotransmitters are secreted from the numerous varicosities present along the axon, and then diffuse to the adjacent tissues.

2. Since the release of neurotransmitters is not confined to the axon terminals, highly specialized neuromuscular junctions are absent in smooth muscles.

3. Unlike the skeletal muscle, the receptors for neurotransmitters are not gathered at the neuromuscular junctions, they are scattered along the postsynaptic membrane.

Electrical Properties
Unlike the skeletal muscle where the stimulus arrives in the form of an all-or-none action potential, in smooth muscles the contraction may or may not be preceded by an action potential. The smooth muscles react to a variety of stimuli, which may be neural (sympathetic or parasympathetic); hormonal (circulating catecholamines, serotonin, histamine, angiotensin, vasopressin, oxytocin, estrogen, and progesterone); chemical (hypoxia, hypercapnia, and \( \text{H}^+ \)); cold; and stretch. The transmission of nerve impulse in sympathetic and parasympathetic nerves supplying visceral muscles and properties of visceral muscle were studied in detail by Dale.

Scientists contributed

Sir Henry Hallett Dale (1875–1968) and Otto Loewi (1873–1961)

The Nobel Prize in Physiology or Medicine 1936 was awarded jointly to neurophysiologist from UK Sir Henry Hallett Dale and neurophysiologist from USA Prof. Otto Loewi “for their discoveries relating to transmission of chemical nerve impulses, especially in sympathetic and parasympathetic nerves”. Their pioneering works were in the field of transmission of nerve impulses, especially its chemical basis. Dale had also studied the details of smooth muscle functions.
**Characteristic Electrical Activities**

1. When the stimulus is excitatory, the membrane potential decreases and it increases in response to an inhibitory stimulus.
2. The membrane potential of visceral smooth muscles is variable (no fixed resting potential), ranging from −30 mV to −70 mV with an average of −50 mV.
3. The visceral smooth muscle shows spontaneous oscillations in a wave-like pattern. Some of the oscillations lead to action potentials that may occur in the rising or falling phase of the wave.
4. The multiunit smooth muscles do not discharge spontaneously; they also do not respond to stretch.
5. Most of the visceral smooth muscles generate action potentials inherently; any stimulus acting on it only alters the rate and pattern of action potentials formed.
6. The shapes of action potentials vary widely, which may be a spike, or action potential with a prolonged plateau, or multiple spikes, superimposed on the membrane potential (Fig. 29.4). Other forms of spikes or action potentials of smooth muscle have been described in GI system).
7. In some cells, in response to stimuli like hormones or mechanical stretch, the membrane potential shows a graded change that may culminate into an action potential.

**Action Potential**

The action potentials are generally of low amplitude, which is about 60 mV, and the duration is around 100 ms. 

The depolarization phase is caused by influx of calcium from ECF due to the opening of voltage-gated Ca$^{++}$ channels. Compared to the striated muscles, the upstroke of action potential is prolonged in smooth muscles, because the Ca$^{++}$ channels take more time to open than the Na$^+$ channels. The repolarization phase occurs due to closure of Ca$^{++}$ channels, which occurs slowly. Opening of voltage-gated K$^+$ channels contributes toward the later part of repolarization. Action potentials are generally observed in visceral smooth muscles. Some smooth muscle cells contract without any change in membrane potential.

**Pacemaker Potential**

In addition to action potentials, pacemaker potentials are recorded in visceral smooth muscle. However, unlike cardiac muscle, the pacemaker activity is not generated at a fixed location; rather it shifts from place to place. In visceral smooth muscles like intestine, pacemaker activities occur at several sites at the same time and then they travel for a short distance in the muscle.

**Mechanism of Contraction**

**Role of Calcium**

As in other muscle types, changes in sarcoplasmic calcium ion concentration plays a major role in smooth muscle contraction.

1. The strength of the stimulus (the degree of stretch or the amount of neurotransmitter/hormone reaching the cell membrane) acting on the smooth muscle fiber can be graded. Accordingly, the change in cytosolic calcium concentration may be minute, more or very high, producing different degrees of contraction.
2. The cytosolic calcium concentration is increased mainly by entry of calcium from the interstitial fluid (calcium influx) or to some extent by its release from cytoplasmic calcium stores (calcium release).
3. However, the concentration of calcium in cytosol decreases due to binding of calcium to calmodulin, pumping back of calcium to the SR and calcium efflux.

**Calcium Influx**

Calcium influx occurs through voltage-gated Ca$^{++}$ channels, ligand-gated Ca$^{++}$ channels and a few leaky channels. The ECF calcium concentration is about 12,000 times higher than that of the cytoplasm (1,200,000 nmol/L versus 100 nmol/L). This high inwardly directed gradient favors calcium entry into the smooth muscle as soon as calcium channels open.

**Calcium Release**

Calcium release from the SR takes place by two mechanisms.
1. $IP_3$-mediated pathway activated by second messengers.
2. Calcium-induced calcium release (CICR) mechanism (as described above) in which, some amount of calcium influx stimulates further calcium release from the SR.
Binding of Calcium to Calmodulin
Binding of calcium to calmodulin starts when cytoplasmic calcium concentration is above $10^{-7}$ M and is completed at $10^{-4}$ M.

Pumping Back of Calcium to the SR
Pumping back of calcium to SR is mediated via a Ca$^{++}$ ATPase present on the membrane of SR.

Calcium Efflux
Calcium efflux to the ECF occurs by Na$^+$-Ca$^{++}$ antiport, a process of secondary active transport and by Ca$^{++}$-ATPase, both the mechanisms being present on the cell membrane.

In smooth muscles, tension is generated in the same manner as in skeletal muscles, by the attachment of energized cross-bridges to actin, followed by detachment from and reattachment to actin.

Molecular Basis of Contraction
As described above, calcium concentration increases in the cell mainly by influx from ECF, and partly by release from SR. The following step-wise processes take place sequentially to culminate in muscle contraction.

1. Rise in cytosolic calcium mainly due to calcium influx from ECF.
2. Calcium binds to calmodulin following which activation of myosin light-chain kinase (MLCK) occurs. Calmodulin is a small protein associated with MLCK, which is a Ca$^{++}$-calmodulin dependent protein kinase. When calmodulin binds with four calcium ions, the calcium-calcmodulin complex activates MLCK.
3. Activated MLCK brings about phosphorylation of myosin head and stimulates myosin ATPase activity. MLCK utilizes one molecule of ATP and causes phosphorylation of the regulatory light chains of myosin, on serine at position 19. This phosphorylation process is critical for the activation of myosin ATPase.
4. Increased myosin ATPase activity and myosin-actin interaction initiates the cross-bridge cycle and shortening occurs due to the sliding-filament mechanism. The myosin ATPase enzyme is activated in the phosphorylated myosin head causing hydrolysis of one ATP molecule. The energized myosin head (Myosin-ADP-Pi complex) binds with actin producing the power stroke and pulls the thin filament toward the center of the thick filament. ADP and Pi are released during this process.
5. The cross-bridge detaches from actin when another ATP molecule attaches to myosin reducing its affinity for actin. Then, the myosin ATPase enzyme hydrolyses ATP resulting in the attachment of myosin head with actin.
6. Thus, the cross-bridge cycle continues, provided that the myosin head remains phosphorylated.
7. The cross-bridge cycle continues as long as the required cytoplasmic calcium concentration is maintained, because phosphorylation depends on cytosolic calcium level (Flowchart 29.1).

In skeletal muscles, contraction proceeds with activation of thin filament proteins (actin-linked regulation), whereas in smooth muscles, the process of contraction is myosin based (myosin-linked regulation).

Mechanism of Relaxation
Relaxation is brought about by dephosphorylation of myosin light-chain by the enzyme myosin light-chain phosphatase (MLCP). This enzyme is constantly active during the periods of relaxation or contraction.

1. During contraction, the rate of MLCK-induced phosphorylation exceeds the rate of MLCP-induced dephosphorylation and therefore, amount of phosphorylated myosin increases in the cell, which allows the contraction to proceed.
2. When the cytoplasmic calcium concentration decreases, calcium is detached from calmodulin resulting in decline in MLCK activity. Consequently, the activity of MLCP predominates causing dephosphorylation of myosin; the myosin ATPase enzyme remains inactive and relaxation ensues.
3. Thus, phosphorylation and dephosphorylation of the myosin light-chain is regulated by respective rise and fall of cytosolic calcium concentration.
Phasic and Tonic Contractions: Unlike the skeletal muscles that demonstrate either twitch or tetanic contraction waves on stimulation, the smooth muscles exhibit phasic or tonic type of contraction.

Phasic Contraction
In phasic type, the degree of phosphorylation, the rate of cross-bridge cycle and the cytoplasmic calcium concentration return to the resting value following the phase of contraction.

Tonic Contraction
During tonic contraction, the above-mentioned parameters do not return to the resting value, rather they remain elevated above the baseline. However, their activity remains less than their peak activity, producing a sustained tension in the muscle at a lower level of MLCK phosphorylation that occurs with less expenditure of energy. This is possible due to the operation of latch-bridge mechanism.

Latch-Bridge Mechanism
Muscle in this state is known as the latch-state, in which dephosphorylation of attached cross-bridges occurs in an environment of elevated cytosolic calcium level.
1. When MLCP-induced dephosphorylation of myosin light-chain takes place, it does not bring about dissociation of myosin from actin until the cytoplasmic calcium concentration falls below a critical level.
2. This attached cross-bridges following dephosphorylation are known as the latch-bridges. In this process the rate of cross-bridge cycle is decreased due to slower rate of detachment of cross-bridges. That means the cross-bridges spend more time in an attached state.
3. Therefore, the ATP utilization is less, which enables the visceral smooth muscles to have sustained contractions with less expenditure of energy.
4. The force of this sustained contraction can be graded over a wide range because the cytosolic calcium concentration can be finely regulated over a wide range. From the latch-state, the relaxation of the muscle occurs when the cytoplasmic calcium concentration falls below $10^{-7} \text{ M}$.

Properties of Smooth Muscle
The properties of visceral smooth muscles may be divided into 3 categories (Table 29.1):

<table>
<thead>
<tr>
<th>Table 29.1: Properties of smooth muscle.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Morphological properties</td>
</tr>
<tr>
<td>1. Lack of striations</td>
</tr>
<tr>
<td>2. Absence of troponin</td>
</tr>
<tr>
<td>3. Absence of T-tubules</td>
</tr>
<tr>
<td>4. Absence of well-developed neuromuscular junctions</td>
</tr>
<tr>
<td>B. Electrical properties</td>
</tr>
<tr>
<td>5. Presence of gap junctions (syncytial property)</td>
</tr>
<tr>
<td>6. Excitability</td>
</tr>
<tr>
<td>7. Variable RMP and shifting pacemakers</td>
</tr>
<tr>
<td>8. Variable action potentials</td>
</tr>
<tr>
<td>C. Mechanical properties</td>
</tr>
<tr>
<td>9. Tonus</td>
</tr>
<tr>
<td>10. Plasticity</td>
</tr>
<tr>
<td>11. Slow and prolonged contractile response</td>
</tr>
<tr>
<td>12. Length–tension relationship</td>
</tr>
<tr>
<td>13. Force–velocity relationship</td>
</tr>
<tr>
<td>14. Muscle hypertrophy</td>
</tr>
</tbody>
</table>

Muscle Tone
Visceral smooth muscles are continuously in a state of spontaneous irregular contraction known as tonus, which occurs due to generation of basic slow wave rhythm. In blood vessels, especially the arterioles, this muscle tone helps to maintain the blood pressure without much expenditure of energy. The multiunit smooth muscles do not exhibit the inherent muscle tone.

Length–Tension Relationship
Unlike the skeletal and cardiac muscles, where the tension changes proportionately to the change in length, the length–tension relationship is variable in smooth muscles.
1. When a smooth muscle is stretched, it initially shortens and exerts tension. However, if the stretch is maintained, gradually the tension decreases and if the stretch is increased, the tension may not rise proportionately or at a particular length, the tension may increase considerably. Thus the relationship is highly inconsistent.
2. The adaptability of the smooth muscle to the new length with less increase in tension is known as plasticity, which is based on the latch-bridge phenomenon.
3. In blood vessels, this feature is also known as stress relaxation that helps to maintain BP within the normal range in spite of sudden rise in pressure.
4. In hollow viscera like stomach, large volume of food and fluid can be accommodated without an undue increase in internal pressure, called receptive relaxation.

Force–Velocity Relationship
Compared to the skeletal muscles, smooth muscles have a low myosin ATPase activity, less number of cross-bridges and a slower rate of cross-bridge cycling. These factors contribute to a slower contraction velocity. Moreover, a variety of force-velocity graphs can be obtained in response to different stimuli, which can alter the plasma...
Ca++ in a graded manner. Thus, the degree of phosphorylation of the cross-bridges changes over a wide range.

**Smooth Muscle Hypertrophy**

Growth and proliferation of vascular smooth muscles are stimulated by a variety of growth factors. This is typically seen during pregnancy and in hypertension.

In pregnancy: Toward term, estrogen stimulates the hypertrophy (increase in cell size) and hyperplasia (increase in cell number) of myometrium as well as the growth of the connective tissue mass. There is increase in the amount of contractile proteins and the number of gap junctions that helps for an effective and coordinated contraction. Stretch of the uterine wall by the growing fetus also induces expansion of the myometrium.

In hypertension: When blood pressure is chronically elevated, the pressure load acts as a stimulus and the walls of the blood vessels undergo hypertrophy and hyperplasia. In addition, if there is sympathetic hyperactivity, increased catecholamines also stimulate the vessel wall proliferation by trophic effect. Angiotensin II stimulates, whereas glucocorticoids inhibit growth of vascular smooth muscles. Some other factors like arachidonic acid derivatives, adenosine, serotonin and heparin like substances also affect smooth muscle hypertrophy.

Other examples: When a hollow viscus like small intestine gets obstructed, the prolonged elevation of the intraluminal pressure stimulates hypertrophy of its muscle layer. Another example is hypertrophy of urinary bladder in men due to enlargement of the prostate gland.

**Neural and Hormonal Influences**

**Autonomic Control**

The autonomic nervous system influences the intrinsic rhythmic or pacemaker activity of the visceral smooth muscles. The effect of sympathetic or parasympathetic stimulation depends on the type of receptor involved and the mechanisms of the second messenger(s) released subsequently.

**Parasympathetic control:** Parasympathetic stimulation is excitatory. It releases acetylcholine. In intestine, acetylcholine is stimulatory.
1. It decreases the membrane potential, which makes the tissue more excitable.
2. It also increases the frequency of action potentials. As a result, there is increase in the number of rhythmic contractions as well as the duration of tonic contractions.
3. Ach acts by increasing the cytoplasmic Ca++ through phospholipase C and IP3.

**Sympathetic control:** Sympathetic stimulation is inhibitory. It releases catecholamines. The effects of catecholamines on intestine are just opposite to that of Ach. Catecholamines increase the membrane potential and decrease the frequency of spikes producing relaxation of the muscle. Norepinephrine (NE) acts through both α and β receptors.

- **Action through α receptors:** Acting via α receptors, NE increases calcium efflux from the cell. This leads to decline in cytoplasmic Ca++ and relaxation of the muscle.
- **Action through β receptors:** Acting via β receptors, NE stimulates adenyl cyclase that increases formation of cAMP, which activates the enzyme cAMP-dependent protein kinase. This protein kinase as well as cAMP increase calcium uptake by the sarcoplasmic reticulum. This results in fall in cytoplasmic concentration of Ca++. Thus relaxation occurs. In addition, the cAMP-dependent protein kinase decreases the activity of MLCK. Therefore, the cross-bridge formation is reduced that also produces relaxation.

The multiunit smooth muscles do not exhibit the inherent muscle tone. In response to the stimulation of the autonomic nervous system, their contraction is regulated in a precise, graded fashion.

**Hormonal Control**

Nitric oxide (NO): In response to the shearing stress caused by flow of blood, NO is released from the endothelial cells lining the arteries. It diffuses to the vascular smooth muscle fibers and binds to the receptors present on the sarcolemma. The NO-receptor complex stimulates guanylyl cyclase and forms cGMP, which activates cGMP-dependent protein kinase. This protein kinase produces relaxation by increasing calcium reuptake by SR, opening of Ca++-activated K+ channels and decreasing IP3-induced Ca++ release by inhibiting phospholipases C.

Other hormones: Angiotensin II acting via AT II receptors, vasopressin and endothelin stimulate contraction of smooth muscles by releasing IP3. Adenosine causes relaxation by increasing the level of cAMP.

**CARDIAC MUSCLE**

Muscles present in heart are cardiac muscles. Though they are structurally close to skeletal muscle they demonstrate many properties of smooth muscles. The details of structure, properties and functions of cardiac muscles are given in “Heart” section of CVS. Here, salient features are given to differentiate it from skeletal muscles and smooth muscles (Table 29.2).

**General features of cardiac muscles are:**
1. Cardiac muscle is an involuntary, but striated muscle. The muscle cell is 100 µm long and 15 µm wide.
2. The fibers are branched and attached with each other closely.
3. Intercalated discs are present at the point of contact of two muscle fibers.
4. Numerous gap junctions are present in the intercalated disc that make cardiac tissue a functional syncytium. There are two such separate syncytia in the heart. The
### Table 29.2: Differences between skeletal, cardiac, and smooth muscles.

<table>
<thead>
<tr>
<th></th>
<th>Skeletal muscle</th>
<th>Cardiac muscle</th>
<th>Smooth muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Morphological properties</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Distribution</td>
<td>Fixed to bones, form body muscle</td>
<td>Heart only, not attached to bone</td>
<td>(A) Single (visceral) unit smooth muscle in hollowed viscera, e.g.: intestine, bronchi, uterus, ureters. (B) Multi unit smooth muscle in capillary muscle. Iris in eye, pilometer muscle of skin; muscle in blood vessels</td>
</tr>
<tr>
<td>2. Striations</td>
<td>It has well-developed striations, therefore, also called striated muscle</td>
<td>Also show cross</td>
<td>Lack cross striations, therefore also called “plain” muscles</td>
</tr>
<tr>
<td>3. Size and shape</td>
<td>Cylindrical, 1–40 mm long, 50-100 µm diameter; multinucleated cells</td>
<td>Short, cylindrical, 100 µm long, and 15 µm diameter; single nucleus; forms a branching network</td>
<td>Spindle (Elongated), single nucleus, variable sizes</td>
</tr>
<tr>
<td>4. Sarcoplasmic reticulum</td>
<td>Well developed</td>
<td>Well developed more than in the skeletal muscle</td>
<td>Poorly developed</td>
</tr>
<tr>
<td>5. Syncytial structure</td>
<td>Non-syncytial</td>
<td>It is functionally syncytial in character</td>
<td>Functionally syncytial in character</td>
</tr>
<tr>
<td>6. Sarcotubular system</td>
<td>Present; T system at A-I junction; terminal cistern prominent.</td>
<td>Present with poorly developed terminal cistern. T system prominent and present at Z lines</td>
<td>Present, but not so characteristic</td>
</tr>
<tr>
<td>7. Nerve supply</td>
<td>By somatic nerves and by special nerve endings</td>
<td>Via two branches of ANS with ganglia and free nerve terminals</td>
<td>Same as in the cardiac muscle</td>
</tr>
<tr>
<td>8. Control and rhythmicity</td>
<td>Voluntary control, no rhythmicity</td>
<td>It contracts rhythmically and spontaneously in the absence of external innervation, by pacemaker drive</td>
<td>Rhythmicity: two types (i) regular and (ii) irregularly discharging pacemaker</td>
</tr>
<tr>
<td>9. Blood supply and oxygen consumption</td>
<td>840 mL/min (3–4 mL 100 gm/min) with moderate oxygen consumption</td>
<td>Abundant, 250 mL/min (80 mL/100 gm/min) with high O₂ consumption</td>
<td>350 mL/min (1.4 mL per 100 gm/min) with less O₂ consumption</td>
</tr>
<tr>
<td><strong>B. Electrical properties</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10. RMP</td>
<td>−90 mV</td>
<td>−90 mV</td>
<td>−50 mV (Range between −30 mV and −50 mV), Fluctuating</td>
</tr>
<tr>
<td>11. Action potential (AP)</td>
<td>Rapid depolarization</td>
<td>Rapid depolarization, 15 to 20 mV produces, AP duration 250 ms, Amplitude 100 Mv, variable speed of conduction</td>
<td>Variable (rapid rise and fall in action potential). Total duration 50 msec. Total amplitude up to 60 mV, variable speed of conduction</td>
</tr>
<tr>
<td><strong>C. Mechanical properties</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12. Mechanical events</td>
<td>Contraction starts 2 msec after the start of depolarization, before repolarization ends. Can summate the contractile response, Tetanization occurs</td>
<td>More than half of mechanical contraction is over, during absolute Tetanization not seen</td>
<td>Muscle starts to contract approximately 200 msec after the start of the spike (150 msec after the spike is over). Peak contraction is reached approximately 500 msec after the spike. Tetanus is seen</td>
</tr>
<tr>
<td>13. Duration of muscle twitch</td>
<td>Varies with types of muscle fibers; 7.5 msec in fast muscles, 100 msec in slow muscles</td>
<td>One and half times the total duration of action potential</td>
<td>Approximately 1,000 msec</td>
</tr>
<tr>
<td>14. Excitation contraction coupling</td>
<td>Rapid process, time from initial depolarization to initiation of contraction is 10 msec</td>
<td>More rapid process time (&lt; 10 msec)</td>
<td>Very slow process</td>
</tr>
<tr>
<td>15. All or none law</td>
<td>Applicable, true for single muscle fiber</td>
<td>Applicable, true for whole of the atria or ventricles</td>
<td>Applicable, true for single muscle fibre</td>
</tr>
<tr>
<td>16. Length tension relationship</td>
<td>Maximum active tension is developed at the optimal length</td>
<td>Same as in skeletal muscle</td>
<td>Shows property of plasticity</td>
</tr>
<tr>
<td>17. Phenomenon of fatigue</td>
<td>Occurs</td>
<td>Not seen</td>
<td>Possible but difficult to demonstrate</td>
</tr>
<tr>
<td><strong>D. Metabolic properties</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18. Energy</td>
<td>Under basal state 20% by fats. &gt;60% from carbohydrates; 20% by proteins</td>
<td>65% by fats, 25% by carbohydrates; and 10% by ketones bodies and amino acids.</td>
<td>Low, mainly provided by the utilization carbohydrates and fats</td>
</tr>
</tbody>
</table>
CHAPTER SUMMARY

Key Concepts

1. Though smooth muscles do not have organized sarcomeres, they have myofilaments for muscle contraction.
2. Membrane potential fluctuates between −30 mV and −70 mV.
3. Cytosolic calcium concentration plays a great role in muscle contraction. Calcium from sarcoplasmic reticulum mainly determines to cytosolic level of calcium, though calcium influx by voltage-gated and ligand-gated channels contribute.
4. Phosphorylation of myosin and latch-bridge mechanism, are special characteristics.
5. There is no definitive length-tension relationship. Plasticity is the main feature.
6. Control of smooth muscle is by autonomic nerves (sympathetic inhibitory and parasympathetic excitatory), and hormones.

Important to Know (Must Read)

1. In examinations, “Mechanism of smooth muscle contraction, and properties of smooth muscle” may come as a Long Question.
2. Special morphological features of smooth muscle, Electrical properties of smooth muscle, Mechanism of smooth muscle contraction, Role of calcium in smooth muscle contraction, Latch bridge mechanism, Properties of smooth muscle, Plasticity, Regulation of smooth muscle activity, and Differences between skeletal muscle, cardiac muscle and smooth muscle, come as Short Questions in exams.
SECTION 4

Autonomic Nervous System

30. Functional Organization of Autonomic Nervous System
31. Sympathetic System
32. Parasympathetic System
33. Control of Autonomic Functions and Applied Aspects
34. Autonomic Function Tests
35. Heart Rate Variability
"A soul shall wake in the Inconscient's house:  
The mind shall be God-vision's tabernacle,  
The body intuition's instrument,  
And life a channel for God's visible power."

Sri Aurobindo (in 'SAVITRI')
The term ‘autonomic’ is derived from the words *auto* means ‘self’ and *nomos* means ‘control’. Emotional responses of the body and responses to environment occur without conscious knowledge of the individual. These responses are therefore, called autonomic responses that are executed by the autonomic part of the nervous system. The ANS controls functions of the involuntary organs of the body that include heart and blood vessels, exocrine and endocrine glands, and all visceral organs. Thus, ANS controls all major functions of the body such as circulation, respiration, digestion, excretion and reproduction. Circulating hormones and locally produced chemicals assist in mediating these autonomic functions.

The functions of ANS can be broadly categorized into five parts:
1. Maintenance of homeostatic conditions of the body.
2. Regulation of visceral activities.
3. Smoothening body’s responses to environmental changes.
4. Coordination of body’s responses to exercise and stress.
5. Assisting the endocrine system to regulate reproductive functions.

**Scientists contributed**

**WH Gaskell** (1847–1914)

Walter Holbrook Gaskell was the first scientist who thoroughly investigated the innervation of the cardiovascular system and viscera. He discovered the cardioaccelerator nerve and laid the foundation for an understanding of autonomic nervous system.

**Sources:**

**JN Langley** (1852–1925)

John Newport Langley analyzed the functional significance of autonomic nervous system (ANS) in vertebrates. He was the first scientist to classify ANS into the sympathetic and parasympathetic divisions, indicating their anatomical, physiological and pharmacological relations and characteristics.

**Source:** Heffer W. *The Autonomic Nervous System.* Cambridge Publication: Cambridge, 1921.
FUNCTIONAL OVERVIEW

Divisions of Autonomic Nervous System

Autonomic nervous system is traditionally divided into two subdivisions: sympathetic and parasympathetic, based on their anatomic, functional and neurochemical properties. Enteric nervous system is considered to be the third division of ANS.

Sympathetic System

When the body requires support to meet emergency conditions and stressful situations, sympathetic system provides the desirable assistance. For its sympathy to help the body to face and overcome such critical situations in life, this division of ANS is called sympathetic system. Sympathetic system also helps to control normal functions of the body such as regulation of blood pressure, respiration, metabolism, etc. The importance of sympathetic system is realized in its dysfunctions. For example, a person with sympathetic imbalance will not be able to stand up from sitting or supine posture and will not be able to maintain the standing posture. Thus, sympathetic system is a friend of the body not only in emergency needs but also in its routine deeds.

1. The cell bodies of sympathetic system are located in the thoracic and abdominal segments of the spinal cord and sympathetic ganglia are present as a chain close to the vertebral column.
2. This system is also termed as the thoracolumbar division of the ANS for the location of the ganglia and cell bodies of neurons at thoracic and lumbar segments of spinal cord (Fig. 30.1).

Parasympathetic System

The component of ANS that works by the side of the sympathetic system is the parasympathetic division of ANS. It has a reciprocal influence on organ functions to that of sympathetic influence. In fact, it checks the overactivity of sympathetic system and smoothens the autonomic responses.

1. The cell bodies are found in the brainstem cranial nerve nuclei (cranial component), and in the most caudal part of the spinal cord (spinal or sacral component).
2. Hence, it is also termed as craniosacral division of ANS (Fig. 30.1).

Enteric Nervous System

The enteric nervous system (ENS) is the local neural network in GI system which has strong anatomical and physiological link with ANS. Therefore, ENS is considered as the ‘Third division of ANS’. It is concerned with the regulation of gastrointestinal function.

FUNCTIONAL SPECIALIZATION

The neurochemical differences between two divisions of sympathetic system led to the description of the sympathetic system as adrenergic for adrenaline (or noradrenaline) like actions resulting from sympathetic activation; and the parasympathetic system as cholinergic for acetylcholine like actions from parasympathetic activation. Some of the important functional characteristics of sympathetic and parasympathetic divisions are as follows:

1. The sympathetic division is primarily meant for utilization of metabolic resources and emergency responses of the body, whereas the parasympathetic division helps in restoration and build-up of the body’s reserves and the elimination of waste products.
2. Almost all the organs are supplied by both sympathetic and parasympathetic nerves and usually they are activated in a reciprocal fashion. Therefore, when the discharge rate in one division is increased, the rate in the other division is decreased. For example, when sympathetic activation occurs during exercise to increase heart rate, simultaneous decrease vagal activity also contributes to achieve the target increase in heart rate.
3. In some organs, sympathetic and parasympathetic systems work synergistically. For example, to enhance gastrointestinal secretions, when parasympathetic stimulation increases volume and enzyme content, simultaneous sympathetic activation contributes to increased mucus content of the secretory product.
4. Also, few structures such as blood vessels and skin receive only sympathetic innervation and therefore, their functions are regulated by alteration in the rate of sympathetic discharge to these structures.

**Somatic vs Autonomic Nervous System**

The nervous system contacts all organs and tissues of the body via the sensory system (afferent innervation), motor system (efferent innervation) and the ANS.

**Central Connections**

For efferent innervation of somatic system (i.e. the motor pathway), axons originate from cell body of neurons in the motor cortex and terminate directly or indirectly on motor neurons in the ventral horn of the spinal cord or in the brainstem motor nuclei, and usually the second order neuron (or sometimes the third order neuron) innervates the skeletal muscle. Similarly, in ANS, efferent pathway consists of two or three-neuron tract with one or two synapses interposed between the center and the effector cells.

1. The cell bodies of autonomic motor neurons are located in the intermediolateral horn of spinal cord or in the specific brainstem cranial nerve nuclei.
2. The efferent fiber emerges from CNS as the preganglionic axon and then synapses with the cell bodies of neurons located in a peripheral ganglion. The neuron from the ganglion then projects as postganglionic axon to the effector cells (usually a visceral organ).

**Reflex Arc**

The somatic nervous system collects information from the external environment, whereas the ANS collects information usually from inside the body, i.e. the changes in visceral structures (Fig. 30.2).

**Receptors**

Receptors are located in the body surface or in the musculoskeletal system in somatic system. In ANS, the receptors are present in the visceral structures.

**Afferent Pathway**

The afferent neurons of somatic system enter spinal cord via dorsal root with their cell bodies in the dorsal root ganglion and terminate on interneurons in deeper layers of dorsal horn or on motor neurons in the ventral horn. The afferent neurons of ANS enter spinal cord in a similar fashion, but they terminate on autonomic efferent neurons having their cell bodies in intermediolateral horn (ILH) of spinal cord.

**Central Neurons**

Central component in somatic system consists of the cell body of a motor neuron in the ventral horn of the spinal cord on which the afferent neuron directly terminates monosynaptically. The afferent neuron may also contact motor neuron through interneurons via disynaptic or polysynaptic connections.

In ANS, the cell bodies of central neurons are located in intermediolateral horn of spinal cord on which the afferent neuron directly terminates.
Efferent Neurons

The efferent neurons in the somatic system are motor neurons that originate from ventral horn of spinal cord and directly terminate on effector organ, which is usually a skeletal muscle. Thus, there is a single efferent neuron between the CNS and the effector organ in the somatic system.

In ANS, there are two neurons between the CNS and the effector organ (Fig. 30.3).
1. The first efferent neuron is the preganglionic neuron that has its cell body in the intermediolateral horn of spinal cord or in the cranial nerve nuclei in brainstem.
2. The second efferent neuron is the postganglionic neuron that has its cell body in the ganglia outside the spinal cord or in the effector organ.

Effector Organs

Somatic motor nerves innervate skeletal muscles. The efferent neurons of ANS innervate visceral organs (smooth muscle, cardiac muscle, glandular tissues, etc.).

Types of Efferent Pathways in ANS

Sympathetic System

In sympathetic system, there are three types of efferent pathways (Fig. 30.4):
1. The preganglionic neurons leave spinal cord via white rami communicantes to the paravertebral sympathetic ganglion where they contact cell bodies of postganglionic neuron, the axons of which terminate on effector organ. The postganglionic sympathetic neurons to head originate from superior and middle cervical ganglia and stellate ganglion. These ganglia are in fact extension of sympathetic ganglion chain into the neck.
2. Axons of preganglionic neurons give collaterals to terminate on another set of cell bodies in the paravertebral ganglion chain. Axons of these postganglionic neurons enter gray rami communicantes and from there enter into the spinal nerve to finally innervate the effector organs.
3. Some of the preganglionic neurons do not relay in paravertebral ganglion chain, rather they come out directly of the ganglion chain to terminate on the cell bodies of postganglionic neurons located in collateral ganglion that are present close to the effector organ. In this efferent pathway of sympathetic system, the preganglionic fibers are longer than the postganglionic fibers. Thus, the postganglionic neurons in this system form short noradrenergic neurons.

Parasympathetic System

In parasympathetic system, the preganglionic neurons of cranial part originate from cell bodies in the cranial nerve......
nuclei (III, VII, IX and X) in the brainstem and terminate in
the postganglionic neuron located very close to or in the
visceral organ. The preganglionic neurons of sacral part
follow a similar route except that the fibers originate from
the cell bodies in the intermediolateral horn of spinal cord and
traverse in spinal nerve.

In general, in sympathetic system, the preganglionic
fibers are smaller than the postganglionic fibers (Application Box 30.1), whereas in parasympathetic system, the
preganglionic fibers are much longer than the postgangli-
onic fibers (Fig. 30.5).

Application Box 30.1

Intrinsic cardiac adrenergic cells: Evidences of recent research studies
indicate that few postganglionic sympathetic neurons are embedded in
the cardiac tissue like that of postganglionic neurons of parasympathetic
systems that are usually located in the visceral organs. These neurons
form intrinsic cardiac adrenergic cells (ICAC). Secretion from ICAC
accounts for about 15% of adrenaline and noradrenaline content of the
heart. It is believed that during fetal and early childhood, catecholamine
content of ICAC plays an essential role in development of the heart.

Neurotransmitters in ANS

In the somatic nervous system, the efferent fibers terminate
on motor end plate usually with one axon terminal to one
skeletal muscle fiber. The neurotransmitter is acetylcholine.
1. In ANS, postganglionic axons terminate in varicosities,
the swellings enriched in synaptic vesicles that release
the transmitter into the extracellular space surround-
ing the effector cells.
2. The electrical activity for discharge of autonomic fibers
originates in some of the effector cells and then propa-
gates to cells of rest of the tissue via gap junctions.
3. The neurotransmitters in ANS are acetylcholine,
noradrenaline and others (Fig. 30.6).

Acetylcholine

Acetylcholine is the neurotransmitter at the following
nerve terminals (cholinergic fibers) in ANS:
1. Preganglionic nerve terminals of both the sympathetic
and the parasympathetic divisions.
2. All parasympathetic postganglionic neurons.
3. Postganglionic sympathetic neurons to sweat glands
and blood vessels in skeletal muscle.

The synapse at preganglionic nerve terminals utilizes
nicotinic receptors similar to that found at neuromuscular
junction. The synapse between the postganglionic neuron
and the target tissues utilizes muscarinic receptors. The
receptor classification is based on the response of the syn-
apses to the alkaloids nicotine and muscarine at respec-
tive type of synapse.
1. The nicotinic receptor is blocked by hexamethonium in
autonomic ganglion, in contrast to blockade by curare
at neuromuscular junction. The nicotinic cholinergic
receptor is of direct ligand-gated type as it contains
ion channel in it.
2. The muscarinic receptor is blocked by atropine. The
muscarinic receptor is of indirect ligand-gated type
as it uses a G protein to mediate effector functions.
The action of acetylcholine is terminated by acetylcholinesterase, the enzyme present at the cholinergic synapses.

**Norepinephrine**

Noradrenaline (NA) or norepinephrine is the neurotransmitter at postganglionic sympathetic nerve terminals (adrenergic fibers). NA released from adrenergic synapses utilize adrenergic receptors that can also be activated by adrenaline or epinephrine released from adrenal medulla. Therefore, these receptors are designated as adrenergic.

1. **Adrenergic receptors** are broadly classified into two types: α and β. The α receptors respond more to NA and less to epinephrine, and least to isoproterenol, the synthetic catecholamine.
2. The β receptors respond best to isoproterenol and epinephrine and less to NA. Propranolol is the antagonist for β receptors. Each class of receptors is further classified as α₁ or α₂, and β₁, β₂ or β₃ (for details, refer ‘Adrenal Medulla’ in ‘Endocrine Physiology’).
3. The adrenergic receptors are of the indirect ligand-gated type as they utilize G protein for their effects. The α₁ receptors activate phospholipase C. The α₂ receptors inhibit adenyl cyclase, whereas β receptors stimulate it.

**Other Neurotransmitters**

In ANS, especially in the intrinsic plexuses of the gut many amines, amino acids and active peptides are widely distributed as co-neurotransmitters. For example, neuropeptide Y is co-released by vasoconstrictor nerve, vasoactive intestinal polypeptide (VIP) and calcitonin-gene-related peptide (CGRP) are released along with cholinergic nerve terminals. Nitric oxide is another neurotransmitter produced by autonomic fibers. The terms nonadrenergic noncholinergic fibers apply to such nerves.

**GENERAL ORGANIZATION OF ANS**

Organization of ANS occurs at five different levels: cortical organization, hypothalamic organization, brainstem organization, spinal organization and peripheral organization.

**Cortical Organization**

Cortical areas controlling functions of ANS are mainly limbic areas and prefrontal cortex.

1. The sympathetic responses to emotion originate in the limbic and prefrontal cortical areas.
2. These areas activate sympathetic system by stimulating hypothalamic and brainstem areas that have influence on the system.

**Hypothalamic Organization**

Hypothalamus considerably influences autonomic functions. Hypothalamus via hypothalamo-pituitary axis controls secretions of major endocrine glands.

1. Hypothalamus receives collaterals from ascending pathways, especially from spinothalamic tracts that transmit pain impulses.
2. Thus, hypothalamus integrates somatosensory, endocrine and autonomic responses that are essential components of homeostatic mechanisms during stressful situations like major surgical procedures, exposures to extreme weathers, trauma and hemorrhage.
3. For its profound influence on autonomic responses, Sherrington had correctly pointed out that hypothalamus is the head-ganglion of sympathetic nervous system.
Brainstem Organization

Brainstem areas contain major nuclei of ANS. These are broadly classified into two categories: parasympathetic nuclei and sympathetic nuclei.

Parasympathetic Nuclei

The cranial outflow of parasympathetic system originates from cranial nerve nuclei that are located in the brainstem.

1. The cranial nerves that carry parasympathetic fibers are oculomotor (III cranial nerve), facial (VII cranial nerve), glossopharyngeal (IX cranial nerve) and vagus (X cranial nerve).
2. Nucleus tractus solitarius (NTS) in the medulla receives general visceral sensation via IX and X cranial nerves. It is also closely connected with reticular formation containing cardio-respiratory centers. Therefore, NTS mediates respiratory and cardiovascular responses to autonomic activation. Special visceral sensations in VII, IX and X cranial nerves reach NTS.
3. Edinger-Westphal nucleus in the midbrain is the nucleus of III cranial nerve.
4. Salivary nucleus is located in pons and dorsal motor nucleus of vagus in medulla.

Sympathetic Nuclei

Sympathetic fibers originate from vasomotor center in the medulla and project to the intermediolateral horn of spinal cord via bulbospinal pathway.

1. Nucleus gigantocellularis and parvocellularis in the reticular formation on stimulation depress activity of vasomotor center.
2. Stimulation of NTS inhibits sympathetic outflow and stimulates vagal activity.

Spinal Organization

Cell bodies of autonomic efferent neurons are located in the intermediolateral column of the spinal cord.

Sympathetic Outflow

Sympathetic fibers originate from thoracic and lumbar segments (T1 to L2) of spinal cord. Hence, sympathetic system is called thoracolumbar outflow of ANS.

Parasympathetic Outflow

Spinal component of parasympathetic system originates from sacral segments (S2 to S4) of spinal cord. For its cranial and sacral origin, parasympathetic system is called craniosacral outflow of ANS.

Peripheral Organization

Ganglia

Autonomic fibers from CNS reach visceral organs in the cranial nerves and somatic nerves. They are distributed to target organs via various ganglia (Table 30.1). The ganglia of sympathetic system are close to spinal cord and the ganglia of parasympathetic system are close to the organs (Fig. 30.7). There are two sets of neurons in the efferent pathway: preganglionic and postganglionic neurons.
Preganglionic Neurons
Preganglionic neurons of sympathetic system originate from intermediolateral horn of thoracolumbar segments of spinal cord. They terminate in sympathetic chain of ganglion from where postganglionic fibers originate and innervate the viscera.
1. In sympathetic system, preganglionic neurons are shorter than postganglionic neurons. Preganglionic neurons of parasympathetic system originate from cranial nerve nuclei in brainstem and intermediolateral horn of sacral segments of spinal cord.

2. In parasympathetic system, preganglionic neurons are much longer than postganglionic neurons, as the ganglion is located either near the viscera or in the viscera.

Postganglionic Neurons
1. In sympathetic system, postganglionic fibers originate from sympathetic chain of ganglia and terminate in the viscera.
2. In parasympathetic system, postganglionic fibers are located close to or in the effector organs. Hence, postganglionic parasympathetic fibers are very small.

CHAPTER SUMMARY

Key Concepts
1. In sympathetic system, postganglionic fibers originate from sympathetic chain of ganglia close to spinal cord. Therefore, preganglionic fibers are small, whereas postganglionic fibers are long.
2. In parasympathetic system, ganglia are close to the viscera. Therefore, preganglionic fibers are long, whereas postganglionic fibers are very small.
3. Preganglionic fibers of both the division of ANS are cholinergic. Postganglionic parasympathetic is cholinergic, and postganglionic sympathetic is adrenergic.

Important to Know (Must Read)
1. In examinations, usually there will be no Long Question from this chapter.
2. Difference between spinal organization of somatic system and sympathetic system, Neurotransmitters of ANS, General organization of ANS, may come as Short Questions in exams.
3. In Viva, examiners usually ask… What the components of ANS, General organization of ANS, General functions of sympathetic and parasympathetic systems, Say the differences between spinal organization of somatic system and ANS Organization of pre-ganglionic and postganglionic fibers of sympathetic and parasympathetic systems, Location of ganglia in sympathetic and parasympathetic systems, List the neurotransmitters of sympathetic and parasympathetic systems.
Sympathetic System

CHAPTER 31

Sympathetic system is the system of energy utilization. At the time of activity, urgency, anxiety, emotion, excitement and combating stressful situations, sympathetic system is activated to provide energy to the body. Excessive and chronic stimulation of this system leads to leanness, degeneration ad decay, and underutilization of it leads to lethargy and adiposity.

FUNCTIONAL ANATOMY

Sympathetic Neurons

Preganglionic Neurons

Cell bodies of preganglionic neurons of the sympathetic division are located in the intermediolateral horn of the thoracic (T1 to T12) and upper lumbar (L1 to L3) segments of spinal cord. Hence, sympathetic division is known as thoracolumbar outflow of ANS. The preganglionic neurons come out of the spinal cord via ventral roots. After the merger of dorsal and ventral roots, spinal nerve emerges. Sympathetic preganglionic axons leave the spinal nerve via

Scientists contributed

Ernst Heinrich Weber (1795–1878)
Wilhelm Eduard Weber (1804–1891)
Eduard Friedrich Wilhelm Weber (1806–1871)

In 19th century, three Weber brothers (Ernst Heinrich Weber, Wilhelm Eduard Weber, and Eduard Friedrich Wilhelm Weber) had contributed greatly to Physiology, especially in the field of autonomous nervous system (ANS). Ernst Heinrich first made a comparative study of sympathetic nerves. With his younger brother, Eduard Friedrich measured the speed of pulse wave and correctly explained the nature of arterial pulse. Eduard Friedrich studied the hearing mechanism, Wilhelm and Eduard completed the full study of locomotion and demonstrated that vagal stimulation stops heart.


Learning Objectives

On completion of study of this chapter, the student MUST be able to:
1. Understand the importance of sympathetic system in regulation of body functions.
2. Appreciate the anatomical and functional specializations of sympathetic divisions of autonomous nervous system (ANS).
3. Trace the course of pre- and postganglionic neurons of sympathetic system.
4. Name the sympathetic ganglia and give the segmental distribution of sympathetic fibers to various visceral organs.
5. Understand the importance of basal sympathetic tone.
6. List the widespread responses to sympathetic stimulation.
7. Give the effects of sympathetic stimulation to various organs and name the receptors that mediate these effects.
8. Comprehend the features, mechanism and importance of fight-or-flight responses.
9. List the differences between sympathetic and parasympathetic systems.

The student MAY also be able to:
1. Describe the detailed widespread responses to sympathetic stimulation.
2. Describe the effects of sympathetic stimulation to various organs.
3. Explain the basis of fight-or-flight responses.
the white rami communicantes and enter the paravertebral sympathetic ganglia, which is an interconnected chain located on both sides of the vertebral column.

Preganglionic fibers have any of the following destinations (refer to Fig. 30.4, Chapter 30):

1. Preganglionic fibers emerging from spinal cord synapse with cell bodies of postganglionic neuron located in the paravertebral sympathetic ganglion at the same spinal cord level. The postganglionic sympathetic axons then travel in the cervical and lumbar-sacral spinal nerves. However, few fibers of sympathetic ganglion chain extend above and below the spinal level.

2. The chain of sympathetic ganglia extends above and below the thoracolumbar spinal levels, in which branches of preganglionic fibers ascend to the cervical levels or descend to the sacral level. Thus, preganglionic axons may synapse with postganglionic neurons in the paravertebral ganglion at the same level, or ascend up or descend down to the several spinal segments and then synapse with the postganglionic neuron (Fig. 31.1).

   - **Preganglionic axons that ascend to cervical level** arise from T1 to T5 and form three major ganglia: superior, middle, and inferior cervical ganglia.
   - **Preganglionic axons descend below L3**, form two additional lumbar and at least four sacral ganglia. Preganglionic fibers **synapse with postganglionic neurons in these sympathetic ganglia** that are present beyond the thoracolumbar segments of spinal cord.

3. The preganglionic axons may pass through the paravertebral ganglia en route without synapsing there to terminate in a prevertebral ganglion (collateral ganglion), which is located close to the organ.

### Postganglionic Neurons

Postganglionic neurons for somatic structures such as sweat glands, piloerector muscles, cutaneous blood vessels and blood vessels of skeletal muscles leave the paravertebral ganglion in the gray rami communicantes and reenter the spinal nerve to supply the target tissues.

Postganglionic neurons to head, heart and lungs originate in the cervical or upper thoracic paravertebral ganglia and proceed to the organs as separate nerves, for example the cardiac nerve to the heart, or as perivascular plexuses of axons that accompany arteries.

### Sympathetic Ganglia

Sympathetic ganglia are of **three types**: paravertebral, prevertebral, and terminal.

### Paravertebral Ganglia

There are two paravertebral chains of ganglia on either side of the spinal cord. Each chain has 22 or 23 ganglia (Fig. 31.2).

### Cervical Ganglia

There are three cervical ganglia: superior, middle, and inferior.

1. The **superior cervical ganglion** provides sympathetic fibers that innervate the structures in the head. These sympathetic fibers travel in the perivascular plexus along the carotid arteries and innervate radial muscle of the iris that causes dilation of the pupil, supply Müller’s muscle that assists in elevating the eyelid, and innervate lacrimal and salivary glands. Therefore, diseases involving this pathway produce prominent ophthalmic signs.

2. The **middle and inferior cervical ganglia** innervate structures in the chest, including the trachea, esophagus, heart and lungs. Often, inferior cervical ganglion and first cervical ganglion fuse to form **stellate ganglion**.

### Thoracic Ganglia

There are about 12 thoracic ganglia. Fibers from these ganglia supply mainly thoracic structures. Preganglionic fibers from T1 and T2 supply structures in head and neck, from T3 and T4 supply thoracic viscera, from T5 to T9 supply structures in upper limb, and from T10 to T12 supply upper abdominal viscera (Table 31.1).

### Lumbar and Sacral Ganglia

There are three lumbar ganglia for three lumbar segments. However, there are **two additional lumbar** and at
least four sacral ganglia that are present below the lumbar segments. Preganglionic fibers from T10 to L2 supply structures in lower limbs, and from L1 and L2 supply lower abdominal viscera (Table 31.1).

**Prevertebral Ganglia**

Postsynaptic neurons for the abdominal and pelvic visceral organs arise from the prevertebral ganglia. They are also called collateral ganglia. There are three major prevertebral ganglia: celiac, superior mesenteric, and inferior mesenteric (Fig. 31.2). They are so named as they overlie the celiac, superior mesenteric and inferior mesenteric arteries at their origin from the aorta respectively.

1. **Celiac ganglion**: The preganglionic axons for celiac ganglion originate in the T5 to T12 spinal levels and provide innervation to the stomach, small intestine, liver, pancreas, gallbladder, spleen and kidneys.

2. **Superior mesenteric ganglion**: The preganglionic fibers for superior mesenteric ganglion originate primarily in T10 to T12 and innervate the small and large intestines.

3. **Inferior mesenteric ganglion**: The preganglionic fibers for inferior mesenteric ganglion originate from L1 to L3 and innervate the lower part of colon, rectum, urinary bladder, and reproductive organs.

**Terminal Ganglia**

These are located in the organ innervated by sympathetic fibers. Examples are adrenal medulla, heart, pancreas, and urinary bladder.

**Adrenal Medulla**

Adrenal medulla is a neuroendocrine structure. It forms the inner core of the adrenal gland.

1. Cells of the adrenal medulla are innervated by preganglionic sympathetic fibers originating in the lower thoracic spinal segments that travel in lesser splanchnic
nerve. Therefore, adrenal medulla is considered as a modified sympathetic ganglion that contains postganglionic cells.

2. Preganglionic fibers terminate on the chromaffin cells that represent modified ganglion cells. Chromaffin cells synthesize both epinephrine and norepinephrine. However, unlike neurons, these cells have no axons though they function as neuroendocrine cells and release hormone in response to preganglionic neuron activation.

**Other Terminal Ganglia**

Some of the cells in heart, pancreas and urinary bladder are modified postganglionic cells. The postganglionic cells in heart (intrinsic cardiac adrenergic cells) influence development of heart during fetal life.

**Neurotransmitters**

All preganglionic fibers are cholinergic and sympathetic postganglionic fibers are adrenergic that secrete either noradrenaline or adrenaline (for details, refer previous chapter). However, there are few sets of postganglionic sympathetic cholinergic fibers. These are postganglionic sympathetic fibers supplying sweat glands and blood vessels of skeletal muscles. Evidences suggest that blood vessels of heart, lungs, kidney and uterus also receive some cholinergic innervation.

**Scientists contributed**

The Nobel Prize in Physiology or Medicine 1936 was awarded jointly to neurophysiologist from UK Sir Henry Hallett Dale and neurophysiologist from USA Prof. Otto Loewi “for their discoveries relating to chemical transmission of nerve impulses”. Their pioneering works were in the field of neurotransmission in ANS. HH Dale had studied the details of neurotransmitters of sympathetic nervous system and sympathetic visceral responses.

**SYMPATHETIC RESPONSES**

Sympathetic activities are broadly two types: basal level activity at rest and widespread responses following activation.

**Basal Sympathetic Activity**

The sympathetic fibers impart a basal influence on many organs they innervate. This basal rate of discharge is called basal sympathetic tone.

1. Usually, functions of many viscera can be altered by changing the basal level of sympathetic discharge to the organs. Many such changes occur during normal physiological activities. For example, change in heart rate and blood pressure in response to change in posture.
2. But, if situations warrant for greater changes, basal firing rate in sympathetic nerves can be increased or decreased profoundly to achieve the target modulation in function. For example, to achieve a target increase in cardiac output during exercise, sympathetic stimulation considerably increases heart rate and myocardial contractility.

**Widespread Sympathetic Response**

Another characteristic of sympathetic stimulation is that it produces widespread organ responses. Example of a widespread response is fight or flight reaction (see below). The widespread response to sympathetic activation is due to two fundamental properties: divergence of sympathetic outflow and activation of adrenal medulla.

**Sympathetic Divergence**

The number of postganglionic axons emerging from the paravertebral chain of ganglia is greater than the number of preganglionic neurons originating from the spinal cord, the ratio of postganglionic to preganglionic neurons being 100:1. Therefore, effector tissues innervated by sympathetic fibers are more.

1. This basic principle of divergence enables the sympathetic system to produce widespread responses by simultaneously modulating functions of many effector organs.
2. The divergence is due to branching of the preganglionic sympathetic axons in the paravertebral chain of ganglia that makes synaptic connections with multiple postganglionic neurons both above and below their original level of emergence from the spinal cord.

**Role of Adrenal Medulla**

The adrenal medulla mediates many sympathetic responses. In addition to its anatomical divergence of postganglionic neurons, sympathetic system activates hormonal mechanism to achieve its widespread responses. These are mediated by catecholamine secreted from adrenal medulla.

1. The adrenal medulla is a neuroendocrine gland, which is basically a modified sympathetic ganglion.
2. The chromaffin cells of the adrenal medulla secrete both epinephrine and norepinephrine in a ratio of about 8:1 and store them in their secretory vesicles.
3. They release hormone directly into the bloodstream in response to activation by sympathetic preganglionic fibers. Catecholamines released from adrenal medulla by sympathetic stimulation modulate many organ functions and therefore, further promote sympathetic effects.
4. Thus, adrenomedullary secretion by sympathetic activation forms the physiological basis of divergence for widespread sympathetic responses. Circulating epinephrine plays a greater role than norepinephrine in physiologically mediating widespread responses for three reasons:
1. From adrenal medulla, epinephrine secretion is considerably more than norepinephrine.
2. Norepinephrine secretion is limited only to the axon terminals of sympathetic fibers, and therefore its effects are restricted only to the postsynaptic receptors in the target tissues, whereas circulating epinephrine reaches almost all tissues of the body.
3. Epinephrine potentiates sympathetic effects with greater efficacy than norepinephrine as it is more effective in stimulating both α and β-adrenergic receptors (though in general, epinephrine is a better β receptor agonist and norepinephrine is a better α receptor agonist).

### EFFECTS OF SYMPATHETIC STIMULATION

Effects of sympathetic stimulation are summarized in Table 31.2. Effects are mediated by release of noradrenaline from sympathetic nerve endings and adrenaline from adrenal medulla.

#### Effects via Adrenergic Receptors

Catecholamines elicit their effects by acting on adrenergic receptors. Adrenergic receptors are broadly divided into two types: α and β. The α receptor has two subtypes: α₁ and α₂; and β receptor has three subtypes: β₁, β₂, and β₃. Generally, β receptors are more sensitive to adrenaline and α receptors to noradrenaline.

#### Effects of α Receptor Stimulation

**Effects of α₁ Stimulation:** The α₁ receptors are present in vascular smooth muscles of cutaneous and splanchnic circulation, sphincters of bladder and GI tract and radial muscles of iris. Stimulation of these receptors causes contraction or constriction of the structures in which they are present. They are equally sensitive to adrenaline and noradrenaline. The effects are mediated by formation of intracellular IP₃.

**Effects of α₂ Stimulation:** α₂ receptors are present in presynaptic nerve endings, wall of GI tract, platelets and adipocytes. Stimulation of these receptors often causes relaxation or inhibition of the structure. The effects are mediated by decreased formation of intracellular cAMP.

#### Effects of β Receptor Stimulation

**Effects of β₁ Stimulation:** β₁ receptors are present in SA node, AV node and ventricular muscle. Stimulation of these receptors causes excitation of these structures. They are more sensitive to adrenaline than noradrenaline. The effects are mediated by increased formation of intracellular cAMP.

**Effects of β₂ Stimulation:** β₂ receptors are present in blood vessels of skeletal muscles, bronchial smooth muscles

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Table 31.2: Effects of sympathetic and parasympathetic stimulation.

<table>
<thead>
<tr>
<th>Effector organ</th>
<th>Sympathetic</th>
<th>Parasympathetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilator pupillae</td>
<td>Contraction (α₁) (dilation of pupil)</td>
<td>–</td>
</tr>
<tr>
<td>Sphincter pupillae</td>
<td>–</td>
<td>Contraction (constriction of pupil)</td>
</tr>
<tr>
<td>Ciliary muscle</td>
<td>Relaxation (β₂)</td>
<td>Contraction</td>
</tr>
<tr>
<td>Muller’s muscle</td>
<td>Contraction (α₁)</td>
<td>–</td>
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<tr>
<td><strong>Glands</strong></td>
<td></td>
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<tr>
<td>Lacrimal gland</td>
<td>–</td>
<td>↑ secretion</td>
</tr>
<tr>
<td>Nasal glands</td>
<td>↓ secretion (α₁)</td>
<td>↑ secretion</td>
</tr>
<tr>
<td>Salivary glands</td>
<td>Constriction (α₁, α₂)</td>
<td>Dilation</td>
</tr>
<tr>
<td><strong>Heart</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SA node</td>
<td>↑ heart rate (β₁, β₂)</td>
<td>↓ heart rate</td>
</tr>
<tr>
<td>AV node</td>
<td>↑ conductivity (β₁, β₂)</td>
<td>↓ conductivity</td>
</tr>
<tr>
<td>Conduction paths</td>
<td>↑ conductivity (β₁, β₂)</td>
<td>↓ conductivity</td>
</tr>
<tr>
<td>Atria</td>
<td>↑ contractility (β₁, β₂)</td>
<td>↓ contractility</td>
</tr>
<tr>
<td>Ventricles</td>
<td>↑ contractility (β₁, β₂)</td>
<td>↓ contractility</td>
</tr>
<tr>
<td><strong>Blood vessels</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cutaneous</td>
<td>Constriction (α₁)</td>
<td>No supply</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>Dilation (β₂)</td>
<td>No supply</td>
</tr>
<tr>
<td></td>
<td>Constriction (α)</td>
<td></td>
</tr>
<tr>
<td>Visceral</td>
<td>Constriction (α₁)</td>
<td>No supply</td>
</tr>
<tr>
<td>Coronary</td>
<td>Dilation (β₂)</td>
<td></td>
</tr>
<tr>
<td>Constriction (α₁, α₂)</td>
<td>Dilation</td>
<td></td>
</tr>
<tr>
<td>Cerebral</td>
<td>Constriction (α₁)</td>
<td>Dilation</td>
</tr>
<tr>
<td>Pulmonary</td>
<td>Constriction (α₁)</td>
<td>Dilation (β₂)</td>
</tr>
<tr>
<td></td>
<td>Dilation</td>
<td></td>
</tr>
<tr>
<td>Renal</td>
<td>Constriction (α₁, α₂)</td>
<td>Dilation (β₁, β₂)</td>
</tr>
<tr>
<td></td>
<td>No supply</td>
<td></td>
</tr>
<tr>
<td>Systemic veins</td>
<td>Constriction (α₁, α₂)</td>
<td>Dilation (β₁, β₂)</td>
</tr>
<tr>
<td></td>
<td>No supply</td>
<td></td>
</tr>
<tr>
<td><strong>Lungs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bronchioles</td>
<td>Dilation (β₂)</td>
<td>Constriction</td>
</tr>
<tr>
<td>Glands</td>
<td>↑ secretion (β₂)</td>
<td>↓ secretion (α₁)</td>
</tr>
<tr>
<td><strong>Skin</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweat glands</td>
<td>Less secretion</td>
<td>More secretion</td>
</tr>
<tr>
<td>Pilomotor muscle</td>
<td>Contraction (α₁)</td>
<td>No supply</td>
</tr>
<tr>
<td><strong>GI tract</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Motility and tone</td>
<td>Decrease (α₁, β₁, β₂)</td>
<td>Increase</td>
</tr>
<tr>
<td>Sphincter</td>
<td>Contraction (α₁)</td>
<td>Relaxation</td>
</tr>
<tr>
<td>Secretion</td>
<td>Inhibition (α₂)</td>
<td>Stimulation</td>
</tr>
<tr>
<td>Gallbladder</td>
<td>Relaxation (β₂)</td>
<td>Contraction</td>
</tr>
<tr>
<td>Liver</td>
<td>Glycogenolysis and gluconeogenesis(α₁, β₂)</td>
<td>–</td>
</tr>
</tbody>
</table>

Contd…
and wall of GI tract. Stimulation of these receptors causes relaxation of these structures. They are more sensitive to adrenaline than noradrenaline. The effects are mediated by change in the level of intracellular cAMP.

**Effects of β3 Stimulation:** β3 receptors are present in adipose tissues. Stimulation of these receptors causes lipolysis. The effect is mediated by increase in the level of intracellular cAMP.

**Fight-or-Flight Response**

The fight-or-flight response is a typical widespread response of sympathetic activation. This occurs in critical situations of life when one has to either fight the situation or flee from the situation. Though many components of response are due to direct effects of sympathetic stimulation, secretion of catecholamine from adrenal medulla contributes considerably. The effects are as follows:

1. **Sympathetic stimulation of CVS increases blood pressure** due to increased cardiac output and vasoconstriction. Also, redistribution of the blood flow occurs to skeletal muscles and heart from splanchnic and cutaneous territories so that performance enhances.
2. In lungs, **increased exchange of blood gases** occurs due to stimulation of the respiratory rate and dilation of bronchiolar tree. This increases supply of oxygen to the tissues.
3. Sympathetic stimulation to salivary gland **decreases salivary secretion.** However, secretion of mucus increases proportionately, permitting lubrication of the mouth despite increased ventilation and reduced salivation.
4. **Supply of metabolic substrates increases,** which is an essential component of effective stress reaction. The demand for increased supply of substrates like glucose and fatty acids is met by the actions of circulating epinephrine on hepatocytes and adipocytes. **Glycogenolysis** increases plasma glucose concentration and lipolysis promotes plasma free fatty acid level.
5. **Sympathetic stimulation to sweat glands** causes secretion of a watery fluid, and evaporation of body heat. **Cutaneous vasoconstriction** with concurrent sweat gland activation causes cold, clammy skin of a frightened individual.
6. **Activation of piloerector muscles** of hair follicles causes hair-standing-on the skin. The hair erection helps in preservation of body temperature or gives a ferocious appearance to threaten the enemy.
7. **Pupillary dilation enhances visual acuity** and perception to make the individual environmentally maximal alert.
8. **Stimulation of brainstem reticular system** makes the individual maximally alert and mentally conscious to take appropriate decisions in quick successions.
9. Activity of **bowel and bladder temporarily ceases** due to constriction of sphincters.

### CHAPTER SUMMARY

**Key Concepts**

1. In sympathetic system (thoracolumbar outflow), preganglionic fibers in the paravertebral chain can ascend up or descend down to terminate in the postganglionic neurons of many segments in spinal cord, which can activate many visceral structures.
2. Sympathetic activation results in widespread sympathetic response that occurs due to divergence (more postganglionic fibers than preganglionic fibers), and secretion of catecholamines from adrenal medulla.
3. Generally, β receptors are more sensitive to adrenaline and α receptors to noradrenaline.

**Important to Know (Must Read)**

1. In examinations, usually there will be no Long Question from this chapter.
2. Sympathetic ganglia, Sympathetic neurons, Sympathetic neurotransmitters, Effects of sympathetic stimulation, Fight-or-flight response, may come as Short Questions in exams.
3. In Viva, examiners usually ask…Organization of sympathetic system, How preganglionic fibers come out of spinal cord, Sympathetic pre- and postganglionic neurons, Name the sympathetic ganglia, Specialities of sympathetic responses, Effects of sympathetic stimulation, Sympathetic neurotransmitters, Receptors for sympathetic neurotransmitters, Details of effects of sympathetic stimulation on each organ, What is Fight-or-flight response, and its features.
The parasympathetic system is the system of energy restoration. This system works when the body is at rest. It helps in generating and restoring the energy and recovering the body from energy loss. Thus, this helps in repair and renovation of the body systems. In general, parasympathetic activity is synonymous with the vagal activity, as vagus nerve controls most of the visceral functions.

Structural Organization

The parasympathetic system is the craniosacral outflow of autonomic nervous system.

1. The cranial component emanates from the brainstem, and the sacral component originates from intermediolateral gray column of sacral segments of spinal cord.

2. In contrast to the stimulation of sympathetic system that causes widespread responses, parasympathetic activation causes localized responses (Table 32.1). The divergence of parasympathetic outflow is minimal. The divergence ratio of the presynaptic output to postsynaptic output is about 1:15, in contrast to 1:100 in sympathetic system.

3. There is no circulating hormonal mechanism to aid to the divergence of parasympathetic activation. Hence, stimulation of parasympathetic nerve to an organ-system results in a limited activation of the concerned structures. For example, vagal stimulation to heart slows the heart rate without significantly altering the vagal influence on stomach.

Parasympathetic ganglia are located either close to the organ or embedded in the organ. Therefore, preganglionic neurons are much longer than postganglionic neurons. The differences between sympathetic and parasympathetic systems are summarized in Table 32.1.

Cranial Component

Cell bodies of preganglionic neurons of cranial component of parasympathetic system are located in the brainstem. Hence, this component is also called brainstem parasympathetic division. Brainstem parasympathetic neurons innervate structures in the head, neck, thorax and abdomen. Parasympathetic axons from brainstem travel in III, VII, IX, and X cranial nerves (Fig. 32.1). Nuclei of these cranial nerves are present in the midbrain in tegmentum, pons and medulla. Therefore, these nuclei serve as the centers for the integration of autonomic reflexes for the organ systems they innervate.
### Table 32.1: Differences between sympathetic and parasympathetic systems.

<table>
<thead>
<tr>
<th><strong>A. General Features</strong></th>
<th><strong>Sympathetic system</strong></th>
<th><strong>Parasympathetic system</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Origin</strong></td>
<td>Thoracolumbar outflow (cell bodies of preganglionic fibers are located in IML horn of thoracic and lumbar segments of spinal cord).</td>
<td>Craniosacral outflow (cell bodies of preganglionic fibers are located in brainstem cranial nerve nuclei and sacral segments of spinal cord).</td>
</tr>
<tr>
<td><strong>Preganglionic fibers</strong></td>
<td>Short and cholinergic, along with ventral nerve roots of thoracolumbar nerves</td>
<td>Long and cholinergic, along with cranial nerves III, VII, IX, X and ventral nerve roots of sacral nerves</td>
</tr>
<tr>
<td><strong>Ganglia</strong></td>
<td>Located close to spinal cord, forms chain of paravertebral ganglia.</td>
<td>Located close to the target organ or in the organ</td>
</tr>
<tr>
<td><strong>Postganglionic fibers</strong></td>
<td>Long, unmyelinated and adrenergic, along spinal nerves, blood vessels and visceral branches of paravertebral chain</td>
<td>Short, myelinated and cholinergic, through branches of trigeminal in head region; and direct ganglionated branches</td>
</tr>
<tr>
<td><strong>Ratio of pre to postganglionic fibers</strong></td>
<td>1:100</td>
<td>1:1 to 1:15</td>
</tr>
<tr>
<td><strong>Organs innervated</strong></td>
<td>Almost all organs of the body</td>
<td>Mainly visceral organs</td>
</tr>
<tr>
<td><strong>Responses</strong></td>
<td>Widespread</td>
<td>Localized</td>
</tr>
<tr>
<td><strong>Impact on body energy</strong></td>
<td>Utilizes energy</td>
<td>Restores energy</td>
</tr>
<tr>
<td><strong>Highest modulators</strong></td>
<td>Limbic region</td>
<td>Limbic region</td>
</tr>
<tr>
<td><strong>Hypothalamus</strong></td>
<td>Caudal</td>
<td>Rostral</td>
</tr>
<tr>
<td><strong>Brainstem control</strong></td>
<td>Reticular formation</td>
<td>Reticular formation</td>
</tr>
<tr>
<td><strong>Supraspinal fibers</strong></td>
<td>Hypothalamus fibers</td>
<td>Dorsal longitudinal fasciculate and hypothalamospinal fibers</td>
</tr>
<tr>
<td><strong>Preganglionic neurons (connector neurons)</strong></td>
<td>Intermediolateral grey column of T₁ to T₁₂</td>
<td>General visceral efferent nuclei and intermediolateral grey column of cranial nerves III, VII, IX, X and S₂ to S₄.</td>
</tr>
<tr>
<td><strong>Myelination of preganglionic fibers</strong></td>
<td>Myelinated (white ramus communicans)</td>
<td>Myelinated</td>
</tr>
<tr>
<td><strong>Length of preganglionic fibers</strong></td>
<td>Relatively short</td>
<td>Relatively long</td>
</tr>
<tr>
<td><strong>Preganglionic neuron terminal (and receptor) /neurotransmitter</strong></td>
<td>Acetylcholine (nicotinic receptor)</td>
<td>Acetylcholine (nicotinic receptor)</td>
</tr>
<tr>
<td><strong>Ganglia of relay effector neuron</strong></td>
<td>Paravertebral and plexus along the abdominal aorta and internal iliac artery</td>
<td>Ciliary, pterygopalati submandibular, otic, cardio-pulmonary plexus and in the wall of the viscera</td>
</tr>
<tr>
<td><strong>Ratio of preganglionic fibers to neurons of ganglia</strong></td>
<td>One is many (therefore mass discharge)</td>
<td>One is to a few (therefore localized effect)</td>
</tr>
<tr>
<td><strong>Myelination of postganglionic fiber</strong></td>
<td>Unmyelinated</td>
<td>Unmyelinated</td>
</tr>
<tr>
<td><strong>Length of postganglionic fiber</strong></td>
<td>Relatively long</td>
<td>Relatively short</td>
</tr>
<tr>
<td><strong>Preganglionic neuron terminal (and receptor) /neurotransmitter</strong></td>
<td>Noradrenaline (α and β adrenergic receptor) and acetylcholine (muscarinic receptor to sweat gland and some blood vessels to skeletal muscle)</td>
<td>Acetylcholine (muscarinic receptor)</td>
</tr>
<tr>
<td><strong>Effect</strong></td>
<td>Response as in ‘fright-flight-fight’ response</td>
<td>Responsible for homeostasis</td>
</tr>
<tr>
<td><strong>Metabolism</strong></td>
<td>Catabolic</td>
<td>Anabolic</td>
</tr>
</tbody>
</table>

### B. Effects of organ stimulation

| **Eye**                  | Dilatation of pupils and contraction of orbitalis and smooth muscles of tarsals | Constriction of pupils and ciliary muscle for accommodation |
| **Lacrimal gland**       | ------- | Secretion |
| **Salivary glands**      | Thick, viscous secretion | Profuse, watery secretion |
| **Heart**                | Increases heart rate, increases contractility | Decreases heart rate, decreases Contractility |
| **Lung**                 | Bronchial smooth muscle relaxation | Bronchial smooth muscle relaxation |
| **Gastrointestinal tract** | Decreases motility, contraction of sphincters and inhibition of secretion | Increases motility, relaxation of sphincters and stimulation of secretion |
| **Urinary bladder**      | Relaxation of detrusor and contraction of involuntary sphincter vesicae | Contraction of detrusor and relaxation of involuntary sphincter vesicae |
| **Male sex organs**      | Ejaculation | Erection |
| **Skin**                 | Contraction of erector pili and secretion of sweat glands | ------- |
| **Blood vessels**        | Vasoconstriction, dilation in some vessels | |
**Cranial Nerve III**

Cranial nerve III is the oculomotor nerve that originates from tectum of the midbrain. The cell bodies are located in the Edinger-Westphal nucleus.

1. The preganglionic axons travel in the III cranial nerve to terminate on the cell bodies of postganglionic neuron in ciliary ganglion, which is located inside the orbit of the eye.
2. The postganglionic axons enter eyeball near the optic nerve and innervate sphincter muscle of the iris (sphincter pupillae) that control the diameter of the pupil, the ciliary muscle that focuses the lens for accommodation for near vision, and the choroidal blood vessels.
3. The postganglionic fibers project mainly to the iris muscles. About 90% of fibers innervate ciliary muscle, 3 to 5% innervate sphincter pupillae and rest of the fibers terminates on blood vessels of choroid. Optic nerve projects to tectum and provides input for ocular reflexes.

**Cranial Nerve VII**

The preganglionic fibers in facial nerve emerge from the superior salivary nuclei in the rostral medulla and then pass from the facial nerve into the greater superficial petrosal nerve to synapse in the pterygopalatine ganglion.

1. The postganglionic fibers innervate the lacrimal gland and the glands of nasal and palatal mucosa. Another set of preganglionic fibers in the facial nerve travel via the chorda tympani nerve to synapse in the submandibular ganglion.
2. These postganglionic fibers from submandibular ganglion innervate submandibular and sublingual glands. Stimulation of this nerve increases saliva production.

**Cranial Nerve IX**

The preganglionic axons of the glossopharyngeal nerve originate form the inferior salivary nuclei in the medulla. The fibers take a tortuous course through the lesser petrosal nerve to terminate in otic ganglion.
1. The postganglionic axons join the auriculotemporal branch of 5th cranial nerve to innervate parotid gland, where they facilitate secretion of saliva.

2. Chemosensory information of blood gasses from carotid bodies and baroreceptor information of blood pressure from carotid sinuses are transmitted to the medullary cardiovascular centers via glossopharyngeal afferents.

Cranial Nerve X

Vagus nerve arises from the nucleus ambiguous and dorsal motor nucleus of vagus in the medulla. This nerve has an extensive autonomic component. It has been estimated that vagal output comprises up to 75% of total parasympathetic activity.

1. Preganglionic fibers travel in the vagus nerve to ganglia located in the organ, i.e. in the heart and lungs and in the intrinsic plexuses of the GI tract. Sympathetic postganglionic fibers intermingle and travel in the same nerve trunk with the parasympathetic preganglionic fibers to the target tissues. This forms the vago-sympathetic trunk.

2. The right vagus nerve has more influence on SA node and left vagus on AV node. Thus, vagal stimulation slows the heart rate.

3. The vagal efferents to the lung control bronchial smooth muscle (constriction of bronchioles), and secretory cell activity.

4. Vagal fibers innervate esophagus, stomach, small intestine and large intestine up to two-third of transverse colon.

5. On GI tract, vagus nerve stimulates motility and secretory functions. Acetylcholine and VIP are the transmitters of the postganglionic neurons.

6. There are vagal innervations of kidneys, liver, spleen and pancreas.

Sacral Component

Sacral parasympathetic neurons innervate structures in the pelvis.

1. Preganglionic fibers originate in the intermediolateral gray column of the sacral segments S₂, S₃, and S₄ of spinal cord.

2. The preganglionic fibers terminate in ganglia in or near the viscera that include descending colon, sigmoid colon, rectum, internal anal sphincter, urinary bladder and the reproductive organs.

PARASYMPATHETIC FUNCTIONS

Parasympathetic system restores body’s energy reserve. Favorable conditions in both external and internal environments herald parasympathetic activation. Except on cardiovascular system, most parasympathetic effects are stimulatory, especially for the processes that facilitate energy storage and growth. They stimulate intestinal motility, secretion, digestion and absorption. They promote reproductive functions (Application Box 32.1).

<table>
<thead>
<tr>
<th>Application Box 32.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adequate parasympathetic tone ensures good health: Parasympathetic activity is more in resting conditions and sympathetic activity is more in active conditions. The energy lost during activities must be restored by adequate rest that stimulates parasympathetic system. It is not only the physical rest, but also the mental rest that promotes vagal activity. Therefore, both mental and physical relaxations as occurs by regular practice of yoga are advised for improving health in general, apart from achieving stable cardiac functions and blood pressure. People having hypersympathetic personality (always anxious and restless) are usually thin and do not maintain good health, and in the contrary, individuals with hyperparasympathetic personality (lazy and lethargic) develop obesity. Therefore, a balanced sympathetic and parasympathetic state (stable sympathovagal balance) is required to preserve good health.</td>
</tr>
</tbody>
</table>

Neurotransmitters and Receptors

Most of the parasympathetic fibers are cholinergic. Acetylcholine is the neurotransmitter in both pre- and post-ganglionic fibers. There are two sets of cholinergic receptors on the target cells: muscarinic and nicotinic.

Muscarinic Receptors

Cholinergic muscarinic receptors are present in heart, smooth muscles and glands. These receptors are activated by acetylcholine and muscarine.

1. Activation of these receptors produce inhibitory effects on heart, for example, decreased heart rate, and excitatory effects on smooth muscle and glands, for example, increased GI motility and secretion etc.

2. Effects are mediated by decreased cAMP formation in the cytosol of cardiac cells and direct opening of K⁺ channels in nodal tissues of heart.

3. In smooth muscles and glandular tissues, effects are mediated by intracellular IP₃ and Ca²⁺. Muscarinic receptors are blocked by atropine.

Nicotinic Receptors

Cholinergic nicotinic receptors are present in autonomic ganglia (both sympathetic and parasympathetic), neuromuscular junctions and adrenal medulla. These receptors are activated by acetylcholine and nicotine.

1. Activation of these receptors produces excitatory effects on target tissue. Effects are mediated by direct binding of acetylcholine to α subunits of the receptors.

2. Receptors also contain Na⁺ and K⁺ channels.

3. Nicotinic effects are blocked by ganglion blockers such as hexamethonium that prevent action of acetylcholine in the ganglia and at neuromuscular junction by curare drugs.
Chapter 32: Parasympathetic System

CHAPTER SUMMARY

**Key Concepts**

1. In parasympathetic system (craniosacral outflow), preganglionic fibers are very long as ganglions are located close to the organs.
2. Parasympathetic activation is considered to be similar with vagal activation, as 75% of parasympathetic functions are mediated by vagus nerve.
3. Greater vagal tone is essential for good health.

**Important to Know (Must Read)**

1. In examinations, usually there will be no Long Question from this chapter.
2. Parasympathetic ganglia, Parasympathetic cranial nerves, Parasympathetic neurotransmitters and receptors, Effects of parasympathetic stimulation, Parasympathetic functions, Vagal tone, may come as Short Questions in exams.
3. In Viva, examiners may ask… Name parasympathetic ganglia, Name parasympathetic cranial nerves, Parasympathetic neurotransmitters and receptors, Effects of parasympathetic stimulation on different organs, especially on CVS, Parasympathetic functions, Importance of vagal tone, and the differences between sympathetic and parasympathetic systems.
LEARNING OBJECTIVES

On completion of study of this chapter, the student MUST be able to:
1. List the control mechanisms of autonomic functions.
2. Understand the importance of regulation of autonomic functions.
3. Classify autonomic disorders.
4. Name the features of common autonomic disorders.

The student MAY also be able to:
1. Describe the regulation of autonomic functions.

CONTROL OF AUTONOMIC FUNCTIONS

Visceral organs are innervated by sympathetic and parasympathetic divisions of autonomic nervous system (ANS). Effects of stimulation of sympathetic and parasympathetic systems are usually opposite. But due to robust regulatory mechanisms, the process that activates one division of ANS usually inhibits and moderates the function of the other. Therefore, sympathetic and parasympathetic divisions are also reciprocal to each other. Consequently, under normal circumstances excessive stimulation or activation of both the systems, which might have deleterious effects, is avoided. Autonomic functions are regulated by various reflexes and supraspinal mechanisms.

Reflex Regulation

Previously, ANS was regarded as an efferent system, and the sensory neurons (afferents) that innervate autonomic structures were not considered as part of ANS. However, afferent fibers also constitute an important component of autonomic system and sensory input from visceral structures is part of autonomic organization.

1. The sensory innervation to the visceral organs including blood vessels and cutaneous structures forms the afferent limb of autonomic reflexes. The information from visceral organs reach the spinal cord through afferent fibers and, from there, the second order of neurons convey information to the higher centers via ascending pathways (Fig. 33.1).
2. Sensory information in the afferent pathways may not always reach higher center for finer integration and
conscious perception, but definitely reaches different levels of autonomic neuraxis up to the level of subcortical structures, especially the thalamus (Figs. 33.2A and B).

3. Thus, ANS integrates a hierarchy of reflexes to control organ functions. According to their level of integration, the autonomic reflexes are subjected to control by various part of CNS.

**Local Reflexes**

A sensory neuron (afferent autonomic fiber) has many terminal branches peripherally.

1. As a result, sensory action potential that propagates orthodromically in one of the afferent branch to CNS may also enter a branch of the same axon and then conduct antidromically to release neurotransmitters at the sensory terminals that end on other structure.

2. The process results in spreading initial reaction produced by the stimulus.

3. For example, when the branch of a sensory neuron innervates blood vessels, the response is the spread of reddening of skin as a result of arteriolar dilation (refer to the ‘Axon Reflex’ in ‘Cutaneous circulation’ in CVS), or if sensory neuron innervates sweat glands, the response will be local sweating as a result of sweat gland activation. This process is called ‘Local axon reflex’.

4. Persistent activation of such reflexes in nociceptive afferents following trauma can produce dramatic features.

---

**Role of Autonomic Ganglia**

Ganglia in ANS serves as relay stations for preganglionic and postganglionic neurons. Recent evidences suggest that synaptic activity in the ganglia may influence final efferent output.

1. Inputs arriving from many preganglionic neurons alter the activity in ganglia, which in turn alter the visceral activity. The best example is the regulation of GI functions by ganglia of GI tract (Fig. 33.3). Chemoreceptors
and mechanoreceptors in the gut generate afferent action potentials that pass to the spinal cord and then from there to the celiac and mesenteric ganglia. The output from these ganglia changes the GI motility and secretion required during digestion.

2. Also, ganglia serve as integrative centers for autonomic reflexes. The example for such integrative functions of ganglia is the parasympathetic ganglia in the wall of GI tract that finally control GI motility and secretion required during digestion.

3. The intrinsic plexuses in the wall of GI tract serve as the centers for integration of local or short reflex activities where input from parasympathetic preganglionic neurons, postganglionic sympathetic neurons and local sensory neurons converge and interact. The intrinsic plexuses also mediate the central or long gastrointestinal reflexes.

**Spinal Autonomic Reflexes**

Many autonomic reflexes are integrated in the spinal cord. The examples are micturition, defecation and sexual reflexes. These reflexes are coordinated by centers in the lumbar and sacral spinal cord.

1. Micturition reflex causes emptying of urinary bladder, defecation reflex causes emptying of rectum, and sexual responses in male cause erection and ejaculation and in female produce vaginal lubrication (Fig. 33.4). Sensory input from the wall of the bladder and bowel inform the degree of distension of these hollow viscera.

2. Coordination between sympathetic and parasympathetic systems is required for many of these responses.

3. Higher centers usually inhibit spinal cord reflex centers.

4. Therefore, following spinal injury, or in spinal preparation in animals, micturition first becomes involuntary and later becomes abnormal. Episodes of hypertension and piloerection in such patients are other examples.

**Supraspinal Regulation**

**Role of Brainstem**

Brainstem plays important role in autonomic functions.

1. The periaqueductal gray in midbrain coordinates autonomic responses to painful stimuli and induces endogenous analgesia system.

2. The nucleus parabrachialis in pons contribute to respiratory and cardiovascular control. The locus ceruleus influences micturition reflexes.

3. The medullary centers are the key areas for control of many visceral functions.

4. The nucleus tractus solitarius receives sensory information from cardiovascular, respiratory and gastrointestinal receptors. Vagal efferent fibers arise from this area in the medulla.

5. The vasomotor center in the ventrolateral medulla is the key center for sympathetic output as it directly controls preganglionic sympathetic neurons in the spinal cord.

Major autonomic reflexes integrated in the brainstem include:

1. Pupillary light reflex
2. Accommodation reflex
3. Reflex salivation
4. Reflex lacrimation
5. Deglutition reflex
6. Vomiting reflex
7. Reflexes for regulation of heart rate and blood pressure
8. Reflexes for regulation of respiration.

**Role of Hypothalamus and Cortex**

Hypothalamus and cortex provide the highest levels of autonomic control.

1. The periventricular hypothalamus, and medial and lateral hypothalamic areas control homeostatic functions such as thermoregulation, appetite behaviors etc.

2. Stimulation of lateral and posterior hypothalamus causes sympathetic activation, and stimulation of posterior hypothalamus causes parasympathetic activation.

3. Hypothalamus controls circadian rhythms that influence many autonomic functions.

4. Hypothalamus also contributes to the regulation of blood pressure and blood volume.

5. For its major role in autonomic functions, hypothalamus was designated (by Charles Sherrington) in the past as ‘head ganglion of the ANS’.
6. Hypothalamus, due to its close proximity and reciprocal interaction with limbic system, is influenced by limbic activities. Therefore, autonomic functions are easily affected by limbic functions and dysfunctions.
7. The **prefrontal cortex** is involved in the regulation of autonomic function.
8. The **amygdala** coordinates the autonomic components of emotional responses.
9. Cortex, limbic structures, brainstem and their spinal connections for control of autonomic functions are collectively referred to as **central network for autonomic control**.

### AUTONOMIC DYSFUNCTIONS

Following are the two fundamental types of lesion of autonomic nervous system
1. Diseases
2. Injury

#### Autonomic Diseases

Autonomic disorders are broadly termed as **autonomic failures**. Autonomic failures are of two types: primary and secondary.

**Primary Autonomic Failure**

Primary autonomic failure is **idiopathic autonomic dysfunction** that invariably manifests as **orthostatic hypotension**.

**Secondary Autonomic Failure**

Secondary autonomic failure occurs in various diseases.
1. Commonly seen in **diabetes**, amyloidosis, beriberi, syringomyelia, tabes dorsalis and subacute combined degeneration of spinal cord.
2. It also occurs in patients receiving **sympatholytic drugs** of those who have undergone surgical sympatheticomasty.
3. Autonomic imbalance also occurs in patients with **prolonged bed rest**.

#### Features of Autonomic Dysfunctions

Autonomic dysfunctions manifest as multiple organ dysfunctions as autonomic nerves innervate many visceral organs.

- **Cardiovascular features**: Usual manifestation is **orthostatic hypotension**. Tachycardia may also be seen.
- **Gastrointestinal features**: Either **constipation or diarrhea** can occur. Dysphagia is not uncommon. Dryness of mouth occurs frequently.
- **Renal features**: Increased **frequency of micturition** is the usual feature. Nocturia and incontinence may occur.
- **Reproductive features**: **Erectile dysfunction** is common in male. **Premature ejaculation** is also common. In females, decreased vaginal lubrication during sexual act is common.

**Sudomotor features**: **Anhydrosis** is common. Heat intolerance and hyperhidrosis may occur.

**Neurological symptoms**: Weakness, lethargy and giddiness.

#### Treatment

Treatment should start immediately for the cause of the disease, if it is of secondary variety of autonomic failure. Treatment otherwise is mostly symptomatic depending on the nature of the failure.

### Diseases affecting Sympathetic System

**Horner’s Syndrome**

This is a syndrome (described by Johan Horner) of autonomic dysfunction, characterized mainly by:

1. Presence of **ptosis** (drooping of the upper eyelid)
2. **Miosis** (constriction of pupil)
3. **Facial anhydrosis** (lack of sweating on the affected side of face and neck).

#### Etiology

Horner’s syndrome is a condition of **oculo-sympathetic paralysis**. Horner’s syndrome commonly occurs in malignancy of cervical lymph nodes that presses on cervical sympathetic chain, and **Pancoast tumor of lungs**.

#### Pathophysiology

Clinical manifestations of this syndrome occur due to interruption of sympathetic nerve supply to the head and neck.

1. Venter (connector neurons) for the sympathetic outflow to head and neck lies in lateral horn cells of first thoracic segment of spinal gray matter. Proximally, it gets supraspinal control through reticulospinal tract descending from brainstem reticular formation.
2. Preganglionic sympathetic fibres for head and neck arising from 1st thoracic segment ascend through cervical part of sympathetic chain.
3. After relay in cervical sympathetic ganglia, postganglionic fibres for head and neck arising from 1st thoracic segment ascend through cervical part of sympathetic chain.

A patient may suffer from Horner syndrome due to lesion of anyone of following three levels of sympathetic pathway for head and neck.
Physiological Basis of Clinical Features

Ptosis occurs due to paralysis of Muller’s muscle, and miosis occurs due to paralysis of dilator pupillae. Anhidrosis is due to decreased sympathetic activity.

1. **Miosis**: Constriction of pupil due to unopposed action of sphincter pupillae for non-functioning dilator pupillae.
2. **Ptosis**: Partial dropping of upper eyelid due to paralysis of levator palpebrae superioris.
3. **Anhidrosis**: Dryness of one half of the face with head and neck due to impaired secretion of sweat gland.
4. **Flushing or blanching of same half of face** due to loss of vasoconstrictor effect on skin.

<table>
<thead>
<tr>
<th>Scientist contributed</th>
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<tr>
<td><strong>Horner’s syndrome</strong>, a disorder of the sympathetic nervous system, was named after Johann Friedrich Horner, a 19th century Swiss ophthalmologist following his description of the condition in 1869. His name is also associated with “Horner’s muscle”, the lacrimal portion of the orbicularis oculi muscle that is sometimes referred to as the tensor tarsi muscle. With Alexis Trantas (1867–1960), the “Horner-Trantas spots” are named, being defined as small whitish-yellow chalky concretions of the conjunctiva around the corneal limbus.</td>
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Raynaud Disease

It is a vasospastic disease due to hyperactivity of vasoconstrictor sympathetic fibres affecting digital arteries of fingers. It is a bilateral disorder which is precipitated by exposure to cold and smoking. In case of smokers, nicotine aggravates vasospasm. Clinical manifestations are pain, pallor and cyanosis due to impaired vascular supply. Fingertips show black discoloration with formation of dry gangrene.

Buerger Disease

It is arterial occlusive disease of lower limb. Ischemia of muscle of leg causes pain due to muscular cramps intermittently. That is why the disorder is named as intermittent claudication.

Diseases affecting Parasympathetic System

1. **Argyll Robertson pupil**: It is a disorder in a patient of neurosyphilis due to lesion of pretectal nucleus of midbrain which is one of the cell stations in light reflex pathway. The disease is characterized by narrow pupil with no reaction to light due to interruption of light reflex pathway which is as follows:

   | Due to degeneration of diseases like |
   | Multiple myeloma, Syringomyelia |
   | Penetrating injury at root of neck |
   | Traction by cervical rib |

   - **Retina — optic nerve — optic chiasma — optic tract — lateral geniculate body — superior brachium — pretectal nucleus — Edinger-Westphal nucleus — ciliary ganglion — short ciliary nerve — sphincter pupillae.**

   In case of Argyll Robertson pupil, accommodation reflex is not disrupted as it is not passing through pretectal nucleus and its pathway id as follows.

   - **Retina — optic nerve — optic chiasma — optic tract — lateral geniculate body — optic radiation — primary visual cortex — superior — longitudinal fasciculus frontal eye lid — corticonuclear tract — occulomotor nucleus — (somatic as well as visceral efferent) — occulomotor nerve to supply medial rectus, sphincter pupillae and ciliaris for accommodation.**

   A simple formula mentioned below may be helpful to remember manifestation of Argyll Robertson pupil.

   - ARP (Pupillary reflex absent)
   - PRA (Pupillary reflex present)

2. **Adie tonic pupil**: This is a syndrome characterized by following clinical presentations.
   i. Diminished or absent light reflex due to disorder of function sphincter pupillae.
   ii. Slow or delayed dilation of pupil in the dark.
   iii. Slow or delayed accommodation to near vision because of ciliary muscle which is responsible for increase of curvature of lens.

   All the above features are supposed to be due to suppression of parasympathetic ocular function.

3. **Frey syndrome**: It is a clinical condition that is found to occur following healing of a penetrating wound of face over parotid gland. During healing process, injured nerves of this area of face communicate with one another, as done by auriculotemporal nerve supplying parasympathetic postganglionic secretomotor fibers to parotid gland with great auricular nerve supplying sweat glands of this area of face. So stimulation of salivary secretion during mastication of food causes sweating of area of face supplied by great auricular nerve.

4. **Hirschsprung disease**: This disease is also called ‘megalocolon’. It is a congenital disease characterized by failure of development of Auerbach (myenteric) plexus with absence of postganglionic parasympathetic neurons in the wall of distal part of colon. So, this part of colon does not show peristaltic activity.
for which part of the colon proximal to it presents huge dilation with stagnant fecal matter.

**Injuries to Autonomic Nervous System**

**Injury to Parasympathetic System**

It may be cranial or spinal. Causes of damage to the cranial component of parasympathetic system are head injury. Head injury may cause impairment of function of following components of parasympathetic system.

**Occulomotor Nerve Injury**

It is affected when head injury is associated with herniation of uncus of temporal lobe. Visceral afferent fibers of the nerve supply of sphincter papillae and ciliary muscles. So, damage of the nerve causes loss of light reflex with dilation of pupil due to non-functioning of sphincter papillae. Accommodation reflex is also affected due to non-functioning of ciliary muscle along with medial rectus and sphincter papillae.

**Facial Nerve Injury**

Facial nerve containing visceral efferent fibers with other functional components may be affected in fracture of base of skull affecting internal auditory meatus of petrous part of temporal lobe. Lesion of preganglionic secretomotor fibers to the lacrimal gland causes impaired lacrimation. Salivary secretion is not fully impaired, as parotid gland remains functioning, because it is supplied by visceral efferent fibers through glossopharyngeal nerve.

**Spinal Injury**

Spinal injury affecting the parasympathetic system alone with sympathetic system leads to disorders of bladder, bowel and sexual function.

**Injury to Sympathetic System**

It is the sympathetic trunk which is injured opposite the level of cervicothoracic (stellate) ganglion at the root of neck.

1. This injury may occur due to stab or gunshot wound.
2. It may also occur due to traction by cervical rib. Beside injury, metastatic lesion at the root of neck may affect stellate ganglion.

Clinical condition arising from this lesion is known as Horner syndrome which is described earlier.

### CHAPTER SUMMARY

#### Key Concepts

1. All visceral functions are controlled by both sympathetic and parasympathetic divisions of ANS. Though sympathetic and parasympathetic systems have opposite effects, they are reciprocal to each other.
2. Regulation of sympathetic and parasympathetic systems occurs at spinal cord, brainstem, hypothalamic, limbic and cortical levels.
3. As hypothalamus is the head ganglion of ANS, hypothalamic control is important. Nevertheless, all medullary visceral centers influence autonomic functions that could in turn be controlled by limbic-hypothalamic projections.
4. Autonomic functions are disturbed by metabolic diseases such as diabetes.

#### Important to Know (Must Read)

1. In examinations, Control of autonomic functions may sometimes come as a Long Question.
2. Control of autonomic functions, supraspinal control of autonomic functions, autonomic dysfunction, Horner syndrome, orthostatic hypotension may come as Short Questions in exams.
3. In Viva, examiners may ask… Name levels of control of autonomic functions, Name autonomic reflexes, Name supraspinal control mechanisms of autonomic functions, Classify autonomic dysfunction, Causes and features of Horner syndrome, Causes and features of orthostatic hypotension.
CHAPTER 34

Autonomic Function Tests

LEARNING OBJECTIVES

On completion of study of this chapter, the student **MUST** be able to:
1. Classify autonomic function tests (AFTs).
2. Appreciate the application of AFTs in clinical physiology.
3. List the AFTs for assessment of sympathetic and of parasympathetic functions.

The student **MAY** also be able to:
1. Describe the physiological basis, method and merits and demerits of AFTs.

Sympathetic and parasympathetic systems control all visceral functions. Neurons of sympathetic and parasympathetic systems are affected in metabolic diseases. Therefore, autonomic dysfunctions are common in metabolic diseases such as diabetes, obesity, hypertension, hyperlipidemia, hyper and hypothyroidism, etc. India is the epicenter of diabetes, hypertension and heart diseases. Therefore, assessment of sympathetic and parasympathetic functions by conventional **autonomic function tests** (AFTs) is now part of routine clinical management.

CLASSIFICATION OF AFTs

Autonomic function tests (AFTs) are performed to confirm the clinical diagnosis of autonomic dysfunctions and to assess the degree of sympathetic and parasympathetic involvement in the process of dysfunction.

AFTs are classified as:

A. Tests for cardiovascular autonomic functions
   1. Heart rate and Blood pressure (BP) response to standing
   2. Heart rate and BP response to passive tilting
   3. Assessing baroreceptor sensitivity (BRS)
   4. Heart rate response to deep breathing
   5. Valsalva ratio
   6. BP response to hand grip
   7. Cold pressure test
   8. Nor-epinephrine spillage technique
   10. Standing to lying ratio
   11. Spectral analysis of heart rate variability (HRV)

B. Tests for sudomotor functions
   1. Sympathetic skin response
   2. Thermoregulatory sweat test (TST)
   3. Quantitative sudomotor axon reflex test (QSART)

C. Vasomotor test
   1. Laser Doppler Velocimetry for skin blood flow measurement
   2. Cold pressor test

D. Tests for pupillary functions
   1. Cocaine test
   2. Adrenaline test

E. Other methods
   1. Muscle sympathetic nerve activity (MSNA)

Tests for Cardiovascular Autonomic Functions

Heart Rate Response to Standing

Changing the posture from supine to standing, heart rate increases immediately by about 10 to 20 beats per minute.
1. On standing, the heart rate increases until it reaches a maximum at about the 15th beat, after which it slows down to a stable state at about 30th beat.
2. The ratio of R-R intervals corresponding to the 30th and 15th heart beat is called the **30:15 ratio**.
3. The 30:15 ratio is a measure of parasympathetic function.
4. However, relative bradycardia at 30th beat depends also on the sympathetic reactivity.
5. The normal 30:15 ratio is 1.15–1.12 at 21 to 30 years and 1.12 to 1.10 at 31 to 40 years of age.
6. This ratio decreases with age. Ratio less than 1.04 is considered abnormal.

**Blood Pressure Response to Standing**
The changes in blood pressure on standing are studied to assess the integrity of the sympathetic system.
1. Immediately on standing, blood pressure falls, which activates baroreceptor reflex and blood pressure returns to normal within 15 seconds.
2. When there is a sustained fall in systolic pressure more than 20 mm Hg or diastolic fall more than 10 mm Hg within three minutes of standing, orthostatic hypotension is said to be present.

**Assessing Baroreceptor Reflex Sensitivity (BRS)**
Baroreceptor reflex sensitivity is a newer method of assessing autonomic reactivity to various stimuli, such as orthostatic challenge and injection of chemicals and drugs that change the blood pressure.
1. This is assessed by continuous blood pressure variability (BPV) measurement. Sensitivity of baroreceptors to change in dynamic component (fluctuations) of blood pressure is an important marker of sympathetic and parasympathetic systems. Thus, it is a reflection of integration of both the components.
2. BRS is expressed as ms/mm Hg.
3. BRS less than 20 ms/mm Hg reflect poor cardiovascular (CV) health, and BRS less than 15 ms/mm Hg is an indicator of increased CV risk.
4. BRS 25 ms/mm Hg or more indicates enhanced autonomic tone and improved CV health.

**Norepinephrine (NE) Spillage Technique**
Norepinephrine level in plasma is measured in supine position and after 5 minutes of standing. The difference in level of NE represents alteration in sympathetic-hormonal reactivity.

**Heart Rate Response to Tilting**
Heart rate response to head up tilt (HUT) is a useful tool in the diagnosis of autonomic dysfunctions. It is more accurate because the active change of position is avoided by passively tilting the subject on a tilt-table. Moreover, variation in time taken by individuals to stand and the manner in which they stand from supine position is avoided in this method.
1. On changing from recumbent to operate position on a tilt table to 60 to 80 degrees HUT, pooling of about 30% venous blood occurs in the peripheral vascular compartment, especially in lower limbs.
2. This decreases cardiac filling pressure and stroke volume by about 40%.
3. Heart rate rises immediately due to withdrawal of parasympathetic activity and afterward due to increased sympathetic activity.

**Standing to Lying Ratio (SLR)**
Heart rate (RR interval) response to lying down from standing posture is assessed by continuous recording of ECG.
1. Following lying from standing position, increase in venous return produces reflex bradycardia.
2. Longest RR interval in standing to shortest RR interval in lying down is calculated as SLR.
3. Value of SLR below 1 is considered as abnormal.

**Heart Rate Response to Deep Breathing**
The variation of heart rate with respiration is known as sinus arrhythmia. Inspiration increases and expiration decreases heart rate.
1. This is primarily mediated via parasympathetic innervation of heart. Pulmonary stretch receptor, and cardiac mechanoreceptors and baroreceptors contribute to sinus arrhythmia.
2. The difference between the maximum and minimum heart rate during a deep breathing is called deep breathing difference (DBD).
3. DBD is more than 15 beats per minute in normal individual. It assesses the parasympathetic activity. DBD decreases with age.
4. It is one of the best parasympathetic reactivity test. Normal values of DBD at different age group are:
   - 10 to 40 years: > 18 beats per minute
   - 41 to 50 years: > 16 beats per minute
   - 51 to 60 years: > 12 beats per minute
   - 61 to 70 years: > 8 beats per minute
   Usually, subject is asked to inhale deeply for five seconds and then exhale for five seconds for six cycles. The ratio of shortest RR interval in inspiration to longest RR interval in expiration is calculated for each, which is called expiration-inspiration ratio (E/I ratio). The average E/I ratio of six cycles in a normal young individual is about 1:20.
   The E/I ratio decreases with age (Table 34.1).
   Normally, instead of DBD expressed in terms of beats per minute, E/I ratio is usually considered for assessing parasympathetic reactivity to deep breathing.
   DBD is abnormal in multisystem atrophy, progressive autonomic failure, diabetes mellitus, autonomic neuropathy and CNS depression.

**Valsalva Ratio**
The Valsalva ratio is a measure of parasympathetic and sympathetic function.
1. In Valsalva maneuver (named after scientist A M Valsalva, who described it), parasympathetic is the afferent and the efferent, and sympathetic is the part
of the efferent pathway. Therefore, Valsalva ratio assesses more of parasympathetic (cardiovagal) than sympathetic functions.

2. The procedure is performed by closing both nostrils and then blowing into a tube connected to sphygmomanometer. By putting strain, blowing pressure is maintained at 40 mm Hg for 15 seconds.

Valsalva maneuver has four phases:

- **Phase I**: Phase I consists of the onset of strain. In this phase, there is transient increase in blood pressure that lasts for a few seconds. This occurs due to increased intrathoracic pressure and mechanical compression of the great vessels. However, heart rate does not change much.

- **Phase II**: This is the phase of straining. In the early part of this phase, venous return decreases, which in turn decreases cardiac output and blood pressure. This change persists for 4 seconds. In the later part of this phase, blood pressure returns towards normal, which occurs due to increased peripheral resistance as a result of sympathetic vasoconstriction. However, heart rate increases steadily throughout this phase due to vagal withdrawal (in the early phase) and sympathetic activation (in the later phase).

- **Phase III**: This phase occurs following the release of strain during which there is transient decrease in blood pressure lasting for a few seconds. This is caused by mechanical displacement of blood to pulmonary vascular bed, which was under increased intrathoracic pressure. There is little change in heart rate.

- **Phase IV**: This is the phase that occurs with further release of strain. The blood pressure slowly increases and heart rate proportionately decreases. It occurs following 15 to 20 seconds after release of strain and lasts for about 1 minute or more. The cardiovascular changes occur due to increase in venous return, stroke volume and cardiac output.

**Valsalva ratio** is the ratio of minimal heart rate in phase IV to maximum heart rate in phase II as depicted in terms of RR interval.

\[
\text{Valsalva ratio} = \frac{\text{Longest R-R interval during phase IV}}{\text{Shortest R-R interval during phase II}}
\]

**Clinical Correlation**

Valsalva ratio more than 1.45 is considered to be normal.
1. Ratio 1.2–1.45 is considered borderline, and ratio less than 1.2 is regarded abnormal.
2. The normal ratio is different at different age groups (Table 34.2).
3. Valsalva ratio is also affected by gender, posture of subject in which recording is done, expiratory pressure, duration of strain and level of yoga practice of the subject.

Changes in Valsalva ratio occur due to changes in cardiac vagal efferent and sympathetic vasomotor activity, which are stimulated by carotid sinus and aortic arch baroreceptors and other intrathoracic stretch receptors. Failure of heart rate to increase during strain suggests sympathetic dysfunction and failure of heart rate to slow down after the strain suggests parasympathetic dysfunction. If the cardiovascular response to Valsalva maneuver is abnormal but that to cold pressure test (see blow) is normal, the lesion is supposed to be present in the baroreceptors or their afferent nerves. Such types of abnormalities occur commonly in diabetes, other neuropathies, multisystem atrophy and autonomic failure.

**BP Response to Sustained Hand Grip**

In hand-grip test is an isometric exercise in which the subject is asked to maintain hand grip against resistance.
1. Resistance usually offered by using a hand grip dynamometer at a 30% of maximum voluntary contraction for 5 min. BP and heart (HR) are recorded before and after the hand grip.
2. In hand-grip test, heart rate and blood pressure increase.
3. These cardiovascular responses to isometric exercise are mediated partly by central motor command and partly by mechanical changes or both, in response to contraction of the muscles that activate small fibers in the afferent limb of the reflex arch.
4. The normal response is rise in diastolic pressure more than 15 mm Hg and rise in heart rate by about 30%.
5. The blood pressure rise is due to increased sympathetic activity and heart rate rise is due to decreased parasympathetic activity.
6. The responses to hand grip test are usually not dependent on age.
7. Isometric handgrip test is one of the best sympathetic reactivity tests.

Cold Pressure Test
This test is performed by submerging the upper limb of the subject in ice cold water at 4°C for 30 to 60 sec. and BP is recorded before and after the procedure. The submer- sion of hand in cold water increases systolic pressure by about 20 mm Hg and diastolic pressure by 10 mm Hg.
1. The afferent limb of the reflex pathway is somatic fibers whereas the efferent pathway is the sympathetic fibers.
2. Thus, it assesses sympathetic activity.
3. Cold pressor test is one of the best sympathetic reactivity tests.

Tests for Sudomotor Functions

Sympathetic Skin Response
Sympathetic skin response (SSR) helps in studying the functions of peripheral sympathetic cholinergic (sudomotor) fibers by evaluating the changes in resistance of skin in response to electrical stimuli.
1. SSR is age dependent and is present in both hands and feet till the age of 60.
2. Composition of surface electrodes, stimulus frequency, skin temperature, and mental state of the subject affect the parameters of SSR.
3. The latency and amplitude of SSR are measured.
4. The amplitude of SSR in hand is 1.6 mV and in feet is 2.1 mV. SSR is helpful in diagnosing multisystem atrophy, progressive autonomic failure, diabetes, uremic patients and alcoholic neuropathy.

Thermoregulatory Sweat Test (TST)
Assessment of sweating response to heat also assesses sudomotor functions.
1. The subject’s body temperature is raised to by 1°C by exposing to heat of the electric heater.
2. Sweating response is studied by demarcating the area of sweating with the help of iodide starch or quinizarin powder that changes the color of the moist skin.
3. Absence of sweating in TST indicates sympathetic pre- and post-ganglionic lesions.

Quantitative Sudomotor Axon Reflex Test
Quantitative sudomotor axon reflex test (QSART) is a measure of regional autonomic function by Ach-induced sweating.
1. In this test, Ach is injected intradermally and the sweat production rate is assessed.
2. Reduced or absence of sweating indicates post-ganglionic lesion of sudomotor fibers (sympathetic fibers concerned with sweating).

Tests for Pupillary Functions
Pupillary function tests assess the function of sympathetic nerve supplying iris. Two tests usually performed are: cocaine test and adrenaline test.

Cocaine Test
Dilation of pupil is observed following instillation of 4% cocaine on both eyes. Cocaine prevents reuptake of nor- epinephrine at adrenergic nerve endings. Therefore, pupils dilate in response to cocaine, but, Horner’s pupils do not.

Adrenaline Test
Instillation of 1:100 or 1% noradrenaline on eyes dilate Horner’s pupil more than normal pupil. This is due to the mechanism of denervation hypersensitivity of Horner’s pupil.

Tests for Bladder Function
Cystometrogram (CMG) is performed to detect autonomic dysfunctions of urinary bladder. CMG reveals decreased ability of bladder to accommodate urine. Absence of accommodation to filling indicates autonomic dysfunction. Also, contraction of bladder muscle is poor in response to the act of micturition (evacuation).

Spectral Analysis of HRV
Recently, spectral analysis of heart rate variability (HRV) has evolved as a sensitive tool for assessing integrity of sympathetic and parasympathetic functions and for determining the sympathovagal balance. (Details are given in the next chapter)

AFTs to Assess Sympathetic and Parasympathetic Functions
A. AFTs for assessment of sympathetic functions:
1. BP response to standing/tilt
2. Cold pressor test
3. Isometric hand grip
4. Galvanic/sympathetic skin response
5. Thermoregulatory sweat test
6. Tachycardia ratio
7. Valsalva ratio
8. NE spillage test
9. LF and LFnu of HRV
B. AFTs for assessment of parasympathetic functions:
1. Resting heart rate: Basal heart rate is a good index of parasympathetic functions as heart rate in resting conditions is a measure of vagal tone. Resting HR more 75 indicates poor vagal tone and is presently considered as a CV risk.
2. 30:15 ratio
3. E/I ratio
4. Valsalva ratio
5. Bradycardia ratio
6. Baroreceptor sensitivity
7. Standing to lying ratio
8. HF and HFnu of HRV

**Concept of Reactivity and Activity Tests and CAFTs**

**Reactivity Tests**
Tests that are based on stimuli or disturbances such as change in position (standing, lying, dipping finger in cold water, hand grip against resistance, Valsalva maneuver etc.) are called reactivity tests. Accordingly, they are grouped as sympathetic and parasympathetic reactivity tests.

**Activity Tests**
Tests that are performed without disturbing the subject (subject at rest usually lying on couch in a comfortable room for 15 to 20 min) are called activity tests.
1. Recording of Resting HR and BP, and HRV analysis are examples.
2. Accordingly, they are grouped as sympathetic and parasympathetic activity tests.
3. Resting heart rate is parasympathetic test and resting BP is sympathetic test.

**CAFTs**
CAFTs refer to conventional autonomic function tests. HR and BP response to standing, HR response to deep breathing, isometric hand grip, cold pressor test and Valsalva maneuver are CAFTs.
Heart Rate Variability

Learning Objectives

On completion of study of this chapter, the student MUST be able to:

1. Define and explain the term heart rate variability (HRV).
2. List different spectral components (time domain and frequency domain) of HRV.
3. Mention physiological significance of each HRV component.
4. Understand the principle of short-term HRV measurement.
5. Comprehend the concept of sympathovagal balance and understand the importance of HRV recording in assessing sympathovagal balance.
6. Explain the importance of HRV analysis and its clinical utility in health and diseases.

The student MAY also be able to:

1. Describe different methods of measurement of HRV indices.
2. Explain the technical aspects of HRV components.
3. Describe the clinical application of HRV in health and disease.

Physiological Aspects

Heart rate variability (HRV) is the cardiac beat-to-beat variation (variation in cardiac cycle length), a physiological phenomenon that occurs mainly due to variation in cardiac activity during the respiratory cycle (respiratory sinus arrhythmia) at rest, though the circadian rhythm, environmental factors and exercise also contribute to it. Resting heart rates can vary; some have rates of 100 beats/min while others beat at only 60 beats/min for no obvious reason.

1. The rate of the heart and its beat-to-beat variations are dependent on the rate of discharge of the primary pacemaker, the SA node, which is influenced by autonomic activities that are controlled in a complex way by a variety of reflexes, central irradiations and cortical factors.
2. As SA nodal discharge is largely controlled by parasympathetic (vagal) influence, and sinus arrhythmia is primarily due to alteration in vagal tone in inspiration and expiration, HRV is mainly influenced by vagal activity, though both the divisions of ANS influence it.
3. Recently, HRV has been proposed as the most sensitive indicator of autonomic function, especially for the assessment of sympathovagal balance, the balance between the sympathetic and parasympathetic activity of the individual at any given time.
4. The state of sympathovagal balance is used for the prediction of many cardiovascular (CV) dysfunctions and other dysfunctions affecting cardiovascular function, its main use is in the CV risk stratification. However, the use of HRV analysis is limited in the diagnosis and management of CV and other diseases.

Technical Terms

Heart rate variability can be quantified in time and frequency domains.

1. Time domain measures include the usual tools of assessment of variations, as done in statistics. Time domain is easier to assess but finer aspects of variations are not appreciated. Shortly, the overall magnitude of HRV is assessed well but the individual contribution of various factors is not elucidated.
2. On the other hand, the variations in the instantaneous heart rate can be assessed spectrally. That is, an RR tachogram is plotted using the RR intervals in the five-minute lead II ECG.
3. The RR tachogram is considered as a non-periodic signal which is transformed to its frequency spectrum using fast-Fourier transformation algorithm or autoregressive modeling.

4. The biggest advantage of this complex mathematical transformation is that the distribution of magnitude of variations in different frequency bands corresponds to the activity of different physiological systems. The entire frequency spectrum 0 to 0.4 Hz is divided as follows:

**HRV Components**

The power spectrum of HRV in mammals usually reveals three spectral components. These components are (Fig. 35.1):

1. A high frequency band (HF) 0.15–0.4 Hz
2. A low-frequency band (LF) 0.04–0.15 Hz
3. A very low-frequency band (VLF) 0–0.04 Hz

**HF Component**

HF component is caused by vagal tone during the respiratory cycle.

1. The inspiratory inhibition of vagal activity is evoked centrally in the cardiovascular center and explains why heart rate fluctuates with the respiratory frequency.
2. In addition, peripheral reflexes arising from thoracic stretch receptors also contribute to this so-called respiratory sinus arrhythmia (RSA).
3. As RSA is clearly abolished by atropine or vagotomy, the power of the HF component has been used as an index of the vagal drive.

**LF Component**

LF component of HRV is usually characterized by an oscillatory pattern with a period of 10 seconds.

1. This rhythm originates from self-oscillation in the vasomotor part (sympathetic component) of the baroreflex loop as a result of negative feedback and it is commonly associated with synchronous fluctuations in blood pressure, the so-called Mayer waves.
2. Thus, LF component mainly represents sympathetic power. However, parasympathetic drive also contributes to it.

**VLF Component**

VLF component accounts for all other heart rate changes, including those associated with thermoregulation and humoral (especially, rennin-angiotensin mechanism) and local factors.

**Power Spectrum Analysis of HRV**

Power spectrum of HRV is analyzed by two methods: Fast-Fourier transform and autoregressive modeling.

**Fast Fourier Transform**

Any electrophysiological signal can be described as a sum of sine waves and this decomposition is called the Fast Fourier Transform (FFT). An efficient algorithm to carry out this transformation is the FFT, which, with some improvements and modifications, is still in use in many applications, such as voice analysis or vibration studies.

1. The analysis of short-term HRV (SHRV) is another one of these applications.
2. FFT algorithms impose some constraints on the signal to analyze because an evenly sampled, infinite and stationary time series is required.

**Autoregressive Modeling**

An alternative method to the FFT is the autoregressive (AR) identification algorithm combined with power spectral estimation for the assessment of SHRV. This method fits the data to a prior defined model and estimates the parameters of the model. The power spectrum implied by the model is then computed.

1. FFT or AR modeling methods share a common goal: the estimation of the power spectrum of a signal.
2. FFT-based methods are also called nonparametric methods because the time domain prior to spectral analysis is greatly simplified.

**Physiological basis**:

The FFT and autoregressive algorithms are the most commonly used tools to study the SHRV.

1. The final step in SHRV analysis includes the application of power spectrum estimation methods to characterize the frequency components associated with vagal and/or sympathetic outflow.
2. AR methods are parametric because they require prior information of the system under study. Thus, it was suggested that FFT-based methods are still the best
choice for the assessment of SHRV in comparative studies, where no previous knowledge of the system is available.
3. In addition, FFT algorithms are readily available in many different languages, even in commercial statistical packages.
4. Once the basic spectral content of the system is known and an initial model of the signal can be formulated, AR algorithms should be a better choice because they provide better frequency resolution and avoid the problems of spectral leakage.
5. The electrocardiogram (ECG) is the most appropriate signal to study SHRV because it offers the most accurate representation of the electrical cardiac events.
6. In particular, the QRS complex of the ECG sharply defines the onset of ventricular electrical depolarization and is the closest approach to time the occurrence of pacemaker potentials, which, in turn, are modulated by the autonomic outflow.

HRV Indices

Analysis of HRV has two parts: time-domain and frequency-domain.
1. HRV assessed by calculation of indices is based on statistical operations on R-R intervals (time domain analysis) or by spectral analysis of an array of R-R intervals (frequency domain analysis).
2. Both methods require accurate timing of R waves.
3. The analysis can be performed on short electrocardiogram (ECG) segments (lasting from 0.5 to 5 minutes) or on 24-hour ECG recordings.
4. The analysis of 5 min ECG recording is called short-term HRV and of 24 h ECG recording is called long-term HRV.

Time Domain Analysis

Two types of heart rate variability indices are distinguished in time domain analysis. Beat-to-beat or short-term variability (STV) indices represent fast changes in heart rate. Long-term variability (LTV) indices are slower fluctuations (fewer than 6 per minute). Both types of indices are calculated from the R-R intervals occurring in a chosen time window (usually between 0.5 and 5 minutes).
1. An example of a simple STV index is the standard deviation (SD) of beat-to-beat R-R interval differences within the time window.
2. Examples of LTV indices are the SD of all the R-R intervals, or the difference between the maximum and minimum R-R interval length, within the window.
3. With calculated heart rate variability indices, respiratory sinus arrhythmia contributes to STV, and baroreflex- and thermoregulation-related heart rate variabilities contributes to LTV.

Frequency Domain Analysis

Ever since spectral analysis was introduced as a method to study heart rate variability, an increasing number of investigators have preferred this method to time domain analysis for the calculation of heart rate variability indices.
1. The main advantage of spectral analysis of signals is the possibility to study their frequency-specific oscillations.
2. Thus, not only the amount of variability but also the oscillation frequency (number of heart rate fluctuations per second) can be obtained.
3. Spectral analysis involves decomposing the series of sequential R-R intervals into a sum of sinusoidal functions of different amplitudes and frequencies by the Fourier transform algorithm.
4. The result can be displayed (power spectrum) with the magnitude of variability as a function of frequency. Thus, the power spectrum reflects the amplitude of the heart rate fluctuations present at different oscillation frequencies.

Measurement of HRV Indices

Time Domain Methods

The variations in heart rate may be evaluated by a number of methods. Perhaps the simplest to perform are the time domain measures.
1. In these methods, either the heart rate at any point in time or the intervals between successive normal complexes are determined.
2. In a continuous ECG record, each QRS complex is detected, and the so-called normal-to-normal (NN) intervals, i.e. all intervals between adjacent QRS complexes resulting from sinus node depolarization or in the instantaneous heart rate are determined.
3. Simple time domain variables that can be calculated include the mean NN interval, the mean heart rate, the difference between the longest and shortest NN interval, the difference between night and day heart rate and so forth.

Statistical Methods

From a series of instantaneous heart rates or cycle intervals, particularly those recorded over longer periods, traditionally 24 hours, more complex statistical time domain measures can be calculated.
1. These may be divided into two classes: (a) Those derived from direct measurements of the NN intervals or instantaneous heart rate, and (b) those derived from the differences between NN intervals.
2. These variables may be derived from analysis of the total ECG recording or may be calculated using smaller segments of the recording period.
3. The most commonly used measures derived from interval differences include RMSSD, the square root of
the mean squared differences of successive NN intervals; NN50, the number of interval differences of successive NN intervals greater than 50 ms; and pNN50, the proportion derived by dividing NN50 by the total number of NN intervals (Table 35.1).

4. All of these measurements of the short-term variation estimate high-frequency variations in heart rate and, thus, are highly correlated.

### Geometrical Methods

A series of NN intervals also can be converted into a geometric pattern, such as the sample density distribution of NN interval durations, sample density distribution of difference between adjacent NN intervals, Lorenz plot of NN or RR intervals and so forth.

1. A simple formula is used that judges the variability on the basis of the geometric and/or graphics properties of the resulting pattern.
2. The HRV triangular index measurement is the integral of the density distribution (that is, the number of all NN intervals) divided by the maximum of the density distribution.
3. The major advantage of the geometric methods lies in their relative insensitivity to the analytical quality of the series of NN intervals.
4. The major disadvantage of the geometric methods is the need for a reasonable number of NN intervals to construct the geometric pattern.

The methods expressing overall HRV and its long-and short-term components cannot replace each other. The selection of method used should correspond to the aim of each particular study.

### Frequency Domain Methods

Various spectral methods for the analysis of the tachogram have been applied since the late 1960s.

1. **Power spectral density (PSD)** analysis provides the basic information of how power (variance) distributes as a function of frequency.
2. Independent of the method used, only an estimate of the true PSD of the signal can be obtained by proper mathematical algorithms.

### Nonparametric and Parametric Methods

Methods for the calculation of PSD may be generally classified as nonparametric and parametric. In most instances, both methods provide comparable results.

**The advantages of nonparametric methods** are:

1. The simplicity of the algorithm used [fast Fourier transform (FFT)] in most of the cases.
2. The high processing speed.

**The advantages of parametric methods** are:

1. Smoother spectral components that can be distinguished independent of preselected frequency bands.
2. Easy post processing of the spectrum with an automatic calculation of low- and high-frequency power components with easy identification of the central frequency of each components.
3. An accurate estimation of PSD even on a small number of samples on which the signal is supposed to remain stationary.

The basic disadvantage of parametric methods is the need for verification of the suitability of the chosen model and of its complexity (that is, the order of the model).

### Spectral Components of Frequency Domain

#### Short-term Recordings

Three main spectral components are distinguished in a spectrum calculated from short term recordings of 2 to 5 minutes: VLF, LF and HF components (Table 35.2). The distribution of the power and the central frequency of LF and HF are not fixed but may vary in relation to changes in autonomic modulations of heart period. The physiological explanation of the VLF component is much less defined and the existence of a specific process attributable to these changes might even be questioned. The non-harmonic component, which does not have coherent properties and is affected by algorithms of baseline or trend removal, is commonly accepted as a major constituent of VLF. Thus VLF assessed from short-term recordings (≤ 5 minutes) is a dubious measure and should be avoided when the PSD of short-term ECG is interpreted.

1. The measurement of VLF, LF and HF power components is usually made in absolute values of power (milliseconds squared).
2. LF and HF may also be measured in normalized units, which represent the relative value of each power component in proportion to the total power minus the VLF component.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Units</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDNN</td>
<td>ms</td>
<td>Standard deviation of all NN intervals</td>
</tr>
<tr>
<td>SDANN</td>
<td>ms</td>
<td>Standard deviation of the averages of NN intervals in all 5 min segments of the entire recording</td>
</tr>
<tr>
<td>RMSSD</td>
<td>ms</td>
<td>The square root of the mean of the sum of the squares of the differences between adjacent NN intervals</td>
</tr>
<tr>
<td>SDNN index</td>
<td>ms</td>
<td>Mean of the standard deviations of all NN intervals for all 5 min segments of the entire recording</td>
</tr>
<tr>
<td>SDSD</td>
<td>ms</td>
<td>Standard deviation of differences between adjacent NN intervals</td>
</tr>
<tr>
<td>NN50 count</td>
<td></td>
<td>Number of pairs of adjacent NN interval differing by more than 50 ms in the entire recording</td>
</tr>
<tr>
<td>pNN50</td>
<td>%</td>
<td>NN50 count divided by the total number of all NN intervals</td>
</tr>
</tbody>
</table>
3. The representation of LF and HF in normalized units (LFnu and HFnu) emphasizes the controlled and balanced behavior of the two branches of the autonomic nervous system. Moreover, the normalization tends to minimize the effect of the changes in total power on the values of LF and HF components.

4. Nevertheless, normalized units should always be quoted with absolute values of LF and HF power in order to describe completely the distribution of power in spectral components.

5. LF-HF ratio provides a better indicator of spectral powers.

**Long-term Recordings**

Spectral analysis also may be used to analyze the sequence of NN intervals of the entire 24-hour period. The result then includes an ultra-low frequency (ULF) component, in addition to VLF, LF and HF components. The slope of the 24-hour spectrum also can be assessed on a log-log scale by linear fitting the spectral values. Frequency domain measures are summarized below.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Units</th>
<th>Description analysis of short-term recordings (5 mins)</th>
<th>Frequency range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total power</td>
<td>ms²</td>
<td>The variance of NN intervals over the temporal segment.</td>
<td>Approximately ≤ 0.4 Hz</td>
</tr>
<tr>
<td>VLF</td>
<td>ms²</td>
<td>Power in very low-frequency range</td>
<td>0-0.04 Hz</td>
</tr>
<tr>
<td>LF</td>
<td>ms²</td>
<td>Power in low frequency range</td>
<td>0.04 – 0.15 Hz</td>
</tr>
<tr>
<td>LF norm</td>
<td>nu</td>
<td>LF power in normalized units</td>
<td>LF/(Total Power – VLF) x 100</td>
</tr>
<tr>
<td>HF</td>
<td>ms²</td>
<td>Power in high frequency range</td>
<td>0.15-0.4 Hz</td>
</tr>
<tr>
<td>HF norm</td>
<td>nu</td>
<td>HF power in normalized units</td>
<td>HF/(Total Power – VLF) x 100</td>
</tr>
<tr>
<td>LF/HF</td>
<td></td>
<td>Ratio LF (ms²)/HF (ms²)</td>
<td></td>
</tr>
</tbody>
</table>

**Technical Aspects**

The basic principle is that beat-to-beat variation in SA nodal discharge as recorded by ECG is computed and analyzed by the software for determination of spectral indices of HRV.

**Brief methodology:** There are two types of HRV recordings: the short-term 5 min HRV recording and day-night long-term HRV recording. Though long-term HRV recording is the ideal one, **short-term HRV recording** is usually performed for research and clinical investigations. We shall briefly describe the procedure of short-term recording as depicted in the **Task Force Report on HRV**.

1. The subject is asked to lie down comfortably in supine position in the laboratory, and ECG electrodes are connected for Lead II ECG recording.

2. After 5 minutes of supine rest, ECG signals are acquired at a rate of 1000 samples/second during supine rest using data acquisition system, such as BIOPAC MP 100 (BIOPAC Inc., USA) (minimum 250 Hz sampling rate). The raw ECG signal and the RR intervals are acquired on a moving time base.

3. Data from BIOPAC are transferred to a windows-based PC loaded with software for HRV analysis, such as Acknowledge software version 3.8.2. Ectopics and artifacts are removed from the recorded ECG.

4. **RR tachogram** is extracted from the edited 256 sec ECG using the R wave detector in the Acknowledge software and saved in ASC-II format, which is later used offline for short-term HRV analysis (RR tachogram should have minimum 288 RR intervals) (Fig. 35.2).

5. HRV analysis is performed by using the HRV analysis software version 1.1 (Bio-signal Analysis group, Finland).

**Calculation of Time Domain Indices**

In a continuous ECG record, each QRS complex is detected, and the so-called normal to-normal (NN) intervals (i.e. all intervals between adjacent QRS complexes resulting from sinus node depolarizations) or instantaneous heart rate is
determined. Simple time domain variables that are calculated include:
1. The mean RR
2. Standard deviation of normal-to-normal interval (SDNN)
3. Square root of the mean squared differences of successive normal-to-normal intervals (RMSSD) of HRV.
4. NN50
5. pNN50

**Calculation of Frequency Domain Indices**

Frequency domain variables that are usually calculated include **total power** (TP), **low frequency** (LF) component, LF component expressed as **normalized unit** (LFnu), **high frequency** (HF) component, HF component expressed as **normalized unit** (HFnu) and **LF/HF ratio** (Table 35.2).

Normalizing spectral powers are calculated by the formula as follows:
1. LF nu = LF x 100 (TP – VLF)
2. HF nu = HF x 100 (TP – VLF)
3. LF/ HF ratio = Ratio of LF to HF spectral powers

**Importance of HRV Analysis**

**Physiological Significance**

HRV analysis is used to precisely assess the efficiency of vagal control of the individual, as it reflects the heart rate variability that occurs mainly due to sinus arrhythmia.
1. Due to inspiratory inhibition of the vagal tone, the heart rate shows fluctuations with a frequency similar to the respiratory rate.
2. The inspiratory inhibition is evoked primarily by central irradiation of impulses from the medullary respiratory to the cardiovascular center.
3. Respiratory sinus arrhythmia can be abolished by atropine or vagotomy as it is parasympathetically mediated.

**HRV Analysis for Assessment of Sympathovagal Balance**

HRV, that is, the degree of heart rate fluctuations around the mean heart rate, can be used as a mirror of the cardiorespiratory control system. It is a valuable tool to investigate the sympathetic and parasympathetic function of the autonomic nervous system. SA nodal activity at any particular time is determined by the balance between vagal activity, which slows it, and sympathetic activity, which accelerates it. Generally, if the rate is lower than the intrinsic rate of the pacemaker, it implies predominant vagal activity, while high heart rates are achieved by increased sympathetic drive.

1. The **HF component of HRV indicates the cardiac vagal drive** of the individual. Increased HF power (or more specifically, increased HFnu) represents increased vagal drive and decreased HF power (decreased HF nu) represents decreased vagal drive to the heart.
2. The **LF component of HRV mainly indicates the cardiac sympathetic drive** of the individual. Increased LF power (or more specifically, increased LFnu) represents increased sympathetic drive while decreased LF power (decreased LFnu) represents decreased sympathetic drive.
3. The **sympathovagal balance** is assessed by the LF–HF ratio. Increased LF–HF ratio reflects increased sympathetic activity, while decreased LF–HF ratio indicates increased parasympathetic and decreased sympathetic activity.

**Physiological basis:** The relationship between vagal stimulation frequency and the resulting change in heart rate is hyperbolic, with changes in frequency at low heart rates having a much greater effect that does not directly control the heart rate, but which regulates the interval between successive beats.

1. The effect of vagal stimulation is rapid. Vagal stimulation releases the neurotransmitter acetylcholine, which inhibits the pacemaker potentials.
2. **Sympathetic responses** differ from vagal effects in that they develop much more slowly. Hence, responses with longer latency are likely to be mainly sympathetic.
3. **Peripheral vascular resistance** exhibits intrinsic oscillations with a low frequency. These oscillations can be influenced by thermal skin stimulation and are thought to arise from thermoregulatory peripheral blood flow adjustments.
4. The fluctuations in peripheral vascular resistance are accompanied by fluctuations with the same frequency in blood pressure and heart rate and are mediated by the sympathetic nervous system.
5. Hence, analysis of HRV also indicates the tone of sympathetic outflow and, therefore, reflects the individual’s state of sympathetic function and susceptibility to sympathetic dysfunction.

**Importance of LF-HF Ratio and Sympathovagal Balance**

1. The **HF component** of HRV, which indicates the cardiac vagal drive to the heart, represents parasympathetic activity. The **LF component** of HRV, which mainly indicates the cardiac sympathetic drive, represents sympathetic activity, though parasympathetic drive also contributes to it.
2. In healthy individuals, **HF constitutes about 60%, and LF constitutes about 40%** of the total power (TP) of HRV.
3. Therefore, **LF-HF ratio less than 1 indicates good cardiovascular health.** However, LF-HF ratio in normal population varies from 0.5 to 1.5.
4. The sympathovagal balance is assessed by the LF-HF ratio.
5. Increased LF-HF ratio reflects increased sympathetic activity (Figs. 35.3A and B) that is invariably associated with decreased TP.

6. Decreased LF-HF ratio indicates increased parasympathetic and decreased sympathetic activity that is invariably associated with increased TP (Fig. 35.4).

**Clinical Application**

Though there is considerable discussion regarding the physiology of HRV, it is well correlated and studied in many physiological and pathological conditions:

1. Total power (TP) of HRV indicates the magnitude of heart rate variability.
2. Decreased TP (decreased overall cardiac vagal modulation) has been implicated with future adverse cardiovascular (CV) morbidities and mortalities.
3. Decreased HRV (decreased total power of HRV) is observed in many cardiovascular disease conditions and generally indicated poor prognosis in these conditions.
4. Much before the onset of clinical symptoms of the cardiovascular disease, alterations are observed in HRV, indicating that HRV could be used as sensitive tool in the prediction of CV risks. However, more research is required to establish the predictive value of HRV in CV dysfunctions.
5. Presently, HRV is used as a prognostic tool in conditions like postmyocardial infarction and cardiac transplantation.
6. The most important application of HRV analysis is the surveillance of postinfarction and diabetic patients.
7. HRV gives information about the sympathetic-parasympathetic autonomic drives, and used as tool for assessment of autonomic imbalance.
8. As HRV analysis is used to assess the state of sympathovagal balance of the individual, it can be used to determine the individual’s susceptibility to developing autonomic dysfunctions in conditions like prehypertension and hypertension.

9. Decreased HRV is well correlated with the risk of sudden cardiac death in patients with heart disease.

10. Improvement in HRV and CV health is observed in interventions, like exercise, yoga and relaxation exercises. Hence, this can be used in future research works for improvement of holistic health.

The clinical applicability is still limited for lack of established normative data of HRV for different ages, genders and ethnic groups due to its demanding technical and mathematical comprehensibility. However, with increasing use of automation and computers in medicine, the clinical applicability of HRV is bound to be appreciated by researchers and clinicians.

CHAPTER SUMMARY

**Key Concepts**

1. Though HRV is a sensitive tool for assessing autonomic functions and dysfunctions, due to lack of wide range of normative data, its application in clinical medicine is limited.

2. HRV has less diagnostic application, though it is widely used in assessing prognosis of the diseases and in prediction of CV risks for morbidity and mortality.

3. TP of HRV depicts overall vagal modulation of heart. HF and HFnu indicate cardiac vagal drive, and LF and LFnu indicate sympathetic drive in general.

4. LF-HF ratio more than 1.5 indicates less vagal and more cardiac sympathetic drive, LF-HF ratio less than 0.5 indicates more vagal and less cardiac sympathetic drives. Less LF-HF ratio and more TP are indicators of good CV health.

**Important to Know (Must Read)**

1. In examinations, ‘Physiological basis, procedure, clinical application and merits and demerits of HRV’ may come as a Long Question.


3. In Viva, examiner may ask……. What is power spectral density, What are the HRV indices and how are they measured, Name Time-domain indices and mention their significance, What are the normalized units and how are they calculated, What LFnu and HFnu represent, Name frequency-domain indices and mention their significance, What is normal LH-HF ratio and what it indicates, What is the importance of sympathovagal balance, What are clinical utilities of HRV.
SECTION–5
Gastrointestinal System

**Part A: Introduction to GI System**
36. Functional Organization of GI System and Principles of GI Regulations
37. Gastrointestinal Hormones

**Part B: GI Secretions**
38. Principles of GI Secretion and Secretion of Saliva
39. Gastric Secretion
40. Pancreatic Secretion
41. Physiology of Liver, Liver Function Tests and Pathophysiology of Jaundice
42. Biliary Secretion
43. Intestinal Secretion
44. Secretion of Large Intestine

**Part C: GI Motility**
45. Introduction to GI Motility
46. Chewing and Deglutition
47. Esophageal Motility
48. Gastric Motility
49. Small Intestinal Motility
50. Motility of Large Intestine

**Part D: Digestion and Absorption**
51. Principles of Digestion and Absorption
“Where ignorance is, there suffering too must come.  
...Your grief is a cry of darkness to the Light above;  
Pain was the first-born of the Inconscience  
Which was the body’s dumb original base”

Sri Aurobindo (in ‘SAVITRI’)
CHAPTER 36

Functional Organization of GI System and Principles of GI Regulations

**Learning Objectives**

On completion of study of this chapter, the student **MUST** be able to:
1. Appreciate the importance of GI physiology in learning medicine.
2. List the functions of GI tract and correlate the functional anatomy with functions of GI tract.
3. Name the layers of wall of GI tract, and give their functional importance.
4. Understand the difference in sympathetic and parasympathetic stimulation on GI functions.
5. Appreciate the organization of enteric nervous system.
6. Learn the principles of GI regulations.

The student **MAY** also be able to:
1. Describe the innervation of GI tract
2. Describe the principle of GI regulations

Gastrointestinal (GI) system is a fundamental design of the nature to provide nutrition to the individual by ingestion. The food particles that enter GI tract as larger molecules like polysaccharides, proteins and fats must be broken down into smaller absorbable molecules in the lumen of stomach and intestine to cross the GI epithelium and enter blood. Though, a person can survive on parenteral (usually, intravenous) nutrition, this restricts the mobil- ity of the individual and requires assistance for connecting intravenous infusion. Therefore, parenteral nutrition is advocated temporarily in patients who cannot eat, or in whom oral feeding is prohibited due to some other cause. It is also difficult to provide all nutritional ingredients for a longer duration through parenteral route. Therefore, gastrointestinal dysfunctions invariably lead to malnutrition.

1. When food enters GI system, starting from the mouth cavity, exocrine secretions (GI secretions) pour into the tract.
2. GI secretions (mainly salivary, gastric, pancreatic, biliary and intestinal) contain enzymes that split various food materials into their absorbable form. This is called digestion of food.
3. For adequate enzymatic digestion to take place, food should be thoroughly grinded and then mixed with GI secretions.
4. The process of grinding and mixing is initiated when food is present in oral cavity. Contraction of smooth muscle in the wall of the gastrointestinal tract produces various movement of the tract. This is called GI motility.
5. Chewing, and gastric and intestinal motilities help in grinding food particles and facilitate their mixing with the digestive juices.
6. GI motility also exposes chyme (digested food) to the epithelial surface of the intestine for their transfer into general circulation. This is called absorption of food.
7. GI motility is essential for propulsion of food in aboral direction.

Keeping the sequence of GI activities in mind, while describing GI physiology, we shall first discuss GI secretions, followed by GI motility and finally, the digestion and absorption of food.

**Scientist contributed**

RENÉ ANTONIE FERCHAULT DE REAUMUR, the French entomologist explored the comparative physiology in insects and birds. He studied the digestive process in birds and isolated gastric juice. He demonstrated the solvent action of gastric juice on food. His pioneering works in comparative physiology included regeneration in crustacean, electric organs of torpedo, star-fish movement, marine phosphorescence, formation of silk from silkworms and digestion in birds.

FUNCTIONAL ORGANIZATION

Functions of GI Tract

Gastrointestinal system, in addition to its principal functions of digestion and absorption of nutrients, carry out many other functions of the body. The GI functions may be listed as follows:

1. **Digestion and absorption of food**: The primary functions of GI system are to digest food materials and absorb essential nutrients of digestive products into the bloodstream.

2. **Excretion of waste materials**: Digestive tract excretes waste materials in the form of feces. Fecal matter includes mainly the excretory products of digestion and undigested food particles.

3. **Fluid and electrolyte balance**: On average, an adult consumes 1 kg of food and 1.5 liters of water per day. An additional amount of 7 liters of fluid is secreted into GI tract in the form of secretions from various exocrine glands. Thus, intestine is presented with about 8.5 liters of fluid per day. About 99% of this fluid and 90% of solids are absorbed in small and large intestines along with absorption of various electrolytes, so that only about 100 ml of liquid and 100 g of solids are excreted in feces per day. Decreased absorption of this water content results in dehydration. **Diarrhea occurs when capacity of intestine to absorb fluid is decreased or secretion of fluid is increased.**

4. **Immunity**: The GI tract is approximately a 15 feet long tube, which is open at both the ends. Therefore, anatomically it is continuous with the external environment. Consequently, it provides a fine channel for organisms from external world to pass through it. However, the gut associated lymphoid tissues (GALTs) protect the body from pathogenic organisms. GALTs are of two types: Peyer’s patches (aggregates of lymphoid tissue in the submucosa) and diffuse population of immune cells (discussed below). GALTs prevent entry of organisms from gut into the body. **Acidic secretion of stomach** also prevents entry of organisms through oral route.

5. **Intestinal bacterial flora**: Many nonpathogenic bacteria inhabit intestine. They form the normal bacterial flora of intestine. This normal flora is essential for many intestinal functions like epithelial cell permeability to electrolytes and water, synthesis and absorption of vitamins, stimulation of enzymatic activity, peristalsis and mucus secretion. Decreased flora impairs intestinal functions and increased flora increases the susceptibility to diarrhea and steatorrhea. These bacteria also **detoxify many toxins** present in the chyme and prevent their absorption into the bloodstream.

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Parts of GI System

Gastrointestinal system consists of gastrointestinal tract (GIT) and its associated glandular structures that produce exocrine secretions essential for digestion and absorption of various nutrients. The parts of GIT include **mouth, pharynx, esophagus, stomach, small intestine (duodenum, jejunum, and ileum), colon (ascending, transverse, descending and sigmoid colons), rectum, and anus** (Fig. 36.1). The major associated glandular structures are salivary glands, liver, exocrine pancreas and intestinal glands.

1. GI tract begins with the **mouth** (oral cavity). Digestion of food starts in mouth with chewing. **Salivary glands** open into the oral cavity and pour their secretion (saliva) into it. Salivary amylase helps in partial digestion of complex sugars (polysaccharides).

2. Oral cavity opens into **pharynx** which in turn is continuous with the esophagus.

3. **Esophagus** transmits food from oropharynx into stomach.

4. **Stomach** is a bag like structure that stores and grinds food, and mixes food with gastric juice. HCl and pepsin secreted in stomach help in partial digestion of proteins and stomach converts food into chyme. Chyme from stomach enters intestine through pyloric sphincter by controlled gastric emptying.

5. **Small intestine** is a 9 feet tube that extends from pyloric sphincter to the ileocecal valve. It consists of three parts: duodenum, jejunum and ileum.

6. **Biliary and pancreatic secretions** are poured into duodenum.

7. Intestinal glands secrete **sucus entericus**.

8. **Pancreatic and intestinal secretions** are rich in various enzymes that cause digestion of carbohydrates, fat and proteins.

9. Absorption of most of the nutrients takes place in jejunum and ileum.
10. Small intestinal motility propels food into large intestine through ileocecal sphincter.
11. Large intestine absorbs water and electrolyte and temporarily stores the concentrated and undigested material, which then enters the rectum.
12. Rectum, the final segment of large intestine stores fecal matter and contracts when distended. Defecation reflex relaxes the anal sphincters and expels feces from rectum to outside through anal canal.

**Structure of the Wall of GI Tract**
The basic nature of arrangement of tissues in the wall of GI tract starting from esophagus to the rectum is similar. In general, there are four layers from interior to exterior: mucosa, submucosa, muscle layer, and serosa (Fig. 36.2).

**Mucosa**
The innermost layer of GI tract is the mucosa, which has an epithelium, lamina propria and muscularis mucosa (Fig. 36.3).

**Epithelium**
A single layer of epithelial cells line the mucosa. The cells are tightly bound to each other at their edges by tight junctions.
1. The epithelium is mainly a stratified squamous epithelium.
2. In stomach and intestine, the mucosa is lined by simple columnar epithelium that facilitates secretion and absorption.
3. At places, the epithelium contains endocrine cells (enterochromaffin cells) that secrete local hormones.

**Lamina Propria**
Just below the epithelium is the lamina propria consisting of mainly loose connective tissue that contains collagen and elastin fibers.
1. This sub-layer is rich in blood vessels, lymph nodes and lymphatic ducts, nerve fibers, and capillaries. This layer supports the epithelium and also binds epithelium to the muscularis mucosa.
2. The lymphatic tissues present in this layer form the local immune system and prevent the entry of pathogens that try to penetrate mucous membrane to enter into the body.

**Muscularis Mucosa**
This is the innermost layer of the intestinal smooth muscle present in the mucosa. The muscle fibers are arranged in two layers: an inner circular and outer longitudinal layer.
1. Contraction of muscularis mucosa in the stomach and intestine throws the mucous membrane into folds and ridges.
2. In the intestine, these mucosal folds called plica increase the surface area for absorption (Fig. 36.4).
3. Contraction of muscularis mucosa in small intestine produces local movements that facilitate the process of digestion and absorption.

**Submucosa**
Next to the mucosa is the submucosa. It consists mainly of loose connective tissue and is highly vascular.
1. The submucosal layer contains a network of nerve fibers known as submucosal plexus or plexus of Meissner.
2. Autonomic nerve fibers supplying muscles and blood vessels of GI tract closely interact with Meissner’s plexus.
3. In some regions, especially in intestine, submucosa contains lymphatic tissue.

**Muscular Layer**
This is called muscularis externa. It consists of two layers of smooth muscles: the inner circular and the outer longitudinal layers.
1. A nerve plexus is located between the muscle layers, called as myenteric plexus or plexus of Auerbach.
2. Auerbach’s plexus contains fibers from both divisions of ANS.
3. Muscularis externa primarily controls the motility of GI tract that mixes and circulates the gastrointestinal contents and propels them along the lumen of the tract.
Fig. 36.4: Differences in the layers of the wall in different parts of GIT. Note that mucosa is simple columnar in stomach and intestine. In jejunum, the muscularis mucosa is folded to form plica that increases the surface area for absorption.

Courtesy: Figure 13.1, Basic Histology, by V Subhadra Devi, 1st edition, 2016; Jaypee Brothers Medical Publishers (P) Ltd.
Serous

This is the outermost layer of the GIT. It consists mainly of a thin layer of connective tissue covered with a layer of squamous mesothelial cells. It provides external protection to the GIT.

Intestinal Modifications

Villi

In small intestine, from the luminal surface, finger like projections extend into the lumen. These are called villi. Surface of each villus is covered with an epithelial cell layer.
1. Small projections arise from the surface of these epithelial cells, known as microvilli (Fig. 36.5).
2. Microvilli are collectively called as brush border.
3. Folded mucosa, villi and microvilli increase the surface area of the intestinal luminal epithelium by 600 fold.
4. The total luminal surface area of human intestine is about 300 m², which is about the area of a tennis court.
5. Intestinal epithelial cells are continuously replaced by new cells. About 17 billions of epithelial cells are replaced every day and the entire intestinal epithelium is replaced almost every five days.
6. Center of each villus is occupied by lymphatics called lacteals, and capillaries, venules and nerve fibers.

Peyer’s Patches

These are the aggregates of lymphoid tissue in the mucosal-submucosal regions of the intestinal wall.
1. They serve local immune functions in the intestine. They prevent organisms to enter blood stream from the intestinal lumen.
2. They also secrete mediators of inflammation (cytokines) that alter motility. These cytokines play role in genesis of inflammatory bowel disease and Crohn’s disease.

Innervation of GI Tract

Gastrointestinal tract is innervated by sympathetic and parasympathetic fibers. Also, it has its own neural circuits (enteric nervous system).

Sympathetic Innervation

Stimulation of sympathetic fibers to GI tract is generally inhibitory. Sympathetic innervation is via noradrenergic fibers having their cell bodies in the prevertebral and paravertebral chain of ganglia. The postganglionic fibers mainly originate from celiac, superior and inferior mesenteric ganglia (Refer to Fig. 31.2; Chapter 31).

Usually, sympathetic fibers do not directly innervate the target GI tissues. Instead, they terminate on neurons of intrinsic nerve plexuses that in turn contact the target structures (Fig. 36.6). However, the vasoconstrictor fibers directly innervate blood vessels of GI tract.

Sympathetic stimulation results in:
1. Inhibition of motor activity resulting in relaxation of GI smooth muscles.
2. Stimulation or contraction of sphincters.
3. Inhibition of GI secretions.

Parasympathetic Innervation

Parasympathetic innervation is mainly via vagus nerve, which innervates GI tract from oral cavity upto transverse colon. Remaining parts (descending and sigmoid colons, rectum, and anal canal) receive parasympathetic fibers from pelvic nerves (see Fig. 32.1; Chapter 32).

1. Parasympathetic fibers are cholinergic and generally excitatory.
2. They terminate mainly on the intrinsic nerve plexuses.
3. Parasympathetic stimulation leads to increased motility and exocrine secretions of GI tract.

Enteric Nervous System

Enteric nervous system (ENS) is the intrinsic nervous system of the GI tract.

1. This includes myenteric and submucosal nerve plexuses in the GI tract (Fig. 36.6). These two plexuses are the network of nerve fibers and ganglion cells.
2. The neurons of these plexuses are small interneurons that connect afferent neurons and efferent neurons to smooth muscles, secretory cells and epithelial cells (Flowchart 36.1), and they form the anatomical basis of local GI reflexes. Therefore, the reflex arc for local GI reflexes is located within the GI tract. Hence, ENS is capable of coordinating GI activity in the absence of external innervation.
3. The neurons of ENS closely interact with the autonomic nervous system. The sympathetic and parasympathetic fibers project to the neurons of the myenteric and submucosal plexuses, and control GI activities via these neurons.
4. Therefore, ENS is also known as **third division of ANS**. However, it should be remembered that **regulation of GI functions by ENS is independent of autonomic control**.

**ENS as the Mini-brain of gut**: ENS consists approximately of 100 million neurons, the number of neurons that are roughly present in spinal cord or in entire ANS. These neurons are clustered exclusively in the gut.

1. They have sensory neurons, interneurons and motor neurons. Therefore, ENS is loosely called the ‘**mini brain**’ for the gut.

2. The ENS afferent neurons monitor changes in luminal activity like distension, alteration in osmolality, pH etc. and activate gut interneurons.

3. The interneurons signal the changes in ENS motor fibers that alter the activities of effector tissues like smooth muscle cells, glandular cells, epithelial cells and vascular cells.

**Neurotransmitters in the ENS**: Many neurotransmitters in the ENS have been recently identified.

1. **Acetylcholine** (ACh) is the primary neurotransmitter in preganglionic and postganglionic neurons that regulate secretory and motor activities of GI tract.

2. **VIP, serotonin, enkephalins, substance P, norepinephrine, GABA, ATP, NO and CO** have also been described as neurotransmitters in ENS, and these chemicals play important role in regulation of GI functions.

**GI Sphincters**

Sphincters are **specialized circular muscles** that are present at the **beginning or at the end of a gut structure**. Sphincters regulate antegrade (forward) movement of food and prevent retrograde (reverse) expulsion of the same. There are **six sphincters** in GI tract:

1. Upper esophageal sphincter
2. Lower esophageal sphincter
3. Pyloric sphincter
4. Ileocecal sphincter
5. Internal anal sphincter
6. External anal sphincter

There is another sphincter located in the wall of the duodenum called **Sphincter of Oddi** that regulates secretion of bile and pancreatic juice into the intestine.

**GI Immune System**

An extensive immune mechanism is developed in GI system, which is called **gastrointestinal immune system**. This system includes:

1. **Mesenteric lymph nodes**
2. **Peyer’s patches** (aggregation of lymphatic tissues in the submucosa)
3. **Phagocytic cells and immunocytes** present in the mucosa and submucosa throughout the GI tract (For details, refer to Chapter 18).

**Immunological Cells in GI System**

The immunological cells in GI system constitute about **50% of the total immunocytes in the body**.

1. Immunocytes in GI tract are **B and T cells**, **epithelial lymphocytes**, **plasma cells**, mast cells and macrophages.
2. These cells **secrete chemicals** that mediate immunological and inflammatory responses in GI tract.
3. They secrete **antibodies** locally in response to antigens that enter through GI tract.
4. **Other chemicals** secreted by immunocytes are **leukotrienes, cytokines, prostaglandins and histamine**. These chemicals not only mediate immunological responses, but also influence activities of smooth muscles of GI tract.
5. GI immune system plays an important role in the pathogenesis of inflammatory bowel diseases like ulcerative colitis.

**PRINCIPLES OF GI REGULATIONS**

The functions of GI tract are regulated mainly by neural and hormonal influences. Interestingly, the hormonal and neural control systems of GI functions are mostly intrinsic to GI tract.

**Neural Regulation**

The digestive system is regulated by the nerves arising locally in the GI tract (intrinsic neural control) and the nerves innervating the gut (extrinsic neural control). The GI reflexes modify activities of GI system depending on the state of digestion and absorption taking place in the tract.

**Intrinsic Neural Regulation**

The intrinsic nerves of the GI system form the enteric nervous system, sometimes called as third component of ANS.

1. The nerves are arranged in the submucosal layer as Meissner’s plexus and in the muscle layer as Auerbach’s plexus.

2. These nerve plexuses control local secretion and movement of GI tract.
3. The Meissner’s plexus performs sensory functions and Auerbach’s plexus performs motor functions.
4. The intrinsic neurons mediate local or short reflexes.
5. The intrinsic nerve plexuses are connected and controlled by extrinsic nerves.

**Extrinsic Neural Regulation**

Extrinsic nerves regulating GI functions belong to sympathetic and parasympathetic systems.

1. In general, the sympathetic system to the GI tract is inhibitory and the parasympathetic system is stimulatory.
2. The autonomic innervations also control GI functions by altering the activity of neurons in the enteric nervous system.
3. Extrinsic nerves mediate long reflexes.

**Reflex Control**

There are two types of neural reflexes that operate in GI system: short or local reflexes and long or central reflexes (Flowchart 36.2).

1. Afferent pathway in the GIT forms afferent limb for both local and central reflexes.
2. There are many chemoreceptors and mechanoreceptors in the mucosa and muscularis mucosa that are extensively connected to intrinsic plexuses. Actions of these receptors mediate local reflex activity.
3. Enterogastric reflex is an example of a short reflex. Receptors also provide signals to the CNS that control GI functions via central reflexes. The efferent fibers for central reflexes are present in the autonomic fibers that terminate in enteric nerve plexuses. Reflex secretion of saliva in response to smell of food is an example of a long reflex.

**Hormonal Regulation**

The hormones controlling GI functions are also of two types: intrinsic hormones, and extrinsic hormones.

### Intrinsic Hormones

Many hormones are secreted from endocrine cells of GI tract. These hormones are called GI hormones (for details, refer next chapter). They mainly act in a paracrine fashion. For example, histamine secreted from neuroendocrine cells of the stomach greatly influences gastric acid secretion. However, many hormones also have systemic functions. Therefore, GI tract is sometimes designated as an endocrine organ.

### Extrinsic Hormones

GI functions are also influenced by hormones secreted from other endocrine glands like thyroxine and cortisol. Thyroxine stimulates intestinal motility, whereas cortisol stimulates acid secretion from parietal cells of stomach.

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**CHAPTER SUMMARY**

**Key Concepts**

1. The layers in the wall of GI tract are modified at parts to suit the functions of that part.
2. Interneurons from sympathetic and parasympathetic system (form extensive neural plexuses in muscle layer and mucosal layer) innervate and control the epithelial cells, smooth muscle cell and secretory cell in GIT.
3. Immunocytes in the GIT (Peyer’s patches, Gut associated lymphatic tissues, and other lymphocytes) provide local immunity.
4. Short and long reflexes control GI functions.

**Important to Know (Must Read)**

1. In examinations, usually Long Questions are not asked from this chapter.
2. Layers of GIT, Innervation of GIT, Immune system of GIT, Enteric nervous system, and Reflex control of GI functions, may come as Short Questions in exams.
3. In Viva, examiner may ask… What are the parts of GIT and what are their main functions, What the layers of GIT and what are their functions, What is the system of innervation of GIT, What are the effects of stimulation of sympathetic and parasympathetic system to GIT, Name the immune mechanisms in GIT, How is enteric nervous system formed and how it works, What are neurotransmitters secreted by GI neurons, What are short and long reflexes and how they work.
4. If a student fails to answer the part of GIT, their organization and functions, it becomes difficult for examiner to give pass mark.
The hormones secreted from the endocrine cells of the GI tract are collectively known as gastrointestinal hormones (GI hormones). Endocrine cells are scattered throughout epithelium of GI tract, especially in stomach and small intestine. The apical surface of epithelial endocrine cells is continuously exposed to chemical changes in the luminal contents of the gut, which directly stimulates the release of hormones from these cells.

1. Endocrine cells that secrete GI hormones are called enteroendocrine cells.
2. Cells that secrete serotonin are called enterochromaffin cells, and cells that secrete amines and polypeptides are called APUD cells (amine precursor uptake and decarboxylation).
3. APUD cells are also found in other organs like lungs. They are also called neuroendocrine cells. Carcinoid tumors originate from these neuroendocrine cells.

Families of GI Hormones: GI hormones are broadly divided into three categories:

1. **Gastrin family** that includes cholecystokinin and gastrin.
2. **Secretin family** that includes GIP, glucagon, secretin, and VIP.
3. **Other polypeptides**.

**GI Hormones of Gastrin Family**

**Gastrin**

**Source**

Gastrin is produced by G cells in the stomach that are located mainly in the antral region.

1. G cells are conical cells with apex projecting toward the lumen. Apical surface of G cells contains numerous microvilli. Microvilli of G cells contain receptors for chemicals that mediate gastrin release.
2. **Gastrin producing cells are also present** in hypothalamus, anterior pituitary, medulla, and fetal pancreas.
3. Gastrin as a neurotransmitter is also secreted from vagus and sciatic nerve.

**Structure**

Gastrin is a polypeptide hormone. Gastrin has marked heterogeneity; but its physiological significance is not clearly known.

1. Gastrin exhibits both macroheterogeneity (gastrins having different polypeptide lengths) and microheterogeneity (gastrins having different molecular structures).
2. Though different types of gastrins have been described, three types of gastrin are physiologically important. Depending on the number of amino acids they possess, they are named as G 34, G 17, and G 14. Other types of gastrins are C-tetrapeptide (carboxyl terminal tetrapeptide, which is also called minigastrin), and gastrin containing 45 amino acids (megagastrin).

3. However, G 17 is the principal gastrin secreted from the stomach and is the major stimulator of gastric acid secretion.

4. Though C-tetrapeptide executes all the actions of G-17, it has only 10% of its physiological strength.

**Metabolism**

Gastrin secreted from G cells enters general circulation. In blood, half-life of gastrin is less. Half-life of G 14 and G 17 is 2–5 min and of G 34 is about 15 min. Gastrin is inactivated in the intestine and degraded in the kidney.

**Functions**

1. Primary function of gastrin is the stimulation of gastric acid and pepsin secretion. In fact, gastrin is the most potent natural stimulator of HCl secretion from parietal cells of stomach. Therefore, hypergastrinemia causes peptic ulcer.

2. Gastrin stimulates growth of gastric mucosa and mucosa of intestine. This is called trophic action of gastrin.

3. It stimulates gastric motility.

4. It causes contraction of muscles at the gastroesophageal junction (lower esophageal sphincter). Therefore, it prevents reflux esophagitis.

5. It stimulates exocrine pancreatic secretion.

6. It also stimulates insulin secretion.

7. It stimulates mass movement of large intestine.

8. It causes colonic contraction that initiates gastrocolic reflex after a meal. Therefore, usually defecation is activated after a meal.

9. It stimulates histamine secretion from ECL (enterochromaffin like cells) in GI mucosa.

**Mechanism of Action**

The primary function of gastrin is to stimulate acid secretion from parietal cells of the stomach. Gastrin acts on gastrin or CCK receptors on parietal cells and increases intracellular calcium concentration via second messenger, IP3. Increased cytosolic calcium activates protein kinase that stimulates H⁺–K⁺ ATPase to promote acid secretion.

**Control of Gastrin Secretion**

1. Stimuli that increase gastrin secretion: Gastric distention, products of protein digestion (peptides and amino acids) in the stomach, increased vagal discharge via GRP (non-cholinergic), epinephrine and calcium (Flowchart 37.1). Hypergastrinemia occurs in ZE syndrome (Clinical Box 37.1). Gastrin secretion is also elevated in pernicious anemia, in which acid secretion in the stomach is less as parietal cells are damaged. This causes feedback release of gastrin from G cells.

2. Stimuli that decrease gastrin release: Acid in the stomach, somatostatin, secretin, GIP, VIP, calcitonin, and glucagon. Acid in the antrum inhibits gastric secretion by negative feedback mechanism, which is partly by direct action of acid on G cells and partly by release of somatostatin.

**Cholecystokinin**

**Source**

Secreted from I cells in the mucosa of upper small intestine. CCK is present as neurotransmitter in cerebral cortex, in somatic nerves and in nerves of distal ileum and colon.

**Structure**

Cholecystokinin (CCK) is a polypeptide hormone. There are different forms of CCK depending on the number of amino acids present, like CCK 58, CCK 39, CCK 33, CCK 12, CCK 8, and CCK 4 (carboxyl terminal tetrapeptide). CCK secreted from duodenum and jejunum are usually CCK 12 and CCK 8. CCK in the enteric and pancreatic nerves is mainly CCK 4. The forms of CCK in brain are primarily CCK 58 and 8. Half-life of CCK is about 5 minutes.
**Chapter 37: Gastrointestinal Hormones**

**Functions**
1. CCK causes contraction of gallbladder. Therefore, CCK increases bile release into the intestine following a meal.
2. It stimulates pancreatic secretion rich in enzymes. Therefore, CCK is also called *cholecystokinin-pancreozymin* (CCK-PZ).
3. It also augments the action of secretin to produce alkaline pancreatic secretion.
4. It inhibits gastric acid secretion.
5. It inhibits gastric motility, thereby delays gastric emptying.
6. Causes relaxation of sphincter of Oddi that allows both bile and pancreatic juice to enter duodenum.
7. Stimulates growth of pancreas.
8. Increases secretion of enterokinase.
10. Stimulates colonic movements.
11. Causes contraction of pyloric sphincter. Therefore, prevents reflux of duodenal contents into the stomach.
12. Stimulates glucagon secretion.
13. In brain, it acts as an anorexigenic neurotransmitter. It inhibits food intake.
14. It also produces analgesia and anxiety.

**GI HORMONES OF SECRETIN FAMILY**

**Secretin**
Secretin has an important place in the history of endocrine physiology, as it was the *first hormone to be discovered* (Bayliss and Starling; 1902).

**Source**
Secretin is secreted from S cells located in the mucosa of upper part of small intestine.

**Structure**
Secretin is a polypeptide hormone containing 27 amino acids.

**Functions**
1. Secretin increases secretion of pancreatic juice rich in bicarbonate (watery and alkaline pancreatic secretion).
2. It also increases alkaline bile secretion.
3. Augments the action of CCK to produce pancreatic secretion rich in enzymes.
4. Decreases gastric acid secretion and motility.
5. Causes contraction of pyloric sphincter.

**Mechanism of Action**
Secretin acts on adenylate cyclase on the cell membrane and increases cytosolic formation of cAMP.

**Regulation of Secretion**
Secretion of secretin is increased by acidic chyme and products of protein digestion entering the upper part of intestine. Secretin stimulates watery and alkaline pancreatic secretion. When watery and alkaline pancreatic juice enters intestine, the acidic content of upper small intestine is neutralized. The increased pH of duodenal and upper jejunal content decreases secretin secretion by feedback mechanism (Flowchart 37.3).

**Flowchart 37.2:** Regulation of cholecystokinin secretion.

**Flowchart 37.3:** Regulation of secretin secretion.
Scientists contributed

Ernest Henry Starling (1866–1927) an English physiologist worked with his brother-in-law, Sir William Maddock Bayliss. Both of them had discovered the first hormone, Secretin. Starling is most famous for developing the “Frank–Starling law of the heart”, presented in 1915 and modified in 1919. In 1891, when he was 25, Starling married Florence Amelia Wooldridge, the widow of Leonard Charles Wooldridge, who had been his physiology teacher at Guy’s and died at the age of 32. She was a great support to Starling as a sounding board, secretary, and manager of his affairs as well as mother of their four children. To be noted, Bayliss was born on 2nd May and Starling died on 2nd May.

GIP

Structure

Glucose-dependent insulinotropic polypeptide (GIP) is a polypeptide hormone containing 42 amino acids. This is also called gastric inhibitory peptide (GIP).

Source

GIP is produced by K cells present in the mucosa of duodenum and jejunum.

Functions

1. It inhibits gastric secretion and motility.
2. It stimulates insulin secretion. For this function, GIP is considered as an important physiological regulator of insulin secretion. Though other hormones like gastrin, CCK, secretin and glucagon also stimulate insulin secretion, plasma insulin level of insulin in response to GIP resembles the concentration of insulin attained following oral glucose ingestion.
   - Therefore, GIP is called glucose-like insulinotropic polypeptide.
   - However, GLP-1, derived from glucagon appears to be more potent than GIP in promoting insulin secretion.
   - Hence, both GIP and GLP-1 are among the important physiological regulator of insulin secretion from beta cells of pancreas.

Regulation of Secretion

Secretion of GIP is increased by glucose and fat in the duodenum.

VIP

Structure

VIP is a polypeptide containing 28 amino acids. It is formed from prepro-VIP that contains both VIP and PHM-27, a closely related peptide.

Source

VIP is secreted from mucosal cells of entire GIT, starting from stomach to colon. However, the secretion is more in the colon. It is found in the nerves of GIT, other autonomic nerves, blood and also in brain. Its half-life is 2 minutes.

Functions

1. It markedly increases intestinal secretion rich in electrolytes and water. Therefore, in excess it produces watery diarrhea.
2. It causes vasodilation. Therefore, it decreases blood pressure.
3. It decreases GI motility. It causes relaxation of intestinal smooth muscle including sphincters.
4. It potentiates the action of acetylcholine on salivary glands.
5. It inhibits gastric acid secretion.

Clinical Significance

The tumor of VIP secreting cells is called VIPoma. Profuse watery diarrhea and hypotension are major features of VIPoma.

Glucagon

This is structurally similar with glucagon secreted from A cells of pancreas. In GIT, it is secreted from A cells in the stomach and L cells in intestine, where it is known as enteroglucagon. This produces hyperglycemia.

Glucagon-like Polypeptides

Glucagon is mainly produced from A cells in pancreas and L cells in intestine.

1. In A cells, preproglucagon is processed to form glucagon and major proglucagon fragments (MPGF), whereas in L cells it is processed to form glucagon, glicentin and glucagon-like peptides (GLP).
2. There are two GLPs: GLP 1 and GLP 2, and both are also produced in brain.
3. GLP 1 is a potent stimulator of insulin secretion.
4. GLP 2 does not have definitive biological activity.
5. However, GLP 2 produced in the brain inhibits food intake and acts as a neurotransmitter in the neurons that project from NTS to dorsomedial nucleus of hypothalamus.
6. Both A and L cells also produce oxyntomodulin and glicentin related polypeptide (GRPP).
7. Oxyntomodulin inhibits gastric acid secretion. Exact function of GRPP is not known.
MISCELLANEOUS FAMILY

Motilin

Structure and Source
This is a polypeptide hormone containing 22 amino acids. It is secreted from enterochromaffin cells and Mo cells present in the mucosa of all parts of GIT, except esophagus and rectum. It acts on G-protein coupled receptors on enteric neurons.

Functions
1. It causes contraction of intestinal smooth muscle and therefore, increases GI motility, especially in the interdigestive phase.
2. It is a major regulator of migrating motor complex (MMC), that regulates GI motility between meals.
3. Secretion of motilin is decreased following ingestion of a meal and its concentration remains low until the digestion and absorption of that meal is complete. Then the concentration increases and activates MMC that sweeps and cleans the intestine. Thus, it prepares the intestine for next meal.

Applied Physiology
Erythromycin can be used in patients having hypomotility of GI tract, as this antibiotic and its derivative bind to motilin receptors and facilitate intestinal motility.

Other Hormones

Neurotensin
This is a polypeptide hormone containing 13 amino acids. It is produced by neurons and mucosal cells of intestinal epithelium, mainly in ileum. It inhibits GI motility but increases ileal blood flow.

GRP
Gastrin releasing polypeptide (GRP) is a polypeptide containing 27 amino acids.
1. It is secreted from non-cholinergic vagal fibers.
2. It mediates gastrin release via non-cholinergic vagal stimulation.
3. The 10 amino acid residues at the carboxyl terminal of GRP is almost similar to the bombesin of amphibians.

Somatostatin
This is a polypeptide containing either 14 (SS 14) or 28 (SS 28) amino acids.
1. Somatostatin is secreted from GIT (starting from stomach to colon), hypothalamus and D cells of pancreas.
2. It inhibits gastrin secretion.
3. It also inhibits secretion of VIP, GIP, secretin, and motilin.
4. It is an inhibitory neurotransmitter in many parts of brain, especially in hypothalamus and pituitary.

Guanylin

Structure and Source
This is a polypeptide hormone containing 15 amino acids. It is secreted from the cells of intestinal mucosa. In human, it is produced by Paneth cells (endocrine cells located in the crypts of Lieberkuhn of small intestine).

Mechanism of Action
It acts by stimulating the activity of guanylyl cyclase (hence called guanylin) which increases the concentration of cGMP. The cGMP in turn increases activity of chloride channels and increases chloride secretion into the intestine.

Functions
Guanylin increases secretion of chloride ions into the intestinal lumen and therefore regulates fluid movement across intestinal tract. Guanylin receptors are present in kidney, liver and female reproductive tract. In these organs, guanylin appears to control fluid movement and particularly integrates the actions of intestine and kidneys.

Applied Physiology
Enterotoxins of some strains of E. coli that cause diarrhea have structural similarity with guanylin. They activate guanylin receptors in intestine and produce fluid secretion into the intestinal lumen.

TRH
This is structurally similar to the hypothalamic TRH. But, as it does not enter circulation, it does not produce any effect on thyroid. However, it is involved in the regulation of secretory immunity of intestine.

ACTH
Structurally, it is similar to the ACTH of anterior-pituitary. The function of intestinal ACTH is not clearly known.

Ghrelin
It is a 28 amino acid polypeptide secreted mainly from stomach. It has more systemic effects than local actions. It is a strong orexigenic agent that increases food intake by acting on arcuate nucleus of hypothalamus. It stimulates secretion of growth hormone from anterior pituitary.

Peptide YY
It is a polypeptide hormone secreted from small intestine and colon. It inhibits gastric secretion and motility.
Hence, it is proposed to be an effective gastric inhibitory peptide. Its secretion is stimulated by presence of fat in jejunum. Though, structurally it resembles neuropeptide Y that stimulates food intake, peptide YY inhibits feeding.

**Substance P**
Substance P is secreted from endocrine cells and neurons of entire GIT starting from stomach to colon. It increases intestinal motility. Its role in modulation of pain is discussed in “Physiology of Pain” in Sensory System.

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### CHAPTER SUMMARY

#### Key Concepts
1. Though of GI hormones have local effects, and they are called local hormones, many of them have systemic effects.
2. In addition to influence on GI secretion and motility, they affect food intake, metabolic functions, insulin and glucagon secretion, cardiovascular function and many brain functions.

**Important to Know (Must Read)**
1. In examinations, “Classify GI hormones and describe their functions” may come as a Long Question.
2. GI hormones of Gastrin family, GI hormones of secretin family, Gastrin, Cholecystokinin, Secretin, VIP, GIP, Enteroglucagon, Guanylin, Motilin, may come as Short Questions in exams.
3. In Viva, examiner may ask... Classification of GI hormones, structure and functions of any GI hormone, Applied aspect of any GI hormone.
4. GI hormones are usually asked in oral examination.
Secretions from exocrine glands associated with GI tract are called GI secretions. The major GI secretions include secretion from:
1. Salivary glands (salivary secretion)
2. Gastric glands (gastric secretion)
3. Exocrine pancreas (pancreatic secretion)
4. Liver (bile secretion)
5. Intestinal glands (intestinal secretion).

The major objectives of GI secretions are to facilitate digestion of food in the gut lumen, promote absorption of nutrients from the GI tract, and to assist in passage of luminal contents in aboral direction.

**PRINCIPLES OF GI SECRETION**

**Phases of GI Secretions**

Most of the GI secretions occur in three phases: cephalic, gastric, and intestinal. These phases are classified based on stimulus location.

**Cephalic Phase**

When stimuli activate the brain mechanisms (cephalic means “head”) to alter GI secretions, the phase is called cephalic phase.
1. **Sight, smell, and thought** of food initiates cephalic phase of secretion. **Taste of food** (food in mouth) and chewing of food that increase GI secretions are also stimuli for cephalic phase.
2. Various **emotional states** also activate cephalic phase of secretions.
3. The efferent pathway is the **vagus nerve** that activates neurons in the ENS, which in turn influence gastric secretion and motility.

**Physiological Importance of Cephalic Phase**

Cephalic phase is important for salivary, gastric, pancreatic, and bile secretions.
1. It accounts for about 90% of volume of salivary secretion. It contributes to about 40% of gastric and pancreatic secretions. This simply indicates that GI tract prepares for digestion even before food enters into stomach and intestine.
2. In fact, sufficient GI secretions **accumulate in advance** in this phase so that the process of digestion starts as soon as food arrives in the gut, especially in the intestine.
3. Therefore, **it is desirable to spend some time in taking soup and starters before taking a major meal.**
Physiological Importance of Gastric Phase
Distension of stomach is the major mechanical event of the gastric phase.
1. **Gastric distension** is the primary regulator of satiety.
2. It also greatly influences digestion of food by controlling GI secretions.
3. Gastric distension along with acidic chyme in the stomach and gastrin secreted from stomach, activate short and long GI reflexes to influence gastric, pancreatic, biliary, and intestinal secretions.
4. These secretions control the processes of digestion and absorption.

Intestinal Phase
Intestinal phase is initiated when chyme enters duodenum.
1. Products of digestion (fatty acids, peptides, etc.), presence of acidic chyme, intestinal distension and osmolality of intestinal contents are important stimuli in the intestinal phase of GI secretions.
2. They activate various intestinal reflexes to alter GI secretions.
3. CCK, secretin, GIP, VIP, and many other hormones are secreted during this phase.

Physiological Importance of Intestinal Phase
Chyme in the intestine requires enzymes for digestion of food particles in which the first step is to hydrolyze macromolecules into their smaller absorbable forms. This is achieved mainly by pancreatic and intestinal secretions.
1. Acidic chyme in duodenum stimulates secretion of CCK and secretin from upper part of small intestine that profoundly influence pancreatic and bile secretions.
2. Through reflex mechanisms, food in intestine inhibits gastric secretion and motility.

Principles of Regulation
Regulation of GI secretions occurs mainly by neural and hormonal factors. However, mechanisms specifically controlling these regulations depend on the phase of GI secretion.
1. For example, cephalic phase is predominantly regulated by neural mechanisms, whereas gastric and intestinal phases are regulated by both hormonal and neural mechanisms.
2. Accordingly, tests detecting the integrity of GI secretions are different for different phases.

General Functions of GI Secretion
1. **Digestion:** Enzymes in GI secretion are key elements for digestion of food particles in the GI tract. Broadly, these enzymes are amylases that digest carbohydrates, peptidases that digest proteins and lipases that digest lipids.
2. **Protection from infections:** Chemicals present in GI secretions protect the body from infections. For example, lysozyme in secretions is antibacterial, IgA in saliva provides local immunity and HCl in gastric secretion kills all types of organisms.
3. **Mixing:** The aqueous part of secretions help in mixing of food with chemicals and enzymes in the secretions. This is essential for thorough exposure of food particles with chemicals for proper digestion of each ingredient in the food.
4. **Lubrication:** Mucus secretions facilitates passage of bolus of food or chyme along the GI tract in aboral direction. This especially helps in propulsion of food.
5. **Protection of mucosa:** The mucus and alkaline pH of secretions protect mucosal epithelium of gut from the harmful effects of HCl, bile acids, and toxins in the food.
6. **Absorption:** GI secretions present digested food materials to the epithelium of GI tract for absorption. Many food particles are dissolved and carried in GI secretions to the absorptive surface of the GI tract for absorption into blood and lymph.
7. **Appetite regulation:** Chemicals in GI secretions stimulate appetite. For example, bile acids and HCl are appetitizers in physiological concentration. Therefore, patients with achlorohydria (gastric atrophy) and bile deficiency (liver disease) develop anorexia. However, in high concentration these chemicals may also inhibit appetite.

**SALIVARY SECRETION**
The secretion from the salivary glands is called salivary secretion. It is secreted from a heterogeneous group of salivary glands located in and around the mouth cavity. Salivary secretion is distinctive for its exclusive neural regulation.

**Scientists contributed**

*Niels Stensen (1638–1686)* was a prominent Danish scientist who laid the foundations of paleontology, geology, and crystallography. Though he is known eponymously for the discovery of the duct of the parotid gland (Stensen’s duct) and study of salivary secretion, he researched more on the anatomy of the brain, and did seminal investigation on methods in neuroscience. His scientific letter on a hydrocephalic calf represents an early pathophysiological investigation on hydrocephalus. Source: Perrini P, Lanzino G, Parenti GF. Niels Stensen (1638–1686): scientist, neuroanatomist, and saint. Neurosurgery. 2010 Jul;67(1):3-9; doi: 10.1227/01.NEU.0000379610.79429.2B.

*Thomas Wharton (1614–1673)*, a great physician from Cambridge, received fellowship of Royal College of Physicians for his extensive studies on salivary and pancreatic secretions. Wharton described the glands more accurately relying on his dissection and experiment. He was the discoverer of the duct of the sub-mandibulary gland for the conveyance of the saliva into the mouth, which bears his name (Wharton’s duct). He made a special study of the minute anatomy of the pancreas.
Salivary Glands

Types
There are major and minor salivary glands.
1. Major salivary glands are three pairs: parotid, sublingual and submandibular glands.
2. There are many minor salivary glands located in the mucosa of oral cavity, at the pharyngeal outlet, in the palates and in buccal pouches.

Based on nature of secretion: Salivary glands may be serous that exclusively release watery secretions, mucous that secrete viscous secretion mainly containing mucus and mixed that secrete moderately viscous secretions.

Parotid Glands
Parotid glands are the largest salivary glands.
1. They are entirely serous glands. Serous cells are usually arranged in the form of rounded acini.
2. The parotid secretion is rich in water and electrolytes, and lacks mucins.
3. They pour their secretion into the mouth cavity by means of parotid duct (duct of Stensen), which opens into the oral cavity at the level of second molar tooth (Fig. 38.1).

Sublingual Glands
These glands are situated below the tongue in the floor of the mouth.
1. Secretions from these glands drain directly into the mouth by means of sublingual ducts (ducts of Rivinus).
2. There are about 10 sublingual ducts. Sublingual glands are predominantly mucous glands.
3. They secrete thick viscous saliva.

Submandibular Glands
Submandibular, also called submaxillary glands are situated below the inner ramus of mandible on both sides.
1. They pour their secretion into the mouth cavity by means of submandibular ducts (ducts of Wharton).
2. These glands are mixed type (both serous and mucous).

Histology of Salivary Glands
Salivary gland consists of base units called salivon. Each salivon consists of acinus, intercalated duct and striated duct (Fig. 38.2).

Acinus
Acinus is a sac like structure containing many pyramidal cells. Myoepithelial cells are present at places surrounding the pyramidal cells.
1. Serous cells of acinus contain many endoplasmic reticulum and zymogen granules, and secrete digestive enzyme, whereas mucous cells contain mucin droplets and secrete mucin (Fig. 38.3).
2. The secretion in the acinus is called primary secretion. Electrolyte composition of primary secretion is similar to that of plasma.
Intercalated and Striated Ducts

These ducts are lined by cuboidal cells. Secretion from these duct epithelial cells modifies the ionic composition of secretion from acinus and determines the final composition of saliva. Secretion coming out of duct is called modified or final secretion.

Innervation of Salivary Glands

Salivary glands are innervated by both the divisions of autonomic nervous system. In normal situation, parasympathetic innervation is the major neural factor for salivary secretion.

Parasympathetic Innervation

The centers for parasympathetic fibers are located in medulla. 1. Preganglionic fibers for parotid gland are present in 9th cranial nerve that originate in inferior salivary nucleus and terminate in otic ganglion from where postganglionic fibers originate and innervate the gland (Fig. 38.4).
2. Fibers for submandibular and sublingual glands are present in the 7th cranial nerve that originate from superior salivary nucleus and terminate in submandibular ganglion from where postganglionic fibers come out and supply the glands (Fig. 38.5).
3. In general, parasympathetic stimulation is excitatory.

Sympathetic Innervation

Sympathetic fibers originate from upper cervical segments and terminate in superior cervical ganglion. Postganglionic fibers leave the ganglion and innervate acini, duct and blood vessels. In general, sympathetic stimulation is inhibitory.
**Salivary Blood Flow**

Salivary glands have a high rate of metabolism and a high blood flow. Blood flow and metabolism are proportionate to the rate of saliva formation.

1. The rate of salivary secretion in human is about 50 mL/min/100 g of salivary tissue.
2. Blood flow to salivary glands is about 10 times the blood flow to that of active skeletal muscles.
3. Stimulation of parasympathetic nerve to salivary gland increases blood flow by about 10 times by causing vasodilation, which occurs due to secretion of VIP, bradykinin, and acetylcholine at nerve terminals.
4. Sympathetic stimulation decreases blood flow.

**Salivary Secretion**

**Rate of Secretion**

About 1.5 liters (1,000–1,800 mL) of saliva is secreted every day. Normally, we never realize the amount of saliva secreted as it is a continuous and slow process. Salivary secretion even continues in minimum amount during sleep. However, an orexigenic stimulus, especially sight, smell or thought of food causes immediate and profuse salivation. We realize the importance of salivation when the secretion becomes less and mouth becomes dry. Salivary secretion increases in response to feeding.

**Composition of Saliva**

Saliva contains mainly water (99.5%) and some solids (0.5%). Solids are organic and inorganic.

**Organic Solids**

Organic solids are mainly enzymes such as ptyalin, lysozyme, lactoperoxidase, carbonic anhydrase, lingual lipase, RNAase, and DNAase. Other organic solids include kallikrein, blood group substances, secretory immunoglobulin (IgA), and nerve growth factor.

**Inorganic Solids**

Cations like sodium, calcium, potassium, and magnesium ions, and anions like chloride, bicarbonate, phosphate, sulfate, and bromide ions constitute the inorganic solids.

**Tonicity of Saliva**

In human beings, saliva is always hypotonic to plasma. The concentration of sodium and chloride ions in saliva is less than that of plasma. The tonicity of saliva depends on the rate at which saliva is produced. Greater the rate of secretion, higher is the tonicity. The tonicity of saliva is about 70% of that of plasma.

**The pH and K+ Content of Saliva**

The pH of saliva is alkaline (about 8).

1. The pH of the original secretion in the salivary gland is slightly acidic. But as saliva flows down the salivary ducts, it becomes alkaline because of secretion of bicarbonate from the duct epithelium.
2. The decrease in flow rate in duct increases bicarbonate concentration as time to add more bicarbonate increases, and therefore, this increases pH.
3. However, if the increase in flow is due to parasympathetic stimulation, secretion of bicarbonate from duct cells is more that increases salivary content of bicarbonate.
4. Therefore, invariably with increased flow rate, bicarbonate content becomes high.
5. The concentration of K+ in saliva is always greater than that in plasma, but with increase in flow rate, K+ concentration decreases.

**Functions of Saliva**

Saliva performs many important digestive and non-digestive functions.

1. Saliva contains ptyalin. This is an enzyme called salivary amylase, which causes splitting of starch. Salivary amylase reduces starch to oligosaccharide molecules. However, digestion of starch is not impaired in the absence of salivary amylase (if pancreas is intact). Action of salivary amylase is maximum at pH 6.8. But, digestion by ptyalin takes place in the stomach, as food remains for a short duration in mouth. In the stomach, digestion occurs at the center of food bolus which is still alkaline, until the food is thoroughly mixed with the acidic gastric secretion of the stomach.
2. Saliva has many protective functions. Saliva keeps the mouth clean and therefore prevents oral infection. Saliva contains lysozyme, which is anti-bacterial. IgA in saliva provides local immunity and lactoferrin in saliva is bacteriostatic.
3. Saliva keeps the mouth cavity and tongue moist, which facilitates speech. The importance of this function of saliva is realized when mouth becomes dry due to decreased salivary secretion that impairs speech. Dryness of mouth is usually felt before appearing for an interview, especially for learners.
4. Saliva increases the taste of food. Taste is perceived by the taste buds present in the tongue. For taste of food to be well appreciated, food particles should better be present in solution. Saliva dissolves the food and makes the solution of food.
5. Saliva helps in mastication and swallowing. This is due to lubricant property of saliva. The mucin in saliva lubricates food. In the absence of saliva, deglutition becomes impossible, especially for dry foods.
6. Saliva contains bicarbonate which buffers gastric acid to some extent in the stomach, and therefore reduces heart burn.
7. Proline rich protein in saliva prevents enamel of the teeth and thus provides them strength. Proline also binds with tannin & reduces its toxicity.
8. Saliva prevents injury to buccal mucosa by diluting the hot and irritant food materials.
9. In animals, salivation (panting) is an important process of dissipation of heat and therefore, has contribution in temperature regulation.

10. Saliva excretes certain heavy metals, thiocyanate and morphine from the body.

**Mechanism of Secretion**

Salivary secretion occurs in two stages: secretion in the acinus and secretion in the duct.

**Secretion in Acinus of Gland**

In the gland acini, the secretion is called primary secretion in which amylase concentration is more.

1. The enzyme is produced and stored in the zymogen granules.
2. On stimulation, granules are exocytosed and the enzyme is released.
3. The electrolytes secreted from the epithelial cells of the end pieces are Na\(^+\), K\(^+\), HCO\(_3\)\(^-\), and Cl\(^-\).
4. The fluid of primary secretion is almost isotonic to that of plasma.

**Secretion in Ducts**

The composition of secretion in the ducts when the fluid passes through the intercalated and striated ducts, is modified. This is called modified or final secretion.

1. This is because Na\(^+\) and Cl\(^-\) are extracted from the fluid and K\(^+\) and HCO\(_3\)\(^-\) are added to the fluid.
2. The ducts do not change the volume of saliva but only modify the composition of the primary secretion (Fig. 38.6).

**Stimuli for Salivary Secretion**

Saliva is continuously secreted. However, a large number of stimuli increase the secretion. The important stimuli are anticipation, thought, sight and smell of food, discussion on food, and presence of food in the mouth cavity. Chewing is also an important stimulus for salivary secretion.

**Control of Salivary Secretion**

Salivary secretion is controlled exclusively by the neural mechanisms. Both sympathetic and parasympathetic stimuli influence salivary secretion.

**Neural Control**

**Parasympathetic Stimulation**

Parasympathetic stimulation occurs by sight, smell, and taste of food.

1. Chewing of food is a potent stimulus for salivary secretion. These factors act through salivary nucleus in the medulla (Flowchart 38.1).

Flowchart 38.1: Mechanism of increased salivary secretion by parasympathetic stimulation.

(CN: Cranial nerve).
2. Parasympathetic activation increases salivary secretion rich in enzymes, and mucin.
3. It increases secretion by causing vasodilation (via bradykinin, VIP, and acetylcholine), and stimulation of glandular tissue.

**Sympathetic Stimulation**
Stimulation of sympathetic fibers (sympathetic fibers to salivary gland originate from superior cervical ganglion) temporarily increases secretion but finally decreases it.
1. The *transient increase* is due to contraction of myoepithelial cells of the glandular tissue.
2. However, sympathetic stimulation causes vasoconstriction that decreases salivation and makes the secretion thick.

**Reflex Secretion**
Saliva is secreted reflexly by contact of food with the mouth cavity. This reflex secretion is *unconditioned* as this is present since birth (does not need learning). However, salivary secretion due to smell or thought of food is a *condition reflex* as these are learnt by social and environmental factors. Conditioned salivary secretion was described by Pavlov.

Salivary secretion exclusively occurs in the **cephalic phase** (sight, smell and thought of food, presence of food in mouth, chewing food, etc.). Secretion is almost nil in gastric and intestinal phase of digestion of food. Hormonal regulation of saliva is negligible.

**CHAPTER SUMMARY**

**Key Concepts**
1. Though salivary secretion does not help much in digestion, it is very essential for chewing, deglutition, speech and oral and dental hygiene.
2. Parasympathetic stimulates and sympathetic inhibits salivary secretion.
3. Xerostomia (dryness of mouth) is a common in acute stressful situation that happens due to sympathetic stimulation.

**Important to Know (Must Read)**
1. In examinations, “Composition, mechanism and regulation of salivary secretion” may sometimes come as a Long Question.
2. Phases of GI secretions, Salivary secretion, Composition and function of salivary secretion, Mechanism of salivary secretion, Regulation of salivary secretion.
3. In Viva, examiner may ask… What are the Phases of GI secretions and how are they generally controlled, Amount of salivary secretion/day, Name of salivary glands, Histologic types of salivary glands, Innervation of salivary glands by parasympathetic and sympathetic fibers, Composition and function of salivary secretion, Function of each constituent of saliva, Mechanism of salivary secretion, What are the differences between primary and secondary salivary secretion, Regulation of salivary secretion, What are the effects of parasympathetic and sympathetic stimulation, What is Xerostomia, silaorrhea and sialolithiasis.
4. Composition and functions of saliva are usually asked in oral examination. A student is expected to answer this; otherwise it may be difficult for him to pass.

**Applied Physiology**

**Xerostomia**
This is a condition in which there is consistent decreased secretion of saliva. This causes dryness of mouth, and predisposes to oral infections. Xerostomia (dryness of mouth) is a common in acute stressful situation that happens due to sympathetic stimulation.

**Sialorrhea**
In this condition, salivary secretion is increased persistently.

**Sialolithiasis**
This is the condition in which stone is formed in the ducts of salivary gland.

**Infections**
Viral infection of parotid gland is seen commonly in children (mumps).
Stomach is an important part of GI system. Though the major function of stomach is the storage of food, it regulates controlled emptying of food into the duodenum and prepares the chyme for digestion and absorption in the small intestine. Chyme results from grinding of food into smaller particles and mixing of food with gastric secretion.

FUNCTIONS AND FUNCTIONAL ANATOMY OF STOMACH

**Functions of Stomach**

1. **Temporary storage of food:** The primary function of stomach is the temporary storage of food. It serves as a **reservoir for food** so that ingestion of a large meal at a time becomes possible.
2. **Grinding and mixing of food:** Stomach causes grinding and mixing of food. It churns the food and breaks larger food particles (macromolecules) into smaller molecules. It also mixes food with gastric juice. The content of stomach after proper mixing with gastric juice is called **chyme.**
3. **Controlled emptying of food:** Stomach performs its **own controlled emptying (gastric emptying).** It allows food to enter into duodenum and jejunum at a lesser but controlled rate so that load on the upper part of the small intestine is not more than the amount that it can handle. This allows intestine to digest and absorb nutrients slowly, steadily and adequately.
4. **Secretion of hydrochloric acid:** Stomach secretes hydrochloric acid (HCl), which is essential for digestion of food. Though the exact nature of digestion by HCl is not clearly known, hypochlorohydria decreases the process of digestion and impairs appetite.
5. **HCl in the stomach converts ferric form of iron into ferrous form.** Iron is absorbed from gut only in the ferrous form. Therefore, **HCl deficiency in the stomach results in iron deficiency anemia.**
6. **Kills the microorganism:** Gastric HCl kills the bacteria and other ingested microorganisms. Therefore, entry of microbes into body through GI system is prevented in the stomach.
7. **Activates pepsinogen to pepsin:** HCl activates pepsinogen to pepsin, an **endopeptidase.** Pepsin cleaves protein molecules to form smaller peptides. The optimal pH
for endopeptidase activity is 1.8 to 3.5. Thus, HCl not only activates pepsin, but also provides acidic environment for action of pepsin.

8. **Secretes intrinsic factor:** Stomach secretes intrinsic factor (IF) which is essential for reabsorption of vitamin B\(_{12}\) (the extrinsic factor) in the terminal ileum. Vitamin B\(_{12}\) binds with intrinsic factor to form IF-B\(_{12}\) complex, which is taken up by cubilin, a lipoprotein present in the terminal part of ileum and then absorbed by endocytosis. Therefore, gastrectomy or chronic gastric atrophy results in megaloblastic anemia (Clinical Box 39.1).

9. Stomach helps in absorption of water and lipid soluble substances, such as alcohol and few drugs.

**Clinical Box 39.1**

**Dimorphic anemia:** When red cell morphologies in an anemia have both microcytic and macrocytic features, the type of anemia is called dimorphic anemia. Anemia following gastrectomy or chronic gastric atrophy results in such type of anemia, as HCl deficiency causes microcytic hypochromic anemia due to iron deficiency and intrinsic factor deficiency causes megaloblastic anemia due to B\(_{12}\) deficiency.

**Functional Anatomy**

Anatomically, stomach is divided into three major parts: fundus, body and antrum (Fig. 39.1). Esophagus opens into stomach through gastro-esophageal junction that contains lower esophageal sphincter (LES). The initial portion of the stomach close to gastroesophageal junction is called cardia. The proximal part of stomach is called fundus, the middle and major part of stomach is the body or corpus, and the distal portion of the stomach is the antrum. Antrum opens into the duodenum through pylorus which contains pyloric sphincter:

1. The capacity of the stomach depends on age, gender and eating habit. On average, it is about 1.5 liter in adults, though it varies from 1 to 4 liters.
2. The mucous membrane of stomach is thick and thrown into large folds, called gastric rugae. These rugae are more prominent in empty stomach (Fig. 39.2).
3. The mucosal epithelium is formed by simple columnar epithelial cells that secrete mucous and alkaline fluid. These mucous and alkaline fluids protect gastric epithelium from acidic content and mechanical injury.
4. The gastric glands are situated deep in the mucosal infoldings that open into the pits.

**Gastric Glands**

The mucosal lining of the stomach is a glandular mucosa that contains surface mucous cells in the gastric pit and glands deep in the mucosal infoldings. There are three types of gastric glands:

1. **The cardiac glands:** Located below the lower esophageal sphincter and contain mainly mucous secreting cells. They secrete mucous and bicarbonate ions.
2. **The oxyntic glands:** Located in the fundus and body of the stomach and contain mainly the oxyntic cells.
3. **Pyloric glands:** Present in the pyloric-antral region and consist mainly of mucous neck cells that secrete mucous and G cells that secrete gastrin.

**Oxyntic Gland**

The acid secreting oxyntic gland is typically a tubular and straight gland (Fig. 39.4). It consists of neck, body and base. The cells in the neck are mainly mucous secreting cell. The oxyntic cells are present mainly in the body of the gland. Chief or peptic cells are present at the base.
Different cell types in the oxyntic glands are:

1. **Mucous cells**: They are present in the neck region of the gland. The mucous and bicarbonate ions secreted by them protect the stomach epithelium from acidic gastric secretion.

2. **Oxyntic or Parietal cells**: Oxyntic cells are present mainly in the body part of the gland. They secrete hydrochloric acid and intrinsic factor.

3. **Chief or Peptic cells**: Chief cells are present towards the base of the gland. They secrete pepsinogen.

4. **Endocrine cells**: The endocrine cells are also called enterochromaffin cells. The major endocrine cells in oxyntic glands are enterochromaffin-like cells (ECL) or mast-like cells that secrete histamine. There are also other endocrine cells that secrete somatostatin, VIP, glucagon, and enkephalin. **G cells** are present in antral region that secrete gastrin. Antrum also contains **D cells** that secrete somatostatin.

**Structure of Oxyntic Cells**

Oxyntic cells or parietal cells are present in the body and neck of the oxyntic glands. They secrete hydrochloric acid and intrinsic factor:

1. They have extensive **tubulovesicular system** and open **canalicular system**.

2. The apex of the parietal cells faces towards the lumen of the gastric glands. The canaliculi extend from apical surface into the cell. The tubules and vesicles open into the canaliculi.

3. **In resting cells**, tubules and vesicles are present abundantly (Fig. 39.5) and microvilli are rudimentary. The membrane of these tubulovesicular structures contains $H^+\cdot K^+\text{ATPase}$.

4. **On activation**, the tubulovesicular membranes fuse with the cell membrane and microvilli that project into the canaliculi (Fig. 39.6), so that the **area of cell membrane in contact with gastric lumen is greatly increased**.

5. Fusion of tubules and vesicle with microvillar or canalicular membrane ensures **increased $H^+\cdot K^+\text{ATPase}$ activity of the membrane** in the active state.

**Nerve Supply of Stomach**

Stomach is supplied by both divisions of ANS:

1. **Parasympathetic innervation** is by vagus nerve.

2. **Vagal stimulation facilitates** and sympathetic stimulation inhibits gastric secretion and motility.

**Gastric Juice**

**Composition of Gastric Juice**

The amount of gastric secretion per day varies from 1 to 2.5 liters. The gastric juice is **highly acidic**, having pH 0.7–4. It has following constituents:
1. Water (99.5%)
2. Solids (0.5%)
   Solids contain inorganic and organic substances.
   **Inorganic constituents:** Anions are Cl\(^-\), PO\(_4\)\(^3-\), SO\(_4\)\(^2-\), and HCO\(_3\)\(^-\); and cations are H\(^+\), Na\(^+\), K\(^+\), Ca\(^{2+}\), and Mg\(^{2+}\).
   **Organic constituents:** Pepsinogen, intrinsic factor, mucin, rennin, gastric lipase, gelatinase, carbonic anhydrase, and lysozyme.

**Functions of Constituents**

Normal concentration of HCl is 40–60 mEq/L. The maximum concentration can increase up to 150 mEq/L:

1. **Pepsinogen** is secreted from the peptic cells. There are two types of pepsinogens:
   - **Type-I pepsinogen** is found in chief cells in fundus and body.
   - **Type-II pepsinogen** is found in the chief cells throughout the stomach.

2. Pepsinogen is converted into pepsin by HCl. Pepsin is a proteolytic enzyme that breaks down protein molecules into peptones. Pepsin acts best at pH of 2–4.

3. The **mucin** secreted by mucous cells is of two types: the insoluble mucin, and the soluble mucin. Mucin forms a protective layer on the gastric epithelium. It retains bicarbonate and has alkaline pH. Thus, it protects the stomach from acid peptic digestion, as it buffers HCl.

4. **Intrinsic factor helps in vitamin B\(_{12}\) absorption** from terminal ileum.

**GASTRIC SECRETION**

**Mechanism of HCl Secretion**

HCl is secreted from **parietal cells** that are located in the fundus and body of the stomach. H\(^+\) secretion is possible in stomach due to the presence of numerous **H\(^+\)-K\(^+\) ATPases** in apical membrane of the parietal cells:

1. H\(^+\)-K\(^+\) pump actively pumps H\(^+\) (against its concentration gradient) out of the cell into the gastric lumen. In exchange for H\(^+\), the K\(^+\) enters the cell (Fig. 39.7). As this is an active transport, energy is provided for the process by hydrolysis of ATP.

2. In the cytosol of parietal cell, H\(^+\) is derived from the breakdown of carbonic acid (H\(_2\)CO\(_3\)). H\(_2\)CO\(_3\) is formed by combination of CO\(_2\) and H\(_2\)O in a reaction catalyzed by **carbonic anhydrase**. This step can be blocked by acetazolamide, the carbonic anhydrase inhibitor.

3. CO\(_2\) utilized for formation of H\(_2\)CO\(_3\) is derived from intracellular metabolisms and plasma.

4. HCO\(_3\)\(^-\) formed by break down of H\(_2\)CO\(_3\) is exchanged for Cl\(^-\) on the basolateral membrane of the cell by **HCO\(_3\)\(^-\)-Cl\(^-\) exchanger**.

5. The Cl\(^-\) that enters parietal cell is transported into the gastric lumen.

6. In the lumen, Cl\(^-\) combines with H\(^+\) to form HCl.

7. The **HCO\(_3\)\(^-\) enters blood stream** from the interstitial fluid.

   Thus, for each H\(^+\) secreted into the gastric lumen, one HCO\(_3\)\(^-\) is reabsorbed into the plasma. Therefore, with **increase in the rate of HCl secretion**, HCO\(_3\)\(^-\) level increases in the blood. Consequently, following a meal that stimulates gastric acid secretion, pH of blood increases. As blood becomes alkaline, the urine becomes alkaline. This is called **post-prandial alkaline tide**.

K\(^+\) that enters the oxyntic cell by H\(^+\)-K\(^+\) ATPase is transported back into the gastric lumen, which is **reutilized** for further H\(^+\) secretion (recycling of K\(^+\)). K\(^+\) entering the
Secretion of Other Constituents

Pepsinogen Secretion

Pepsinogen is secreted from chief cells. It is synthesized in the cell like other proteins and stored in the zymogen granules:

1. There are two types of pepsinogens: **type-I pepsinogen**, found in chief cells in fundus and body and **type-II pepsinogen** found in the chief cells throughout the stomach.
2. Pepsinogen is converted into pepsin by HCl.
3. Pepsin is a strong proteolytic enzyme.
4. Pepsinogen secretion is stimulated by gastrin and histamine.

Mucus Secretion

Mucus is secreted by mucus secreting cells that are plentiful available in the neck region of gastric glands:

1. The **mucin** secreted by mucous cells is of two types: the **insoluble mucin**, which is secreted by mucus secreting cells of entire gastric mucosa and the **soluble mucin**, secreted from mainly cardiac and pyloric mucosal cells.
2. Mucin forms a protective layer on the gastric epithelium. It retains bicarbonate and has alkaline pH. Thus, it protects the stomach from acid peptic digestion, as it buffers HCl.
3. Mucus secretion is stimulated by increased blood flow to the stomach.

Intrinsic Factor Secretion

Intrinsic factor (IF) is secreted from parietal cells along with HCl. It is a glycoprotein:

1. It is synthesized like other glycoproteins and its secretion is stimulated by factors that stimulate HCl secretion like histamine and gastrin.
2. IF helps in vitamin B₁₂ absorption from terminal ileum.

Factors that Influence HCl Secretion

Factors that Stimulate Gastric Acid Secretion

Factors that increase HCl secretion from the parietal cells of stomach mainly act through locally altering the concentration of three hormones: acetylcholine, gastrin and histamine. In the parietal cells, there are specific receptors for these hormones and other hormones. Hormones bind with the receptors and change the intracellular second messenger concentration that finally stimulate HCl secretion (Fig. 39.9).

Acetylcholine

Acetylcholine is an effective stimulator of gastric acid secretion. It is released at the nerve endings of **vagal cholinergic fibers** that innervate parietal cells:

1. Acetylcholine acts on the **M₃ cholinergic receptors** on the parietal cells and increases intracellular Ca²⁺.
2. It acts directly on the parietal cell to increase HCl secretion and acts indirectly by secreting histamine and gastrin from ECL cells and G cells respectively that in turn stimulate parietal cells.

Gastrin

Gastrin is the **most potent stimulus for HCl secretion**:

1. Gastrin acts on **gastrin receptors** on the parietal cells and increases HCl secretion by **increasing intracellular Ca²⁺** (Fig. 39.8).
2. Gastrin is secreted from G cells that are present in the antral mucosa of the stomach.
3. Vagal fibers innervate G cells and vagal stimulation increases gastrin release. However, vagal fibers that mediate gastrin release are **non-cholinergic** (Fig. 39.9) as the neurotransmitter is GRP (**gastrin releasing peptide**).
4. Gastrin also stimulates histamine release from ECL cells that in turn increases secretion of HCl from parietal cells. Gastrin secretion from stomach is increased by gastric distension, noncholinergic vagal stimulation, protein rich food, and catecholamines. **Pentagastrin** is used for assessing gastric acid output (Application Box 39.1).
Application Box 39.1

Pentagastrin test; and Gastrectomy: As gastrin is the most potent stimulator of HCl secretion, exogenously administered synthetic gastrin (pentagastrin) assesses the degree of acid output from parietal cell mass of the stomach. This forms the physiological basis of pentagastrin test. As G cells are present in antral part of stomach and gastrin is the strong stimulus for parietal cells, antrectomy (partial antral gastrectomy) is performed for surgical treatment for protracted peptic ulcer. It is useful in patients with protracted peptic ulcer.

Histamine

Histamine is a powerful stimulator of HCl secretion from stomach:
1. It acts on H2 receptors on the parietal cells and increases intracellular cyclic AMP as second messenger. Cyclic AMP stimulates protein kinase, which increases the activity of H+–K+ ATPase and HCl secretion.
2. Histamine is secreted from enterochromaffin-like (ECL) cells.
3. Histamine release is stimulated by both acetylcholine and gastrin.
4. Thus, histamine is considered as a major mediator of HCl secretion. Therefore, patients with peptic ulcer are usually first treated with histamine type-2 receptor antagonists.

Mechanical and Chemical Factors

Accumulation of food in the stomach increases acid secretion. This mainly occurs due to mechanical distension that stretches G cells and stimulates gastrin release. Also, products of protein digestion (peptides and amino acids) increase gastrin secretion, and hot and spicy foods facilitate HCl secretion from the stomach (Table 39.1).

Factors that Inhibit Gastric Acid Secretion

Increased acid output, somatostatin and acidic content of duodenum decrease gastric acid secretion.

pH of Gastric Luminal Content

Decreased pH of gastric content is an important and natural inhibitor of HCl secretion:
1. When secretion of acid is high enough to decrease pH of gastric content to below 2, secretion of HCl is inhibited by negative feedback mechanism. Hence, this is called as autoregulation of acid secretion.
2. The highly acidic gastric pH does not inhibit parietal cell directly; rather inhibition of acid secretion is mediated by gastrin and somatostatin.
3. The highly acidic chyme directly inhibits gastrin secretion from G cells, and stimulates somatostatin secretion from D cells of stomach.
4. Somatostatin inhibits secretion of gastrin from G cells that decreases acid secretion.

Somatostatin

Somatostatin is secreted from the D cells of the gastric mucosa:
1. Decreased pH of gastric content (pH less than 2) increases the secretion of somatostatin, which inhibits gastrin release.
2. As gastrin is the most potent stimulator of acid secretion, decreased gastrin release decreases HCl secretion from the stomach.
**Chyme in the Duodenum**

When acidic chyme enters duodenum, **secretin** is secreted from upper intestinal mucosa:

1. **Secretin inhibits gastric secretion** and motility, and gastrin release from G cells.
2. Products of carbohydrate and lipid digestion in the duodenum, and hyperosmolality of duodenal content inhibit acid secretion by increasing the release of an **enterogastrone** called GIP (**gastric inhibitory peptide**).
3. There are other enterogastrones also that inhibit gastric secretion.

**Regulation of Gastric Secretion**

Gastric secretion is regulated by neural and humoral mechanisms. The mechanism of regulation depends on the phase of gastric acid secretion. The **neural mechanisms** are autonomic influences, short and long GI reflexes, and central influences mediated by vagus nerve. The **hormonal mechanisms** regulating gastric secretion are discussed above.

Gastric secretion occurs in **three phases** (cephalic, gastric and intestinal) and the mechanisms regulating secretion are different for each phase of secretion.

**Cephalic Phase**

The cephalic phase of gastric secretion is elicited by smell, sight, thought, taste and chewing of food. This is called cephalic phase as impulses to increase acid secretion originate mainly in the brain:

1. The sensory stimuli activate dorsal motor nucleus of vagus in the medulla. Therefore, cephalic phase of gastric secretion is **entirely mediated by vagus nerve** and the fibers are both **cholinergic and noncholinergic** (see Fig. 39.9).
2. The vagal fibers that directly contact parietal cells are cholinergic and fibers that contact G cells are **noncholinergic** (**neurotransmitter is GRP**).
3. As noncholinergic effects are stronger than cholinergic effects, **atropine can not effectively prevent vagally mediated acid secretion**. Therefore, atropine is not prescribed in the management of peptic ulcer.

The gastric juice secreted in the cephalic phase occurs before food reaches the stomach. Therefore, this is also called **pregastric phase**. This phase accounts for 40–50% of total gastric secretion (Application Box 39.2).

**Experimental Design to Study Cephalic Phase**

The usual experimental design to assess the integrity of the cephalic phase is the **sham feeding**. In this procedure, a dog is taken as the experimental animal:

1. A fistula is made in the esophagus of the dog; so that when animal eats the food comes out of the neck **through the fistula** (food is not allowed to reach stomach). Simultaneously, the gastric juice is collected from the stomach by placing a cannula into it (Fig. 39.10). Gastric juice obtained during the cephalic phase is analyzed for volume and composition.
2. Then, **bilateral vagotomy** is performed and the gastric juice is collected following vagotomy for analysis.
3. **Vagotomy abolishes gastric secretion during cephalic phase**, which proves that this phase is primarily vagally mediated.

**Application Box 39.2**

**Appetite juice**: The gastric juice secreted during cephalic phase stimulates the appetite, and therefore, this is called **appetite juice**, as it occurs in anticipation of food. Usually in parties and special dinners, enough time is spent initially in taking soup and appetizers before actual dinner is served. This is meant to stimulate appetite for food and to collect enough gastric juice in stomach before food enters the stomach, so that digestion becomes easier.

**Gastric Phase**

The gastric phase of gastric secretion starts when food enters the stomach:

1. The primary stimulus for secretion in this phase is the **distension of the stomach**.
2. Also, products of digestion like peptides and amino acids produced by pepsin action that breaks proteins stimulate gastric secretion.
3. The distension of the stomach elicits acid secretion by both local as well as central reflexes (Fig. 39.11). The **central reflex** is mediated by vagus nerve (**vagovagal reflex**), and is both cholinergic and noncholinergic. The **local reflex** releases acetylcholine that directly stimulates parietal cell. Distension of stomach per se also increases gastrin release from the antral G cells that in turn increases HCl secretion.
4. Amino acid and peptides stimulate G cells to release gastrin.
5. Therefore, mechanisms of acid secretion in the gastric phase are **mechanical, neural, and chemical**.
6. Gastric phase accounts for 50–60% of gastric secretion.
HCl secretion in this phase is decreased by the acidic pH of the chyme bathing in the gastric mucosa. When pH of gastric content becomes less than 2, HCl secretion is effectively reduced. This is called autoregulation of gastric acid secretion (discussed above). HCl inhibits both G cells and parietal cells.

**Experimental Designs to Study Gastric Phase**

Gastric phase of gastric secretion is studied by making five different types of pouches: Pavlov pouch, Heidenhain pouch, Bickel pouch, Farrell pouch and Ivy pouch. However, the pouch experiment to study gastric secretion is not performed nowadays. This is mainly of academic and historical importance.

1. **Pavlov pouch** is a small pouch separated from the main body of the stomach by a double layer of mucous membrane. This gastric pouch of mucous membrane has intact nerve and blood supply. Therefore, it helps to study both neural and chemical factors of gastric acid secretion.

2. **Heidenhain pouch** is a denervated pouch, which helps to study the influence of neural factors on gastric secretion.

**Scientist contributed**

Rudolf Peter Heinrich Heidenhain (1834–1897) pioneered in the study of salivary and gastric secretions. He developed experimental designs to study secretion of salivary and gastric juices. The pouch made in experimental animals to study gastric secretion is named as Heidenhain’s pouch. He also studied the functions of secretory and trophic nerves of glands and promoted the intracellular theory of secretory phenomena.

**Intestinal Phase**

When chyme enters the intestine, intestinal phase of gastric secretion starts. This is also called postgastric phase. Initial part of intestinal phase is stimulatory to gastric secretion, but later part is inhibitory.

**Stimulation of Secretion**

1. Distension of duodenum increases gastric acid secretion by activating vagovagal reflex.

2. The chemical composition of chyme, especially the products of protein digestion like amino acids and peptide stimulate G and other endocrine cells in the duodenum and upper jejunum to secrete entero-oxynin, which in turn stimulates gastric acid secretion (Fig. 39.12).

**Inhibition of Secretion**

1. Acidic chyme in the duodenum (decreased pH of duodenal content) inhibits gastric secretion via enterogastric reflex.

2. Acid also stimulates release of secretin, which inhibits gastrin secretion from G cells. Secretin also decreases the response of parietal cells to gastrin and histamine.

3. Acid in the duodenum and hyperosmolality of duodenal content also secrete a hormone called bulbogastrone that inhibits acid secretion from parietal cells of the stomach.

4. Products of fat digestion especially fatty acids and triglycerides stimulate secretion of GIP and CCK from upper part of small intestine. These hormones inhibit secretion of acid from parietal cells.

Thus, the net effect of intestinal phase is the inhibition of gastric secretion. Hence, intestinal phase accounts for about 10% of gastric secretion.

The differences between cephalic, gastric and intestinal phase of gastric secretion are summarized in Table 39.2.
GASTRIC FUNCTION TESTS

To diagnose various diseases of stomach, the laboratory tests measuring gastric secretion, serum gastrin, etc carry much importance. Therefore, gastric function tests (GFTs) are performed to diagnose various gastric and duodenal anomalies and to monitor the effectiveness of the therapy. It includes:

1. Examination of gastric contents
2. Test for gastric acid secretion
3. Tests for pepsin, mucous, intrinsic factor
4. Tests for gastrin
5. Visualization of the interior of stomach
6. Tests for gastric motility and electrical activities (gastrography)
7. Obtaining biopsy from the suspected tissue.

Classification

Gastric content is examined for the normal and abnormal constituents. The following parameters are looked for in the gastric content sample: Volume of acid (acid output), pH, colour, acidity (total and free), and presence of blood, mucous and food particles.

Examination of gastric contents can be divided into three types according to the time of examination:

1. At rest: Gastric juice is collected after the patient awakes in the morning, but still lying on the bed.
2. After a meal in the post absorptive phase: A specific diet is provided, which has a fixed composition (also known as fractional test meal analysis) followed by that gastric content is collected for analysis.
3. After a specific stimulus: A specific stimulus for induction of acid secretion is applied following which the gastric content is withdrawn. Mainly the maximum amount of acid output is checked in these type of tests.

Measurement of Acid Output

Two types of acid outputs are assessed: basal acid output (BAO), and maximal acid output (MAO). Acid output is measured to assess the size of parietal cell mass or the ability of parietal cells to secrete HCl.

BAO

Usually, it is measured in the interdigestive phase (between two meals, when stomach is supposed to be at rest). The gastric juice is collected for about 60 minutes through a Ryle’s tube introduced into patient’s empty stomach following an overnight fast and complete sleep.

Normal value: Usually, it is 10–40 mEq/L or 0.5–2 mEq/hour.

Significance: Increased BAO indicates high gastrin activity as occurs in Zollinger-Ellison syndrome. A pH more than 2.5 for the acid, rules out this syndrome.

MAO

This is the maximum quantity of acid that can be secreted by the stomach. Usually, estimation of MAO is performed by a stimulation test in which a stimulant is injected to increase acid secretion. The chemical is injected and gastric juice is collected by Ryle’s tube. Usual tests performed are given below.

Normal value: The MAO is usually 12–60 mEq/hour.

Special Test to Detect Acid Output

Histamine Test

Historically, histamine was the first standard stimulant used for gastric acid secretion test. 0.01 mg/kg bodyweight of histamine phosphate is injected subcutaneously with simultaneous administration of antihistaminic agent to prevent the untoward side effects. Then the acid output is measured, every 10 minutes for 1 hour. Upto 10 ml of gastric content is aspirated and analyzed. It is helpful in the diagnosis of pernicious anemia, subacute combined degeneration of spinal cord and assessing the maximum acid output following duodenal surgery.

Histalog (Betazole) Test

Histalog, is a better replacement for histamine as it does not require the simultaneous administration of antihistaminic agent due to its fewer side effects. 1-15 mg/kg of
Radioimmunoassay (RIA) is the diagnosis of gastric malignancy (Achlorhydria in the presence of normal or slightly below normal. Higher values are found in:

- Duodenal ulcer
- Zollinger-Ellison syndrome (gastrinoma)
- Anastomotic ulcer
  Higher values are found:
  - Pernicious anemia (atrophic gastritis)
  - Gastric malignancy (Achlorhydria in the presence of gastric ulcer is highly suggestive of gastric malignancy)

Tests for other Gastric Secretory Products

Test for Pepsin

Pepsin inhibitors are used for analysis of pepsin derived from pepsinogen for research purposes. The level of pepsin is low in atrophic gastritis.

Test for Mucous

Protein content of gastric mucous is measured, normal value being 1.8 mg/ml. The level is increased in chronic hypertrophic gastritis (Menetrier disease).

Test for Intrinsic factor

Intrinsic factor (IF) is essential for vitamin B₁₂ absorption from the small intestine. In its absence, the absorption of vitamin B₁₂ is impaired as occurs in chronic atrophic gastritis and gastric atrophy. Schilling test is used for evaluation of patients with suspected pernicious anemia but can also be used as diagnostic test for pancreatic efficiency resulting in impaired absorption of vitamin B₁₂ since gastric R binder protein is not cleared from intrinsic factor due to reduced pancreatic proteolytic activity.

Test for Gastrin

Gastrin is secreted by G cells present in the antralpyloric and proximal duodenal mucosa. The circulating gastrin level is normally 0-200 pg/mL. It can be tested by following methods:

1. Serum gastrin level: Radioimmunoassay (RIA) is the commonly used method for gastrin measurement. Normal fasting level of gastrin is 20-150 pg/mL. Its levels are higher in:
   - Atrophic gastritis (with low gastric acid secretion)
   - Zollinger-Ellison syndrome or gastrinoma (with high gastric acid secretion)
   - Following surgery of the stomach
2. Gastrin provocative tests: These tests are used to differentiate between hypergastrinaemia and gastric acid hypersecretion as follows:
   - Secretin test: an intravenous injection of secretin (1 U/kg body weight) is given. If the serum level rises by more than 50% of basal value in 5-15 minutes, it is diagnostic of Zollinger-Ellison syndrome (gastrinoma).
     - This rise does not occur in other conditions.
   - Calcium infusion test: Intravenous infusion of calcium (5 mg/kg per hour) is given for 3 hours. Rise in serum gastrin levels by more than 50% of basal value is diagnostic of Zollinger-Ellison syndrome (gastrinoma).

Other Tests

Barium-Meal X-Ray

A barium-mixed food is swallowed by the subject following which X-rays of upper GI tract are obtained. This gives
the details of the contour of the lower esophagus, stomach and upper small intestine. The **location and size of the ulcer and tumor** can be delineated.

**Endoscopy (Gastroscopy)**

A fiber-optic gastroscope is introduced into the stomach to study the details of the ulcer or other pathologies. The advantages are:

1. It gives an opportunity to visualize the lumen of the stomach, so that the ulcer details can be seen.
2. A **biopsy of the ulcer** can be taken to study the type of ulcer (to exclude malignancy).
3. The tissue is also cultured to study the organism (to confirm *H. pylori* infection).
4. The gastroscope can be introduced into the duodenum and biliary tract to study further details.
5. **Urease test** is performed from the biopsy sample for *H. pylori* confirmation.

The major tests for analysis of gastric secretory functions are summarized in Table 39.3.

### Applied Aspects

The common diseases related to gastric secretion are gastritis and peptic ulcer.

**Gastritis**

The term gastritis is commonly employed for any clinical condition with upper abdominal discomfort like indigestion or dyspepsia in which the specific clinical signs and radiological abnormalities are absent. The condition is of great importance due to its relationship with peptic ulcer and gastric cancer. Broadly, gastritis is of 2 types: acute and chronic. Chronic gastritis can further be of various types.

A simple classification of various types of gastritis is presented in Table 39.4.

### Etiopathogenesis

A variety of etiologic agents have been implicated in the cause of acute gastritis. These are as follows:

1. **Diet and personal habits**
2. **Infections**
3. **Drugs**
4. **Chemical and physical agents**
5. **Severe stress**

Chronic gastritis, if untreated leads to peptic ulcer.

**Peptic Ulcers**

Peptic ulcer means ulcer in the stomach (**gastric ulcer**) or duodenum (**duodenal ulcer**). Acid of the gastric juice or pepsin in the gastric secretion produces damage to the gastroduodenal mucosa in abnormal conditions. Therefore, peptic ulcer is called **acid-peptic disease**.

### Pathophysiology

Peptic ulcer is caused either by decreased mucosal defense, or by hypersecretion of acid or infection.

**A. Diminished Effectiveness of Mucosal Barrier**

The defense barrier of the stomach is the mucous coat on the gastric epithelium. This is called **mucosal defense barrier**:

1. The mucus is secreted by mucous cells. Mucus is a viscous gel that contains mucin, phospholipid, electrolytes (mainly HCO₃⁻) and water.
2. The **mucous gel layer** is about 0.2 mm thick and effectively separates the bicarbonate rich secretion of epithelial cells from the acidic content of the stomach (Fig. 39.13).
3. This allows the **pH of the epithelial cells to remain alkaline** despite acidic pH of gastric content. It protects mucosal epithelium from injury caused by acidic chyme.
4. However, when secretion of mucus is impaired, or bicarbonate production is decreased or when the mucosal coat is mechanically damaged, acid and pepsin cause ulcer formation.
5. Therefore, antibiotic therapy to kill *H. pylori* is frequently successful in the treatment of peptic ulcer.

**B. Hypersecretion of Acid**

Gastric acid secretion increases in chronic anxiety. Peptic ulcer is common in business executives as most of them lead a life either in hurry or in worry. Intake of *more spicy food* is known to increase acid secretion. Therefore, it is generally believed that *hurry, worry, and curry* are the causes of peptic ulcer. Chronically increased secretion of acid (hyperchlorhydria) produces peptic ulcer by damaging the mucosal barrier.

Conditions that cause hyperchlorhydria are:

- Zollinger-Ellison syndrome
- Gastric outlet obstruction syndrome (as occurs in pyloric stenosis)
- Systemic mastocytosis (histamine secreted from mast cell increases HCl secretion)

**Zollinger-Ellison syndrome** is a non-α non-β *gastrin secreting tumor of pancreas*.

**C. Helicobacter Pylori Infection**

Recently it is observed that infection by *H. pylori* is the major cause of peptic ulcer:

1. This is a *Gram-negative bacillus* that secretes an enzyme called *urease* that converts urea into carbon dioxide and ammonia. Ammonia buffers the acid surrounding the bacteria.

2. *H. pylori* colonizes the antral mucosa, where it causes *local inflammation* and disrupts immune responses. It also *inhibits somatostatin secretion* from D cells that facilitates gastrin release and consequently increased HCl secretion.

3. Therefore, serum gastrin level is moderately elevated in duodenal ulcer.

4. *H. pylori* causes gastritis initially, but later, ulcer is produced.

**Features**

In most of the cases ulcer is located in the duodenum (Fig. 39.14), usually above the ampulla of Vater, as in this area acidic chyme is not neutralized by alkaline pancreatic juice. The main feature of peptic ulcer is *upper abdominal pain* (*epigastric pain*). Typically, pain is experienced in empty stomach and is relieved by taking water, food or antacid. If disease is untreated, *hematemesis* (vomiting of blood) or *malena* (dark, tarry stool), *vomiting* (due to pyloric obstruction), and peritonitis due to perforation of ulcer into the peritoneal cavity occurs.

**Treatment**

**Specific Treatment**

The specific treatment includes use of following drugs:

1. **H₂ receptor antagonists**: Ranitidine, cimetidine, famotidine, and nizatidine are different generations of H₂ receptor blockers. These drugs block the H₂ receptor and inhibit histamine secretion. As histamine is a potent stimulator of HCl release from parietal cells, H₂ receptor blockers inhibit HCl secretion.
2. Proton-pump blocker (H⁺-K⁺ATPase inhibitor): This drug inhibits the activity of proton-pump, i.e., the activity of H⁺-K⁺ATPase. Therefore, the final step of acid secretion is inhibited. This is the most effective medicine for the treatment of peptic ulcer. The usual proton-pump blocker used is omeprazole.

3. Sucralfate: This is a sucrose octasulfate. It provides a protective layer on the ulcer. Therefore, it promotes ulcer healing.

4. Muscarinic blockers: Atropine and pirenzepine are used to block the M₁ and M₃ receptors. Therefore, acetylcholine does not act on parietal cells. However, as the noncholinergic vagal innervation dominates over the cholinergic innervation for acid secretion, atropine does not produce encouraging results. Therefore, muscarinic blockers are not used in peptic ulcer treatment.

5. Gastrin blockers: As gastrin is the most potent stimulator of acid secretion, effort has been made to discover gastrin antagonists. However, a successful gastrin blocker has not yet been discovered. Proglumide, a gastrin blocker is used recently for the purpose.

6. Antibiotics: Recently, high dose of antibiotics such as amoxicillin to kill H. pylori has provided promising result.

Nonspecific Measures

Antacids
Antacids give immediate and temporary relief from pain. As the disease is mostly due to stress, measures to reduce the stress level are very helpful.

Yoga Therapy and Other Measures

Yoga therapy like practice of relaxation techniques, adequate sleep and rest, regulation of diet, and withdrawal of drugs like aspirin and NSAID improve the condition. Use of cold milk and avoidance of spicy food & alcohol also help in curing the disease.

Surgical Treatment

Sometimes inspite of effective use of medicines, the disease is not cured. In such patients, surgery is advocated. The usual surgical procedures performed are:

1. Vagotomy: There are different types of vagotomy such as truncal vagotomy (cutting the trunk of vagus nerves in abdomen just below the diaphragm), selective vagotomy (cutting the vagus nerve that supplies only stomach), and highly selective vagotomy (cutting the vagus nerve that preferentially innervate the parietal cell) (Fig. 39.15). Usually, the parietal cell vagotomy is preferred as other two types are associated with complications.

2. Gastrectomy: Partial gastrectomy removes the antral portion of stomach, as this part contains G cells. Antrum is the pump of the stomach that propels food into duodenum. Therefore, antrectomy results in stasis of food. To avoid such complication, usually gastroduodenostomy or gastrojejunostomy (the drainage procedures) is performed with gastrectomy (Fig. 39.16). Gastrojejunostomy is also performed with truncal vagotomy.
CHAPTER SUMMARY

KEY CONCEPTS

1. Though the primary function of stomach is temporary storage, grinding and mixing of food and controlled emptying of chyme into the intestine, gastric acid secretion is useful for acid-peptic digestion of food, providing IF for Vitamin B₁₂ absorption and killing microorganisms in food in stomach.

2. Gastric distension, spicy food, emotion and stress are important stimulant for gastric secretion. Mental relaxation, healthy food, Yoga and adequate sleep are important to have control over secretion of gastric acid.

3. *H. Pylori* is an important causative factor for gastritis and peptic ulcer.

4. Though endoscopy is the surest method for diagnosis of gastritis and peptic ulcer, estimation of gastrin level is useful in the management.

Important to Know (Must Read)

1. In examinations, 'Mechanism and regulation of gastric secretion' comes as a Long Question.

2. Phases of gastric secretion, Composition and functions of gastric secretion, Mechanism of gastric secretion, Regulation of gastric secretion, Gastric function tests, can come as Short Questions.

3. In Viva, examiner may ask……. Structure and functions of stomach, Amount of gastric secretion/day, Names of gastric glands, Innervation of stomach, Composition and function of gastric secretion, Function of each constituent of gastric secretion, Mechanism of gastric secretion, What are the Phases of gastric secretions and how are they regulated, What are the stimuli for different phases of gastric secretion, How different phases of gastric secretion can be studied, What are the gastric pouches and how they differ from each other, What are the effects of parasympathetic and sympathetic stimulation on gastric secretion, What is appetite juice, Classify gastric function tests, Procedure and normal values of important gastric function tests, Causes of gastritis and peptic ulcer, Who got Nobel prize for discovery of *H. pylori*, What is the role of *H. pylori* in peptic ulcer.

4. Functions of stomach, and Composition and functions of gastric juice are usually asked in viva. Mechanism of HCl secretion, and Regulation of different phases of gastric secretion are usually asked in theory exam. A student is expected to answer these questions; otherwise it may be difficult for him to pass.
The exocrine pancreas plays a major role in digestion and absorption of all essential nutrients from the GI tract. The exocrine pancreas constitutes about 80% of the total mass of the pancreas (12% by ducts and blood vessels, and 2% by endocrine tissues). This is a unique organ in the body having both major endocrine and exocrine tissues in it. The endocrine pancreas is involved in energy metabolism, deficiency of which results in diabetes mellitus, exocrine pancreatic deficiency results in severe indigestion, malabsorption and malnutrition.

History and Secretory Apparatus

Histologically, exocrine pancreas resembles salivary glands. The exocrine tissue of pancreas consists of lobules that contain multiple acini (Fig. 40.2).

1. Acini are sac-like dilatations composed of single layer of pyramidal (acinar) cells. Acinar cells contain multiple endoplasmic reticulum, Golgi apparatus and zymogen granules that are located in the apical region of the acinar cells (Fig. 40.3).
2. A few centroacinar cells line the lumen of the acinus.
3. Acini open to intercalated duct which in turn empty into intralobular duct.
4. Intralobular duct drains into extralobular duct that finally open to the main collecting duct, the duct of Wirsung.
5. Sometimes an accessory pancreatic duct, the duct of Santorini, drains separately from the head of the pancreas into the duodenum.
6. Collecting ducts combine to form pancreatic duct that drains into the common bile duct and form hepatopancreatic duct with an ampulla (Ampulla of Vater), which opens to the second part of duodenum through the sphincter of Oddi.
7. The acini secrete enzymes of the pancreatic juice.
Chapter 40: Pancreatic Secretion

8. The aqueous component of pancreatic juice is produced by epithelial cells that line the pancreatic ducts.

Scientist contributed

**Ruggero Ferdinando Antonio Giuseppe Vincenzo Oddi** (1864–1913) was an Italian physiologist, studied medicine at Perugia, Bologna and Florence, and in 1894 was appointed as Head of the Physiology Institute at the University of Genoa. While still a student, Oddi described a small group of circular and longitudinal muscle fibers that wrapped around the end of the bile and pancreatic ducts, and he demonstrated the physiology of these muscles. This structure was later named as the eponymous "sphincter of Oddi." Though this sphincter was initially identified by English physician Francis Glisson, it was Oddi who was first to characterize its physiological properties. Inflammation of the junction of the duodenum and common bile duct at the sphincter of Oddi is referred to as "Odditis".

**Nerve supply**

Pancreas is supplied by both parasympathetic (vagal fibers) and sympathetic fibers.

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**Vagal Fibers**

These fibers innervate both the acinar cells (exocrine tissue) and islet cells (endocrine tissue). Stimulation of parasympathetic fibers increases exocrine pancreatic secretion.

**Sympathetic Fibers**

The postganglionic sympathetic fibers from celiac and superior mesenteric plexuses innervate pancreatic blood vessel and tissue. Stimulation of sympathetic fibers inhibits pancreatic secretion.

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**PANCREATIC SECRETION**

**Composition and Functions**

**Composition**

The pancreatic secretion has aqueous component (98%) and enzyme component (2%).

**Aqueous Component**

This contains mainly water and ions. The cations are Na\(^+\), K\(^+\), Ca\(^{2+}\), Mg\(^{2+}\), and Zn\(^{2+}\), and the anions are HCO\(_3\)\(^-\), Cl\(^-\), PO\(_4\)\(^{3-}\), and SO\(_4\)\(^{2-}\).
Enzyme Component

Enzymes for Lipid Digestion
Enzymes for lipid digestion are pancreatic lipase (triacylglycerol hydrolase, cholesterol ester hydrolase, and phospholipase A₂), colipase and phospholipases.
1. The pancreatic lipase digests neutral lipids into fatty acids and monoglycerides.
2. The colipase facilitates the action of lipase.
3. The phospholipase acts on phospholipids.
4. Cholesterol hydrolase splits cholesterol esters into cholesterol and fatty acids.

Enzymes for Protein Digestion
Enzymes for protein digestion are trypsin, chymotrypsin, carboxypeptidase, procollagenase, proelastase, and nucleases.
1. These proteolytic enzymes are secreted in the inactive forms like trypsinogen, chymotrypsinogen, procarboxypeptidase, procollagenases, proelastase, and pronucleases (Table 40.1).
2. The enzymes are activated in the intestine.
3. The first step in the process of activation is the activation of trypsinogen to trypsin, which is mediated by the intestinal enzyme enteropeptidase (enterokinase).
4. Once trypsin is formed, it converts other proenzymes to their active forms (Flowchart 40.1).
5. Trypsin is strongly proteolytic. Therefore, once it is activated in the pancreas, it digests the pancreatic tissue (Clinical Box 40.1). However, normally it remains inactivated in pancreas.

Enzymes for Carbohydrate Digestion
Pancreatic amylase splits starch at α-1,4-glycosidic linkage. This is an α-amylase that acts on starch to produce maltose, α-limit dextrin, etc. It differs from salivary amylase by its action on both boiled and unboiled starch (salivary amylase acts only on boiled starch).

Functions of Pancreatic Secretion
1. Pancreatic secretion contains enzymes that help in digestion of fat, protein and carbohydrate as listed in Table 40.1. Pancreatic enzymes are the primary requirement for digestion and absorption. Therefore, pancreatic deficiency leads to severe malabsorption syndrome.
2. Pancreatic secretion contains bicarbonate and water that neutralizes acidic chyme entering intestine from stomach. It also neutralizes the effects of bile acids. Thus, it prevents formation of duodenal ulcer (Clinical Box 40.2).

Factors that normally prevent autodigestion of pancreas are:
1. Enzymes are present in inactive form.
2. Trypsin inhibitor in pancreatic tissue does not allow active trypsin to be formed (trypsin remains inhibited).
3. The wall of acini is thick, and therefore does not allow pancreatic enzymes to escape into the pancreatic tissue.

Clinical Box 40.1
Autodigestion of pancreas in acute pancreatitis: Normally, trypsin is not formed in the pancreas due to the presence of trypsin inhibitor in the pancreatic juice which prevents the activation of trypsinogen to trypsin. In acute pancreatitis, the trypsin inhibitor is deficient. Therefore, activation of trypsin results in autodigestion of pancreatic tissue.

Flowchart 40.1: Steps of activation of pancreatic proteolytic enzymes.

Clinical Box 40.2
Upper part of duodenum is the commonest site for duodenal ulcer: Acidic chyme from stomach is neutralized by alkaline pancreatic juice. However, as acidic chyme in the 1st part of duodenum (part above the ampulla of Vater, the site of opening of major pancreatic duct) remains mostly unaffected by alkaline pancreatic secretion, it is the commonest site of peptic ulcer.
Chapter 40: Pancreatic Secretion

Mechanism of Secretion

Secretion of Aqueous Component

Aqueous component of pancreatic secretion is mainly produced by the columnar epithelial cells that line the pancreatic ducts.

1. Pancreatic juice is nearly isotonic with plasma at any rate of formation and flow. The ionic composition includes mainly cations (Na\(^+\) and K\(^+\)) and the anions (HCO\(_3\)\(^-\) and Cl\(^-\)). The Na\(^+\) and K\(^+\) concentration of pancreatic juice is similar to that of plasma. But, HCO\(_3\)\(^-\) and Cl\(^-\) concentration varies according to the rate of secretion.
   - At lower rate of secretion, concentration of HCO\(_3\)\(^-\) and Cl\(^-\) is about 70% of plasma.
   - At higher rate of secretion, their concentration is more than 100% of plasma (Fig. 40.4).

2. The aqueous component secreted by the duct cell is hypertonic to plasma as it contains more HCO\(_3\)\(^-\).

3. But, when the secretion passes through the ducts, water moves into the duct lumen to make the pancreatic juice isotonic, during which HCO\(_3\)\(^-\) is partly exchanged with Cl\(^-\) by HCO\(_3\)\(^-\)-Cl\(^-\) exchanger present on the luminal membrane (Fig. 40.5).

4. In the resting state (interdigestive phase), the secretion of aqueous component occurs mainly from intercalated and intralobular ducts, but in the stimulated state (after eating) the secretion occurs additionally from extralobular ducts, which has higher HCO\(_3\)\(^-\) concentration.

Defect in the chloride channel causes thick exocrine secretion in the duct (Clinical Box 40.3)

Secretion of Enzyme Components

Pancreatic enzymes are synthesized in the acinar cells and stored in the zymogen granules. The granules are located toward the apical region of the cells. In response to appropriate stimulation, the granules are released by exocytosis into the lumen of acinus.

Clinical Box 40.3

Defect in CFTR produces viscid secretion: The Cl\(^-\) enters the acinar cells and duct epithelial cells via voltage gated Cl\(^-\) channels. The primary defect in cystic fibrosis is the mutation of the gene that encodes for Cl\(^-\) channel. Cystic fibrosis gene is located on chromosome 7, mutation of which alters the function of its product, cystic fibrosis transmembrane conductance regulator (CFTR). CFTR is localized in the apical membrane of epithelial cells in the pancreatic ducts. Alteration of CFTR results in decreased number of Cl\(^-\) channel. Therefore, Cl\(^-\) transport into duct lumen is impaired. This also impairs the transport of Na\(^+\) and water. As a result, the secretion becomes thick causing duct obstruction which in turn causes destruction of acinar cells and duct system. This leads to severe pancreatic deficiency causing severe impairment of absorption of important nutrients. This is also accompanied by progressive pulmonary disease causing lung infection, especially in children.

Factor That Influence Secretion

Pancreatic secretion is influenced by neural and hormonal factors.
Section 5: Gastrointestinal System

1. The hormonal factors are mainly cholecystokinin (CCK) and secretin. Cholecystokinin stimulates enzymatic pancreatic secretion, whereas secretin stimulates pancreatic secretion rich in water and bicarbonate.

2. The neural factors are sympathetic and parasympathetic. Parasympathetic stimulation increases secretion whereas sympathetic stimulation decreases secretion. However, the control mechanism varies according to the phases of secretion (described below).

### Hormonal Factors

1. CCK: CCK has two receptors. (CCK A and CCK B receptors, gastrin acts through CCK B receptors)
2. Secretin
3. Gastrin: Gastrin receptors, CCK B receptors
4. GRP
5. GIP
6. VIP

### Table 40.2: Mechanisms of pancreatic secretion in its different phases.

<table>
<thead>
<tr>
<th>Stimuli</th>
<th>Mechanisms</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cephalic phase</td>
<td>Sight, smell, thought, and taste of food. Chewing and swallowing of food</td>
<td>Vagal stimulation</td>
</tr>
<tr>
<td>Gastric phase</td>
<td>Gastric distension</td>
<td>Vagovagal reflex</td>
</tr>
<tr>
<td>Products of protein digestion</td>
<td>Gastrin</td>
<td>Increased secretion containing more enzymes</td>
</tr>
<tr>
<td>Intestinal phase</td>
<td>Acidic chyme in duodenum</td>
<td>Secretin</td>
</tr>
<tr>
<td>Fatty acids and amino acids</td>
<td>CCK and vagovagal reflex</td>
<td>Increased secretion containing more enzymes</td>
</tr>
</tbody>
</table>

(CCK: Cholecystokinin).

### Neural Factors

1. Parasympathetic: stimulates secretion
2. Sympathetic: inhibits secretion

### Regulation of Secretion

### Phases of Pancreatic Secretion

There are three phases of pancreatic secretion: cephalic, gastric, and intestinal. Mechanisms of pancreatic secretion are different in different phases of secretion (Table 40.2).

#### Cephalic Phase

The cephalic phase is induced by smell, sight, thought and taste of food, and by chewing and swallowing of food.

1. Cephalic phase is mediated by vagus nerve. The efferent impulses originating from brain directly stimulate pancreatic secretion. The fibers mediating this response are cholinergic vagal fibers.
2. Also, gastrin released from the stomach in response to vagal stimulation increases pancreatic secretion.
3. The pancreatic secretion in cephalic phase is rich in enzymes. Cephalic phase contributes to 15–30% of pancreatic secretion.
**Gastric Phase**

When food enters the stomach, gastric phase is initiated.

1. Distension of stomach elicits vagovagal reflex that stimulates pancreatic secretion.
2. Presence of amino acids and peptides in the gastric content stimulate gastrin release from stomach that in turn stimulate pancreatic secretion (Fig. 40.7).
3. Gastric phase has greater effects on enzyme secretion.
4. About 40% of pancreatic secretion occurs in this phase.

**Intestinal Phase**

When chyme enters the duodenum, intestinal phase starts.

1. Acidic chyme increases release of secretin from S cells present in the mucosa of upper part of small intestine. Secretin stimulates pancreatic juice rich in aqueous (water and bicarbonate) component.
2. The presence of products of protein digestion like amino acids and peptides, and the products of fat digestion like fatty acids and monoglycerides in chyme evoke pancreatic secretion rich in enzymes, which is mediated by CCK secreted from I cells in intestinal mucosa (Fig. 40.7).
3. CCK potentiates the effect of secretin on ducts and secretin potentiates the effect of CCK on acinar cells.
4. The vagovagal reflex also mediates pancreatic secretion during gastric and intestinal phases. Intestinal phase contributes to 40–60% of pancreatic secretion.

**APPLIED PHYSIOLOGY**

**Acute Pancreatitis**

**Pathophysiology**

This is a serious condition of acute abdomen that occurs due to inflammation of the pancreas.

1. The disease is characterized by severe pain in the epigastric or periumbilical region that often radiates to back.
2. The physiological alteration in the disease is the activation of trypsin inside the pancreatic tissue that causes autodigestion of the gland.
3. Trypsin also activates phospholipase-A₂ which forms lyssolecithin from fatty acid of lecithin.
4. Lyssolecithin damages pancreatic tissue and produces necrosis of surrounding fat.
5. In severe case, hemorrhage occurs into abdominal cavity (hemorrhagic pancreatitis).
Etiology
The common causes of acute pancreatitis are gall stone causing obstruction of sphincter of Oddi, chronic alcoholism, hypertriglyceridemia, blunt abdominal trauma and drugs like azathioprine, sulfonamides, tetracycline, valproic acid, etc.

Diagnosis
Acute pancreatitis is diagnosed by its typical presentation of abdominal pain associated with increased plasma level of serum amylase.

Treatment
In 90% of patients, symptoms disappear with conservative treatment with i.v. fluid, nil orally, analgesics, antibiotics and nasogastric suction. In severe cases, especially in hemorrhagic form, abdomen is opened, and blood is collected from abdominal cavity of the patient and filtered, abdomen is thoroughly washed, bleeding points are closed, and the filtered blood is transfused (autotransfusion).

Chronic Pancreatitis
Slow and chronic inflammation of pancreas occurs without any definite etiology.
1. It may be due to improper recovery from pancreatitis or persistence of low grade acute pancreatitis.
2. Patient develops steatorrhea due to pancreatic lipase deficiency and malnutrition due to indigestion and malabsorption of various nutrients.
3. Patient may develop diabetes mellitus due to associated endocrine deficiency.

Cystic Fibrosis
This is an autosomal recessive monogenic disorder, in which the major defect is in the Cl– channel.
1. The disease occurs due to mutation in the CFTR gene (cystic fibrosis transmembrane-conductance regulator gene), located on chromosome 7.
2. The disease usually starts in childhood.
3. Lung is the most common organ affected and decreased nasociliary clearance is the common pathology.
4. Respiratory features are chronic sinusitis, nasal obstruction, rhinorrhea and cough with expectoration of viscous-purulent sputum.
5. Common gastrointestinal features are intestinal obstruction and exocrine pancreatic deficiency.

PANCREATIC FUNCTION TESTS
Exocrine pancreatic function tests are based on assessment of pancreatic secretion in response to hormonal stimulation, or a meal or measurement of production of digestion in the stool that assesses digestion and absorption of nutrients. Apart from color, odor, pH (8–8.3), volume (maximum 3 L), specific gravity (1.007–1.042) and bicarbonate concentration of pancreatic secretion, the important parameters analyzed are serum and fecal enzymes such as trypsin, amylase, lipase, etc.

Classification
Pancreatic function tests are described in detail in biochemistry books. However, in this section, we describe the physiological principles of these tests. These tests are usually employed to diagnose pancreatic deficiencies as in pancreatitis, cystic fibrosis, etc. These are of two types: invasive and non-invasive tests.

Invasive Tests
Invasive tests are of two types.
1. First type uses stimulation of pancreatic secretion by meals such as Lundh meal or duodenal infusion of amino acids.
2. Second type makes use of hormonal injection intravenously such as secretin, CCK, etc.

Secretin Stimulation Test
Secretin is injected IV at a dose of 1 unit/kg and then the duodenal samples are collected for 80 minutes. It increases rate of formation and flow, and aqueous component of pancreatic juice.

CCK Stimulation Test
CCK increases enzymatic component of pancreatic secretion.

Meal Stimulation Test
The test meal is called Lundh meal (Lundh and Borgstrom, 1962). It consists of milk protein, corn oil, and dextrose. Following ingestion of the meal, duodenal content is aspirated for analysis.

Non-Invasive Tests
These are simpler and cheaper. Therefore, noninvasive tests are preferred to invasive tests. However, they are not as sensitive and specific as the invasive tests.

Visualization of pancreas and scanning by ultrasonography, CT scan, MRI, cholangiopancreatography, etc., are usually performed before opting for biochemical pancreatic function tests. These tests are of three types.
1. First type includes estimations in stool for food particles that are not absorbed (fecal estimation of fat) and fecal enzymes (trypsin, chymotrypsin and elastase).
2. Second type includes estimations in blood, urine and breath for products of digested food due to action of pancreatic enzymes that are absorbed into systemic circulation.
3. **Third type** includes estimations in plasma or blood for hormones (Schilling test) and enzymes, amino acids, etc.

**Fecal Estimation of Fat**

Normally, about 5 g of fat is excreted per day in the feces. In pancreatic deficiency, excretion **more than 20 g of fat** is common. In severe deficiency, fat excretion may be 50 g or more.

**Fecal Estimation of Nitrogen**

About **7 g of nitrogen** is excreted in stool per day in a healthy individual. In pancreatic deficiency, due to decreased proteolytic enzyme activity, nitrogen excretion increases in stool.

**D Xylose Absorption Test**

A pentose sugar called xylose is administered (25 g, usually) and its recovery in urine is measured over next 5 hours and in blood in next 2 hours. This is a test that distinguishes between malabsorption due to pancreatic (normal absorption) and jejunal (low absorption) insufficiencies. It also helps evaluate response to therapy.

**Schilling Test**

It is done to assess the absorption of **vitamin B₁₂**, which requires intrinsic factor. Vitamin B₁₂ deficiency is seen in chronic pancreatitis because trypsin helps in binding B₁₂ to intrinsic factor after which the complex is reabsorbed.

**Serum Amylase Estimation**

Normal level of serum amylase is **60–120 units** per liter. In acute pancreatitis, serum amylase concentration becomes very high. This differentiates pancreatitis from other conditions of acute abdomen.

**Cholangiopancreatography**

This is a special radiodiagnostic investigation in which the details of the contour of hepatopancreatic duct system are visualized by introducing an endoscope. The procedure is called endoscopic retrograde cholangiopancreatography (ERCP). This is the most accurate test for assessing pancreatic structure and function.

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**CHAPTER SUMMARY**

**Key Concepts**

1. Pancreas secretes enzymes for digestion of all types of nutrients. Therefore, pancreatic deficiency leads to severe indigestion and malabsorption.
2. Pancreatic juice is alkaline. It helps in protecting the mucosa of intestine from acidic chyme that may damage the intestinal epithelium.
3. CCK stimulates pancreatic juice rich in enzymes, and secretin increases secretion of aqueous component.
4. Trypsin inhibitors prevent activation of trypsin inside pancreas. It is activated in duodenum by enterokinase. Activation of trypsin inside pancreas causes acute pancreatitis and autodigestion of pancreas, as trypsin is strong proteolytic agent.

**Important to Know (Must Read)**

1. In examinations, “Composition and function of pancreatic secretion, and mechanism and regulation of pancreatic secretion” may come as a **Long Question**.
2. Phases of pancreatic secretion, Composition and functions of pancreatic secretion, Mechanism of pancreatic secretion, Regulation of pancreatic secretion, Pancreatic function tests, can come as **Short Questions**.
3. In **Viva**, examiner may ask… Structure and functions of pancreatic acini, Amount of pancreatic secretion/day, Composition pancreatic juice and function of each constituent pancreatic secretion, Name pancreatic enzymes and tell their functions, Mechanism of gastric secretion, What are the Phases of pancreatic secretions and how are they regulated, What are the stimuli for different phases of pancreatic secretion, What are the effects of parasympathetic and sympathetic stimulation on pancreatic secretion, Classify pancreatic function tests, Procedure and normal values of important pancreatic function tests, Causes of acute and chronic pancreatitis, What is cystic fibrosis and which ion channel is defective in this disease.
4. Composition pancreatic juice and functions of each constituent, and Pancreatic function tests, are usually asked in viva. A student is expected to answer these questions.
Learning Objectives
On completion of study of this chapter, the student MUST be able to:
1. List the functions of liver.
2. Understand the importance of knowing hepatic physiology in learning medicine.
4. Outline the bilirubin metabolism.
5. Understand the physiological basis of classification of jaundice.
6. Appreciate the differences in laboratory findings of different types of jaundice.
The student MAY also be able to:
1. Describe details of bilirubin metabolism.
2. Explain the pathophysiology of jaundice.

Physiology of Liver
Liver is an essential organ of the body as it is the center of all metabolisms and crucial for many other vital functions. Therefore, liver dysfunctions result in major abnormalities of the body. In adults, liver weighs about 1.5 kg. Liver is protected by a thin but strong capsule, called Glisson's capsule.

Scientist contributed
Francis Glisson (1599–1677) was a British physician, anatomist, physiologist, and writer on medical subjects. He did important work on the anatomy of the liver, and wrote on rickets. A physiology experiment he performed that helped debunk the balloonist theory of muscle contraction, as he demonstrated that when a muscle contracts under water, the water level does not rise, and thus no air or fluid could be entering the muscle. Glisson is a well-known medical eponym, the Glisson's capsule of Liver, named after him.

Functional Anatomy
The main function of the liver is the bile synthesis and secretion. The bile is secreted into hepatic ducts. The hepatic ducts join to form common hepatic duct. Bile is continuously formed and delivered to the gallbladder, the process known as bile secretion (described in detail in next chapter). In the gallbladder, the bile is stored and concentrated and delivered via the common bile duct into second part of duodenum in response to chyme entering the duodenum.

Blood Supply
Liver receives blood supply from two sources.

Portal Vein
Portal vein is formed by the union of two veins, viz. superior mesenteric vein and splenic vein. Thus portal vein supplies blood from most parts of the stomach and intestine as well as from the spleen and pancreas. Therefore, portal blood is rich in end products of digestion, GI hormones and pancreatic hormones. However, this blood is comparatively poor in oxygen.

Hepatic Artery
Hepatic artery contains pure arterial blood and is rich in oxygen.

Portal Circulation
The portal vein and hepatic artery break up into large number of branches.
1. Blood of these two sets of vessels get mixed up in the sinusoids of the liver. The hepatic cells receive oxygen and nutrients from the sinusoids.
2. The various substances produced by the liver, products of metabolism, the waste products and the CO₂ are discharged into the sinusoids.
3. The sinusoids drain into the central vein of the lobule.
4. Central veins from different lobules unite to form larger veins, which in turn join ultimately and drain into hepatic vein.
5. The hepatic vein opens into the inferior vena cava.

**Bile Secretion by Liver Cells**
The liver cells synthesize bile, which is first secreted to small canaliculi. The smaller canaliculi join and ultimately form two hepatic ducts (right and left).

**Histology of Liver**
Liver is formed by large number of lobules (Fig. 41.1). Each lobule is delineated by a connective tissue sheath.
1. The individual lobule is polygonal in shape with a central vein at the center.
2. From the central vein, plates of liver cells radiate like spokes of a bicycle wheel to the periphery of the lobule (Fig. 41.2).
3. The plates are one cell thick and are separated by liver sinusoids. Liver cells have the capacity to regenerate (Application Box 41.1).

**Sinusoids and Bile Canaliculi**
In a typical lobule, between the plates of hepatic cells, the sinusoids are present that carry blood.

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**Fig. 41.1:** Histology of liver showing hepatic lobules. 1: Central vein; 2: Radiating cord of hepatocytes; 3: Branch of portal vein; 4: Branch of hepatic artery; 6: Interlobular duct. Note, 3, 4, 5 form the portal triad.

**Fig. 41.2:** Detailed structure of liver lobule.

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1. Each sinusoid receives blood from a branch of portal vein and hepatic artery of the portal tract, and drains into the central vein.
2. Wall of the sinusoids are mostly made up of endothelial cells, but at places macrophage cells, called Kupffer cells, are occasionally present.
3. The bile canaliculi run in between the two layers of cells (layer of cells that make the thickness of each plate) (Fig. 41.3). The liver cells (hepatocytes) synthesize the bile acids and transfer them into bile canaliculi.
4. The space that lies between sinusoid and hepatocytes is the space of Disse (perisinusoidal space). The space of Disse serves as a route through which hepatocytes remove certain substances from blood and discharge certain products into the blood.

**Portal triad**
Portal triads are typically found at the angles of liver lobules. Each portal triad consists of a branch of portal vein, a branch of hepatic artery and a bile ductule (Figs. 41.2 and 41.3).
1. The angular space that contains portal triad and its surrounding connective tissue is called portal canal.
2. Portal sinusoids originating from portal veins remain in close proximity with bile canaliculi that drain into interlobular bile duct (Fig. 41.4).
3. A small space between hepatocyte and portal canal is called space of Mall, the site of origin of lymph in liver.

**Application Box 41.1**

**Hepatic regeneration:** Despite its slow rate of cell renewal, liver has the capacity of regeneration. The loss of hepatic tissue by surgical removal, injury or effect of toxin triggers a mechanism by which hepatocytes begin to divide and grow till the normal size is attained. This property of liver tissue is used in liver transplantation, in which a small portion of transplanted liver tissue proliferates to become the normal size liver.
Section 5: Gastrointestinal System

Functions of Liver

Liver is the center for most of the metabolisms. It synthesizes many essential proteins, stores nutrients that are released into circulation at the time of starvation, and detoxifies harmful substances. It plays an essential role in maintaining blood glucose level. Therefore, liver is among the vital organs of the body.

1. **Secretory functions**: Liver forms and secretes bile into the biliary tract.

2. **Metabolic functions**: Liver is involved in metabolism of major nutrients such as carbohydrate, fat, proteins, and fat soluble and water soluble vitamins. Liver plays a central role in the metabolisms of urea, iron, and alcohol.

3. **Synthetic functions**: Liver is the major organ for the synthesis of proteins that include clotting factors, acute phase proteins that mediate inflammation, hormone binding proteins, lipids, carbohydrates, vitamins and bile salts.

4. **Storage functions**: Liver stores glucose, protein, fat, and vitamins. These nutrients are released from liver and utilized during their scarcity, and are stored in liver when they are in excess.

5. **Detoxifying action**: Liver detoxifies many chemicals. Toxins released from infecting organisms are neutralized in liver.

6. **Degradation of drugs and chemicals**: Liver is the site of inactivation of many drugs. Liver degrades enzymes, hormones, cytokines, and various other chemicals.

7. **Excretory function**: Liver excretes bile pigments, cholesterol, and some metals.

8. **Immunity**: Kupffer cells of liver are part of mononuclear phagocyte system (reticuloendothelial system) that forms the nonspecific defenses of the body. These cells phagocytose and kill microorganisms.

9. **Endocrine functions**: Liver converts vitamin D₃ to 25-hydroxyvitamin D₃. Liver is a major site for conversion of T₄ to T₃. Somatomedin (insulin-like growth factor) that mediates important functions of growth factor is secreted from liver. Many hormones like insulin, glucagon, growth hormone, GI hormones, etc., are degraded in liver.

**LIVER FUNCTION TESTS**

In view of multiplicity and complexity of the liver functions, it is obvious that no single test can establish the disturbance in liver function. Thus a battery of liver function tests is employed for accurate diagnosis, to access the severity of damage, to judge prognosis and to evaluate therapy. These tests are listed below in relation to major liver functions. A summary of various liver function tests and their significance is depicted in Table 41.1.

1. **Tests for manufacture and excretion of bile**
   1. Bilirubin estimation
### Table 41.1: Liver function tests and their significances.

<table>
<thead>
<tr>
<th>Tests</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Tests for manufacture and excretion of bile</strong></td>
<td></td>
</tr>
<tr>
<td>1. Bilirubin estimation</td>
<td></td>
</tr>
<tr>
<td>i. In serum (0.2–0.8 mg/dL)</td>
<td>Increased in hepatocellular, obstructive, and hemolytic disease, Gilbert disease</td>
</tr>
<tr>
<td>ii. In feces</td>
<td>Absent in biliary obstruction</td>
</tr>
<tr>
<td>iii. In urine</td>
<td>Conjugated bilirubinuria in patients of hepatitis</td>
</tr>
<tr>
<td>2. Urobilinogen</td>
<td>Increased in hepatocellular and hemolytic disease, absent in biliary obstruction</td>
</tr>
<tr>
<td>3. Bile acids (bile salts)</td>
<td>Increased in serum and detectable in urine in cholestasis</td>
</tr>
<tr>
<td>4. Bromsulphalein excretion</td>
<td>Helps in diagnosis of Dubin-Johnson syndrome</td>
</tr>
<tr>
<td><strong>B. Serum enzyme assays</strong></td>
<td></td>
</tr>
<tr>
<td>1. Alkaline phosphatase (ALP) (33–96 U/L)</td>
<td>Increased in hepatobiliary disease (highest in biliary obstruction), bone diseases, pregnancy</td>
</tr>
<tr>
<td>2. γ-Glutamyl transpeptidase (γ-GT) (9–58 IU/L)</td>
<td>Rise parallels ALP but is specific for hepatobiliary diseases</td>
</tr>
<tr>
<td>3. Transaminases (aminotransferases)</td>
<td></td>
</tr>
<tr>
<td>i. Aspartate transaminases (SGOT or AST)</td>
<td>Increased in tissue injury to liver as well as to other tissues like in myocardial infarction</td>
</tr>
<tr>
<td>ii. Alanine transaminases (SGPT or ALT)</td>
<td>Increase is fairly specific for liver cell injury</td>
</tr>
<tr>
<td>4. Other serum enzymes</td>
<td></td>
</tr>
<tr>
<td>i. 5’ Nucleotidase</td>
<td>Rise parallels ALP but more specific for diseases of hepatic origin</td>
</tr>
<tr>
<td>ii. Lactate dehydrogenase</td>
<td>Increased in tumors involving liver</td>
</tr>
<tr>
<td>iii. Choline esterase</td>
<td>Decreased in hepatocellular disease, malnutrition</td>
</tr>
<tr>
<td><strong>C. Tests for metabolic functions</strong></td>
<td></td>
</tr>
<tr>
<td>1. Amino acid and plasma protein metabolism</td>
<td></td>
</tr>
<tr>
<td>i. Serum proteins (total = 6.7–8.6 g/dL; A:G ratio = 1.5 to 3.1)</td>
<td>Hypoalbuminemia in hepatocellular diseases; hyperglobulinemia in cirrhosis and chronic active hepatitis</td>
</tr>
<tr>
<td>ii. Immunoglobulins</td>
<td>Nonspecific alterations in IgA, IgG, and IgM</td>
</tr>
<tr>
<td>iii. Clotting factors</td>
<td>Prothrombin time and partial thromboplastin time prolonged in patients with hepatocellular diseases</td>
</tr>
<tr>
<td>iii. Serum ammonia (19–60 µg/dL)</td>
<td>Increased in acute fulminant hepatitis, cirrhosis, hepatic encephalopathy</td>
</tr>
<tr>
<td>iv. Aminoacudria</td>
<td>In fulminant hepatitis</td>
</tr>
<tr>
<td>2. Lipid and lipoprotein metabolism Blood lipids</td>
<td>Increased in cholestasis, decreased in acute and chronic diffuse liver disease and in malnutrition</td>
</tr>
<tr>
<td>(total serum cholesterol &lt; 200 mg/dL; triglycerides &lt; 150 mg/dL; and lipoprotein fractions)</td>
<td></td>
</tr>
<tr>
<td>3. Carbohydrate metabolism Blood glucose and GTT</td>
<td>Decreased in hepatic necrosis</td>
</tr>
<tr>
<td><strong>D. Immunologic tests</strong></td>
<td></td>
</tr>
<tr>
<td>1. Nonspecific immunologic reactions</td>
<td></td>
</tr>
<tr>
<td>i. Smooth muscle antibody</td>
<td>In hepatic necrosis</td>
</tr>
<tr>
<td>ii. Mitochondrial antibody</td>
<td>In primary biliary cirrhosis</td>
</tr>
<tr>
<td>iii. Antinuclear Ab and LE cell test</td>
<td>In chronic active hepatitis</td>
</tr>
<tr>
<td>2. Antibodies to specific etiologic agents</td>
<td></td>
</tr>
<tr>
<td>i. Antibodies to hepatitis B (HBsAg, Hbc, HBeAg)</td>
<td>In hepatitis B</td>
</tr>
<tr>
<td>ii. Amoeba antibodies</td>
<td>Amoebic liver abscess</td>
</tr>
<tr>
<td><strong>E. Ancillary diagnostic tests</strong></td>
<td></td>
</tr>
<tr>
<td>1. Ultrasound examination</td>
<td>Cholestasis of various etiologies; SOLs, US-guide-FNAC/liver biopsy</td>
</tr>
<tr>
<td>2. FNAC and/or percutaneous liver biopsy</td>
<td>Unknown cause of hepatocellular disease, hepatomegaly and splenomegaly, long-standing hepatitis, PUO and SOLs of the liver</td>
</tr>
<tr>
<td>i. In serum</td>
<td></td>
</tr>
<tr>
<td>ii. In faeces</td>
<td></td>
</tr>
<tr>
<td>iii. In urine</td>
<td></td>
</tr>
<tr>
<td>2. Urobilinogen</td>
<td></td>
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<tr>
<td>3. Bile acids (bile salts)</td>
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<tr>
<td>4. Bromsulphalein excretion</td>
<td></td>
</tr>
</tbody>
</table>
B. Serum enzyme assays
   1. Alkaline phosphatase
   2. γ-Glutamyl transpeptidase
   3. Transaminases (aminotransferases)
      i. Aspartate transaminases or AST
      ii. Alanine transaminases or ALT
   4. Other serum enzymes
      i. 5′Nucleotidase
      ii. Lactate dehydrogenase
      iii. Choline esterase
C. Tests for metabolic functions
   1. Amino acid and plasma protein metabolism
      i. Serum proteins
      ii. Immunoglobulins
      iii. Clotting factors
      iv. Serum ammonia
   2. Lipid and lipoprotein metabolism
   3. Carbohydrate metabolism
D. Immunologic tests
   i. Nonspecific immunologic reactions
   ii. Antibodies to specific etiologic agents
E. Ancillary diagnostic tests
   i. Ultrasonography
   ii. FNAC and/or percutaneous liver biopsy

**PATHOPHYSIOLOGY OF JAUNDICE**

Jaundice is the yellowish discoloration of sclera, skin and mucous membrane due to the deposition of bilirubin. This happens when concentration of bilirubin increases in blood. Bilirubin is the product of hemoglobin breakdown. Therefore, to understand pathophysiology of jaundice, it is necessary to learn the process of hemolysis and hemoglobin breakdown.

**Red Cell Breakdown**

Red cells have an average lifespan of 120 days. The aged red cells are trapped by the macrophages of mononuclear phagocytic system (MPS) that are present in spleen, liver, lymph nodes, and bone marrow. Macrophages, especially in the spleen, after destroying red cells and hemoglobin, release their components into blood. For details of red cell destruction and bilirubin metabolism, refer to Chapter 13.

**Hemoglobin Catabolism**

Macrophages split hemoglobin into heme and globin.
1. **Globin** is the protein component, which is degraded into amino acids that enter the amino acid pool of the body and are reutilized whenever required.
2. **Heme** is catabolized by the microsomal oxygenase system to release iron, which joins the iron pool of the body.
3. Some of the heme molecules are simultaneously oxidized to biliverdin.
4. Then, biliverdin is reduced to bilirubin by the enzyme biliverdin reductase. Bilirubin for its lipophilic nature crosses cell membrane easily. However, for its water-insolubility, it is transported in body fluids only after conjugation in the liver or in combination with albumin.

**Bilirubin Metabolism**

Bilirubin released from macrophages enters the blood stream, where it forms a complex with albumin. Bilirubin bound to albumin (**albumin-bilirubin complex**) is not excreted in urine, as albumin molecule is large enough not to get filtered in renal glomeruli. Bilirubin-albumin complex is split in the liver, bilirubin enters liver cells and albumin stays back in the blood.

In liver cells, bilirubin undergoes three-step metabolism: uptake, conjugation, and excretion.

1. **Uptake:** Bilirubin after splitting from albumin-bilirubin complex is transported into the hepatocyte, where it forms a complex with a cytoplasmic protein, called ligandin. Formation of bilirubin-ligandin complex prevents bilirubin to return back to the blood as the complex is too big to pass through the liver cell membrane.
2. **Conjugation:** Hepatocytes conjugate bilirubin with glucuronic acid, which takes place in the endoplasmic reticulum and involves UDP-glucuronic acid and glucuronyl transferase. This forms **bilirubin glucuronide** (Fig. 41.5).
3. **Excretion:** Bilirubin glucuronide is excreted from hepatocytes into the biliary canaliculi. This is an active process, and is the rate-limiting step in the hepatic bilirubin metabolism.

**Fate of Conjugated Bilirubin**

Bilirubin glucuronide is excreted in bile to be discharged into the small intestine. In large intestine, bilirubin is acted upon by the bacterial flora, where glucuronic acid is split and bilirubin undergoes a series of reduction reactions to form **stercobilinogen**.

Stercobilinogen, a colorless compound has two fates:

1. In the intestine, 80% of stercobilinogen is oxidized to **stercobilin**, which is excreted in stool. Stercobilin is a brownish compound that imparts brown color to the stool.
2. The remaining 20% stercobilinogen is absorbed into the portal circulation and re-excreted by liver into the bile, and re-enters the intestine. A part of the absorbed stercobilinogen enters the general circulation and is filtered and excreted by the kidneys. In urine, the stercobilinogen is called urobilinogen.
3. Conjugated bilirubin is not reabsorbable, whereas it metabolic products, urobilinogen is reabsorbed and enters enterohepatic circulation. The major differences between conjugated and unconjugated bilirubin are summarized in Table 41.2.
Normal Plasma Bilirubin Level: The normal concentration of bilirubin in plasma is 0.2–0.8 mg/dL. Jaundice is detected clinically only when the bilirubin level exceeds 2 mg/dL.

Types of Jaundice

Clinically, jaundice is detected when bilirubin level is more than 2 mg/dL. Bilirubin level between 0.8 and 2 mg% is called latent or subclinical jaundice. Jaundice first appears in sclera (Fig. 41.6), because bilirubin has an extremely high affinity for scleral protein called elastin.

Physiologically, the causes of jaundice are divided broadly into two categories: increased production of bilirubin and decreased excretion of bilirubin.

Increased Production of Bilirubin

Production of bilirubin is increased in hemolysis. Therefore, the jaundice is called hemolytic jaundice. This is seen in hemolytic anemias (for details, refer “Pathophysiology of Anemia, Chapter 15”).

Decreased Excretion of Bilirubin

The excretion of bilirubin is impaired when liver cannot conjugate bilirubin efficiently, which is called hepatic jaundice, or when conjugated bilirubin cannot be excreted in bile due to biliary obstruction, which is called obstructive jaundice.

1. Hepatic jaundice commonly occurs in viral hepatitis and obstructive jaundice is commonly seen in gallstones (stone in the common bile duct) or stricture of bile duct. However, hepatic and obstructive jaundice overlap in their pathophysiologic processes.
2. In hepatic jaundice, narrowing of biliary canaliculi occurs very often resulting in \textit{intrahepatic obstruction (stasis)}. This adds an obstructive element to the hepatic jaundice.

3. In obstructive jaundice, \textit{biliary stasis} behind the site of obstruction causes damage to the hepatocytes. This adds hepatocellular element to the obstructive jaundice.

### Laboratory Diagnosis of Jaundice

#### Hemolytic Jaundice

In hemolytic jaundice, \textit{excessive production of bilirubin} allows liver to conjugate more than the normal quantity of bilirubin.

1. Therefore, more quantity of \textit{bilirubin glucuronide} is delivered to the intestine.
2. Consequently, the amount of \textit{stercobilinogen} formed in the intestine is increased.
3. This leads to \textit{increased excretion of fecal stercobilinogen and urinary urobilinogen} (Table 41.3).
4. Bilirubin in plasma forms a complex with albumin, which cannot be excreted in the urine. Therefore, hemolytic jaundice is \textit{acholuric jaundice} (absence of bilirubin in urine). Liver function tests are usually normal.

#### Hepatic Jaundice

In hepatic jaundice, all three steps of bilirubin metabolism (\textit{uptake, conjugation, and excretion}) are affected. But as mentioned earlier, the \textit{rate-limiting step is excretion}, and therefore that may be the most affected. Therefore, not only the conjugation of bilirubin is impaired, but also some amount of conjugated bilirubin is not excreted in bile.

1. The conjugated bilirubin that accumulates in liver cells diffuses across the cell membrane into the bloodstream. Thus, in hepatic jaundice the \textit{blood contains excess of bilirubin-albumin complex} as diseased liver may not be able to conjugate all the load of bilirubin. Also, conjugated bilirubin diffuses back into the bloodstream.

2. Consequently, \textit{conjugated bilirubin (bilirubin glucuronide) is excreted in the urine}. This makes urine \textit{yellow} due to the presence of urinary bilirubin.

3. Bilirubin excreted in bile is reduced. Hence \textit{fecal stercobilinogen and urinary urobilinogen are reduced}.

4. Plasma albumin may be low as diseased liver cannot synthesize the normal amount of albumin. Plasma globulins are high in liver disease because of a \textit{rise in the gamma-globulin fraction}. Thus, albumin-globulin ratio is altered.

5. \textit{Neonatal jaundice} could be due to defective conjugation of bilirubin (Clinical Box 41.1).

#### Obstructive Jaundice

Obstructive jaundice occurs due to obstruction to bile secretion into intestine.

1. In obstructive jaundice, no bile reaches the intestine. Hence, neither bilirubin nor bile salts is present in the intestine. Therefore, \textit{no fecal stercobilinogen is formed}, and stool becomes clay colored.

2. Also, \textit{urinary urobilinogen is absent}.

3. As bile salt is reduced in intestine, there is an increased fecal excretion of fat \textit{(steatorrhea)}.

4. The conjugated bilirubin accumulates proximal to the obstruction, and is regurgitated by the liver cells into the bloodstream. Therefore, the level of \textit{conjugated bilirubin in the blood is high}, which is excreted in urine and causes \textit{deep yellow urine}.

5. Like conjugated bilirubin, bile salts are also regurgitated into the blood stream, and excreted in urine. Initially liver function tests are normal. But, later, prolonged biliary stasis damages the liver and impairs liver functions.

\textit{van den Bergh test}: Van den Bergh test detects whether \textit{bilirubin is conjugated or not}. The test is based on the principle that excess of water soluble bilirubin-glucuronide gives a reddish-violet color when brought in contact with \textit{diazo reagent}.

1. If the color appears immediately, the test is said to be \textit{direct positive}.
2. If the color appears late, or only after addition of alcohol, the test is said to be \textit{indirect positive}.
3. In hemolytic jaundice, the Van den Bergh test is \textit{indirect positive}.
4. In obstructive jaundice, the Van den Bergh test is \textit{direct positive}.
5. In hepatic jaundice, the test may be \textit{biphasic}, i.e. an atypical color develops almost immediately.

**Clinical Box 41.1**

\textbf{Physiological jaundice}: This is seen in some newborns and therefore this is also known as \textit{neonatal jaundice}. It is common in premature babies and neonates having low birth weight. The jaundice usually appears on the second or third day of life and disappears within a week. It occurs due to subnormal activity of glucuronyl transferase that impairs conjugation of bilirubin in hepatocyte. It is unlikely to be due to hemolysis.
## CHAPTER SUMMARY

### Key Concepts

1. Liver is the site of all metabolisms.
2. Bilirubin released from hemolysis is conjugated in liver and conjugated bilirubin is secreted in bile into intestine.
3. Excess production of bilirubin by hemolysis leads to hemolytic (Prehepatic) jaundice, diseases of liver (defect in conjugation) causes hepatic jaundice, accumulation of bilirubin due to obstruction to flow of bile causes obstructive (posthepatic) jaundice.

### Important to Know (Must Read)

1. In examinations, **Long Questions** usually do not come from this chapter. However, Liver function tests may come as a question.
2. Functions of liver, Bilirubin metabolism, Pathophysiology of jaundice, Differences in laboratory diagnosis of types of jaundice, Liver function tests, can come as **Short Questions**.
3. In **Viva**, Structure of a hepatic lobule, Portal triad, Relationship between sinusoidal capillary and bile canaliculi, Functions of liver, Steps of bilirubin metabolism, How is bilirubin metabolized in liver and excreted from the body, Differences between conjugated and unconjugated bilirubin, Pathophysiology of hemolytic, hepatic, and obstructive jaundice, Common causes of hemolytic, hepatic, and obstructive jaundice, Differences in laboratory diagnosis of three types of jaundice, Classification of liver function tests (LFT), Common values and interpretation of LFT, What is physiological jaundice.
4. “Functions of liver” and “name important LFT” are very common questions in viva. A student, who fails to answer these questions satisfactorily, may get fail mark.
Learning Objectives

On completion of study of this chapter, the student **MUST** be able to:
1. Give the composition and functions of bile.
2. List the differences between hepatic and gallbladder bile.
3. Name bile salts and acids and give their functions.
4. Understand the importance of enterohepatic circulation.
5. Outline the mechanism and regulation of bile secretion.
6. Learn the physiological abnormality in gallstone formation.

The student **MAY** also be able to:
1. Describe the regulation of bile secretion.
2. Explain the mechanism of enterohepatic circulation.

Hepatobiliary System

Bile is secreted from liver. Human liver secretes about **0.5 to 1 liter of bile per day**. Secretion of bile is important for digestion and absorption of fat.

Functional Anatomy

Bile is formed in the liver and is excreted through the bile ductules. The bile ductules along with the branches of portal vein and hepatic artery form the **portal triad**. The portal triad opens into the hepatic sinusoids. The sinusoids are surrounded by hepatocytes. The hepatocytes are in intimate contact with the sinusoidal blood. The **bile canaliculi** start at the space between two hepatocytes (refer to Figs. 41.3 and 41.4, Chapter 41), which opens into bile ductules. Bile ductules then form the bile duct which leads to **hepatic duct**. The right and left hepatic ducts join to form **common hepatic duct** (Fig. 42.1). The **cystic duct** from gallbladder combines with hepatic duct to form **common bile duct**. Common bile duct combines with pancreatic duct and opens to second part of duodenum through sphincter of Oddi.

Composition of Bile

Bile is a greenish yellow fluid formed in the liver and stored in gallbladder. It is composed of water (98%), and solids that mainly include bile salts and pigments and different ions (Table 42.1).
Chapter 42: Biliary Secretion

Biliary Secretion

The color of bile is greenish yellow due to the presence of glucuronides of bile pigments. The bile pigments are bilirubin and biliverdin.

Hepatic and Gallbladder Bile

There are differences between hepatic bile (bile formed in the liver) and gallbladder bile (bile stored in gallbladder) as bile is concentrated and acidified in gallbladder (Table 42.2).

1. Water absorption is the major mechanism for concentration of bile that occurs secondary to Na⁺ absorption (secondary active transport).
2. Na⁺-K⁺ ATPase in the basolateral membrane of epithelial cell lining transports Na⁺ actively out of the cell and creates gradient for Na⁺ transport into the cell.
3. Water is reabsorbed passively by osmotic gradient created by Na⁺ movement.
4. H⁺ is exchanged with Na⁺ that causes acidification of bile.

Bile Acids and Salts

Bile Acids

There are two types of bile acids: primary and secondary.
1. The primary bile acids are cholic acid and chenodeoxycholic acid. The primary bile acids are formed in the hepatocytes from cholesterol.
2. Secondary bile acids are deoxycholic acid and lithocholic acid. The secondary bile acids are produced in the intestine where intestinal bacteria convert primary bile acids into secondary bile acids.

Bile Salts

Bile salts are Na⁺ and K⁺ salts of bile acids. Bile acids are conjugated with taurine or glycine to form taurocholic acid or glycocholic acid. These acids then combine with sodium and potassium salts to form Na⁺-taurocholate, Na⁺-glycocholate, K⁺-taurocholate, and K⁺-glycocholate, respectively.

Functions of Bile Salts

Bile salts are primarily responsible for absorption of fat and fat soluble nutrients. Therefore, bile deficiency causes steatorrhea. Bile salts perform following important functions.

1. Absorption of fat: Bile salts are essential for absorption of fat from intestine. This depends on formation of micelles by bile salts in the intestine. For their amphipathic (both hydrophilic and hydrophobic domains) property, along with lecithin and cholesterol, bile salts form cylindrical disks, called as micelles (for details, refer the chapter “Principles of Digestion and Absorption : Chapter 51”. Lipids are transported in micelles from the lumen to the membrane of intestinal mucusosal epithelial cells where micelle dissociates and lipids are absorbed.

2. Emulsification of fat: Bile salts are surface tension reducing agents. Along with phospholipids and monoglycerides, they cause emulsification of fat, which is essential for digestion and absorption of fat.

3. Source of bile acid: Bile salts are converted to bile acids in the intestine, which are then absorbed into portal blood. Thus, bile salts are important sources of bile acids that add to the bile acid pool of the body.

4. Bile secretion: Bile salts are important cholericins. They are constituent of bile and also they stimulate bile secretion.


7. Prevention of gallstone formation: Bile salts along with lecithin solubilize cholesterol. Thus, they prevent formation of stones in the gallbladder.

8. Physiological purgatives: Bile salts act as physiological purgatives. Constipation occurs in conditions of deficiency of delivery of bile into intestine as occurs in obstructive jaundice.

9. Stool color: Bile salts add natural brownish color to the stool.


Enterohepatic Circulation

Bile acids and salts are absorbed from the intestine and re-excreted in the bile, and this cycle is repeated so many times, which is called enterohepatic circulation of bile acids and salts.
Bile salts are produced in liver (200 to 500 mg/day) and conjugated. Conjugated bile salts (CBS) through biliary tract enter intestine.

From intestine, they are absorbed into the portal blood to reach liver.

Also, in the intestine CBS is deconjugated and unconjugated bile salt is absorbed into portal circulation.

The free bile acids in terminal ileum and colon by the action of bacteria are converted into secondary bile acids (SBAs), i.e., deoxycholic acid and lithocholic acid.

SBAs are also reabsorbed into portal circulation.

Thus, from gut, about 95% of secreted bile salts and bile acids are transferred back to liver via portal circulation, and only 200 to 500 mg/day of bile acids are excreted from stool.

From liver, again they are excreted through biliary tract into the intestine. This forms the loop-pathway for enterohepatic circulation (Fig. 42.2).

Enterohepatic circulation ensures preservation and reutilization of various substances. For example, bile salts are recirculated 4–12 times a day. The substances that undergo enterohepatic circulation include bile salts, bile acids, bile pigments, vitamin D, vitamin B₁₂, thyroxine, drugs, etc.

Importance of Enterohepatic Circulation

The primary function of enterohepatic circulation of bile acids and salts is to maintain the total bile acid pool of the body.

Total amount of bile acids (conjugated and unconjugated, and primary and secondary) in the body ranges from 2 to 4 g.

Bile acid is cycled several times a day during meals, so that a small pool of bile acid can efficiently provide enough bile salts to facilitate adequate lipid absorption from small intestine.

Though absorption of bile acids and salts from intestine occurs efficiently, some amount is lost with every cycle of passage through intestine. This accounts for loss of about 500 mg of bile acid daily, which is replenished by synthesis of new bile acids from cholesterol.

Thus, fecal excretion of bile acid accounts for an efficient medium for loss of body cholesterol.

Determinants of Enterohepatic Circulation

The major determinants of enterohepatic circulation of bile acids and salts are integrity of intestinal epithelium to reabsorb them and efficiency of hepatocytes to pick them up from portal blood.

Integrity of Intestinal Epithelium

Bile salts are absorbed mainly in the terminal ileum by an efficient carrier mediated process so that only a 5% of intestinal bile salts enter the colon. Also in the intestine, bacteria deconjugate the bile salts to bile acids, and bile acids are absorbed passively and easily as they are more lipophilic than bile salts.
1. Thus, *intestinal absorption of bile acids and salts into portal blood is an important determinant* of enterohepatic circulation of these substances.
2. Further, bacteria convert primary bile acids to secondary bile acids that are also absorbed into portal circulation. Therefore, lipid malabsorption occurs in chronic inflammatory conditions of intestine (Clinical Box 42.1).

**Effectiveness of Hepatic Uptake**

In the portal blood, bile salts are transported bound to HDL or albumin. Once they reach liver, hepatocytes efficiently pick-up bile salts from hepatic blood, which accounts for removal of more than 80% of portal bile salts.

1. This *hepatic uptake of bile salts is an important determinant* of secretion of bile salts in the bile.
2. Thus hepatic uptake is the determinant of efficiency of enterohepatic circulation of bile salts.

**Clinical Box 42.1**

Enteritis and hepatitis cause lipid malabsorption: Bile salts are absorbed mainly in the distal ileum and bile salts promote lipid absorption. Therefore, inflammation of lower part of small intestine (enteritis) that inhibits absorption of bile salts in intestine results in loss of large quantity of lipid in the stool (due to malabsorption of fat). Similarly, inflammation of liver (hepatitis) impairs the uptake of bile acid and salts from portal blood that in turn decreases their secretion in the bile. This also can cause intestinal malabsorption of fat.

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**BILE SECRETION**

**Mechanism of Secretion**

The secretion of bile consists of *canalicular flow* and *ductular secretion*. In the canaliculi, bile acids and Na⁺-K⁺-ATPase contribute to bile flow. In the ducts, epithelial cells secrete HCO₃⁻ and Cl⁻ actively into the lumen.

**Mechanism of Bile Acid Secretion**

Bile is formed in hepatocytes. Bile acids are taken up from the sinusoidal blood by transport proteins that are present on the basolateral membrane of the hepatocytes.

1. In cytoplasm of hepatocytes, bile acids bind with *bile acid binding proteins*. This *prevents bile acids to accumulate in higher concentration* in cytoplasm. It should be noted that high concentration of free bile acids destroys the organelles of hepatocytes.
2. Bile acids are *conjugated with glycine or taurine* and then *secreted into the lumen of hepatic canaliculi by facilitated diffusion*.
3. Bile acids move into the bile down their electrochemical gradient. The concentration gradient is maintained by formation of *micelles in the bile* present in the canaliculi.

**Mechanism of Water and Electrolyte Secretion**

Water and electrolyte concentration of bile in the bile canaliculi is same with that of plasma.

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1. The bile duct epithelial cells secrete bicarbonate rich fluid into the lumen.
2. The ions are transported in a similar way to that of pancreatic extralobular ducts.

**Modification of Bile in Gallbladder**

Bile is continuously synthesized and secreted from liver and is stored in the gallbladder. Gallbladder is a small sac like structure with the capacity of 30–60 mL. Gallbladder performs **three functions**:

1. **Storage of bile**
2. **Acidification of bile**: The hydrogen ion is secreted from gallbladder epithelial cell into the bile to make the bile acidic.
3. **Concentration of bile**: H⁺ is secreted into the lumen of gallbladder in exchange for Na⁺ (Na⁺-H⁺ exchanger). For Na⁺ transport into the cell, the gradient is created by Na⁺-K⁺ pump located in the basolateral membrane of the epithelial cells. Water is reabsorbed from the gallbladder bile so that bile is concentrated (Fig. 42.3).

**Regulation of Bile Secretion**

Bile secretion is influenced by choleretics and cholagogues.

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**Clinical Box 42.1**

Enteritis and hepatitis cause lipid malabsorption: Bile salts are absorbed mainly in the distal ileum and bile salts promote lipid absorption. Therefore, inflammation of lower part of small intestine (enteritis) that inhibits absorption of bile salts in intestine results in loss of large quantity of lipid in the stool (due to malabsorption of fat). Similarly, inflammation of liver (hepatitis) impairs the uptake of bile acid and salts from portal blood that in turn decreases their secretion in the bile. This also can cause intestinal malabsorption of fat.
Choleretics
The substances that enhance the secretion of bile are called choleretics. The important choleretics are:
1. Bile salts
2. Secretin
3. Vagal stimulation.
   Secretin increases aqueous component of bile.

Cholagogues
The substances that cause contraction of gallbladder to increase the output of bile from the structure are called cholagogues. The important cholagogues are:
1. Cholecystokinin
2. Fatty acids.
   Cholecystokinin causes contraction of smooth muscles of gallbladder and empties its contents.

Functions of Bile
Functions of bile are mainly due to presence of bile salts in it.
1. Bile helps in absorption of lipids from intestine by forming micelles.
2. Bile salts in bile are important choleretics (increase bile secretion).
5. Bile pigments (bilirubin and biliverdin) are responsible for greenish-yellow coloration of gallbladder that helps to identify the organ especially in small animals.
6. Bile salts are physiological purgative.

APPLIED PHYSIOLOGY

Gallstones
Presence of stone in the biliary tract (gallbladder, and bile ducts) is called gallstone or cholelithiasis. Gallstone is commonly associated with five Fs: females, forty to fifty age, fair looking individuals, fertile ladies, and fat people.

Types
There are two types of stones: cholesterol stones and calcium bilirubinate stones. In USA and Europe, 85% of stones are cholesterol stones (pigment stones). Bile salts along with lecithin solubilize cholesterol. Thus, they prevent formation of stones in the gallbladder. When cholesterol concentration is high or bile concentration is less, cholesterol stone develops.
   Factors that favor stone formation are:
1. Bile stasis. Decreased bile flow or decreased gallbladder emptying facilitates stone formation.
2. Supersaturation of bile with cholesterol.
3. Nucleation factor that favors formation of stones from the supersaturated bile. Glycoproteins in gallbladder mucous form the nucleus surrounding which gallstones are formed.
   Usually cholesterol stones are big and may be one or two stones are present in gallbladder. The calcium bilirubinate stones are multiple, and more than 50 stones may be present in gallbladder of the patient (Fig. 42.4).

Features
Usually, gallstones are silent stones. But, when stones come out of the gallbladder and pass through the biliary ducts, severe colicky or spasmodic acute abdominal pain occurs.
1. Chronic gallstones may present with acute cholecystitis, bile stasis inducing inflammation of gallbladder.
2. The stone may induce inflammation and edema of gallbladder, resulting in chronic cholecystitis (Fig. 42.4).
3. Gallstones may also present with obstructive jaundice.

Diagnosis
Diagnosis is usually made by ultrasound or cholecystography. Plain X-ray may sometime detect stones.

Treatment
There is no effective medical treatment, though many drugs have been advocated to dissolve stone. Gallstones are treated surgically by removal of gallbladder (cholecystectomy).

Effects of Cholecystectomy
Gallbladder is not absolutely essential for digestive functions. The patients those who have undergone cholecystectomy, maintain normal health and usually their nutrition is not affected. Only problem they face is the steatorrhea, if they eat excess of fat food. Cholecystectomized patients tolerate fat foods to some extent. It decreases body fat and may be beneficial for obese people.
CHAPTER SUMMARY

Key Concepts
1. Bile is required for absorption of fat from intestine.
2. Enterohepatic circulation of bile salts and bile acids prevents loss of important metabolizes from the body and ensures their reutilization.
3. Cholecystectomy is not detrimental to health, and may be useful in the treatment of obesity.

Important to Know (Must Read)
1. In examinations, usually Long Questions are not asked from this chapter. However, ‘Mechanism and regulation of bile secretion’ may sometime come as a question.
2. Bile salts, Bile acids, Functions of bile salts, Enterohepatic circulation, Mechanism of bile secretion, Regulation of bile secretion, Choleretics and cholagogues, may come as Short Questions.
3. In Viva, examiner may ask…… Name bile salts and tell their functions, Name bile acids and tell their functions, Composition of bile and functions of bile, What is enterohepatic circulation and what is its importance, What are the common substances that undergo enterohepatic circulation, Mechanism of bile secretion, Regulation of bile secretion, Name choleretics and cholagogues, Cause, types and features of gallstones.
4. “Enterohepatic circulation” is usually asked as a short note, or in viva.
Learning Objectives

On completion of study of this chapter, the student MUST be able to:
1. Appreciate the importance of intestinal secretion in digestion and absorption of nutrients.
2. Understand the mucosal modifications in intestinal epithelium to increase the surface area for absorption.
3. Give the composition and functions of intestinal secretion.
4. Understand the importance of intestinal flora in GI physiology.
5. Learn the physiological basis of malabsorption syndrome.
The student MAY be also able to:
1. Describe the details of mechanism and regulation of intestinal secretion.

Functional Anatomy

Small intestine consists of duodenum, jejunum and ileum. It is the longest segment of GI tract, which is about 285 cm (duodenum 25 cm + jejunum-ileum 260 cm) in adults. Though duodenum is well demarcated from jejunum, there is no distinct separation between jejunum and ileum. For its length and capacity, small intestine is the most important segment of GI system for digestion and absorption of all nutrients.

1. Secretion of small intestine is called succus entericus.
2. It contains most enzymes for carbohydrate, protein and fat digestion.
3. Small intestine secretes about 2–3 liters of isotonic fluid per day.

Intestinal Glands

As discussed in chapter 36, the intestinal wall has all the layers of the gut (Refer to Fig. 36.3): serosa, outer longitudinal and inner circular muscle layers, submucosa, muscularis mucosa, and mucosa (Figs. 43.1A and B). The mucosa is highly developed to contain villi and intestinal glands.

Intestinal secretion is produced by the glands in the small intestine. The intestinal glands are called crypts of Lieberkühn:

1. These are tubular glands present at the base of the villi. Throughout the length of small intestine, the mucous membrane is covered by villi. There are about 20–40 villi per square millimeter of mucosa (Fig. 43.2).
2. Each villus is a fingerlike projection covered by a layer of columnar epithelium and contains a network of capillaries and lacteals (lymphatics).
3. The villi have numerous microvilli on their surface that are called brush border. Brush border considerably increases the surface area for absorption.
4. Some quantity of secretion is also added by Brunner’s glands that are coiled acinotubular glands present in the duodenum in addition to crypts of Lieberkühn.
5. The intestinal mucosa is supported externally by thin layer of smooth muscle fibres, muscularis mucosae. The mucous membrane is thrown into folds or plicae which are more in the jejunum and less in the ileum, thus increasing the absorptive surface enormously.

Scientist contributed

Johann Nathanael Lieberkühn (1711–1756) was a German physician and physiologist. Initially he studied theology, then physics (mechanics) and then medicine. He served as a MEDICAL professor and medical doctor. Besides his physiological work, Lieberkühn was most known for his preparation of medical specimens—these were still presented up to the nineteenth century, especially in Moscow, as masterpieces. The Crypts of Lieberkühn (intestinal glands) are named after him, which he first described these in detail. He had developed microscopes for studying blood vessels were called ‘Wundergläser’, ‘wonder-glasses’ by his contemporaries.
Chapter 43: Intestinal Secretion

**Intestinal Mucosa**

The mucosa of small intestine contains lymphatic nodules (Peyer’s patches). There are also enterochromaffin cells, Paneth cells and undifferentiated cells in the intestinal mucosa (Fig. 43.3):

1. The mucous membrane of the intestine contains many valve-like folds called **valvulae conniventes**, which add to the surface area for absorption.
2. In intestine, the surface area for absorption is **increased by about 600 fold** by villi, brush border and valvulae conniventes.
3. **Paneth cells** are endocrine cells present in the crypts of Lieberkühn in their deeper part. They secrete **defensins**, the naturally occurring antibiotics that protect developing enterocytes against infections. Paneth cells also secrete **guanylin**.
4. The **undifferentiated cells** are the progenitor cells in the mucosa present in the crypts of Lieberkühn that...
form new epithelial cells, which migrate to the surface. The epithelial cells are continuously renewed by progenitor cells.

5. After the life span of about 2–5 days, enterocytes are sloughed along with mucosal cells. Shedding of these epithelial cells accounts for daily excretion of about 30 g of protein as the cells are protein-rich.

6. Goblet cells secrete mucus that form a gel solution on the surface of mucosa.

**Types of Cells in Villi**

The absorptive surface of intestinal mucosa is increased by the intestinal villi. Villi are finger like or leaf like projections which contain 3 types of cells:

i. **Simple columnar cells:** They perform absorptive function due to the presence of brush border consisting of large number of villi.

ii. **Goblet cells:** These are mucous secreting cells and are interspersed between the columnar cells.

iii. **Endocrine cells:** These are scattered in the villi as well as are widely distributed throughout the gastrointestinal tract. These cells are:
   a. Kulchitsky cells: After the name of its discoverer
   b. Enterochromaffin cells: Due to their resemblance to chromaffin cells of the adrenal medulla
   c. Argentaffin cells: As the intracytoplasmic granules stain positively with silver salts by reduction reaction (agyrophil cells, on the other hand, require the addition of exogenous reducing substances for staining)
   d. Enterochromaffin cells: As these specialized cells are considered to be part of APUD cell system (having common properties as amine content, Amine Precursor Uptake and Decarboxylation). APUD cells are considered to be endodermal in origin, while previously they were thought to be neural crest derivative. Other endocrine cells belonging to the APUD cell system are C-cells of the thyroid, chromaffin cells of the adrenal medulla, certain cells of the carotid body, bronchi, hypothalamus, pituitary and sympathetic ganglia.

Endocrine cells are heavily populated in the proximal small bowel as this is the most active site for absorption and secretory activities. They are sparse in the colon which is less active site for such functions.

**INTESTINAL SECRETION**

**Composition**

Daily secretion from small intestine varies from 1–2 liters:

1. Intestinal secretion is isotonic, watery and alkaline.
2. The pH is about 8. It consists of water (98.5%) and solids (1.5%).
3. The solids contain anions (HCO$_3^-$, Cl$^-$, PO$_4^{3-}$, and SO$_4^{2-}$), cations (Na$^+$, K$^+$, Ca$^{2+}$ and Mg$^{2+}$), enzymes (Table 43.1), and mucous.

**Table 43.1: Actions of Intestinal enzymes.**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterokinase</td>
<td>Trypsinogen</td>
<td>Trypsin</td>
</tr>
<tr>
<td>α-dextrinase</td>
<td>α-dextrins</td>
<td>Glucose</td>
</tr>
<tr>
<td>Malatase</td>
<td>Malate</td>
<td>Glucose</td>
</tr>
<tr>
<td>Sucrase</td>
<td>Sucrose</td>
<td>Glucose and fructose</td>
</tr>
<tr>
<td>Lactase</td>
<td>Lactose</td>
<td>Glucose and galactose</td>
</tr>
<tr>
<td>Peptidases</td>
<td>Terminal amino</td>
<td>Peptides and amino acids at amino end of peptides</td>
</tr>
<tr>
<td>Nucleotidases</td>
<td>Nucleotides</td>
<td>Nitrogenous bases, pentoses, and phosphates</td>
</tr>
</tbody>
</table>

4. Enterokinase is present in the brush border of enterocytes and is extruded with denudation of mucosal epithelium.

**Mechanism and Regulation of Secretion**

**Mechanism**

The exact mechanism of intestinal secretion is not clear:

1. The cations are secreted by active transport and anions are transported along with cations to maintain electroneutrality.
2. Water follows the ions to maintain osmotic balance.
3. Mucus is secreted by surface epithelial cells, Brunner’s gland and goblet cells are present in the mucosa of the intestine.
4. Mucin is the major component of mucus that forms a gel to cover the mucosal epithelium.
5. Mucous protects the intestinal epithelium and helps in smooth passage of chyme through the intestinal lumen. Mucous also traps bacteria and holds immunoglobulin.
6. Secretion of Brunner’s gland, a thick alkaline mucus protects duodenal mucosa from acidic chyme.

**Regulation of Secretion**

Intestinal secretion is mainly controlled by GI hormones and vagus nerve:

1. VIP stimulates secretion. Therefore, diarrhea is a major feature of VIPomas.
2. The vagal stimulation increases intestinal secretion.
3. Many toxins stimulate intestinal secretion (Clinical Box 43.1).

**Clinical Box 43.1**

**Cholera is a secretory diarrhea:** Cholera toxin increases cyclic AMP concentration in the enterocytes that stimulate active secretion of HCO$_3^-$ and Cl$^-$ into the lumen and produces watery diarrhea.

**Experiment to Study Intestinal Secretion**

In animal models, experiments are performed to study the rate and composition of intestinal secretion. In these animals, a loop of intestine is resected and both ends of the loop are connected to anterior abdominal wall in such a way that they open to outside. Thereafter, various stimuli are applied on the loop and their effects are studied. Such an intestinal loop is called Thiry-Vella loop.
Functions of Intestinal Secretion

1. Intestinal secretion helps in proper mixing of chyme. This provides suitable environment for digestion and absorption of food materials in the intestine.
2. Intestinal secretion contains enzymes for digestion of various nutrients.
3. Mucus of intestinal secretion protects intestinal epithelium from damage. It also traps and kills bacteria.
4. Mucus in the intestinal secretion contains immunoglobulins that play an important role in local defenses.
5. The alkaline secretion of Brunner’s gland protects upper intestinal mucosa from damage by acidic chyme.
6. Intestinal secretion helps in forward propulsion of chyme in the intestine.

Absorption of Water

Normally, about 8.5 liters of fluid is added to GI tract daily. However, only about 100 ml of water is excreted in the stool. Thus, GI tract absorbs more than 99.5% of water load presented to it per day. 75% of water absorption takes place in the distal part of small intestine (For details, see next chapter).

Intestinal Bacteria

Normally, a group of bacteria are present in the intestine. They constitute normal intestinal flora. These microorganisms are present mainly in the ileum than in upper part of the intestine. This is because the acidic content of duodenum and upper jejunum do not favor growth of bacteria:

1. The organisms in the flora mainly include E. coli, Enterobacter aerogenes and Bacteroids fragilis.
2. There are also few other bacteria like, cocci and bacilli of various types.
3. Normally, bacteria are lost in the stool and replaced in the intestine by their natural growth. However, excess loss of bacterial flora in diseases like acute diarrhea results in improper digestion and absorption (Clinical Box 43.2).

Functions of Intestinal Flora

Intestinal bacteria are essential for many gut functions:

1. Normal bacterial flora is essential for digestion and absorption of essential nutrients including vitamins, minerals, and water.
2. They synthesize vitamin K and B complexes.
3. They produce chemicals that help in formation of short chain fatty acids, which help in growth of the intestinal mucosa.
4. Some bacteria species like Salmonella inhibit inflammatory processes. They do so by inhibiting ubiquitination of I Ba, the step that helps the transcription of factor NF B to initiate inflammation. Bacteria also secrete anti-inflammatory cytokines like IL-10. Recently, commensal bacteria are genetically programmed to produce IL-10.
5. Some bacteria utilize substances like ascorbic acid and cyanocobalamin. Therefore, these vitamins are usually given in higher dosages.
6. Bile salts are converted to bile acids by intestinal bacteria, which are then absorbed into portal blood from intestine and colon. Thus, intestinal flora maintains bile acid pool of the body.
7. They impart brown color to the stool by forming pigments from bile pigments. Therefore, acholic (pale) stool is seen in biliary obstruction.
8. They produce gases that cause normal flatus. Normal flatus is required for normal distension and motility of intestine. Exaggeration or decrease flatus indicates abnormality in intestinal flora.
9. They also contribute to the odor of the feces. This occurs due to production of amines like indole and skatole by intestinal bacteria.
10. They contribute to plasma lipid level by interfering in absorption of LDL and cholesterol. Therefore, poorly absorbable antibiotic like neomycin that modifies bacterial flora decreases plasma cholesterol.

Though these bacteria are nonpathogenic and beneficial, their entry into systemic circulation can cause systemic sepsis as occurs in ionizing radiation that breaks the intestinal defense barrier. This results in radiation poisoning.

Clinical Box 43.2

Lactobacilli is used in treatment of diarrhea: Apart from antibiotics, lactobacilli are included as part of the treatment of severe acute diarrhea that causes loss of intestinal flora. Bacterial flora is not well developed in infants and is slowly established during early childhood. Therefore, lactobacilli treatment is a must in any acute gastroenteritis in children. Many antibiotics interfere in growth of intestinal bacteria. Hence, lactobacilli supplement is given along with higher antibiotics.

Applied Physiology

Malabsorption Syndrome

The commonest abnormality due to inappropriate intestinal secretion is malabsorption syndrome. However, malabsorption also occurs due to gastric, hepatic and pancreatic deficiencies.

1. In malabsorption due to intestinal causes, the digestive and absorptive functions of small intestine are impaired. The small intestine is very long (about 285 cm in adults). Therefore, unless the disease process significantly affects the adequate length of intestine, malabsorption does not develop. Similarly, only in surgical procedure that removes or bypasses more than 50% of the intestine, significant malabsorption occurs.
2. The common intestinal conditions that produce malabsorption are sprue (tropical sprue), celiac disease (gluten enteropathy), Crohn’s disease, Whipple’s disease and radiation enteritis (for details of malabsorption, refer the Chapter “Principles of Digestion and Absorption”).
3. In these conditions, hypoproteinemia develops early due to deficient absorption of amino acids.
Absorption of carbohydrate and fat is also impaired. Fat soluble vitamins (A, D, E, and K) are also not properly absorbed due to defective fat absorption. Excretion of large amount of fat (steatorrhea) results in bulky, pale and foul-smelling stool.

**Blind Loop Syndrome**

Though normal bacterial flora is essential for health, overgrowth of bacteria is harmful:

1. Such overgrowths occur when there is stasis of intestinal contents.
2. This condition is commonly observed in patients with **surgically created blind loops** of small intestine, which is popularly known as blind loop syndrome.
3. It causes **macrocytic anemia, malabsorption of vitamin B₁₂**, and steatorrhea.
4. Steatorrhea occurs due to excessive hydrolysis of conjugated bile salts by the bacteria.

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**CHAPTER SUMMARY**

**Key Concepts**

1. Intestinal secretion is very useful for digestion and absorption of nutrients from intestine, as it contain enzymes for digestion of all types of nutrients. Therefore, nature has given a vast surface area for absorption by providing microvilli and brush borders.
2. Intestinal bacteria facilitate the process of digestion and absorption and help in synthesis of few vitamins.
3. 75% of water is absorbed in distal part of small intestine. Diarrhea occurs, if the secretion of water is increased or absorption of water is decreased.

**Important to Know (Must Read)**

1. In examinations, usually **Long Questions** are not asked from this chapter.
2. Intestinal glands, Intestinal mucosa, Intestinal endocrine cells, Mechanism and regulation of intestinal secretion, Composition and functions of intestinal secretion, Bacterial flora of intestine, may come as **Short Questions**.
CHAPTER 44

Secretion of Large Intestine

Learning Objectives

On completion of study of this chapter, the student MUST be able to:
1. Appreciate the importance of physiology of the colon.
2. Understand the importance of colonic bacteria.
3. Learn the physiology of water absorption and secretion through GI tract.

The student MAY also be able to:
1. Describe the details of secretion from colon.

The secretion of large intestine is not considered so important physiologically as it does not help in digestion. The major function of large intestine is the absorption of water and electrolytes. It absorbs about 90% of its load (mainly water is absorbed) in the form of chyme presented to it from the ileum. Of about 2 liters of isotonic chyme that enters large intestine, only about 200 ml is excreted as feces.

Physiology of Colon

Functional Anatomy

Large intestine consists of cecum, colon and rectum (Fig. 44.1). Colon forms the major part of the large gut:
1. Length of colon (ascending, transverse, descending and sigmoid colon) in adult is about 110 cm.
2. The diameter of large intestine is bigger than small intestine, which favours slow passage of chyme through it and also absorption of maximum quantity of water during its passage.
3. There are no villi, though the mucosal epithelium is folded to give villi like appearance to some extent.
4. There are many crypts of Lieberkühn in the mucous membrane that secrete a solution isotonic to that of plasma.
5. There are numerous goblet cells in the mucosal epithelium that secretes mucous.
6. Lamina propria contains intestinal glands and submucosa contains lymphatic nodules (Fig. 44.2).
7. No digestive enzymes are secreted in the colon.

Colonic Secretion

Composition of Secretion

Secretion of large intestine contains mainly mucous secreted by goblet cells. It contains $\text{HCO}_3^-$ and $\text{K}^+$ in large amount and some amount of sodium and water. The pH is about 8.

Mechanism of Secretion

There are crypts in the mucous membrane that secrete a solution isotonic to that of plasma.
1. There are plenty of goblet cells that secrete mucous. Mucous secreted from goblet cells has following functions:
   - Protects intestinal mucosa
   - Help in stool formation
   - Lubricate the intestinal mass
   - Neutralize the acid which is formed by bacteria in the large intestine.
2. Water and bicarbonate are secreted in significant amount.
3. $\text{Na}^+$ is reabsorbed and $\text{K}^+$ is secreted in large amount.
4. The bacterial flora of large intestine is similar to that of small intestine.
5. About 90% water is reabsorbed from the intestinal contents:
   - Therefore, when content of large intestine passes very slowly or there is stagnation, constipation occurs due to absorption of more water that causes solidification of stool.
   - Conversely, when the contents pass faster due to increased motility, diarrhea occurs. Secretion of water and electrolyte in large quantity can also cause diarrhea.

Transfer of electrolytes and water: Large intestine can absorb \( \text{Na}^+ \), \( \text{K}^+ \), \( \text{Cl}^- \), glucose and certain vitamins. However, secretion of \( \text{K}^+ \) and \( \text{HCO}_3^- \) into the colon is more. Colon cannot absorb protein, fat or Ca\(^{2+}\). \( \text{Na}^+ \) is actively absorbed from the colon and water follows along the osmotic gradient generated by absorption of \( \text{Na}^+ \) and \( \text{Cl}^- \).

**Colonic Bacteria**

Colonic bacteria resemble the intestinal flora inhabiting ileum. They produce about 8 liter of gas per day that contributes to flatus and colonic motility. They have trophic effects on colonic mucosal growth. Ammonia is produced by colonic bacteria, which is detoxified in the liver. Hence, in severe liver disease, hepatic encephalopathy (suppression of brain activities) occurs.

**Organisms**

At birth, the colon is sterile but the colonic bacterial flora becomes established early in life. The micro-organisms habituating the colon are bacilli such as mainly *Escherichia coli* and *Enterobacter aerogenes*. Gas gangrene bacilli may be present.

**Functions of Colonic Bacteria**

The physiological role of colonic bacterial flora are:

1. **Synthesis of the vitamins**: Vitamins e.g. vitamin K, number of B complex vitamins and folic acid are produced by these colonic organisms.
2. **Production of gas**: \( \text{CO}_2 \), hydrogen sulphide, hydrogen and methane which contribute to the flatus, are produced by bacterial flora (Application Box 44.1)
3. **Smell of stool**: The smell of stool is largely due to sulphides.
4. **Acidic reaction of stool**: Organic acids formed from carbohydrates by bacteria causes slightly acidic reaction of the stool (pH 5 to 7):
   a. **Production of amines**: A number of amines are formed in the colon by bacterial enzymes that decarboxylate amino acids. These amines are *histamine* and *tyramine* which may be harmful if produced in excess quantity. *Indole* and *skatole* are responsible for the odour of the faeces.
5. **Colour of stool**: Pigments biliverdin and stercobilinogen formed from the bile pigments by the intestinal bacteria are responsible for the brown colour of the stool.
6. **Decrease blood lipids**: Intestinal bacteria play some role in *cholesterol metabolism* and decrease plasma cholesterol and LDL levels.

**Application Box 44.1**

**Excess gas production causes borborygmi**: The volume of gas normally found in the human gut is approximately 200 mL, and the daily production is 500–1,500 mL. In some individuals, more gas is produced in the intestine that causes cramps, borborygmi (rumbling noise) and abdominal discomfort.
Absorption of Water

Normally, about 1.5 liters of water is ingested per day and 7 liters is added from GI secretions per day. However, only about 100 ml of water is excreted in the stool daily. Thus, **GI tract absorbs more than 99.5% of water load** presented to it per day:

1. Water is not absorbed from mouth, esophagus, and stomach.
2. As most of the nutrients are absorbed mainly in the duodenum and jejunum, the osmolality of intestinal content which is about 600 mOsm/kg H₂O, decreases to about 200 mOsm/kg H₂O in the ileum and colon.
3. Therefore, **water is absorbed mainly from terminal ileum and colon** (Fig. 44.3) by osmotic gradient as osmolality of blood is about 290 mOsm/kg H₂O.

**PHYSIOLOGY OF RECTUM**

Rectum stores fecal materials. Role of rectum in defecation reflex is discussed in “Motility of Large Intestine”. Though, normally absorption of water from rectum is not significant, the **absorptive capacity is more** (Clinical Box 44.1)

**Clinical Box 44.1**

Rectal administration of drugs: The absorptive capacity of mucosa of colon and rectum is large. Therefore, sometimes drugs are administered through rectum. Rectal administration is more preferred in children. Drugs that are administered rectally include purgatives, sedatives, anesthetics, and tranquilizers. However, care should be taken to control the volume of enema introduced into rectum in children as excess water absorption can cause water intoxication.

**Fig. 44.3:** Water absorption in small and large intestines. Note, out of 8.5 liters of load of fluid to the intestine per day, about 75% of absorption takes place in small intestine (6.8 L) and about 25% occurs in large intestine (1.6 L). Thus, total 99.5% of water presented to gut, is absorbed to blood by intestine. Thus, intestine plays an important role in water homeostasis of the body.
CHAPTER SUMMARY

**Key Concepts**

1. Large intestine does not produce any enzyme for digestion of nutrients. However, significant quantity of water is reabsorbed from colon.
2. Colonic bacteria are involved in gas production that facilitates the process of bowel movement by distending the bowel and also they help in synthesis of few vitamins.

**Important to Know (Must Read)**

1. In examinations, no Long Question is asked from this chapter.
2. Colonic bacterial flora, Colonic secretion, Role of colon in water and electrolyte absorption, Absorption of water by intestine, may come as Short Questions.
3. In Viva, examiner may ask... Layers of wall of large intestine, Structure of large intestinal glands, Structure of large intestinal mucosa, Mechanism and regulation of colonic secretion, Types of bacteria in bacterial flora of intestine, Functions of bacterial flora in large intestine, How is water secreted and absorbed in different parts of the colon.
CHAPTER 45

Introduction to GI Motility

**LEARNING OBJECTIVES**

On completion of study of this chapter, the student **MUST** be able to:
1. Correlate the electrophysiology of smooth muscle with GI movements.
2. Draw a labeled diagram of slow wave of GI smooth muscle.
3. List the types of GI motilities and give their functions.
4. Understand the role of VARIOUS sphincters in GI tract.
5. Appreciate the applied aspects of physiology of GI motility.

The student **MAY** also be able to:
1. Describe the electrophysiological properties of GI smooth muscle.

**PHYSIOLOGY OF GI SMOOTH MUSCLES**

Movements of GI tract assist in propelling foodstuffs in the forward direction, breaking down foodstuffs into smaller particles and mixing of food with GI secretions for proper digestion and absorption. This is possible due to the presence of **smooth muscles** in the wall of the GI tract and presence of a developed system of **enteric motor neurons**.

1. GI smooth muscles are **unitary type** of smooth muscles.
2. The unique feature of GI smooth muscles is that they **contract spontaneously without external neural and humoral influences**.
3. They also **contract when stretched** without external innervation.
4. There is no structured neuromuscular junction.
5. GI smooth muscles are long (about 500 mm) and slender (5–20 mm in width).
6. They are arranged in bundles.

**Electrophysiology of GI Smooth Muscle**

**Types of Couplings**

There are three types of couplings: electromechanical, pharmacomechanical, and mechanomechanical.

**Electromechanical Coupling**

Like skeletal muscle, smooth muscle have electromechanical coupling. That means, depolarization of the membrane causes opening of voltage-gated Ca\(^{++}\) channels and increases cytosolic calcium, which leads to muscle contraction.

**Pharmacomechanical Coupling**

In addition, smooth muscles have an additional mechanism of pharmacomechanical coupling, in which binding of a ligand to its receptor on the muscle membrane leads to opening of Ca\(^{++}\) channels and increase in cytosolic calcium without change in membrane potential.

**Mechanomechanical Coupling**

Stretch of smooth muscle causes muscle contraction, which is known as mechanomechanical coupling. Stretch on muscle opens stretch-sensitive calcium channel on the muscle cell membrane. Calcium influx causes muscle contraction.

**Syncytial Tissues and Pacemaker Tissues**

GI smooth muscles have numerous gap junctions that easily transmit electrical impulse from cell to cell. This accounts for the syncytial nature of smooth muscle in GI...
tract. There are also pacemaker tissues in GI tract that generate and spread electrical impulses.

**Basic Electrical Rhythm and Slow Waves**

Resting membrane potential (RMP) of smooth muscle of GI tract exhibit wide range of fluctuation.

1. The electrical slow waves are wide spontaneous rhythmic fluctuations in the membrane potential ranging between –65 and –40 mV (Fig. 45.1).
2. The slow waves, which oscillate significantly are called basal electrical rhythm (BER) that occurs due to wide variation in the RMP of GI smooth muscles.
3. The BER is present in all parts of GIT except in the esophagus.

**Slow Waves**

Slow waves in many regions of GI tract represent only as BER, whereas at other places like gastric antrum and intestine, they trigger action potential.

1. Slow waves are generated by the interstitial cells of Cajal located between the longitudinal and circular muscle layers.
2. Interstitial cells have long processes that form gap junctions with longitudinal and circular muscle cells. This enables easy and rapid conduction of slow waves from the interstitial cells into the smooth muscles.
3. The slow waves spread throughout the smooth muscles of each segment of GI tract.

**Phases of Slow Waves**

A typical slow wave has four phases (Fig. 45.2).

**Phase 1:** This is the rising phase (depolarization), which occurs due to opening of voltage gated Ca\(^{2+}\) channel.

**Phase 2:** This is the plateau phase that occurs due to balance between inward Ca\(^{2+}\) current and outward K\(^+\) current.

**Phase 3:** This is the falling phase (repolarization) occurring due to activation of voltage gated K\(^+\) channel.

**Phase 4:** Return to membrane potential.

**Frequency:** The frequency of slow waves is 3/min in stomach, 6 to 10/min in colon, 15/min in jejunum and ileum, and 18/min in duodenum (Fig. 45.3).

- The amplitude and the frequency of slow waves are influenced by the activity of extrinsic and intrinsic nerves, and by hormones.
- Usually, sympathetic stimulation decreases the amplitude and parasympathetic stimulation increases the amplitude of slow waves.

**Action Potentials**

When the peak of a slow wave exceeds the threshold, action potential is triggered from the peak (Fig. 45.4). These action potentials facilitate the force of contraction of smooth muscle.

1. Action potentials have longer duration (10–20 ms). Usually, they do not overshoot.
2. The rising phase of the action potential is caused by influx of Ca\(^{2+}\) and Na\(^+\).
Chapter 45: Introduction to GI Motility

3. The repolarization phase is due to $K^+$ efflux.
4. The magnitude and frequency of action potential are enhanced by vagal stimulation and inhibited by sympathetic stimulation.

Relationship between electrical and mechanical responses:
Slow waves that are not accompanied by action potentials do not elicit contraction, whereas, slow waves that are accompanied by action potentials evoke contraction. The greater the frequency of action potential (that occur at the peak of slow waves), the more intense is the contraction (Fig. 45.5).

**Electrical Coupling between Cells**
The smooth muscle cells of the GIT are well coupled (better coordinated). Electrical charge spreads rapidly from cell to cell. However, the electrical coupling between circular cells is better than the longitudinal cells because the circular cells have more gap junctions.

**Enteric Motor Neurons**
Enteric motor neurons innervate the smooth muscles of GI tract and are the final pathway for output of information from autonomic nervous system (ANS) and enteric nervous system (ENS) to the muscle cells (Fig. 45.6).
1. They neither form regular neuromuscular junctions nor do they release neurotransmitters at their axon terminal. Rather, most of motor axons release neurotransmitters from varicosities that occur all along their axon, during propagation of an action potential, and the neurotransmitter diffuses to reach the muscle or interstitial cells (refer to Fig. 36.6, Chapter 36).
2. The major excitatory neurotransmitters are acetylcholine and substance P, and inhibitory neurotransmitters are catecholamines, ATP, VIP, and NO.
3. Cell bodies of excitatory motor neurons are present in the myenteric plexus and the axons project aborally to innervate muscle fibers.
4. There are also secretomotor neurons that stimulate secretion of water, electrolytes and mucous from GI tract. The cell bodies of secretomotor neurons are present in the submucosal plexus.
5. Stimulation of secretomotor neurons release histamine during allergic reactions and produce neurogenic secretory diarrhea during stress.

**BASIC PATTERNS OF GI MOTILITY**
GI motility is required for propulsion, trituration (crushing and grinding), and mixing of food. Propulsion of food is achieved by peristaltic movement; trituration and mixing are accomplished by retropulsive and segmental movements.
Section 5: Gastrointestinal System

Peristalsis

**Definition**

This is a reflex response of GI tract to stretch, which results in organized propulsion of luminal content of the gut in forward direction. This response is present in all parts of GIT starting from esophagus to rectum. When the gut wall is stretched by the contents of its lumen, peristalsis is initiated.

**Mechanism**

Stretch initiates a circular ring like contraction behind the stimulus (i.e. the part before the area of distension) and relaxation in front of it.

1. The part behind the stimulus is called **propulsive segment** as it propels the bolus of food or chyme and the part ahead of the stimulus is called **receiving segment** as it receives the bolus (Fig. 45.7).

2. In the propulsive segment, the circular muscles contract and longitudinal muscles relax, and in receiving segment the circular muscles relax and longitudinal muscles contract.

3. The contraction wave then moves in forward direction, i.e. from oral to anal direction. It helps in propelling the contents aborally.

**Role of Cholinergic Neurons**

The local **cholinergic neurons** play an important role in the transmission of peristaltic waves.

1. Cholinergic neurons that are **present in a retrograde direction** (with cell bodies toward anus and terminals toward oral cavity) activate the neurons that release **substance P and acetylcholine**. These neurons **stimulate circular muscle contraction**, and help in formation and progression of contractile ring behind the stimulus.

2. Cholinergic neurons that are **present in antegrade direction** (cell bodies toward oral cavity and terminals toward anus) activate the neurons that secrete **VIP and nitric oxide**. These neurons assist in ensuing **relaxation of circular muscle** in the portion of the gut ahead of the stimulus.

**Factors Influencing Peristalsis**

The normal rate of transmission of peristaltic wave varies in different parts of GI tract, ranging between 2–15 cm/s. This is influenced by intrinsic and extrinsic nerve supply.

1. **Sympathetic stimulation** inhibits peristalsis and **parasympathetic stimulation** enhances it. Though the transmission of peristalsis is affected by autonomic influence, its **genesis is independent of it**.

2. It is affected by the **hormones** secreted locally. **Serotonin**, secreted by local stretch of gut enhances peristalsis.

**Migrating Motor Complex**

During the interdigestive period, the electromechanical activity of GI smooth muscles is altered. A cycle of motor activity migrates from stomach to distal ileum. This is called migrating motor complex (MMC) or **long (mass) peristalsis**.

**Phases**

MMC has three phases:

**Phase-I:** The quiescent phase

**Phase-II:** The period of irregular electrical and mechanical activity.

**Phase-III:** The period of burst of electrical activities.

Usually it originates in the stomach and transmitted aborally at a rate of 5 cm/min. It occurs at an interval of 90 min, after which the cycle is repeated.

**Significance**

MMC occurs in the **interdigestive phase**.

1. The phase III of MMC results in long peristalsis, which starts from stomach and terminates in distal part of the ileum. Therefore, it sweeps the gastric and intestinal contents into the large intestine. Consequently, MMC is alternatively called **sweeper of GI tract**.

2. This clears the stomach and intestine of their luminal contents, so that they are ready to receive and digest the next meal whenever ingested.

3. Characteristically, MMC immediately stops when food is ingested.
Retroperistalsis

Normally, peristalsis moves in forward direction. However, if there is an obstruction in the lumen, reverse peristalsis occurs. This is called retroperistalsis.

1. Vomiting occurs due to retroperistalsis.
2. Retroperistalsis is also initiated by emotional factors or due to stimulation of medullary vomiting centers.

Segmentation

This commonly occurs in small intestine.

1. It is characterized by closely spaced contraction of the circular muscle layer.
2. These contractions divide the small intestine into many segments. Therefore, the movement is called segmentation.
3. It is primarily meant to mix chyme with pancreatic and intestinal secretions and also bring the fresh chyme into contact with the mucosal surface.
4. Therefore, segmentation is also called mixing movement (for details, refer to “Intestinal Motility”).

ROLE OF SPHINCTERS

Sphincters are rings of smooth muscles that remain in a state of continuous contraction. There are anatomical and physiological sphincters. Upper esophageal sphincter is an example of physiological sphincter, which prevents esophageal content to regurgitate back into oral cavity. Anatomical sphincters are located at gastroesophageal, gastroduodenal and ileocecal junctions, and at the opening of bile duct in the duodenum and opening of rectum into anus. Thus, sphincters:

1. Separate two specialized compartments,
2. Regulate movement of content from upper compartment to lower compartment,
3. Prevent backward movement of content from lower to upper compartment.

Usually, when wall of the gut contracts sphincters relax and when gut wall relaxes sphincters relax. Hence, sphincters regulate coordinated propulsion of contents aborally. They also mediate reflex activities like gastrocolic, gastroileal reflexes, etc.

CHAPTER SUMMARY

Key Concepts

1. Electrical slow waves that oscillate significantly in GI smooth muscles are called basal electrical rhythm (BER). BER is due to the wide variation in RMP in GI smooth muscle.
2. Number of spikes on slow waves determines the magnitude of contraction.
3. Peristalsis is the propulsive movement and segmentation is the mixing movement.

Important to Know (Must Read)

1. In examinations, no Long Question is asked from this chapter. However, “Describe the electrical properties of GI smooth muscles”, may come as a long question.
2. Basal electrical rhythm of GI smooth muscle, Slow waves of GI smooth muscle, Enteric motor neurons, Migrating motor complex, may come as Short Questions.
3. In Viva, examiner may ask…. What are the specialities of electrical activity of GI smooth muscles, What is basal electrical rhythm of GI smooth muscle, Slow waves of GI smooth muscle, Organization of enteric motor neurons, Function and mechanism of peristalsis, segmentation, retroperistalsis, and migrating motor complex.
LEARNING OBJECTIVES

On completion of study of this chapter, the student MUST be able to:
1. List the functions of mastication.
2. Understand the mechanism of deglutition.
3. Appreciate why one should not speak while eating.

The student MAY also be able to:
1. Describe the mechanism of deglutition reflex.

CHEWING (MASTICATION)

Chewing or mastication is mostly a voluntary activity. The process of mastication is initiated usually reflexly following the arrival of food in the mouth.

Mastication has following functions:
1. Mastication cuts and grinds larger food particles into smaller particles.
2. It increases salivary secretion (Application Box 46.1).
3. It mixes food with saliva.
4. It lubricates content of the oral cavity, so that swallowing becomes easier.
5. It breaks down starch and allows saliva to mix with the broken starch particles. Therefore, chewing improves the taste of starch containing foods.
6. It improves dental strength and hygiene. To keep the teeth strong and in good health, dentists always encourage chewing of food adequately before swallowing. Chewing occurs due to forceful contraction of four groups of muscles of mastication.

- Masseter elevates mandible and helps in clenching of teeth.
- Temporalis helps in retracting mandible.
- Pterygoids (external and internal) protrude mandible and depress chin. This helps in opening the mouth. Alternative contractions of right and left pterygoids help in grinding movements.
- Buccinator prevents accumulation of food between cheek and mouth.

Application Box 46.1

Food should be chewed adequately before swallowing: Mastication breaks the larger food particles into smaller particles. Mastication is the major stimulus for salivary secretion. More saliva is required for a smooth swallowing. Digestion of salivary amylase takes place in the stomach. More quantity of saliva facilitates more breaking down of food particles so that the work load on stomach for grinding food becomes less, and grinding and mixing of becomes easier. This helps in easy digestion of food. Therefore, it has been said that one should chew 32 times before swallowing. As it is always not possible to chew 32 times, chewing should be done at least 10 times.

SWALLOWING (DEGLUTITION)

Swallowing or deglutition is the process by which the food material from oral cavity is transported into esophagus. Though it is initiated voluntarily, most part of it is involuntary or reflexive. Therefore, this is also called deglutition reflex.

1. During the reflex act of deglutition, respiration is inhibited, which prevents entry of food into the trachea.
2. The receptors for deglutition reflex are present near the opening of the pharynx.
3. The afferent impulses are transmitted to deglutition centers in medulla and pons.
4. The efferent impulses are directed to the muscles of pharynx and upper esophagus via cranial nerves.
Chapter 46: Chewing and Deglutition

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Stages of Deglutition
Swallowing is divided into three stages (phases): oral phase, pharyngeal phase, and esophageal phase. Oral phase is voluntary and pharyngeal and esophageal phases are involuntary.

Oral Phase
This is also called buccal phase. This is the voluntary phase of swallowing. It is initiated when the tongue separates a bolus of food from the mass of foodstuff present in the mouth. At the beginning, the tip of the tongue presses against the hard palate and then the body of tongue presses on it. This action of the tongue moves the food backward in the oral cavity. Thus, the food bolus is forced into the pharynx. Once the food touches the receptors at the pharyngeal opening, swallowing reflex is initiated.

Pharyngeal Phase
The major objective of pharyngeal phase is to push food bolus into the esophagus without its entry into respiratory passage.

Reflex Pathway
Receptors: Receptors are present around the pharyngeal opening.

Afferent: Impulses from pharyngeal receptors are transmitted to centers through trigeminal, glossopharyngeal, and vagus nerves.

Centers: Centers for deglutition are nucleus tractus solitarius and nucleus ambiguous located in the medulla.

Efferents: Efferent organs are muscles of pharynx and tongue that are innervated by trigeminal, glossopharyngeal, vagus and hypoglossal nerves.

Events
The events in pharyngeal phase occur in sequence within few milliseconds (Figs. 46.1A to C).

1. The soft palate is pulled in upward direction. The palatopharyngeal folds move in inward direction. These movements prevent entry of food into the nasopharynx. This also provides a small passage for food to enter the pharynx.

2. Laryngeal opening is closed by vocal cords pulled together. The larynx moves forward and upward against the epiglottis. These events prevent entry of food into trachea (Clinical Box 46.1).

3. The superior constrictor muscles of pharynx constrict, which forces food to enter deep into the pharynx and then from there into the esophagus. Simultaneously, relaxation of upper esophageal sphincter (UES) occurs that allows the food to easily enter the esophagus.

4. During this phase, respiration is reflexly inhibited due to inhibition of respiratory centers. This is called deglutition apnea.

Esophageal Phase
Once food enters into esophagus, which is facilitated by relaxation of UES, the peristaltic wave (primary peristalsis) is initiated just below the UES.

1. Reflexly, UES contracts that prevent regurgitation of food back into the pharynx.

2. The peristaltic wave travels at about 3–5 cm/s. It takes about 10 seconds for food bolus to pass through the entire esophagus.

3. If the primary peristalsis is ineffective, a second peristaltic (secondary peristalsis) wave is initiated to push the food bolus. Motility of esophagus is discussed in detail in next chapter “Esophageal Motility”.

Clinical Box 46.1
Do not speak while eating: During, deglutition, respiration is temporarily arrested and food does not enter the respiratory passage. Laryngeal opening is closed by vocal cords pulled together. Larynx moves forward and upward against the epiglottis. Speaking or coughing during swallowing keeps the laryngeal outlet opened and therefore there is risk of food particle entering into the respiratory passage. Food or water entering into respiratory passage causes laryngeal and bronchial spasm. Sometimes it may cause sudden respiratory arrest and death. Therefore, one should eat in silence and concentrate on the universal energy that he receives through the food.

Disorders of Deglutition
1. Decreased or Absence of deglutition reflex: If deglutition reflex is impaired or abolished, regurgitation of
### Chapter Summary

#### Key Concepts

1. Though swallowing is a voluntary process, most of it is a reflex phenomenon.
2. Laryngeal opening closes during deglutition, preventing entry of food into the respiratory passage. Speaking or coughing during eating allows food to enter the trachea and may cause choking in severe cases.
3. Mastication increases salivary secretion and facilitates swallowing and digestion in the stomach. Therefore, food should be chewed enough before swallowing.

#### Important to Know (Must Read)

1. In examinations, no Long Question is asked from this chapter.
2. Deglutition reflex may come as Short Questions.
3. In Viva, examiner may ask... Functions of mastication, phases of deglutition, afferent and efferent pathways and mechanism of deglutition in each phase, Problems of deglutition.

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- Air swallowed is usually transferred to lower part of GI tract, but partly air is regurgitated into the oral cavity (belching). Most of it passes down to the colon and is then expelled as flatus through the anus.

2. **Aerophagia**
   - Aerophagia is an unavoidable swallowing of air along with swallowing of food bolus and liquids.
   - It is seen in nervous individuals who have decreased tone of the upper oesophageal sphincter.

3. **Dysphagia**: Dysphagia is difficulty in swallowing due to any cause. Usually it occurs in acute and severe pharyngitis. But it can occur due to motor problems in pharynx, upper esophagus or foreign body, acute inflammation in oral cavity.
Esophageal Motility

CHAPTER 47

Learning Objectives

On completion of study of this chapter, the student MUST be able to:

1. Understand the functions of lower and upper esophageal sphincters.
2. Learn the mechanism of esophageal peristalsis.
3. Understand the physiological abnormalities in reflux esophagitis and achalasia cardia.

Esophagus serves as a conduit to transfer food from pharynx into the stomach. Once food enters the esophagus, reflex contraction of esophagus is initiated that transports food into the stomach.

Functional Anatomy

Parts of Esophagus

Esophagus is divided into three parts: upper esophageal sphincter, body of esophagus and lower esophageal sphincter.

1. In the upper part of esophagus, the muscles are striated (like skeletal muscles) muscles, in the lower part the muscles are smooth muscles and in the middle part, there is mixture of smooth and skeletal muscles (Fig. 47.1).

2. However, all esophageal muscles are mainly innervated by vagal fibers. Even the striated esophageal muscles receive vagal motor fibers.

Special Features

1. Esophagus differs from other parts of GI system for not having electrical slow waves to trigger contraction. Therefore, excitability of its muscle is low.
2. Muscles in the body of the esophagus are relaxed always, except during deglutition.
3. All muscle fibers including striated fibers are innervated by vagus nerve.
4. Activation of deglutition reflex initiates peristaltic contraction by neural mechanism (primary peristalsis) or presence of food bolus locally stimulates peristalsis (secondary peristalsis).

Esophageal Sphincters

Upper Esophageal Sphincter

Upper esophageal sphincter is mainly a physiological sphincter.
1. It reflexly relaxes to allow the food from pharynx to enter the esophagus.
2. However, once food has entered the esophagus, it constricts to prevent regurgitation of food back into the pharynx.

**Lower Esophageal Sphincter**

Lower esophageal sphincter remains tonically contracted. It relaxes only when food from the esophagus enters the stomach. Therefore, it always prevents reflux of food back into the esophagus from the stomach.

1. The significant fraction of basal tone of lower esophageal sphincter is contributed by vagal cholinergic fibers.
2. However, vagal fibers inhibit circular muscles of the sphincter in response to primary peristalsis that relays the sphincter and allows food to enter the stomach. This inhibitory action is mediated by stimulation of other neurons through activation of cholinergic vagal neurons that secrete VIP and nitric oxide (NO).

**ESOPHAGEAL PERISTALSIS**

Two types of peristalsis occur in esophagus: primary and secondary.

**Primary Peristalsis**

Primary peristalsis is initiated by deglutition reflex. In the third phase of deglutition, when food bolus enters esophagus, a ring of contraction appears just cephalad to the bolus, which pushes the bolus towards stomach. This is the primary peristalsis and it appears due to the act of swallowing whether or not food is present in the mouth. Even swallowing of saliva activates it.

**Secondary Peristalsis**

When primary peristalsis fails to push the bolus from the body of the esophagus, secondary peristalsis starts. Secondary peristalsis is initiated by activation of mechanoreceptors due to presence of food bolus in the esophagus. Experimentally, it can be induced by inflating a balloon in the body of the esophagus.

**Applied Physiology**

**Reflux Esophagitis and Barrett’s Esophagus**

Lower esophageal sphincter (LES) remains always tonically contracted except during swallowing when food in the esophagus stimulates esophageal peristalsis. The tonic contraction of LES prevents regurgitation of gastric content back into the esophagus.

1. When LES becomes incompetent, reflux of acid gastric content into esophagus produces esophagitis, known as reflux esophagitis.
2. Popularly, it is known as “heartburn” as the patient describes the pain in the retrosternal area. In the long run, it can cause stricture or ulceration of esophagus.

3. It is treated by proton pump blocker or H₂ receptor blocker.
4. Fundoplication may also be tried in this condition, in which a portion of the fundus of the stomach is wrapped around the lower esophagus so that LES remains inside a narrow tunnel of the stomach.

**Barrett’s Esophagus**

In few cases of chronic reflux esophagitis, the normal squamous epithelium of esophagus is replaced by columnar epithelium. This is called columnar metaplasia. This is a premalignant condition.

**Achalasia Cardia**

‘Achalasia’ means failure to relax. Normally, when food enters esophagus, esophageal peristalsis pushes food into the stomach. During this process, LES relaxes so that food easily passes into the stomach. However, in some individuals, resting tone of LES is high. Therefore, the sphincter fails to relax completely during deglutition. This causes inadequate emptying of esophagus and food collects in the esophagus and the organ is massively dilated. The condition is called achalasia cardia.

**Causes**

It occurs due to deficiency of myenteric plexus at LES in the esophagus. Also, the secretion of VIP and NO, the neurotransmitters that cause relaxation of GI muscles, is defective in this condition. Thus, resting tone of LES becomes high.

**Diagnosis**

Diagnosis is made by demonstrating rat tail in barium meal X-ray (body of esophagus is dilated and lower part is narrowed giving appearance of a rat) (Fig. 47.2) or by demonstrating retained food and fluid in esophagoscopy (Fig. 47.3)

**Treatment**

Treatment for achalasia is mechanical pneumatic dilatation of the sphincter.

1. In severe cases, surgical weakening of the sphincter by myotomy (incision of the esophageal muscle) is performed.
2. Also, administration of drugs that decrease tone of the sphincter has been successfully tried. For example, injection of botulinum toxin into LES that inhibits acetylcholine release produces relaxation of the sphincter.

**Aerophagia**

While eating and drinking, some volume of air is automatically swallowed. This is called aerophagia. A small volume of the swallowed air is removed by belching (regurgitation of air) and a volume of it enters into the intestine.
1. Intestinal and colonic bacteria add hydrogen, hydrogen sulfide, CO$_2$ and methane to this air that are expelled from rectum as flatus.
2. The daily production of gas by GI tract is about one liter.
3. The volume of gas present in GIT at any given time is about 200 mL as a large volume of produced and swallowed air is removed regularly as flatus.
4. The smell of the flatus is mainly due to the presence of sulfides.
5. Some individuals have more aerophagia or more production of gas. Disturbance in intestinal flora also leads to more gas production. These individuals develop abdominal discomfort and borborygmi (rumbling noises in the intestine and colon).

**CHAPTER SUMMARY**

**Key Concepts**
1. Esophagus is a unique structure having both striated and smooth muscle, and both the muscle are innervated by vagus nerve.
2. Esophagus does not exhibit electrical slow waves.
3. Esophageal peristalsis is initiated by swallowing, which may be air, or saliva, and need not be food.
4. Relaxation of LES mediated by VIP and NO released from vagal neurons.

**Important to Know (Must Read)**
1. In examinations, no Long Question is asked from this chapter.
2. Esophageal peristalsis may come as Short Questions.
3. In Viva, examiner may ask…… Special features of esophageal muscles, Esophageal sphincters, Types and mechanism of esophageal peristalsis, Causes and treatment of achalasia cardia and reflux esophagitis.
Functionally, stomach is divided into two parts: the **proximal stomach** (consisting mainly the body of the stomach), which acts as a reservoir, and the **distal stomach** (consisting mainly the antrum of the stomach), which acts as a pump.

1. The **proximal stomach**, for its property of **receptive relaxation**, receives and stores food, and for its **tonic contractions** pushes food toward the antrum.
2. The **distal stomach** for its property of **phasic contractions**, mixes, grinds, and breaks down food into smaller particles and regulates emptying of food into small intestine through gastroduodenal junctions.

Thus, **major objectives** of gastric motility are:
1. To allow the stomach to act as a **reservoir for storage of large amount of food** of a single meal.
2. To cut foodstuff into smaller particles and mix the food with gastric juice, the process in which **food is converted into chyme**.
3. To allow gastric contents to **enter the duodenum at a slow but controlled rate**, so that duodenum and jejunum being narrower tubes, handle the chyme appropriately.

**Scientist contributed**

William Beaumont (1785–1853) was a surgeon in the US Army who became known as the “Father of Gastric Physiology” following his extensive research on human digestion, gastric juice and gastric functions. He was the first scientist to systematically study the digestive processes in human beings. He was first physiologist to study gastric motility in a patient with a gastric fistula.


**FUNCTIONAL ANATOMY**

**Parts of Stomach**

As described above, for understanding gastric motility, stomach is divided into **two parts**: the proximal stomach and distal stomach (Fig. 48.1).

**Proximal Stomach**

The proximal part of stomach accommodates large volume of food (as large as 2 to 4 liters) without much increase in intragastric pressure.
Chapter 48: Gastric Motility

1. This becomes possible due to the phenomenon of receptive relaxation.
2. The contractile ability of fundus and body of stomach is normally poor. Therefore, food in fundus and body remains relatively unmixed for a longer duration.
3. Thus, the proximal stomach serves mainly as reservoir of food.

Distal Stomach
It consists mainly of the antrum.

1. The antrum, physiologically acts as a mechanical pump, which propels food towards the pylorus and helps in grinding and mixing of food.
2. Antral contractions break foodstuff into smaller particles and mix food thoroughly with gastric juice that help in partial digestion of food.
3. *Pyloric sphincter remains partially closed* and does not allow easy entry of gastric contents into the duodenum.
4. However, vigorous contractions of gastric antrum help gastric content enter the duodenum at a slow but controlled rate.
5. After grinding and mixing of food with gastric juice, the gastric content is now called chyme.

Structure of Stomach Wall
Stomach wall contains all general components of the wall of the GI tract.

1. However, the *circular muscle layer of the muscularis externa is more prominent* than the longitudinal layer.
2. In general, muscularis externa in fundus and body is thin and in antrum and pylorus is considerably thick. The *thickness of muscularis externa increases from body toward pylorus* (Figs. 48.2A and B). The highly developed muscle coat helps antrum work as effective pump.

Gastroduodenal Junction
The junction between the stomach and duodenum is called gastroduodenal junction. The pylorus separates antrum and duodenum. There is a sphincter in the pylorus consisting of a ring like thickening of circular muscle fibers, known as *pyloric sphincter*.

**Important functions** of gastroduodenal junction are:
1. To allow gastric contents to enter the duodenum at a slow and controlled rate (the rate at which duodenum is capable of possessing the chyme).
2. To prevent regurgitation of duodenal contents back into the stomach.

Gastroduodenal Mucosa
The duodenal mucosa is relatively resistant to bile acids but sensitive to gastric acid, whereas gastric mucosa is apparently resistant to gastric acid but sensitive to bile acids.

1. Therefore, in case of *incompetent pyloric sphincter*, regurgitation of duodenal content (containing bile acids) into the stomach usually results in *gastric ulcer*.
2. On the other hand, *rapid emptying of gastric contents* (containing acidic chyme) into the duodenum promptly causes *duodenal ulcer*.
3. Normally, as soon as acidic chyme from stomach enters the duodenum, the acidic pH stimulates release of secretin from S cells of duodenum and upper jejunum. Secretin stimulates pancreatic secretion rich in water and bicarbonate. Therefore, immediately the *acidic chyme is neutralized* by the aqueous component of pancreatic juice. This is the physiological mechanism that prevents formation of duodenal ulcer.
4. However, when gastric emptying is faster or secretion of pancreatic juice is less, the possibility of duodenal ulcer is more. Moreover, the content in the part of the duodenum above the sphincter Oddi does not get well mixed with pancreatic secretion.

Neural Control of Pyloric Part
The pylorus is richly innervated by parasympathetic (vagal) and sympathetic fibers.

1. Normally, *sympathetic stimulation causes constriction of pyloric sphincter*.
2. However, activation of *parasympathetic fibers have both stimulating and inhibiting effects*.
3. The stimulatory effects are mediated by cholinergic vagal fibers and the inhibitory effects are mediated by the *vagal fibers* that release VIP or nitric oxide (NO).
4. Constriction of pyloric sphincter also occurs in response to hormones like CCK, gastrin, GIP, and secretin. Therefore, these hormones slow gastric emptying.

Innervation of Stomach
Stomach is densely innervated by both the division of ANS and the neurons of the enteric nervous system.
1. Parasympathetic innervation (vagal fibers) stimulates whereas sympathetic innervation (fibers originate from celiac plexus) inhibits gastric motility and secretion.

2. Axons arising from intramural plexuses innervate smooth muscles and secretory cells.

3. Sensory fibers from stomach travel to CNS via vagal and sympathetic fibers. Some of the sensory fibers act as afferent link between the sensory receptors of the gastric mucosa and the intramural plexuses.

4. Few of these afferent fibers provide information about an intragastric pressure, gastric distention, chemical composition and pH of gastric content, and pain sensation originating in the stomach.

5. All these stimuli influence gastric motility.

**Electrophysiology of Gastric Motility**

The peristaltic waves in the stomach occur usually at the frequency of gastric slow waves. These peristaltic waves are generated by a pacemaker zone located in the middle of the body of the stomach. The frequency of peristaltic wave is about 3 per minute in human being and the waves are conducted from body toward pylorus.

1. The gastric slow wave has four phases that resemble the action potentials of cardiac muscle (Fig. 45.2; Chapter 45). However, it does not overshoot and last for a longer period (10 times that of cardiac action potential).

2. The smooth muscles of stomach contract when the depolarization of the slow wave exceeds the threshold for contraction.

3. The force of contraction depends on the degree, frequency and duration of depolarization.

4. Greater the depolarization and longer the muscle cells remain depolarized (above threshold), greater is the force of contraction.

5. In antrum of the stomach, action potential spikes occur in the plateau phase. The contractions that result from these action potentials are stronger than the contractions that occur in the absence of these action potentials.

6. Acetylcholine and gastrin improve gastric contractility by enhancing the amplitude and duration of the plateau phase of gastric slow waves. Norepinephrine inhibits by the opposite mechanism.

**TYPES OF GASTRIC MOTILITY**

The types of gastric movements are hunger contractions (movements of empty stomach), receptive relaxation, peristalsis, migrating motor complex and reverse peristalsis. The peristalsis of stomach results in a regulated gastric emptying.

**Hunger Contractions**

When, stomach is empty, motility of the stomach increases. If stomach is allowed to remain empty for a longer duration, contractions become vigorous. These contractions are called hunger contractions.

1. Usually, hunger contractions are distressing and painful.

2. The antral contractions are intense in such a situation and are associated with the relaxation of pyloric sphincter.

**Gastric Relaxations**

**Receptive Relaxation**

This is the relaxation of the fundus and body of the stomach in response to chewing and swallowing of food. Thus, stomach prepares itself to receive food (Fig. 48.3). The

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Figs. 48.2A and B: Layers of the stomach wall. (A) Fundus and body of the stomach; (B) Antrum of the stomach. Note, muscularis externa is more developed in the antrum of the stomach (B) compared to the body of the stomach (A).

relaxation continues till food continues to enter the stomach, so that a large meal is easily accommodated in it.

1. Because of receptive relaxation, the intragastric pressure does not rise in spite of accumulation of a large volume of food.
2. The receptive relaxation is mediated by vagus nerve (Fig. 48.4). Normally, gastric motility induced by vagal stimulation is mediated by cholinergic fibers. However, receptive relaxation mediated by vagus is due to release of neurotransmitters like VIP and NO (non-cholinergic fibers) at its nerve ending.

Adaptive and Feedback Relaxations

There are other two types of gastric relaxations: adaptive and feedback.
1. The adaptive relaxation is the relaxation of stomach triggered by distension of stomach. Receptive relaxation starts even before food reaches stomach whereas adaptive relaxation occurs in response to stretching of stomach wall. This is mediated by vagovagal reflex (Fig. 48.4) and is meant to accommodate a greater volume of food.
2. The feedback relaxation of stomach is a reflexive relaxation that occurs due to presence of food in proximal segment of small intestine. Acidic chyme and fatty acid in intestine inhibit gastric motility by both hormonal and neural mechanisms that cause feedback relaxation of stomach (Fig. 48.4).

Peristalsis

After about half an hour following gastric filling, gastric peristalsis starts.
1. The peristaltic movements are initiated by gastric slow waves.
2. The pacemaker for gastric peristalsis is located in the middle of the stomach close toward greater curvature.

Migrating Motor Complex

During the interdigestive phase, antrum of the stomach remains silent for about 75–90 minutes, after which a burst of electrical and motor activities occurs.
1. This causes intense antral contraction with relaxation of pylorus. Therefore, the remaining gastric content is emptied into the duodenum.
2. The wave of contraction progresses from stomach toward terminal ileum. Thus, it helps in cleaning the stomach and intestine and keeps the GI tract ready for the next meal.
3. Usually, it is repeated every 90 minutes during interdigestive phase.

Reverse Peristalsis

Sometimes in abnormal situations, peristalsis occurs in reverse direction, which starts in the lower parts of the body and proceeds toward esophagus.
1. The lower and upper esophageal sphincters relax so that gastric content is forced out of esophagus and oral cavity.
2. This results in vomiting (described below).
GASTRIC EMPTYING

This is the process by which the content of the stomach is emptied into the duodenum. Usually, it occurs at a slow but controlled rate so that duodenum and jejunum comfortably accommodate and process the chyme at a desired rate.

Mechanism of Gastric Emptying

When food enters stomach, stomach relaxes due to receptive and adaptive relaxations. Later, after about half an hour, slowly gastric motility begins. Gastric emptying occurs by three mechanisms: peristaltic contraction, antral contraction, and retropulsion (Figs. 48.5A to D).

Peristaltic Contractions

The peristaltic contractions usually begin in the middle of the stomach and proceeds in a ring like fashion toward pylorus (Fig. 48.5A). These contractions mainly push food into the antral part of the stomach.

1. The velocity and magnitude of contraction increase as the contractile waves approach pylorus.
2. As contractions are weak in the fundus and body of the stomach, the proximal portion of stomach mainly serves the reservoir function. Therefore, usually proper mixing of food with gastric secretion does not occur in these parts of the stomach.
3. Major amount of mixing takes place in the antrum, as antrum contracts vigorously.

Antral Contractions

Antral contractions help thorough mixing of food with the gastric juice.

1. The forceful contraction of antrum forces gastric contents toward the pylorus. But, as the pyloric sphincter remains closed, peristaltic wave fails to push food into the duodenum, rather food returns back into the body of the stomach (Fig. 48.5B).
2. After few such contractions, pylorus opens partially with a narrow opening at the center.
3. Therefore, stomach empties in small squirts with each peristaltic wave.

Retropulsion

The terminal part of antrum exhibits rapid and forceful contractions that forces the chyme to be propelled back toward the proximal part of the antrum and body of the stomach (Fig. 48.5C). This movement is called retropulsion.

1. Retropulsion is very effective in mixing and grinding the larger food particles into smaller ones.
2. Then, pyloric sphincter partially opens and gastric pump slowly pushes food into duodenum (Fig. 48.5D).

Physiological Significance

As the muscle layers in the fundus and body are thin, contractions in these parts of the stomach are weak. Therefore, gastric content in body of stomach settles into different layers based on their density.

1. Fat content of the food forms an oily layer on the top of the other gastric contents. This is why fat is emptied slower than the carbohydrate and protein (Application Box 48.1).
2. Liquid portion of the food flow around the mass and enter the antrum, and from there into the duodenum. Therefore, liquid is emptied faster than the solid.

Application Box 48.1

A cup of fat is taken in cocktail party: As fat decreases gastric emptying, usually a cup of fat is ingested before drinking alcohol in cocktail party. Fat ensures slow gastric emptying and slow absorption of alcohol from intestine, and therefore the person drives his car back after the party, even after a heavy drink.

Regulation of Gastric Emptying

Gastric emptying is regulated by both neural and hormonal mechanisms. The upper part of the small intestine (duodenum and jejunum) contains receptors that detect change in pH, osmotic pressure, and products of fat and protein digestion. The chyme that enters duodenum is highly acidic and hypertonic and contains products of protein and fat digestion. All these stimuli influence gastric emptying.

1. Acid in the duodenum: With decrease in the pH of the duodenal content the rate of gastric emptying decreases.
This response is mediated by both neural and hormonal mechanisms. The acidic chyme in the duodenum releases secretin that decreases gastric emptying by inhibiting contraction of the antrum and by stimulating the contraction of pyloric sphincter.

2. **Products of fat digestion:** Products of fat digestion like fatty acids and also some fat molecules in the duodenal content inhibit gastric emptying. This response is mediated by CCK and GIP. CCK is secreted from duodenum and jejunum in response to fatty acids and it inhibits gastric emptying. GIP, which is released in response to fatty acids also inhibits gastric emptying.

3. **Osmolality of duodenal content:** The chyme entering into duodenum has higher osmolality. There are osmoreceptors in the mucosa of duodenum and jejunum that detect change in osmolality of the duodenal content. Hypertonic solutions in the duodenum release hormones that inhibit the rate of gastric emptying.

4. **Products of protein digestion:** Presence of peptides and amino acids in the duodenum release gastrin from the G cells located in the duodenum. Gastrin increases antral contraction but at the same time also causes constriction of pyloric sphincter. Therefore, the net effect is decreased rate of gastric emptying.

- The products of protein digestion also release CCK and GIP from duodenum and jejunum that inhibit gastric emptying.

5. **Volume of the meal:** Whenever a large amount of food is taken in a meal, the time taken for gastric emptying prolongs. However, if the volume is mainly due to liquid then emptying is faster.

6. **Stretching of duodenum:** Entry of chyme into the duodenum stretches the wall of duodenum. This initiates enterogastric reflex that inhibits gastric emptying. Enterogastric reflex is also activated by acid in the duodenum.

7. **Neural factors:** Vagal stimulation promotes gastric emptying. Therefore, **vagotomy produces gastric stasis.** Consequently, whenever vagotomy is performed for the treatment of peptic ulcer, usually a **drainage procedure** like pyloroplasty or gastrojejunostomy is also performed to ensure proper passage of food from the stomach into the duodenum (Clinical Box 48.1). **Sympathetic stimulation inhibits** gastric emptying.

8. **Hormonal factors:** Most of the hormones liberated from duodenum and jejunum like CCK, GIP, secretin, etc., inhibit gastric emptying.

**Clinical Box 48.1**

**Drainage procedure is done with vagotomy:** As vagotomy decreases gastric motility and produces gastric stasis, whenever vagotomy is performed as done for the treatment of peptic ulcer, usually a drainage procedure like gastrojejunostomy is also performed to ensure proper passage of food from the stomach into the duodenum.

### APPLIED PHYSIOLOGY

#### Dysfunctions of Gastric Emptying

**Delayed Gastric Emptying**

1. Gastric emptying is delayed in **autonomic neuropathy** as occurs in diabetes mellitus.
2. Paralysis of propulsive movements occurs following **vagotomy,** which is called **gastroparesis.** Therefore, a drainage procedure like pyloroplasty is performed to overcome post-vagotomy gastric stasis.
3. **Hypertrophic pyloric stenosis** can cause gastric stasis.

**Rapid Gastric Emptying**

Normally, vagus stimulation promotes gastric emptying.

1. Therefore, states of increased vagal activity increase emptying.
2. Conversely, **sympathetic stimulation inhibits emptying.** Therefore, loss of appetite is a feature of acute stress, a state of sympathetic overactivity.
3. Hormones like **thyroxine** stimulate gastric emptying and intestinal motility. Hence, increased appetite and hyperdefecation are features of hyperthyroidism.
4. Increased liquid content of food increases gastric emptying.

**Vomiting**

**Definition**

Vomiting is the expulsion of gastroduodenal content from GIT to the external environment via mouth.

**Associated Features**

Vomiting is usually preceded by the feeling of **nausea,** **tachycardia,** **sweating,** **pallor,** **dizziness,** and dilatation of pupils. It is associated with retching that forces contents of the stomach into the esophagus.

**Stimuli and Vomiting Centers**

Vomiting is a reflex phenomenon, the center for which is located in the medulla (Fig. 48.6).

1. The receptors present in many parts of the body provide inputs to the **vomiting center,** in the brainstem. Vomiting center present in the reticular formation of medulla consists of various scattered group of neurons that control different aspects of vomiting.
2. **Vestibular nuclei** mediate vomiting in response to motion sickness.
3. **Pharyngeal stimulation induces vomiting by activating nucleus tractus solitarius.**
4. **Area postrema** mediate vomiting activated by drugs (opiates, chemotherapeutic agents, etc.) and hormones (as in pregnancy).
5. Vomiting activated by emotion influenced by limbic and diencephalic inputs.

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**Chapter 48: Gastric Motility**
The important stimuli that activate these afferent fibers are:
1. Distension of stomach
2. Tickling the back of throat
3. Painful injury of the genitourinary tract
4. Conditions associated with dizziness and nausea
5. Many chemicals or drugs can elicit vomiting.

**Emetics and Antiemetics**

Drugs that induce vomiting are called emetics. The common emetics are apomorphine, ipecac, etc.
1. These chemicals stimulate the receptors that are present in the CTZ (chemoreceptor trigger zone), located in area postrema, a V-shaped band of tissue on the lateral wall of the fourth ventricle.
2. Many antiemetics prevent vomiting by inhibiting CTZ.
3. There are 5-HT$_3$ and D$_2$ receptors in area postrema. Serotonin stimulates vomiting through 5-HT$_3$ receptors. Ondansetron, a 5-HT$_3$ receptor antagonist is an antiemetic.
4. Chlorpromazine, a D$_2$ receptor antagonist and haloperidol are also effective antiemetics.
5. Corticosteroids, cannabinoids, and benzodiazepines are useful antiemetics for chemotherapy-induced vomiting.

**Mechanism of Vomiting**

Vomiting reflex is executed in a sequence of events.

The steps of vomiting are:
1. Genesis of reverse peristalsis that starts from the middle of the jejunum. This sweeps the content of intestine and duodenum into the stomach.
2. Relaxation of the pyloric sphincter that allows the intestinal content to enter the stomach.
3. Forced inspiration occurs against the closed glottis. Lowering of the diaphragm increases intra-abdominal pressure.
4. This is followed by vigorous contraction of abdominal muscles. Thus, intra-abdominal pressure sharply increases, which forces the gastric contents to enter the esophagus.
5. Relaxation of the lower esophageal sphincter allows gastric content to enter the esophagus.
6. Normally, the upper esophageal sphincter remains closed and prevents regurgitation of food into the pharynx. With stronger retching and sharp rise in intra-abdominal pressure, upper esophageal sphincter relaxes, which allows chyme to enter the pharynx and then from there into the mouth. Entry of vomitus into the trachea is prevented by central movement of vocal cords, closure of glottis and inhibition of respiration.

**Dumping Syndrome**

This is a distressing syndrome consisting mainly of weakness, dizziness and sweating that develop in about **two hours after meal** in persons who have undergone gastrectomy or gastrojejunostomy (that bypasses stomach). This occurs due to **two causes**:
1. Hypoglycemia: Rapid entry of food into intestine causes quick absorption of glucose from intestine and produces prompt hyperglycemia, which in turn increases insulin secretion. Insulin produces hypoglycemia that produces weakness, dizziness, and sweating.
2. Hypovolemia: The speedy entry of hypertonic meal from esophagus directly into intestine produces **high osmotic load on intestine that transfers water from blood into the gut**. This produces hypovolemia, dehydration and hypotension that lead to weakness, dizziness and sweating.
CHAPTER SUMMARY

**KEY CONCEPTS**

1. The proximal stomach is mainly for receiving and storing food, and the distal stomach is meant for mixing and emptying food.
2. Antral contractions help in proper mixing and grinding food that finally becomes chyme.
3. LES sphincter partially opens in response to every antral pump activity so as to allow small quantity of chyme to be delivered to duodenum at a time.
4. Degree of distension and type of food mainly contribute to gastric emptying. Vagal fibers stimulate and sympathetic fibers inhibit gastric emptying.
5. Vomiting is mainly a central phenomenon initiated by stimulation of vomiting center in medulla, though local factors contribute to it.

**Important to Know (Must Read)**

1. In examinations, “Mechanism and factors affecting gastric emptying” may come as a Long Question.
2. Gastric relaxations, Reverse peristalsis of stomach, Electrophysiology of gastric smooth muscles, Gastric emptying, Vomiting, may come as Short Questions.
3. In Viva, examiner may ask... What are the parts of the stomach and what are their functions, Who is the Father of Gastric Physiology, What are the special electrophysiological properties of gastric smooth muscles, Types of gastric relaxations, What is the importance of receptive and adaptive relaxation, What is the mechanism of gastric emptying, What are the factor affecting gastric emptying, What is Retropulsion, What is the specialty of gastric peristalsis, Who is the pacemaker of gastric contractions, What is hunger contraction, What are the causes of rapid and delayed gastric emptying, Reverse peristalsis of stomach, Mechanism of vomiting, What is dumping syndrome and how is it treated.
Learning Objectives

On completion of study of this chapter, the student MUST be able to:

1. Understand the importance of intestinal movements in mixing, propelling, and absorption of food materials in GI tract.
2. Name intestinal motilities, and give their mechanisms and functions.
3. Name the intestinal reflexes and give their functions.
4. Appreciate the physiological basis of motility disorders.

The student MAY also be able to:

1. Describe the detailed mechanism of intestinal movements.

The main objective of the motility of the small intestine is to **thoroughly mix the chyme** with the pancreatic, bile and intestinal juices so that proper digestion and absorption of the essential nutrients can take place. Intestinal motility also **propels chyme** into the colon.

**FUNCTIONAL ASPECTS**

The length of small intestine is about **three-fourths of the length** of the whole of the GI tract. It measures 285 cm.

1. It takes 2–4 hours for the chyme to traverse the small intestine.
2. Small intestine consists of duodenum, jejunum, and ileum. Duodenum is the first part of the intestine and constitutes 5% of it. Jejunum constitutes 40% and ileum constitutes more than 55% of the small intestine. However, there is no definite demarcation between jejunum and ileum.
3. The wall of small intestine have all the layers of the gut (refer to Figs. 36.3 and 36.4, Chapter 36). However, the **muscularis externa** consisting of outer longitudinal and inner circular muscles is well developed in small intestine (Fig. 49.1). This helps in effective intestinal motility.
4. Though the **lymphatic nodules** are less in jejunum, they are more in number in ileum, and they extend into the submucosa (Fig. 49.2).
Electrophysiology of Intestinal Smooth Muscles

The frequency of slow wave is maximum in small intestine that occurs regularly. The frequency is highest in duodenum (about 15 per minute) and decreases slowly toward ileum where it is about 8–10 per minute.

1. Slow waves are not always accompanied by bursts of action potential spikes. When slow wave is associated with spike, the contraction is stronger and in its absence (no spike), contraction is weaker or absent (refer to Fig. 45.5; Chapter 45).

2. A characteristic feature of slow waves of intestine is that they are localized to a short segment of the intestine. Therefore, contraction is also localized to the segments. This results in segmentation type of movement in the intestine.

3. The basic electrical rhythm is purely intrinsic.

4. The frequency of action potential spike, which determines the strength of muscle contraction, depends on excitability of the smooth muscles. The excitability in turn depends on autonomic innervation, activity of the enteric neurons and the circulating hormones.

5. Parasympathetic stimulation enhances and sympathetic stimulation inhibits intestinal contractility.

TYPES OF INTESTINAL MOVEMENTS

Intestinal movements carry out three primary functions:

1. **Mixing the chyme** with digestive secretions.
2. Bringing the chyme in contact with the absorptive surface of the microvilli to increase absorption.
3. Propelling the chyme toward colon.

These functions are achieved by various small intestinal motilities. The motilities are segmentation, peristalsis, migrating myoelectric complex, contraction of the muscularis mucosa, villus contractions, and movements due to intestinal reflexes.

**Segmentation**

This is the most common variety of movement of the small intestine.

1. It is characterized by closely spaced contraction of the circular muscle layer. These contractions divide the small intestine into many segments (Fig. 49.3A).

2. The rate of segmental contraction is same as the frequency of slow waves. It is about 18/min in duodenum, 15/min in jejunum, and 12/min in ileum.

3. Segmentation contractions effectively mix chyme with pancreatic and intestinal secretions.

4. Two nearby propulsive segments force the chyme toward each other into the receiving segment (Fig. 49.3B), which efficiently helps in mixing.

5. They also bring the fresh chyme into the contact with the mucosal surface.

6. Therefore, segmentation movements are also called mixing movements. They help in digestion and absorption of nutrients.

**Peristalsis**

Peristalsis is the progressive contraction of successive portions of circular smooth muscles of the small intestine.

1. The wave of contraction moves in orthograde direction, i.e. toward colon. In fact, peristaltic wave spreads in both directions. However, wave toward oral cavity (oral spread) dies out after a short distance, and wave...
toward colon (aboral spread) continues progressively. This is called law of the intestine.
2. Peristaltic waves involve only a short length of intestine. When chyme enters the intestine, the bolus of the chyme stretches its wall. The part of the intestine behind the chyme contracts and the part of the intestine in front of the chyme relaxes and the ring of contraction proceeds in forward direction (Figs. 49.4A and B).
3. The primary function of peristaltic movements is to propel chyme in forward direction. Sometimes, the frequency of peristalsis increases to an extent that intestinal contents are emptied very fast (with a speed of 20 cm/s) into the colon. This is called rush peristalsis. This is typically seen in acute diarrhea.
4. There are also antiperistaltic contractions of intestine. Antiperistalsis results in vomiting.

Short Range Peristalsis
Short range peristalsis also occurs in the intestine, but less frequently.
1. Short range peristalsis along with segmentation contraction decreases the net rate of propulsion of chyme in forward direction.
2. This allows the chyme to stay more time in intestine to facilitate digestion and absorption.

Clinical Significance
1. Administration of codeine decreases the motility of the intestine, therefore decreases the frequency of defecation.
2. The decreased motility also prolongs the transit time for the intestinal contents so that more water and nutrients are reabsorbed. Therefore, this also decreases the volume of stool.

Other Motilities

Migrating Myoelectric Motor Complex
In the interdigestive phase, the pattern of motility of small intestine changes.
1. There are bursts of intense electrical and contractile activity, once in about every 90 minutes. This is called migrating motor complex or migrating myoelectric complex (MMC).
2. MMC starts in the stomach and is propagated throughout the intestine to the terminal part of the ileum. Once, a MMC reaches the distal end of the ileum, a next MMC begins in the stomach. Likewise, MMC is repeated every 75–90 minutes.
3. MMC results in vigorous and strong propulsive contraction; therefore, this sweeps the intestine and empties the remaining contents into the colon.
4. MMC cleans the entire lumen of the stomach and intestine, to keep the house ready for the next meal. This is why the MMC is known as housekeeper of small intestine.
5. MMC also inhibits the migration of colonic bacteria into the intestine.

Contraction of Muscularis Mucosa
The muscularis mucosa of small intestine contracts irregularly. These contractions alter the patterns of the mucosal folds.
1. Contractions occur at a frequency of about 3 per minute.
2. Such contractions help in mixing the luminal contents and also in bringing the fresh chyme in contact with mucosal surface.

Villus Contraction
The villi of small intestine also contract irregularly. This is called villus contraction.
1. This is typically seen in upper part of the small intestine.
2. These contractions are especially meant for emptying the central lacteals of the villi.
3. They also increase intestinal lymph flow.

Intestinal Reflexes
There are two reflexes observed in the intestine: intestino-intestinal reflex and gastroileal reflex.
**Intestinointestinal Reflex**

When a part of the intestine is over-distended, the rest of the intestine relaxes. This is called intestinointestinal reflex. It is mediated by local enteric neurons and vagovagal pathways.

**Gastroileal Reflex**

When food enters the stomach (stretching of stomach), the motility of the terminal part of the ileum is enhanced. This increases entry of contents of ileum into the colon through ileocecal sphincter. This reflex is believed to be mediated by vagus nerve.

**Law of the Intestine**

When a bolus of chyme enters the intestine, the part of the intestine behind the bolus contracts and the portion of the intestine ahead of it relaxes. This helps in propagation of ring of contraction in aboral direction. This response is known as law of the intestine. This is meant to propel the intestinal content in the forward direction as occurs in peristalsis.

**Function of the Ileocecal Sphincter**

This is also known as ileocecal valve. It separates terminal part of the ileum from the cecum.

1. Normally, the ileocecal sphincter is tonically contracted, and therefore the sphincter remains closed most of the time and prevents small intestinal emptying.
2. When a peristaltic wave reaches the terminal part of the ileum, the sphincter relaxes so that the ileal content enters the cecum.
3. Distention of ileum also causes opening of ileocecal sphincter. On the other hand, distention of cecum causes closure of the ileocecal sphincter.
4. Ileocecal sphincter is also controlled by extrinsic nerve fibers. For example relaxation of the sphincter occurs by vagal stimulation as seen in gastroileal reflex.

**APPLIED PHYSIOLOGY**

**Adynamic Ileus**

When the intestine is injured, excessively handled or mishandled, the smooth muscles of the intestine are inhibited. This decreases the intestinal motility, sometimes even resulting in paralysis of the gut. This is called adynamic ileus or paralytic ileus.

1. This usually occurs following abdominal surgery. Following such procedures, GI tract remains paralyzed for about 6 hours to few days. Peristalsis first starts in the small intestine (6–8 hours later) followed by in the stomach (8–12 hours) and finally in the colon (2–3 days).
2. Adynamic ileus is also observed in peritonitis.
3. This occurs due to increased discharge of non-adrenergic fibers in the splanchnic nerves.

**Intestinal Colic**

Severe abdominal cramps are experienced in localized obstruction of small intestine.

1. The segment proximal to the obstruction dilates and gets filled with fluid and gas. This increases the pressure inside the lumen that causes compression of blood vessels in the intestinal wall. Resulting local ischemia of the intestinal wall produces severe cramping pain.
2. Abdominal cramps are also experienced in other diseases that result in distention of the intestine.

**CHAPTER SUMMARY**

**Key Concepts**

1. The primary function of small intestine is to adequately mix the chyme with intestinal and pancreatic juice. This is archived by Segmentation. Therefore, segmentation is called mixing movement.
2. The other important function of intestine is to push the chyme into the colon. This is achieved by peristalsis. Therefore, peristalsis is called propulsive movement.
3. The peristaltic movements move in aboral (away from oral cavity) direction. This is called the law of the gut.

**Important to Know (Must Read)**

1. In examinations, “Describe the mechanism and significance of intestinal motilities” may come as a Long Question.
2. Segmentation, Peristalsis, MMC, Villous contraction, Intestinal reflexes, Gastroileal reflex, Law of the gut, may come as Short Questions.
3. In Viva, examiner may ask… What are the special electrophysiological properties of intestinal, Name the types of intestinal movements, What is segmentation and peristalsis and what are their functions, What is MMC, What is Villous contraction, Name the intestinal reflexes and what are their functions, What is law of the gut, What is inflammatory bowel disease.
Large intestine consists of cecum, colon, rectum, and anal canal. Colon constitutes about 90% of large intestine and consists of ascending, transverse, descending, and sigmoid colons. Anatomically, cecum is considered as part of the ascending colon (refer to Fig. 44.1; Chapter 44).

1. Small intestine receives chyme of meals sequentially with no mixing of individual meals, whereas large intestine contains mixture of chymes of many meals of one to three days.
2. On average the total transit time of chyme of a meal through large intestine as recorded from passage of radiopaque markers is about 30 to 48 hours.

   The transit time of chyme through different parts of gut is as follows:
   1. In small intestine: 4 hours
   2. In ascending colon: 6 hours
   3. In transverse colon: 8 hours
   4. In descending and pelvic colons: 12 hours
   5. From pelvic colon to rectum, the transit is very slow, which may take 2 to 3 days.

   However, transit time depends on the fiber content of diet. Transit time is less in high fiber diet, sometimes may even be reduced to 6 hours through the entire gut.

### Motility of Colon

The major functions of colon are storage of chyme that arrives from small intestine, and absorption of salt and water from the chyme. Therefore, though colon receives about 2 liters of chyme per day from small intestine, its output is only about 200 mL.

1. The objectives of colonic contractions are to mix the chyme and circulate it across the mucosal surface of the colon so that maximum contact occurs between the chyme and the mucosal epithelium.
2. This, plus the slow movement of the chyme across the colon, which is about 5–10 cm/hour allows maximum absorption of salt and water.

### Physiological Anatomy

Structure of the wall of large gut resembles the general structure of the GI tract. However, the special features are:

1. Longitudinal muscle layer of muscularis externa is concentrated into three bands, called as tenia coli. These bands are shorter than rest of the colon. Therefore, the wall of the colon forms haustra (outpouchings).
2. There are no villi on the mucosa. There are many goblet cells.
3. The colonic glands are small inward projections of the mucosa. They secrete mucus.
4. The circular muscles usually do not exhibit action potentials.
5. outer longitudinal muscle layer is specialized into tenia coli (Fig. 50.1).
Innervation of Large Intestine

1. **Parasympathetic innervation** to cecum, ascending, transverse and most part of descending colon comes via vagus nerve, whereas innervation to the sigmoid colon, rectum, and anal canal comes via pelvic nerves that arise from the sacral spinal cord.

2. **Sympathetic fibers** to large gut come via superior and inferior mesenteric plexuses, and superior and inferior hypogastric plexuses.

3. Parasympathetic stimulation increases and sympathetic stimulation decreases colonic movements.

**Electrophysiology of Colonic Muscle**

Colon consists of both circular and longitudinal muscle.

**Circular Muscle**

There are two types of pace making (rhythm generating) cells in the colon. They are made up of interstitial cells.

1. The one set of cells that are present near the inner border of circular muscles produce regular slow waves of high amplitude like that of gastric slow waves.

2. The second set of interstitial cell is present near outer border of the circular muscle that produces waves with low amplitude and high frequency oscillations called myenteric potential oscillations (MPO).

3. Circular muscles **do not fire action potentials**.

**Longitudinal Muscle**

These muscles also exhibit myenteric potential oscillations MPO. However, longitudinal muscles sometimes fire **action potentials** at the peak of MPO that elicit contractions of the large intestine.

**Colonic Movements**

Colonic movements include haustral contractions, propulsive movements, mass peristalsis, and colonic reflexes.

**Haustral Contractions**

These are contractions similar to that of segmentation. Haustra become more prominent in these contractions. Therefore, they are called haustral contractions. They help in mixing and circulation of chyme within the large intestine.

**Propulsive Movements**

These are peristaltic movements that propel colonic contents toward the rectum. The mechanism of colonic peristalsis is same as intestinal peristalsis.

**Mass Peristalsis**

Mass colonic peristalsis is a stronger peristaltic contraction that forcefully pushes the contents from colon into the rectum. Activation of mass peristalsis in colon finally leads to the initiation of defecation reflex. It also cleans the large intestine. It occurs 3 to 4 times a day, and is initiated usually after a meal. Mass peristalsis is not affected after colostomy (Clinical Box 50.1).

**Colonic Reflexes**

Colonic reflexes include colonocolonic reflex and gastrocolic reflex.

**Colonocolonic Reflex**

Colonocolonic reflex is the relaxation of the entire colon in response to distention of one part of the colon. This is partly mediated by sympathetic fibers.

**Gastrocolic Reflex**

Gastrocolic reflex is initiated when food accumulates in the stomach.

1. Distention of the stomach causes motility of proximal and distal colon.

2. This pushes colonic content into the rectum, which stimulates the desire for defecation. Therefore, usually after taking a large meal, the urge for defecation is enhanced.

3. This reflex is more developed in children.

4. Gastrocolic reflex is proposed to be mediated by gastrin secreted from stomach in response to gastric distension, and not by neural factors.
Section 5: Gastrointestinal System

Clinical Box 50.1

Colostomy: When a large part of colon is removed (colectomy), the terminal part of ileum or proximal part of colon is brought to the anterior abdominal wall through an opening. This is called ileostomy or colostomy. The chyme that comes out from the gut is collected in a colostomy bag fastened around the colostomy opening. Usually, colectomy or hemicolectomy is performed for cancer of colon. This procedure per se does not affect the health of the individual if water and electrolyte balance are maintained.

MOTILITY OF RECTUM AND ANAL CANAL

Normally, rectum remains empty. Mass colonic peristalsis pushes contents in the colon into the rectum.

1. Anal canal always remains closed by the tonic contractions of internal and external anal sphincters. The internal anal sphincter is made up of thickening of circular smooth muscle of the anal canal.

2. The external anal sphincter consists of striated muscle (Fig. 50.2). Thus, external sphincter is innervated by somatic motor fibers via pudendal nerves, which brings it under voluntary control.

Before initiation of the defecation reflex, colonic peristalsis pushes colonic contents into the rectum. This causes filling and distension of the rectum that initiates relaxation of internal anal sphincter and constriction of external anal sphincter.

- **With initiation of defecation reflex,** the external sphincter opens and the person defecates.
- **However,** the reflex relaxation of the internal sphincter in response to rectal distention is a temporary phenomenon.
- **If defecation is delayed,** the internal sphincter regains its normal tone and the reflex is inhibited.

Defecation

This is a reflex phenomenon with a voluntary control. Thus, it has both reflexive (automatic) and voluntary components. The center for defecation is present in the sacral portion of the spinal cord, which is influenced by higher centers. The efferent pathway involves cholinergic parasympathetic fibers in the pelvic nerves.

**Stimulus**

Defecation reflex is initiated when mass peristaltic movement of the descending and sigmoid colons pushes the colonic content into the rectum (filling of the rectum).

1. As the external anal sphincter is innervated by somatic nerves, the voluntary effort is also important in initiating defecation.
2. However, normally defecation is inhibited by the acute anorectal angle, which is about 90°, and contraction of external sphincter and puborectalis muscle.

**Pathway**

1. **Receptors** for defecation reflex are stretch receptors located in the wall of rectal rectum.
2. **Afferent information** from the wall of rectum is conveyed to sacral segment (S3) of spinal cord via pelvic nerve.
3. **Effferent input** from spinal cord to rectum and internal anal sphincter comes via pelvic nerve and to external anal sphincter via somatic nerve (Fig. 50.3).
4. **Higher center,** especially cortex influences spinal cord center via corticospinal pathway.
5. Relaxation of internal anal sphincter is due to inhibitory signals that originate in myenteric plexus in response to peristaltic wave approaching anus. This allows the fecal matter to press onto the anal canal.
Mechanism

The individual sits on toilet and strains. This increases intra-abdominal pressure, which forcefully expels the rectal contents through the anal canal. This is assisted by relaxation of external anal sphincter, decreased anorectal angle and relaxation of puborectalis muscle.
1. Evacuation of bladder is preceded by a deep breathing that pushes the diaphragm downward.
2. Contraction of respiratory muscles increases intrathoracic and intra-abdominal pressures.
3. Contraction of abdominal wall muscle further increases abdominal pressure.
4. When all these mechanisms elevate the intra-abdominal pressure to about 200 cm of H2O, the feces is forced out through the external anal sphincter.

Applied Aspect

Defecation reflex is a spinal reflex. Therefore, following spinal transection, defecation is never complete. However, evacuation of bowel and bladder can be achieved by activating mass reflex in paraplegic patients.

Feces

Stool is a semisolid mass of about 200–250 mL excreted form large gut per day. It contains inorganic material, undigested fibers, bacteria, and water. Water constitutes 75% and solids 25% of the total volume. The undigested fibers include cellulose and other fibers. Bacteria and inorganic materials constitute 30% and 15% of the total solids respectively. The composition of feces is relatively not affected by diet as a large fraction of it comes from non-diary origin. Even in fasting, a good amount of feces is passed. The color of the stool is due to the presence of bile pigments. The smell of feces is due to presence of indole and skatole, the amines that are produced by colonic bacterial flora.

Applied Physiology

Hirschsprung’s Disease

Cause

This is also known as congenital megacolon or aganglionosis. In this condition, the entire neuronal plexuses in the wall of the colon are congenitally absent.
1. The enteric neurons are usually markedly absent in the anus and distal part of the rectum.
2. Failure of migration of neural crest from cranial to caudal region results in absence of ganglion in both myenteric and submucosal plexuses in distal part of colon and rectum.
3. This prevents relaxation of rectal outlet and internal anal sphincter in response to rectal filling. Thus, obstruction occurs to the outflow of feces and feces accumulate behind the obstruction.
4. Consequently, distention of the colon ensues.

Features

Clinically, it manifests as abdominal distention, anorexia and lassitude. In severe cases, symptoms appear in newborns as early as third day after birth.

Treatment

Usually, it is treated by surgical dilation.

Diarrhea

Diarrhea occurs in many conditions.
1. Physiologically it may be due to either increased secretion as occurs in cholera or increased GI motility.
2. In any case, acute diarrhea results in dehydration and hypovolemia.
3. Oral rehydration therapy is the immediate treatment to prevent volume and electrolyte loss. Oral rehydration solution (ORS) contains salt, electrolytes, and glucose. ORS contains both Na+ and glucose so that Na+ is absorbed via SGLT 1 (Na+-glucose cotransporter). Glucose facilitates Na+ transport.

Constipation

Constipation occurs due to many causes. However, the physiological basis is the decreased intestinal motility that causes stasis of chyme in the large intestine, which facilitates water absorption and dehydration of intestinal contents. The chyme hardens and feces become more solid. Usually it is treated by distending rectum by inert material.

Irritable Bowel Syndrome

This condition has been known by several synonyms such as mucous colitis, spastic colon, irritable colon, and colonic neurosis. In the West 20–30% of gastrointestinal disorders are constituted by IBS. In India this is very common and many cases used to be misdiagnosed as chronic amebiasis in the past.
1. IBS is a functional disorder of the intestine characterized by alteration of the bowel habits and abdominal pain in the absence of any detectable organic pathology.
2. There is no morphologic, histologic, microbiologic, or biochemical abnormalities in IBS. Changes in gut motility are observed in several studies though they poorly correlate with the symptoms.
3. In the constipated variety the frequency of high altitude peristaltic contraction is less whereas nonpropulsive segmentation contractions are more.
4. Moreover food induced hypermotility of the colon occurring normally about one hour after the meal is reduced in many patients. This may account for their postprandial symptoms.
5. Emotional stress is seen to aggravate the motility disorder.

Electrical Abnormalities

Normally gut has two types of myoelectrical activity, the basal electric rhythm (BER) and spike activity (SA). The BER
is in the continuous wave form at the rate of 6 cycles per minute. The SA is in the form of electrical bursts superimposed on BER and this is responsible for the mechanical contraction of the gut.

1. BS patients have a slow BER at the rate of 3 cycles per minute.
2. In normal people, feeding induces SA immediately which peaks in 30 minutes and lasts for about 50 minutes. But in the IBS patients the feeding induced SA is dampened in the first 50 minutes, but it becomes stronger later on.

**Clinical Features**

In India the female to male ratio is 1:3, though in the West, female suffer more. The clinical spectrum is wide. The presentations include

i. Painless functional diarrhea
ii. Painless simple constipation
iii. Alternating diarrhea and constipation
iv. Bloating of abdomen
v. Pain due to spasm of colon and small intestine.

The common age group is 20–40 years. Symptoms are vague and these include abnormal bowel habits ranging from constipation to diarrhea (often alternating irregularly), pallet like stools, increased gastrocolic reflex, vague abdominal pain ranging from dull ache to severe colic, flatulence relieved by belching, capricious appetite and insomnia. Around 20% of subjects complain of weight loss. All patients are emotionally tense and they tend to exaggerate the disability.

**Inflammatory Bowel Disease (Crohn’s Disease and Ulcerative Colitis)**

The term “inflammatory bowel disease (IBD)” is commonly used to include 2 idiopathic bowel diseases having many similarities but the conditions usually have distinctive morphological appearances.

These 2 conditions are: Crohn’s disease (regional enteritis) and ulcerative colitis.

1. **Crohn’s disease or regional enteritis** is an idiopathic chronic ulcerative IBD, characterized by transmural, non-caseating granulomatous inflammation, affecting most commonly the segment of terminal ileum and/or colon, though any part of the gastrointestinal tract may be involved.

2. **Ulcerative colitis** is an idiopathic form of acute and chronic ulcero-inflammatory colitis affecting chiefly the mucosa and submucosa of the rectum and descending colon, though sometimes it may involve the entire length of the large bowel.

Both these disorders primarily affect the bowel but may have systemic involvement in the form of polyarthritits, uveitis, ankylosing spondylitis, skin lesions, and hepatic involvement. Both diseases can occur at any age but are more frequent in 2nd and 3rd decades of life. Females are affected more than males.
CHAPTER 51

Principles of Digestion and Absorption

LEARNING OBJECTIVES

On completion of study of this chapter, the student MUST be able to:
1. Understand the principle of digestion and absorption of carbohydrate, protein, fat, and other nutrients from GIT.
2. Appreciate the physiological basis of common malabsorption syndromes.
3. Learn the importance of digestion and absorption of various nutrients.

The student MAY also be able to:
1. Describe the mechanism of digestion and absorption of each category of nutrient.
2. Explain the physiological basis of problems in malabsorption syndrome.

PHYSIOLOGICAL ASPECTS

Digestion is the process by which foodstuff are broken down into smaller particles that can be absorbed from the GIT. Absorption is the process by which the products of digestion, vitamins, minerals, and water are transported from the lumen of the GI tract into the blood and the lymph draining the GI tract.

Digestion is a complex process involving breaking down of foodstuffs by many enzymes into their absorbable form. Though the major part of digestion takes place in the small intestine, digestion starts as soon as food enters the mouth. The mucosal epithelial cells of the small intestine are called enterocytes. Enterocytes have brush border, which is rich in digestive enzymes. The nutrients first pass from lumen of GI tract to the interstitial fluid and then from there into the blood or lymph.

Scientist contributed

Herman Boerhaave (1668–1738) was a Dutch botanist, physician and physiologist of European fame. He is regarded as the founder of clinical teaching and is sometimes referred to as “the father of ancient physiology”; along with his pupil Albrecht von Haller. He is best known for demonstrating the relation of symptoms to lesions and, in addition, he was the first to isolate the chemical urea from urine. He had emphasized the role chemical factors in functional activity, especially in digestive process.

Digestion and Absorption of Carbohydrates

Digestion of Carbohydrate

The dietary carbohydrates are mainly polysaccharides, disaccharides, and monosaccharides. Starch is the polysaccharide that is usually digested in human GI tract.

1. Digestion of carbohydrate begins in the mouth by the action of salivary amylase which catalyses the hydrolysis of α-1, 4 linkage (but not α-1, 6 linkage) to form α-dextrins.
2. The pancreatic amylase also does the same function. The products of amylase digestion are maltose, maltotriose and α-dextrins.
3. The further digestion of starch is carried out by oligosaccharidases that are present in the brush border of the intestine. These membrane enzymes are α-dextrinase that causes hydrolysis of α-1,6 linkage, maltase that causes hydrolysis of maltotriose and maltose, lactase that causes hydrolysis of lactose into glucose and galactose, glucoamylase that splits malto-oligosaccharides into single glucose molecules and sucrase that causes hydrolysis of sucrose into fructose and glucose.
4. The end product of these membrane digestions are glucose, galactose, and fructose (monosaccharides).
Absorption of Carbohydrates

The carbohydrate absorption is maximum in duodenum and jejunum, which progressively decreases toward the terminal part of ileum. The easily absorbable forms of carbohydrate are monosaccharides that are glucose, galactose, and fructose.

1. They are first transported from the lumen into the epithelial cells. The apical membrane of epithelial cells contains SGLT (sodium dependent glucose transporter).

2. The transport is greatly influenced by the concentration of Na\(^+\) in the intestinal lumen. The higher the concentration of Na\(^+\), the greater the degree of glucose absorption. This is because both glucose and Na\(^+\) share the same transport protein, the SGLT. The SGLT that transports glucose and Na\(^+\) is SGLT 1.

3. The concentration of Na\(^+\) is less in the enterocytes, which is created by Na\(^+\)-K\(^+\) pump present on the basolateral membrane of the cells that pumps Na\(^+\) into the lateral intercellular space from the enterocytes in exchange for K\(^+\). Therefore, Na\(^+\) moves into the cells along the concentration gradient (Fig. 51.1). Glucose enters along with Na\(^+\) using the same co-transporter (SGLT 1).

4. From the cell, glucose is transported by GLUT 2 (glucose transporter) into the interstitium (ECF). From the ECF glucose enters the blood.

5. As the glucose transport depends on Na\(^+\)-K\(^+\) pump present on the basolateral membrane that creates gradient for Na\(^+\), this mechanism of glucose transport is a secondary active transport.

6. Galactose uses the same carrier protein (SGLT 1) for absorption.

7. The entry of fructose into the enterocytes is facilitated by GLUT 5, which is present only in the brush border of matured enterocytes.

Applied Aspects

Carbohydrate Malabsorption Syndromes

Carbohydrate malabsorption occurs due to deficiency of oligosaccharidases, the membrane enzymes of enterocytes.

Lactose Intolerance

This occurs due to deficiency of lactase in the brush border of enterocytes. Therefore, lactose is not digested and absorbed from the intestinal lumen. The lactose enters the colon where it is utilized by colonic bacteria that produce gas, and different metabolites. The metabolites increase colonic motility and diarrhea. Excess gas production results in borborygmi (gurgling sounds in the intestine) and abdominal distention.

Sucrase-Isomaltase Deficiency

Deficiency of sucrase and isomaltase in the brush border of enterocytes is an autosomal recessive disease. It is present in 10% of Eskimos (in Greenland) and few North Americans. Ingestion of fructose in this condition results in diarrhea and flatulence.

Glucose-Galactose Malabsorption Syndrome

This is a rare hereditary disorder due to deficiency of SGLT 1 in the brush border of enterocytes. In this condition, ingestion of glucose or galactose or starch produces diarrhea and flatulence. Fructose is well tolerated.

Oral Sugar Tolerance Test

In carbohydrate malabsorption diseases oral sugar tolerance test is performed to diagnose the disease. An oral dose of sugar (in question) is given to the patient following which the concentration of the sugar in patients, blood and stool is monitored. If the patient is intolerant to the sugar, the ingestion of sugar causes diarrhea and flatulence, the concentration of sugar in stool increases, but characteristically the sugar concentration fails to increase in the blood.

Digestion and Absorption of Proteins

The quality and quantity of dietary protein depend on the socioeconomic status and food habit of the individual. Ten to thirty gram of protein is added to the intestinal content from the GI secretions and the ex-foliated intestinal cells. Normally, all ingested protein is digested and absorbed. However, excess protein intake causes excretion of protein in the stool.
Chapter 51: Principles of Digestion and Absorption

**Digestion of Proteins**

Digestion of protein starts in the stomach.

1. **Pepsinogen secreted from chief cells** is converted to pepsin by the action of HCl. Pepsin hydrolyzes proteins into peptides and amino acids.

2. **In the duodenum and small intestine**, the proteins are digested by proteases secreted in the pancreatic secretion. The proteases are trypsin, chymotrypsin, carboxyptidases and elastase.

3. The **enterokinase** secreted from mucosal cells of duodenum and jejunum converts trypsinogen into trypsin, which then acts as an enzyme to convert other proteases.

4. **Peptidases** are also present in the brush border of enterocytes. These enzymes hydrolyze the peptides produced by pancreatic proteases into oligopeptides and amino acids. The **brush border peptidases** are amino peptidases, and dipeptidases.

5. The **products of protein digestion** are small peptides and amino acids.

**Absorption of Proteins**

There are many transport systems (at least seven such systems are known) for absorption of amino acids and peptides. The five protein transport systems require Na⁺ like that of Na⁺-glucose co-transporter. The other two transporters use Cl⁻ (independent of Na⁺). Absorption of amino acid is greater in the duodenum and jejunum and slower in ileum.

**Defects of Protein Digestion**

**Hartnup Disease**

This is a hereditary disorder in which absorption of amino acids in the intestinal and renal epithelial cells is defective. The neutral amino acids appear in the stool and urine.

**Cystinuria**

This is a congenital disease in which the amino acid cystine appears in the urine. The defect is in the protein transporter in the mucosal cells of intestine and epithelial cells of PCT of kidney.

**Prolinuria**

This is a rare disorder in which proline is not reabsorbed from intestine and kidney. This causes prolinuria and hydroxyprolinuria.

**Digestion and Absorption of Lipids**

**Digestion**

Digestion of lipid starts in the mouth.

1. In oral cavity **lingual lipase** is secreted from Ebner’s glands in the tongue.

2. **Gastric lipase** also helps in digestion of fat. However, deficiency of lingual and gastric lipase does not result in malabsorption of fat as pancreatic lipase is actually important for lipid digestion.

3. **Principal fat digestion starts in the duodenum by the pancreatic lipase**, which hydrolyzes 1 and 3 bonds of triglycerides that results in formation of free fatty acid and 2-monoglycerides (2-monoglycerols). Pancreatic lipase acts on lipids that have been emulsified (emulsification by bile acids).

4. Fat digestion is facilitated by pancreatic co-lipase.

5. There is another lipase secreted from pancreas called **bile salt activated lipase**, which also assists in lipid digestion.

6. The dietary cholesterol is hydrolyzed by **cholesteryl ester hydrolase**.

**Absorption**

Lipids are absorbed by passive diffusion and carrier mediated transport. As soon as lipids enter the cell they are esterified, therefore a gradient is maintained for their entry into the cell.

1. For their absorption, fats are emulsified in the intestine by detergent action of bile salts, lecithin, and monoglycerides. With the help of bile salt, lipids form micelles.

2. **Micelles** are cylindrical aggregates of lipids like fatty acids, monoglycerides, and cholesterol with their hydrophobic ends at the center.

3. Micelle solubilizes the lipids and provides a medium for their transport to the intestinal epithelial cells. Thus, micelles help in transport of lipids to the enterocytes, where they disintegrate into the individual lipids and passively diffuse into the cells.

**Short chain fatty acids** are produced in the colon by the colonic bacteria and absorbed there. About 60% of short chain fatty acid is acetate, 25% propionate, and 15% butyrate. Short chain fatty acids are absorbed in exchange for H⁺, therefore help in acid base balance. They also promote the absorption of Na⁺.

**Cholesterol** is easily absorbed from the intestine in the presence of bile, fatty acid and pancreatic juice. The absorbed cholesterol is incorporated into the chylomicrons that enter circulation via lymphatics.

The vitamins **A, D, E, and K** are fat soluble vitamins. Their absorption is facilitated by presence of bile acids and products of lipid digestion in the intestine. Therefore, in the absence of bile acids or malabsorption of fat, deficiency of these vitamins occurs.

**Disorders of Fat Digestion**

**Steatorrhea**

This is a condition in which there is passage of fatty, bulky, and clay colored stool.
1. This occurs due to deficiency of exocrine pancreas. Pancreatic lipase deficiency results in impairment of fat digestion.

2. This is also sometimes seen in patients with excess secretion of gastric acid in which decreased duodenal pH inhibits pancreatic lipase. Acid also precipitates bile salt.

3. Another cause of steatorrhea is impaired reabsorption of bile salts in the distal ileum.

4. Steatorrhea can also occur due to intestinal diseases.

Tropical Sprue

In this condition, the enterocytes are distorted and the density of microvilli is decreased. This causes lipid malabsorption probably due to decreased surface area for absorption of lipids.

Absorption of Water and Electrolytes

Absorption of Water

Normally, about 1–2 liters of water is ingested per day and 7 liters is added from GI secretions. However, only about 100 mL of water is excreted in the stool. Thus, GI tract absorbs more than 99.5% of water load presented to it per day.

1. Water is not absorbed from mouth, esophagus, and stomach.

2. Due to hyperosmolality of duodenal contents, water is secreted from blood into duodenum. As most of the nutrients are absorbed mainly in the duodenum and jejunum, the osmolality of intestinal content which is about 600 mosm/kg H₂O, decreases to about 200 mosm/kg H₂O in the ileum and colon.

3. Water is absorbed from intestinal lumen into blood as osmolality of blood is about 290 mosm/kg H₂O. Thus, water is mainly absorbed in the small intestine and colon.

4. The absorption of water depends on the absorption of electrolytes, especially that of Na⁺ and Cl⁻.

5. In the colon, part of water absorption occurs against an osmotic pressure gradient, the mechanism is known as standing gradient osmosis.

Absorption of Sodium

Sodium is absorbed along the entire length of the intestine. Sodium absorption is mainly a secondary active transport in which, Na⁺–K⁺ ATPase on the basolateral surface pumps sodium out of the enterocytes, which creates low concentration of sodium in the cell. Therefore, sodium enters the enterocytes by facilitated diffusion and via SGLT.

Physiological Importance

The sodium and glucose reabsorption are facilitatory to each other. This physiological process is utilized in oral rehydration therapy (ORT). ORT is commonly prescribed for condition of acute dehydration. ORT includes solution containing sugar (glucose), salt (sodium chloride), and lime juice (Application Box 51.1).

Application Box 51.1

Sodium and glucose facilitate absorption of each other in ORT: The glucose in ORT helps in absorption of sodium and sodium helps in the absorption of glucose. Thus, glucose and sodium content in ORT facilitate each others absorption in the intestine.

Absorption of Other Nutrients

Absorption of Potassium

The average intake of K⁺ per day is 4 g. K⁺ is absorbed mainly passively in the intestine (jejunum and ileum).

1. In small intestine, as water is reabsorbed, the concentration of K⁺ increases in the lumen. This causes diffusion of potassium across the concentration gradient.

2. The mechanism for active transport of K⁺ is absent in the intestine.

3. In the large intestine, potassium is both secreted and reabsorbed. Hypokalemia produces cardiac abnormalities (Clinical Box 51.1)

Clinical Box 51.1

Prolonged diarrhea can cause cardiac arrhythmias: As the K⁺ absorption is dependent on water absorption, significant K⁺ loss occurs in diarrhea. Therefore, prolonged diarrhea, especially in infants and children makes them susceptible to hypokalemia (low plasma K⁺), which may be life threatening. Hypokalemia can cause cardiac complications such as arrhythmias, and muscular dysfunctions.

Absorption of Cl⁻ and HCO₃⁻

HCO₃⁻ is secreted mainly in pancreatic juice into duodenum. Cl⁻ is secreted in gastric, pancreatic and intestinal juices.

1. Cl⁻ and HCO₃⁻ are reabsorbed mainly in the jejunum.

2. In ileum, HCO₃⁻ is secreted and Cl⁻ is absorbed.

3. In colon, Cl⁻ absorption occurs through special Cl⁻ channels in the enterocytes.

Physiological Significance

1. In acute diarrhea like cholera (secretory diarrhea), Cl⁻, Na⁺ and water are secreted into the lumen. The cholera toxin produced by Vibrio cholerae activates adenylate cyclase, therefore increases cAMP concentration in the mucosal cells. The cAMP in turn activates Cl⁻ channels and Cl⁻ is actively secreted into the lumen. This facilitates the secretion of Na⁺ and water resulting in profuse watery diarrhea (Clinical Box 51.2).

2. In cystic fibrosis, an autosomal disorder, the defective gene for Cl⁻ channel causes reduction in these channels in the mucosal cells of intestinal epithelium. Therefore, such patients suffer from less severe secretory diarrheas as compared to normal individual.
Chapter 51: Principles of Digestion and Absorption

Absorption of Vitamins

Absorption of Water Soluble Vitamins

Water soluble vitamins are absorbed mostly by simple diffusion though there are specific transport mechanisms available for them, especially for absorption of B<sub>12</sub>.

Absorption of Vitamin B<sub>12</sub>

Vitamin B<sub>12</sub> present in food is mostly bound to proteins.
1. In the stomach, low pH and pepsin release vitamin B<sub>12</sub> from the protein. The free B<sub>12</sub> then binds with R protein (Cobalophilin secreted in the saliva), which is a glycoprotein, forming R-B<sub>12</sub>-complex (Fig. 51.2).
2. However, vitamin B<sub>12</sub> also binds with intrinsic factor (IF) in the stomach, though it has less affinity for IF than the R proteins.
3. In the intestine, pancreatic proteases degrade the R-B<sub>12</sub>-complex and decrease the affinity of vitamin B<sub>12</sub> for R proteins. This facilitates the binding of vitamin B<sub>12</sub> with IF, forming B<sub>12</sub>-IF complex.
4. B<sub>12</sub>-IF complex resists degradation by pancreatic proteases. Therefore, in pancreatic deficiency, vitamin B<sub>12</sub> absorption is impaired as degradation of R protein is not adequate.
5. Binding of IF with B<sub>12</sub> brings structural change in IF resulting in formation of dimers.
6. Each dimer of IF binds two vitamin B<sub>12</sub> molecules. There are specific receptors on the brush border of the epithelial cells of terminal ileum for IF-B<sub>12</sub> complex that do not recognize free vitamin B<sub>12</sub> or IF alone.
7. The binding of IF-B<sub>12</sub> complex to the receptor is facilitated by cubilin, a high affinity apolipoprotein component of receptor. Then the complex is absorbed by endocytosis.
8. The transport of vitamin B<sub>12</sub> from the epithelial cells into the blood is a remarkably slow process. Therefore, vitamin B<sub>12</sub> concentration in plasma rises after 6–8 hours after a meal.
9. Vitamin B<sub>12</sub> then dissociates from IF and enters the mitochondria of epithelial cells where it binds with transcobalamin II (TC II) forming TC II-B<sub>12</sub> complex. This complex is rapidly cleared from portal blood by the liver by receptor mediated endocytosis.

Absorption of vitamin B<sub>12</sub> is very less (2%) in the absence of IF. However, the IF-independent absorption of B<sub>12</sub> is faster and there is no saturation in this absorption process (Clinical Box 51.3).

Clinical Box 51.3

IF-independent absorption of B<sub>12</sub>: IF-independent absorption of B<sub>12</sub> is faster. Therefore, for treatment of pernicious anemia (megaloblastic anemia due to IF deficiency), a high dose of orally administered vitamin B<sub>12</sub> (1 mg/day) can be given in addition to IV/IM B<sub>12</sub> therapy.

Clinical Significance

Deficiency of IF causes impaired absorption of vitamin B<sub>12</sub> from distal ileum. As vitamin B<sub>12</sub> is essential for maturation of red cells, IF deficiency results in pernicious anemia, a type of megaloblastic anemia.

There are three types of pernicious anemia.
1. Autoimmune pernicious anemia: This is caused by autoimmune atrophy of gastric mucosa that decreases secretion of IF from parietal cells. In most patients, antibodies against parietal cells are detected in the serum.
2. Congenital IF deficiency pernicious anemia: In this condition, IF secretion is deficient despite normal secretion of HCl from parietal cells.
3. Congenital vitamin B<sub>12</sub> deficiency pernicious anemia: This is due to congenital problem in malabsorption of vitamin B<sub>12</sub>, in which gastric function and IF secretions are normal. The disease occurs due to deficiency of ileal receptors that recognize IF-B<sub>12</sub> complex.

Absorption of Fat Soluble Vitamins

The fat soluble vitamins are A, D, E, and K. Most of these vitamins are absorbed in the upper small intestine. Absorption of these vitamins requires intactness of the mechanisms for absorption of fat, which depends on other fat soluble vitamins.
normal pancreatic secretion and bile secretions. Therefore, deficiency of A, D, E, and K are seen in pancreatic deficiency or biliary obstruction that prevents flow of bile into the intestine.

**Absorption of Minerals**

**Absorption of Calcium**

About 50% of dietary calcium is absorbed. Absorption occurs primarily in the upper segments of small intestine.

1. The absorption is mostly by an **active transport**, which is facilitated by **1,25-dihydroxycholecalciferol** that increases expression of calcium binding protein in the mucosal cells.

2. Decreased serum calcium concentration increases 1,25-dihydroxycholecalciferol and increased serum calcium decreases it. This **feedback mechanism** controls calcium absorption according to the need of the body.

3. Calcium absorption is inhibited by phosphates and oxalates, as they form insoluble salts with calcium in the intestine.

**Absorption of Iron**

The normal plasma level of iron is 130 µg/100 mL in males and 110 µg/100 mL in females. Normally, absorption of iron ranges from 3–6% of the iron ingested.

1. Iron is absorbed in the **ferrous (Fe²⁺) form**.

2. The iron ingested in the diet is present in the ferric (Fe³⁺) form. In the stomach, ferric form is converted to ferrous form by the action of **hydrochloric acid**. Gastric secretion dissolves the iron and allows it to form soluble complexes with ascorbic acid, which facilitates iron absorption. Therefore, iron deficiency anemia occurs in chronic gastric disease (Clinical Box 51.4).

3. Iron is mostly absorbed in the **upper part of the small intestine**. There is also the enzyme **ferric reductase** in the brush border of intestine, which converts ferric form of iron to ferrous form. Iron is absorbed in heme-ferrous and nonheme-ferrous form. The heme binds with a transport protein present in the brush border of the enterocytes (Fig. 51.3). This transport protein transfers heme into the enterocytes.

4. In the cytoplasm of enterocytes, **heme oxygenase** removes Fe³⁺ from the porphyrin. Fe³⁺ then is actively transported across the basolateral membrane of the cells to enter the blood where it binds with **transferrin** (the iron bound to transferrin is in the form of Fe⁴⁺).

5. Some of the Fe³⁺ in the enterocytes is **oxidized to the ferric form**, which bind with **apoferitin** to form **ferritin**. This serves as **storage of iron in the form of ferritin**.

6. But it is difficult to release iron from this storage form. They are also lost in the stool with the loss of epithelial cells. Normally, transferrin is only 35% saturated with iron. Therefore, **transport mechanism is usually not exhausted**.

**Clinical Box 51.4**

Gastrectomy or gastric atrophy causes iron deficiency anemia: Gastric secretion dissolves the iron and allows it to form soluble complexes with ascorbic acid, which facilitates iron absorption. Therefore, though iron is not actually absorbed in the stomach, gastrectomy or gastric atrophy causes iron deficiency anemia.

**Hemosiderosis and Hemochromatosis**

Absorption of iron increases when body iron store is less or when there is increased demand for iron like increased erythropoiesis. In conditions of **iron overload**, more ferritin is formed in the enterocytes, which is then excreted in the stool.

1. Ferritin is the principal storage form of iron in the tissue. In the tissue, ferritin molecules aggregate in the lysosomal membranes, which is called **hemosiderin**. Hemosiderin accumulates in the tissue when iron overload is prolonged. The condition is called **hemosiderosis**.

2. Deposition of large of hemosiderin in the tissue causes damage to the tissue. This condition is called **hemochromatosis**, which is characterized by skin pigmentation, diabetes (due to damage to the pancreatic tissue; bronze diabetes), cirrhosis of liver, and gonadal atrophy (testicular atrophy in males).

Iron present in the body is mainly in the form of hemoglobin (70% of the total iron). About 23% is present in the ferritin (in the store form) and only 3% is present in the form of myoglobin.
CHAPTER SUMMARY

**Key Concepts**

1. The major part of absorption of nutrients takes place in the small intestine. Therefore, chronic intestinal disease or intestinal resection leads to malabsorption syndrome.
2. Though the mechanisms are specific for specific nutrient, few of them facilitate each other.

**Important to Know (Must Read)**

1. In examinations, “Describe the mechanism of digestion and absorption of carbohydrate, fat and proteins from intestine” may come as a Long Question.
3. In Viva, examiner may ask… mechanism of digestion and absorption of each nutrient and clinical conditions that occur due to their deficiencies.
4. Usually questions are asked about malabsorption syndrome, in oral.
52. Introduction to Endocrinology
53. Mechanisms of Hormone Action
54. Hypothalamus and Hypothalamo-pituitary Axis
55. Pituitary Gland: The Anterior Pituitary
56. Posterior Pituitary
57. Thyroid Gland
58. Adrenal Gland: The Adrenal Medulla
59. Adrenal Cortex
60. Endocrine Pancreas
61. Calcium and Phosphate Metabolism and Physiology of Bone
62. Parathyroid Gland, Calcitonin and Vitamin D
63. Pineal Gland
64. Local Hormones
“But when the hour of the Divine draws near
The Mighty mother shall take birth in Time
And God be born into the human clay
In forms made ready by your human lives.
Then shall the Truth supreme be given to men.”

Sri Aurobindo (in ‘SAVITRI’
Introduction to Endocrinology

CHAPTER 52

Learning Objectives

On completion of study of this chapter, the student **MUST** be able to:

1. Appreciate the importance of learning endocrine physiology in understanding medicine.
2. Classify hormones and give examples.
3. Understand the principles of hormone synthesis.
4. Understand regulation of hormone secretion, especially the feedback mechanisms.
5. Learn the physiological importance of binding of hormones to plasma proteins.
6. Know different mechanisms of hormone signaling and intercellular communications, and give examples for each.

The student **MAY** also be able to:

1. Describe the principle of synthesis, secretion and metabolism of hormones.
2. Understand how are the hormones are degraded in the body.
3. Briefly describe the procedure of estimation of hormones.

Endocrine physiology is the branch of physiology that deals with the study of processes involved in regulation and integration of cells and organ systems by specialized chemical substances called hormones. The word ‘hormone’ is derived from ‘hormaein’ - a Greek word, which means ‘to excite’.

1. **Hormone** is defined as specialized organic molecules that are synthesized by endocrine glands or tissues in response to specific stimuli and exert their precise effects on particular target cells.
2. An endocrine gland is a ductless gland that pours its secretion directly into general circulation. Hormones circulate in blood to reach the target tissues on which they act.

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2. An endocrine gland is a ductless gland that pours its secretion directly into general circulation. Hormones circulate in blood to reach the target tissues on which they act.

**Scientist contributed**

**John Jacob Abel** (1857–1938) was pioneered in the study of endocrine secretions. He received his PhD in physiology from Johns Hopkins University and studied in Germany under Carl Ludwig. His work on the blood-pressure-raising constituent of the adrenal medulla led to the identification and synthesis of catecholamines, and further to the development of many derivatives of catecholamines. Similarly, his studies on the anterior pituitary gland resulted in the isolation of its **oxytocic, pressor and diuretic principles**. He also pioneered with crystalline insulin. With Leonard G Rowntree, he introduced kidney and liver function tests.

3. Target cells are conferred with **receptors that are specific in their affinity** for hormones. Actions of hormones on the target tissues are diverse in nature. Broadly, **functions of hormone** include:

1. Change in cell function
2. Control of growth and development
3. Alteration in body mass and its composition
4. Reproductive functions
5. Digestion, utilization and storage of nutrients
6. Regulation of volume and composition of fluid compartments
7. Behavioral changes
8. Control of senescence.

Hormones to accomplish their functions, **bind to receptors** on the target tissues. **Activation of these receptors** leads to alteration in multiple intracellular mechanisms and signaling pathways that change cell functions.

**ENDOCRINE GLANDS**

**Types**

Endocrine structures can broadly be divided into major endocrine glands (Fig. 52.1) and other endocrine organs:
Section 6: Endocrine Physiology

A. Major endocrine glands
   1. Hypothalamus
   2. Pituitary (anterior and posterior pituitaries)
   3. Thyroid
   4. Adrenals (adrenal cortex and medulla)
   5. Parathyroid
   6. Endocrine pancreas
   7. Gonads (testis and ovary)
   8. Pineal gland.

B. Other endocrine organs
   1. Thymus (secretes thymosin, especially in childhood)
   2. Kidney (secretes erythropoietin, renin, etc.)
   3. Heart (secretes ANP)
   4. Lungs (secrete prostaglandins and activate angiotensin)
   5. GI tract (secretes many GI hormones)
   6. Placenta (secretes many hormones during pregnancy).

Analyzing with Nervous and Immune Systems

The endocrine system is closely associated with the nervous and immune systems of the body and also resembles them in many aspects.

Similarity with Nervous System

Endocrine system like that of nervous system is a major system of communication. In its various physiological aspects, it is analogous to the nervous system:

1. Nervous system requires signaling by a stimulus that evokes a response and a feedback mechanism that controls the response. Similarly, endocrine system needs signaling for hormone secretion and feedback mechanisms for regulation of secretion. The control mechanisms that regulate hormone secretion operate mainly on feedback principle.

2. Moreover, neurons secrete chemicals at their axon terminals, the neurotransmitters, which are basically...
hormones in their chemical structure. In fact, many hormones act locally as neurotransmitters in the nervous system.

Thus, nervous and endocrine systems integrate with each other to bring about desirable effects in response to changes in external or internal environment. Even some of the hormones are called neurohormones.

**Similarity with Immune System**

The immuno-competent cells (immunocytes or immune cells) secrete many chemicals that are grouped as ‘cytokines’:

1. Cytokines resemble hormones in structure and functions. Like hormones, cytokines act on specific receptors on the target tissues to achieve desirable changes.
2. In fact, many hormones are synthesized and secreted by immune cells, though they act locally.
3. Also, cytokines modulate functions of endocrine glands and hormones modulate functions of immunocytes.

**Types of Hormones**

Hormones are broadly classified into three types: peptides, amino acids and steroids.

**Proteins or Peptides**

Peptide hormones include a large group of hormones secreted from a variety of endocrine tissues. These hormones are made up of peptide chains:

1. Depending on the number of amino acids in the chain, they may be **oligopeptides** (less than 10 amino acids) like oxytocin and ADH, and **polypeptides** (10 or more amino acids) like insulin and growth hormone.
2. They originate from a common ancestral gene during evolution.
3. Based on their structure and functions, they are grouped into a **number of families** like insulin, glycoprotein, growth hormone and secretin families (Table 52.1).

**Amino Acids**

These hormones are amines. Many of them are derived from a **common amino acid**, for example dopamine, epinephrine, norepinephrine and thyroxine are derived from tyrosine. They are usually hydrophilic.

**Steroids**

Steroid hormones are **synthesized from cholesterol**, and are lipid soluble and hydrophobic:

1. This group includes hormones of **adrenal cortex** and **many gonadal hormones**.
2. The biological activities of this group are determined by modification of their side chains, hydroxylation and ring aromatization at various sites.

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**Table 52.1: Classification of hormones.**

<table>
<thead>
<tr>
<th>A. Peptide Hormones</th>
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<tbody>
<tr>
<td>1. Insulin family</td>
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<tr>
<td>– Insulin</td>
</tr>
<tr>
<td>– Insulin like growth factors</td>
</tr>
<tr>
<td>– Relaxin</td>
</tr>
<tr>
<td>2. Glycoproteins</td>
</tr>
<tr>
<td>– LH</td>
</tr>
<tr>
<td>– FSH</td>
</tr>
<tr>
<td>– TSH</td>
</tr>
<tr>
<td>– hCG</td>
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<tr>
<td>3. Growth hormone family</td>
</tr>
<tr>
<td>– GH</td>
</tr>
<tr>
<td>– Prolactin</td>
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<tr>
<td>– Human placental lactogen</td>
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<td>4. Secretin family</td>
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<tr>
<td>– Secretin</td>
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<tr>
<td>– Glucagon</td>
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<tr>
<td>– VIP</td>
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<tr>
<td>– GIP</td>
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<tr>
<td>5. Others</td>
</tr>
<tr>
<td>– ANP</td>
</tr>
<tr>
<td>– Calcitonin</td>
</tr>
<tr>
<td>– CCK</td>
</tr>
<tr>
<td>– ADH</td>
</tr>
<tr>
<td>– Inhibin</td>
</tr>
<tr>
<td>– Somatostatin</td>
</tr>
<tr>
<td>– ACTH</td>
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<td>– Parathormone</td>
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<table>
<thead>
<tr>
<th>B. Amino Acid Derivative</th>
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</thead>
<tbody>
<tr>
<td>1. Amines</td>
</tr>
<tr>
<td>– Epinephrine</td>
</tr>
<tr>
<td>– Norepinephrine</td>
</tr>
<tr>
<td>– Dopamine</td>
</tr>
<tr>
<td>– Serotonin</td>
</tr>
<tr>
<td>2. Iodinated amino acid</td>
</tr>
<tr>
<td>– Thyroxine (T&lt;sub&gt;4&lt;/sub&gt;)</td>
</tr>
<tr>
<td>– Triiodothyronine (T&lt;sub&gt;3&lt;/sub&gt;)</td>
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<tr>
<th>C. Steroid Hormones</th>
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<tbody>
<tr>
<td>– Glucocorticoids</td>
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<tr>
<td>– Mineralocorticoids</td>
</tr>
<tr>
<td>– Estrogen</td>
</tr>
<tr>
<td>– Progesterone</td>
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<tr>
<td>– Testosterone</td>
</tr>
<tr>
<td>– 1,25-dihydroxycholecalciferol</td>
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</tbody>
</table>

**GENERAL PHYSIOLOGY OF HORMONES**

**Principles of Synthesis and Secretion**

**Peptide Hormone Synthesis**

In general, protein hormones are synthesized in the **rough endoplasmic reticulum** of endocrine cells:

1. They are first synthesized as a signal peptide called **preprohormone**, which is then cleaved to form **prohormone**.
2. Prohormone is then transported into the Golgi apparatus, where it is converted into hormone. Hormone is packaged in the secretory granules for storage (Flowchart 52.1).
3. The hormone is secreted from stored granules on appropriate stimulation. Granules are released by calcium-mediated exocytosis.
4. Hormones are also secreted from neoplastic tissues (Application Box 52.1).

Application Box 52.1

Ectopic Hormone Secretion: Sometimes in pathological conditions, nonendocrine tissues secrete hormones. For example, neoplastic tissues of lungs like small cell pulmonary carcinoma produce several hormones. The condition is called paraneoplastic syndrome. Hormones are usually secreted in such syndromes are ACTH, ADH and parathormone, resulting in Cushing syndrome, water retention and hypercalcemia respectively. Hormones are also secreted from gastrointestinal tumors and the condition is called carcinoid syndrome. Carcinoid tumors are also associated with melanoma, lymphoma and neural tumors.

Pathways of Synthesis

Peptide Hormone Synthesis
Synthesis and secretion of peptide hormones occur in two pathways: the regulated and constitutive pathways.

Regulated Pathway
In regulated pathway, external stimuli trigger release of hormone which is already synthesized and stored in secretory granules, and also promote synthesis of some additional hormones. For example, GnRH from hypothalamus stimulates release of gonadotropins and additional synthesis of gonadotropins from anterior pituitary.

Constitutive Pathway
In constitutive pathway, secretion of hormones occurs more directly from endoplasmic reticulum or vesicles formed from the Golgi apparatus, in which additional hormones are formed simultaneously.

Thus, regulated pathway is capable of secretion of large amount of hormones, whereas constitutive pathway promotes secretory reserve. In any case, stimuli that trigger secretion also increase synthesis of hormones.

Amine Hormone Synthesis
All amine hormones except serotonin are synthesized from the amino acid tyrosine that requires a series of enzymatic reactions. Serotonin is synthesized from 5-HT. Catecholamines are stored in granules and secreted by calcium mediated exocytosis of granules in which they are stored.

Steroid Hormone Synthesis
Steroid hormones are synthesized from cholesterol. Many enzymatic reactions are involved in the process of steroid hormone synthesis:
1. These hormones are not stored in the cell as granules.
2. They are usually present in the cytosol bound to proteins.
3. On stimulation, the hormones become free form intracellular proteins and are transported outside the cell usually by diffusion.

Regulation of Hormone Secretion

Normally, concentration of a hormone in circulation is maintained within a narrow range. Increase or decrease in hormone concentration for a longer period results in major dysfunctions. Alteration in hormone concentration is mainly checked by regulation of hormone secretion. Hormone secretion is principally controlled by four mechanisms:
1. Feedback control,
2. Neural control,
3. Rhythmic or chronotropic control, and
4. Humoral control.

Of these control processes, feedback control is the most common and developed mechanism for regulation of hormone secretion. However, many factors arriving from variety of stimuli play simultaneously to achieve an integrated response of hormone secretion. This is called multiplicity of regulation of hormone secretion.

Feedback Control
When the change in concentration of a hormone in plasma alters its rate of secretion, the mechanism is called feedback control. There are two feedback mechanisms: The positive and the negative feedbacks.

Negative Feedback
When increased concentration of a hormone and its metabolites provide feedback inhibitory signal to the gland that secretes the hormone, the mechanism is called negative feedback mechanism. This is the common mechanism of hormone homeostasis. Depending on the circuit or the pathway involved in the feedback control, the mechanism may be simple feedback or complex feedback.

Simple Feedback Control
This is the first order of feedback control in which the hormone secreted from a gland controls its secretion through
the physiological effects. The endocrine cells that secrete the hormone also sense the biological activity produced by the hormone:

1. When the biological effects are more, the hormone secretion decreases appropriately to maintain normal function of the hormone (Flowchart 52.2). For example, β cells of pancreas secrete insulin that acts on liver and skeletal muscles to regulate blood glucose concentration. β cells in turn sense the alteration in plasma glucose and accordingly adjust their insulin secretion to maintain the plasma glucose concentration within normal range.

**Complex or Hierarchical Feedback Control**

When the feedback regulation involves second or third order feedback loop or both, the control mechanism is called complex or hierarchical control. This multiorper or complex control system is the usual mechanism for regulation of many hormone secretions:

1. In this system, the hormone secreted by first (upper) order gland stimulates secretion of second (middle) order gland.
2. Secretion (usually, trophic hormones) of middle order gland stimulates secretion of final (lower) order gland or target gland.
3. Secretion of target gland (target gland hormone) inhibits the secretion of first order or middle order glands (Flowchart 52.3).
4. Also, secretion of middle order gland (trophic hormone) inhibits secretion of first order gland.
5. This system operates mainly for control of hypothalamo-pituitary-target endocrine gland axis.
6. As this is an integrated system of control of endocrine functions, disorder at any level of hierarchy influences the function of other levels.

The major hormone axes regulated by hierarchical system of feedback control are:

- Hypothalamic-pituitary-thyroid axis
- Hypothalamic-pituitary-adrenal axis
- Hypothalamic-pituitary-gonadal axis.

For example, in hypothalamic-pituitary-adrenal axis, excess cortisol in plasma inhibits secretion of adrenal cortex by decreasing ACTH secretion from anterior pituitary or by inhibiting hypothalamic release of CRH.

**Loops of Negative Feedback Control**

Depending on the distance from which the hormone of the target gland inhibits the upper order glands, the inhibition is classified into long loop, short loop and ultrashort loop. For example, in the same hypothalamic-pituitary adrenal axis, cortisol inhibiting CRH release is the long loop of negative feedback, ACTH inhibiting CRH release is the short loop of negative feedback and CRH inhibiting its own release is the ultrashort loop of negative feedback (Flowchart 52.3).

**Positive Feedback**

This is a less common mechanism of regulation of hormone secretion. In this control system, increase in hormone concentration in plasma stimulates further secretion of that hormone so that the hormone concentration increases steadily to reach a peak plasma level:

1. The best example of positive feedback regulation is the LH surge that occurs just before ovulation. Normally, estrogen inhibits LH secretion; however, just before ovulation, increased estrogen concentration...
in plasma provides a positive feedback for LH release from anterior pituitary that results in LH surge (for details, refer ‘Menstrual Cycle’).
2. Other examples are oxytocin secretion during parturition, release of oxytocin during breastfeeding and release of melatonin in response to darkness.

**Neural Control**

Endocrine glands are usually innervated by both the components of autonomic nervous system:
1. **Stimulation of sympathetic or parasympathetic system** therefore alters the endocrine secretions. Secretion of catecholamines from adrenal medulla in response to sympathetic stimulation is an example.
2. However, the receptor types present in the endocrine tissue determine the final secretion from the gland. Besides, innervation of the endocrine tissues may also be cholinergic, serotonergic or dopaminergic depending on the neurotransmitter released at the nerve ending.
3. Secretion of hormones in response to various stimuli like visual, olfactory, gustatory, tactile, etc. is also neurally mediated. One of the examples is the milk ejection reflex in which suckling by the baby increases secretion of oxytocin that causes contraction of myoepithelial cells of the mammary gland.

**Rhythmic or Chronotropic Control**

Chronotropic control of hormone secretion is the regulatory mechanism operated by various rhythms of biologic phenomena that either cycle at regular intervals like circadian rhythm, sleep-wake cycle, seasonal rhythm and menstrual cycle, or appear at different phases of development like hormonal changes occurring in pre-pubertal children.

Influenced by chronotropic control mechanism, hormones are secreted in a definable and rhythmic pattern, which may be:
1. episodic or pulsatile lasting for few minutes to hours
2. diurnal lasting for several hours in a light-dark cycle
3. periodic lasting for many days
4. developmental occurring at different phases of development
5. seasonal, in different seasons.

**Examples of chronotropic control:**
1. **Example of episodic (pulsatile) hormone secretion** is the secretion of GnRH. Normally, GnRH is secreted in episodic bursts that cause circchral peaks of LH secretion (for details, refer, “Female Reproduction”).
2. **Example of diurnal hormone secretion** is the change in ACTH or cortisol at different times of day and night. Another example of day-night variation is melatonin secretion.
3. **Example of periodical secretion** is alteration in sex hormones or gonadotropins in different phases of menstrual cycle.
4. Alteration in secretion of gonadal hormones at puberty in both boys and girls is the example of developmental hormonal secretion.
5. **Example of seasonal variation** is change in hormone concentration in different times in a year that mostly occurs due to environmental changes. This is more prominent in birds.

**Mechanisms**

The variation in hormone secretion is due to many mechanisms such as change in secretory pattern influenced by photic stimuli (light-dark variation), change influenced by sleep (sleep-wake variation) or change subjected to environmental alteration (seasonal variation).

**Humoral Control**

Humoral control is the control by hormones and chemicals.

**Hormonal Control**

Many hormones influence secretion of other hormones. Examples are glucagon stimulating insulin secretion, angiotensin stimulating aldosterone secretion, somatostatin inhibiting growth hormone secretion and so on.

**Chemical Control**

Secretion of hormone is influenced by various chemical stimuli such as concentration of blood gasses, acids, ions and osmolality. Examples are hypokalemia inhibiting insulin secretion, hyperkalemia or hyponatremia stimulating aldosterone secretion, etc.

**Hormone Signaling**

The chemical signaling of hormone occurs through three pathways: endocrine, paracrine, and autocrine.

**Endocrine Signaling**

Hormone secreted from the endocrine gland reaches distant target tissues via bloodstream. Most of the hormones signal target tissues located far away from the gland via this route. Thus, cells of hormone secreting tissue communicate with cells of other tissues of the body via endocrine signaling. Hence, this type of intercellular communication is called endocrine communication.

**Paracrine Signaling**

Hormone secreted from endocrine tissue diffuses into extracellular space and signals the neighboring tissue. This is called paracrine signaling. For example, somatostatin secreted from D cells of pancreatic islets influences the secretion of insulin and glucagon from same islet cells (Flowchart 52.4).
Autocrine Signaling
Hormone secreted from an endocrine cell binds with the receptor located in the same cell that secretes the hormone. Thus, hormone modifies the function of its parent cell. The example of autocrine signaling is the platelet activating factor secreted from platelet, which activates the platelet.

Intercellular Communications
Cells communicate with each other by five major mechanisms: direct, neural, endocrine, paracrine and neurocrine (Table 52.2). Autocrine and juxtacrine communications are strictly not part of the intercellular communications, as in these two systems cells influence their own activities or the other cells in the vicinity:
1. Direct communication occurs between cells via gap junction (electrical synapses). Example is rapid transmission of impulse between cardiac myocytes via gap junctions.
2. Neural communication is the major mechanism of intercellular communication. Examples are neurons communicating through synapses.
3. Endocrine, paracrine and autocrine communications are described above in ‘Hormone signaling’.
4. Juxtacrine communication is the communication through cell adhesion to growth factors expressed on cell surface. For example, many cells having receptors for transforming growth factor \( \alpha \) (TGF\( \alpha \)) interact with each other by attaching themselves to the transforming growth factor \( \alpha \) (TGF\( \alpha \)) present on their cell surface.
5. Neurocrine communication is via secretion of chemicals at nerve ending. For example, cholinergic vagal fibers to oxyntic cells in stomach influence parietal cell function by releasing acetylcholine at their terminals, and non-cholinergic vagal fibers to G cells in the antrum of stomach influence G cell function by releasing gastrin releasing peptide (GRP) at their terminals.

Transport of Hormones
By definition, hormones are secretions of ductless glands, transported via blood stream to reach their target tissues. After entering the blood they either remain in free form or bind with a specific carrier protein. Usually, amine and protein hormones remain in unbound (circulate in free) form, and thyroxine and steroid hormones (and vitamin D) circulate in the bound form.

Transport of Amine and Peptide Hormones
Amino acid-derived and peptide hormones readily dissolve in the plasma and do not require special mechanism for their transport.

Transport of Steroid Hormones
Thyroxine, steroid hormones and vitamin D are relatively insoluble in plasma and circulate in the bound form. In blood, more than 90% of thyroid and steroid hormones are bound to plasma proteins. The transport proteins are specific for hormones (Table 52.3). However, few nonspecific proteins are also involved in the process of hormone transport.

Importance of Hormone Binding
Binding of hormone with carrier proteins influences important biological properties of hormones, especially, hormone action, metabolism, storage and removal:
1. Hormone action: The intensity of the hormone action depends on the extent to which hormone is bound to the proteins. Usually, hormones that bind to transport proteins remain 1 to 10% only in the free form, which is called as the biologically active hormone. Thus, about 90% of these hormones, i.e. the hormone in bound form constitutes the inactive pool of hormone. Any condition that decreases hormone binding by decreasing the concentration of proteins available for binding or by any other mechanism, increases the hormone...
activity as plasma level of free hormone rises; and conversely, condition that increases binding decreases hormone activity.

2. **Hormone reservoir**: Transport proteins also provide reservoir of hormones that is essential for buffering rapid change in hormone concentration in plasma.

3. **Hormone metabolism and clearance**: Binding with carrier protein influences metabolism and removal of hormone from circulation. This determines the half-life of the hormone. For example, the plasma half-life of thyroxine is 6 days as it is 99.9% protein bound, whereas half life of aldosterone is 25 minutes as only 15% of it is bound to protein.

4. **Diagnostic importance**: From diagnostic viewpoint, it is important to know both free and bound form of the hormone. Assay of total hormone concentration may sometimes be misleading as total concentration may be normal in the presence of actual hormonal deficiency or excess. For example, binding proteins may be increased in various conditions such as pregnancy, drug therapy, etc. that decrease the free form, and therefore lead to functional hormone deficiency though the total concentration of hormone remains normal. Therefore, free concentration of hormone (free hormone index), or the ratio of free form to bound form of hormone reflects the actual physiological state of hormone. Hence, often, in the diagnosis of hormonal disorder, measurement of free hormone index is preferred to total hormone estimation to evaluate the degree of dysfunction.

### Degradation and Disposal of Hormone

Liver and kidney are the major sites for extraction and degradation of hormones. Therefore, diseases of liver and kidney alter hormonal status of the body by impairing removal of hormones from the body. Hormone degradation also depends on the rapidity of uptake of the hormone by the target cells like receptor-mediated endocytosis of peptide hormones. Also, metabolic degradation of hormones occurs by various enzymes.

#### Physiological Importance

Hormone disposal is physiologically important for two reasons: measurement of hormone secretion and metabolic clearance rate.

1. **Measurement of hormone secretion**: Urine is the major route of excretion for many hormones, and few hormones are excreted in bile. Therefore, for many hormones, measurement of hormone metabolites in urine is a better indicator of the rate of production of hormones. For example, urinary excretion of metanephrine or VMA is an index of catecholamine secretion, which provides a simple and noninvasive test for assessment of adrenomedullary function.

2. **Estimation of metabolic clearance rate (MCR)**: Sometimes estimation of rate of metabolism is clinically useful for assessing hormone dysfunction. The rate at which the hormone is removed from the body is one of the indices of MCR. MCR is the hormone removed per unit time divided by the plasma concentration of the hormone.

\[
MCR = \frac{\text{Hormone removed per unit time (mg/min)}}{\text{Plasma conc. of hormone (mg/mL)}}
\]

MCR is reported as ml of plasma per min. Half-life of hormone and MCR are inversely related. Thus, shorter the half life, greater is the MCR.

### Estimation of Hormone Concentration

Measurement of concentration of hormone in biological fluids is often performed for confirmation of clinical diagnosis. Hormone estimation can be done by bioassay, radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA).

#### Bioassay

Bioassay is the assay of hormone’s ability to produce a characteristic biological response:

1. In this method, hormone is estimated in terms of units, which is defined as an amount sufficient to produce a response of specified magnitude under standard conditions.

2. Bioassay was the earlier method of assessment of hormone concentrations.

### Table: 52.3: Transport proteins for hormones.

<table>
<thead>
<tr>
<th>Specific proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Thyroxine binding globulin (TBG)</td>
</tr>
<tr>
<td>– Transports T3 and T4</td>
</tr>
<tr>
<td>2. Corticosteroid binding globulin (CBG)</td>
</tr>
<tr>
<td>– Transports cortisol and aldosterone</td>
</tr>
<tr>
<td>3. Sex-hormone binding globulin (SHBG)</td>
</tr>
<tr>
<td>– Transports testosterone and estrogen</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nonspecific proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Serum albumin</td>
</tr>
<tr>
<td>– Transports many steroid and thyroid hormones</td>
</tr>
<tr>
<td>2. Prealbumin</td>
</tr>
<tr>
<td>– Transports thyroxine</td>
</tr>
</tbody>
</table>

Alteration of Hormone

Some of the hormones are transformed in the peripheral blood, which is required for their full biological activity. Examples are the conversion of T₄ to T₃ and testosterone to dihydrotestosterone.
3. After the discovery of RIA and ELISA, bioassay is rarely used at present for the purpose.
4. Also, the method is slow and often expensive.

Radioimmunoassay (RIA)

RIA is usually used for estimation of hormones, proteins, drugs and vitamins in body fluids like plasma, urine and CSF. RIA is a type of competitive binding assays. It is based on the theory of competitive binding. In RIA, two components are used: a specific antibody (Ab) that is produced against the hormone to be assayed, and a radioactively labeled hormone (H):

1. When the hormone to be measured is a peptide, the molecule is commonly labeled with a radioactive iodine atom (¹²⁵I or ¹³¹I) that can be easily attached to the tyrosine residue of peptide chain, and if the hormone is a steroid (that lack tyrosine residue), labeling is done by radioactive carbon (¹⁴C) or hydrogen (³H).
2. The principle of RIA is that the labeled and unlabeled hormones compete for a limited number of antibody binding sites. The quantity of each hormone bound to antibody is the ratio of that present in solution. The hormone and the radioactive hormone bind to antibody, and the amount of radioactivity present as Ab-H' is determined.
3. The response produced by the standards is used to generate a standard curve and the response produced by the unknown samples is then compared to the standard curve to determine the amount of hormone present in the unknowns.
4. The major drawback of the RIA is that it measures immunoreactivity, not the biological activity of the hormone.
5. Therefore, recently RIA is modified to receptor assay (RRA), in which specific receptors of hormone instead of antibodies are used as hormone-binding reagent. In RRA, as the receptor binding (the hormone that binds to its own receptors) is assessed, it measures biologically active hormone.

Enzyme-linked Immunosorbent Assay (ELISA)

This is an enzyme-based colorimetric or fluorometric assay of hormones that does not produce radioactive wastes. Therefore, the environmental hazard is reduced. Moreover, as it is a solid-phase assay, it is automated to a large extent.

Hormone Actions

Most of the hormones have several effects on target tissues that are called as pleiotropic effects of hormones. For example, acting on liver, cortisol not only decreases glucose uptake, but also influences glycogenolysis, glyco genesis, neoglucogenesis, lipolysis, protein synthesis, etc. Similarly, many hormones have multiple actions on several other tissues simultaneously. For example, the same cortisol apart from acting on liver, also acts on other tissues like skeletal muscle, intestine, heart, brain, bones, blood cells etc for various other functions:

1. As hormones act on different body systems for different actions, some of the actions are complementary and some are antagonistic.
2. However, both complementary and antagonistic actions of hormones are meant for integration of body functions in normal conditions as well as in different other situations.

Complementary Actions

When actions of different hormones facilitate a function of the body in a particular situation, it is called complementary actions of hormone. Complementary actions may be for a short-term regulation or for a long-term modification:

1. The example for short-term complimentary action is the acute physical exercise, during which secretion of epinephrine, cortisol and glucagon contributes to defend plasma glucose concentration. Deficiency of one or more hormones in such a condition results in severe hypoglycemia.
2. The example of long-term complementary actions is the regulation of growth by growth hormone, thyroxine, insulin like growth factors and sex steroids.

Antagonistic Actions

When the action of a hormone on a target organ is opposed by another hormone, the process is called antagonistic action. The example is the effect of insulin and glucagon on liver to regulate plasma glucose concentration. Insulin lowers plasma glucose by inhibiting hepatic glycogenolysis and gluconeogenesis, whereas glucagon increases blood glucose by stimulating glycogenolysis and gluconeogenesis. Thus, glucose homeostasis depends on the balance of actions of insulin and glucagon and other hyperglycemic hormones. Therefore, antagonistic actions of hormones, is an important regulatory process of the nature to fine-tune the physiological activities of the body.

CHAPTER SUMMARY

**Key Concepts**

1. Regulation of hormone secretion occurs mainly by feedback control mechanism, in which negative feedback system is the usual process.
2. Hormone concentration in the blood depends mainly on the free hormone available (the quantity of the hormone not bound to binding proteins).
3. Also, the concentration remains elevated when the degradation of the hormone is less, in addition to increased production.
4. Paracrine signaling is for controlling of neighboring cells, and endocrine signaling is for control of all the cells including the distant cells.

**Important to Know (Must Read)**

1. In examinations, ‘Mechanism of regulation of hormone secretion’ may come as a **Long Question**.
2. Classification of hormones, Pathways of hormone synthesis, Types of feedback control of hormone secretion, Negative feedback mechanism of hormone control, Hormone signaling mechanisms, Intercellular communications, Types of hormone actions, are usual **Short Questions** in exams.
3. In **Viva**, examiner may ask… Define hormone, Classify hormones and give example of each category, Name the endocrine glands, How is endocrine system analogous with nervous and immune system, What is the basic principle of hormone synthesis, What are the pathways of hormone synthesis, What is the meaning of ectopic hormone secretion, In what conditions ectopic hormone secretion occurs, What is paraneoplastic syndrome, What is the meaning and role of a feedback system, What are types of feedback control of hormone secretion, and give example for each, What is negative and positive feedback mechanism of hormone control, give examples, What are the hierarchical systems of negative feedback process, What are the hormone signaling mechanisms and give example for each, What are the intercellular communications and give example for each type of communication, How are the hormones transported, What is the importance of hormone binding, What are the processes of hormone degradation, How are hormones estimated, What are types of hormone actions.
Mechanisms of Hormone Action

Learning Objectives

On completion of study of this chapter, the student **MUST** be able to:
1. Understand the concept of receptor up-regulation and down-regulation.
2. Appreciate the role of G proteins in hormone action.
3. List the second messengers produced by first messengers (hormones).
4. List the important hormones that act through different second messengers.
5. Describe the mechanism of hormone action via adenylyl cyclase-cyclic AMP system, membrane phospholipid-phospholipase system, cyclic-GMP system and transcription of mRNA system.

The student **MAY** also be able to:
1. Explain the role of G proteins in health and disease.
2. Describe the effects of various second messengers on cells and tissues.

Receptors

Hormones to exert their effects on tissues bind with the **specific receptors** located in the target cell. The receptor may be present on the surface of the cell, in the cytoplasm or nucleus of the cell.

1. The binding of hormone with the receptor that forms hormone-receptor complex (HR complex), activates a series of signal generating mechanisms via a cascade of enzymatic reactions in the target cell.
2. The generated signal molecules increase in number in each step leading to manifold increase in final effect of the hormone on the cell. This is called **signal amplification**.
3. Normally, concentration of hormones in the body fluid is exceedingly low, which is usually in the range of $10^{-9}$ to $10^{-12} \, \text{mol/L}$. In spite of their very low concentration, hormones effectively alter cell functions through the process of signal amplification.
4. The peptide hormones bind to the cell surface receptors and activate a series of intracellular signal transduction systems.
5. Amine hormones also act via surface receptors.
6. Steroid and thyroid hormones bind to the intracellular receptors that regulate gene transcription.

Scientist contributed

The **Nobel Prize in Physiology or Medicine 1971** was awarded to American physiologist and endocrinologist, **Earl W Sutherland, Jr** “for his discoveries concerning the mechanisms of the action of hormones”. He worked extensively on the physiology of cyclic AMP.

Receptor Functions

The receptors located on the membrane are usually **large glycoproteins** with molecular weight of 50,000–200,000 dalton. They usually span the membrane seven times. Following binding of hormone with the receptor, **HR complex** is formed, which is **internalized by endocytosis**. Inside the cell, **HR complex is degraded by lysosomal enzymes**.

1. Usually, the receptor molecule is **recycled back to the cell membrane**. However, degradation of the receptor within the cell is not uncommon.
2. Mutation of receptors produces receptor diseases (Application Box 53.1).
Quantity and Sensitivity of Receptor

The receptor quantity and sensitivity are usually regulated by the concentration of the hormone that acts specifically on that set of receptors. Accordingly, there is up-regulation, down-regulation, and desensitization of receptors.

Up-regulation

When, concentration of a hormone decreases in plasma for a longer period, the number of receptors for that hormone usually increases in the target tissue. Also, sensitivity of receptor to the hormone increases. This is called up-regulation.

1. The exact mechanism by which deficiency of hormone results in increased recruitment of its own receptors is not clearly understood.
2. Up-regulation of receptors forms the physiological basis of denervation hypersensitivity.

Down-regulation

When, a hormone is present in excess in blood for a longer period, the number of receptors for that hormone in the target tissue decreases. This is called down-regulation.

Desensitization

When cells are chronically exposed to the excess concentration of a hormone, they become less responsive on subsequent exposures. The process is called desensitization. In this process, the sensitivity of receptors for the hormone decreases.

1. Chronic exposure to one hormone can also cause desensitization of receptors to other hormones.
2. If, desensitization occurs for the same hormone, the effect is called homologous desensitization, and if desensitization occurs for other hormones, the effect is called heterologous desensitization.

Application Box 53.1

Mutation of receptors: Receptor diseases are produced by mutation of receptors. Examples are Hirschsprung disease due to mutation of endothelin B receptors, familial hypothyroidism due to mutation of TSH receptors, color blindness due to mutation of receptors for cone opsins, X-liked nephrogenic diabetes insipidus due to mutation of V2 vasopressin receptors and so on.

G PROTEINS

Peptide hormones with the exception of IGF I and IGF II usually circulate freely as they do not have binding proteins for them in plasma. They bind with surface receptors on the target cells. Receptors are coupled with G proteins in the cell membrane.

1. G proteins belong to several families of intrinsic membrane proteins that link receptors to the nearby effector molecules in the membrane. Also, there are different subunits of each family of G proteins. In fact, specific subunit of G protein of a family relay specific signal from the receptors to the effectors that are usually specific ion channels or enzymes.
2. The effector molecules in turn generate second messengers that produce changes in cell functions when hormone binds with the receptors. Thus, G proteins convert the signal into biological activities.
3. These membrane proteins are called G proteins as they are attached to GDP. When the signal binds with the G protein, G protein exchanges GDP for GTP.
4. The GTP-protein complex influences subsequent signal transductions that results in generation of many intracellular signals, which mediate physiological effects of the hormone (Flowchart 53.1).
5. G protein has the inherent GTPase activity, which converts GTP to GDP. Therefore, once the hormone action is over, resting state of the G protein is restored.


Scientists contributed


Alfred Goodman Gilman (1941–2015) was an American pharmacologist and biochemist. He and Martin Rodbell shared the 1994 Nobel Prize in Physiology or Medicine for their discovery of G-proteins and the role of these proteins in signal transduction in cells. Martin Rodbell (1925–1998) an American biochemist and molecular endocrinologist discovered that ATP could reverse the binding action of glucagon to the cell receptor. He then noted that traces of GTP (guanosine triphosphate) could reverse the binding process almost one thousand times faster than ATP. This GTP, he found, stimulated the activity in the guanine nucleotide protein (later called the G protein), which, in turn, produced profound metabolic effects in the cell.
Types of G Proteins

Broadly, G proteins are classified into two categories based on their molecular size: small G proteins and large G proteins.

Small G Proteins

There are six different subfamilies of small G proteins. The major categories among them are Rab, Rac, and Ras families. A number of small G proteins contain lipid modifications that help them to adhere to membranes, whereas other small G proteins diffuse throughout the cytosol.

GAPs and GEFs

The GTPase activating proteins (GAPs) inactivate small G proteins by facilitating the hydrolysis of GTP to GDP in the central binding site. Guanine exchange factors (GEFs) activate small G proteins by facilitating exchange of GDP for GTP in the active site.

Functions of Small G Proteins

The small G proteins regulate many cellular activities.

The Rab family regulates rate of vesicle movement between cell organelles and the cell membrane.

The Rho/Rac family mediates interaction between cytoskeleton and the cell membrane.

The Ras family controls growth by regulating transmission of signals from the cell membrane to the nucleus.

Large G Proteins

These are the membrane proteins coupled to the receptors. Presently, five families of large G proteins have been discovered: Gs, Gi, Gq, Gv, and G13. In addition, there are three genes for G proteins: 20α, 6β, and 12γ genes. Therefore, a large number of subunits of G protein families are produced. Usually, G proteins are trimeric proteins consisting of three subunits. Therefore, they are called heterotrimeric G proteins. The subunits are called α, β, and γ subunits. Normally, α subunit is bound to GDP.

Mechanism of Action

When HR complex binds to the G protein couple receptors (GPCR), GDP is exchanged for GTP and α subunit is separated from βγ subunits of the G protein. This separation of α subunits from βγ subunits bring about physiological activities (Figs. 53.1A and B). The β and γ subunits do not separate from each other. The α subunit has the intrinsic GTPase activity that converts GTP to GDP. This results in reassociation of α subunit with the β and γ subunits of the G proteins. The GTPase activity of α subunit can be facilitated by regulators of G protein signaling (RGS).

The α subunit of three types of G proteins (Gs, Gi, and Gq) are physiologically most important. They are αs, αi, and αq subunits. Their activation produces following effects:

1. Activation of αs subunit activates the membrane enzyme adenylyl cyclase that promotes cAMP formation.
2. Activation of αi subunit inhibits the membrane enzyme adenylyl cyclase that decreases cAMP formation.
3. Activation of αq subunit activates membrane bound phospholipases like phospholipase C.

G Protein Coupled Receptors

G protein coupled receptors (GPCR) are membrane proteins that span the membrane seven times (Fig. 53.2). Hence, they are called serpentine receptors (seven-helix receptors). A large number of GPCR have been cloned recently. They have many diverse functions.

1. When a ligand binds with GPCR, conformational change occurs in GPCR that activates the large G protein attached to the cytoplasmic surface of the cell membrane.
2. Activation of a single GPCR can lead to activation of multiple large G proteins that transduces and amplifies the action of first messenger.
Scientists contributed

<table>
<thead>
<tr>
<th>Scientists</th>
<th>Image</th>
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<tbody>
<tr>
<td>Robert Joseph Lefkowitz (1943)</td>
<td><img src="image1.jpg" alt="Image of Robert Joseph Lefkowitz" /></td>
</tr>
<tr>
<td>Brian Kobilka (Born 1955)</td>
<td><img src="image2.jpg" alt="Image of Brian Kobilka" /></td>
</tr>
</tbody>
</table>

Robert Joseph Lefkowitz (1943) is an American physician (cardiologist). He was awarded the 2012 Nobel Prize for Chemistry with American Physiologist Brian Kobilka. Lefkowitz studies receptor biology and signal transduction and is most well known for his detailed characterizations of the sequence, structure and function of the β-adrenergic and related receptors and for the discovery and characterization of the two families of proteins which regulate them, the G protein-coupled receptor (GPCR) kinases and β-arrestins.

3. The GPCR bound to a ligand can be inactivated by phosphorylation of cytoplasmic side of the receptor to limit the process of cellular signaling.

There are many ligands (hormones/chemicals) for receptors coupled to G proteins (Table 53.1).

### G Protein Diseases

Mutation of G protein resulting in diseases is not uncommon. G protein responses may be increased or decreased by mutations.

#### Dysfunction due to Increased G Protein Responses

An example of increased G protein activity is somatotroph tumor causing acromegaly, in which mutation of Goαs decreases its intrinsic GTPase activity, therefore G protein activity is prolonged and more cAMP is produced. This leads to hyperplasia of somatotrophs of anterior pituitary producing acromegaly in about 40% of patients.

Another example is McCune-Albright syndrome, in which mutation of Goαs increases G protein activity producing areas of skin with hyperpigmentation and the state of hypercortisolism.

### Table 53.1: Ligands (hormones/chemicals) that act through G proteins coupled receptors (GPCRs).

<table>
<thead>
<tr>
<th>A. Neurotransmitters</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Catecholamines</td>
<td></td>
</tr>
<tr>
<td>1. Epinephrine</td>
<td></td>
</tr>
<tr>
<td>2. Norepinephrine</td>
<td></td>
</tr>
<tr>
<td>3. Dopamine</td>
<td></td>
</tr>
<tr>
<td>2. Acetylcholine</td>
<td></td>
</tr>
<tr>
<td>3. Histamine</td>
<td></td>
</tr>
<tr>
<td>4. Serotonin</td>
<td></td>
</tr>
<tr>
<td>5. Adenosine</td>
<td></td>
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<tr>
<td>6. Opioids</td>
<td></td>
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<tr>
<td>B. Other peptides</td>
<td></td>
</tr>
<tr>
<td>1. Angiotensin II</td>
<td></td>
</tr>
<tr>
<td>2. ADH, Oxytocin</td>
<td></td>
</tr>
<tr>
<td>3. GRP, VIP, PTH, TRH</td>
<td></td>
</tr>
<tr>
<td>C. Glycoprotein hormones</td>
<td></td>
</tr>
<tr>
<td>1. TSH, FSH, LH</td>
<td></td>
</tr>
<tr>
<td>2. hCG</td>
<td></td>
</tr>
<tr>
<td>D. Arachidonic acid derivative</td>
<td></td>
</tr>
<tr>
<td>1. Thromboxane A2</td>
<td></td>
</tr>
<tr>
<td>E. Tachykinins</td>
<td></td>
</tr>
<tr>
<td>1. Neuropeptide K</td>
<td></td>
</tr>
<tr>
<td>2. Neurokinin A</td>
<td></td>
</tr>
<tr>
<td>3. Substance P</td>
<td></td>
</tr>
<tr>
<td>F. Other ligands</td>
<td></td>
</tr>
<tr>
<td>1. Endothelins</td>
<td></td>
</tr>
<tr>
<td>2. PAF</td>
<td></td>
</tr>
<tr>
<td>3. Testants, Odorants</td>
<td></td>
</tr>
</tbody>
</table>

### Dysfunction due to Decreased G Protein Responses

An example of decreased G protein responses is type 1 pseudohypoparathyroidism, in which mutation of Goαs leads to failure of G protein to respond to parathyroid hormone. This results in features of hypoparathyroidism without actual decrease in parathyroid hormone.

#### SECOND MESSENGERS (Signal Transduction Pathways)

Hormones are considered as first messengers that bind with the receptors. The hormone (or the ligand) binding with receptors forms the complex called hormone-receptor (HR) complex. Formation of HR complex is the first step in the hormone action that eventually leads to formation of second messengers.

In general, the processes of second messenger formation are broadly categorized into 4 types (Table 53.2):

1. Activation of ion channels
2. Activation of G proteins
Chapter 53: Mechanisms of Hormone Action

3. Activation of intracellular or membrane enzymes
4. Activation of transcription process.

**Role of phosphorylation**: Phosphorylation is an important **post-translational event** in the cell signaling pathway. Phosphorylation in the cell is controlled by two group of proteins namely kinases and phosphatases.

1. **Kinases** catalyze the phosphorylation of tyrosine or threonine residues in proteins and **phosphatases** remove phosphates from proteins.
2. More than 300 protein kinases have been described (Table 53.3).
3. The process of phosphorylation-dephosphorylation of cellular proteins is important in control of some ligand signaling pathway, which is referred to as **phosphate timer**.

### Second Messengers

The intracellular signal molecules that are formed by a series of enzymatic reactions subsequent to the formation of HR complex are designated as **second messengers**.

1. Second messengers are formed depending on the hormone signaling of the effector cells.
2. The signal transduction pathways are activated depending on G protein activation of membrane enzymes (Table 53.4).
3. **The major second messengers are**: cyclic AMP, diacylglycerol (DAG), inositol triphosphate (IP₃), cyclic GMP, phosphoproteins, transcripted new mRNAs, and intracellular calcium.

### Second messengers are formed by activation of **four main transduction systems**:

1. **Adenylyl cyclase–cyclic AMP system**
2. **Membrane phospholipase–phospholipid system**
3. **Guanylyl cyclase–cyclic GMP system**
4. **Transcription of mRNAs**.

#### Cyclase–Cyclic AMP System

Cyclic adenosine monophosphate (cAMP) is an important **second messenger for many peptide and amine hormones**. It also causes **lymphocyte activation and mast cell degranulation**. Formation of cAMP and alteration in cell functions by it involve a series of events following interaction of hormone with receptor on the cell membrane (Fig. 53.3).

### Steps of Signal Transduction

The steps of signal transduction are as follows:

1. The hormone binds with the receptor present on the cell membrane and forms hormone-receptor complex (HR complex formation).
2. HR complex is formed that results in activation of G proteins (either αᵣ or αᵢ).
3. The membrane enzyme **adenyl cyclase is activated (if αᵣ is stimulated)**. Adenylyl cyclase is a membrane protein that spans the membrane 12 times. When the binding of hormone to receptor stimulates the stimulatory G protein (αᵣ subunit), adenylyl cyclase is activated. If the hormone binding with receptor stimulates the inhibitory G protein the (αᵢ subunit), adenylyl cyclase is inhibited. (Accordingly, in the next step, CAMP formation is either increased or decreased in the cell).
4. Adenylyl cyclase catalyzes the conversion of ATP to cyclic AMP.

---

**Table 53.2**: Broad mechanisms of ligands bringing about change in cell functions.

<table>
<thead>
<tr>
<th>A. Opening or closure of ion channels</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Ach on nicotinic receptors</td>
</tr>
<tr>
<td>2. NE on K⁺ channel in heart</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B. Via adenylate cyclase (cAMP formation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. NE via β₁ receptor († cAMP)</td>
</tr>
<tr>
<td>2. NE via α₂ receptor (+ cAMP)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>C. Activation of phospholipase C (DAG, IP₃ formation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Angiotensin II</td>
</tr>
<tr>
<td>2. NE via α₁ receptor</td>
</tr>
<tr>
<td>3. ADH via V₁ receptor</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>D. Via cyclic GMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>– ANP, NO</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>E. Via tyrosine kinase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>– Insulin, PDGF, M-CSF, EGF</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>F. Via serine or threonine kinase</th>
</tr>
</thead>
<tbody>
<tr>
<td>– Inhibin, Activin, TGF-β</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>G. Via nuclear receptors (m-RNA transcription)</th>
</tr>
</thead>
<tbody>
<tr>
<td>– Steroid hormones</td>
</tr>
<tr>
<td>– Thyroid hormones</td>
</tr>
</tbody>
</table>

**Table 53.3**: Important protein kinases in the cell.

<table>
<thead>
<tr>
<th>A. Kinases that phosphorylate serine or threonine residues or both</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Calmodulin-dependent kinases</td>
</tr>
<tr>
<td>– Myosin-light chain kinase</td>
</tr>
<tr>
<td>– Phosphorylase kinase</td>
</tr>
<tr>
<td>– Ca²⁺-calmodulin kinase I, II and III</td>
</tr>
<tr>
<td>2. Calcium-phospholipid dependent kinases</td>
</tr>
<tr>
<td>– Protein kinase C</td>
</tr>
<tr>
<td>3. Cyclic nucleotide dependent kinase</td>
</tr>
<tr>
<td>– Protein kinase A (cAMP dependent)</td>
</tr>
<tr>
<td>– cGMP dependent kinase</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B. Kinases that phosphorylate tyrosine residues</th>
</tr>
</thead>
<tbody>
<tr>
<td>– Insulin receptor</td>
</tr>
<tr>
<td>– EGF receptor</td>
</tr>
<tr>
<td>– PDGF receptor</td>
</tr>
<tr>
<td>– M-CSF receptor</td>
</tr>
</tbody>
</table>

---
5. The cAMP activates protein kinase A (PKA). 
**Protein kinase A** (PKA) has two catalytic and two regulatory subunits. cAMP separates catalytic from regulatory subunits. This separation of subunits leads to activation of PKA.

6. Free catalytic subunits phosphorylate the serine and threonine residues on many cellular enzymes and other proteins. This process of phosphorylation of intracellular proteins results in formation of various phosphoproteins.

7. New phosphoproteins induce cell functions (mediate the physiological effects of hormone). The PKA also migrates to the nucleus where it phosphorylates CREB (cAMP-responsive element-binding protein). CREB binds to DNA and influences transcription of a number of genes. Thus, cAMP also alters genetic functions of the cell.

### Termination of cAMP Actions
Actions of cAMP in the cell are terminated in two ways.
1. cAMP is degraded to 5′-AMP in the cell by the cytoplasmic enzyme phosphodiesterase (PDE).
2. Serine or threonine specific phosphatases dephosphorylate the proteins already phosphorylated by PKA.

### Clinical Importance
Not only, many hormones act through cAMP (Table 53.5), but also, toxins released by various pathogens produce...
Toxic features by altering cAMP concentration in the cell. **Cholera and pertussis toxins** are examples of stimulation and inhibition of cAMP respectively.

**Stimulation of cAMP**

In cholera, the highly infective acute diarrheal disease caused by Vibrio cholerae, the **cholera toxin** (especially, subunit A of the toxin) irreversibly transfers ADP-ribose from NAD to its specific target protein, the GTP-binding regulatory component of adenylate cyclase in intestinal epithelial cells. The ADP-ribosylated G protein **up-regulates activity of adenylate cyclase**. Therefore, cAMP production is increased. In intestinal epithelial cells, **cAMP decreases Na⁺ absorption and increases Cl⁻ secretion**. Increased NaCl content in the intestinal lumen osmotically retains water and produces **isotonic fluid diarrhea**. Tea and coffee stimulate cyclic AMP formation (Application Box 53.2).

**Inhibition of cAMP**

In pertussis (whooping cough), an acute respiratory tract infection caused by *Bordetella pertussis*, a variety of toxins are produced by the pathogen. A component of pertussis toxin decreases adenylyl cyclase activity by **activating inhibitory G protein**. This impairs the host's defense ability.

**Application Box 53.2**

Tea and coffee is good for heart: Normally, PDE degrades cAMP in the cells. Therefore, the agents that inhibit the action of PDE like caffeine and theophylline increase the concentration of intracellular cAMP. These chemicals increase myocardial contractility by increasing intracellular cAMP. Caffeine and theophylline are active principles in coffee and tea. Hence, moderate intake of tea and coffee is good for heart, and tea is considered better than coffee in this regard.

**Membrane Phospholipid-Phospholipase System (via IP₃ and DAG)**

This is an important system for transduction of hormone signals into physiological activities. In this system, binding of hormone with the receptor initiates the following cascade of reactions (Fig. 53.4).

1. **Hormone binding with receptor results in HR complex formation.**
2. **HR complex activates Gαq.**
3. **Activated Gαq activates the membrane enzyme phospholipase C.**

**Phospholipase C** (PLC) is attached to protein Gαq and is located on the inner surface of the membrane. There are eight isoforms of PLC. Usually, **PLC β₁ and β₂ isoforms** are activated by Gαq. They catalyze the cleavage of membrane phosphatidylinositols, the phospholipids present in the inner lamella of cell membrane.

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**Table 53.5: Important hormones that act by altering cAMP concentration in the cell.**

<table>
<thead>
<tr>
<th>A. Hormones that act by increasing cAMP formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. GHRH</td>
</tr>
<tr>
<td>2. CRH</td>
</tr>
<tr>
<td>3. GnRH</td>
</tr>
<tr>
<td>4. FSH</td>
</tr>
<tr>
<td>5. LH</td>
</tr>
<tr>
<td>6. Norepinephrine via β₁ receptor</td>
</tr>
<tr>
<td>7. ADH via V₂ receptor</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B. Hormones that act by inhibiting cAMP formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Norepinephrine via α₂ receptor</td>
</tr>
<tr>
<td>2. Somatostatin</td>
</tr>
</tbody>
</table>

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**Fig. 53.4: Mechanism of hormone action through membrane phospholipid-phospholipase system.** (H: Hormone; R: Receptor; Gq: Gαq protein; PIP₂: Phosphatidylinositol bisphosphate; IP₃: Inositol triphosphate; DAG: Diacylglycerol; CaBP: Calcium binding proteins).
4. PLC β₁ or β₂ causes hydrolysis of phosphatidylinositols 4,5-bisphosphate (PIP₂) that generates two important signal molecules: inositol 1,4,5-triphosphate (IP₃) and diacylglycerol (DAG).
   - IP₃ releases calcium from endoplasmic reticulum.
   - DAG stimulates intracellular (submembrane) enzyme protein kinase C.

5. The increased protein kinase C activity activates many intracellular enzymes that bring about change in cell functions. Thus, two subsystems are formed in this pathway: the IP₃ subsystem and the DAG subsystem.

**In IP₃ Subsystem:**
5a. IP₃ binds with its receptors located on cytosolic surface of endoplasmic reticulum (ER). This binding facilitates release of Ca²⁺ from ER, which causes cytosolic Ca²⁺ to increase several-fold.
6a. Increased intracellular Ca²⁺ facilitates binding of Ca²⁺ with calcium-binding proteins (CaBP) like calmodulin, and also activates CaBP. Ca²⁺-calmodulin dependent protein kinases are also activated in this process.
7a. CaBP alters cell functions.

**In DAG Subsystem:**
5b. DAG stimulates the enzyme protein kinase C (PKC).
6b. PKC causes phosphorylation of a variety of intracellular proteins leading to formation of phosphoproteins.
7b. Phosphoproteins alter cell functions.

Examples of hormones that act via IP₃ and DAG are: norepinephrine acting via α₁ receptors, ADH acting via V₁ receptors, angiotensin II, TRH, etc.

2. The HR complex activates guanylyl cyclase in the membrane.

**Guanylyl cyclase** is the sub-membrane enzyme that forms part of the receptor. In fact, it is the cytoplasmic domain of the receptor. Therefore, binding of hormone with receptor automatically activates this enzyme. There are various isoforms of the guanylyl cyclase.

3. Guanylyl cyclase converts cytoplasmic guanosine triphosphate (GTP) to cGMP.

4. cGMP activates cGMP-dependent kinases and phosphatases that cause phosphorylation of different intracellular proteins. cGMP also directly opens ion channels. In rods and cones, cGMP operated mechanisms mediate physiologic effects.

**Ligands that act through cGMP:** The hormones that act through cGMP are ANP, nitric oxide (NO). The enterotoxin of E. Coli and GI polypeptide hormone guanylin also act through cGMP.

**Intracellular Receptor (Transcription of mRNA) System**

The receptors for thyroid and steroid hormones, 1,25-dihydroxycholecalciferol and retinoids are located inside the cell. The hormone diffuses through cell membrane into the cytoplasm and combines with the receptor, which is present either in cytoplasm or in nucleus. The receptor for glucocorticoid is present in cytoplasm, whereas the receptor for thyroxine and other steroid hormones is located in nucleus.
proteins are intracellular proteins and they are so named as their concentration increases on exposure of cells to heat and stress. Therefore, they are also called stress proteins. They protect cells from various stresses.

3. The binding of active HR complex to DNA facilitates transcription of mRNAs.
4. The mRNAs are then translated in the ribosomes to form new proteins.
5. Formation of new proteins in the cell alters cell function.

**Other Mechanisms of Signal Generation**

**Tyrosine Kinase Activation**

Some peptide hormones do not require G protein for inducing the signal transduction system. The hormone binds with the receptor, which has intrinsic tyrosine kinase activity. The receptor has three domains: extracellular, membrane and intracellular domains. The extracellular domain possesses the binding site for hormone and the intracellular domain possesses tyrosine kinase activity.

The steps of signal induction are as follows:

1. The binding of hormone with the receptor causes conformational change in the receptor that exposes the intracellular sites of the receptor for autophosphorylation (Fig. 53.8).
2. The kinase autophosphorylates tyrosine residue within the receptor and tyrosine residues on intracellular protein substrates.
3. The phosphorylation of tyrosine residue initiates cascade of phosphorylation reactions that phosphorlyates various enzymes like serine and threonine kinases, and phosphatases. Phosphorylated enzymes change cellular activities.

The hormones that act through tyrosine kinase are insulin, IGF I, IGF II, other growth factors like PDGF, and EGF, and M-CSF.

**JAK-STAT Pathway**

The other method of tyrosine kinase activation is that the hormone binding causes conformational change in the intracytoplasmic tip of the receptor that exposes the sites to which cytoplasmic tyrosine kinase binds.

1. The activation of tyrosine kinase such as Jannus tyrosine kinase (JAK) phosphorylates signal transducers and activator of transcription proteins (STAT proteins).
2. Activation of JAK-STAT pathway further activates intracellular enzymes that change cell function. Example of the hormone acting through JAK-STAT pathway is the growth hormone (for details of mechanism, refer “Growth Hormone”).

**Calcium–Calmodulin System**

This is the system of transduction of the hormone signal in which binding of hormone to a receptor on the cell

---

**Fig. 53.6:** Structure of steroid receptor. C and N at both end of the receptor depict carboxy terminal and amino terminal, respectively.

**Receptors**

The receptors for steroid hormones are monomeric phosphoproteins. They have amazing resemblance with the receptors for 1,25-dihydroxycholecalciferol, thyroid hormones, and retinoic acids. Receptors for all these diverse hormones are considered to be part of a single gene superfamily.

1. They have five domains (A to E), and the homology is mainly seen for the C domain, especially C1 sub-domain (Fig. 53.6). The estrogen receptor has an extra domain called F domain. The C1 sub-domain of the receptor is responsible for binding to DNA.
2. The receptors dimerize when they bind with DNA, which is essential for gene transcription.
3. The receptors have an amino terminal and a carboxy terminal.
4. The hormone binding domain is present close to the carboxy terminal.
5. A cysteine-rich DNA binding domain is located close to the hormone-binding domain.
6. When hormone binds with receptor, C1 DNA binding domain binds with DNA through two zinc fingers.

**Steps of Signal Transduction**

Binding of hormone with the receptor triggers following series of events:

1. The binding of hormone with the receptor causes conformational change in the receptor protein. The DNA binding domain of the receptor is exposed.
2. The hormone and active-receptor complex then moves to the DNA and binds with the enhancer elements (also called hormone response elements or steroid response elements; SRE) in the DNA. Normally, steroid receptors are bound to heat shock protein (HSP) that covers the DNA binding domain (Fig. 53.7). When hormone binds with receptors, the conformational change in receptor releases the heat shock protein from it, which exposes the DNA binding domain. Heat shock proteins are intracellular proteins and they are so named as their concentration increases on exposure of cells to heat and stress. Therefore, they are also called stress proteins. They protect cells from various stresses.
3. The binding of active HR complex to DNA facilitates transcription of mRNAs.
4. The mRNAs are then translated in the ribosomes to form new proteins.
5. Formation of new proteins in the cell alters cell function.
Increased intracellular calcium changes cell function by various mechanisms.
1. Thus, in this system, calcium is the second messenger for hormone action.
2. Calcium is also mobilized from the intracellular storage sites like mitochondria and endoplasmic reticulum (Fig. 53.9).
3. In many tissues, the secretion of calcium from the intracellular storage sites triggers opening of calcium channels in the cell membrane. This is called store-operated calcium influx. This further increases the calcium concentration in the cell and replaces calcium in endoplasmic reticulum and mitochondria.
4. Calcium then binds with many intracellular calcium binding proteins. One of the important calcium binding proteins is the calmodulin.

**Calmodulin-dependent Kinases**
Calmodulin is a polypeptide containing 148 amino acids and has four calcium binding domains. When calcium binds with calmodulin, it activates different calmodulin-dependent kinases. There are various calmodulin-dependent kinases in the cell. The important kinases are:
1. Myosin light change kinase that causes phosphorylation of myosin.
2. Phosphorylase kinase that causes phosphorylation of many intracellular proteins.
3. Other important kinases are calcium-calmodulin kinase I, II, and III. Kinases I and II are concerned with synaptic function and kinase III is involved in protein synthesis.
Likewise, there are many kinases that are involved in various cell functions.

**Calcium Binding Proteins**

The calcium binding proteins in the cells are calmodulin, troponin, and calbindin.

1. The troponin is the calcium binding protein present in the skeletal muscle involved in muscle contraction.
2. Calcineurin, a calmodulin-activated protein, is a phosphatase, which inactivates calcium channels through dephosphorylation. Calcineurin also participates in immunity by activating T cell.

**Rapid Actions of Steroids**

Usually steroids act through the intracellular receptor that causes translation and transcription of mRNA to form new proteins. As transcription of mRNA is a time-consuming process, actions (genomic actions) of steroids are normally delayed. However, some actions of steroid hormones manifest more rapidly than the transcription process. These are categorized as nongenomic actions of steroids.

1. For nongenomic actions, the hormone binds with the surface receptors that either changes membrane permeability to calcium or other ions, or stimulates formation of second messengers like cAMP in the cell. An example of nongenomic action is the rapid neuronal changes induced by steroid.
2. Thus, steroids also act through intracellular second messengers that are activated by other hormones.
3. This forms the physiological basis of interaction of steroid hormones with other hormones. For example, estrogen and dopamine interact at the second messenger level for nongenomic actions of estrogen.

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**CHAPTER SUMMARY**

**Key Concepts**

1. Protein and amino acid hormones act by altering intracellular levels of cAMP, cGMP, IP$_3$, DAG, and Ca$^{2+}$. Few hormones act directly by altering ion channels in the membrane and many through the G proteins.
2. Steroid hormones and thyroid hormones act by altering cellular transcription and translation mechanisms.
3. Few hormones like insulin do not require G proteins. Binding with the receptor, activate the intrinsic tyrosine kinase activity of the receptor that triggers the induction of intracellular signaling pathway.
4. Up-regulation and down-regulation of receptors occurs with sustained decrease or increase in the level of hormone in the blood.
Important to Know (Must Read)

1. In examination, “Describe the mechanisms of hormone action”, “Describe the mechanism of hormone action via adenylate cyclase-cyclic AMP and membrane phospholipid-phospholipase pathways” may come as **Long Questions**.

2. Hormone signaling, G proteins, adenylate cyclase-cyclic AMP pathway of hormone action, Membrane phospholipid-phospholipase system, cGMP, Calcium-calmodulin system, Intracellular receptors, Hormone action via mRNA transcription mechanism, Hormone action via tyrosine kinase system, may be asked as **Short Questions**.

3. In **Viva**, examiner may ask… Up-regulation, Down-regulation, and Desensitization of receptors, Types, functions and dysfunctions of G proteins, list of second messengers, Steps and mechanism of action of cyclic AMP, Steps and mechanism of action of IP$_3$-DAG system, Steps and mechanism of action of cyclic GMP, Steps and mechanism of action of thyroid and steroid hormones, Steps following tyrosine kinase activation, Steps following tyrosine JAK-STAT pathway activation, Calmodulin-dependent kinases, Calcium binding proteins and their actions, Examples of hormones acting through the various pathways, and Rapid action of steroids.
CHAPTER 54
Hypothalamus and Hypothalmo‑pituitary Axis

LEARNING OBJECTIVES
On completion of study of this chapter, the student MUST be able to:
1. Appreciate the importance of hypothalamus as an endocrine organ.
2. List the hormones secreted from hypothalamus and mention their functions.
3. Understand the functional organization of hypothalmo‑pituitary axis (HPA).
4. Brief the role of HPA in the regulation of pituitary functions.

The student MAY also be able to:
1. Describe the functions of hypothalamic hormones.
2. Explain the function of HPA in the control of pituitary function.

HYPOTHALAMUS
Hypothalamus plays a vital role in the regulation of visceral functions. For its central location in the brain, hypothalamus is closely connected with the limbic system, autonomic nervous system and pituitary gland. Therefore, hypothalamus is the main neural structure for the integration of visceral homeostatic mechanisms. Through the regulation of pituitary secretions, hypothalamus plays a master role in the control of many endocrine functions of the body. As the hormones secreted from hypothalamus (especially to posterior pituitary) are synthesized in hypothalamic neurons, they are called as neurohormones.

Scientists contributed

Roger Charles Louis Guillemin (Born, 1924) and Andrew V Schally (Born, 1926)

Roger Charles Louis Guillemin received the National Medal of Science in 1976, and the Nobel prize in Physiology and Medicine for the year 1977 for his work on neurohormones (peptide hormones produced in the brain), sharing the prize with Andrew V Schally. Guillemin and Schally discovered the structures of TRH and GnRH in separate laboratories.

Hypothalamus as an Endocrine Gland
The hypothalamus plays an important role in the regulation of endocrine functions. It controls all the secretions from pituitary gland.
1. Through its connection with anterior pituitary gland, it controls:
   - thyroid functions (hypothalamo‑pituitary‑thyroid axis),
   - adrenocortical secretions (hypothalamo‑pituitary‑adrenocortical‑axis), and
   - gonadal functions (hypothalamo‑pituitary‑gonadal‑axis).
2. Via its influence on sympathetic output, hypothalamus controls secretions of adrenal medulla (hypothalamo‑sympatho‑adrenal‑axis).
3. It directly controls the secretions from posterior pituitary.
   Thus, hypothalamus regulates functions of major endocrine glands. Therefore, hypothalamus is apparently designated as the “master of endocrine orchestra”.

Integration with Neural Structures
Anatomically and physiologically, hypothalamus is situated at the center of the brain, below the corpus callosum and thalamus (Fig. 54.1). Therefore, hypothalamus collects and integrates information from various parts of
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Fig. 54.1: Location of hypothalamus in the brain.

Fig. 54.2: Connection of hypothalamus with other brain centers. Note, after receiving inputs from all these structures hypothalamus funnels these inputs to brainstem and pituitary for regulation of visceral functions.

the brain and funnels them through the pituitary gland for modification of endocrine functions. Thus, hypothalamus is the primary link between nervous and endocrine systems. It receives afferent signals from:
1. Reticular activating system
2. Thalamus
3. Neocortex
4. Eyes
5. Limbic system, especially from amygdala, septum, olfactory bulb, and hippocampus (Fig. 54.2).

These neural inputs convey the information regarding alertness, application of nociceptive stimuli, sleep-wakefulness, changes in environment, emotion, visual, olfactory and gustatory sensations and so on. After gathering these information, hypothalamus appropriately alters body functions partly through its influences on endocrine secretions and partly through its control on major neural outputs from brain like projections from brainstem cardio-respiratory centers, and autonomic and limbic outputs (Flowchart 54.1).

Hypothalamus, via its endocrine influences also controls thirst, appetite, energy store, body fat composition, immunity, behavior and visceral functions. Thus, hypothalamus through its extensive neuroendocrine connections, controls major body functions. Hence, diseases affecting hypothalamic nuclei result in many pathological syndromes.

Hypothalamic Hormones

Hypothalamic hormones are secreted from various nuclei of the brain (Fig. 54.3). Hypothalamic hormones can be classified broadly into three categories: anterior pituitary regulating hormones, posterior pituitary hormones and other hormones.

Anterior Pituitary Regulating Hormones

Hypothalamic hormones that regulate anterior pituitary secretions are known as releasing or release inhibiting hormones as they stimulate or inhibit the release of various hormones from this part of the pituitary. The hormones are: Thyrotropin releasing hormone, gonadotropin releasing hormone, corticotropin releasing hormone, growth hormone releasing and inhibiting hormones, and prolactin...
releasing and inhibiting hormones (Flowchart 54.2). These are peptide hormones that act on target cells mostly through cyclic AMP and IP3 or DAG as second messengers. They regulate secretions from anterior pituitary.

**A. Releasing Hormones**

**Thyrotropin Releasing Hormone (TRH)**

TRH is an oligopeptide containing 3 amino acids. 

**Source:** TRH is secreted from paraventricular nucleus of hypothalamus.

**Functions:**

1. TRH stimulates secretion of thyroid stimulating hormone (TSH) or thyrotropin from thyrotrophs of anterior pituitary.
2. It stimulates expression of genes for α and β subunits of TSH in thyrotrophs.
3. It also promotes secretion of prolactin and growth hormone (GH).

**Gonadotropin Releasing Hormone (GnRH)**

GnRH is a peptide containing 10 amino acids. 

**Source:** GnRH is secreted from the arcuate nucleus of hypothalamus.

**Functions:**

1. GnRH stimulates secretion of luteinizing hormone (LH); therefore, GnRH is known as luteinizing hormone releasing hormone (LHRH). It also stimulates secretion of follicular stimulating hormone (FSH). Together, it is known as gonadotropins releasing hormone as it stimulates secretion of both LH and FSH.
2. GnRH also stimulates secretion of GH.

**Corticotropin Releasing Hormone (CRH)**

CRH is a polypeptide having 41 amino acids. 

**Source:** CRH is secreted from paraventricular nucleus of hypothalamus.

**Functions:**

1. CRH stimulates the secretion of adrenocorticotropic hormone (ACTH) or corticotropin from corticotrophs of anterior pituitary.
2. It also stimulates the expression of proopiomelanocortin (POMC) gene in corticotrophs.
3. CRH promotes secretion of β- and γ-lipotropin and β-endorphins.

**Growth Hormone Releasing Hormone (GHRH)**

GHRH appears in two forms in humans, one containing 40 and other 44 amino acids. 

**Source:** GHRH is secreted from the arcuate nucleus of hypothalamus.

**Functions:**

1. GHRH stimulates secretion of growth hormone from somatotrophs of anterior pituitary.
2. It also stimulates the expression of GH gene in corticotrophs.
Section 6: Endocrine Physiology


**Prolactin Releasing Factor (PRF)**
The exact site of synthesis of PRF in hypothalamus is not known. It stimulates prolactin synthesis and secretion from lactotrophs of anterior pituitary.

**B. Release Inhibiting Hormones**

*Growth Hormone Inhibiting Hormone (Somatostatin)*
Somatostatin is a peptide having 14 amino acids. It is secreted from anterior periventricular nucleus of hypothalamus. It inhibits secretion of growth hormone, prolactin and TSH.

*Prolactin Inhibiting Hormone (PIH)*
PIH is the dopamine, which is secreted from arcuate nucleus of hypothalamus. It inhibits secretion of prolactin, TSH, and growth hormone from anterior pituitary.

**Posterior Pituitary Hormones**
ADH and oxytocin secreted from posterior pituitary are actually not formed in this gland. They are synthesized in supraoptic and paraventricular nuclei of hypothalamus and stored in posterior pituitary (for details, see below and refer the chapter on “posterior pituitary”).

**Other Hypothalamic Hormones**
Other hormones of hypothalamus are neuropeptide Y, orexins, MCH, ghrelin, MSH and CART. They are mainly involved in the control of ingestive behaviors and regulation of body weight and composition. Ghrelin is secreted mainly from GI tract, and less from hypothalamus.

*Neuropeptide Y*
This polypeptide hormone containing 36 amino acids is secreted from hypothalamus. The cell bodies of neurons that synthesize neuropeptide Y are present in the arcuate nucleus, and the axons project to the paraventricular nucleus.

1. It is the principal neurohormone that stimulates food intake. It has also been observed that neuropeptide Y secretion in hypothalamus increases during feeding and decreases during cessation of feeding (satiety).
2. Many neurotransmitters that stimulate food intake increase neuropeptide Y release and neurochemicals that inhibit feeding decrease neuropeptide Y release from hypothalamus.

**Orexins**
There are two types of orexins: orexin-A and orexin-B. Orexins are synthesized in the lateral hypothalamus. They stimulate food intake.

**Melanin-Concentrating Hormones (MCH)**
This is a polypeptide containing 19 amino acids. It is secreted by the pituitary, lateral hypothalamus, and zona incerta. There are receptors for MCH in the hypothalamus. It increases food intake.

**Cocaine and Amphetamine-Regulated Transcript**
The hypothalamic hormone CART (cocaine and amphetamine-regulated transcript) inhibits food intake.

**Applied Physiology**
Endocrine hypothalamic dysfunctions lead to abnormalities of hypothalamo-endocrine axes described above. This results in irregularities in anterior and pituitary secretions and many target organ dysfunctions. These are called hypothalamic syndromes (for details, refer the Chapter “Hypothalamic Functions” in Neurophysiology).

**HYPOTHALAMO-PITUITARY AXIS**
The hypothalamus is closely linked with pituitary gland both anatomically and functionally. The connection between hypothalamus and pituitary is called hypothalamo-pituitary axis. Hypothalamus is connected separately and differently with anterior and posterior pituitaries. The connection with anterior pituitary is by means of blood vessels (portal-hypophyseal vessels) and with posterior pituitary is through the neurons (hypothalamo-hypophyseal tract).
Chapter 54: Hypothalamus and Hypothalamo-pituitary Axis

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Portal Hypophyseal Vessels

The connection between the anterior pituitary and hypothalamus is vascular. Anterior pituitary develops from the Rathke’s pouch, an evagination from the roof of the pharynx. The portal hypophyseal vessels directly connect between hypothalamus and anterior pituitary.

1. The blood vessels arise from capillaries on the ventral surface of hypothalamus (primary capillary plexus) that derive blood from superior hypophyseal artery (Fig. 54.4).

2. The capillaries drain into the sinusoidal portal hypophyseal vessels. These long portal hypophyseal vessels carry blood down the pituitary stalk to the anterior lobe of the pituitary where they end in another set of capillaries. Thus, the system of blood vessels begins with capillaries and ends with capillaries without passing through the heart. Therefore, they are examples of true portal system of blood vessels (a portal vessel starts and ends with capillaries without traversing through the heart).

3. The releasing hormones secreted from hypothalamus reach anterior pituitary via hypophyseal portal circulation. Therefore, these hormones are also called hypophysiotropic hormones.

4. Hypophysiotropic hormones are synthesized in small hypothalamic neurons (parvocellular neurons), the axon terminals of which contact the capillary network in the median eminence and infundibulum in hypothalamus that give rise to long portal vessels in the pituitary stalk.

5. Thus, hypophysiotropic hormones are transported to anterior pituitary via hypophyseal portal blood.

6. Short portal hypophyseal vessels communicate the capillaries of anterior pituitary with capillaries of posterior pituitary, which derives blood from inferior hypophyseal artery.

Hypothalamo-hypophyseal Tract

The connection between hypothalamus and posterior pituitary is neural. Posterior pituitary develops as an evagination of the floor of the third ventricle. It is formed by the terminals of the nerves, the cell bodies of which are present in the supraoptic and paraventricular nuclei of hypothalamus.

1. The cell bodies of these neurons are larger than the cell bodies of other hypothalamic neurons. Therefore, they are called magnocellular neurons.

2. The axons arise from these cell bodies and descend down to terminate on the capillary bed in the posterior pituitary. They form the hypothalamo-hypophyseal tract (Fig. 54.4).

3. Because of its neural origin and connections, posterior pituitary is known as neurohypophysis.

4. Most of the fibers originating from supraoptic nucleus terminate in the posterior pituitary whereas some of the fibers originating from the paraventricular nucleus in addition to their termination on posterior pituitary also terminate in the median eminence.
CHAPTER SUMMARY

**Key Concepts**

1. Hypothalamus is closely connected with limbic structures and secretes neurohormones.
2. It controls all pituitary secretions. Posterior pituitary hormones are formed in hypothalamus.
3. For its limbic connections, and control of pituitary functions, hypothalamus mediates body’s responses to emotion, stress and environmental changes.

**Important to Know (Must Read)**

1. **Long Questions** may not be asked from this chapter.
2. In examination, ‘Hypothalamic hormones, Hypothalamo‑hypophyseal tract, Hypothalamo‑pituitary axis’, are asked as **Short Questions**.
3. In **Viva**, examiner may ask... name of hormones secreted from hypothalamus, the hormones of anterior pituitary stimulated or inhibited by each hypothalamic hormone, Source and functions of TRH, GnRH, CRH, GHRH, PRF, PIH, and Somatostatin, name and functions of other hypothalamic hormones like Neuropeptide Y, Orexins, MCH, and CART, How is hypothalamo‑pituitary axis formed, What is the main difference between connections of hypothalamus with anterior pituitary and posterior pituitary. Dysfunctions associated with each hypothalamic hormones.
Learning Objectives

On completion of study of this chapter, the student MUST be able to:
1. Appreciate the importance of anterior pituitary as an important endocrine gland and its role in regulation of body functions.
2. List the hormones secreted from anterior and name the cells that produce them.
3. Describe the regulation, mechanism of action, functions and dysfunctions of growth hormone (GH).
4. Understand the role of GH in regulation of growth and development.
5. Mention the functions and dysfunctions of other anterior pituitary hormones.

The student MAY also be able to:
1. Describe the regulation of GH secretion.
2. Explain the mechanism of action of GH.
3. Describe the details of the functions and diseases of GH.

Pituitary Gland: The Anterior Pituitary

The pituitary gland or hypophysis controls many aspects of human physiology starting from the birth (delivery of the fetus) and feeding of the baby to the growth and development of the individual to adulthood and reproduce. It controls various aspects of metabolism. It contributes to regulation of blood volume and pressure and body’s reaction to stress. Therefore, dysfunctions of pituitary gland result in various clinicopathological disorders.

Scientist contributed

Bernardo Alberto Houssay (1887–1971) was an Argentine physiologist who, in 1947, received one-half of Nobel Prize for Physiology or Medicine for his discovery of the role played by anterior pituitary in regulating the amount of blood sugar (glucose) in animals. He shared the prize with Carl Ferdinand Cori and Gerty Cori, who won for their discoveries of catalytic conversion of glycogen in carbohydrate metabolism. The animals used for pituitary related experiments are called as ‘Houssay animals’.

Functional Anatomy

Pituitary gland is situated at the base of the brain in sella turcica, a small cavity on sphenoid bone. It consists of two lobes: anterior and posterior lobes (Fig. 55.1). Though,
intermediate lobe of pituitary is present in many species, in human beings, it is rudimentary. Anterior and posterior lobes of pituitary are two separate and distinct glands.

1. The anterior pituitary is rich in various endocrine cells. Hence, anterior pituitary is called adenohypophysis.
2. The posterior pituitary contains neurons that secrete hormones. Hence, posterior pituitary is called neurohypophysis.

Development
Embryologically, anterior and posterior pituitaries develop from different sources:

1. Anterior pituitary develops as an evagination from Rathke’s pouch and neurohypophysis develops as part of developing hypothalamus that later merges with Rathke’s pouch.
2. Later, endocrine tissue of anterior lobe becomes purely glandular (adenohypophysis) and posterior lobe becomes neuroendocrine (neurohypophysis).

Blood Supply
Blood supply to pituitary is achieved by means of superior and inferior hypophyseal arteries:

1. The superior hypophyseal artery terminates in rich capillary network in the median eminence, from where long hypophyseal portal vessel arises and descends down the pituitary stalk to end in capillaries in the anterior lobe.
2. As the major hypophyseal vessels start and end with capillaries, the arrangement is called hypophyseal portal circulation.
3. The inferior hypophyseal artery supplies blood to the posterior lobe from where short hypophyseal portal vessels arise and terminate locally (Fig. 55.2).

Hormone Secretion
The anterior pituitary has extensive network of sinusoidal capillaries. The endothelium of capillaries is fenestrated:

1. The endocrine cells contain numerous granules that store hormones.
2. Hormones are secreted by exocytosis of these granules, following which they immediately enter circulation through the capillaries.

**ANTHORIAL PITUTARY**

**Hormones of Anterior Pituitary**

Six important hormones secreted from anterior pituitary are:

1. Growth hormone (GH) or somatotropin
2. Thyroid-stimulating hormone (TSH) or thyrotropin
3. Adrenocorticotropic hormone (ACTH) or corticotropin
4. Follicle-stimulating hormone (FSH) or gonadotropin
5. Luteinizing hormone (LH) or gonadotropin
6. Prolactin

Other hormones are: β-lipotropin (β-LPH), α-melanocyte-stimulating hormone (α-MSH) and interleukins.

**Structure**
Anterior pituitary hormones are polypeptides. LH, FSH, and TSH are glycoproteins containing two subunits: the α and β subunits, similar to that of hCG secreted from placenta. The α subunit of these hormones have similar amino acid pattern. Therefore, the β subunit confers specificity to the hormones.

Control of Secretion and Major Effects
Secretion of anterior pituitary is controlled by hypothalamic hormones (Fig. 55.3). Anterior pituitary hormones in turn control secretion of major endocrine glands of the body except pancreas. They also control growth and development and all aspects of metabolism (Flowchart 55.1). Therefore, diseases of anterior pituitary manifest with widespread and extensive abnormalities.

**Cell Types of Anterior Pituitary**
The cell types of anterior pituitary are divided into two broad categories: chromophobes and chromophils.

**Chromophil Cells**
The chromophil cells are subdivided into acidophils (α cells) and basophils (β cells):

1. The acidophils are the cells that stain with acidic dyes, and basophils are the cells that stain with basic dyes (Fig. 55.4).
2. There are five types of chromophil cells (Table 55.1):
Chapter 55: Pituitary Gland: The Anterior Pituitary

**Folliculostellate Cells**
Recently, **folliculostellate cells** have been identified in the anterior pituitary that secrete cytokines, especially IL-6.

**GROWTH HORMONE**
Growth hormone (GH) is secreted from **somatotrophs** that constitute about 50% of the total endocrine cells of the anterior pituitary. As GH promotes postnatal somatic growth, it is also known as **somatotropin**. It does not stimulate fetal growth. It maintains normal lean body and bone mass in adults. It has many metabolic effects.

**Structure**
Human GH is a polypeptide containing 191 amino acids. It has structural resemblance with human prolactin and placental lactogen.

**Flowchart 55.1:** Hypothalamic-pituitary-target organ axis. Plus sign (+) indicates stimulation and minus sign (−) indicates inhibition.
### Types, Secretion and Metabolism of GH

There are **two forms** of GH: **22 K GH** (molecular weight of 22000) that constitutes 90%, **20 K GH** (molecular weight of 20000) that constitutes 10% of the total circulating GH.

#### Synthesis of GH

GH is synthesized as a **larger prohormone** in rough endoplasmic reticulum. The prohormone consists of N-terminal signal peptide and 191 amino acid-peptide hormone. Subsequently, the **signal peptide** is removed when the prohormone traverses through the Golgi apparatus. Finally, the hormone is packaged and stored in the granules of somatotrophs.

#### Regulation of GH Synthesis

Two hypothalamic hormones control the synthesis of GH: GHRH and somatostatin. GHRH stimulates and somatostatin inhibits synthesis of GH. GHRH promotes GH synthesis by stimulating expression of **GH gene in the somatotrophs**. **TSH also stimulates** expression of GH gene. Therefore, patient with thyroid hormone gene deficiency also develops GH deficiency.

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### Regulation of GH Secretion

The normal basal plasma GH concentration ranges from 0–5 ng/mL in adults. The rate of GH secretion primarily depends on the balance between the action of GHRH and somatostatin on somatotrophs of anterior pituitary:

1. GHRH stimulates and somatostatin inhibits GH secretion. GH in turn controls its own secretion by **feedback mechanisms** (Fig. 55.5).
2. GH has a **negative feedback effect** on GHRH and a positive feedback effect on somatostatin secretion. GH stimulates production of **insulin like growth factor I (IGF-1)** or somatomedin C, which in turn has a negative feedback effect on GH secretion.
3. IGF I has effects both at pituitary and hypothalamus levels. Various factors regulate GH secretion (Table 55.2) by influencing secretion of GHRH, somatostatin and IGF I.

Recently, a new growth hormone releasing factor (GHRF) secreted from hypothalamus has been identified, which profoundly modulates GH secretion. Ghrelin is proposed to be the new GHRF.

**GH secretion throughout life**: Exercise, stress, high protein meal and fasting facilitate GH secretion:

1. GH secretion **increases during infancy** and then the secretion is maintained throughout childhood.
2. GH secretion **increases enormously at puberty** (Fig. 55.6), which is associated with a sudden increase in height and general growth of the body. The final height of the individual is mainly determined by their inherent GH secreting capacity.
3. After puberty, GH secretion decreases to adult level and maintains till senescence. GH secretion is less in old age.

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| Table 55.1: Hormone-secreting cells of the human anterior pituitary gland. |
|---|---|---|---|
| Cell type | % of total cells | Staining property | Hormone secreted |
| Somatotrophs | 40–50 | Acidophilic | Growth hormone |
| Lactotrophs | 10–25 | Acidophilic | Prolactin |
| Corticotrophs | 10–20 | Basophilic | ACTH |
| Thyrotrophs | 3–5 | Basophilic | TSH |
| Gonadotrophs | 10–15 | Basophilic | LH and FSH |

**Fig. 55.5**: Feedback regulation of GH secretion. Plus sign (+) indicates stimulation and minus sign (−) indicates inhibition.

<table>
<thead>
<tr>
<th>Table 55.2: Factors affecting GH secretion.</th>
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<tr>
<td>A. Factors that increase secretion</td>
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<td>1. Hypoglycemia, as occurs in exercise and fasting</td>
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<tr>
<td>2. Hyperaminoacidemia, as occurs after a high protein meal.</td>
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<td>3. Hormones: Estrogens, androgens, acetylcholine, serotonin and glucagon</td>
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<td>4. Stress like fever, surgery, etc., or any psychological trauma</td>
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<td>5. Puberty</td>
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<td>6. Stage IV sleep</td>
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<td>7. Drugs: L-dopa, α-receptor agonists, apomorphine</td>
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<td>8. Enkephalins</td>
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<td>B. Factors that decrease secretion</td>
</tr>
<tr>
<td>1. Increase in glucose and free fatty acid</td>
</tr>
<tr>
<td>2. Hormones: Cortisol, GH, medroxyprogesterone</td>
</tr>
<tr>
<td>3. REM sleep</td>
</tr>
<tr>
<td>4. Obesity</td>
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<tr>
<td>5. Pregnancy</td>
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</table>
GH secretion in sleep: It is not clearly known why GH secretion occurs in episodes or bursts throughout the day when growth occurs slowly over months and years. The pulsatile secretion becomes more prominent in the midnight, especially within first few hours of deep sleep (Fig. 55.7). Approximately, 70% of total 24 hours GH secretion occurs during slow wave sleep (stages 3 and 4) (Application Box 55.1).

**Application Box 55.1**

Adequate sleep promotes growth: In a normal sleep, maximum time is spent in slow wave sleep. As GH secretion is maximum in slow wave sleep, individuals who sleep adequate usually remain healthy and those who sleep less do not gain weight. Especially, children (this is the growing phase of life) are advised to sleep more to attain normal growth. Those who have sleeplessness, they do not gain weight.

**Metabolism**

GH binds with a plasma protein, which is a fragment of the extracellular domain of GH receptor. The concentration of this plasma protein (GH receptor fragment) is therefore an index of the number of GH receptors in the tissue:

1. About 50% of the GH is bound to this protein that provides a reservoir of GH in the plasma.
2. GH is metabolized rapidly by the liver.
3. The half life of GH is about 6–20 minutes.
4. Normally, in adult, less than 3 ng/mL of GH is present in the plasma in basal conditions.
5. Though, only a small quantity of GH is excreted unaltered in urine, daily urinary output of GH reflects its 24 hours profile.

**Mechanisms of Action**

Growth hormone binds with the receptor called growth hormone receptor, which is a membrane polypeptide containing 620 amino acids. This receptor neither resemble any of the G protein-linked receptor, nor is it similar to receptors with intrinsic tyrosine kinase activity. Rather, it is a tyrosine kinase associated receptor that belongs to the member of cytokine receptor superfamily:

1. It has three domains: a large extracellular domain, a transmembrane domain, and a large cytoplasmic domain.
2. GH receptor has two subunits and there is binding site on each subunit.
3. When GH binds to one receptor subunit, the binding site on other subunit is attracted and subunits come close to each other. This produces a homodimer of GH receptor.
4. This dimerization is an essential component of receptor activation as it initiates the activation of a number of enzyme cascades including JAK-STAT pathway of hormone action (Fig. 55.8).
5. The hormone binding with receptor activates following four signaling pathways:

   a. JAK-STAT pathway: JAK-2 is the member of the family of cytoplasmic tyrosine kinases, known as Janus kinase. It is associated with but not an integral part of GH receptor. STAT is the signal transducers and activators of transcription, which belongs to a family of inactive cytoplasmic transcription factor:
      • Binding of GH with GH receptor activates cytoplasmic domain of it, which induces JAK kinase.
      • STAT on phosphorylation by JAK kinases migrate to the nucleus.
      • This results in induction of various genes that mediate hormone action.
      • Prolactin and other growth factors also act through the JAK-STAT pathway.

   b. SHC-Grb pathway: JAK 2 phosphorylates intracellular protein SHC which in turn activates Grb2 proteins. Grb2 proteins stimulate MAP kinase that induces various gene transcriptions.
c. **TK-IRS pathway**: Activation of JAK-tyrosine kinase causes **phosphorylation of IRS** (insulin receptor substrate) which in turn induces various intracellular enzymes.

d. **Phospholipase-C pathway**: HR complex also stimulates membrane enzyme **phospholipase C**, which results in production of DAG. **DAG initiates calcium influx** into the cell and probably, also initiates gene transcription.

### Physiological Actions of GH

Growth hormone is an anabolic hormone with profound **short-term metabolic effects** and **long-term growth-promoting effects**:

1. **Acute metabolic effects** of GH like lipolysis in adipose tissue, decreased glucose uptake by muscle and stimulation of gluconeogenesis by hepatocytes are due to its **direct actions** on target tissues.

2. Whereas, many **long-term effects** of GH are mediated by **somatomedins** (IGF-1 and IGF-2) produced by it. Growth promoting effects are also part of the direct effects of GH (Flowchart 55.2).

### Direct Actions of GH

**Effects on Growth**

The most striking and specific effect of GH is the *stimulation of linear growth*, which occurs due to its action on the epiphysial cartilage of long bones. GH stimulates **all aspects of metabolism of chondrocytes** (the cartilage forming cells). The major actions of GH on chondrocytes include:

1. Incorporation of **proline into collagen** and its conversion into **hydroxyproline** (Application Box 55.2).

2. Incorporation of sulfate into the proteoglycan chondroitin. The hydroxyproline and chondroitin together form the extracellular matrix of cartilage. Thus, GH **promotes cartilage matrix formation**.

3. Increased amino acid uptake and **protein synthesis**, and increased RNA and DNA synthesis in chondrocytes.

4. Increase in **size and number of chondrocytes**.

5. GH also acts directly on progenitor or stem cells such as **prechondrocytes** in growth plates of bone. It facilitates the **differentiation of prechondrocytes to chondrocytes**. All these actions result in **growth of epiphyseal cartilage** that increases the length of the bone and consequently promotes linear growth of the body. Thus, GH increases the height of the individual before closure of epiphysis.
Application Box 55.2

**Hydroxyprolinuria reflects GH activity:** Increased secretion of GH manifests as hydroxyprolinuria (excretion of hydroxyproline in the urine). Hydroxyproline is derived from collagen. As GH increases the soluble collagen synthesis, hydroxyproline is formed and excreted. Thus, hydroxyprolinuria is an index of GH activity. However, hydroxyprolinuria is also associated with diseases that cause collagen destruction.

**Other growth promoting effects of GH include:**

1. **On bone:** GH stimulates the activity of bone modeling units. It increases activity of both osteoclasts and osteoblasts. However, osteoblastic activity predominates, and therefore bone formation becomes more. The total bone mass and mineral content of bone increase.

2. **On skeletal muscle:** GH increases the growth of skeletal muscle by increasing protein synthesis and causing hypertrophy of skeletal muscle cell. It also promotes activity of satellite cells of skeletal muscle. Satellite cells are progenitor cells in skeletal muscles.

3. **On visceral organs:** GH also stimulates growth of many visceral organs like liver, kidney, heart, pancreas, intestine, etc., many endocrine organs, skin, and connective tissue. All these organs increase in size and function due to hypertrophy and hyperplasia in response to GH. Organs grow in size due to:
   - increased protein synthesis,
   - increased RNA and DNA synthesis and
   - increase in cell size and number.
   The general visceral functions like digestion and specific visceral functions like cardiac output and GFR improve.

4. **Pubertal and gonadal growth:** GH increases height during puberty. It also sensitizes gonads to the actions of LH and FSH. Thus, GH promotes prepubertal sexual maturation.

**Effects on Protein Metabolism**

The effects of GH on general somatic growth are primarily due to the protein anabolic effects. These include:

1. Positive nitrogen and phosphorus balance.
2. Increased amino acid entry into the cells in many tissues.
3. Increased RNA and DNA synthesis in the cell.
4. Increased protein synthesis.
5. Decreased plasma level of amino acid and urea that occur secondary to amino acid entry into the cells and increased protein synthesis.

**Effects on Carbohydrate Metabolism**

Growth hormone is a prodiabetogenic hormone as it causes hyperglycemia by following actions:

1. It increases release of glucose from liver by facilitating hepatic neoglucogenesis.
2. It decreases glucose uptake by skeletal muscle.
3. Decreases insulin sensitivity.

However, GH stimulates insulin secretion indirectly by increasing the ability of pancreas to various insulinogenic stimuli. IGF-I, secreted mainly from liver under the influence of GH has insulin like activity. Thus, indirectly GH also promotes growth, as insulin is a growth stimulating hormone.

**Effects on Fat Metabolism**

Growth hormone causes lipolysis and increases plasma level of FFA. By increasing FFA, it promotes ketogenesis. As it increases FFA and ketoacids in plasma, GH provides energy in various conditions of hypoglycemia and stress.

**Effects on Electrolyte and Water Metabolism**

Growth hormone influences concentration of various electrolytes by following mechanisms:
GH increases plasma Ca\(^{++}\) by increasing its absorption from GI tract. GH also potentiates the effect of vitamin D on the intestine.

2. GH causes Na\(^{+}\) retention. It decreases Na\(^{+}\) and K\(^{+}\) excretion in urine.

3. It maintains ECF volume indirectly by stimulating renin-angiotensin-aldosterone system, and suppressing the action of ANP (atrial natriuretic peptide) on kidney. Thus, GH decreases Na\(^{+}\) and water excretion.

4. It increases plasma phosphate level by increasing its reabsorption from proximal tubules of kidney.

**Effects Mediated through IGFs**

The effects of growth hormone on growth, cartilage, and protein metabolism depend partly on the production of insulin-like growth factors (IGFs) (see Flowchart 55.2). Initially, they were called somatomedins, as they were found to mediate the somatic effects of GH:

1. The principal circulating somatomedins are insulin-like growth factors-I (IGF-I, which is also called as somatomedin C) and insulin like growth factors-II (IGF-II).

2. IGF-I and II have similar actions as insulin and therefore, are called insulin like growth factors.

3. IGFs are polypeptide growth factors synthesized mainly in the liver.

4. They are also formed in cartilages and other tissues.

**Applied Physiology**

**IGF-I**

IGF-I is more potent in mediating the effects of GH than IGF-II. It is a polypeptide containing 70 amino acids:

1. **IGF-I receptor** is very similar to insulin receptor.

2. IGF-I is secreted independent of GH before birth. However, after birth, IGF-I secretion and actions are dependent on GH. Its plasma concentration is 10–700 ng/mL, which peaks at puberty. The concentration of IGF-I rises in childhood, attains peak at puberty and declines in old age.

3. It binds with plasma protein called insulin-growth factor binding proteins (IGFBP). There are six IGFBPs. **IGFBP-3 is the most prevalent** IGFBP, which serves as reservoir for IGFs in plasma.

4. IGF-I mainly causes skeletal and cartilage growth.

**IGF-II**

IGF-II is independent of GH action and plays an important role in the fetal growth. It is a polypeptide containing 67 amino acids:

1. IGF-II receptor is a mannose-6-phosphate receptor.

2. In adults, IGF-II genes are expressed only in the choroid plexus and meninges.

3. The plasma concentration of IGF II is 300–800 ng/mL.

**Hypersecretion of GH**

The hypersecretion of GH in adults (after fusion of epiphyses) results in acromegaly and in children (before fusion of epiphyses) results in gigantism.

**Acromegaly**

Acromegaly means enlargement of acral (peripheral) parts of the body. ‘Acromegaly’ is derived from the Greek words, ‘akron’ means top, and ‘megas’ means large). Hypersecretion of GH after the fusion of epiphysis results in acromegaly.

**Etiology**

Acromegaly occurs due to tumor of the somatotrophs of anterior pituitary:

1. Usually, tumor of the somatotrophs is associated with proliferation of lactotrophs also; therefore, prolactin secretion is increased in 20–40% of patients.

2. Acromegaly can also occur due to extra-pituitary causes like increased hypothalamic secretion of GRH (in hypothalamic tumors).

**Features**

- Enlargement of acral parts of the body, especially of hands and feet, prognathism (protrusion of lower jaw), acromegalic facies (overgrowth of malar, frontal and facial bones), increased amount body hair, osteoarthritis (due to skeletal changes), glucose intolerance, hirsutism, gynecomastia and lactation, increased heel pad thickness, and visual field changes (bitemporal hemianopia) are common features (Fig. 55.9).

1. Visceromegaly increases the size and function of the viscera. Macroglossia may be seen.

2. Enlargement of acral parts and skeletal deformities occur as bones enlarge in width. Bones cannot grow in length as epiphyses are already closed.

3. The visual defects are due to the effect of the pituitary tumor that enlarges the sella turcica and compresses on optic chiasma. This also causes headache.

**Management**

Disease is diagnosed by typical clinical presentation, demonstration of pituitary tumor by CT scan or MRI and demonstration of high GH level in plasma by estimation of the hormone. Surgical removal of tumor is the best option to cure the disease.

**Gigantism**

Hypersecretion of GH before fusion of epiphysis results in gigantism.

**Causes**

Pituitary tumor (of somatotroph) during childhood resulting in increased GH secretion causes gigantism. Hypothalamic tumor causing excess GHRH secretion can also produce the disease.
Features
Increased GH secretion before closure of epiphysis increases the epiphyseal growth of long bones. Therefore, the patient becomes abnormally tall, with giant stature (Fig. 55.10). Other features are same as acromegaly except the acromegalic facies.

Management
Disease is diagnosed by typical clinical presentation, demonstration of pituitary tumor by CT scan or MRI and demonstration of high GH level in plasma by estimation of the hormone. Surgical removal of tumor at the earliest cures the disease.

Hypossecretion of GH
Deficiency of GH results in dwarfism. This may occur due to four causes:
1. Decreased GRH deficiency (hypothalamic failure)
2. Decreased GH secretion (pituitary failure)
3. Decreased IGF synthesis by the liver (as seen in African pygmies)
4. Decreased or unresponsive GH receptors (Laron dwarfism).

Pituitary Dwarfism
A pituitary dwarf (dwarfism due to GH deficiency) is characterized by short stature and correspondingly delayed bone and sexual maturation (Fig. 55.10):
1. Decreased muscle and total body mass, decreased cardiac function and decreased bone density are common.
2. Mental power relatively remains normal in a pituitary dwarf, which helps in differentiating from a thyroid dwarf in whom mental retardation is an outstanding feature.

Fig. 55.9: Features of Acromegaly. Note the presence of acromegalic facies (malar and frontal prominence) and prognathism (enlarged and protruded mandible as shown in skull X-ray), enlarged acral parts of the body (increased hand and finger size as compared with a normal hand), osteoarthritis, macroglossia, increased heel pad thickness and increased size of visceral organs (visceromegaly).

Courtesy: Figure 10.1, page 757, Manual of Practical Medicine, by R Alagappan, 5th edition, 2014; Jaypee Brothers Medical Publishers (P) Ltd.
3. It is diagnosed by low level of GH or IGF-I in plasma.
4. Pituitary dwarfism is treated by administration of GH preparations (Clinical Box 55.1). Details of dwarfisms are discussed in the chapter “Physiology of Growth and Development” in Section 13.

Clinical Box 55.1
Recombinant human GH: Due to difference in structure, there is wide species variation in GH. Therefore, GH extracted from animals does not work in GH deficient patients. The amount of GH collected from human pituitaries at the time of autopsy is not adequate for treatment of GH deficient dwarfs. Presently, large quantity of human GH is produced by recombinant DNA technology that meets the need of the GH deficient children.

OTHER HORMONES

Prolactin
Prolactin is the hormone for milk synthesis. The term ‘prolactin’ refers to a hormone that favors lactogenesis (‘pro’ favoring, and ‘lactin’ lactogenesis). It also influences development of the mammary gland, reproductive functions and immune responses.

Source
Prolactin is secreted from the lactotrophs of anterior pituitary, which constitutes 10–25% of the total secreting cells of the gland. However, lactotroph population increases during pregnancy, lactation, and estrogen therapy.

Structure
This is a polypeptide hormone containing 198 amino acids with molecular weight of 23,000. It has considerable structural similarity with human GH.

Synthesis
Prolactin is synthesized like other peptide hormones, i.e. preproprolactin that forms proprolactin, which finally forms prolactin. After synthesis, the hormone is stored in the granules of the lactotrophs and on appropriate stimulation, secreted into circulation.

Regulation of Secretion
Prolactin secretion increases steadily during later part of pregnancy and attains peak at term. The increased prolactin secretion correlates with increase in plasma estrogen concentration during pregnancy:
1. In fact, estrogen causes hyperplasia of lactotrophs though it does not stimulate prolactin synthesis.
2. Estrogen also increases responsiveness of lactotrophs to other stimuli that increase prolactin synthesis and secretion.

Factors that Increase Prolactin Secretion
Prolactin releasing factor, TRH, pregnancy, estrogen therapy, nursing (breastfeeding), sleep, stress, angiotensin-II, oxytocin, dopamine antagonists, serotonin, and opioids promote prolactin secretion.

Factors that Decrease Prolactin Secretion
Dopamine and its agonists, somatostatin, prolactin and GABA inhibit prolactin secretion.

Recently, it has been found that a prolactin releasing factor is secreted from hypothalamus. However, the exact nature of the chemical is not yet known. TRH stimulates prolactin secretion. Dopamine has been identified as the prolactin inhibiting factor (PIF) secreted from hypothalamus (Clinical Box 55.2). Somatostatin also inhibits prolactin secretion. Prolactin stimulates secretion of dopamine and somatostatin, those in turn inhibit prolactin secretion. Thus, prolactin controls its own secretion by this feedback mechanism (Fig. 55.11).

Clinical Box 55.2
Dopamine is used in hyperprolactinemia: Dopamine is a potent inhibitor of prolactin secretion. Therefore dopamine is used in the treatment for conditions of hyperprolactinemia and dopamine antagonists are used in conditions of hypoprolactinemia.

Mechanism of Action
Prolactin binds with the prolactin receptors that are homologous with GH receptors in their extracellular domain. Binding of hormone with the receptor activates the cytoplasmic domain of the receptor, which in turn
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activates cytoplasmic tyrosine kinases (Fig. 55.12). Activation of JAK-STAT signal induction pathway stimulates formation of transcription factors that induces DNA and mRNA synthesis. Increased formation of mRNA promotes specific protein synthesis in the ribosomes, which in turn causes formation of casein, lipid and lactose, the ingredient of milk. Thus, milk production is stimulated.

Physiological Effects

Effects on Milk Synthesis and Secretion

The primary function of prolactin is to stimulate milk synthesis and secretion (therefore, the name pro-lactin). Therefore, prolactin secretion increases during lactation.

Effects on Breast Development

Prolactin causes hyperplasia of breast tissue before and after puberty. It also causes hyperplasia of breast tissue during pregnancy and lactation:

1. Together with estrogen, progesterone, cortisol and GH, it stimulates development (branching and proliferation of ducts) of the female mammary gland.
2. Along with estrogen and progesterone, especially during pregnancy, it increases the lobules of alveoli of the mammary gland in which milk is produced.
3. During lactation, together with insulin and cortisol, it increases milk synthesis and secretion in the mammary gland.

Effects on Reproduction

In Females

Increased prolactin concentration in plasma inhibits hypothalamic GnRH secretion. Therefore, the concentration of LH and FSH decreases during lactation:

1. This prevents ovulation and causes amenorrhea in lactating mothers (Clinical Box 55.3). This is called lactational amenorrhea.
2. Prolactin also controls some reproductive behaviors such as inhibition of libido in human beings and stimulation of maternal behavior (love, affection and protective behavior of the mother for the newborns).

In Males

In males, prolactin decreases spermatogenesis.

Clinical Box 55.3

Lactation prevents pregnancy: After delivery, pregnancy does not occur till active breast feeding continues. Lactation prevents pregnancy by maintaining high prolactin concentration that in turn inhibits GnRH secretion. Decreased GnRH inhibits ovulation. Therefore, conception does not occur till breastfeeding continues (physiological contraception during lactation). Breastfeeding has two important objectives: First, to provide best food for the baby, and second, to prevent pregnancy, so that automatically adequate spacing occurs between two children. The first one is important for the educated mothers as now-a-days breastfeeding is practiced less amongst them, the second one is essential for uneducated mothers.
Effects on Immunity

Prolactin is synthesized by immunocytes. The immunocytes number increases during pregnancy. It is believed that prolactin brings the immunologic balance required for acceptance of fetal tissue by the mother.

Effects on Liver

Prolactin increases synthesis of synlactin, an intermediary growth factor secreted from the liver. Synlactin is structurally analogous to somatomedin and functionally analogous to prolactin. Therefore, it is believed that prolactin indirectly stimulates growth.

Clinical Correlation

Amenorrhea–Galactorrhea Syndrome

Excess secretion of prolactin occurs in tumors of lactotrophs. Hyperprolactinemia causes amenorrhea and infertility. This also causes increased milk secretion in the absence of pregnancy and postpartum lactational state. Therefore, the condition is called amenorrhea-galactorrhea syndrome:

1. This is seen in hypothalamic or pituitary tumors that increase prolactin secretion.
2. The diagnosis is established by detecting high plasma prolactin level or by demonstrating tumor of lactotroph by CT scan or MRI.
3. The disease is treated by dopaminergic drugs that inhibit the secretion of prolactin. This restores fertility and libido.

Thyroid Stimulating Hormone

Structure

Thyroid stimulating hormone (TSH) is a glycoprotein hormone that controls the growth and function of thyroid gland. It mainly controls the secretion of thyroid hormones especially $T_4$ and $T_3$. It has two subunits: $\alpha$ and $\beta$. The $\alpha$ subunit is nonspecific as it is similar in structure with that of the LH and FSH because it is derived from the same gene for these hormones. It contains 96 amino acids. The $\beta$ subunit contains 110 amino acids and provides specificity to TSH. The molecular weight of TSH is 28,000.

Source

Thyroid stimulating hormone is secreted from the thyrotrophs of anterior pituitary that account for 3–5% of the cell population of the gland. These cells develop at about 13 weeks of gestation. Fetal thyroid gland starts secreting hormones in response to TSH almost during the same time in pregnancy.

Synthesis

Like other peptide hormones, TSH is synthesized as preprohormone, which is converted to prohormone. The $\alpha$ and $\beta$ subunits are synthesized separately from separate mRNA molecule:

1. Separate genes on different chromosomes code for synthesis of $\alpha$ and $\beta$ subunits.
2. The peptide chains of $\alpha$ and $\beta$ subunits undergo glycosylation in rough endoplasmic reticulum. The carbohydrate moiety, which is rich in mannose, is added to the prohormone molecule.
3. During packaging in the Golgi apparatus the carbohydrate unit undergoes modification by addition of sialic acid and sulphate to it, which allows two subunits to combine to form TSH molecule.
4. The TSH molecule is stored in the secretory granules of thyrotrophs. Thyrotrophs normally synthesize more $\alpha$ subunits than $\beta$ subunits. Therefore, secretory granules of thyrotrophs contain extra $\alpha$ subunits along with TSH, which is released into circulation along with the hormone at the time of secretion.

Regulation of Secretion

The normal plasma concentration of TSH is 0.3 to 5 µU/mL. The secretion of TSH is mainly controlled by two factors:

1. TRH, secreted from hypothalamus is the major stimulant for TSH secretion.
2. Somatostatin, released from hypothalamus inhibits TSH secretion.

The secretion of TRH is inhibited by $T_3$ and $T_4$ secreted from thyroid gland, by negative feedback mechanism (Fig. 55.13). Dopamine, another hypothalamic hormone also inhibits TSH secretion. TSH secretion exhibits some
diurnal variation with maximum secretion occurring in the night, which may be influenced by cortisol that inhibits both TRH and TSH secretion. Growth hormone also inhibits TSH secretion.

**Mechanism of Action**

TSH exerts its effects on thyroid cells by increasing concentration of intracellular cyclic AMP.

**Functions**

Thyroid stimulating hormone promotes growth of the thyroid gland and stimulates thyroid hormone synthesis and secretion. TSH acts almost in all the steps of thyroid hormone synthesis:
1. It facilitates iodide uptake and its organification in the thyroid gland. Iodide trapping is increased in few hours of TSH injections.
2. It enhances intermolecular coupling (coupling reaction), and thyroglobulin synthesis and secretion into the colloid.
3. It stimulates release of thyroid hormones and iodothyrosines from the gland.
4. It promotes endocytosis of colloid.
5. It increases blood flow to the thyroid gland.
6. It causes growth and hypertrophy of the thyroid gland.

**Applied Physiology**

Decreased secretion of TSH due to pituitary diseases results in thyroid atrophy and decreased secretion of thyroid hormones (secondary hypothyroidism or hypopituitary hypothyroidism). Chronic increase in TSH secretion results in hypertrophy of thyroid gland, called goiter.

**Adrenocorticotropic Hormone**

Adrenocorticotropic hormone (ACTH) is an important hormone that controls growth and secretion of adrenal cortex. It mainly influences the secretion of cortisol. ACTH plays vital role in physiology of stress and pathophysiology of stress disorders.

**Source and Structure**

Adrenocorticotropic hormone is secreted from corticotrophs of anterior pituitary that constitute 10–20% of its total cell population. ACTH is the smallest peptide hormone of anterior pituitary containing 39 amino acids. It has molecular weight of 4500.

**Synthesis**

Like other peptide hormones ACTH is synthesized as a preprohormone. It is synthesized in the corticotrophs as part of a larger molecule called pro-opiomelanocortin (POMC), which cleaves to form β-lipotropin, and ACTH in human beings (Fig. 55.14). In other species, POMC also forms β-endorphins. In the intermediate lobe of pituitary, POMC forms α-MSH (melanocyte stimulating hormone), especially in lower vertebrates. Intermediate lobe is rudimentary in humans. However, in humans, ACTH contains α-MSH sequence at its N-terminal, and therefore, it possesses intrinsic α-MSH activity.

**Circadian Rhythm of ACTH Secretion**

Normally, ACTH secretion occurs in irregular bursts. The pulsatile secretion of ACTH is due to several bursts of CRH (corticotropin releasing hormone) secretion in 24 hours. However, there is a prominent diurnal rhythm for ACTH secretion, in which secretion is more in the early morning, which constitutes about 75% of the total 24 hours secretion:
1. The secretion begins to rise at about 4 am and reaches a peak between 7 and 10 am and then falls slowly to basal level by noon (Fig. 55.15). Another small peak occurs in the evening between 4 and 6 pm.
2. The secretion is minimal when the person sleeps, especially in the midnight when the individual is in deep sleep. Again, ACTH secretion starts increasing at about 2 hours before awakening.
3. This diurnal variation in ACTH secretion is due to the natural sleep-wake cycle, controlled by inherent biological rhythm of suprachiasmatic nucleus of hypothalamus.
4. The pattern of secretion is reversed in individuals who sleep in the day and remain awake during night. The pattern of glucocorticoid secretion closely follows the ACTH secretion (for details, refer the chapter “Adrenal Cortex”).

**Regulation of ACTH Secretion**

Adrenocorticotropic hormone secretion from anterior pituitary is controlled by various factors. However, the
primary factor controlling the ACTH secretion is CRH secreted from hypothalamus. Many factors influence ACTH secretion by regulating CRH secretion from hypothalamus (Fig. 55.16). CRH in addition to its stimulation of ACTH secretion, also stimulates sympathetic activity, increases blood pressure and activates brainstem reticular activating system (causes arousal). Also, ADH is an important regulator of ACTH secretion.

**Factors that Increase ACTH Secretion**
Corticotropin releasing hormone, ADH, sleep-wake transition, stress (hypoglycemia, surgery, anesthesia, injury, infection, fever, etc.), anxiety, depression, α receptor agonist, β receptor antagonist, serotonin, acetylcholine, interleukins, and GI hormones.

**Factors that Inhibit ACTH Secretion**
Cortisol, ACTH, somatostatin, GABA, brain natriuretic peptide and opioids.

**ACTH and Stress**
Stress and hypothalamo-pituitary-adrenocortical axis are closely associated. Stress profoundly influences this neuro-endocrine axis. Increased ACTH secretion is an immediate response of the body to any form of stress. Therefore, stress is defined as a condition that results in increased ACTH level in plasma.
1. Due to increased ACTH secretion, glucocorticoid secretion increases rapidly. Inspite of increased cortisol secretion, ACTH secretion continues to remain high due to direct stimulation of hypothalamo-pituitary-adrenocortical axis by neurally mediated stimulation of hypothalamic paraventricular nucleus that secretes CRH.
2. In stress, higher brain centers stimulate hypothalamic nucleus to secrete CRH at a higher rate. Thus, stress induced CRH release overrides the negative feedback control of ACTH secretion.
3. If stress continues, glucocorticoid concentration remains high and mechanism of negative feedback control operates at a higher set point.

**Mechanism of Action**
ACTH acts primarily by increasing cAMP in the target cells.

**Functions**
1. ACTH stimulates synthesis and secretion of cortisol and other steroid hormones from adrenal cortex by stimulating growth of specific zone of the adrenal cortex. ACTH increases adrenocortical secretion by producing hypertrophy rather than hyperplasia of adrenal cells. The impact of ACTH is more on glucocorticoid secretion than on other steroids.
2. ACTH has intrinsic MSH activity; therefore, it stimulates growth and activity of melanocytes. This results in hyperpigmentation of the skin due to increased synthesis of melanin (Clinical Box 55.4).
3. The receptors for ACTH are also present in the GI tract and brain, where it acts as a local neurotransmitter.
4. ACTH influences immunity by controlling secretion of cytokines from lymphocytes. Cytokines also stimulate ACTH secretion.
Clinical Box 55.4

High ACTH level causes Hyperpigmentation: Patients with high ACTH level, which may be due to either Addison’s disease or ACTH secreting tumor of anterior pituitary, are hyperpigmented (appear black). In Addison’s disease, hyposecretion of cortisol increases ACTH production by negative feedback mechanism. ACTH promotes melanocyte activity as it has inherent MSH actions, and therefore causes skin hyperpigmentation.

LH and FSH

Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are called gonadotropins as they regulate growth and development of gonads, pubertal maturation and secretion of sex steroids.

Source and Structure

Gonadotropic hormones are secreted from gonadotrophs of anterior pituitary that constitute 10–15% of cell population of the gland. LH and FSH are glycoproteins. Both of them have α and β subunits.

1. The α subunit is nonspecific as it is similar to the α subunit of TSH and hCG.
2. The β subunit confers specificity to LH and FSH. The peptide chain of β subunit of FSH contains 111 amino acids and of LH contains 121 amino acids.
3. The carbohydrate moiety constitutes 15–25% of the hormones.
4. The molecular weight of LH is 28,000 and of FSH is 38,000.

Synthesis

Both LH and FSH are produced by the same gonadotrophs. There are separate genes that code the synthesis of α and β subunits of gonadotropins. The addition of carbohydrate moiety to the molecules allows variation in biological activity of LH and FSH in different conditions.

Regulation of Secretion

The regulation of LH and FSH is a complex phenomenon. The secretion of gonadotropins is pulsatile, periodic, diurnal, cyclic, and seasonal. However, secretion of both the hormones is mainly controlled by gonadotropin releasing hormone (GnRH) secreted from hypothalamus. GnRH secretion is influenced by various psychological, emotional and chemical factors. Among chemicals, important are norepinephrine that stimulates, and dopamine and endorphins that inhibit GnRH secretion.

LH and FSH secretion is regulated by feedback inhibition through gonadal hormones such as estrogen, testosterone, activin, inhibin, and follistatin (Clinical Box 55.5):

1. Testosterone in males and estrogen in females provide negative feedback signal to inhibit gonadotropin secretion from anterior pituitary (Fig. 55.17).
2. Inhibin, a hormone secreted by gonads inhibits GnRH and gonadotropin secretion.
3. Activin which is structurally similar to inhibin stimulates FSH synthesis and secretion.
4. Follistatin inhibits FSH secretion.
5. Prolactin inhibits LH and FSH secretion by inhibiting GnRH secretion from hypothalamus.

Clinical Box 55.5

Oral contraceptives: Oral contraceptives (estrogen and progesterone preparations) use negative feedback influence of gonadal hormones to interfere with LH and FSH secretion in both timing and concentrations. This prevents ovulation by interfering in balanced action of LH and FSH on ovary.

Functions

Luteinizing hormone and FSH exert their effects by increasing cyclic AMP concentration in the target cells.

CHAPTER SUMMARY

Key Concepts

1. Secretion of interior pituitary hormones is controlled primarily by hypothalamic hormones. Therefore, disorders of anterior pituitary hormones can occur in hypothalamic diseases.
2. Though visceral, metabolic and growth effects of GH are due to direct action of GH on target tissues, many of these effects are also mediated through somatomedins, that are mainly produces in liver. Therefore, liver diseases in childhood may considerably affect growth and development.

3. Secretion of ACTH is more in the early morning. Therefore, getting up early in the morning and do activities, help in keeping good health.

**Important to Know (Must Read)**

1. In examination, ‘Mechanism of action and functions growth hormone (GH)’ comes as a **Long Question**.

2. Mechanism of action of GH, Regulation of GH secretion, Insulin-like growth factors, Somatomedins, Acromegaly, Gigantism, Dwarfism, Prolactin, are usual **Short Questions** in exam.

3. In **Viva**, examiner may ask… Name the hormones secreted anterior pituitary, What is hypothalamopituitary axis, functions of GH, Direct action of GH, Actions mediated by somatomedins, Etiology, features and management of Acromegaly, Etiology, features and management of Gigantism, Types/causes of dwarfism and its treatment, Differences between pituitary dwarf and thyroid dwarf, Factors that increase and decrease GH secretion, Hormones of anterior pituitary and their target gland and major functions, Hormone secreting cell types of anterior pituitary, Factors affecting prolactin secretion, Functions of prolactin, How lactation prevents pregnancy, Amenorrhea-Galactorrhea syndrome, Functions of TSH and Functions of ACTH.
Learning Objectives

On completion of study of this chapter, the student MUST be able to:
1. Name the hormones secreted from posterior pituitary.
2. Describe the mechanism of action, physiological effects and dysfunctions of ADH and oxytocin.
3. Understand the meaning and importance of a neurohumoral reflex.
4. Understand the pathophysiological basis of diabetes insipidus.

The student MAY also be able to:
1. Describe the synthesis, metabolism and regulation of posterior pituitary hormones.
2. Explain the detailed mechanisms of milk ejection reflex, parturition reflex and SIADH.

Posterior pituitary secretes two important hormones: ADH and oxytocin. These hormones are formed in supraoptic and paraventricular nuclei of hypothalamus and released into posterior pituitary. They are called neurohormones as they are synthesized and secreted by neurons into the circulation.

**ANTIDIURETIC HORMONE**

Antidiuretic hormone (ADH) plays an important role in regulation of blood volume and pressure.

**Structure, Synthesis and Secretion**

**Structure and Source**

ADH, also known as arginine-vasopressin (AVP) or vasopressin, is an oligopeptide containing nine amino acids. It is synthesized in the magnocellular cells of supraoptic and paraventricular nuclei of hypothalamus. The synthesis of ADH largely occurs in the supraoptic nucleus.

**Important Note**

Roger CL Guillemin received Nobel Prize in Physiology and Medicine for the year 1977 for his discovery of and extensive work on neurohormones (For details, refer to Chapter 54).

**Synthesis of ADH**

The hormone is synthesized in the cell bodies of the supraoptic and paraventricular nuclei of hypothalamus (Fig. 56.1). It is synthesized as preprohormone which becomes prohormone and then forms the hormone. The genes that cause ADH synthesis are located on chromosome 20. They code for synthesis of a larger molecule that includes a distinct larger protein called as neurophysin:

1. There are two neurophysins: neurophysin I, meant for oxytocin, and neurophysin II, meant for ADH. The neurophysins are almost identical.
2. The precursor molecule for ADH is prepropressophysin having 19 amino acids that contains ADH, neurophysin II and a glycoprotein (Fig. 56.2).

**Fig. 56.1:** Hypothalamic nuclei (supraoptic and paraventricular) synthesize and secrete posterior pituitary hormones (ADH and oxytocin). Note the Herring bodies at the end of these neurons in posterior pituitary.
3. Prepropressophysin is cleaved enzymatically to release ADH, neurophysin II and glycoprotein molecules.
4. The glycoprotein and ADH are packaged in the Golgi apparatus as secretory granules along with the neurophysin II.
5. **Neurophysin serves as carrier protein** to transport the hormone from the cell body to the axon terminals. The secretory granules are called as **Herring bodies** that are transported to axon terminal by slow axoplasmic flow. The axon terminals of the neurons are located in the posterior pituitary.

**Regulation of Secretion**

ADH secretion is primarily controlled by **two important stimuli**: plasma osmolality and ECF volume.

**Plasma Osmolality**

Increased plasma osmolality above 285 mOsm/kg increases ADH secretion proportionate to the osmolality rise (Fig. 56.3):

1. The increased osmolality is sensed by **hypothalamic osmoreceptors** that increases the synthesis of ADH in supraoptic and paraventricular nuclei and increase ADH release from posterior pituitary. ADH causes water retention and brings osmolality back to normal.
2. Osmolality of plasma is a very **sensitive regulator** of ADH secretion. Even, **change in 1% osmolality** leads to appreciable increase in plasma ADH level.
3. The hypothalamic osmoreceptors are located in the anterior hypothalamus.

**Blood Volume and Pressure**

Decrease in blood pressure and blood volume stimulates ADH secretion:
1. Percentage **fall in mean arterial pressure** (MAP) causes linear increase in ADH release (Fig. 56.4).
2. **Decreased ECF volume** also stimulates ADH secretion. However, change in osmolality is a stronger stimulus than the change in volume of plasma. Hypovolemia sensitizes ADH response to hyperosmolality (Fig. 56.5).
3. Effect of hypovolemia on blood volume and blood pressure is partly mediated by activation of **renin-angiotensin system** (RAS).
4. Angiotensin II (AII) formed by stimulation of RAS acts on **subfornical organ** (SFO) and **organum vasculosum of lamina terminalis** (OVLT) that are part of thirst center to stimulate thirst and water intake. SFO, OVLT, area postrema and posterior pituitary are **circum ventricular organs** that are located outside blood-brain barrier.

**Factors Affecting ADH Secretion**

**Factors that Increase ADH Secretion**

Increased plasma osmolality, decreased ECF volume, decreased blood pressure, angiotensin II, pain, nausea and vomiting, emotion, hypoglycemia, standing, increased temperature and stress increase ADH secretion.
Stimuli that Inhibit ADH Secretion
Decreased plasma osmolality, increased ECF volume, decreased temperature, ethanol, cortisol, ANP, thyroxine and α-adrenergic agonist decrease ADH secretion.

Metabolism
ADH is rapidly inactivated in kidneys and liver. In humans, the biologic half-life of ADH is about 18 min.

Mechanism of Action
ADH acts on kidney to conserve water and on blood vessel to regulate blood pressure. There are receptors for ADH on these organs:

ADH Receptors
There are two types of ADH receptors: V₁ and V₂.

V₂ Receptors
V₂ receptors are located on the kidney tubules. ADH increases permeability of the tubular cells by acting on the V₂ receptors.
1. It acts by increasing the cyclic AMP concentration on the target cells. Cyclic AMP activates protein kinase, which causes phosphorylation of different membrane and cellular proteins.
2. This leads to transport of vesicles or endosomes by the microfilaments and microtubules from the cytosol to the luminal membrane of the epithelial cells of the tubule.
3. The endosomes contain water channels (aquaporins). Thus, water channels are incorporated into the cell membrane of epithelial cells on the luminal side of the tubules. This increases the permeability of the collecting duct (CD) and distal convoluted tube (DCT) to water (Flowchart 56.1). The water channels are called aquaporins.
4. Aquaporin 2 is responsible for water reabsorption from DCT by ADH.

Aquaporins
13 types of aquaporins have been identified till date: AQP0 to AQP12. Aquaporins 1, 2 and 3 are found in kidney, aquaporin 4 is found in brain and aquaporin 5 is present in salivary and lacrimal glands and in respiratory tract. Aquaporin 9 has been described in human leucocytes. According to recent observations, aquaporins are distributed in all tissues of the body.

V₁ Receptors
For its vasoconstriction effect, ADH acts on V₁ receptors present in the smooth muscles of blood vessels. There are two types of V₁ receptors: V₁A and V₁B. The V₁A receptors cause constriction of smooth muscles of blood vessels. ADH increases calcium concentration in the smooth muscles by activating the membrane enzyme phospholipase C that causes hydrolysis of phosphatidylinositol (increases intracellular Ca²⁺ concentration). Via its action through V₁B receptors (that are also called V₃ receptors), ADH increases CRH and ACTH secretions.
Flowchart 56.2: Restoration of fluid volume of the body by ADH in volume depletion state.

- Diarrhea, vomiting, etc.
- Loss of water
- Increased plasma osmolality
- Decreased ECF volume
- Decreased BP
- Osmoreceptors in hypothalamus
- Low pressure (or volume) receptors in atria and veins
- High pressure (arterial) receptors
- Decreased vagal activity
- ADH secreting neurons in hypothalamus
- Increased plasma ADH
- Water reabsorption from kidney
- Restoration of ECF volume
- NTS in medulla

NTS: Nucleus tractus solitarius.

Scientist contributed
The Nobel Prize in Chemistry 2003 was awarded to Peter Agre “for the discovery of water channels” called aquaporins that revolutionized the mechanism of water absorption by many cells, tissues and organs in the body, including the process in the renal tubules.

Peter Agre (Born, 1949)

Functions of ADH
1. ADH acts mainly on DCT and collecting duct of renal tubule to increase water reabsorption. It is secreted in conditions of hypovolemia, hypotension and hyperosmolality. It is an important hormone for restoration of ECF volume in conditions of depletion like acute diarrhea, vomiting, etc. (Flowchart 56.2).
2. Acting on blood vessels, ADH causes vasoconstriction. Thus, in higher concentration it increases blood pressure. However, water conservation occurs at physiological concentration whereas vasoconstriction occurs at supraphysiological concentration of ADH.
3. ADH facilitates memory by acting as a neurotransmitter in memory areas of the brain.
4. It causes contraction of smooth muscles of spermatic cord. Therefore, it facilitates ejaculation of sperm. This action is mediated via V1 receptors.
5. ADH increases CRH release and therefore increases ACTH secretion. It also directly stimulates corticotrophs to secrete ACTH. This action of ADH is mediated via V1 receptors.
6. ADH acts on area postrema to decrease cardiac output.
7. It causes glycogenolysis in liver.
8. ADH acts as a neurotransmitter in some areas of brain and spinal cord.

Applied Physiology

Diabetes Insipidus
Deficiency of ADH causes diabetes insipidus (DI), a syndrome characterized by production of abnormally large volume of dilute urine. Usually, it occurs due to hypothalamic or posterior pituitary dysfunctions:
1. The main clinical features are polyuria and polydipsia. Polyuria occurs due to ADH deficiency that promotes water diuresis. Polydipsia occurs secondary to dehydration caused by polyuria.
2. Polyuria and polydipsia are also features of diabetes mellitus. However, the main distinguishing characteristic is the difference in osmolality of the urine; in diabetes insipidus urine is dilute (pure water loss) and urine osmolality is <300 mOsm/L, whereas in diabetes mellitus the urine is hyperosmolar due to loss of glucose in urine (>1200 mOsm/L). In diabetes insipidus, the urine is tasteless (insipidus, means tasteless), whereas in diabetes mellitus urine is tasty due to presence of more glucose in it (mellitus, means sweet taste).
There are two types of DI: nephrogenic (kidneys fail to respond to ADH) and neurogenic (deficiency of ADH secretion). Neurogenic DI is also called central DI.

Nephrogenic DI
Etiology and Types
In nephrogenic DI, ADH secretion is normal, but kidney is unresponsive to it due to receptor deficiency or abnormality. It may be acquired or genetic:
1. Acquired causes are usually due to drugs such as demeclocycline, rifampicin, aminoglycoside, lithium, cisplatin and amphotericin B. Other acquired causes are ischemia resulting in acute tubular necrosis, metabolic disorders such as hypercalcemia and hypokalemia, and infiltrative diseases like neurosarcoidosis and amyloidosis.
2. Genetic disorders are X-linked recessive defect in which V2 receptor gene is deficient and autosomal defect in which aquaporin gene is deficient.

Treatment
Chlorpropamide is used for treatment of the disease as it increases the renal response to ADH.

Neurogenic DI
Etiology and Types
This occurs in diseases of CNS in which hypothalamus, hypothalamo-hypophyseal tract or posterior pituitary are...
affected. Accordingly, the disease is referred to as, central, neurohypophyseal or pituitary DI. It occurs in head injury, tumors such as craniopharyngioma and suprasellar pituitary tumors, infections such as meningitis and encephalitis, vascular lesions such as Sheehan’s syndrome and aneurysm of internal carotid artery, and congenital or genetic defects. ADH secretion is deficient in these conditions.

Treatment
Uncomplicated pituitary DI is cured completely by treatment with DDAVP, a synthetic analogue of AVP that acts selectively on V₂ receptors to increase water reabsorption from tubular fluid. Injection of vasopressin is useful. Clofibrate therapy also improves the condition.

Syndrome of Inappropriate ADH Secretion
Excessive secretion of ADH occurs in a clinical syndrome called as SIADH (syndrome of inappropriate ADH secretion). The term SIADH is used as ADH secretion is inappropriately high relative to serum osmolality.

SIADH is seen in:
1. Head injury
2. Ectopic production of ADH by some malignant tumors such as carcinoma of lungs, pancreas, ovary and bladder.
3. Neurologic diseases like multiple sclerosis, Guillain-Barré syndrome, brain abscess, meningitis, encephalitis, etc.
4. Drugs such as desmopressin, chlorpropamide, high dose of oxytocin, vincristine, phenothiazine, carbamazepine, etc.

In SIADH, not only there is dilutional hyponatremia due to increased absorption of large quantity of water from kidney, but also there is natriuresis (loss of sodium in urine) secondary to decreased aldosterone secretion activated by expansion of ECF (Application Box 56.1). If SIADH is due to brain diseases, the condition is called cerebral salt wasting, and if due to lung diseases the condition is called pulmonary salt wasting.

Application Box 56.1
Vasopressin escape: In lung cancers and other malignant tumors, ADH secretion is very high. In such conditions, the water retaining action of ADH is counteracted by a process called vasopressin escape that limits the degree of hyponatremia. This escape phenomenon occurs due to down regulation of aquaporins production in collecting duct. Thus, urine output increases despite high ADH levels in plasma, which indicates that the kidney has escaped from the effects of vasopressin (hence, the term ‘vasopressin escape’).

OXYTOCIN
Structure, Synthesis and Secretion
Source and Synthesis
Oxytocin is an oligopeptide containing nine amino acids. The hormone is synthesized in magnocellular neurons of hypothalamus. Oxytocin is mainly synthesized in the paraventricular cells of hypothalamus though synthesis also occurs in supraoptic nucleus (see Fig. 56.1):
1. After synthesis, oxytocin is secreted into the posterior pituitary where it is stored.
2. The precursor molecule is prepro-oxyphysin that contains oxytocin, neurophysin I and a glycoprotein. Otherwise, the steps of synthesis and secretion of oxytocin are similar to that of ADH, as described above.

Regulation of Secretion
Oxytocin secretion occurs in response to two important physiological stimuli:
1. Suckling at the time of breastfeeding, and
2. Cervical dilatation at the time of parturition.

Genital stimulation in females during coitus increases oxytocin release. Stressful stimuli facilitate oxytocin release. Oxytocin secretion is inhibited by alcohol.

Functions
Oxytocin mediates two physiological reflexes: 1. milk ejection reflex and 2. parturition reflex.

Milk Ejection Reflex
Discharge or expulsion of milk from the breast of the mother into mouth of the baby when baby suckles during breastfeeding, is called milk ejection reflex.

Receptors
Tactile receptors in and around the nipple.

Reflex Arc
Nipple is stimulated by suckling of the baby:
1. The afferent neural impulses are transmitted from the nipple to the spinal cord and from there via spinothalamic tract to the brainstem (Fig. 56.6).
2. In the midbrain, the information reaches paraventricular nucleus of hypothalamus via collaterals arising from the ascending sensory pathway. The neurons terminating on hypothalamus are cholinergic.
3. These fibers stimulate oxytocin secreting magnocellular neurons of hypothalamus.
4. Via hypothalamopituitary axis, oxytocin is released from posterior pituitary and enters circulation.
5. Oxytocin causes contraction of myoepithelial cells of the milk-laden alveoli of the mammary gland. Therefore, milk is forced from the alveoli into the ducts and from there to the mouth of the infant.

Effects
Due to initiation of this reflex, milk from the mother’s breast is forcefully discharged into the mouth of the baby. Therefore, the reflex is called milk ejection reflex.
Physiological Significance

This is an example of neurohumoral reflex (Application Box 56.2). Ovulation that occurs during mating in some animals like rabbit is also an example of neurohumoral reflex.

Application Box 56.2

A neurohumoral reflex: Normally, a reflex is purely a neural phenomenon, the reflex arc having neurons in both afferent and efferent limbs. When, a hormone forms part of the efferent limb, the reflex becomes a neurohumoral reflex. Thus, the neurohumoral reflex involves both neurons and hormones in the reflex pathway. Therefore, milk ejection by oxytocin is a neurohumoral reflex.

Parturition Reflex

Oxytocin causes contraction of uterine muscle:
1. Toward term, oxytocin secretion gradually increases and reaches its peak just before parturition. Also, number of oxytocin receptor increases in the uterine muscle.
2. Due to increased plasma level of oxytocin and increased sensitivity of uterus to oxytocin, uterus contracts vigorously leading to expulsion of the fetus.
3. Thus, oxytocin initiates and completes parturition. Parturition reflex is also a neurohumoral reflex.

Other functions of oxytocin are as follows:
1. Oxytocin receptors are also present in the ovary. It is believed that oxytocin facilitates degeneration of corpus luteum at the end of the menstrual cycle.
2. Oxytocin secretion increases in males during orgasm, though the exact physiological significance of it is not known. At the time of ejaculation in male, possibly oxytocin causes contraction of smooth muscles of vas deferens that helps in propelling sperms toward the urethra.
3. In females, following ejaculation of sperms into female genital tract, oxytocin causes contraction of nonpregnant uterus to facilitate sperm transport into fallopian tubes. Though sperm transport to the fallopian tube for fertilization depends on motility of sperm, uterine contraction facilitates the process.

Applied Physiology

Oxytocics (preparations of oxytocin) are used routinely for:
1. Induction of labor, if labor pain does not start effectively at term.
2. Oxytocic infusion is given to facilitate the progress of labor.
3. It is routinely injected immediately following delivery of placenta, to prevent excessive postpartum hemorrhage, in which uterus contracts severely in response to oxytocin and bleeding vessels are compressed in the contracted uterus that prevents bleeding.

CHAPTER SUMMARY

Key Concepts

1. Hormones of posterior pituitary are synthesized in hypothalamic neurons. Therefore, they are called neurohormones. Therefore, the reflexes caused by ADH and oxytocin are called neurohumoral reflex.
2. Osmolality is a better stimulus than hypotension and hypovolemia for secretion of ADH.
3. SIADH occurs in paraneoplastic syndrome.

Important to Know (Must Read)

1. In examination, 'Mechanism of action, and function of hormones of posterior pituitary/ may come as a Long Question.
2. In examination, ADH, Oxytocin, Diabetes insipidus, Neurohumoral reflex, may be asked as Short Questions.
Learning Objectives

On completion of study of this chapter, the student **MUST** be able to:

1. Appreciate the role of thyroid hormones in the integration of various body functions.
2. Name the hormones secreted from thyroid gland.
3. Briefly describe the steps of thyroid hormone synthesis.
4. Comprehend the role of TSH in regulation of thyroid hormone secretion.
5. Know the important aspects of thyroid hormone metabolism.
6. Describe the mechanism of action and physiological effects of thyroid hormones.
7. Understand physiological basis of dysfunctions in hyperthyroidism and hypothyroidism.
8. Learn the physiological basis of thyroid function tests.

The student **MAY** also be able to:

1. Describe the synthesis of thyroid hormones.
2. Explain the mechanisms of action of thyroid hormones.
3. Describe the regulation of thyroid hormone secretion.
4. Describe the thyroid function tests.

Thyroid is an important endocrine gland that primarily governs the rate at which metabolism occurs in the individual cells. Thyroid hormones profoundly influence normal growth and development of the individual. They are essential for mental and psychological development in infancy and early childhood. Though, thyroid hormones are not very essential for immediate maintenance of vital functions of life, their deficiency causes severe deficit in mental and physical growth, and extreme decrease in body metabolism. Thyroid gland also influences calcium metabolism by secreting calcitonin from its parafollicular cells.

Scientist contributed

Emil Theodor Kocher (1841–1917) was a Swiss surgeon, medical researcher and physiologist who, in 1909, received Nobel Prize in Physiology and Medicine for his outstanding contribution in physiology, pathology and surgery of the thyroid gland. His studies on goiter began the systematic work on the functions of thyroid. Among his many accomplishments are the introduction and promotion of aseptic surgery and scientific methods in surgery, specifically reducing the mortality of thyroidectomies below 1% in his operations. He was the first Swiss citizen and the first surgeon to ever receive a Nobel Prize.

FUNCTIONAL ANATOMY

General Aspects

Development

Thyroid gland develops from the floor of primitive pharynx during third week of gestation. Along with thyroglossal duct, it migrates from floor of the tongue to the neck. Therefore, ectopic thyroid gland may be located at the base of the tongue, which is called as lingual thyroid. Normally, thyroid hormone synthesis begins at about 11th week of gestation.

Location

Thyroid gland is present on the anterior aspect of the neck. It is located anterior to trachea, between the cricoid cartilage and the suprasternal notch. It consists of two lobes that are connected by a band of thyroid tissue called isthmus. Sometimes an extra thyroidal tissue arises from the isthmus, which is known as pyramidal lobe. The weight of thyroid gland in a healthy adult is between 15 and 20 g. Four tiny parathyroid glands are located posteriorly at each pole of thyroid gland (Clinical Box 57.1).
Section 6: Endocrine Physiology

Clinical Box 57.1
Surgical precautions: During thyroid surgeries, utmost care is taken not to remove parathyroid glands that are embedded in thyroid tissue; otherwise that leads to acute hypoparathyroidism (hypocalcemic tetany) following thyroidectomy. Also, recurrent laryngeal nerves traverse beneath the lateral borders of the thyroid gland on both sides. Therefore, care is also taken to prevent damage to this nerve to avoid vocal cord paralysis during thyroid surgery.

Blood Supply
Thyroid gland has rich blood supply, which is maximal among all endocrine organs. Blood flow per gram is even more than that of kidneys. The blood supply to thyroid gland is derived from superior and inferior thyroid arteries that originate from external carotid and subclavian arteries respectively (Fig. 57.1). Thyroid veins drain into external jugular and innominate veins.

Innervation
Thyroid gland is innervated by autonomic nerves. The fibers for sympathetic innervation originate from cervical ganglia and fibers for parasympathetic innervation travel in vagus nerve. Autonomic innervation regulates delivery of TSH and iodide to the gland by controlling vasomotor tone. Sympathetic innervation plays important role as it has direct influence on functions of thyroid cells.

Histology
Thyroid gland is formed by aggregates of acini or follicles (Fig. 57.2). The thyroid follicles are spherical in shape and are formed by a single layer of epithelial cells that surround a central thick solution called colloid, which is a viscus-gel like substance containing thyroglobulin in it.

SYNTHESIS, SECRETION AND METABOLISM OF THYROID HORMONES

The principal hormones synthesized and secreted by thyroid gland are:

1. $T_4$ (thyroxine)
2. $T_3$ (triiodothyronine).
3. $RT_3$ (reverse-triiodothyronine), secreted in small quantity.
4. Calcitonin

$T_4$, $T_3$, and $RT_3$ are secreted from thyroid follicles and calcitonin is secreted from the parafollicular cells (C cells) of thyroid gland.

Normally, $T_3$ is more active than $T_4$, though $T_4$ is secreted in more quantity from thyroid gland. $T_3$ is converted to $T_3$ in the peripheral blood and tissues. Therefore, $T_4$ is mainly a prohormone. $RT_3$ is physiologically inactive.

The raw materials required for thyroid hormone synthesis are the iodine and tyrosine. Therefore, iodine
deficiency or excess produces thyroid dysfunction. Before discussing thyroid hormone synthesis, it is desirable to know the basics of iodine metabolism.

**Iodine Metabolism**

Iodide uptake is the first and crucial step in the thyroid hormone synthesis.

1. About 500 µg of iodine is ingested daily in the food, which is converted to iodide and absorbed from GI tract.
2. Ingested iodine binds with albumin and unbound iodine is mainly excreted in urine.
3. However, daily ingestion of 150 µg of iodine in an adult maintains normal thyroid function. The normal plasma concentration of iodide is 0.3 µg/dL. Thyroid gland is the principal organ that takes up iodine to form thyroid hormone.
4. Normally, about 120 µg of iodide is taken up by the thyroid gland per day for thyroid hormone synthesis.
5. Kidney is the principal organ that excretes iodide in the urine.
6. The thyroid gland secretes 80 µg of iodine in the form of T₃ and T₄, and 40 µg of iodine directly into ECF per day (Fig. 57.5).
7. T₃ and T₄ are primarily metabolized in liver. Daily, liver releases about 60 µg of iodine into the ECF and 20 µg of iodine into bile, which is excreted in stool.
8. On average, 480 µg of iodine is excreted in the urine and 20 µg is excreted in the stool.

**Iodine deficiency** is prevalent in developing countries and in mountainous regions all over the world. When iodine intake is less than 50 µg per day, thyroid hormone synthesis decreases. The consequent increase in TSH results in goiter, hypothyroidism and cretinism.

**Thyroid Hormone Synthesis**

**Steps of Thyroid Hormone Synthesis**

The thyroid hormone synthesis involves following steps:

1. Iodide trapping
2. Conversion of iodide into iodine
3. Thyroglobulin synthesis
4. The coupling reaction
5. Proteolysis of thyroglobulin
6. Secretion of thyroid hormones

**Iodide Trapping**

Thyroid gland takes up iodide by active mechanisms. The active transport of iodide from circulation into the colloid of the thyroid follicles is known as iodide trapping or iodide pump.

1. The transport process is a secondary active transport. Na⁺ and I⁻ are co-transported into the thyroid cell by means of Na⁺-I⁻ symporter (NIS) or cotransporter (Clinical Box 57.2). NIS can cause 20 to 40 times intracellular iodine accumulation, compared to the concentration of iodine in plasma.
2. TSH induces expression and retention of NIS in the basolateral membrane of thyrocytes where it facilitates sustained iodine uptake.
3. The Na⁺ is pumped back into the interstitium by Na⁺-K⁺ pump (Fig. 57.6).
4. The RMP of thyroid cell is −50 mV and iodide is negatively charged. Therefore, iodide is pumped into the cells against the electrical gradient.
5. The iodide concentration inside the colloid is usually very high. Therefore, iodide is also pumped against its own chemical gradient.

6. ATP is used in iodide uptake by thyroid cells. This enables the follicular cells to accumulate more iodide than its concentration in blood.

7. The other tissues that accumulate iodide are salivary gland, placenta, choroid plexus, mammary gland, gastric mucosa, and ciliary body; however, they do not form thyroid hormones. Iodide trapping is stimulated by TSH.

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**Clinical Box 57.2**

NIS deficiency: Mutation of NIS (Na⁺-iodide symporter) gene, an autosomal recessive condition results in decreased thyroid uptake and causes congenital hypothyroidism.

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**Conversion of Iodide into Iodine**

Iodide that is actively transported into the colloids of thyroid follicles is immediately converted to iodine by means of oxidation. This oxidation of I⁻ to I, also known as organification (Clinical Box 57.3) is facilitated by the enzyme thyroid peroxidase.
Clinical Box 57.3

Pendred syndrome: An iodine transporter called pendrin is located on the apical surface of the thyroid cells that transports iodine actively from thyroid cells into the colloid. Pendrin is an anion exchanger (Cl⁻–I⁻ exchanger). Mutation of pendrin gene leads to Pendred syndrome, which is characterized by defective organification of iodine, goiter and sensorineural deafness.

Thyroglobulin Synthesis
Thyroglobulin is the glycoprotein with two subunits and the molecular weight of 660,000. It contains 123 tyrosine residues. It is synthesized in the endoplasmic reticulum of thyroid cells, packaged in Golgi apparatus and then, secreted into the colloid by exocytosis. This secretion is facilitated by thyroid peroxidase. The thyroid hormones are synthesized in the thyroglobulin molecule and remain bound with the thyroglobulin till they are secreted.

Binding of Iodine to Thyroglobulin
Once reactive iodine is formed (by oxidation of iodide to iodine), it binds immediately with the tyrosine molecule, which is attached to thyroglobulin molecule at 3 position. This binding of iodine to thyroglobulin is facilitated by thyroid peroxidase.

Coupling Reaction
Binding of iodine with tyrosine at 3 positions forms mono-iodotyrosine (MIT), which is again iodinated in the 5 position to form diiodotyrosine (DIT). Two DIT molecules undergo oxidative condensation to form thyroxine (T₄). This is called coupling reaction. There are two theories of coupling reaction: intramolecular coupling and intermolecular coupling.

Intramolecular Coupling
In this process, coupling occurs with both DIT molecules attached to thyroglobulin.

Intermolecular Coupling
In this process, DIT that forms the outer ring is detached from the thyroglobulin.

The coupling reaction is also facilitated by thyroid peroxidase. The condensation of MIT with DIT forms triiodothyronine (T₃). When condensation of DIT occurs with MIT, reverse-T₃ (RT₃) is formed. Normally, distribution of MIT, DIT, T₄, and T₂ in the thyroid cell is 23%, 33%, 35%, and 7% respectively. RT₃ is present in very minor quantity.

Secretion of Thyroid Hormones
The thyroid cells ingest colloid by endocytosis.
1. The lysosomal enzymes digest the peptide bonds between iodinated residues and thyroglobulin. This is called proteolysis of thyroglobulin molecule.
2. This results in formation of free T₄, T₃, DIT, and MIT in the cytoplasm. The iodinated tyrosines are deiodinated by the microsomal enzyme iodotyrosine deiodinase.

3. The T₄ and T₃ are then released from thyroid cells into the general circulation.
4. The iodine released by deiodination of MIT and DIT is usually reutilized by the gland for further synthesis of thyroid hormone.
5. This provides more supply of iodine for thyroid hormone synthesis in comparison to the iodine available by iodide pump.
6. MIT and DIT are not secreted by the thyroid gland.

Thus, thyroid cells serve three important functions:
1. They actively accumulate iodide from blood for synthesis of thyroid hormones.
2. They synthesize thyroglobulin molecules and secrete them into the colloid.
3. They digest the colloid and free thyroid hormones from thyroglobulin molecules to secrete them into the circulation.

Structure of T₃ and T₄
Thyroxine contains four iodine atoms, each one at 3, 5, 3’ and 5’ positions, whereas triiodothyronine contains three iodine atoms at 3, 5 and 3’ positions of the thyronine ring structures (Fig. 57.7). For the positions of iodine atoms in thyroid hormones, thyroxine and triiodothyronine are abbreviated as T₄ and T₃ respectively.

Metabolism of Thyroid Hormones

Normal Concentration
In a normal individual, total plasma T₄ concentration is about 8 µg/dL, and plasma T₃ is about 0.15 µg/dL. In circulation, most of the thyroid hormones bind with the plasma proteins. About 99.9% of T₄ and 99.8% of T₃ are bound to
plasma proteins. Therefore, the free T4 level in plasma is about 2 ng/dL, and free T3 is about 0.3 ng/dL (Application Box 57.1).

**Application Box 57.1**

**Measurements of free and bound forms:** Concentration of thyroid hormone is measured by RIA. As physiological effects of thyroid hormones depend on their free-form level in plasma, and the free hormone component influences feedback control of hypothalamo-pituitary-thyroid axis, in thyroid dysfunctions, mainly the free hormone concentrations are measured. However, it is ideal to measure both total and free level of hormones in blood for diagnosing thyroid dysfunctions.

**Protein Binding**

Thyroid hormones bind with three types of plasma proteins:
1. Thyroxine binding globulin (TBG)
2. Thyroxine binding prealbumin (TBPA), also called transthyretin.
3. Albumin.

Normally, T4 binds mainly with TBG and TBPA, and T3 binds with albumin and TBG.

**TBG**

TBG is a glycoprotein synthesized by liver. Its molecular weight is 54000. Each TBG molecule has a single binding site on it for a molecule of thyroid hormone.
1. About 70% of T4 and 45% of T3 are bound to TBG.
2. TBG level in plasma increases in pregnancy and in patients receiving estrogen, major tranquilizers, clofibrate, etc.
3. Plasma TBG level decreases in patients receiving glucocorticoids, androgens, danazol and L-asparaginase (cancer chemotherapeutic drug). Salicylates, phenytoin (anticonvulsant), mitotane (cancer chemotherapeutic drug) and 5-fluorouracil inhibit binding of T3 and T4 to TBG.

**TBPA**

TBPA, though named as prealbumin is not an albumin, rather a globulin. Recently, TBPA has been designated as transthyretin. The binding of T3 with TBPA is very less (less than 1%), whereas about 20% of T4 binds to it.

**Albumin**

T3 mainly binds with albumin. About 55% of T3 and 10% of T4 bind with albumin.

The concentration of free T3 and T4 depends on the concentration of plasma proteins that bind these hormones.
1. When, concentration of thyroid binding proteins increases in plasma, the free level of thyroid hormones decreases. However, the decreased hormone level provides feedback signals to secrete more TSH, which in turn increases secretion of thyroid hormones.

2. The opposite phenomenon occurs when level of plasma binding protein decreases in plasma.
3. Thus, initial change observed in thyroid hormones due to change in plasma proteins is a temporary effect. Therefore, thyroid activity remains normal (euthyroid state) in patients with altered binding proteins.

**Importance of protein binding:**
1. Protein-bound forms of T3 and T4 serve as reservoir of these hormones. This can replenish the circulating hormone, when due to some reasons or the other, the secretion is decreased or metabolism is increased suddenly and temporarily.
2. Protein binding protects the hormone against metabolic degradation. Therefore, the half-lives of circulating T3 and T4 are more (1 day for T3 and 7 days for T4).
3. Circulating free form concentration provides feedback effect on regulation of hormone secretion.

**Peripheral Conversion**

The major product of thyroid gland secretion is T4.
1. Normally, about 35% of secreted T4 is converted to T3 in the circulation by the enzyme 5’-deiodinase (Flowchart 57.1) present in liver and kidney, and this accounts for about 85% of circulating T3.
2. T3 mainly acts on the target cells. Therefore, physiologically T3 is considered as the most active hormone.
3. About 45% of T4 is also converted to RT3 by the enzyme 5-deiodinase, which accounts for 95% of circulating RT3. However, RT3 is physiologically inert.
4. As 5’-deiodinase is present in high concentration in pituitary and cerebral cortex, the ratio of T3/T4 is very high in these structures. Remaining about 20% is conjugated with sulphates and glucuronides.

**Effect of Diet**

Fasting decreases conversion of T4 to T3. Therefore, T3 concentration in plasma decreases in fasted individuals. However, conversion of T4 to RT3 is facilitated. Therefore, RT3 is increased and T4 remains normal in fasting. In starvation (chronic and severe fasting), RT3 returns to normal, but T3 continues to remain low. In overfeeding, opposite happens; T3 is increased and RT3 is decreased.
Section 57: Thyroid Gland

Flowchart 57.2: Metabolism of thyroxine.

Effect of Drugs

Various drugs inhibit deiodinase, and therefore, they decrease $T_3$ and increase $RT_3$ level in plasma.

Effects of Diseases

In many nonthyroidal conditions such as chronic febrile illness, trauma, burns, liver and kidney failure, myocardial infarction, selenium deficiency, advanced stage of cancers and other cachectic states, activity of deiodinases is suppressed. Therefore, level of $T_3$ decreases in these conditions. However, $T_3$ becomes normal when patient recovers.

Peripheral Degradation

Thyroid hormones are metabolized mainly by enzymes deiodinases (Flowchart 57.2).
1. There are three types of deiodinases: Type 1 ($D_1$), type 2 ($D_2$) and type 3 ($D_3$).
2. All the deiodinases contain a special amino acid selenocysteine. This rare amino acid makes the enzyme potent to catalyze oxidoreductive reactions. In fact, the selenium in the amino acid is essential for their enzyme activity.
3. $D_1$ is present in liver, kidney, thyroid and pituitary. $D_2$ is present in skeletal muscle, brain, pituitary gland, placenta and brown fat. $D_3$ is present in brain and reproductive tissues.
4. $D_1$ and $D_2$ are 5’-deiodinase that convert $T_4$ to $T_3$ and maintain the intracellular $T_3$ in their target tissues.
5. $D_3$ promotes conversion of $T_4$ to $RT_3$.
6. Especially in brain, $D_2$ maintains the supply of $T_3$ to the neurons. $T_3$ is degraded to diiodothyronines by deiodinases that are present in the liver and kidney.
7. In liver, $T_2$ and $T_3$ are metabolized by conjugation with sulphates and glucuronic acid. The conjugated forms are secreted in the bile into the intestine to reenter the enterohepatic circulation. Thus, iodine is lost minimally, about 4% in stool.
8. In many tissues, $T_4$ is also metabolized to tetradiodoacetic acid (tetrac).

Regulation of Secretion

Secretion of thyroid hormones is regulated by a feedback control mechanism. Hypothalamus secretes thyrotropin releasing hormone (TRH), which stimulates thyrotrophs of anterior pituitary to secrete thyroid stimulating hormones (TSH). TSH stimulates thyroid gland to secrete $T_3$ and $T_4$.
1. The plasma concentration of free $T_3$ and $T_4$ provides feedback signal for regulated release of TRH and TSH (refer to, Fig. 55.17; Chapter 55).
2. When thyroid hormones decrease in plasma, TSH secretion increases, and conversely, when thyroid hormones increase, TSH secretion decreases.
3. This counter regulation of TSH by thyroid hormones is partly attributed to their inhibitory effect on thyrotrrophs to directly inhibit TSH secretion and partly to their effects on hypothalamus to inhibit the secretion of TRH.
4. Thus, concentration of TSH is a better index of thyroid activity. Dopamine, somatostatin and glucocorticoid also inhibit TSH secretion from the anterior pituitary.

TSH

Structure and Secretion

TSH is a glycoprotein containing 211 amino acids. It has two subunits: $\alpha$ and $\beta$.
1. The $\alpha$-subunit of TSH is structurally similar to the $\alpha$-subunits of LH, FSH, and hCG (Application Box 57.2).
2. Therefore, the specificity of TSH is conferred by its $\beta$-subunit.
3. Normally, TSH is secreted at the rate of 110 µg/day and its plasma concentration is about 2 µU/mL.
4. Secretion of TSH is usually pulsatile. The mean secretion starts increasing at about 9 PM, reaches its peak at midnight and then decreases slowly. The secretion is less during the day.

Functions of TSH

TSH stimulates thyroid hormone synthesis and secretion by influencing almost all the steps involved in it.
1. This includes iodide trapping, iodide binding, synthesis of $T_3$ and $T_4$ (coupling reaction), secretion of thyroglobulin into the colloid and endocytosis of colloid.
2. TSH also increases blood supply to the thyroid gland.
3. TSH increases the size and number of follicles. Therefore, chronic rise in TSH causes thyroid hypertrophy or goiter (for details of TSH, refer Chapter “Anterior Pituitary”).

TSH Receptor and Other Receptors

The receptor for TSH on thyroid cells is a glycoprotein receptor with an extended glycosylated extracellular domain. It is a G protein-coupled receptor having typical seven transmembrane segments.
1. It activates adenylyl cyclase via Gs protein.
2. It also acts via phospholipase-C pathway.

Apart from having TSH receptors, thyrocytes also have receptors for IGF-1, EGF, TNF-α and γ-interferon and other growth factors.

1. IGF-1 and EGF promote growth and TNF-α and γ-interferon inhibit growth of thyroid tissue.
2. Cachexia in chronic inflammation could be due to decreased thyroid activity mediated by action of inflammatory cytokines (TNF-α and γ-interferon) on thyrocytes.

**Application Box 57.2**

Placental tumors can cause hyperthyroidism: As α-subunit of hCG is identical to the α-subunit of TSH, the hypersecretion of hCG stimulates thyroid activity. Major source of hCG is placent. Therefore, mild to moderate hyperthyroidism is observed in tumors of placental origin like choriocarcinoma.

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**PHYSIOLOGICAL EFFECTS OF THYROID HORMONES**

### Mechanism of Action

The mechanism of action of thyroid hormones is similar to that of steroid hormones. They act by binding with the intracellular receptors. With few exceptions like adult brain and gonads, receptors for thyroid hormones are present in all tissues and organs. Though the developing neurons in infants and children are highly sensitive to thyroid hormones, it is not clear why the adult neurons are not so sensitive.

The steps of mechanism of action are as follows:

1. T₃ and T₄ enter the cells of the target organs by carrier mediated (energy dependant) transport.
2. Inside the cell, most of the T₄ is converted to T₃, which then binds with the thyroid-hormone receptors (TR) present on nucleus. The thyroid receptor protein binds to thyroid-hormone response elements (TRE) in the DNA via zinc fingers.
3. Binding of T₃ with thyroid hormone receptor-TRE elements cause translation of DNA that in turn increases the transcription of mRNA (Fig. 57.8).
4. Increased mRNA causes increased intracellular protein synthesis that stimulates cellular growth and maturation, increases intracellular enzyme synthesis, increases mitochondria formation and respiratory enzyme synthesis, and increases Na⁺-K⁺ ATPase activity.
5. The increased Na⁺-K⁺ ATPase activity increases cellular oxygen consumption and increased mitochondrial activity increases general metabolism of the cell.

**Thyroid-Hormone Receptors (TR)**

There are two human TR genes: α and β. The α receptor gene is located on chromosome 17 and β receptor gene is located on chromosome 3.

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1. Each of them synthesizes two different mRNAs. Therefore, two different receptor proteins are formed: TRα₁, TRα₂, TRβ₁ and TRβ₂. TRα₁, TRβ₁ and TRβ₂ bind with T₃.
2. TRα₁ and TRβ₁ are widely distributed in tissues.
3. TRα₂ does not bind with T₃, though widely distributed and its function is not clearly known. TRβ₂ is found only in the brain.
4. There are many activators and repressors of TR, which makes thyroid hormones, exert their diverse effects in various tissues. T₃ binds more rapidly and avidly to TR. Therefore, T₃ is more potent than T₄.
5. In myocytes, especially in heart T₃ is not formed from T₄. However, T₃ directly enters myocyte to combine with nuclear receptors and promotes expression or inhibition of genes.

**Physiological Actions**

In general, thyroid hormones control basal energy metabolism, and development of CNS and normal body growth in childhood.
General Effects on Basal Metabolism

The metabolism of a cell depends on the rate of its oxygen consumption. Oxygen is essential for oxidative phosphorylation of ADP to ATP that takes place in mitochondria.

1. Thyroid hormones increase the basal rate of oxygen consumption and therefore, the basal metabolism of the tissues. This increased metabolism increases the rate of heat production. This is called calorogenic or thermogenic actions of thyroid hormones.

2. The cellular metabolism is also activated by increased Na⁺-K⁺ ATPase activity. Increased oxygen consumption by the cells parallels their increase in Na⁺-K⁺ ATPase activity and in some of the tissues, inhibition of Na⁺-K⁺ pump decreases oxygen consumption.

3. T₃ also stimulates the transcription of genes for both α and β subunits of Na⁺-K⁺ pump. Therefore, it is strongly perceived that increased oxygen consumption stimulated by thyroid hormones is mediated by Na⁺-K⁺ ATPase activity, at least partly.

Target tissues: The increased consumption of oxygen by thyroid hormone is observed in all tissues of the body, which is prominent especially in skeletal muscle, liver, heart, kidney and connective tissues. However, exceptions are anterior pituitary, adult brain, gonads (testis and ovary), uterus, lymph nodes, and spleen that show little thermogenic response. Receptors for thyroid hormones are almost absent in adult brain.

Basal metabolic rate: In the resting stage, oxygen consumption in human is about 250 mL/min. In hyperthyroid state, it increases to about 400 mL/min. Therefore, the basal metabolic rate (BMR) increases to about +80% in hyperthyroidism. Conversely, in hypothyroidism BMR decreases to as low as -40%. Increased BMR increases body temperature.

Mitochondrial mechanism: Metabolism is increased by thyroid hormones not only by increased oxygen consumption and Na⁺-K⁺ ATPase activity, but also by increase in synthesis of mitochondrial cytochromes and promote cytochrome oxidase activity.

1. Thus, thyroid hormones regulate the number of respiratory unit in each cell and their capacity to carry out oxidative phosphorylation.

2. Thyroid hormones also stimulate synthesis of uncoupling protein 1 (UCP-1) that contributes significantly to oxidation of nutrients and heat production.

3. The tissue expression of UCP-2, and UCP-3 are also increased by thyroid hormones.

Effects Secondary to Metabolic or Thermogenic Actions

Increased body metabolism increases nitrogen excretion. Therefore, increased food intake should be associated with hypermetabolic states to prevent catabolism of endogenous protein and fat. Thus, significant weight loss occurs promptly in increased thyroid activity, without adequate nutrient supplementation.

1. Increased metabolism increases body temperature.

2. This causes vasodilation that decreases peripheral resistance and consequent changes occur in hemodynamics.

3. Increased metabolism also increases the requirement for vitamins. Therefore, vitamin deficiency is common in increased thyroid activity.

Effects on Nervous System

Thyroid hormones are essential for development of the central nervous system, especially during infancy and early childhood. Development of brain occurs maximally in last six months of fetal life and first six months of postnatal life. During this period, thyroid hormones initiate and facilitate the process of differentiation and maturation of brain cells.

Thyroid hormones are required for following neural functions:

1. Growth of cerebral and cerebellar cortices, and basal ganglia.

2. Proliferation of axons and branching of dendrites.

3. Development of synaptic connections.

4. Development of neurotransmitter systems in CNS. Thyroid hormones induce formation of enzymes essential for neurotransmitter synthesis.

5. Increase in number of receptors on different brain tissues for various neurotransmitters in the brain.

6. Myelination of neurons. Thyroid hormones stimulate galactosyl sialyl transferase activity, which is essential for myelin formation.

7. Synthesis of proteins and various enzymes like succinic dehydrogenase that are required for energy generation in neurons.


9. General alertness and responsiveness to various stimuli.

10. Speed and amplitude of stretch reflexes.

11. Memory, learning and intellectual capacities.

Deficiency of thyroid hormones during infancy results in irreversible retardation in CNS development (Clinical Box 57.4). This is why thyroid deficiency in newborn should be detected early and treated promptly.

Cerebral blood flow, glucose and oxygen utilization by brain remains normal in adult hypothyroidism and hyperthyroidism. Thyroid hormones enter the brain in adults and found in gray matter of various parts of the brain. Also, T₄ is converted to T₃ by astrocytes in brain. After thyroidectomy, D₂ type 2 deiodinase activity in brain increases enormously, which is reversed in 3 to 4 hours following injection of T₃.
Clinical Box 57.4

Mental retardation in hypothyroid children: Thyroid hormones are essential for normal mentation. Therefore, mental retardation is an important feature of a thyroid deficiency in infancy and early childhood (thyroid dwarf). This differentiates it from a pituitary dwarf in whom mental activities are apparently normal. Decreased stretch reflex activities, especially decreased reaction time of Achilles tendon reflex (ankle jerk) is diagnostic in hypothyroidism.

Effects on Growth and Development

Thyroid hormones are essential for normal growth and musculoskeletal maturation.

1. Thyroid hormones promote expression of gene for growth hormone (GH) in somatotrophs of anterior pituitary.
2. They facilitate the effect of GH on tissues.
3. They stimulate linear growth of bones, and endochondral ossification and maturation of epiphyseal bone centers.
4. They enhance the activity of chondrocytes in cartilage.
5. They increase osteoid activity and bone remodeling.
6. They cause eruption and development of teeth.
7. They promote epidermal growth, and growth of nails and hairs.
8. They stimulate synthesis of structural and enzymatic proteins.

In hypothyroid children, bone growth is slowed and epiphyseal closure is delayed.

On tissues: Thyroxine causes alteration in the characteristics of mucopolysaccharides in the subcutaneous tissue. It decreases synthesis and promotes degradation of glycosaminoglycans (mucopolysaccharides).

Cardiovascular Effects

Thyroid hormones increase heart rate and myocardial contractility. Therefore, they increase the cardiac output.

1. Heart rate: Thyroid hormones increase the number of β receptors on the nodal tissues (SA and AV nodes) of the heart and also increase the sensitivity of β receptors to catecholamines. Thus, thyroid hormones increase the heart rate. Tachycardia is a common feature of hyperthyroidism (Clinical Box 57.5).
2. Myocardial contractility: Myocardial contractility increases by following mechanisms:
   i. Thyroid hormones induce myosin heavy chain (MHC) expression in the cardiac muscle. Especially, the α-MHC activity is increased, which has more actin and calcium-activated ATPase activity.
   ii. Thyroid hormones increase the expression of β receptors, G proteins and Na⁺-K⁺ ATPase in myocardiual cells.
   iii. They also increase the calcium-ATPase activity of the sarcoplasmic reticulum in cardiac muscle, which facilitates sequestration of calcium.

All these factors increase the force of contraction that in turn increase stroke volume.

MHCs in heart muscle: Normally, heart muscle contains two MHCs: α and β MHCs. The α MHC has high myosin ATPase activity, and β MHC has low myosin ATPase activity. Thyroid hormones stimulate α MHC and inhibit β MHC activities. In hyperthyroidism, expression of α MHC gene is enhanced and of β MHC gene is suppressed. However, in myocytes, thyroid hormones inhibit phospholamban, adenylyl cyclase, T₃ nuclear receptor and Na⁺-Ca⁺ exchanger, in addition to its inhibition of β MHC.

3. Systolic pressure: Increased heart rate and stroke volume result in increase in cardiac output (Flowchart S7.3). Thus, systolic blood pressure increases significantly.

4. Diastolic pressure: Via its calorigenic action, thyroid hormones increase body temperature that causes thermogenic cutaneous vasodilation and decreases peripheral resistance. Thus, diastolic pressure decreases.
Chapter 57: Thyroid Gland

5. **Pulse pressure and circulation time**: The net effect on blood pressure is increased pulse pressure. Wide pulse pressure is a prominent feature of hyperthyroidism. Increased cardiac output and vasodilation makes the circulation hyperdynamic. Therefore, circulation time is shortened.

**Clinical Box 57.5**

**Tachycardia in sleep**: Tachycardia is an important feature of hyperthyroidism. Tachycardia due to other causes (anxiety, tension, etc.) usually disappears during sleep or heart rate falls significantly. In thyroid excess, tachycardia persists even during sleep. **Sleeping tachycardia is an important diagnostic feature of thyrotoxicosis.** Sometimes atrial arrhythmias such as flutter or fibrillation are also seen in hyperthyroidism, especially in elderly people.

**Effects on Intermediary Metabolism**

Thyroid hormones by increasing oxygen consumption of tissues increase oxidation of substrates in general. They also increase the supply of substrates for metabolism by increasing their absorption from GI tract. By activating genes encoding enzymes of various metabolic pathways, thyroid hormones amplify all intermediary metabolisms.

**On Carbohydrate Metabolism**

Thyroid hormones increase absorption of glucose from intestine.
1. They increase hepatic glucose output by inducing gluconeogenesis. They also stimulate hepatic glycogenolysis. Therefore, plasma glucose level increases.
2. However, thyroid hormones increase the turnover of glucose by increasing its uptake and oxidation. Therefore, the net effect is no substantial change in plasma glucose concentration.
3. In hyperthyroidism, though plasma glucose level rises fast after a carbohydrate meal, it decreases rapidly.
4. Thus, sustained hyperglycemia or glucose intolerance is not a usual feature of hyperthyroidism. However, some patients develop glucose intolerance or even diabetes in the long run.

**On Fat Metabolism**

Thyroid hormones stimulate lipolysis.
1. Thus, thyroxine increases release of free fatty acids and glycerol from adipose tissues. Glycerol is utilized for gluconeogenesis.
2. Thyroid hormones also increase turnover of lipids, by facilitating their oxidation.
3. They stimulate synthesis of cholesterol and simultaneously promote its oxidation and biliary excretion.
4. Hepatic uptake of cholesterol is increased by increased expression of LDL receptors in liver.
5. Therefore, the net effect is significant decrease in plasma cholesterol and total lipid.

**On Protein Metabolism**

Thyroid hormones promote proteolysis in skeletal muscle, increasing the release of amino acid. They increase protein turnover by promoting protein degradation.

**On Metabolic Actions of Other Hormones**

Thyroid hormones potentiate the metabolic actions of catecholamines, glucagons, cortisol and growth hormone, especially their gluconeogenic and lipolytic effects.

**Effects on Sympathetic Nervous Systems**

Thyroid hormones increase the rate of metabolism, heat production, heart rate and neuromuscular functions; the features that are similar to the effects of sympathetic stimulation (Clinical Box 57.6).
1. They also synergize the metabolic effects of catecholamines like lipolysis, glycogenolysis, and gluconeogenesis.
2. In fact, they do not increase the release of catecholamines; rather increase the expression and sensitivity of β receptors in various tissues like heart, skeletal muscle and adipose tissues to catecholamines.
3. Recently, it has been observed that T₃ stimulates the production of thermogenin, a protein in the brown adipose tissue that partly mediates the thermogenic action of catecholamines.

**Clinical Box 57.6**

**Hypersympathetic state**: The features of hyperthyroidism mimic the features of sympathetic stimulation like increased metabolism, increased body temperature, nervousness, increased motor activity, and excessive sweating. Use of sympathetic blockers such as propranolol that blocks β receptors alleviates many features of hyperthyroidism. β blockers have some degree of inhibitory effects on conversion of T₄ to T₃; and therefore, mildly decrease plasma T₃ level.

**Effects on Respiratory System**

Thyroid hormones increase oxygen utilization of tissues. Therefore, the demand for oxygen also increases.
1. Thyroxine meets this demand by stimulating the rate of respiration, minute ventilation, and ventilatory responses to hypercapnia and hypoxia.
2. Thus, arterial PO₂ increases that increase the supply of oxygen to tissues.
3. Thyroid hormones stimulate erythropoiesis by increasing the synthesis of erythropoietin. Thus, the supply of oxygen to the tissues is also increased.

**Effects on GI Tract**

Thyroid hormones enhance the motility GI tract. Therefore, **hyperdefecation** is a feature of hyperthyroidism and constipation is a feature of hypothyroidism. Thyroid hormones also increase appetite and food intake, and reabsorption of glucose from GI tract.
**Effects on Skeletal Muscle**

Thyroid hormones increase the expression of MHC genes in the skeletal muscle. Therefore, hypothyroidism is associated with muscle cramp and weakness. However, muscle weakness also occurs in hyperthyroidism, which may partly be due to increased protein catabolism in the muscle. Myopathy occurs in chronic hyperthyroidism (thyrotoxic myopathy).

**Effects on Reproductive System**

In both males and females, thyroid hormones play an important role in the regulation of reproductive functions.

1. In women, they cause follicular maturation and ovulation. Thyroid abnormalities in women result in irregularities in menstrual cycle, such as menorrhagia (increased menstrual loss) in hypothyroidism and oligomenorrhea (decreased menstrual loss) in hyperthyroidism.

2. In males, they promote spermatogenesis. T₃ promotes differentiation of prepubertal testicular Sertoli cells.

**Effects on Kidney**

Thyroid hormones increase kidney size and promote growth of renal tubular epithelial cells. They increase renal blood flow and GFR. Tubular reabsorption of electrolytes, glucose and water is also increased by thyroid hormones. Increased water reabsorption increases blood volume.

In summary, thyroid hormones have widespread effects on metabolism, growth and development and control of many systemic functions (Table 57.1)

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### APPLIED PHYSIOLOGY

#### Hyperthyroidism

Hyperthyroidism occurs due to hypersecretion of thyroid hormones from thyroid gland or from extrathyroidal tissues, which may be broadly divided into primary and secondary varieties.

**Primary Hyperthyroidism**

When hyperthyroidism occurs due to pathology of the thyroid gland, the condition is called primary hyperthyroidism. The common causes are:

1. Adenoma of thyroid
2. Multinodular goiter
3. Metastatic carcinoma of functioning thyroid gland
4. Graves’ disease
5. Activating mutation of TSH receptors
6. Iodine excess (Jod-Basedow phenomenon)

**Secondary Hyperthyroidism**

When hyperthyroidism occurs due to the pathology outside thyroid gland, the condition is called secondary hyperthyroidism, which may be due to a tumor of the pituitary gland or of extrathyroidal tissue.

#### Pituitary Causes

Tumor of thyrotrrophs of anterior pituitary (secretes excess of TSH).

#### Extrathyroidal Causes

Chronic excess administration of thyroid hormones (iatrogenic), tumor of ectopic thyroid tissue (lingual thyroid), hCG secreting tumors such as choriocarcinoma.

#### Features of Hyperthyroidism

Features of hyperthyroidism in descending order of frequency are:

**Symptoms**

Hyperactivity and irritability, heat intolerance, fatigue, tremor, sweating, palpitation, nervousness, hyperphagia (due to increased appetite), weight loss in spite of hyperphagia, hyperdefecation (due to increased GI motility), loss of libido, and oligomenorrhea (in females).

**Signs**

Sinus tachycardia, atrial fibrillation, systolic hypertension and increased pulse pressure, fine tremor, thyroid swelling (goiter), increased body temperature (warm and moist skin), muscle weakness (proximal myopathy), protrusion of eyeball with retracted lid (exophthalmos) (Fig. 57.9), periorbital edema, and gynecomastia (in males).

**Increased BMR** to as high as +100 is an important and diagnostic laboratory finding of hyperthyroidism.

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### Table 57.1: Summary of physiologic effects of thyroid hormones.

<table>
<thead>
<tr>
<th>Target system</th>
<th>Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolism</td>
<td>Increase in BMR (↑ O₂ consumption in all tissues except testes, uterus, lymph node, spleen and anterior pituitary)</td>
</tr>
</tbody>
</table>
| CVS           | 1. Tachycardia (increased no. and sensitivity of β receptors)  
2. Increased myocardial contractility (increased MHC and myosin ATPase)  
3. Increased SBP (↑ cardiac output)  
4. Decreased DBP (↓ peripheral resistance) |
| CNS           | Brain development |
| Bone          | Skeletal development and ↑ growth |
| Muscle        | ↑ expression of MHC gene, Protein catabolism |
| Adipose tissue| ↑ Lipolysis       |
| GIT           | Stimulates GI motility, ↑ appetite, ↑ Carbohydrate absorption |
| Reproduction  | Follicular maturation and ovulation |
Diagnosis of Hyperthyroidism
Diagnosis is made by estimation of $T_3$, $T_4$, and TSH in plasma.
1. Typically, in primary hyperthyroidism, $T_3$ and $T_4$ are high and TSH is low.
2. In secondary hyperthyroidism due to pituitary cause, TSH is high along with high $T_3$ and $T_4$.

Physiological Basis of Treatment
Administration of antithyroid drugs is the mainstay of treatment of hyperthyroidism. They usually inhibit iodide trapping mechanism or binding of iodide with the tyrosine molecule.

Antithyroid drugs are thionamides, anions, high dose of iodides, nonspecific beta-blockers and radioiodines.

Thionamides
These are thioureylenes, a group of compound related to thiourea. The commonly used thionamides are propylthiouracil, carbimazole and methimazole (active metabolite of carbimazole). The mechanisms of action of thionamides are:
1. They inhibit the function of thyroid peroxidase.
2. They inhibit oxidation and organification of iodide. Thus, they inhibit iodination of monoiodotyrosine and coupling reaction.
3. They reduce the level of thyroid stimulating antibodies.
4. Propylthiouracil reduce the rate of conversion of $T_4$ to $T_3$ by inhibiting $D_2$-deiodinase activity.

Anions
These are chlorate, perchlorate, pertechnetate, periodate, b iodate, etc. They are monovalent anions that compete with iodide for transport into the thyroid via NIS (Na$^+$-I symporter). Thus, they prevent iodide trapping. Thiocyanate is also an anion that inhibits iodide transport, but is less potent.

High Dose of Iodide
Iodide is essential for thyroid hormone synthesis. However, in very high dose it prevents organic binding by itself, and therefore, prevents thyroid hormone synthesis. This inhibition is called Wolff-Chaikoff effect. High dose of iodide also inhibits the effect of TSH on the gland by decreasing cAMP production in the cell, and by inhibiting proteolysis of thyroglobulin.

Propranolol
It is a nonspecific β-blocker. By preventing the action of catecholamines on β receptors, propranolol ameliorates many of the hyperthyroid features. It decreases heart rate, cardiac output, nervousness etc. and is very helpful in the early stages of the disease.

Radioiodine
It causes progressive destruction of thyroid cells and achieves euthyroid state. The dose should be appropriately adjusted to prevent development of hypothyroidism.

However, from the above-listed drugs, most commonly used drugs are propylthiouracil, methimazole and perchlorate.

Special States of Hyperthyroidism

Thyrotoxicosis
Thyrotoxicosis is not synonymous with hyperthyroidism. It is a state of thyroid hormone excess that occurs due to severe hyperthyroidism as seen in Graves’ disease, toxic multinodular goiter and toxic adenoma. This can also occur without hyperthyroidism as seen in thyroiditis or due to excess administration of thyroid hormones (thyrotoxicosis factitia).

Graves’ Disease
Graves’ disease is a condition in which there is diffuse enlargement of thyroid gland, which is usually associated with exophthalmos.
1. This is an autoimmune disease that occurs due to development of autoantibodies against TSH receptors. The antibodies formed against the TSH receptors activate the receptors. Therefore, thyroid gland becomes hypertrophied and hyperactive.
2. There is marked stimulation of secretion of thyroid hormones. Therefore, plasma level of $T_3$ and $T_4$ is very high.
Hypothyroidism

3. **TSH concentration is less** as excess thyroid hormones inhibit TSH secretion.

4. **Exophthalmos** occurs due to swelling of extraocular muscles and proliferation of connecting tissue within the bony walls of the orbit that pushes the eyeball forward.

5. **The disease is treated by** using antithyroid drugs, by decreasing thyroid hormone synthesis, or by reducing the amount of thyroid tissue (with treatment of radioactive iodine like $^{131}$I or by subtotal thyroidectomy).

**Thyroid Storm**

A patient suffering from hyperthyroidism becomes **suddenly extremely ill** with features like high fever, profound tachycardia, restlessness, and sometimes circulatory collapse. This is called thyroid storm. It usually develops when a hyperthyroid patient **undergoes major surgery, or experiences a major trauma or illness**. This is a medical emergency that requires immediate fluid replacement and steroid therapy with antithyroid drugs.

**Hypothyroidism**

**Hypothyroidism in Adult**

Hypothyroidism in adult is usually known as **myxedema**.

**Etiology**

Hypothyroidism, according to the cause may be classified into two categories: Primary and secondary.

**Primary Hypothyroidism**

When hypothyroidism develops due to the diseases or causes that primarily affect thyroid gland, is classified under primary hypothyroidism. The common causes are:

1. Autoimmune hypothyroidism: for example, Hashimoto’s thyroiditis.
2. Iatrogenic: Excess use of radioiodine ($^{131}$I), thyroidectomy, etc.
3. Iodine deficiency
4. Drugs: Excess of iodine, excess use of antithyroid drugs, lithium, etc.
5. Congenital hypothyroidism

**Secondary Hypothyroidism**

When hypothyroidism develops primarily due to a defect outside the thyroid gland, the condition is called secondary hypothyroidism. Usually, it occurs due to a defect either in the pituitary or in the hypothalamus.

1. **Pituitary hypothyroidism**: Pituitary resection, pituitary tumors affecting thyrotrophs, and Sheehan’s syndrome (postpartum necrosis of pituitary). Hypothyroidism occurs due to decreased TSH secretion.
2. **Hypothalamic hypothyroidism**: Brain injury involving hypothalamus, tumors of hypothalamus, etc. in which secretion of TRH is less.
3. Features of hypometabolism similar to hypothyroidism could occur due to resistance of peripheral tissues to thyroid hormones, called **thyroid hormone resistance** (Clinical Box 57.7).

**Clinical Box 57.7**

**Thyroid hormone resistance**: Recently, the condition of thyroid hormone resistance has been described, in which the tissues (usually peripheral tissues and anterior pituitary) become resistant to the effects of $T_3$ and $T_4$. Features of hypothyroidism may not develop, as in this condition mutation of thyroid receptor gene occurs only for $TRb$, whereas $TRa$ remains unaffected. Also, levels of $T_3$ and $T_4$ are maintained that overcome the resistance to some extent. But, features of hypometabolism similar to hypothyroidism occur in peripheral tissues. However, TSH level of plasma remains inappropriately high, which is not suppressed by exogenous thyroxine. The **patients with resistance in pituitary** have hypermetabolism, elevated $T_3$ and $T_4$ and normal nonsuppressible TSH level. The **patients with peripheral tissue resistance** have hypometabolism despite normal levels of $T_3$ and $T_4$. These patients require large doses of thyroid hormone to maintain their BMR. It has been observed that the **attention deficit hyperactive disorder** in children is more associated with thyroid hormone resistance.

**Features of Hypothyroidism**

Following are the features in descending order of frequency:

**Symptoms**

Cold intolerance, weakness and easy-tiredness, dry-thick skin, loss of hair, poor memory and inability to concentrate, constipation (due to decreased GI motility), weight gain in spite of poor appetite, thick and husky voice (typical hoarse voice that helps physician to **diagnose the disease over phone**), yellow skin (occurs due to carotenemia, as thyroxine causes conversion of carotene to vitamin A; therefore deficiency of it causes deposition of carotene), alopecia, psychosis (myxedema madness) menorrhagia (in females), galactorrhea and infertility (Fig. 57.10).

**Signs**

Cool extremities with dry coarse skin, dry hair, bradycardia, puffy face, with edematous hands and feet (myxedema), diffuse alopecia, goiter, bradycardia, hypertension (diastolic), anemia, decreased reaction time of tendon reflexes (especially, delayed Achilles tendon reflex relaxation), carpal tunnel syndrome and periorbital edema.

**BMR decreases** to about −40, and plasma cholesterol is usually elevated.

**Diagnosis of Hypothyroidism**

In primary hypothyroidism, $T_3$ and $T_4$ levels are decreased, but TSH is increased by feedback mechanism. In hypopituitary or hypothalamic hypothyroidism, the TSH concentration is less, in addition to decreased $T_3$ and $T_4$. The TSH response to TRH is usually normal in hypothalamic hypothyroidism, whereas it is increased in hypothyroidism caused by thyroid disease.
**Treatment of Hypothyroidism**

Thyroid hormone replacement is the mainstay of treatment. \( T_4 \) is instituted at a dose (usually 10 to 15 µg/kg/day) to maintain its normal level in plasma.

**Hashimoto’s Thyroiditis**

It is a chronic form of autoimmune thyroiditis in which antibodies are formed against the thyroglobulin and thyroid peroxidase. Therefore, thyroid cells are damaged and hypothyroidism develops. Fine needle aspiration cytology (FNAC) demonstrates sheet of Hürthle cells with lymphoid infiltrate.

**Hypothyroidism in Children (Cretinism)**

Cretinism is the hypothyroidism in children. When hypothyroidism develops from or before birth, the patients are called cretins.

**Causes**

Maternal iodine deficiency during pregnancy, maldevelopment of thyroid gland during fetal life, inborn errors of thyroid hormone synthesis, antithyroid antibodies in mother that crosses placenta and enters fetal circulation, and hypopituitarism in fetal life are usual causes of cretinism.

**Features**

Patients are dwarf and mentally retarded. Typically, they have potbelly and protrusion of tongue (Fig. 57.11). Other features of hypothyroidism are also present.

**THYROID FUNCTION TESTS**

Thyroid function tests include measurement of hormones in plasma, estimation of plasma TSH, measurement of binding proteins, TRH response test, radioactive iodine uptake, thyroid scanning, detection of thyroid antibodies, and determination of cholesterol level in blood.

**Classification**

A. **Isotopic tests:**
   a. Estimation of \( T_3 \) and \( T_4 \)
   b. Estimation of free \( T_3 \) and free \( T_4 \)
   c. TRH stimulation test
   d. Thyroid scan
   e. \(^{125}\text{I} \) uptake studies, TSH stimulation and \( T_3 \) suppression tests. These are seldom done at present.

B. **Non-isotopic tests:**
   a. Demonstration of thyroid autoantibodies
   b. Imaging procedures of the neck. USS, CT and MRI
   c. Biopsy of thyroid: fine needle aspiration cytology (FNAC) or open biopsy.

**Measurement of Thyroid Hormones**

\( T_3 \) and \( T_4 \) concentration in plasma are usually measured by RIA or sometimes by ELISA test. In primary hyperthyroidism, the thyroid hormone levels are increased, but typically the TSH is reduced due to feedback inhibition. In primary
**Plasma TSH Estimation**

Measurement of plasma TSH is an important diagnostic tool for the detection of thyroid abnormalities. In *primary hypothyroidism* TSH label is high but, in secondary hypothyroidism TSH as well as T3 and T4 are low. In *primary hyperthyroidism*, TSH is low. But, in hyperthyroidism due to pituitary causes, TSH, T4 and T3 are high.

**Measurement of Binding Proteins**

Usually, T3 uptake by the resin is measured. The T3 resin uptake indicates the free binding sites on the TBG.

1. In this test, radioactive iodine labeled-T3 is added to the patient’s serum. The labeled-T3 occupies the free binding sites on TBG.
2. Then, a resin is added to the tube, which absorb the excess of labeled-T3. The amount of labeled-T3 absorbed by the resin (T3 resin uptake) is directly proportional to the amount of thyroid hormones present in the serum and is inversely proportional to the free binding sites on the TBG.
3. When the hormone level is high as in hyperthyroidism, the free binding site on TBG is less.
4. Therefore, more T3 is taken up by the resin. In hypothyroidism, due to more of free binding sites on the TBG, T3 resin uptake is low.

**TRH Response Test**

This is a useful test for diagnosing both hypo- and hyperthyroidism. Normally, administration of TRH increases TSH production and therefore, the secretion of T3 and T4. An *abnormal response* is seen in hyperthyroidism because the negative feedback effect of high T4 overrides the stimulant effect of TRH. In hypopituitarism in which pituitary cannot respond to TRH, the TRH response test is also abnormal. An *exaggerated response* is observed in primary hypothyroidism because the negative feedback effect of T4 is reduced.

**Detection of Thyroid Antibodies**

Detection of antibodies against thyroid glands is essential for diagnosing Graves’ disease and Hashimoto’s thyroiditis, as these are autoimmune dysfunctions. In these diseases, specific antibodies are detected in plasma.

In Graves’ disease thyroid stimulating immunoglobulin known as LATS (long acting thyroid stimulator) is increased in plasma. LATS *mimic TSH* by binding to TSH receptors on thyroid gland and stimulates more secretion of thyroid hormones. In this condition, the thyroid secretion is not under the feedback control as TSH is not the actual stimulator for the hormone secretion.

In Hashimoto’s thyroiditis, antibodies against thyroglobulin are detected in circulation.

**Plasma Cholesterol Estimation**

Cholesterol level in plasma is *high in hypothyroidism* and low in hyperthyroidism. This is not diagnostic because change in plasma cholesterol occurs in many diseases. However, plasma cholesterol level is a useful index for monitoring the effectiveness of therapy in thyroid diseases, especially in hypothyroidism. Moreover, hyperlipidemic complications such as coronary artery disease are common in hypothyroidism.

**Estimation of BMR**

BMR is invariably *high in hyperthyroidism* and low in *hypothyroidism*. Though BMR determination is not performed now-a-days, and it is also not diagnostic, its alteration is highly suggestive of thyroid dysfunctions.

**Thyroid Scintiscanning**

Scintiscanning offers a *visual display* of the size and shape of the thyroid gland. It is employed in following situations.

1. It provides an objective assessment of the morphology of the gland.
2. It locates ectopic foci of the thyroid tissue.
3. The functional activity of the thyroid nodules (hot or cold nodules) can be evaluated.
4. In the investigation of masses in the neck or mediastinum, scintiscanning is useful to detect their nature (e.g: retrosternal thyroid).
5. Metastasis from thyroid carcinoma can be visualized.

Originally 131I was used for visualizing the thyroid, the scan being done after 24 hours of administration. At present *technetium pertechnetate*, which has a half life of 6 hours is used for this purpose. With technetium scanning it can be done soon after the injection of the radioisotope.

Additional laboratory investigations include hematological tests and estimation of the levels of serum calcium, sodium, CPK and proteins.

**Fine-Needle Aspiration Cytology**

This biopsy of FNAC is a very *valuable preoperative procedure* done routinely. A correct diagnosis can be obtained in over 80% of cases. False negative results occur...
more frequently than false positive ones. The diagnosis accuracy of FNAC is about 95%, specificity 92% and sensitivity is around 83%.

**Ultrasound/CT/MRI**

Ultrasound scanning provides an accurate indication of the size and is useful for differentiating cystic from solid lesions. CT and MRI are useful in the evaluation of retrosternal and retrotracheal extension of the gland, compression of trachea, extent of intrathoracic extensions of thyroid malignancy and infiltration to adjacent structures. MRI gives better delineation of soft tissue involvement like infiltration of nerves.

**Achilles Tendon Reflex**

Decreased relaxation time of Achilles tendon reflex is an important clinical assessment to suspect hypothyroidism.

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**CHAPTER SUMMARY**

**Key Concepts**

1. Thyroid hormone is the key hormone for body metabolism. In hyperthyroidism there is substantial increase in BMR. Most of the features of hyperthyroidism are due to increased BMR and secondary to increased BMR. Opposite phenomena happens in hypothyroidism.

2. Thyroxine is highly essential for brain development in first year of life. Hypothyroidism in early infancy leads to severe mental retardation. Therefore, thyroxine deficiency if present in newborn, should be detected and treated immediately after birth.

3. Thyroxine profoundly increases body metabolism and HR. Therefore, heat intolerance and tachycardia even during sleep (sleeping tachycardia) are highly indicative of hyperthyroidism.

**Important to Know (Must Read)**

1. In examination, Steps of thyroid hormone synthesis, mechanism of action and physiological actions of thyroid hormone, is usually asked as a Long Question.

2. Mechanism of action of thyroxine, Synthesis and regulation of secretion of thyroxine, Actions of thyroxine on the cardiovascular system, nervous system and general effects on basal metabolism, Hyperthyroidism, Graves’ disease (Features and Physiological basis of treatment) Hypothyroidism (Etiology, features, diagnosis and treatment), Cretinism (Causes, features and treatment), and Thyroid function tests, can come as Short Questions in exam.

3. In Viva, examiner may ask… Name of hormones secreted from thyroid gland, Actions of Thyroxine, specially on the cardiovascular system, nervous system and general effects on basal metabolism, Steps of thyroid hormone synthesis, specially mechanism of iodide trapping, role of Na\(^+\)-I\(^–\) cotransporter, Pendred syndrome, Synthesis and secretion of thyroglobulin, Intramolecular and Intermolecular coupling, Proteins binding to T\(_4\) and importance of protein binding, Factors affecting peripheral conversion of T\(_4\), Regulation of thyroxine secretion, Mechanism of action of thyroid hormone, Functional anatomy of thyroid gland especially blood supply and histology of thyroid follicles, Surgical precautions during thyroid surgery, Functions of TSH, Features of Hyperthyroidism, Diagnosis of hypothyroidism, Graves’ disease, Thyrotoxicosis, Hashimoto’s thyroiditis, Mechanism of action/ Physiological basis of different antithyroid drugs like Thionamides group (Propylthiouracil, Carbimazole, Methimazole), Anions, Iodide in high dose, Radiiodine, Propranolol etc., Etiology, features, diagnosis and management of Hypothyroidism, Etiology, features and management of cretinism, and Classify thyroid function tests and explain few important tests.
ADRENAL GLAND

General Features

There are two adrenal glands, each one located at the upper pole of each kidney (Fig. 58.1). The adrenals consist of two distinct endocrine glands, one surrounding the other: the inner adrenal medulla, surrounded by the outer adrenal cortex that consists of three layers (Fig. 58.2).

1. The outer zone of adrenal gland, i.e. the adrenal cortex forms about 80–90% of the total gland, the inner zone, i.e. the adrenal medulla consists of 10–20% of the gland.
2. Developmentally and functionally, these glands are two separate endocrine organs. Adrenal cortex develops from the mesodermal tissue and secretes steroid hormones.
3. Adrenal medulla develops from neuroectodermal tissue related to sympathetic ganglia, and therefore secretes catecholamines.

Blood Supply

Weight of each adrenal gland is about 5–10 g. The gland is highly vascular and receives blood from three sources: branches of aorta, renal arteries, and phrenic arteries. The arterial blood enters the sinusoidal capillaries in the cortex and then drains into the medullary venules. This arrangement exposes the medulla to the high concentration of corticosteroids secreted from the cortex.

General Importance

The adrenal glands are essential for survival. In general, they play important role in following physiological processes of the body:
1. Homeostasis of energy stores
2. Control of fluid volume and extracellular environment of cells
3. Supply of substrates for generation of ATP in the cells
4. Regulation of intermediary metabolisms
5. Control of immunological mechanisms
6. Regulation of functions of various organ systems.
Therefore, an adrenalectomized animal unlike its normal counterpart, cannot survive prolonged fasting and stress, and dies due to hypoglycemia, inadequate ATP generation in the cells and inefficiency to maintain fluid volume and composition (circulatory collapse). However, the adrenocortical hormones are essential for survival of the being. Though, adrenomedullary hormones are not essential for immediate survival, they help prepare the individual to deal with emergencies.

**ADRENAL MEDULLA**

Unlike hormones of adrenal cortex, adrenomedullary hormones are not very essential for survival. However, they help the individual to cope with emergencies. Adrenal medulla, by secreting catecholamines assist the individual to prepare for fight or flight responses and also assist in meeting the metabolic requirements of the body in hypoglycemic emergencies in addition to their effects on other systemic functions.

**Scientist contributed**

John Jacob Abel (1857–1938), a German scientist, who later worked in USA, was pioneer in the study of endocrine secretions, especially on adrenal medulla. His outstanding works on the blood pressure-raising constituents of the adrenal medulla led to the identification and synthesis of catecholamines, and further to the development of many derivatives of catecholamines. Similarly, his studies on pituitary gland resulted in the isolation of its oxytocic, pressor and diuretic principles. He also pioneered with crystalline insulin. With Leonard G. Rowntree he introduced kidney and liver function tests.

**Cell Types**

Adrenal medulla is the inner zone of the adrenal gland, which constitutes about 20–30% of its mass. Essentially, adrenal medulla represents an enlarged and specialized sympathetic ganglion in which the postganglionic fibers are embedded and modified to become the endocrine cells. The tissue of adrenal medulla weighs about 1 g in adults.

**ADRENOMEDULLARY HORMONES**

The major hormones secreted from adrenal medulla are catecholamines:

1. Epinephrine
2. Norepinephrine
3. Dopamine.

The gland consists of clumps and strands of chromaffin cells, interspersed with venous sinuses. The cells are called chromaffin cells as they have greater affinity for chromium stains. They contain numerous granules that are present in the vesicles that store catecholamines (Fig. 58.3).

There are two types of endocrine cells in the adrenal medulla: epinephrine-secreting and norepinephrine-secreting.

1. **Epinephrine-secreting type:** In humans, 90% of cell types in adrenal medulla are epinephrine-secreting type. The granules in these cells are larger and less dense. Granules completely fill the vesicles.

2. **Norepinephrine-secreting type:** They constitute 10% of the cell mass of adrenal medulla. The granules in these cells are small and very dense. The granules do not fill the vesicles in which they are present.

The types of cells that secrete dopamine are not known, but are believed to be very less in number. Chromaffin cells receive sympathetic preganglionic cholinergic innervation and on stimulation discharge their content into venous sinuses. Small group of chromaffin cells are present near abdominal sympathetic ganglia. These are called paraganglia. They also secrete catecholamines.
In addition to secretion of catecholamines, the gland also secretes following peptides:
1. Adrenomedullin
2. Enkephalins
3. \( \beta \)-endorphin
4. Neuropeptide-Y
5. Chromogranin

Epinephrine secreting cells secrete opioid peptide. Most of the circulating metenkephalins are secreted from adrenal medulla.

The major secretion from adrenal medulla in human is epinephrine. About 85% of adrenomedullary secretion is epinephrine, 10–12% is norepinephrine and 1–3% dopamine, whereas secretion from sympathetic noradrenergic nerve ending is almost entirely norepinephrine.

**Effect of Sympathetic Stimulation**

Adrenal medulla is stimulated with the activation of sympathetic system. Therefore, sympathetic stimulation not only increases norepinephrine secretion from its nerve endings that slowly enters circulation, but also directly increases epinephrine secretion from adrenal medulla that rapidly enters circulation. Thus, sympathetic activation increases the level of both epinephrine and norepinephrine in blood.

**Synthesis, Secretion, and Metabolism of Catecholamines**

**Synthesis and Secretion**

Catecholamines are synthesized from the amino acid phenylalanine and tyrosine (Flowchart 58.1).

1. Conversion of tyrosine to Dopa is catalyzed by tyrosine hydroxylase and dopa to dopamine by dopamine decarboxylase.
2. Norepinephrine is formed by hydroxylation and decarboxylation of tyrosine and epinephrine is formed by methylation of norepinephrine by phenylethanolamine-N-methyltransferase (PNMT).
3. After synthesis, the hormones are stored in the granules of chromaffin cells before they are secreted into venous blood.

**Effects of Glucocorticoid**

The enzyme PNMT is induced by glucocorticoid at higher concentration. It may be noted that, the concentration of glucocorticoids is normally high in blood bathing adrenal medulla as blood from cortex drains into medulla before entering general circulation. Therefore, in adrenocortical deficiency, epinephrine synthesis is also reduced. Also, glucocorticoid promotes normal growth of adrenal medulla.

**Effects of 21β-hydroxylase**

During fetal life, the adrenocortical enzyme, 21β-hydroxylase stimulates development of adrenal medulla. Therefore, fetal deficiency of 21β-hydroxylase results in dysplasia of adrenal medulla, and if enzyme deficiency is not corrected promptly after birth, circulating catecholamines remain permanently low.

**Regulation of Secretion**

Catecholamine secretion from adrenal medulla increases in following conditions.
1. Exercise
2. Hypoglycemia
3. Trauma (Physical injury)
4. Anger and anxiety
5. Pain
6. Cold

The main mechanism of secretion in these conditions is sympathetic stimulation. Hypoglycemia is a strong stimulus for catecholamine release. When blood glucose is less than 60 mg%, CNS receptors monitoring plasma glucose are activated. They stimulate the neural pathways that activate sympathetic fibers to release norepinephrine and adrenal medulla to release mainly epinephrine. These catecholamines in turn increase plasma glucose level.

**Metabolism**

The normal plasma concentration of free hormone in recumbent posture is as follows:

- Norepinephrine: 300 pg/mL
- Epinephrine: 30 pg/mL
- Dopamine: 35 pg/mL

On standing, the hormone level increases by 50–100%. The circulating epinephrine is mainly derived from adrenal medulla and norepinephrine from the sympathetic nerve endings, whereas dopamine is derived equally from adrenal medulla and autonomic nerve endings.

1. About 70% norepinephrine and epinephrine, and 90% of dopamine are conjugated to sulfate. They have very short life span. Half-life is about 1–3 minutes.
2. They are metabolized primarily in the liver and kidney.

**Degradation**

The steps of degradation of epinephrine and norepinephrine are summarized in Flowcharts 58.2A and B respectively. Only 2–3% of catecholamines are excreted unchanged in urine. About 50% of secreted catecholamines appear in urine as metanephrine and normetanephrine, and 35% as 3-methoxy-4-hydroxy-mandelic acid, also called as VMA (Application Box 58.1).

The steps of degradation of dopamine are listed in Flowchart 58.3.

**24 h urinary excretion:** The normal daily urinary excretion of catecholamines and their metabolites are as follows:

- Metanephrine plus normetanephrine: 300 µg
- VMA: 400–600 µg
- MOPG: 200 µg
- Norepinephrine: 30 µg
- Epinephrine: 6 µg

**Application Box 58.1**

VMA is the index of sympathetic activity: Urinary excretion of VMA and MOPG reflect activity of sympathetic nervous system as they are mainly derived from norepinephrine. Epinephrine contributes relatively less to the production of VMA and MOPG. Therefore, the activity of adrenal medulla is assessed primarily by measurement of plasma epinephrine or free urinary epinephrine.

**Mechanism of Action**

Catecholamines act on α and β receptors. There are three types of β receptors: β₁, β₂, and β₃, and two types of α receptors: α₁ and α₂.

1. The α₁ and α₂ receptors have three subtypes each. Epinephrine and norepinephrine act on both α and β receptors.
2. However, in general, epinephrine acts more on β receptors and norepinephrine on α receptors. β₁, β₂, and α₂ are structurally similar.
Physiological Actions of Catecholamines

Catecholamines have profound effects on carbohydrate and fat metabolisms. They supply energy at the time of emergency and prepare the individual for either Fight or flight responses. They also control many autonomic and visceral functions.

Effects on Intermediary Metabolism

On Carbohydrate Metabolism

Catecholamines act on liver to increase glucose production by following mechanisms:

1. Catecholamines stimulate hepatic glycogenolysis by activating the key enzyme glycogen phosphorylase, which causes hydrolysis of stored glycogen. They also activate glycogenolysis in the skeletal muscle.

2. They stimulate gluconeogenesis from lactate and amino acids in the liver.

3. They also inhibit glycogen synthesis by inhibiting glycogen synthase enzyme complex.

4. Epinephrine inhibits insulin mediated glucose uptake by the skeletal muscle and adipose tissue.

5. Catecholamines also stimulate glucagon and inhibit insulin secretion from pancreas.

All these effects are primarily aimed to increase plasma glucose concentration.

On Fat Metabolism

Catecholamines promote lipolysis.

1. Epinephrine activates hormone sensitive lipase in the adipose tissue that causes hydrolysis of triglycerides and increases plasma FFA. FFA provides an alternative substrate for energy metabolism.

2. Catecholamines also promote β oxidation of FFA in muscle and liver to form ketone bodies.

3. Catecholamines are therefore prodiabetogenic and proketogenic hormones.

On Thermogenesis

Epinephrine is more potent than norepinephrine in most of the metabolic effects. Epinephrine increases BMR by 10–15%. It increases nonshivering thermogenesis and diet-induced thermogenesis. Therefore, epinephrine plays an important role in body adjustment mechanisms in response to cold.

During Hypoglycemia

Secretion of catecholamines increases in profound hypoglycemia, as occurs during strenuous exercise or fasting.

1. Catecholamines induce glycogenolysis and gluconeogenesis to combat hypoglycemia.

2. Lipolysis-induced release of FFA is used as alternative fuel.

3. In such situation, catecholamines also stimulate secretion of glucagon and inhibit insulin release.

Various metabolic effects of catecholamines mediated through different receptors are summarized in Table 58.2.

Fight or Flight Response

Norepinephrine and epinephrine have widespread effects on cardiovascular, respiratory and gastrointestinal systems, and intermediary metabolisms to prepare the individual to fight or flight at the time of emergency.

1. They increase cardiac output, promote blood flow to organs, increase ventilation, stimulate energy supply, relax the smooth muscle of GI tract and urinary

### Table 58.2: Various major actions of catecholamines.

<table>
<thead>
<tr>
<th>Beta receptors (E &gt; NE)</th>
<th>Alpha receptors (NE &gt; E)</th>
</tr>
</thead>
<tbody>
<tr>
<td>↑ glycogenolysis</td>
<td>↑ glucoseogenesis (α₁)</td>
</tr>
<tr>
<td>↑ gluconeogenesis (β₁)</td>
<td>↑ glycogenolysis (α₁)</td>
</tr>
<tr>
<td>↑ lipolysis (β₁β₂)</td>
<td>↓ insulin secretion (α₂)</td>
</tr>
<tr>
<td>↑ insulin secretion (β₁)</td>
<td>↓ insulin secretion (α₂)</td>
</tr>
<tr>
<td>↑ Glucagon secretion (β₁)</td>
<td>↑ Glucagon secretion (β₁)</td>
</tr>
<tr>
<td>↑ muscle K⁺ uptake (β₁)</td>
<td>↑ muscle K⁺ uptake (β₁)</td>
</tr>
<tr>
<td>↑ cardiac contractility (β₁)</td>
<td>↑ cardiac contractility (α₁)</td>
</tr>
<tr>
<td>↑ heart rate (β₁)</td>
<td>↑ heart rate (β₁)</td>
</tr>
<tr>
<td>↑ conduction velocity (β₁)</td>
<td>↑ conduction velocity (α₁)</td>
</tr>
<tr>
<td>↑ arteriolar dilation (β₁) (decreased BP)</td>
<td>↑ arteriolar constriction (α₁) (increased BP)</td>
</tr>
<tr>
<td>↑ muscle relaxation (β₁) (GI, urinary and bronchial)</td>
<td>↑ pupillary dilation (α₁)</td>
</tr>
</tbody>
</table>

### Table 58.1: Mechanism of action at various catecholaminergic receptors.

<table>
<thead>
<tr>
<th>Receptors</th>
<th>G proteins</th>
<th>Enzyme</th>
<th>2nd Messengers</th>
</tr>
</thead>
<tbody>
<tr>
<td>α₁</td>
<td>Goₐq</td>
<td>PLC</td>
<td>↑ IP₃, DAG, Ca²⁺</td>
</tr>
<tr>
<td>α₂</td>
<td>Goₐq</td>
<td>AC</td>
<td>↓ cAMP</td>
</tr>
<tr>
<td>β₁</td>
<td>Goₐq</td>
<td>AC</td>
<td>↑ cAMP</td>
</tr>
<tr>
<td>β₂</td>
<td>Goₐq</td>
<td>AC</td>
<td>↑ cAMP</td>
</tr>
<tr>
<td>β₃</td>
<td>Goₐq</td>
<td>AC</td>
<td>↑ cAMP</td>
</tr>
</tbody>
</table>

(PLC: Phospholipase C; AC: Adenylate cyclase).
system, and cause piloerection; all these effects are intended to either Fight with the enemy to finish or escape fast from the enemy to protect.

2. This is called Fight or flight response, which is exclusively mediated by sympathetic stimulation and catecholamines released from adrenal medulla (for details, refer Chapter “Sympathetic System”).

**Effects on Cardiovascular System**

Both epinephrine and norepinephrine increase force and rate of contraction of the heart that are mediated by $\beta_1$ receptors. Catecholamines also increase cardiac excitability and conductivity.

**Effects of Epinephrine**

1. Epinephrine increases heart rate and force of myocardial contraction, and therefore, causes adequate increase in cardiac output. Due to increased cardiac output systolic pressure increases.

2. It causes selective arteriolar constriction in renal, splanchnic and cutaneous vascular bed. However, epinephrine produces vasodilation in the skeletal and hepatic circulation via $\beta_2$ receptors. The vasodilation effect of epinephrine overrides the vasoconstriction effect and therefore, total peripheral resistance falls. Consequently, the diastolic pressure decreases mildly.

3. Thus, pulse pressure widens. This occurs especially during sympathetic stimulation that takes place during exercise.

4. The primary aim of all these changes is to divert blood from splanchnic and cutaneous circulation to the exercising (active) muscles, while maintaining the cerebral and coronary blood flow.

5. This also occurs during stress. These changes ensure delivery of substrate for energy production to the vital organs during Fight or flight situations (Application Box 58.2).

**Effects of Norepinephrine**

1. Norepinephrine produces vasoconstriction in most of the organs via $\alpha_1$ receptors that increases peripheral resistance and therefore, diastolic blood pressure rises.

2. Norepinephrine also produces some degree of tachycardia and increases myocardial contractility; therefore, systolic blood pressure also increases.

3. However, hypertension produced by norepinephrine stimulates baroreceptors in the carotid sinus and aortic arch (activates baroreceptor reflex) that causes reflex bradycardia, and overrides cardioacceleratory effects.

4. Therefore, the net effect is decrease in heart rate and cardiac output inspite of increased peripheral resistance (Fig. 58.4).

**Application Box 58.2**

Chronic catecholamine secretion is not good: In shock or circulatory failure, sympathetic stimulation that causes catecholamines secretion benefits the individual. However, if secretion of catecholamine is prolonged, then the effect on the body is deleterious, because renal vasoconstriction decreases kidney blood flow and compromises kidney function. Also, decreased splanchnic circulation impairs visceral functions. Chronic sympathetic stimulation may even result in intestinal paresis or hepatic failure.

**Effects on Other Systems**

**GI System**

Catecholamines inhibit gastric secretion and motility.

**Respiratory System**

Catecholamines cause bronchial dilation (prevent expiratory airway obstruction) so that gas exchange improves. They also stimulate respiration.
On Eye
They cause pupillary dilation; therefore vision for distant objects improves. This helps to see the surroundings clearly that helps the endangered individual.

On Endocrine Glands
Catecholamines stimulate ADH release through β receptors. They also increase renin secretion from the JG apparatus of kidney. Catecholamines increase thyroid hormone secretion and promote peripheral conversion of T₄ to T₃ which is mediated by β₂ receptors.

On Kidney
Catecholamines increases Na⁺ reabsorption from kidney. Catecholamines also redistribute renal blood flow. They increase renin formation that causes angiotensin and aldosterone synthesis and secretion, which in turn increases Na⁺ and water retention.

On Electrolyte Balance
Catecholamines stimulate the entry of K⁺ into the muscle cell. Therefore, they decrease plasma K⁺ (prevent hyperkalemia). Catecholamines also increase plasma Na⁺ concentration.

Effects of Dopamine
1. It produces renal and mesenteric vasodilation.
2. It causes vasoconstriction in other parts of the body.
3. It has positive inotropic effects (via β₁ receptors), increases cardiac output.
4. It increases systolic pressure, whereas diastolic pressure does not change significantly.
5. It causes natriuresis, by inhibiting Na⁺-K⁺ ATPase in proximal tubule.
   As dopamine increases systolic pressure and at the same time maintains kidney functions, it is very useful for the treatment of traumatic and cardiogenic shocks.

Applied Physiology

Clinical Uses of Catecholamines
Agonists and antagonists of catecholamines are widely used in clinical practice.
1. The agonists are used as nasal decongestant, appetite inhibitor, and for stimulation of general body functions.
2. The antagonists (both α and β receptor antagonists) are also used for the treatment of hypertension.
3. The antagonists (β receptor blockers) are also used for the treatment hyperthyroidism, especially to prepare the patient before thyroid surgery to decrease the heart rate.
4. Catecholamines are used for the treatment of shock.
5. Dopamine is used for treatment of traumatic and cardiogenic shock.

Pheochromocytoma
Pheochromocytoma is a tumor of adrenal medulla that occurs due to hyperplasia of chromaffin cells. Also, proliferation of chromaffin cells of paraganglia is associated with this condition. Most of pheochromocytomas produce both epinephrine and norepinephrine. Therefore, concentration of epinephrine and norepinephrine is very high. However, few pheochromocytomas produce either epinephrine or norepinephrine.

Features
The most common feature is sustained hypertension.
1. The disease is associated with increased metabolic rate, profuse sweating, extreme tachycardia and high BP, hyperglycemia, and loss of appetite and body weight.
2. In this disease, though there is continuously high secretion of catecholamines, typically there are episodes of excess catecholamine release.
3. The burst of catecholamine secretion usually occurs following rapid change in posture or the regular physiological events that stimulate sympathetic system.
4. This manifests with severe headache, tachycardia, palpitation, extreme anxiety, perspiration, either pallor or flushing, severe rise in blood pressure and a feeling of impending death.

Diagnosis
Diagnosis is established by detecting increased concentration of catecholamines in blood when the patient is in recumbent and at rest. Urinary excretion of metanephrine and VMA also increases.

Treatment
Treatment is by surgical removal of the tumor. The systematic improvement occurs with administration of α blockers.

Role of Catecholamines in Stress
Stress activates CRH and ADH secreting neurons of hypothalamus.
1. CRH in turn increases adrenergic discharge. Adrenergic stimulation increases plasma catecholamine concentration.
2. All these hormones together increase glucose concentration in plasma. Catecholamines increase glycogenolysis and cortisol promotes gluconeogenesis.
3. They also shift glucose utilization from peripheral tissues to the neural tissue.
4. Epinephrine increases supply of FFA to the heart.
5. Catecholamines increase cardiac output and blood pressure and promote supply of substrate to the tissue.
   During acute stress, a general state of arousal and vigilance is maintained by catecholamines. In chronic stress,
reproductive functions, sexual activity, and feeding are suppressed that are also mediated by catecholamines. A variety of cytokines are secreted during stress. The hypothalamic-pituitary-adrenal axis works in adaptation to stress. They generally activate the lifesaving mechanisms.

OTHER ADRENOMEDULLARY PEPTIDES

Adrenomedullin

Adrenomedullin is a polypeptide hormone. It is synthesized from proadrenomedullin. It is also formed in other tissues like brain and kidney.

Functions
1. Adrenomedullin decreases blood pressure by causing vasodilation, which is due to the increased production of EDRF by it.
2. It inhibits aldosterone secretion, and therefore, decreases ECF volume.
3. It, along with proadrenomedullin decreases peripheral sympathetic activity.

Chromogranin

This is a granular protein present in the secretory granules of chromaffin cells. The protein makes the dense core vesicles denser. The dominant chromogranin in human is chromogranin B.
1. Chromogranins are secreted along with catecholamines from the granules.
2. Therefore, measurement of their concentration in plasma indicates the secretory activity of chromaffin cells and indirectly the rate of catecholamine secretions.

Other Hormones

Enkephalins, endorphins, and neuropeptide Y are discussed elsewhere in the book.

CHAPTER SUMMARY

Key Concepts
1. Adrenal medulla is a neuroendocrine gland, a modified sympathetic postganglionic tissue. Therefore, sympathetic stimulation results in instantaneous increase in catecholamines level in plasma.
2. Though, epinephrine and norepinephrine act on both α and β receptors, in general, epinephrine acts more on β receptors and norepinephrine on α receptors.
3. Increased level of urinary VMA is an index of increased sympathetic activity.
4. Though sympathetic activation is essential in stressful situations to maintain energy metabolism and cardiovascular parameters, chronic stress (sustained sympathetic stimulation) is not good for body as it leads to depletion and degeneration.

Important to Know (Must Read)
1. In examination, “Describe the synthesis, metabolism and physiological effects of adrenaline and noradrenaline” may come as a Long Question.
2. In examination, Fight or flight response, Pheochromocytoma, Difference in the effects of adrenaline and noradrenaline on cardiovascular system, Effects of catecholamines on intermediary metabolism can be asked as Short Questions.
3. In Viva, examiner may ask… List the hormones secreted from adrenal medulla, Why VMA is an index of sympathetic activity, What is Fight or flight response, Cause, features, diagnosis, and treatment of Pheochromocytoma, Why chronic catecholamine secretion is not good, Types of catecholaminergic receptors, Which catecholamine act more on which receptor, Mechanism of action of catecholamine on different receptors, Effects of catecholamines on cardiovascular system, Effects of catecholamines on intermediary metabolism, Effects of catecholamines on other systems, Effects of Dopamine, Clinical uses of catecholamines, Mechanism of action of catecholamines, Steps of synthesis of adrenomedullary hormones, Factors regulating secretion of catecholamines, Names of other adrenomedullary hormones, Functions of adrenomedullin, and Functions of chromogranin.
CHAPTER 59

Adrenal Cortex

Learning Objectives

On completion of study of this chapter, the student **MUST** be able to:
1. Correlate the histological specialization of different layers of adrenal cortex and medulla for secretion of specific hormones.
2. List the hormones secreted from different layers of adrenal cortex.
3. Give the steps of synthesis of different adrenocortical hormones.
4. Understand the deficiency of adrenocortical enzymes causing a specific dysfunction or syndrome.
5. Describe the regulation of secretion, mechanism of action and functions of glucocorticoids.
6. Describe the regulation of secretion, mechanism of action and functions of mineralocorticoids.
7. Appreciate the physiological importance of cortisol, and indications & contraindication of cortisol use in clinical medicine.
8. Understand the physiological basis of dysfunctions caused by excess or deficiency of glucocorticoids and mineralocorticoids.
9. Outline the functions and dysfunctions of adrenal sex steroids.

The student **MAY** also be able to:
1. Describe the steps of synthesis and regulation of secretion of adrenocortical hormones.
2. Explain the mechanism of action of glucocorticoids and mineralocorticoids.
3. Describe the role of cortisol in stress.
4. Describe the functions and dysfunctions of adrenal sex steroids.

The adrenals consist of **two distinct endocrine glands**, one inside the other. The inner **adrenal medulla** is surrounded by the outer **adrenal cortex** that consists of three layers (Figs. 59.1A to C). The outer zone of adrenal gland, i.e. the adrenal cortex forms about 80–90% of the total gland, the inner zone, i.e. the adrenal medulla consists of 10–20% of the gland. Developmentally and functionally, these glands are **two separate endocrine organs**. Adrenal cortex develops from the mesodermal tissue and secretes steroid hormones. Adrenal medulla develops from neuroectodermal tissue related to sympathetic ganglia, and, therefore, secretes catecholamines.

**FUNCTIONAL ANATOMY**

Adrenal cortex, the outer zone of the adrenal gland constitutes 80–90% of the gland. In fetus, adrenal cortex is larger in size in which **fetal adrenal cortex constitute 80%** of the gland and the future permanent adrenal cortex makes only 20% of the gland.

**Scientists contributed**

EC Kendall (1886–1972)  
T Reichstein (1897–1996)  
PS Hench (1896–1965)

The **Nobel Prize in Physiology or Medicine 1950** was awarded jointly to American biochemist and endocrinologist Edward Calvin Kendall, Swiss biochemist and endocrinologist Tadeus Reichstein, and Jamaican biochemist and endocrinologist Philip Showalter Hench “for their discoveries relating to the hormones of the adrenal cortex, their structure and biological effects”.

1. At term, the fetal adrenal cortex **undergoes rapid degeneration** and almost disappears in early infancy.
2. The remaining permanent cortex remains and constitutes **postnatal adrenal cortex**.
3. During fetal life, the **fetal adrenal cortex synthesizes and secretes sulfate conjugates of androgens** that
are converted to estrogens and progesterone in the placenta, which is essential for maintenance of pregnancy (see below).

**Hormones and their major functions:** Adrenal cortex secretes three categories of hormones: glucocorticoids, mineralocorticoids and sex hormones (Table 59.1).

1. The glucocorticoids are important for their metabolic, permissive, anti-inflammatory and immunosuppressive effects.
2. Mineralocorticoids are important for their role in extracellular fluid volume and electrolyte balance.
3. The sex steroids contribute to establish and maintain secondary sexual characteristics.

Of adrenocortical hormones, glucocorticoids and mineralocorticoids are the indispensable hormones.

**Histology**

Adrenal cortex consists of three layers: the outer zona glomerulosa that secretes mineralocorticoids, the middle zona fasciculata that secretes glucocorticoids and sex steroids, and the inner zona reticularis that secretes sex steroids and glucocorticoids (Figs. 59.1A to C).

**Zona glomerulosa:** This is the outermost and thinnest layer of the gland. It constitutes 20% of the adrenal cortex and consists of small clumps of cells that contain numerous mitochondria. It secretes mineralocorticoids, mainly aldosterone.

**Zona fasciculata:** This is the middle and widest layer of the adrenal cortex. It constitutes 80% of the adrenocortical mass. It consists of columnar cells that form long cords. The cytoplasm is loaded with vacuoles and lipid droplets. The mitochondria are large and contain numerous vesicular cristae. Cells of this layer secrete glucocorticoids (mainly cortisol and corticosterone) and sex steroids.

**Zona reticularis:** This is the innermost layer of the adrenal cortex and constitutes about 10% of its mass. It contains network of intercalated cells. The mitochondria are large and have vesicular cristae. There are no vacuoles and fat droplets. Cells of this layer secrete sex steroids like androgens and estrogens, and to some extent also glucocorticoids.

**SYNTHESIS OF ADRENOCORTICAL HORMONES**

Adrenocortical steroid hormones are synthesized from cholesterol. Cholesterol used for synthesis of steroid hormones in the adrenal cortical cells, are made available from two sources:

1. From blood, and,
2. Synthesized de novo.

**From blood:** Most of the cholesterol used for biosynthesis of steroid hormones are derived from cholesterol esters attached to the LDL (LDL-cholesterol) in circulating blood. Cells of adrenal cortex actively take up cholesterol from plasma by endocytosis of LDL molecules.

1. There are abundant LDL receptors on adrenocortical cells. In the cytoplasm, the cholesterol is esterified immediately and then stored as cholesterol esters in the lipid droplets (Fig. 59.2).
2. Whenever required for synthesis of hormones, cholesterol ester is converted to free cholesterol by the action of the cytosolic enzyme cholesterol ester hydrolase.
3. Cholesterol is then transported to mitochondria by a carrier protein, called sterol carrier protein 2. Sterol carrier protein 2 facilitates entry of cholesterol into mitochondria for hormone synthesis.

**From the cell itself (de novo synthesis):** Cholesterol is synthesized de novo in the cytosol of adrenocortical cells from acetate by the action of the enzyme HMG CoA reductase.

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**Table 59.1: Hormones secreted from adrenal cortex.**

<table>
<thead>
<tr>
<th>Category</th>
<th>Hormones</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mineralocorticoids</td>
<td>- Aldosterone</td>
</tr>
<tr>
<td></td>
<td>- Deoxycorticosterone</td>
</tr>
<tr>
<td>Glucocorticoids</td>
<td>- Cortisol</td>
</tr>
<tr>
<td></td>
<td>- Corticosterone</td>
</tr>
<tr>
<td>Sex steroids</td>
<td>- Dehydroepiandrosterone</td>
</tr>
<tr>
<td></td>
<td>- Androstenedione</td>
</tr>
</tbody>
</table>

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**Figs. 59.1A to C:** (A) Location of adrenal gland; (B) Parts of adrenal gland as seen in cross-section of the gland; (C) Histology of adrenal gland. Note, zona fasciculata constitutes more than 80% of adrenal cortex.
1. Formed cholesterol is then converted to cholesterol ester by the action of acyl-CoA-cholesterol acyltransferase (ACAT). Cholesterol ester is then stored in the lipid droplet, to be used later for hormone synthesis. Though, cells of the adrenal cortex can synthesize cholesterol de novo, under basal conditions plasma cholesterol is the major source for adrenocortical hormone synthesis. However, when hormone synthesis is stimulated by ACTH, the stored cholesterol in the cytosol of adrenocortical cells and newly formed cholesterol in the cytosol become the important source for hormone synthesis. In such conditions, plasma cholesterol without undergoing esterification directly enters mitochondria for biosynthesis of hormones. 

Cytochrome P-450 enzymes: The synthesis of hormones from cholesterol involves cytochrome P-450 enzymes, also known as CYPs (Table 59.2). These are the enzymes that catalyze hydroxylation of steroids. They are located in the membranes of endoplasmic reticulum and mitochondrial cristae.

Steps of Cortisol Synthesis

Steps of cortisol synthesis are depicted in Flowchart 59.1. In the mitochondria, pregnenolone forms 17-hydroxy-pregnenolone by the enzyme 17α-hydroxylase. 17-hydroxy-pregnenolone then moves into endoplasmic reticulum, where it is converted to 17-hydroxyprogesterone by 3β-hydroxysteroid dehydrogenase, which in turn hydroxylated to 11-deoxycortisol by 21β-hydroxylase. ACTH facilitates cortisol synthesis by increasing the supply of cholesterol and by converting the cholesterol to pregnenolone.

Steps of Corticosterone Synthesis

Steps of corticosterone synthesis are depicted in Flowchart 59.2. The cholesterol is converted to pregnenolone by the mitochondrial enzyme cholesterol desmolase. Pregnenolone then moves to endoplasmic reticulum where it is dehydrogenated to form progesterone by the enzyme 3β-hydroxysteroid dehydrogenase. Progesterone is hydroxylated by the 21β-hydroxylase to form 11-deoxycorticosterone, which then moves back to mitochondria to form corticosterone by the enzyme 11β-hydroxylase.
Synthesis of Mineralocorticoids

Mineralocorticoids are formed in the zona glomerulosa of adrenal cortex. Aldosterone is the major mineralocorticoid synthesized in humans.

The steps of aldosterone synthesis are depicted in Flowchart 59.3.

1. Aldosterone synthase is found only in the cells of zona glomerulosa of adrenal cortex.
2. Therefore, aldosterone synthesis is limited to the zona glomerulosa. Zona glomerulosa lacks the enzyme 17α-hydroxylase. Hence, synthesis of glucocorticoid and sex steroid does not occur in this layer of adrenal cortex.

Synthesis of Sex Steroids

In adrenal cortex, sex steroids are formed mainly in the zona reticularis. However, zona glomerulosa also contributes to it. The major sex steroids formed are dehydroepiandrosterone and androstenedione. The steps of androstenedione synthesis are depicted in Flowchart 59.4.
1. Dehydroepiandrosterone is converted to dehydroepiandrosterone sulfate by the enzyme sulfokinase. Though, androstenedione is synthesized from dehydroepiandrosterone, it can also be formed from 17-OH-progesterone.
2. Androstenedione is then converted to testosterone, which later forms estradiol.

The steps of estradiol synthesis are depicted in Flowchart 59.5.

In woman, adrenal gland supplies about 50–60% of the androgenic hormones. The adrenal androgen is not important in males because the testes produce testosterone. Similarly, the conversion of androgen to estrogen in the adrenal cortex in females is not important because ovaries secrete estrogen. However, after menopause secretion...
Section 6: Endocrine Physiology

Effects of Enzyme Deficiency

Deficiency of adrenocortical enzymes results in various syndromes. The congenital deficiency of the adrenal enzymes results in decreased cortisol secretion. Decreased level of cortisol in plasma stimulates ACTH secretion. As the ACTH has growth promoting (trophic) effects on cells of adrenal cortex, the gland size increases. Therefore, the conditions are called **congenital adrenal hyperplasia**. Deficiency of 21\(\beta\)-hydroxylase and 11\(\beta\)-hydroxylase are common.

**Deficiency of 21\(\beta\)-Hydroxylase**

Deficiency of 21\(\beta\)-hydroxylase accounts for **90% of the cases of congenital adrenal hyperplasia**. Deficiency of 21\(\beta\)-hydroxylase decreases the production of glucocorticoids and mineralocorticoids. Decreased cortisol level in...
plasma increases the production of ACTH, which facilitates the production of pregnenolone from cholesterol. Surplus pregnenolone is diverted for the excess production of androgens. Therefore, the condition is mainly characterized by virilization.

1. This leads to characteristic adrenogenital syndrome in females. The features of this syndrome are hirsutism, small breasts, male escutcheon, heavy arms and legs, enlarged clitoris (Clitromegaly), and receding hairline.

2. In severe cases, genitalia of genetic females are masculinized (female pseudohermaphroditism).

3. Many of them develop hyponatremia (salt losing form of congenital virilizing adrenal hyperplasia). Hyponatremia occurs due to urinary loss of Na⁺, which occurs due to lack of mineralocorticoid activity.

4. Hyponatremia causes hypovolemia and hypotension.

**Deficiency of 11β-hydroxylase**

In 11β-hydroxylase deficiency, increased production of androgen is associated with increased secretion of 11-deoxycortisol and 11-deoxycorticosterone. The 11-deoxycorticosterone is a mineralocorticoid. Thus, excess mineralocorticoid activity increases retention of Na⁺ and water. Therefore, patient develops hypertension in addition to virilization (hypertensive form of congenital virilizing adrenal hyperplasia).

In all virilizing forms of adrenal hyperplasia, glucocorticoid therapy is preferred. Exogenously administered glucocorticoid fills the endogenous cortisol deficit, and also suppresses the production of ACTH. Thus, ACTH induced excess production of sex steroid is decreased.

**Deficiency of 17α-hydroxylase**

This is a rare condition. It results in decreased production of sex steroids. Therefore, female external genitalia are present. However, as the production of aldosterone and corticosterone is normal, elevated level of mineralocorticoids result in hypertension and hyperkalemia.

**3β-hydroxysteroid Dehydrogenase**

This is also a rare condition, in which production of dehydroepiandrosterone is increased. There is some degree of masculinization in females. But, as dehydroepiandrosterone is a weak androgen, it does not cause full masculinization of the genitalia in male babies. Therefore, hypospadias (urethral opening on the undersurface of the penis) develops.

**Cholesterol Desmolase Deficiency**

Deficiency of the cholesterol side-chain cleaving enzyme decreases production of all adrenocortical hormones as it converts cholesterol to pregnenolone, the first step in steroidogenesis. The condition is fatal during fetal life. The production of placental progesterone depends on fetal adrenocortical production of androgen. Progesterone is essential for pregnancy to continue. Thus, deficiency of this enzyme results in termination of pregnancy. However, this condition is very rare.

**Metabolism of Adrenocortical Hormones**

**Normal Secretion**

The daily secretion and plasma concentration of adrenocortical hormones are listed in Table 59.3.

**Plasma Protein Binding**

Adrenocortical steroids usually bind with proteins, such as transcortin and albumin.

**Transcortin Binding**

About 80% of adrenal steroids, especially cortisol and corticosterone bind with a plasma protein called transcortin, or corticosteroid binding globulin (CBG). The transcortin is a glycoprotein produced by liver. The normal plasma concentration of transcortin is 3 mg/dL.

1. The concentration increases in pregnancy and estrogen therapy. When transcortin level increases, the quantity of hormone binding to it increases that in turn decreases free cortisol level.

2. The decreased free hormone increases ACTH secretion, which consequently increases cortisol secretion. However, a new state is reached at which the bound form is increased, but the free form remains normal.

3. Thus, the total hormone concentration increases without changing the concentration of the free form. Therefore, in pregnancy features of cortisol excess do not appear inspite of increased total cortisol level.

**Albumin Binding**

About 10–15% of cortisol is bound to albumin. Thus, only 5–10% of cortisol is free in the plasma.

Because of its protein binding, the half life of cortisol is more (60–90 mins), its concentration as free hormone in plasma is less and its excretion in urine is less. As binding of aldosterone to protein is less, the half life of aldosterone is less (about 20 minutes).

**Metabolic Degradation**

Cortisol is metabolized in liver, where it is reduced to dihydrocortisol and tetrahydrocortisol glucuronides.
tetrahydroglucuronide derivative cortisol and corticosterone are water soluble, and therefore is rapidly excreted in urine (Flowchart 59.6).

1. The free cortisol circulating in the plasma is filtered by kidney and about 50 mg is excreted in the urine. The measurement of urinary metabolite of cortisol provides a reliable index of cortisol secretion.
2. The excretion of 17-hydroxycorticoids represents about 50% of the total daily cortisol secretion. Normally 2–12 mg of 17-hydroxycorticoids is excreted per day.
3. The precursors of cortisol (progesterone and 17-OH-progesterone) are metabolized to pregnanediol and pregnanetriol.
4. In adult females, urinary excretion of these metabolites reflects the activities of ovarian-adrenal axis. However, their increased urinary concentration in prepubertal girls indicates specific congenital defect in cortisol secretion (due to disease of the adrenal cortex, not the ovary).

17-Ketosteroids

17-Ketosteroid derivative is formed in the liver from cortisol. However, corticosterone does not form 17-ketosteroid. Dehydroepiandrosterone, the major adrenal androgen is a 17-ketosteroid. Testosterone is converted to 17-ketosteroid.
1. Etiocicholanolone, a metabolite of adrenal androgens and testosterone, forms 17-ketosteroid.
2. 17-ketosteroid is excreted in the urine. Normally, 5–10 mg of 17-ketosteroids in women, and 8–20 mg of 17-ketosteroids in men is excreted in urine per day.
3. About 70% of this amount excreted is normally derived from adrenal cortex and 30% from the gonadal androgens.
4. When they accumulate in blood, they cause episodic fever known as etiocholanolone fever.

Regulation of secretion, mechanism of action, functions and dysfunctions of each category of adrenocortical hormones are discussed separately in the following sections:

GLUCOCORTICOIDS

Regulation of Secretion

Glucocorticoid secretion is controlled mainly by feedback mechanisms of ACTH secretion. Angiotensin II and other hormones also contribute.

Role of ACTH

ACTH is the polypeptide hormone containing 39 amino acids secreted from anterior pituitary. This is the major stimulator of glucocorticoid secretion. ACTH is secreted in irregular bursts throughout the day. Accordingly, the concentration of cortisol alters in the plasma.

1. The bursts are more frequent and prominent in the early morning (4–10 AM), which accounts for about 75% of the total ACTH secreted in a day (for details, refer chapter “Anterior Pituitary”).
2. This circadian rhythm (the diurnal variation) of ACTH secretion is an important regulator of cortisol secretion. Therefore, cortisol secretion also has a definite pattern that follows the pattern of ACTH secretion (Fig. 59.5).

Feedback Control

Glucocorticoid secretion is controlled by feedback release of ACTH from the anterior pituitary.

1. ACTH secretion is stimulated by CRH (corticotropin releasing hormone) secreted from the hypothalamus CRH secretion is influenced by various stimuli originating
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from the limbic system, pain pathways, NTS, reticular formation and suprachiasmatic nucleus (Fig. 59.6).

2. CRH increases ACTH secretion, which in turn increases glucocorticoid secretion from the adrenal cortex.

3. Increased glucocorticoid level in plasma provides negative feedback signal to the hypothalamus and anterior pituitary to inhibit the secretion of CRH and ACTH respectively.

4. Thus, cortisol secretion returns to normal.

5. Conversely, decreased glucocorticoid secretion increases CRH and ACTH release by feedback mechanisms that tend to increase the secretion of cortisol (Clinical Box 59.1).

Clinical Box 59.1

Steroid therapy should not be stopped abruptly: A patient receiving steroid for a long time should not stop the medicine abruptly. This is because, after a prolonged treatment with steroid, the adrenal cortex atrophies and becomes unresponsive to the ACTH for about six months. Also, pituitary secretes less ACTH initially, due to its diminished synthesis. However, pituitary recovers and secretes more ACTH thereafter. Increased level of ACTH slowly stimulates adrenal cortex to secrete glucocorticoids and it takes about 8 to 10 months for cortisol level to return to normal plasma level (Fig. 59.7). ACTH, from its level also returns to normal in about 10 months. Thus, pituitary-adrenocortical axis takes about 10 months to recover from the suppressive effect of prolonged steroid therapy. During this period, an individual fails to cope with stress and may succumb to stressful situations. To prevent such a dangerous complication, steroids should not be stopped abruptly after a prolonged therapy; rather the dose should be decreased gradually over weeks before finally stopping the treatment.

In Stress

In stress, increased cortisol secretion occurs mainly due to increased hypothalamic release of CRH. Fibers projecting from limbic system, especially from amygdala to hypothalamus (amygdalohypothalamic pathway) mediate the responses to emotional stress, fear, and anxiety. Ascending nociceptive fibers give collaterals to hypothalamus and they mediate the stress response to painful stimuli or injury.

Angiotensin II

Normally, angiotensin II is an important stimulator for aldosterone secretion. However, it also induces glucocorticoid secretion.

Other Hormones

ADH, serotonin, and VIP also stimulate glucocorticoid secretion. But their role in the physiologic regulation of glucocorticoid secretion is not clearly known.

Mechanism of Action

Cortisol takes few hours to days to exert its effect.

1. It diffuses into the cell and binds with the glucocorticoid receptors (GR) present in the cytoplasm.

2. Binding of cortisol with the receptor displaces the inhibitory heat shock protein complex from the receptor.

3. This results in alteration in the receptor configuration and causes hyperphosphorylation of the receptor.

4. Then, the hormone-receptor complex enters the nucleus, where it binds with the specific glucocorticoid regulatory elements (GRE) on the target DNA molecule (Fig. 59.8).

5. This leads to transcription of mRNAs that regulate expression of various genes.
Functions of Glucocorticoids

There are receptors for glucocorticoids in almost all tissues of the body and glucocorticoids influence many physiological processes of the body. Cortisol influences metabolisms profoundly, facilitates action of other hormones (permissive actions), influences functions of important organ systems, controls inflammation and immunity, and mediates responses of the body to stress. For its all-around physiological and pharmacological effects, cortisol is used widely in clinical practice.

Effects on Intermediary Metabolisms

On Carbohydrate Metabolism

Glucocorticoid is essential for survival during fasting. In prolonged fasting, the liver glycogen store is depleted and unless there is gluconeogenesis, death occurs due to hypoglycemia. Cortisol increases blood glucose by various mechanisms and, thus, plays an important defensive role in fasting (Clinical Box 59.2), and also against hypoglycemia induced by insulin.

Clinical Box 59.2

Adrenal-deficient patients should not fast: Cortisol increases blood glucose, and therefore is an important defense against hypoglycemia in fasting. In adrenal insufficiency, blood glucose remains normal so long as the food intake is normal. However, fasting induced hypoglycemia becomes fatal in such patients, as cortisol defense of hypoglycemia is lacking. Therefore, patients suffering from adrenocortical insufficiency are advised not to fast.

Cortisol increases plasma glucose by following mechanisms:

1. **Cortisol stimulates hepatic gluconeogenesis**: Cortisol secretion increases in fasting. Cortisol induces gluconeogenic enzymes. Especially, it activates glucose-6-phosphatase, which converts glucose-6-phosphate to glucose and, therefore, increases the release of glucose from liver. Also, by causing proteolysis, it mobilizes amino acids for neoglucogenesis.

2. **Increased secretion of glycogenolytic hormones**: Another defense mechanism of cortisol against hypoglycemia is the increase in secretion of glucagon and epinephrine that cause glycogenolysis. However, cortisol per se facilitates liver glycogen synthesis.

3. **Anti-insulin effect**: Cortisol decreases the utilization of glucose by antagonizing the action of insulin on peripheral tissues. It prevents the mobilization of glucose transporters from cytosol to the cell membrane. Especially, it inhibits the insulin stimulated glucose uptake in skeletal muscle and adipose tissue. This adds to the hyperglycemic effects of cortisol and makes diabetes worse (Clinical Box 59.3). It also reverses insulin suppression of hepatic glucose production.

Clinical Box 59.3

Cortisol complicates diabetes: In normal individuals, rise in blood glucose by cortisol increases insulin secretion, which counteracts ketone bodies and lipids raised by the cortisol. In diabetes, cortisol effects on ketone body and lipid remain unaffected as insulin is lacking. Thus, excess cortisol makes the diabetes worse by promoting ketoacidosis.

On Protein Metabolism

Cortisol facilitates proteolysis, especially in skeletal muscle, and inhibits protein synthesis. It mobilizes muscle protein for gluconeogenesis. The overall action is to facilitate the conversion of protein to glycogen. The chronic administration of cortisol or excess secretion of glucocorticoid causes depletion of protein storage in the body, especially in the muscle, bone, skin, and connective tissue.

On Fat Metabolism

Cortisol causes lipolysis. In fact, the lipolytic effect of epinephrine and growth hormone requires cortisol. During fasting, by promoting lipolysis, cortisol causes rapid release of free fatty acid and glycerol from adipose tissues, which are utilized for gluconeogenesis. Increased free fatty acid formation promotes ketogenesis, especially in diabetes.

On Food Intake and Fat Distribution

Cortisol increases appetite and food intake by stimulating neuropeptide Y secretion from the hypothalamus.

1. It stimulates differentiation of pre-adipocytes to adipocytes in the adipose tissue.

2. It also stimulates lipogenesis by activating lipoprotein lipase and glucose-6-phosphate dehydrogenase activity of the adipocytes in some other parts of the body.
3. Therefore, the action of cortisol varies from tissues to tissues. Thus, cortisol excess causes maldistribution of fat in the body that result in truncal obesity, moon face and buffalo hump with thinning of extremities. The exact cause of peculiar distribution of fat is not known, but it is associated with insulin resistance and/or increase in insulin level.

4. Cortisol also increases leptin synthesis in adipose tissue. Therefore, obesity is restricted by the negative feedback actions of leptin (leptin inhibits feeding).

To summarize the effect of cortisol on metabolisms, it is an important diabetogenic, ketogenic, and anti-insulin hormone (Flowchart 59.7).

**Permissive Actions of Cortisol**

Glucocorticoid is essential (even in small quantity) for some physiological actions of other hormones to take place. This is called permissive action of cortisol (as cortisol allows the specific actions of these hormones to occur, though it does not produce these effects by itself).

Permissive actions of cortisol include the following:

1. **Vasopressor and bronchodilator effects** of catecholamines.
2. **Calorogenic effects** of glucagon and catecholamines.
3. **Lipolytic effects** of catecholamines.
4. Development of **mammary gland** during puberty in females.
5. Development of **hepatic enzyme systems** during fetal life.
6. **Surfactant synthesis** in the fetal lung and maturation of lungs during intrauterine life.
7. Development of **bacterial flora and enzyme** systems in the intestine.

**Effects on Cardiovascular System**

**On Heart**

Cortisol increases myocardial performance by increasing Na⁺–K⁺ ATPase activity and also by increasing expression of β-adrenergic receptors in the heart.

**On Blood Vessels**

Cortisol maintains vascular reactivity. This is an important action of cortisol. It increases the responsiveness of arterioles to catecholamines and angiotensin II.

1. Probably, this action is mediated by inhibition of Na⁺–Ca²⁺ exchanger in the cell membrane so that the Ca²⁺ concentration is maintained in the blood vessel smooth muscle cells. Cortisol also decreases the release of vasodilators like prostaglandins.
2. In adrenal insufficiency, blood vessels become unresponsive to catecholamines (Clinical Box 59.4). This promotes vascular collapse, as already hypovolemia exists in such patients.

**Clinical Box 59.4**

**Injection of cortisol is a must in shock:** Injection of catecholamines in hypovolemic shock is required to restore blood pressure, as they cause vasoconstriction and increase cardiac output. However, it is desirable to inject cortisol along with catecholamines as it maintains vascular reactivity. Also, cortisol mediates responsiveness of catecholamines to vasoconstrictors by its permissive action. Catecholamine injection without administration of cortisol in shock does not ensure adequate vasoconstriction and blood pressure continues to remain low.
Effects on Central Nervous System
Cortisol influences the mood and behavior of individuals. There are abundant glucocorticoid receptors (both type I and type II) in limbic system including prefrontal cortex.

1. Cortisol decreases REM sleep but increases the slow wave sleep. The increased level of cortisol in plasma can cause insomnia.
2. It causes both elevation and depression of mood, and impairs memory.
3. It decreases the responsiveness to gustatory, olfactory, auditory, and visual stimuli.
4. The patients with adrenal insufficiency develop changes in personality, and remain irritable and apprehensive.

Effects on Musculoskeletal System

**On Muscle**
It increases the performance of cardiac and skeletal muscle. The inotropic effect on skeletal muscle is due to increase in acetylcholine synthesis at the muscle-nerve terminals. However, cortisol in excess decreases muscle protein synthesis and promotes proteolysis. Therefore, it decreases the muscle mass and strength.

**On Bone**
Cortisol inhibits bone formation by various mechanisms:
1. It decreases the synthesis of type-I collagen, which is a fundamental component of bone matrix.
2. It inhibits the conversion of osteoprogenitor cells to the osteoblasts.
3. It decreases absorption of Ca**+** from GI tract (by inhibiting the effect of vitamin D on GIT). It also decreases the synthesis of active vitamin D. Thus, it decreases supply of Ca**+** to the bones, which is essential for bone mineralization.
4. Cortisol also facilitates bone resorption. The overall effects are decreased bone mass and mineralization. Therefore, prolonged administration of cortisol may cause osteoporosis (Clinical Box 59.5).

**Clinical Box 59.5**
Bone X-ray should be done to check complications: Cortisol decreases bone mass and promotes osteoporosis. Even, vertebral collapse may occur. Therefore, patients receiving steroid for a longer period should be checked for osteoporotic changes by taking bone X-rays or MRI, especially in elderly patients.

Effects on Connective Tissue
Cortisol inhibits collagen synthesis. Therefore, it decreases skin thickness and causes thinning of capillary walls. The capillaries become fragile (susceptible to rupture). Thus, intracutaneous hemorrhage occurs in cortisol excess.

Effects on Kidney and Water Metabolism
Cortisol increases GFR by increasing glomerular blood flow. It is essential for the rapid clearance of a water load (fast excretion of water from the body) as happens in infusion of saline or glucose solution. Cortisol achieves this partly by inhibiting ADH secretion. Therefore, in the absence of cortisol, free water clearance is impaired and dilution of urine is limited (Clinical Box 59.6). It also increases phosphate excretion by inhibiting reabsorption of it in proximal tubule.

**Clinical Box 59.6**
Be cautious while infusing glucose solution in cortisol deficiency: Patients with adrenocortical insufficiency can not excrete a water load. Sudden infusion of large volume of saline or glucose solution in such patient may result in water intoxication. Following infusion of glucose saline, the glucose is metabolized, but the body can not remove water as cortisol is lacking. Therefore, plasma becomes hypoosmolar due to hemodilution. The cells in the brain swell, that impairs the hypothalamic thermoregulatory centers. Patient develops high fever and collapses. Therefore, such patients should receive cortisol while receiving a water load.

Apparent Mineralocorticoid Excess
In the absence or deficiency of 11-β-OH-steroid dehydrogenase type-2 (that converts cortisol into cortisone), cortisol has marked mineralocorticoid activity (Table 59.4), which causes hyperaldosteronism like situation due to action of cortisol on mineralocorticoid receptors.

1. The patient develops clinical features of hyperaldosteronism, though the plasma aldosterone and plasma renin activity remains low. Hence, the condition is called apparent mineralocorticoid excess.
2. It may occur due to congenital deficiency of the hormone or ingestion of licorice.
3. Licorice contains glycyrrhetinic acid that inhibits 11-β-OH-steroid dehydrogenase type-2. Persons consuming large quantity of licorice may also develop hypertension as sodium absorption through ENaC (epithelial sodium channel) in kidney is high.

Effects on Fetus
Cortisol imparts profound influence on fetal maturation.
1. Cortisol causes maturation of CNS during intrauterine life.
2. It causes growth of retina, GI tract, lungs, and skin of the fetus.

<table>
<thead>
<tr>
<th>Table 59.4: Mineralocorticoid and glucocorticoid activities of various naturally occurring steroids in comparison to cortisol (considering cortisol effect as 1).</th>
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<tbody>
<tr>
<td><strong>Steroid</strong></td>
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<tr>
<td>Cortisol</td>
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<tr>
<td>Corticosterone</td>
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<tr>
<td>Aldosterone</td>
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<td>Deoxycorticosterone</td>
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<tr>
<td>Cortisone</td>
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</table>
3. The intestinal enzyme system of fetal pattern to the postnatal or adult pattern is altered by cortisol, which is essential for disaccharides to be digested by the infants.
4. Cortisol causes lung development. It promotes growth of alveoli and causes flattening of alveolar epithelial cells. Cortisol also facilitates pulmonary surfactant synthesis. All these actions allow the fetal lung to satisfactorily expand at the first breath.

**Effects on Blood Cells**

Though, cortisol influences all the three formed elements of blood, the impact is more on leucocytes.

**On Leucocytes**

Cortisol causes leucocytosis. Though it causes mild neutrophilia and monocytes, it causes profound lymphocytopenia, eosinopenia and basopenia.

**Causes of Lymphocytopenia**

2. It decreases the size of lymph nodes and thymus.
3. It decreases the ability of lymphocytes and monocytes to secrete cytokines that are essential for proliferation of lymphocytes.

**Causes of Eosinopenia**

1. Cortisol causes eosinopenia by stimulating apoptosis (programmed cell death) of eosinophil.
2. By inhibiting the release of cytokines, it prevents eosinophil growth.
3. It facilitates sequestration of eosinophil in spleen and lungs (thus, decreases the peripheral eosinophil count).

**On RBC**

It causes mild erythrocytosis by stimulating erythropoietin production.

**On Platelets**

It produces mild thrombocytosis.

**Effects on Inflammation**

Cortisol has profound anti-inflammatory and anti-allergic actions. Inflammation is the response of tissue to injury. The inflammatory responses include: dilation of capillaries, increased capillary permeability, migration of granulocytes to the site of injury and killing of the organisms or the insulting agents by the granulocytes, mainly neutrophils (for details, refer Chapter 19). These responses are mediated by various chemicals like prostaglandins, thromboxanes, leukotrienes, kinins, histamine, serotonin, lymphokines, EDRF and PAF (platelet activating factor) (refer to Tables 19.2 and 19.5, Chapte 19). Cortisol interferes in most of the mechanisms of inflammation.

1. Cortisol inhibits synthesis of chemical mediators of inflammation. It stimulates synthesis of lipocortins (a family of phosphoproteins) in the target cells. Lipocortin inhibits the activity of phospholipase A₂; therefore, decreases the release of arachidonic acid, which is the precursor for many mediators of inflammation like prostaglandins, thromboxanes, leukotrienes, etc. Thus, cortisol prevents the formation of these mediators of inflammation.
2. Cortisol stabilizes the lysosomal membrane. Therefore, it decreases release of proteolytic enzymes and hyaluronidase from lysosomes that are required for inflammatory reactions to occur.
3. Cortisol inhibits mast cells. It prevents differentiation and proliferation of mast cells. Consequently, it decreases the release of histamine, an important mediator of inflammation (histamine causes vasodilation and increases capillary permeability).
4. Cortisol inhibits leucocyte functions. It prevents migration of leucocytes to the site of inflammation or infection. It prevents the margination of leucocytes and adherence of leucocytes to the capillary endothelial wall. Cortisol inhibits the expression of receptors in the endothelial cells that normally causes chemotactic peptides to interact with leucocytes to stick to the vessel endothelium.
5. Cortisol inhibits phagocytic and bactericidal activity of neutrophils. Though cortisol causes mild neutrophilia, it actually decreases the activity of leucocytes.
6. Cortisol inhibits proliferation of fibroblasts. Thus, it decreases the synthesis and deposition of fibrils at the site of inflammation. This forms the basis of chronic anti-inflammatory action of cortisol to injury. Cortisol prevents walling off of a chronic infection.
7. Recent evidences suggest that a transcription factor, called nuclear factor-κB (NF-κB), plays an important role in inflammation. Normally, NF-κB is bound to another cytoplasmic protein called IκBα, and this binding keeps NF-κB in an inactive state. Viruses and cytokines that induce inflammation separate NF-κB from IκBα by activating IκB kinase. NF-κB then migrates to nucleus and attaches with the DNA. This induces the transcription of genes for formation of various chemicals that participate in inflammation. Thus, NF-κB stimulates synthesis and secretion of mediators of inflammation. Glucocorticoids increase the production of IκBα in the cell, and, therefore, prevent the activation of NF-κB. This is suggested to be the primary mechanism of anti-inflammatory actions of cortisol (Flowchart 59.8). Nevertheless, the physician has to be cautious for use of cortisol to prevent inflammation (Clinical Box 59.7).
Flowchart 59.8: Mechanism of anti-inflammatory actions of cortisol.

(PG: Prostaglandins, LT: Leukotrienes; I₁B₅: A cytoplasmic protein; NF-κB: Nuclear factor-κB).

Clinical Box 59.7
Cortisol should be given with antibiotics: For its strong anti-inflammatory properties, cortisol is very often prescribed for the treatment of inflammatory diseases. However, it should not be prescribed for treating acute inflammations due to infections. If cortisol is given in infections, the toxic features of infection dramatically disappear due to its anti-inflammatory actions. This may give the wrong impression that the infection has subsided. But, actually infection spreads as cortisol suppresses immunity (discussed below). Thus, cortisol in such conditions masks the actual disease and delays the diagnosis. Moreover, it also aggravates the disease, which may become fatal. Therefore, cortisol should not be prescribed in acute infections like pneumonia, cholecystitis, pancreatitis, active tuberculosis etc. If situation warrants cortisol treatment in such conditions, it should always be prescribed with appropriate and adequate antibiotics.

Effects on Allergy
Cortisol has intense anti-allergic effects. Allergy occurs due to antigen-antibody reaction that stimulates the release of histamine from mast cells. There are two types of allergies: local and systemic.

1. In local allergy, release of histamine locally causes redness, itching and swelling.
2. In systemic allergy (anaphylaxis), histamine released into circulation inhibits heart that decreases cardiac output, and causes vasodilation that decreases blood pressure. Most of the effects of allergy are mediated through histamine.

The antiallergy effects of cortisol include the following:

1. Cortisol inhibits degranulation of mast cells and, therefore, prevents release of histamine.
2. It also prevents the growth of mast cells.

Thus, cortisol strongly prevents allergic reactions. Therefore, cortisol is used frequently for the treatment of allergy both locally and systemically (Clinical Box 59.8).

Effects on the Immune System
Cortisol has profound immunosuppressive effects. The influence is more on cellular immunity. When an antigen enters the body, antigen presenting cells (APCs) take it up and present to the T cells. This causes proliferation and activation of T cells. The activated T cells kill organisms, secrete interleukins and activate type 1 helper T cells that mediate immunological responses. Cortisol suppresses immunity by following mechanisms.

1. Cortisol decreases circulating lymphocytes by inhibiting lymphocyte mitotic activity. It mainly decreases T cell population, especially type-I helper T cells by stimulating their apoptosis.
2. Immature T cells in thymus and immature B and T cells in lymph nodes are destroyed by high level of plasma cortisol, which in turn decreases the size of thymus and lymph nodes.
3. Cortisol inhibits the cytotoxic effects of T cells by inhibiting the transport of lymphocytes to the site of antigenic stimulation and by decreasing the production of cytokines from them. Cortisol inhibits cytokine secretion by inhibiting the NF-κB activity of the cells.
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4. Cortisol inhibits production of interleukins (IL-1, IL-2, and IL-6) from the helper T cells, and inhibits synthesis of γ interferon from macrophage and lymphocytes. IL-2 and γ interferon are essential for cellular immunity.
5. Cortisol inhibits both resting as well as activated lymphocytes. It inhibits lymphocyte proliferation. It also inhibits differentiation of monocytes to macrophages (Flowchart 59.9).

**Important Note**

**Indications and contraindications of cortisol use:** Cortisol is used in the treatment of many diseases and also contraindicated for other diseases.

1. Cortisol is used for the treatment of inflammatory diseases like rheumatoid arthritis, etc. When an inflammatory process is life-threatening, administration of cortisol provides immediate relief.
2. Cortisol is frequently used for prevention of transplant rejection as it inhibits cellular immunity. However, cortisol given for a long time decreases body immunity and, therefore, increases the susceptibility to various bacterial, fungal, and viral infections. Therefore, cortisol treatment is supplemented with antibiotics.
3. Cortisol is used in the treatment of both local and systemic allergy. For example, in the management of bronchial asthma, to ensure effective bronchodilation, inhalation of both cortisol and salbutamol is advised. In acute exacerbation of asthma, systemic steroid is very helpful.
4. Cortisol prevents fibroblastic growth. Therefore, it is used in the treatment of keloid (the tumor of scar tissue) by local injections.
5. A person suffering from acute infection should not be treated with steroids without antibiotics, though it suppresses inflammation and dramatically improves the condition. This is because cortisol suppresses immunity and spreads infection (person may die due to severe infection).

Thus, cortisol has profound inhibitory effects on cellular immunity, for which it is frequently used for prevention of transplant rejection. However, in large doses, it also suppresses production of antibodies and, therefore, inhibits humoral immunity. Cortisol does not facilitate degradation of antibodies nor does it interfere in the interaction of antigen and antibodies. The decreased production of interleukin-1 by cortisol suppresses the febrile responses to inflammation.

**Clinical Box 59.8**

**Use and misuse of steroids:** Steroids are used in the treatment of various diseases for their anti-inflammatory, immunosuppressive, metabolic, and permissive actions. However, they are also misused by some sportspersons and others. The usual synthetic steroids in use are:

1. **Prednisolone:** It has predominantly glucocorticoid activity and less mineralocorticoid activity. Its glucocorticoid activity is about four times that of cortisol.
2. **Dexamethasone:** It has only glucocorticoid activity. Its glucocorticoid activity is about six times that of prednisolone.
3. **9α-Flurocortisol:** It has predominantly mineralocorticoid activity and less glucocorticoid activity. Its glucocorticoid activity is about two-and-half times that of prednisolone.

**Effects on GI tract**

1. It stimulates HCl secretion from parietal cells of the stomach. Thus, it causes hyperacidity and gastritis. Given for a long period, it produces gastric ulceration.
   Therefore, cortisol is prescribed cautiously in patients already having history of peptic ulcer.
2. Cortisol decreases Ca++ absorption from GI tract.

**Effects on Endocrine Function**

1. Cortisol inhibits growth hormone secretion. Therefore, it inhibits growth.
2. Cortisol inhibits TSH secretion. Therefore, it inhibits thyroid functions.
3. Glucocorticoids inhibit ACTH secretion. In cortisol excess ACTH secretion is less and in cortisol deficiency ACTH secretion is more. This helps in the diagnosis of adrenocortical dysfunction by estimating plasma ACTH concentration.

4. Cortisol induces expression of PNMT (phenylethanolamine-N-methyltransferase), the enzyme that catalyzes the formation of epinephrine from norepinephrine.

Role in Stress
The most important function of cortisol is to protect the body against stress. In absence of glucocorticoids, body cannot cope with stress even in mild intensity.

1. The glucocorticoid secretion increases due to increased ACTH secretion, which exclusively occurs due to increased release of CRH from hypothalamus.

2. In fact, stress is defined as the condition in which plasma ACTH secretion is high. The stimuli that increase ACTH secretion are called stressors.

3. In stress, glucocorticoid is essential for catecholamines to exert their FFA mobilizing action (FFA is an important source of energy supply in emergency situations) and for maintenance of vascular reactivity to catecholamines (Fig. 59.9).

4. Therefore, in absence of cortisol, the individual succumbs to stress.

Rapid Actions of Steroid
As steroids act though transcription of genes in the cells, it takes hours to days for their full actions to manifest. These are called genomic actions. However, recently, it has been observed that some of the steroid actions occur within few minutes. These rapid actions of steroid are due to their direct action on the membrane channels or enzymes. These are called nongenomic action of steroids. They activate various membrane enzymes that lead to formation of second messengers like cAMP in the cells, and second messengers in turn change cell functions.

Dysfunctions of Glucocorticoids

Cushing's syndrome
This is a pathological condition that occurs due to hypersecretion of glucocorticoids. This is called Cushing’s syndrome as it was described by Harvey Cushing.

Etiology
It occurs due to either a tumor of the adrenal cortex that causes cortisol excess or exogenously administered excess steroids (ACTH independent) or due to the tumor of the pituitary that increases secretion of ACTH (ACTH dependent).

Traditionally, Cushing’s disease refers to the disease that occurs only due to the ACTH producing tumor of pituitary, whereas Cushing’s syndrome refers to all the causes of cortisol excess.

Scientist contributed
Harvey William Cushing (1869-1939) was an American Neurosurgeon, developed neurosurgery as a branch of medicine, and he is often called as the ‘Father of modern neurosurgery’. He had pioneered in the study of functions hypothalamus, pituitary and the hypothalamo-pituitary-adrenocortical axis. During his experiments with Kocher, he first encountered the Cushing reflex which describes the relationship between blood pressure and intracranial pressure. He described the cortisol excess is due to increased pituitary activity, which was later named after him as Cushing’s disease and the cortisol excess is due to adrenocortical tumor as Cushing’s syndrome.


The common causes of Cushing’s syndrome are:
1. Adrenal hyperplasia secondary to pituitary ACTH producing tumors, hypothalamo-pituitary dysfunctions, and ectopic ACTH or CRH producing tumors, such as bronchogenic carcinoma.
2. Adrenal tumors (adenoma or carcinoma)
3. Adrenal macronodular hyperplasia
4. Familial adrenal dysplasia (Carney syndrome)
5. Prolonged and excess use of ACTH or cortisol (iatrogenic)

Features
The features of Cushing’s syndrome according to their frequency of occurrences are:
1. Centripetal obesity and overweight: This is due to peculiar distribution of body fat in which fat is deposited more in the abdomen and upper back. Fat pad in lower neck and interscapular region gives the typical appearance of buffalo hump (Fig. 59.10).
2. **Moon face**: The face becomes round with red cheeks due to fat deposition. Salt and water retention contribute to it.


4. **Hypertension**: Patient develops hypertension due to water accumulation as glucocorticoid in excess has significant mineralocorticoid activity. Increased angiotensinogen secretion by cortisol and direct action of cortisol on blood vessel contribute to it.

5. **Hirsutism and amenorrhea**: Occurs due to associated increased adrenal androgens.

6. **Reddish purple striae**: Striae develop on the abdomen. Excess fat deposition in abdomen causes rapid stretching of the skin that results in formation of striae.

7. **Ecchymoses**: The skin and subcutaneous tissue are thin as a result of protein catabolism. Capillaries of the skin become thin and fragile. Therefore, minor injury causes ecchymoses and bruises (subcutaneous and intracutaneous hemorrhages).

8. **Proximal myopathy**: The legs become thin due to proteolysis in the skeletal muscle and reduced bone mass. The muscle development is poor.

9. **Poor wound healing**: Hyperglycemia promotes growth of the organism at the wound site. Also, decreased immunity favors growth of the organism. Cortisol prevents walling off of a chronic infection.

10. Many patients develop **hyperglycemia** and glucose intolerance, and about 20% of patients develop insulin resistant **diabetes mellitus**.

11. **Osteoporosis** develops due to decreased bone mineralization and decreased bone mass. This may cause collapse of vertebral bodies and pathologic fractures of bones.

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**Fig. 59.10**: Features of Cushing syndrome. Note the presence of centripetal obesity, moon face, buffalo hump, pendulous abdomen with stria over it, poor muscle development and thin limbs, easy bruisability, poor wound healing (infections).

12. Emotional changes may be profound ranging from irritability to frank psychosis.
13. Hyperacidity is common. Some patients may develop peptic ulcer.
14. The hairs are thin and scraggly. Some may have increase in facial hair and acne.

**Diagnosis**

Diagnosis is based on demonstrating increased cortisol production (increased plasma level of cortisol) and failure to suppress cortisol secretion by dexamethasone (dexamethasone suppression test). ACTH in plasma differentiates pituitary (ACTH dependent) causes from adrenal (ACTH independent) causes. In general, in ACTH independent cases, ACTH is less and, in ACTH dependent cases, ACTH is high.

**Treatment**

If the disease is due to tumor of adrenals or pituitary, surgical resection of the tumor is required. Medical adrenalectomy is done in few patients by inhibiting steroidogenesis with administration of high dose of ketokonazole or metyrapone that inhibit cortisol synthesis.

**Adrenocortical Insufficiency**

Hypofunction of adrenal gland may be primary or secondary variety.

**Primary adrenal insufficiency:**
1. Idiopathic atrophy of the gland (usually due to an autoimmune process).
2. Surgical removal of the gland
3. Infection (usually tubercular infection, but fungal and viral infection may also cause)
4. Bilateral hemorrhage into the gland
5. Metastatic invasion of the gland
6. Drugs: ketokonazole, metyrapone, mitotane, etc.

Primary adrenocortical insufficiency is called Addison's disease.

**Secondary adrenal insufficiency:**
1. Pituitary disease that decreases ACTH secretion
2. Hypothalamic disease that decreases CRH secretion

**Addison's Disease**

This is the condition of hyposecretion of glucocorticoid that results from progressive destruction of the adrenals.

**Etiology**

The atrophy of the adrenal cortex is usually idiopathic. An autoimmune mechanism is thought to be responsible. However, tubercular infection of the adrenal gland, secondary metastasis, amyloidosis and cytomegalovirus infection affecting the gland may destroy and produce the disease.

**Clinical Features**

Features, in order of their frequency are as follows:
1. Loss of weight and easy fatigability
2. Pigmentation of skin: The most characteristic feature of the disease is hyperpigmentation of skin, especially marked over the pressure points, sun-exposed areas and on the scar marks. Less plasma cortisol increases ACTH secretion by feedback mechanism. As ACTH has intrinsic MSH activity, pigmentation occurs.
3. Hypotension: Hypotension occurs due to decreased mineralocorticoid activity and decreased vascular reactivity. In sever cases, hypotensive shock occurs.
4. Anorexia, nausea and sometimes abdominal pain.
5. Hyponatremia (depletional), hyperkalemia.
6. Eosinophilia: Cortisol causes eosinopenia. Therefore, deficiency of it produces eosinophilia.
7. Patient develops rapid hypoglycemia on fasting; stress causes collapse.

**Diagnosis**

Decreased plasma level of cortisol with increased ACTH is diagnostic.

**Treatment**

Hormone replacement is mainstay of therapy.

**Adrenogenital Syndrome**

**Etiology**

This occurs due to 21β-hydroxylase deficiency, which decreases the synthesis of glucocorticoids and mineralocorticoids. Decreased plasma level of cortisol increases secretion of ACTH by feedback mechanism that increases the production of pregnenolone. Excess pregnenolone is diverted for the production of androgen. Thus, steroids in this syndrome are converted to excess of androgen that produces virilization.

**Features**

Excess androgen secretion in females causes masculinization and precocious pseudopuberty or female pseudohermaphroditism. The usual features are:
1. Hirsutism
2. Small breast
3. Receding hairline
4. Heavy arms and legs
5. Enlarged clitoris (clitoromegaly)
6. Male escutcheon (male distribution of suprapubic hair)
7. Androgenic flush.

**MINERALOCORTICOIDS**

Aldosterone is the major mineralocorticoid in humans.

### Regulation of Aldosterone Secretion

Aldosterone secretion is regulated mainly by three important stimuli: angiotensin-II, ACTH, and plasma K⁺ concentration. Many conditions alter aldosterone secretion (Table 59.5) by influencing these stimuli.

#### Angiotensin II

Angiotensin II is an important stimulator of aldosterone synthesis and secretion. It is formed from angiotensinogen, an α₂-globulin secreted from liver.

1. Renin, a circulating protease enzyme secreted from JG cells of kidney catalyzes the conversion of angiotensinogen to angiotensin I (A I).
2. A I is converted to angiotensin II (A II) by the action of angiotensin converting enzyme (ACE), present in the endothelial cell of blood vessels, especially in the pulmonary vasculature.
3. A II is further degraded to angiotensin III (A III). A II and A III stimulate the formation of aldosterone (Flowchart 59.10).

Thus, this system of aldosterone formation is called renin-angiotensin-aldosterone system (Details of RAS is described in first chapter of Renal System). A III is as potent as stimulator of aldosterone synthesis and secretion as A II. A I has no such activity. A II also stimulates synthesis of aldosterone. A II causes vasoconstriction and has many other functions (for details, refer to Chapter 75).

### Table 59.5: Conditions that alter aldosterone secretion.

<table>
<thead>
<tr>
<th>A. Conditions that increase secretion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Hemorrhage</td>
</tr>
<tr>
<td>2. Hypovolemia</td>
</tr>
<tr>
<td>3. Hyperkalemia</td>
</tr>
<tr>
<td>4. Hyponatremia</td>
</tr>
<tr>
<td>5. Standing</td>
</tr>
<tr>
<td>6. Anxiety</td>
</tr>
<tr>
<td>7. Physical trauma and surgery</td>
</tr>
<tr>
<td>8. Secondary hyperaldosteronism: Aldosterone secretion may increase in congestive heart failure, cirrhosis of liver, and nephritic syndrome.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B. Conditions that decrease secretion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Expansion of ECF volume</td>
</tr>
<tr>
<td>2. Hypernatremia</td>
</tr>
<tr>
<td>3. Hypokalemia</td>
</tr>
</tbody>
</table>

### Angiotensin Receptors

There are two types of angiotensin receptors: AT₁ and AT₂. AT₁ receptors are present on the zona glomerulosa cells of adrenal cortex. A II binds with AT₁ receptors and increases intracellular IP₃ and DAG production that in turn increase intracellular calcium. Rise in calcium facilitates aldosterone secretion.

**ACTH**

Cells of zona glomerulosa of adrenal cortex have ACTH receptors.

1. ACTH stimulates aldosterone synthesis and secretion, mediated by cyclic AMP and protein kinase.
2. However, the quantity of ACTH required to stimulate aldosterone secretion is more than the concentration required to stimulate cortisol secretion.
3. Moreover, the effect of ACTH on aldosterone secretion is temporary (Application Box 59.2). Aldosterone secretion declines, even if ACTH secretion is elevated, which is partly due to decreased renin secretion secondary to hypervolemia. Interestingly, deoxycorticosterone (another mineralocorticoid) secretion remains high.

#### Application Box 59.2

**ACTH stimulated aldosterone secretion is not transient in GRA:** Though ACTH-mediated aldosterone secretion is a temporary phenomenon; it is persistent in glucocorticoid-remediable aldosteronism (GRA). This is an autosomal dominant disorder, in which ACTH causes persistent hypersecretion of aldosterone that leads to hypertension. GRA occurs due to fusion of genes coding for 11b-hydroxylase and aldosterone synthase (as they are 95% identical and remain close together on chromosome 8) forming a hybrid gene called ACTH-sensitive aldosterone synthase. Therefore, ACTH effect on aldosterone becomes a prolonged phenomenon. Hypertension in GRA is treated by glucocorticoid, as this decreases the production of ACTH, which in turn decreases the synthesis of aldosterone. Hence, this syndrome is called glucocorticoid-remediable aldosteronism.

### Hyperkalemia

Aldosterone secretion is stimulated by increase in K⁺ in ECF.

1. Rise in ECF K⁺ activates voltage gated calcium channels that increases intracellular calcium.
2. Increased cytosolic calcium increases aldosterone synthesis and secretion.
3. Aldosterone, in turn promotes excretion of K⁺. Thus, aldosterone is a natural regulator of K⁺ level in ECF. Also, acute decrease in plasma Na⁺ or increase in plasma K⁺ stimulates the aldosterone synthesis and secretion.

**Mechanism of Action of Aldosterone**

Aldosterone binds with the cytoplasmic receptor. The HR complex moves to the nucleus where it induces transcription of mRNA. This increases new protein synthesis that alters cell function.

Aldosterone has both genomic and nongenomic actions:

**Genomic Action**

The gene activated by aldosterone is *sgk* gene (serum and glucocorticoid regulated kinase gene).

1. This is an early response gene that increases ENaC (Epithelial Na⁺ Channels) activity.
2. Aldosterone also increases mRNA for synthesis of proteins that form subunits of ENaC.
3. Aldosterone increases insertion of these channels on cell membrane and also increases their activity.

**Nongenomic Action**

Nongenomic action occurs rapidly. This is due to stimulation of the activity of Na⁺-K⁺ exchanger, which increases intracellular accumulation of Na⁺. The second messenger that mediates this action is IP₃.

**Physiological Actions of Aldosterone**

**Electrolyte and Water Balance**

The main function of aldosterone is to increase Na⁺ and water reabsorption and to promote K⁺ and H⁺ excretion from the kidney. Na⁺ reabsorption is the primary function, and other effects are mostly secondary to Na⁺ reabsorption. For Na⁺ reabsorption, aldosterone acts primarily on P cells (principal cells) of collecting duct and distal tubule of kidney. In fact, aldosterone controls only 3% of total Na⁺ reabsorption from the kidney. However, aldosterone deficiency results in significant hyponatremia.

Aldosterone increases Na⁺ and water reabsorption by following mechanisms:

1. It increases the number of Na⁺ channels in the epithelial cells of the kidney tubule.
2. It stimulates Na⁺-K⁺ ATPase activity, therefore, Na⁺ is reabsorbed and K⁺ is excreted.
3. It stimulates enzymes of Krebs cycle that facilitate ATP generation and provide energy for removal of Na⁺ from the interstitial fluid into the capillary.

Thus, aldosterone increases plasma Na⁺ concentration and decreases K⁺ concentration. Therefore, in aldosterone deficiency, hyponatremia is associated with hyperkalemia.

As Na⁺ is reabsorbed, Cl⁻ is transported in the same direction to maintain electroneutrality. The reabsorption of NaCl causes osmotic reabsorption of water. Thus, reabsorption of salt and water leads to ECF expansion.

**Aldosterone Escape Phenomenon**

Increased reabsorption of salt and water by aldosterone causes ECF expansion.

1. Increased ECF volume increases venous return to the heart that causes further distention of atria during their filling.
2. Atrial stretching increases synthesis and secretion of ANP from atrial myocytes.
3. ANP causes profound natriuresis and diuresis.
4. Therefore, ECF volume returns back to normal. This is called aldosterone escape phenomenon as kidney escapes from the effect of aldosterone.

**Dysfunctions of Aldosterone**

**Hypersecretion of Aldosterone**

Hyperaldosteronism can be divided broadly into two categories: primary and secondary.

**Primary Hyperaldosteronism**

The cause of excess aldosterone secretion is due to adrenal disease. The usual causes are aldosterone-producing adrenal adenoma (*Conn’s syndrome*), adrenal hyperplasia and adrenal carcinoma. *Renin secretion is decreased* in primary hyperaldosteronism (Fig. 59.11A).

**Conn's Syndrome**

This is the major cause of primary hyperaldosteronism. It occurs due to the tumor (adenoma) of zona glomerulosa of adrenal cortex.

1. The disease manifests with hypertension (due to sodium retention and ECF expansion), muscle weakness and fatigue (due to potassium depletion), and polyuria (due to impairment of urinary concentrating ability).
2. Edema is usually not a feature of primary hyperaldosteronism.
3. Hypokalemia, hypernatremia and low renin in plasma, and metabolic alkalosis are usual laboratory findings.

**Secondary Hyperaldosteronism**

Increased aldosterone secretion due to activation of renin-angiotensin system is called secondary hyperaldosteronism.

1. It is associated with increased renin in plasma (Fig. 59.11B), as decreased volume is the initiating stimulus for renin secretion. This is a major differentiating feature from primary hyperaldosteronism, in which renin secretion is less.
2. It occurs in congestive heart failure, cirrhosis of liver and nephritic syndrome. Renin secreting tumor (primary reninism) can also cause it.
3. **Edema is a usual feature** in secondary hyperaldosteronism. However, secondary hyperaldosteronism in **Bartter syndrome** and **Gitelman syndrome** occurs without edema.

**Bartter Syndrome**

It is a condition of hyperplasia of JG cells (juxtaglomerular hyperplasia).

1. The disease occurs due to **mutation in the Na⁺-K⁺-2Cl⁻ cotransporter gene**, which results in **high renin activity** and increased aldosterone synthesis.
2. Hyperaldosteronism produces **K⁺ depletion**.
3. Major features are **hypokalemic alkalosis and hypercalciuria**.
4. However, **blood pressure remains normal and there is no edema**.

**Hyposcretion of Aldosterone**

Hyposcretion of mineralocorticoid is usually associated with **adrenal insufficiency**. However, **isolated hypoaldosteronism** with normal cortisol production occurs in following conditions:

1. Inherited defect of biosynthesis of aldosterone.
2. Decreased renin production (**hyporeninemic hypoaldosteronism**).
3. Surgical removal of aldosterone secreting adenoma causing more resection of normal adrenocortical tissue.
4. During protracted heparin administration.
5. Selective unresponsiveness of Zona glomerulosa to angiotensin II (**hyperreninemic hypoaldosteronism**).
6. Pretectal disease of nervous system.
7. Severe postural hypotension.

**SEX STEROIDS**

Sex steroids secreted from adrenal gland are called **adrenal androgens**. The major adrenal androgens are **dehydroepiandrosterone** and **androstenedione**.

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**Functions of Adrenal Androgens**

Adrenal androgens have less than 20% of activity of testicular androgen.

1. **Adrenarche**: At the onset of puberty in both boys and girls, in the **stage 1** (before the physical development of puberty), secretion of adrenal androgen becomes **high** before the gonadal secretions are significantly elevated. This is called **adrenarche**. It is proposed that adrenarche **initiates the process of puberty** in which adrenal androgen sensitizes the gonads to secrete more sex hormones and **sensitizes the sex organs** to the effects of sex hormones.
2. They exert **masculinizing effect**: However, the masculinizing effect is less in normal concentration of adrenal androgens. These effects are prominent only when the hormones are secreted in excess amount.
3. **Promote protein synthesis**: They are protein anabolic, increase protein synthesis.
4. **Facilitate growth**: They **facilitate growth**. In adult males, they accentuate the already developed sex characteristics. However, in prepubertal boys, in excess, they develop secondary sex characteristics precociously. This is called **precocious pseudopuberty**.
5. **Source of estrogen**: Adrenal androgens are converted to estrogen in the peripheral tissue and serve as the **major source of estrogen in males and postmenopausal females**.
Dysfunctions of Adrenal Androgens

Excess of adrenal androgens results in **virilization**. However, along with increased secretion of adrenal androgens, other adrenocortical hormones are secreted in larger quantity. Therefore, the features are collectively called as **“virilization syndromes”**.

1. One such example is **“Adrenogenital syndrome”** as described earlier in this chapter.
2. In males, they cause **precocious pseudopuberty**.
3. In females they cause **pseudohermaphroditism** and **adrenogenital syndrome**.

**CHAPTER SUMMARY**

**KEY CONCEPTS**

1. Glucocorticoids have many important functions. Permissive action, metabolic action, effects on blood cells and immunity are examples. However, anti-inflammatory and antiallergic properties of cortisol are very useful in the treatment of many acute and life-threatening conditions. The immunosuppressive effect of cortisol helps in prevention of transplant rejection.
2. Mineralocorticoids regulate serum electrolyte (especially K⁺) and water metabolisms of the body. Thus, aldosterone maintains ECF volume and blood volume. Cortisol maintains vascular reactivity to assist in control of blood volume and pressure.
3. Sex hormones, especially adrenal androgens (mainly DHEA) sensitize gonads for secretion of sex hormones at the time of puberty. Therefore, adrenocortical deficiency leads to delayed puberty or dysfunctions of puberty.

**Important to Know (Must Read)**

1. In examination, Describe the functions of glucocorticoids (or cortisol) usually comes as a **Long Question**. Regulation of secretion, mechanism of action, physiological actions and dysfunctions of aldosterone may come as a **Long Question**.
2. Regulation of cortisol synthesis and secretion, Mechanism of action of cortisol, Effects of cortisol on inflammation, Effects of cortisol on allergy, Effects of cortisol on immunity, Effects of cortisol on blood cells, Permissive actions of cortisol, Cushing's syndrome (etiology, features diagnosis and treatment), Addison's disease (etiology, features diagnosis and treatment), Conn's syndrome, Adrenogenital (Virilization) syndrome, Aldosterone (physiological actions, regulation of secretion, mechanism of action), Mechanism of action of glucocorticoids, Aldosterone escape phenomenon, are usual **Short Questions** in exam.
3. In **Viva**, examiner may ask… List the hormones of adrenal cortex and the layer from where they are secreted, Specific enzymes involved in synthesis of adrenocortical steroids and the diseases produced by their deficiencies, Functions of cortisol especially permissive actions of cortisol, effects of cortisol on inflammation, allergy, immunity and blood cells, Why steroid therapy should not be stopped abruptly? Why adrenal deficient patients should not fast? How cortisol excess complicates diabetes mellitus? Why injection of cortisol is a must during treatment of shock? Why bone X-ray should be checked in patients receiving steroid therapy for a prolonged period? Why one should be cautious while infusing glucose solution in cortisol deficiency? What are the indications and contraindications for cortisol use? Name some important synthetic steroids and their glucocorticoid activity, What is rapid action of steroids? What is the role of cortisol in stress? Effects of cortisol on intermediary metabolism (carbohydrate, protein and fat), Effects of cortisol on cardiovascular system and central nervous system, Effects of cortisol on bone, connective tissue and musculoskeletal system, Effects of cortisol on fetus, Effects of cortisol on gastrointestinal tract and endocrine system, What is Cushing’s syndrome and what are its features? Also, how these features develop? What is Addison's disease and what are its features? Physiological actions of aldosterone, What are the conditions that increase or decrease aldosterone secretion? What is aldosterone escape phenomenon? What is Conn's syndrome and what are its features? Functions of adrenal androgens, What is adrenogenital (Virilization) syndrome and what are its features? Regulation of glucocorticoids secretion, Mechanism of action of glucocorticoids, Regulation of aldosterone secretion, Mechanism of action of aldosterone, What is apparent mineralocorticoid excess? Cause and features of hyposecretion of aldosterone, Why ACTH stimulated aldosterone secretion is not transient in GRA (Glucocorticoid-remediable aldosteronism), Steps of synthesis of Cortisol, Steps of synthesis of Corticosterone, Steps of synthesis of Aldosterone, Steps of synthesis of Androstenedione, Steps of synthesis of Estradiol, and effect of deficiency of key enzymes like 21β-hydroxylase, 11β-hydroxylase, 17α-hydroxylase and 3β-hydroxysteroid dehydrogenase and cholesterol desmolase.
The digestion, storage and utilization of nutrients are processes that require higher degree of regulation. Pancreas plays a crucial role in controlling these processes. Pancreas has two parts: the exocrine pancreas, which constitutes about 80% of the pancreatic tissue that controls digestion, and the endocrine pancreas, which constitutes 1–2% of the pancreatic tissue that controls storage and utilization of nutrients. Endocrine pancreas is an important endocrine organ as it secretes insulin, the only antidiabetogenic and antiketogenic hormone in the body.

Importance of insulin-glucagon ratio: Pancreas secretes another three hormones: glucagon, somatostatin and pancreatic polypeptide. Glucagon has metabolic actions that are opposite to that of insulin:
1. **Insulin is anabolic and promotes storage of nutrients** (glucose, fatty acids and amino acids), whereas glucagon is **catabolic and promotes mobilization of nutrients** from their storage depots into the bloodstream.
2. Insulin and glucagon, by their opposing effects, control metabolism of carbohydrate, fat and protein to ensure energy supply during basal and active states. As insulin and glucagon have reciprocal physiological actions, the **ratio of insulin to glucagon** is more important than their individual concentrations in the regulation of intermediary metabolisms.

**Scientists contributed**

FG Banting (1891–1941) and JJ Rickard MacLeod (1876–1936) were awarded the **Nobel Prize in Physiology or Medicine** 1923 for their discovery of insulin. They studied the role of insulin for the control of carbohydrate metabolism. They acknowledged and shared the prize with Charles H Best and James Bertram Collip, who had also worked with them.
Section 6: Endocrine Physiology

3. Usually, the conditions that stimulate secretion of one inhibit the secretion of other, because when one is essential for the body the other is usually not.

4. **Deficiency of insulin produces diabetes mellitus** and its excess results in hypoglycemia. On the contrary, **deficiency of glucagon produces hypoglycemia** and its excess worsens diabetes mellitus.

5. Usually, insulin deficiency is associated with glucagon excess.

### PHYSIOLOGICAL ANATOMY

**Islets of Langerhans**

The endocrine pancreas, which constitutes less than 2% of the total pancreatic tissue, is formed by **islets of Langerhans** (named after the German medical student, Paul Langerhans who first described it in a dog pancreas in 1869). Islets are more abundant in the tail than the head and body of the pancreas. There are about **1 million islets** in the human pancreas. Islets are oval in shape with diameter ranging from 50 to 300 µm (Fig. 60.1). Each islet contains about **2,500 cells**.

**Scientist contributed**

Paul Langerhans Jr (1847–1888), a German pathologist and physiologist was born into a family of physicians and scientists, known for two medical eponyms. He made his first major contribution before he finished his medical school, with the discovery of epidermal nonpigmentary dendritic cells that are derived from the bone marrow and play a role in cell-mediated immunity. These cells are known as **Langerhans cells**. Second, Langerhans made the first detailed description of the pancreas during his studies for his doctorate degree at the Berlin Pathological Institute in 1869. He described different types of cells in the pancreatic islets, that later became known as the **islets of Langerhans**.

**Fig. 60.1:** Histology of pancreas showing islets of Langerhans.

**Fig. 60.2:** Structure of islet of Langerhans. Note, beta cells constitute majority of cell mass of the islet.

**Four type of cells** present in the islets:
1. **β cells** (B cells): Secrete **insulin**.
2. **α cells** (A cells): Secrete **glucagon**.
3. **δ cells** (D cells): Secrete **somatostatin**.
4. **F cells** (PP cells): Secrete **pancreatic polypeptide**.

**β Cells**

They constitute 70–80% of islet cells that **secrete insulin**. The cells are placed centrally in the islet (Fig. 60.2):
1. The diameter of each cell is 10–20 µm.
2. Packets of insulin are present in the form of **secretory granules** in the cell cytoplasm that measure about 0.25 µm.
3. Each packet of insulin is present in a vesicle and a clear space or halo exists between the wall of the vesicle and the packet.
4. A developed system of microtubules and microfilaments are present in the cytoplasm that facilitates the exocytosis of vesicles.

**α Cells**

They constitute 15–20% of islet cells that **secrete glucagon**. They are present in the periphery of the islet.

**δ Cells**

They constitute about 10% of the islet cells that **secrete somatostatin**. The cells are typically located toward the periphery between the β and α cells.

**F Cells**

They constitute 1–2% of islet cells that **secrete pancreatic polypeptide**. The cells are distributed randomly among the D cells.
**Fate of islet hormones:** The hormones secreted from islets are released into the pancreatic vein from where they are transported into the portal vein and from there they enter general circulation:

1. This special arrangement provides liver the first and maximum access to the proper concentrations of islet hormones as they are secreted, and then to other tissues in the body.
2. Thus, liver being the primary site of substrate metabolism avails maximum advantage of actions of islet hormones, especially of insulin and glucagon.

**Islet blood supply:** Islets are highly vascular:

1. They receive about 10% of the total pancreatic blood supply though they constitute only about 1% of the pancreatic tissue.
2. There is a portal arrangement of the blood vessels of pancreas, which allows hormones secreted from a cell group to reach the other islet cells.
3. This arrangement is essential for paracrine regulation of hormone secretion from the islet cells.

**Innervation:** Islet cells are neuroectodermal in development:

1. They receive both parasympathetic and sympathetic innervations.
2. Usually, stimulation of β adrenergic receptor increases insulin secretion and α adrenergic receptor decreases it.
3. Increased vagal (cholinergic) activity increases insulin secretion.

**INSULIN**

**Structure of Insulin**

Insulin is a peptide hormone consisting of two chains, A and B, which are connected by disulfide bridges. The molecular weight of insulin is 6,000:

1. The A chain contains 21 amino acids and B chain 30 amino acids (Fig. 60.3).
2. The final structure of insulin is determined by the N-terminal and C-terminal amino acids of A chain, and the hydrophobic character of the amino acids at the C-terminal of B chain.
3. However, the hydrophobic character of the amino acids at the C-terminal of B chain is important for biological activity of insulin.
4. Insulin is synthesized as monomers that immediately form the crystalline hexamer unit within two zinc atoms (Application Box 60.1).

**Application Box 60.1**

**Synthetic insulin:** Insulin is synthesized in pharmaceuticals usually as crystalline zinc insulin. This preparation is the fast acting insulin. Usually, insulin is injected subcutaneously as it is absorbed slowly to provide a slow rise in insulin concentration that resembles basal insulin secretion from pancreas.

**Scientist contributed**

Christian de Duve (born, 1917) a Belgian cytologist and biochemist, was awarded the Nobel Prize for Physiology or Medicine in 1974, for his research work on lysosomes (the cell’s digestive system) and peroxisomes (cell metabolic system), and shared his Nobel honors with Albert Claude and George E. Palade. He won Nobel Prize for his research in the field of diabetes mellitus and how his revelations led to the discovery of lysosomes. de Duve demonstrated that hepatectomized dogs needed considerably less glucose to be kept normoglycemic after injection of a supramaximal dose of insulin than did an intact animal. He proved that the liver is the major site of insulin action.

**Synthesis, Secretion and Metabolism**

**Synthesis of Insulin**

Like other peptide hormones, insulin is synthesized as preproinsulin, containing 110 amino acids. The insulin gene belongs to the superfamily of genes that encode synthesis of a variety of insulin like growth factors. The insulin gene is located on the short arm of the chromosome 11. Insulin is first formed as preproinsulin in the ribosome of rough endoplasmic reticulum. The preproinsulin contains four sequential peptides:

1. An N-terminal signal peptides
2. The B chain of insulin
3. A connecting peptide (C peptide), and
4. The A chain of insulin.

The N-terminal signal peptide is immediately cleaved from the preproinsulin molecule to form the proinsulin, a 86 amino acid peptide, which then enters the Golgi apparatus.
1. In the Golgi apparatus, the disulfide bridges are established that allows the proinsulin molecule to be folded (Figs. 60.4A and B). The A and B chains of insulin are linked by the connecting peptide (C peptide). The C peptide not only connects, but also facilitates folding of A and B chains.

2. During packaging in the Golgi apparatus into the granules, the proinsulin is cleaved to form insulin molecules, and C peptides are retained in the granules.

3. When insulin is secreted, the C peptide is also released in equimolar concentration with insulin.

4. Insulin is associated with zinc as molecule matures. The zinc insulin crystals form the dense central core of the granule with a clear space around.

5. The C peptide is present in the clear space between the membrane of the granule and the central dense core.

6. Also, during insulin secretion, some amount of proinsulin is secreted from the granules. Thus, β-cell granules secrete the following substances:
   1. **Insulin**: Insulin constitutes about 95% of secretion from β-cell.
   2. **Proinsulin**: Proinsulin is a 86 amino acid peptide. It has about 10% of biological activity of insulin. It is secreted as only about 3% of the amount of secreted insulin. However, as plasma clearance is slower (half life 15-30 min) than insulin, the plasma concentration in fasting state is about 10-15% of insulin concentration.
   3. **C peptide**: It is a 31 amino acid peptide. The amount of C-peptide secreted is about 7% of the insulin secreted from the gland:
      - It has no biological activity of insulin. Its importance lies in the fact that it is secreted in equimolar ratio with insulin.
      - Measurement of C-peptide has advantages over measurement of insulin. Hepatic metabolism of C-peptide is negligible; therefore its concentration in plasma directly reflects the β-cell activity.

   Moreover, C-peptide assay does not measure exogenous insulin and do not cross-react with insulin antibodies, whereas insulin measurements have these fallacies.

   Therefore, C-peptide assay is the better index of β-cell activity (Application Box 60.2).

**Substances Having Insulin Like Activity**

There are few substances present in human plasma having insulin like activity. However, their insulin activity is very poor. Therefore, insulin deficiency or insulin resistance leads to diabetes. Important among them are nonsuppressible insulin like activities (NSILAs) that are mainly somatomedins (IGF I and II). NSILAs are not suppressed by anti-insulin antibodies. Following are the insulin like activities in human plasma:

1. Insulin
2. Proinsulin
3. NSILA (IGF I, IGF II and IGF bound proteins).

**Application Box 60.2**

**C peptide measurement**: As C peptide is secreted in equimolar concentration with insulin, and is least degraded in liver, its concentration in plasma provides a good index of beta cell secretory capacity. It is measured by RIA.

The major regulator of insulin synthesis is the plasma glucose concentration. Glucose stimulates insulin secretion. Therefore, feeding (hyperglycemia) increases and fasting (hypoglycemia) decreases insulin synthesis and secretion. Glucose increases insulin synthesis by increasing translation of insulin mRNA and transcription of insulin gene.

**Secretion of Insulin**

Insulin secretion is greatly influenced by plasma glucose concentration. Elevation of glucose level in plasma is an important stimulator of insulin secretion. Factors that control insulin secretion are listed in Table 60.1.
Regulation of Insulin Secretion

Insulin secretion is mainly regulated by the feedback control signal provided by nutrients level in plasma. When the nutrients are more, insulin secretion increases to facilitate their metabolism and use, and when nutrients are less, insulin secretion is less.

**Plasma Glucose**

Glucose is the most important stimulator of insulin secretion. With the rise in plasma glucose level, there is almost a linear rise in plasma insulin concentration in the range of 50–300 mg% of plasma glucose (Fig. 60.5). Insulin secretion is almost nil below 50 mg% and no extra secretion above 300 mg% of plasma glucose. The secretion of insulin in response to rise in plasma glucose concentration occurs in two phases.

**First Phase Response**

Immediately following the rise in plasma glucose (in response to i.v. glucose infusion), insulin secretion increases rapidly to reach a peak within 1–2 minutes and then decreases to basal level in another 2 to 3 minutes (Fig. 60.6).

1. This is the first and rapid phase of insulin secretion in response to sudden increase plasma glucose concentration.
2. The first phase response is due to release of already synthesized and stored insulin from granules of β cells.

**Second Phase Response**

In the next phase, the rise in plasma insulin concentration occurs slowly that reaches a peak in about 60 minutes and then remains elevated for 3–5 hours. The second and slow phase insulin response is due to stimulation of insulin synthesis and secretion.

**Mechanism of Glucose-induced Insulin Secretion**

Glucose enters β-cells of pancreas via GLUT 2:

1. In the β-cells, glucose is utilized by glycolytic enzymes (see below) to pyruvate that enters TCA cycle to produce ATP.
2. Increased intracellular ATP inhibits ATP-sensitive K⁺ channels, which increases cytosolic K⁺ by decreasing K⁺ efflux (Fig. 60.7).
3. This depolarizes the β cells, which in turn opens the Ca²⁺ channels. Ca²⁺ influx increases cytoplasmic Ca²⁺ that facilitates Ca²⁺-mediated exocytosis of insulin granules.
4. Plasma K⁺ is a natural regulator of insulin secretion. Hypokalemia decreases insulin secretion (Application Box 60.3).

**Response depends on route of administration:** The insulin response to plasma glucose depends on the route of glucose administration:

1. The response of insulin secretion to orally administered glucose is more than the glucose administered intravenously.
2. When given orally, glucose stimulates secretion of hormones from gastrointestinal tracts. Many GI hormones such as gastrin, secretin, enteroglucagon, GLP1, and GIP are insulinogenic.
3. They stimulate insulin secretion in addition to its secretion that occurs due to rise in plasma glucose.
Therefore, insulin response is higher when glucose is given orally than intravenously.

**Application Box 60.3**

**Effects of hypokalemia:** Hypokalemia decreases insulin secretion. Therefore, patients with hypokalemia as seen in primary hyperaldosteronism develop impaired glucose tolerance. Similarly, patients receiving thiazide diuretics develop glucose intolerance as thiazide causes hypokalemia and may also produce damage to pancreatic islets.

**Other Stimuli**

Insulin secretion is also stimulated by **products of protein digestion.** Arginine, leucine, lysine and alanine are potent stimulator of insulin release:
1. **Glucose and amino acids** facilitate the insulinogenic action of each other.
2. **Triglycerides and fatty acids** also stimulate insulin secretion.
3. **Ketoacids** are also insulinogenic.
4. **Cholinergic vagal stimulation** strongly increases insulin secretion, which provides the basis of secretion in the cephalic phase of digestion.
5. **Catecholamines via β receptors stimulate** and via α receptors inhibit insulin secretion.
6. **During exercise,** sympathetic stimulation causes α-adrenergic inhibition of insulin secretion that tries to prevent hypoglycemia during exercise.

Obesity significantly increases insulin secretion and activity of insulin receptors. However, down-regulation of receptor causes insulin resistance (see below). Therefore, obesity contributes to development of type II diabetes mellitus (NIDDM), which can be prevented by regular physical exercises and maintaining normal weight.

**Metabolism of Insulin**

Insulin circulates freely (unbound to carriers) in the plasma, therefore, its **half-life is 5–8 minutes.** The metabolic clearance rate of insulin is about 800 mL/min.

1. The basal insulin release to the circulation is about 0.5 – 1 unit/h (20–40 µg/h).
2. Following food intake, release of insulin increases to about 10 times.
3. The total release of insulin into peripheral circulation in a day is about 30 units.
4. Insulin is metabolized mainly in the liver and kidney.

**C peptide Activity**

**C peptide** is secreted in equimolar concentration to that of insulin. However, the basal plasma concentration of C peptide is 5 times more than insulin (1 ng/mL). This is because the **metabolic clearance of C peptide is slower** than insulin. Therefore, the concentration of C peptide in plasma is a better index of β cell activity than the insulin (Clinical Box 60.1).

**Clinical Box 60.1**

**C Peptide is a better marker of cell activity:** C peptide is secreted in equimolar concentration with insulin. But, as the metabolic clearance of C peptide is slower than insulin, its concentration in plasma provides better knowledge of β cell activity than the insulin. Therefore, C peptide assessment is done in good laboratories to assess β cell functional status.
Mechanism of Action

Insulin acts on insulin receptors present on various cells. The major target tissues of insulin are liver, skeletal muscle and adipose tissues.

Insulin Receptor

Insulin receptor (IR) is a glycoprotein tetramer consisting of two α and two β subunits. The α subunits are present on the membrane extracellularly, whereas the β subunits traverse the membrane. Thus, β subunits have extracellular domain, membrane domain, and intracellular domain (Fig. 60.8). The α and β subunits are glycosylated.

They are bound to each other by disulfide bridges. The insulin receptor gene is located on the chromosome 19, which belongs to the superfamily of genes that code for other growth factors also.

Down regulation of IR: Insulin binds with α subunits of the insulin receptor, which results in conformational change of the receptor. The HR complex is then internalized by endocytosis:

1. The hormone is degraded in the cytoplasm, whereas the receptor is either degraded, or stored or recycled back to the membrane.
2. Therefore, excess insulin activity down-regulates the insulin receptors.

Fig. 60.8: A: Mechanism of action of insulin. Binding of insulin with α-subunit causes autophosphorylation of intracellular part of β-subunit of the insulin receptor. This causes phosphorylation of intracellular proteins like IRS and Shc that in turn activate phosphorylation cascades and generate other signals. Phosphorylation of Grb 2 proteins activates a series of signals that finally activate mitogen activated protein (MAP) kinase, which induces gene transcription. Phosphorylation of IRS activates intracellular enzymes that alter cell metabolisms, and transfers GLUT from cell to the cell surface. Increased GLUT on cell surface increases glucose uptake of the cell.
3. This is one of the mechanisms by which insulin resistance (decreased sensitivity to insulin) develops in obesity.

**Hormone Mechanisms**

Insulin binding to its receptors trigger following events:

1. The binding of insulin to α subunits brings about conformational change in the β subunits.
2. The intracellular domain of β subunits possesses tyrosine kinase activity. Conformational change of the β subunits activates its tyrosine kinase activity. This produces autophosphorylation of β subunits on tyrosine residues.
3. Autophosphorylation triggers phosphorylation of many intracellular proteins that alter cell functions. Also, dephosphorylation of proteins occurs.
4. The active tyrosine kinase phosphorylates tyrosines on insulin receptor substrates (IRS 1 and IRS 2):
   - IRS proteins are docking proteins to which a variety of downstream proteins bind. Thus IRS phosphotyrosines serve as docking site and activating site for different protein kinases and protein phosphatases (see Fig. 60.8).
   - The IRS also serves as facilitatory proteins that link to membrane G proteins, phospholipases, and ion channels.
5. Phosphorylation of IRS causes activation or deactivation of many target enzymes, translocation of GLUTs (glucose transport proteins) to the cell membranes and induction or suppression of genes in the nucleus. This results in synthesis of different intracellular proteins.
6. The GLUTs that move to the cell membrane facilitates glucose entry into the cell. The insertion of different protein channels on the plasma membrane increases entry of amino acids, potassium, magnesium, and phosphates into the cell.
7. Activation of mitogenic proteins increases transcription of various factors that are essential for stimulation of gene expression, especially concerned with cell growth.
8. IRS also activates growth receptor binding protein-2 (GRB 2), which stimulates binding of GTP to ras oncogene (a proto-oncogene present in plasma membrane). This activates the enzyme glycogen synthase, a key enzyme that modulate the metabolic effects of insulin.

**Action through GLUT 4**

Binding of insulin with receptors rapidly mobilizes glucose transport into the muscle and adipose tissue cells. This process of glucose entry in to the cell is increased by about 20 times by the activation of a glucose carrier system in the plasma membrane:

1. Insulin rapidly recruits the glucose transporter 4 (GLUT-4), which is specifically meant for insulin-stimulated glucose uptake in skeletal and cardiac muscle, adipose tissue and other tissues.

2. In the basal state, GLUT 4 is internalized from cell membrane into the endosomes of the cell and maintained in the cytoplasm in vesicles.
3. On activation by insulin, the vesicles containing GLUT 4 are swiftly transported to the cell membrane.
4. Membrane of the vesicles fuses with the membrane of the cell during which GLUT 4 is inserted into the cell membrane (Fig. 60.9).
5. Insulin facilitates this process of recruitment of GLUT 4 from its intracellular pool to the membrane pool by activating the enzyme phosphoinositole-3-kinase, and perhaps this is the most important function of insulin (Application Box 60.4).

**Application Box 60.4**

Regular exercise prevents diabetes: Exercise facilitates the recruitment of GLUT 4 from its cytoplasmic pool into the cell membrane in all tissues of the body, especially in the skeletal muscle and adipose tissues. This, exercise promotes glucose uptake by the cells and decreases blood glucose level. The increased glucose entry into the cell persists for many hours after exercise. Moreover, exercise-induced glucose entry does not depend on insulin. It is likely that S-AMP-activated kinase facilitates GLUT 4 insertion from endosomes into membrane in exercise. Thus, to check the rise in plasma glucose, one should perform regular exercise. Also, regular exercise can cause prolonged increase in insulin sensitivity. However, diabetic patients should be careful during exercise. As acute exercise significantly decreases blood glucose level by facilitating glucose uptake by skeletal muscles, and during exercise absorption of injected insulin is more rapid, hypoglycemia may be precipitated in diabetic patients during exercise. Therefore, diabetic patients should decrease their insulin dose when they exercise or they should take extra calorie just prior to exercise.
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Physiological Actions of Insulin

Insulin is secreted in fed state, i.e. at the time of nutrient abundance. Therefore, insulin is known as the hormone of abundance. Insulin facilitates storage of nutrients when nutrients are present in excess of the energy need. It also suppresses mobilization of endogenous substrates. The stored nutrients are made available at the time of need like exercise, fasting, etc. Insulin produces its target effects by acting mainly on the liver, adipose tissue, and skeletal muscle.

On Carbohydrate Metabolism

Insulin increases glucose entry into the cell, stimulates its oxidation and promotes also its storage. At the same time insulin inhibits glucose production. Therefore, the primary function of insulin is to lower the plasma glucose concentration. It is the only hormone that decreases plasma glucose level (decreases basal glucose level and also prevents rise in plasma glucose following feeding). Insulin is the only effective anti-diabetogenic hormone. The antidiabetogenic functions of insulin are mediated by its action on liver, adipose tissue and muscle.

In general, insulin increases glucose entry into cells of almost all tissues of the body except brain (excluding ventromedial hypothalamus), RBCs, epithelium of GI tract, and renal tubules.

In Liver

In liver, insulin promotes glucose storage and prevents its production by following mechanisms (Flowchart 60.1):

1. Insulin facilitates glucose entry into the hepatic cell by inducing the action of the enzyme glucokinase. Glucokinase catalyzes phosphorylation of glucose to glucose-6-phosphate. Thus, by facilitating glucose entry into the cells and also simultaneously converting glucose into glucose-6 phosphates, insulin keeps cytoplasmic glucose concentration at lower level. Therefore, facilitated diffusion of glucose into the cell continues.

2. It stimulates glycolysis by activating the enzymes phosphofructokinase and pyruvate kinase. These actions convert glucose into pyruvic and lactic acids. Pyruvate and lactate are also oxidized by insulin as it stimulates pyruvate dehydrogenase activity. Thus, insulin decreases the cellular concentration of glucose and consequently helps in its facilitated diffusion into the cell.

3. It promotes glycogen synthesis in liver. In the liver cells, it activates the enzyme glycogen synthase complex that promotes formation of glycogen. Thus, it promotes storage of glucose in the form of hepatic glycogen.

4. It inhibits hepatic glycogenolysis, and therefore it decreases hepatic glucose output. Insulin achieves it by inhibiting the activity of the enzymes glycogen phosphorylase and glucose-6-phosphatase.

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Table 60.2: Types of glucose transporters and their functions.

<table>
<thead>
<tr>
<th>Types of GLUT</th>
<th>Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Secondary active transport (Na+–Glucose cotransport)</strong></td>
<td></td>
</tr>
<tr>
<td>SGLT 1</td>
<td>Increases absorption of glucose in the intestine and kidney tubules</td>
</tr>
<tr>
<td>SGLT 2</td>
<td>Increases reabsorption of glucose in the kidney tubules</td>
</tr>
<tr>
<td><strong>B. Facilitated diffusion via GLUTs (Na+ independent)</strong></td>
<td></td>
</tr>
<tr>
<td>GLUT 1</td>
<td>Basal glucose uptake in the placenta, brain, RBC, colon, kidney and other organs</td>
</tr>
<tr>
<td>GLUT 2</td>
<td>Acts as a glucose sensor in the β cells of pancreas</td>
</tr>
<tr>
<td>GLUT 3</td>
<td>Same as GLUT 1, i.e., basal glucose uptake in the placenta, brain, kidney and other tissues</td>
</tr>
<tr>
<td>GLUT 4</td>
<td>Causes insulin stimulated glucose uptake in the skeletal and cardiac muscle, adipose tissue, and other tissues</td>
</tr>
<tr>
<td>GLUT 5</td>
<td>Facilitates transport of fructose in the jejunum and into the sperm</td>
</tr>
<tr>
<td>GLUT 6</td>
<td>Acts as a pseudogene</td>
</tr>
<tr>
<td>GLUT 7</td>
<td>Increases transport of glucose 6-phosphate into the endoplasmic reticulum in hepatic and other tissues</td>
</tr>
</tbody>
</table>

6. Once insulin action is over, again they are internalized into the vesicles. In liver, insulin facilitates glucose uptake by activating the enzyme glucokinase not by GLUT 4.

Glucose Transporters

Glucose enters the cell by means of facilitated diffusion or by secondary active transport with sodium in intestine and kidney. In skeletal and cardiac muscle, adipose tissue and other tissues, entry of glucose into the cell is facilitated by insulin by increasing the number of glucose transporters (GLUTs) in the cell membrane. GLUTs promote the facilitated diffusion of glucose into the cell:

1. Glucose transporters are different from sodium dependent glucose transporters (SGLT 1 and SGLT 2) that mediate secondary active transport of glucose in the kidney and GIT.
2. GLUTs are expressed in different tissues. GLUTs are membrane proteins that span membrane 12 times.
3. About 14 different types of glucose transporters have been described (GLUT 1 – GLUT 14). However, the first 7 types have been extensively studied.
4. GLUT 1 and GLUT 3 are meant for basal glucose uptake by the cells.
5. GLUT 4 is primarily meant for insulin stimulated glucose uptake especially in the skeletal and cardiac muscle and adipose tissues (Table 60.2).
6. GLUT 2 acts as the β cell glucose sensor.
7. GLUT 5 facilitates fructose transport in jejunum and sperm.
8. GLUT 7 acts as glucose-6-phosphate transporter in the endoplasmic reticulum of liver and other tissues.
9. GLUT 6 is a pseudogene.
5. It also **inhibits gluconeogenesis**. This is achieved by two mechanisms: (i) insulin inhibits gluconeogenic enzymes (pyruvate carboxylase, phosphoenolpyruvate carboxykinase, and fructose-1, 6-diphosphatase), and (ii) insulin decreases hepatic uptake of gluconeogenic amino acids.

**In Adipose Tissue**
Insulin **stimulates entry of glucose** into the adipose tissue cells by **activating GLUT 4 and hexokinase activity**:
1. In fat cells, glucose is then **converted into α-glycerophosphate** (α-GP). The α-GP is used for the **esterification of fatty acids**.
2. It also promotes storage of fatty acids as triglycerides.

**In Skeletal Muscle**
Insulin **facilitates transport of glucose** into the muscle cells by **activating GLUT 4 and hexokinase activity**:
1. In muscle cell, **glucose is oxidized** by activation of the enzyme *pyruvate dehydrogenase*.
2. Glucose is also **stored as muscle glycogen**, which is stimulated by insulin.

**On Fat Metabolism**
Actions of insulin on fat metabolism are as profound as its influences on carbohydrate metabolism. Insulin **increases storage of fat** and inhibits mobilization and oxidation of fatty acids from fat depots. It decreases the level of free fatty acids and ketoacids in the plasma. Insulin is the **only anti-ketogenic hormone** in the body. The functions of insulin on fat metabolism are achieved by its actions on adipose tissue and liver.

**In Adipose Tissue**
Insulin **promotes storage of fat** in the adipose tissues in various ways:

1. **By inhibiting lipolysis**: It inhibits the activity of hormone sensitive lipase (HSL) in adipose tissues:
   - HSL causes breakdown of triglycerides into free fatty acids (FFA) and glycerol. Thus, insulin inhibits lipolysis and **decreases release of stored FFA**.
   - Decreased plasma FFA decreases the formation of ketoacids. Insulin also facilitates the use of ketoacids by peripheral tissues. Thus, insulin **profoundly decreases ketoacid level** in the plasma (see below).
2. **By promoting lipogenesis**: Lipids absorbed from intestine circulate in plasma in the form of triglycerides. Triglycerides cannot enter adipose tissue as such. They must be split by lipoprotein lipase, which is present in the vascular endothelium to FFA and glycerol. Then, FFA can enter adipose tissue and form TG there:
   - Insulin **promotes the enzyme lipoprotein lipase**, therefore makes FFA available from blood stream for formation of triglyceride in the adipose tissue cell (Fig. 60.10).
   - FFA is immediately **esterified to α-glycerophosphate** in adipose tissue, which is also induced by insulin.

**In Liver**
Insulin is **anti-ketogenic and lipogenic** in liver:
1. The anti-ketogenic function of insulin is achieved by **its stimulation of malonyl-CoA formation**. Normally, acetyl-CoA is converted to malonyl-CoA by the enzyme *acetyl-CoA carboxylase*. Insulin stimulates the activity of acetyl-CoA carboxylase and thus increases malonyl-CoA formation. Malonyl-CoA inhibits the enzyme *carbamoyl transferase* (CAT). CAT transfers FFA from cytoplasm into mitochondria for oxidation and then conversion into ketoacids. Thus, insulin by inhibiting CAT through malonyl-CoA prevents ketoacid formation (Fig. 60.11).
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2. Fatty acid is also synthesized from glucose by insulin. Insulin achieves this by three mechanisms:
   i. The rate-limiting step in fatty acids synthesis is the conversion of acetyl-CoA to malonyl-CoA by the action of acetyl-CoA carboxylase, which is activated by insulin. Malonyl-CoA is used as substrate for FFA synthesis.
   ii. Insulin also induces fatty acids synthase, the final step in fatty acid synthesis.
   iii. Insulin activates the HMP shunt by activating the enzyme glucose-6-phosphate dehydrogenase. HMP shunt increases the supply of reduced triphosphopyridine nucleotide, which is required for fatty acid synthesis. Thus, from circulation, FFA is shunted away from oxidation and ketogenesis to be stored in the liver.

3. Insulin facilitates synthesis of cholesterol in the liver from acetyl-CoA by activating the enzyme HMG-CoA reductase, the rate-limiting step in the process.

On Protein Metabolism
Insulin is an anabolic hormone. It facilitates protein synthesis in the muscles and liver by following mechanisms:
1. It facilitates amino acid entry into the muscle cells. Therefore, it decreases plasma amino acid concentration. Thus, supply of amino acids for gluconeogenesis is also decreased.
2. It promotes protein synthesis in ribosomes. This is achieved by induction of gene transcription for various proteins by insulin (Fig. 60.12).
3. Insulin also decreases degradation of RNA.
4. Insulin inhibits proteolysis by decreasing lysosomal activity. Thus, it decreases release of amino acid from muscle and inhibits their oxidation.

On Plasma K+ Concentration
Insulin facilitates the rapid entry of K+ into the cell by stimulating Na+-K+ ATPase activity in the membrane. Thus, it decreases plasma K+ level:
1. Insulin is considered as a physiological regulator of plasma K+ concentration.
2. Infusion of insulin and glucose markedly decreases plasma $K^+$ level; and therefore is very useful in the management of acute renal failure (Clinical Box 60.2).

3. This is the reason why diabetic patients receiving insulin sometimes develop hypokalemia.

**Clinical Box 60.2**

Insulin is given with glucose in the treatment of hyperkalemia: Hyperkalemia is a feature of acute renal failure (ARF) and it is dangerous if remains untreated for a longer time. Insulin for its rapid and profound hypokalemic effect may be prescribed in the treatment of hyperkalemia as seen in acute renal failure, for prompt relief. Glucose is administered simultaneously, as insulin induces hypoglycemia.

**Other Actions of Insulin**

1. Insulin increases entry of phosphate and magnesium into the cell.
2. It also increases reabsorption of $K^+$, $Na^+$, and phosphate from the kidney.
3. Insulin decreases food intake. This action is partly mediated by its inhibitory effect on neuropeptide Y release from hypothalamus.
4. Chronic insulin excess increases body weight and adipose tissue mass. The leptin level in plasma increases that promotes satiety (decreases food intake).
5. Growth promoting actions: Insulin stimulates synthesis of macromolecules in cartilage and bone that promotes their growth. Insulin stimulates transcription of genes for growth factors such as IGF-I and II. Thus, insulin facilitates growth of the individual, in addition to its stimulation of protein synthesis. Therefore, deficiency of insulin in childhood (as in diabetic children) decreases their height, growth, and maturation.

In summary, insulin by acting on liver, adipose tissue and muscle increases glucose uptake and its storage, decreases lipolysis, promotes lipogenesis and stimulates protein synthesis (Flowchart 60.2).

**Applied Physiology**

**Insulin Deficiency (Diabetes Mellitus)**

Deficiency of insulin results in diabetes mellitus (DM). Diabetes mellitus is the most common endocrine disorder in both developing and developed worlds. Recently, the incidence of DM in India is increasing like an epidemic. Every one in three in urban and every one in five in rural population are found to have latent (glucose intolerance) or frank DM. Change of lifestyle, easy access to food (increased calorie intake), junk foods, sedentary life, environmental factors and stressful life are among the causes for such a spurt in diabetes in India.

**Flowchart 60.2: Summary of insulin actions.**

![Flowchart](image-url)

(GP: Glycerophosphate; HSL: Hormone sensitive lipase; LL: Lipoprotein lipase).
Table 60.3: Etiological classification of diabetes.

<table>
<thead>
<tr>
<th>Type of DM</th>
<th>Etiology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1 DM</td>
<td>Insulin-dependent diabetes mellitus (IDDM)</td>
</tr>
<tr>
<td>Type 2 DM</td>
<td>Non-insulin-dependent diabetes mellitus (NIDDM)</td>
</tr>
</tbody>
</table>

Insulin only matters: Though there are few more chemicals (insulin like substances) in the body having insulin activity, they are not adequate to fulfill metabolic actions of insulin in states of insulin deficiency. These substances are proinsulin and somatomedins (IGF I and IGF II). These chemicals have only about 10% of insulin activity. Therefore, insulin deficiency leads to DM.

Type of DM: Etiologically diabetes has been traditionally classified into two:
1. Type I DM or insulin-dependent DM (IDDM) as it is associated with profound insulin deficiency and requires insulin replacement therapy.
2. Type II DM or non-insulin-dependent DM (NIDDM) as it is associated with normal insulin level in plasma and patient is usually treated without insulin replacement therapy.

However, recently it has been observed that more than 25% of type 2 DM ultimately develop insulin deficiency requiring insulin replacement therapy. Therefore, the classification of IDDM and NIDDM has become obsolete. Etiological classification of diabetes is depicted in Table 60.3.

Major Types of DM

Diabetes is primarily classified into two types: type-1 and type-2.

Type-1 Diabetes Mellitus
Type 1 DM is characterized by insulin deficiency, which is usually caused by selective autoimmune destruction of β-cells of pancreas. Therefore, the disease has traditionally been called insulin dependant diabetes mellitus (IDDM):
1. The disease starts with insulitis. Many patients demonstrate antibodies against β-cell surface antigens.
2. If one of the twins develops the disease the other twin has more chance of developing the disease than people in the general population (the concordance rate is < 50%).
3. The disease usually starts early in childhood, and ketoacidosis is common (Table 60.4).
4. The disease is treated by insulin replacement.
5. Sometimes it occurs due to mutation of proinsulin gene that decreases insulin synthesis.

Type-2 Diabetes Mellitus
Type 2 DM is characterized by impaired ability of the target cells to respond to insulin. This is usually caused by insulin receptor resistance. The insulin secretion may be normal or even more. Therefore, the disease has traditionally been called non-insulin dependant diabetes mellitus (NIDDM):
1. The chance of identical twin developing the disease (concordance rate) is 100%.
2. The disease usually starts late, in third or fourth decade of life.
3. The patients are usually overweight and sedentary in their habit.
4. Feeding habit and environment also contribute.
5. Chronic stress is usually associated with it.
6. Usual complication is hyperosmolal coma.
7. Oral antidiabetic drugs are usually preferred for treatment. However, insulin may also be prescribed.
Mechanism of insulin resistance: The exact mechanism of insulin resistance in type II DM is not known. However, insulin resistance has been highly correlated with body weight gain and few other factors. The following factors contribute to insulin resistance:

1. **Obesity:** In obesity, insulin fails to transport glucose into the tissues. Obesity eventually leads to hyperinsulinemia, hyperlipidemia and accelerated atherosclerosis that are part of metabolic syndrome (Clinical Box 60.3). These are combinely called as metabolic syndrome of obesity. With reduction in body weight, insulin resistance and dyslipidemia decrease.

2. **Adipokines:** The chemical signals originating from adipose tissue are recently proposed to be contributing factors for insulin resistance. Fat cells secrete a group of hormones called adipokines that influence insulin resistance. The known adipokines are:
   - Leptin
   - Adiponectin
   - Resistin
   - Tumor necrosis factor (TNFα) Usually, leptin and adiponectin decrease, and resistin and TNFα increase insulin resistance. Resistin and TNFα are increased significantly in NIDDM.

3. **Decreased second messengers:** Knockout of intracellular second messengers that are usually formed following autophosphorylation of tyrosine kinase has been recently reported to be the major factor in insulin resistance.

4. **Decreased GLUT:** Knock out of glucose transporters in skeletal muscles and adipose tissue has been suggested as a contributing factors.

5. **Free fatty acids:** Increased FFA contributes to insulin resistance.

Secondary Diabetes Mellitus

Diabetes also occurs due to the diseases of pancreas like pancreatitis, or following pancreatectomy. Diabetes can also occur in Cushing’s syndrome (cortisol increases plasma glucose) and acromegaly (growth hormone increases plasma glucose). These forms of diabetes are included under the category of secondary diabetes mellitus. This category constitutes about 5% of the total diabetes.

Maturity Onset Diabetes of the Young (MODY)

MODY constitutes about 5% of all cases of diabetes. This is a form of Type II (maturity-onset) DM in younger individuals:

1. Six types of MODY have recently been described, MODY 1 to MODY 6. Commonest among them is MODY 3 that accounts for 20–75% of all MODYs.
2. It occurs due to mutation of HNF1-α gene (gene for hepatic nuclear factor 1 alpha).
3. Patients with MODY 3 are mostly in their adolescence, diabetic complications are frequent, but they have a greater hypoglycemic response to sulphonylureas than the patients with type II DM.
4. MODY 2 is the next common (10–65%) group that occurs due to mutation of glukokinase gene. They present with mild hyperglycemia from birth, but they are stable and managed by diet alone. Other forms of MODYs are rare.

Gestational DM

This refers to the hyperglycemia occurring for the first time during pregnancy. 80% of women with gestational diabetes develop diabetes permanently.

Features of DM

Diabetes mellitus is characterized by polyphagia, polyuria, polydipsia, weight loss in spite of increased food intake,
hyperglycemia, and glycosuria. If not treated, the disease leads to ketosis, acidosis, and coma. In chronic cases, there may be diabetic retinopathy, neuropathy and nephropathy.

**Polyphagia**

Glucose entry into cells of brain except in ventromedial hypothalamus (VMH) is independent of insulin. Thus, VMH depends on insulin for its glucose uptake:

1. VMH is the *satiety center* that normally inhibits the lateral hypothalamus (LH), the *feeding center*.
2. As VMH depends on insulin for glucose uptake and utilization, *insulin deficiency in diabetes decreases the activity of VMH*.
3. Decreased VMH activity removes its inhibitory effect on LH (Fig. 60.13).
4. Therefore, LH activity increases due to *disinhibition* (abolition of inhibition from VMH).
5. **Facilitation of feeding center** (increased LH activity) increases feeding (polyphagia).

**Polyuria**

In diabetes, when plasma glucose concentration increases above renal threshold (above 180 mg%), glucose appears in urine. Filtration of more glucose increases its tubular load. Increased concentration of glucose in the tubular fluid causes *osmotic diuresis* that result in polyuria.

**Polydipsia**

In diabetes, because of polyuria water is lost in excess from the body. This decreases plasma and ECF volumes. *Dehydration stimulates thirst center* that causes polydipsia.

**Weight Loss**

In diabetes, food intake is more due to stimulation of feeding center. However, in spite of more food intake, *glucose is not utilized by the cells* due to deficiency of insulin. Insulin is essential for entry and utilization of glucose in the tissue. Insulin also facilitates growth. Therefore, insulin deficiency causes weight loss and growth retardation.

**Hyperglycemia and Glycosuria**

Insulin is the only hormone that decreases plasma glucose concentration. Therefore, deficiency of insulin results in hyperglycemia. When plasma glucose concentration exceeds renal threshold (180 mg%) glucose appears in urine (glycosuria).

**Ketosis**

Insulin prevents lipolysis, and therefore, decreases release of free fatty acids into the circulation. Insulin also facilitates utilization of ketone bodies (acetoacetate, acetone, and β-hydroxybutyrate) by the tissue. Therefore, ketosis occurs in insulin deficiency diabetes (IDDM). Presence of *excess acetyl-CoA* also facilitates conversion of aceto-acetyl-CoA to acetoacetate in the liver.

**Acidosis**

The *hydrogen ions liberated from ketone bodies* (acetoacetate and β-hydroxybutyrate) are usually buffered:

1. When ketosis becomes severe, the *buffering capacity decreases*. This causes plasma acidic.
2. Decreased pH of plasma stimulates respiration (*Kussmaul breathing*).
3. Urine also becomes highly acidic.
4. Normally, insulin increases Na+, K+ and phosphate reabsorption from kidney. Therefore, in diabetes, there is *loss of electrolyte and water*.
5. This causes dehydration, hypovolemia, and hypotension.
6. Diabetic acidosis is a *medical emergency* that requires immediate and appropriate replacement of electrolyte and fluid.

**Coma**

Coma in diabetes is of two types: *Acidotic coma* (due to acidosis) and *hyperosmolar coma* (due to hyperosmolality of plasma):

1. In acidosis, loss of water and electrolyte causes dehydration that results in coma (Flowchart 60.3). Accumulation of lactate (lactic acidosis) also causes coma.
2. *Increased glucose concentration* in plasma to a very high level increases its osmolality to the extent that it causes dehydration of brain cells that results in coma without affecting pH.

**Diagnosis**

Diagnosis of diabetes is done by demonstrating persistent hyperglycemia and glycosuria. Estimation of fasting and post-prandial blood glucose is performed to demonstrate hyperglycemia, and estimation of glucose in urine is carried out to demonstrate glycosuria. This is done by GTT, estimation of fasting blood glucose (FBG), and estimation of glycated hemoglobin (HbA1c).

**GTT:** In the past, glucose tolerance test was usually performed for the diagnosis of diabetes:

1. The *oral GTT* was preferred to intravenous GTT. The **GTT curve in diabetes** typically exhibits persistent rise in blood glucose above the normal value (Fig. 60.14).
2. Abnormal GTT also occurs in few other conditions like renal disease, intestinal problems, etc.

**FBG:** Recently, estimation of fasting blood glucose (FBG) has been recommended to be the main criterion to diagnose DM. FBS more than 126 mg% in more than
two occasions confirms DM. The blood glucose levels are classified into 3 types (Table 60.5).

**HbA1c:** This is the **glycated hemoglobin.** HbA1c indicates the state of **persistent hyperglycemia** at least more than three months. The normal range is 4 to 5.6%. The values between 5.7 and 6.4% indicate prediabetes, and 6.5% or above indicates diabetes.

**Treatment**

Diabetes is usually treated by insulin and oral hypoglycemic agents. However, change in life style improves the condition to a greater extent as recently diabetes has been found to be closely associated with chronic stress.

**Insulin Therapy**

Insulin replacement is the cornerstone of treatment of type 1 DM. The maximum decline in plasma glucose occurs following 30 minutes after IV injection of crystalline insulin. Insulin is also injected subcutaneously, but decrease in plasma glucose concentration occurs in 2–3 hours.

Different types of insulin preparations are commercially available. Generally, **insulin preparations** are divided into three categories depending on their duration of action:

1. Rapid acting insulin,
2. Intermediate acting insulin, and
3. Long acting insulin.

**Pork insulin** differs from human insulin only by one amino acid and therefore, has low antigenicity. Therefore, pork insulin is preferred to beef and other insulins. However, **human insulin** is produced nowadays from bacteria by recombinant DNA technology.

**Oral Hypoglycemic Agents**

These are usually used for the treatment of type II diabetes mellitus. The usually administered drugs are:

1. **Sulphonylurea derivatives,** like tolazamide, glipizide, glyburide, etc. These drugs are usually prescribed for
the treatment of type-2 (NIDDM) diabetes. They act by binding to the ATP-inhibited K⁺ channels in the membrane of B cells of pancreas. This increases Ca²⁺ influx and increases insulin secretion.

2. Other oral hypoglycemic agents are biguanides (phenformin and metformin). Metformin acts mainly by decreasing gluconeogenesis; therefore, it decreases hepatic glucose output.

3. Other group of drugs like thiazolidine-diones (troglitazone is an example of this group) are also used. They increase insulin mediated peripheral glucose disposal. Hence, they decrease insulin resistance (used in NIDDM). Troglitazone also binds with peroxisomes proliferator-activated receptor γ (PPAR γ) in the nucleus of the cell. It activates PPAR γ, which induces nuclear transcription that in turn regulate metabolic functions of the cell.

**Change in Lifestyle**

In addition to drugs, it is important to assure lifestyle modification and proper calorie intake in the long-term management of DM:

1. **Calorie intake**: Food intake should be adjusted to ensure appropriate nutritional requirements. Excess intake should be avoided. Diet should have less carbohydrate and fat, more fibers and adequate proteins and vitamins.

2. **Regular exercise**: Morning walk and freehand exercises improve insulin release and decreases insulin resistance.

3. **Relaxation of body and mind**: Healthy body and mind without stress will not only cure diabetes, but also prevent other diseases.

4. **Practice of yoga**: Yoga is very helpful in reducing the intensity of diabetes, and reducing the complications.

**Complications**

Improperly treated or untreated chronic diabetes results in various complications. The disease affects small and larger vessels:

1. The microangiopathy causes retinal edema and scarring, which is often associated with hard exudates in retina, the condition known as diabetic retinopathy, which also occurs due to neovascularization of retina, especially in the advanced stages (Fig. 60.15).

2. The microangiopathy in kidney is known as diabetic nephropathy.

3. The macrovascular complications are primarily due to increased atherosclerosis. Atherosclerosis is accelerated is due to increased plasma LDL. This increases the incidence of heart attack (myocardial infarction) and stroke.

4. Autonomic nervous system and peripheral nerves are also involved in the disease process. This results in autonomic neuropathy (diabetic neuropathy).

5. Hyperglycemia and neuropathy decrease the resistance to infection. This results in chronic ulcer and gangrene formation especially in the foot, called as diabetic foot.

**Insulin Excess**

Insulin excess occurs in insulin secreting tumor of pancreas (insulinoma). This causes hypoglycemia:

1. Chronic hypoglycemia causes incoordination of movement and slurring of speech. This is usually misdiagnosed as drunkenness. It is typically most common in the morning, as toward the early morning blood glucose concentration decreases and hepatic glycogen store is depleted.

2. Hypoglycemia also occurs in excess administration of insulin in the treatment of diabetes. But in this condition hypoglycemia is acute and manifest in the form of sweating, palpitation, anxiety, and other autonomic functions.

**OTHER PANCREATIC HORMONES**

**Glucagon**

Glucagon has opposite metabolic action to that of insulin. It is a prodiabetogenic and ketogenic hormone.
Source and Structure
Glucagon is secreted from α-cells of pancreas which constitutes about 20–25% of the islet cell mass. Glucagon is also secreted in the intestine (enteroglucagon) as local hormone and found in some parts of the brain as neurotransmitter. It is a polypeptide hormone containing 29 amino acids. The molecular weight is 3,485.

Synthesis
Like other peptide hormones, it is synthesized as preproglucagon that has 179 amino acids. Preproglucagon is found in α-cells of pancreas, L cells in terminal part of intestine and in the brain. Preproglucagon molecule in α-cells contains glucagon, major proglucagon fragment (MPGF) and glicentin related polypeptide (GRPP), whereas preproglucagon molecule in L-cells of intestine contains glicentin, glucagon like polypeptides (GLP 1 and 2), oxyntomodulin and MPGF. Accordingly, preproglucagon molecule forms different hormones in α-cells and L cells. In α-cells, it forms proglucagon that finally forms glucagon.

Both glucose and insulin inhibit glucagon synthesis by suppressing transcription of preproglucagon gene.

Functions of products of preproglucagon molecule are as follows:
1. Glicentin has some glucagon activity.
2. GLP-1 and GLP-2 are found in the brain and intestine. GLP1 stimulates insulin secretion and facilitates glucose utilization. GLP 1 is a neurotransmitter in nerve terminals projecting from NTS in the medulla to the dorsomedial hypothalamus.
3. GLP 2 inhibits food intake.
4. Oxyntomodulin inhibits HCl secretion from stomach.
5. Function of GRPP is not clearly known.

Secretion
Glucagon secretion is regulated by following factors:
Factors that increase secretion: Amino acids (especially gluconeogenic amino acids), cortisol, CCK, gastrin, stress, exercise, infection, β receptor agonist and theophylline.
Factors that decrease secretion: Glucose, somatostatin, insulin, secretin, ketone bodies, FFA and α receptor agonist.

Metabolism and Mechanism of Action
It is mainly degraded in the liver. It circulates freely in blood. Its half-life in circulation is about 5–10 minutes. Glucagon acts by increasing cyclic AMP in the cell. It also increases IP₃ in the cell.

Physiological Actions
Physiological actions of glucagon are almost opposite to that of insulin. It facilitates mobilization of nutrients by decreasing their storage. It especially facilitates hepatic glucose output and ketogenesis.

On Carbohydrate Metabolism
Glucagon increases blood glucose by following mechanisms:
1. Stimulates glycogenolysis: It causes immediate and profound glycogenolysis in the liver by activating glycogen phosphorylase. This causes release of glucose 6-phosphate:
   - Glucagon increases cAMP in the cell. cAMP stimulates protein kinase A that in turn activates phosphorylase and other enzymes.
   - Activated protein kinase A also inhibits conversion of fructose-6-phosphate to fructose 1,6-biphosphate, which helps in increasing glucose 6-phosphate in the cell.
   - Accumulated glucose 6-phosphate is converted to glucose.
   - Thus, more glucose is released from liver. It does not cause glycogenolysis in muscle.
2. Gluconeogenesis: It also stimulates gluconeogenesis by activating gluconeogenic enzymes, especially phosphoenolpyruvate carboxykinase. It also promotes hepatic proteolysis and supplies more amino acids for gluconeogenesis.
3. Inhibition of glycogenesis: Glucagon inhibits glycogen synthase. Therefore, conversion of glucose to glycogen is inhibited.

All these actions result in increased output of glucose from the liver. Normally, the metabolic actions of glucagon on muscle and adipose tissue are not significant.

On Fat Metabolism
Glucagon promotes lipolysis and ketogenesis in liver:
1. Lipolysis: In hepatic cells, glucagon facilitates lipolysis. Thus, it increases release of FFA from liver. However, the fat store of liver is not more unlike in adipose tissue. Therefore, the degree of lipolysis is moderate.
2. Ketogenesis: Glucagon increases ketone body formation in liver cells by decreasing the level of malonyl-CoA. Increased FFA also promotes ketogenesis. Glucagon facilitates entry of FFA into mitochondria for their β-oxidation and production of ketone bodies.

On Calorigenesis
Glucagon increases calorigenesis. Calorigenic action of glucagon is not due to increased blood glucose, rather to the hepatic deamination of amino acids.

On Heart
Glucagon in supraphysiological concentration increases myocardial contractility by increasing cyclic AMP level in myocardial cells. Therefore, glucagon is advocated for the treatment of heart disease.

On Other Hormones
Glucagon stimulates secretion of growth hormone, insulin, and pancreatic polypeptide.
Insulin-Glucagon Ratio

Insulin is glucopenic, glycogenic, anti-gluconeogenic, antilipolytic, and anti-ketotic (anti-diabetogenic and anti-ketogenic). Therefore, insulin is the hormone of energy storage. Glucagon is prodiabetogenic and proketogenic. Therefore, glucagon is the hormone of energy release. Because of their opposing effects, a balance should be maintained between the secretion of insulin and glucagons for maintaining normal metabolic functions. Therefore, insulin-glucagon molar ratio (I/G ratio) in plasma is more important than their individual concentration. Normally, the I/G ratio following a balance diet is approximately 3. Following overnight fasting, it decreases to 1, and after prolonged fasting the ratio may be as low as 0.4. Following glucose infusion, the ratio may rise up to 30.

Physiological Significance:
1. During starvation, the low I/G ratio favors glycogen break down and gluconeogenesis that increases nutrient supply. Conversely, during high-fed state, the ratio is high favoring deposition of nutrients in the form glycogen, protein and fat.
2. During neonatal period (immediately after birth), a low I/G ratio is critical for the survival of the neonate. In first few hours to days, the abrupt cessation of maternal fuel supply during which neonate does not receive required amount of fuel from its own GI tract, a low I/G ration maintains internal supply of nutrients.
3. In diabetes, inappropriate I/G ratio influences metabolic status. Secretion of glucagon is inappropriately elevated in insulin deficiency. The metabolic derangements are affected by this abnormal ratio.

Applied Physiology
Glucagon excess is produced by tumor of α-cells of pancreas. This is called glucagonoma. It causes hyperglycemia, ketosis and other metabolic derangements that mimic diabetes mellitus. In fact, insulin deficiency in diabetes is associated with glucagons excess. Glucagon deficiency occurs rarely.

Somatostatin

Source and Structure
Somatostatin is secreted from D cells of pancreas. It is also secreted in hypothalamus and gastrointestinal tract. Somatostatin is a peptide hormone. It has two forms. One contains 14 amino acids and the other 28 amino acids. The 14 amino acid somatostatin is mostly synthesized in pancreas and 28 amino acid peptide in GI tract.

Synthesis and Secretion
Like other pancreatic hormones it is synthesized as preprosomatostatin, which becomes prosomatostatin. Prosomatostatin is converted to somatostatin. Somatostatin secretion is stimulated by glucose, amino acids, free fatty acids, GI hormones, glucagon, vagal stimulation and sympathetic stimulation via β receptors.

Functions
1. Somatostatin profoundly inhibits the secretion of insulin and glucagon from pancreas, by neurocrine and paracrine fashions.
2. It decreases the rate of assimilation of all nutrients from GI tract.
3. It inhibits gastric, duodenal and gallbladder motility.
4. It inhibits HCl and pepsin secretions from stomach, and intestinal secretions.
5. It inhibits secretion of secretin and gastrin.
6. It inhibits pancreatic exocrine secretion.
7. It inhibits absorption of glucose, and triglycerides from the mucosal epithelial cells of intestine.
8. It regulates feedback control of gastric emptying (entry of food from stomach into the intestine). When food enters intestine, somatostatin inhibits gastric emptying. Therefore, it prevents rapid overload of intestine by gastric content.

Clinical Significance
Treatment of Diarrhea: Somatostatin preparations are used in medical practice for the treatment of diarrhea as somatostatin inhibits GI motility.

Somatostatinoma: In tumor of D cells of pancreas (somatostatinoma), excess somatostatin secretion decreases nutrient absorption from GI tract. Therefore, excess of undigested food appear in the stool. This causes weight loss. Moderate hyperglycemia is observed due to low insulin level in the plasma. These patients also develop dyspepsia due to decreased gastric emptying and decreased gastric secretion. They also develop gallstone, which is precipitated by decreased gallbladder contraction as somatostatin inhibits CCK secretion.

Pancreatic Polypeptide

Source and Structure
Pancreatic polypeptide is secreted from F cells of pancreas. It is a polypeptide containing 36 amino acids. It has a characteristic C terminal tyrosine-amide residue. Structurally, it is similar to the neuropeptide Y (NPY) secreted from hypothalamus and polypeptide YY, a GI hormone. However, in functions, it is not very similar to them.

Synthesis and Secretion
It is synthesized like other peptide hormones. It is secreted primarily in response to food intake. When food is ingested and food passes through the GI tract, the GI hormones and vagal stimulation stimulate the secretion of pancreatic...
polypeptide. It is also **stimulated by hypoglycemia and inhibited by hyperglycemia**. Its secretion is also inhibited by **somatostatin**. As pancreatic polypeptide secretion is under the influence of cholinergic control, its secretion decreases after atropine administration. Its secretion is stimulated by protein rich food, fasting and exercise.

**Functions**
1. **It inhibits exocrine pancreatic secretion**. This is partly due to its inhibition of uptake of precursor amino acids by the acinar cells.

**Clinical Significance**
1. Increased secretion of pancreatic polypeptide is invariably **associated with islet cell tumors**. Therefore, its increased level in plasma serves a **tumor marker** for the tumors of endocrine pancreas.
2. Failure of pancreatic polypeptide concentration in plasma to rise in response to hypoglycemia suggests loss of cholinergic innervation of pancreatic islets.

### CHAPTER SUMMARY

#### Key Concepts
1. Insulin has prominent effects on fat metabolism, as it has effects on carbohydrate metabolism. Therefore, in diabetes, in addition to hyperglycemia, there are hyperlipidemia and ketonemia. Diabetic complications are considerably linked to hyperlipidemia.
2. HbA1c reflects the chronicity of hyperglycemia. Therefore, HbA1c should be assessed in all diabetic patients to assess their level of hyperglycemia for last few months, rather than to check FBG or PPBG, which indicates the current blood glucose level.
3. For assessing insulin sensitivity, C peptide estimation should be done, which is a more reliable marker of β cell activity.

#### Important to Know (Must Read)
1. In examination, 'Describe the functions of insulin' usually comes as a **Long Question**.
2. Mechanism of insulin secretion in response to plasma glucose, Mechanism of action of Insulin, Physiological actions of Insulin on fat metabolism, Physiological actions of Insulin on carbohydrate metabolism, Glucose transporters (GLUT), Explain the physiological basis of features of DM, Differences between type I and type II DM, Glucagon, Effect of life style changes including exercise on DM, Insulin-glucagon ratio (I/G ratio), Somatostatin, Pancreatic polypeptide are usual **Short Questions** in exam.
3. In **Viva**, examiner may ask... List the hormones secreted from pancreas and the cell type from which they are secreted, Physiological actions of insulin especially on carbohydrate, fat and protein metabolism, List the factors that increase or decrease insulin secretion, What are the types of GLUTs and what are their functions, What is the mechanism of insertion of GLUT into cell membrane by insulin in insulin- sensitive cells, How is insulin secreted from beta cells in response to glucose, What is the relationship between increase in plasma glucose and rate of insulin secretion, What is first phase response and what is second phase response, Why the secretion of insulin is greater in response to orally administered glucose compared to intravenously administered glucose, Why is insulin given with glucose in the treatment of hyperkalemia, What is the effect of insulin on growth, Classify diabetes mellitus based on etiology, What are IDDM and NIDDM? List the differences between type I and type II DM, What is metabolic syndrome and what are its components, What is secondary DM, What is gestational DM, What is Maturity onset Diabetes of the Young (MODY), Explain the physiological basis of polyphagia in DM, Explain the physiological basis of polydipsia in DM, Explain the physiological basis of polyuria in DM, Explain the physiological basis of hyperglycemia and glycosuria in DM, Explain the physiological basis of weight loss in DM, Explain the physiological basis of ketosis, acidosis and coma in DM, What is the normal blood glucose level, in fasting and post prandial, What do you mean by impaired fasting glucose or impaired glucose tolerance, What is the fasting and post prandial blood glucos level in DM, List some oral hypoglycemic agents and give their mechanism of action, What are the types of insulin preparation used in the treatment of DM, What is the plasma glucose response to oral glucose tolerance test (OGTT), Explain how does regular exercise prevent diabetes, What is the effect of healthy life style on DM, What are the complications of DM, What are the physiological actions of glucagon, What is insulin-glucagon ratio, What are the physiological significances of I/G ratio, What are the functions of somatostatin, What are the functions of pancreatic polypeptide, What is the structure of insulin, How insulin is synthesized, What is C-peptide, What is the importance/ functions of C-peptide, List the substances having insulin like activity, What is the structure of insulin receptor, What is the physiological and clinical significance of Somatostatin, What is the physiological and clinical significance of pancreatic polypeptide.
Learning Objectives

On completion of study of this chapter, the student MUST be able to:
1. Learn the basics of calcium and phosphate metabolism.
2. Understand the importance of learning calcium and phosphate metabolism to learn bone physiology.
3. List the functions of calcium and phosphate.
4. Appreciate various aspects of bone physiology.
5. Understand the mechanism of bone formation and bone resorption.
6. Describe the hormonal regulation of calcium metabolism.
7. Learn the physiological basis of osteoporosis and osteomalacia.

The student MAY also be able to:
1. Describe the role of calcium and phosphate in bone physiology.
2. Describe the mechanism and regulation of bone formation and resorption.

The major hormones involved in the regulation of plasma calcium and phosphorous metabolisms are parathormone (PTH) secreted from parathyroid gland, calcitonin secreted from parafollicular cells of thyroid gland, and vitamin D. Other hormones like glucocorticoids, growth factors, insulin, etc. also influence calcium metabolism. These hormones control calcium metabolism by primarily acting on three structures: GI tract, kidney, and bones. While regulating calcium concentration, many of these factors also influence phosphate level in the plasma. A balance between calcium and phosphate is always maintained in our body and abnormalities in this balance result in severe dysfunctions. To understand the regulation of plasma calcium, it is important to understand the basics of calcium and phosphate metabolisms and also the physiology of bone dynamics.

Calcium and Phosphate Metabolism

Calcium Metabolism

The normal plasma calcium concentration is 9–11 mg% (average 10 mg%). This is among the most tightly regulated physiological parameters of the body. The regulation of plasma calcium concentration within a narrow range of 1–2% indicates the importance of this ion in controlling critical body functions.

Functions of Calcium

Calcium ions are involved in many key physiological processes of the body. These are:
1. Genesis and maintenance of action potentials, especially in cardiac and smooth muscles.
2. Genesis of pacemaker potential in various pacemaking tissues.
3. Excitation-contraction coupling during muscle contractions.
4. Excitability of nerve and muscle: Calcium influences sodium permeability; therefore, influences the ease with which action potentials are triggered. Especially in nerves, low calcium can cause generation of spontaneous action potentials. This causes the characteristic muscle spasm in hypocalcemic tetany.
5. Cell division.
7. Secretion of endocrine and exocrine glands (calcium-mediated exocytosis causes release of hormones and enzymes from the gland cells).
8. **Neurotransmitter release** from nerve terminals.
9. **Blood coagulation**: Calcium is coagulation factor IV and is highly essential for clotting of blood. Many anticoagulants chelate calcium to prevent clotting.
10. Modulation of various enzyme activities (enzymes use calcium as cofactor).
11. Calcium acts as a second or third messenger in various intracellular signaling pathways for hormone actions.
12. GI motility and motility of many other structures.

Therefore, it is essential to maintain calcium concentration within its normal range. A minor deviation in calcium level results in alteration in many physiological functions of the body.

**Distribution of Calcium in the Body**

In the cell, calcium is stored in mitochondria and endoplasmic reticulum. A transient alteration in plasma calcium is balanced by entry or extrusion of calcium from the intracellular or extracellular reservoirs. The total intracellular free calcium is about 0.2 mg, whereas about 9 g is present in the bound form or in the storage sites such as in mitochondria and endoplasmic reticulum. This intracellular calcium provides an immediate source of calcium for cell functions.

<table>
<thead>
<tr>
<th>Table 61.1: Distribution of calcium in the body.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total body content: 1200 g (average)</td>
</tr>
<tr>
<td>In bones and teeth: 99% of total</td>
</tr>
<tr>
<td>In intracellular fluid: 0.9% (11 g approx.)</td>
</tr>
<tr>
<td>In extracellular fluid: 0.1% (1 g approx.)</td>
</tr>
</tbody>
</table>

**Metabolism**

Daily dietary intake of calcium ranges from 200 mg to 2 g. In an adult, the **recommended daily intake** is about 800 mg.
1. Calcium absorption from the intestine is inversely proportional to its intake. This prevents calcium overload when intake is high or maintains calcium concentration even with low intake.
2. With a daily intake of 1 g of calcium, generally 30% (300 mg) of it is absorbed in the intestine and about 70% (700 mg) is excreted in the stool.
3. About 150 mg of calcium is secreted into the intestine, which makes a total of about 850 mg excreted daily in the stool.
4. About 150 mg is excreted in the urine.
5. Thus, about 1 g of calcium is excreted daily from the body to maintain the balance between input and output (Fig. 61.1).
6. Calcium absorption from the intestine is diminished with advancement of age, which contributes to the development of osteoporosis. Calcium absorbed from intestine enters the ECF pool of calcium which is about 1000 mg. This is in equilibrium with the rapidly exchangeable pool (4000 mg) of the body. From the rapidly exchangeable pool of plasma, 500 mg of calcium enters bones for normal bone remodeling and from bone about 500 mg of calcium extrudes back into the plasma.

Phosphate Metabolism

The normal plasma phosphate concentration is 2.5 to 4.5 mg%. Functions of Phosphorus

Phosphorus (usually as phosphate) is important as calcium for biological activities.
1. Phosphate is present in the ATP, creatine phosphate, various co-factors like NAD, NADP, and thiamine pyrophosphate.
2. It is an integral part of second messengers in the cells like cyclic AMP and inositol triphosphate.
3. It is also found in DNA and RNA.

4. It is required for phosphorylation of many intracellular proteins, for formation of phosphoproteins. Phosphoproteins mediate many intracellular metabolic activities.
5. Phosphate acts as covalent modifier of many enzymes.
6. It is a major constituent of the bone and teeth like that of calcium.
7. It serves as an important component of intracellular pH buffering system.

Like calcium metabolism, phosphate metabolism is also closely regulated in the body.

Distribution of Phosphate in the Body

Total body phosphate content is roughly half of the calcium content. Out of 600 g of total phosphate in the body, about 86% is present in the bones, 14% in intracellular fluid and 0.08% in extracellular fluid (Table 61.2). About 6% is present in the muscle and 8% in other cells.

Metabolism

The daily phosphate intake is about 1400 mg, of which 75% is absorbed from the intestine and 25% is excreted in the stool.
1. The phosphate pool in the ECF is about 700 mg, from where about 200 mg is exchanged with the bones for bone remodeling.
2. Normally, about 1000 mg is excreted in urine.
3. Regulation of renal excretion of phosphate mainly controls the concentration of plasma phosphate.
4. Renal (1 g) and fecal (0.4 g) excretion balances the daily intake with daily output of phosphate (Fig. 61.2).
5. Phosphate deficiency results in muscle weakness, cardiac and respiratory dysfunction, abnormal bone formation, and loss of red cell membrane integrity.

Table 61.2: Distribution of phosphate in the body.

<table>
<thead>
<tr>
<th>Total body content</th>
<th>600 g (average)</th>
</tr>
</thead>
<tbody>
<tr>
<td>In bones and teeth</td>
<td>86% of total</td>
</tr>
<tr>
<td>In intracellular fluid</td>
<td>14% (about 84 g)</td>
</tr>
<tr>
<td>In extracellular fluid</td>
<td>0.08% (1.2 g)</td>
</tr>
</tbody>
</table>

Fig. 61.2: Summary of phosphorus metabolism.
Magnesium: Magnesium is closely related with the calcium and phosphate metabolisms.
1. It is essential for neuromuscular transmission and it serves as cofactor for many enzymatic activities.
2. The normal plasma concentration of magnesium is 1.5–2.5 mg%.
3. About 35% of it is bound to protein.
4. There is about 25 g of magnesium in our body of which about 50% is present in the bones and rest 50% in the intracellular fluid.
5. The magnesium depletion causes neuromuscular dysfunction and ventricular arrhythmias.

Regulation of Calcium and Phosphate Homeostasis
Homeostasis of calcium and phosphate is influenced mainly by exchange of these ions between plasma and bone. Also, absorption of calcium and phosphate from intestine and kidney that determines their fecal and urinary excretion respectively greatly contributes to their homeostasis. In general, calcium is excreted more in the stool (850 mg/day) and less in urine (150 mg/day), whereas phosphate is excreted more in urine (1000 mg/day) and less in stool (400 mg/day).

Intestinal Control of Calcium and Phosphate
Absorption of calcium and phosphate from intestine significantly contributes to their plasma concentration.

Absorption of Calcium
About one third of ingested calcium is absorbed from GI tract and remaining two-third is excreted. The percentage absorption is more in growing children, pregnant women and nursing mother.
1. About 150 mg of calcium enters intestine in the form of various GI secretions, and from sloughing of mucosal cells. Intestinal absorption plays an important role in calcium homeostasis.
2. Calcium absorption occurs by active transport in duodenum and jejunum, and passive diffusion in ileum. Active transport is facilitated by 1,25-dihydroxycholecalciferol (metabolite of vitamin D), which increases the expression of calcium binding proteins in the intestinal epithelial cells increasing their capacity to transport more calcium.
3. Calcium is transported across the brush border of intestinal epithelial cells via TRPV6 (transient receptor potential vanilloid type 6) channels.
4. Calbindin sequesters the absorbed calcium, which is then delivered to the basolateral membrane of the epithelial cells and from there enters the blood stream via sodium-calcium exchanger or calcium-dependent ATPase.
5. However, some degree of intestinal calcium uptake persists in the absence of TRPV6 and calbindin. Cholecalciferol and calcitonin influence calcium absorption either directly or indirectly through vitamin D.

Absorption of Phosphate
In contrast to calcium, most of the ingested phosphorous (about 75–85%) is absorbed from intestine as inorganic phosphate (Pi).
1. Pi absorption occurs by a transporter called NaPi-IIb, which is related to the transporter in kidney.
2. Though absorption occurs by both active and passive processes, active transport is the primary mechanism of absorption.
3. However, the regulatory mechanism for phosphate absorption is not well developed. To some extent, it is coupled to calcium absorption.

Renal Control of Calcium and Phosphate
Reabsorption of calcium and phosphate from kidney contributes significantly to their plasma concentration.

Reabsorption of Calcium
About 60% of plasma calcium is filtered in the glomeruli and 40% is bound to plasma proteins.
1. Of the filtered calcium, 1–5% is excreted in urine and about 95% is reabsorbed back into the plasma. Therefore, the total urinary excretion is less.
2. Of the filtered load of calcium, about 65% is reabsorbed in proximal tubule, 25% in loop of Henle and 8% in distal tubule.
3. In proximal tubule, the transport process is active that involves two sodium-dependent Pi co-transporters such as NaPi-IIa and NaPi-IIc.
4. Parathyroid hormone (PTH) inhibits NaPi-IIa. PTH regulates plasma calcium by also acting on the distal tubule.

Reabsorption of Phosphate
Phosphate is mainly excreted in urine. Therefore, kidney plays important role in phosphate homeostasis.
1. Usually, 75–85% of filtered phosphate is reabsorbed.
2. The major site of reabsorption is proximal tubule, where about 70% of the filtered load of phosphate is reabsorbed.
3. Parathyroid hormone controls phosphate excretion by mainly inhibiting its reabsorption in the proximal tubule.

Bone Physiology
Bone is a compact living connective tissue, which is well vascularized. It plays a vital role in calcium and phosphate homeostasis in addition to its other important functions.
Chapter 61: Calcium and Phosphate Metabolism and Physiology of Bone

The major functions of bones are as follows:
1. They form the skeletal framework of the body, which is crucial for changing and maintaining various body postures.
2. By providing stable postural background, bones allow movements to occur.
3. They play important role in metabolism of various minerals, especially, contribute to calcium, phosphate and magnesium homeostasis.
4. Bones protect important structures and viscera in the thoracic and pelvic cavities and in the skull.
5. Bone (bone marrow) is the primary site of hemopoiesis. They produce and supply formed elements of blood.

**Composition of Bone**

Bone is simply a collagen framework in which inorganic minerals are deposited on an organic matrix.

**Inorganic Components**

Inorganic component consists of various minerals such as calcium, phosphate, carbonate, magnesium and sodium. Minerals constitute about 25% of the bone volume, but they comprise half the bone weight because of their high density.
1. Bone is the major reservoir of these minerals in the body.
2. About 99% of calcium, 86% of phosphate, 80% of carbonate and 50% of magnesium of their total body content are present in bones (Table 61.3).

**Organic Matrix**

Organic matrix of the bone is called osteoid.
1. It is formed mainly by the Type I Collagen, which forms 95% of the matrix.
2. The remaining 5% noncollagen part is called the ground substance, which is a mixture of various proteoglycans and high molecular weight compounds, consisting mainly of polysaccharides.

**Parts of Bone**

Long bones consist of epiphysis, the both ends, and diaphysis, the shaft which is separated from epiphyses by epiphyseal plates (Fig. 61.5).
1. The linear growth of the bones occurs at the end of long bones by replacement of cartilage at the epiphyseal plates.
2. Closure of epiphyseal plates occurs towards the end of puberty. This causes cessation of further increase in height of the individual.
3. However, width of the bone continues to increase due to addition of cells to the periosteum, the outer surface of the bone. The nutrients from ECF diffuse into the trabecular bone via canaliculi (see below). In compact bone, nutrients are supplied by Haversian canal that contains blood vessels.

**Remodeling of Bone**
Bone is continuously remodeled throughout the life, which is synchronized by bone formation and resorption (bone destruction).
1. During the growth period, bone formation exceeds bone resorption so that the bone mass increases.
2. During the adulthood, rate of formation and resorption is almost balanced so that the bone mass remains unchanged.
3. After the age of 40 to 50, bone resorption exceeds formation so that bone mass slowly decreases.

**Cell Types in Bone**
There are three types of cells in the bone: these are osteoblasts, osteoclasts, and osteocytes (Figs. 61.6A to C). Osteoblasts and osteocytes are called osteoprogenitor cells as they develop from primitive cells. Osteoclasts develop from the precursors such as monocytes and tissue macrophages.

**Osteoblasts and Osteocytes**
Osteoblasts are bone forming cells. They are modified fibroblasts.
1. Osteoblasts synthesize osteoid, the organic matrix of the bone. They have numerous endoplasmic reticulum and Golgi apparatus. They are present towards the periphery of the bone and their cytoplasmic processes connect osteocytes that are present deeper in the bone.
2. Osteoid produced by osteoblasts is secreted towards the interior of the bone. Slowly the osteoblasts are surrounded by osteoid and osteoid is mineralized.
3. Osteoblasts surrounded by mineralized osteoid gradually lose their ability to form bones and they become osteocytes.
4. The cytoplasmic connection between osteoblasts and osteocytes become canaliculi (Fig. 61.7).
5. The canaliculi are the anatomical link for transfer of nutrients, chemicals and waste products between the surface and interior of the bone.

**Osteoclasts**
Osteoclasts are cells of mononuclear phagocyte system in the bone. Therefore, they are large, multinucleated cells located towards the periphery of the bone.
Mechanism of Bone Formation

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1. They cause bone resorption by secreting proteolytic enzymes into the surroundings.
2. The chemicals secreted by osteoclasts make the environment acidic that enhances the solubility of bone minerals and the enzymes secreted cause degradation of the organic matrix.

Mechanism of Bone Formation

Formation of bone is the function of osteoblasts (Figs. 61.8A to E).

1. Osteoblasts synthesize collagen and extrude it into the adjacent extracellular space (Fig. 61.8A). The collagen fibrils form the organic matrix of the bone, which is called as osteoid (Fig. 61.8B).
2. In the osteoid, calcium-phosphate is deposited which is called as mineralization of bone. Thereafter, to the organic matrix, hydroxides and bicarbonates are added to form the hydroxyapatite crystals (the complete mineralization). Osteoid converted into lamella of the bone (Fig. 61.8C).
3. The mineralized matrix slowly accumulates and surrounds the osteoblasts. As this process of mineralization of matrix continues, the osteoblasts decrease their synthetic activity and become osteocytes (Fig. 61.8D).
4. Osteoblastic activity is seen along the surface of the bones. Lamellae of the bones are formed with osteocytes remaining inside the lamellae (Fig. 61.8E).
5. Vitamin D is essential for bone mineralization. It profoundly influences the availability of calcium and phosphate to the bone matrix.
6. The canaliculi that connect osteocytes from bone interior to the surface transfer calcium from the interior to the exterior bone units and then from there to the ECF.
7. This process of transfer of calcium carried out by osteocytes is known as osteocytic osteolysis.
8. Normally, this process does not decrease the bone mass rather removes calcium from the recently formed bone crystals.

Role of Chondrocytes and Endochondral Ossification

Before epiphyseal closure, the leading edge of the epiphyseal plate contains chondrocytes.

1. The active chondrocytes synthesize cartilage that gradually embeds the chondrocytes.
2. The embedded chondrocytes die and are replaced by new chondrocytes and the formation of cartilage continues.
3. The cartilage is slowly calcified and osteoblasts migrate into it.
4. Osteoblasts secrete osteoid that ultimately mineralize and the new bone is formed.
5. Thus, the bone increases in length as the epiphyseal plate lays down new bone on the end of the shaft. This is called endochondral ossification (Fig. 61.9).
6. IGF I, insulin and thyroid hormones stimulate chondrocyte activity.
7. Within few years from the onset of puberty, the chondrocytes become unresponsive to hormonal stimuli and lose their activity. Finally the epiphyses unite with the shaft and this process is called 

**closure of epiphysis** (Clinical Box 61.1). This is also called ossification. This stops the linear growth of the bone.

Clinical Box 61.1

Epiphyseal closure determines age: The epiphyses of various bones close in an orderly fashion in sequence and the age at which they close is known. Therefore, the age of an individual especially in adolescents and young adults can be determined easily by taking x-rays of bones by determining whether the epiphysis is closed or still open.

**Bone Resorption**

This is process of destruction of the bone matrix. Therefore, resorption of bone decreases bone mass. It also removes calcium from the bone. But, it differs from osteocytic osteolysis in which the calcium is removed without bone destruction. Bone resorption is carried out by osteoclasts.

1. Osteoclasts contain many mitochondria and lysosomes.
2. The phosphatases, type 4-collagenase and lysosomal enzymes cause dissolution of bone matrix.
3. During the process of resorption, the calcium, phosphate, hydroxyproline, and hydroxylysine are released into ECF.
4. Therefore, the rate of excretion of these organic compounds, especially hydroxyproline indicates the rate of bone resorption.

**Phases of Bone Resorption**

Bone resorption occurs in two phases.

**First Phase**

In the first phase, H⁺ is secreted by osteoclasts. There are proton pumps (H⁺—dependent ATPases) in the endosomes present in the cytosol of the osteoclasts.

1. When osteoclasts are activated, proton pumps migrate to the cell surface and are inserted into the osteoclast membrane.
2. Secretion of H⁺ makes the environment acidic; pH decreases to about 4. The acidic pH dissolves the hydroxyapatite and favors action of acidic enzymes.

**Second Phase**

In the second phase, the acid protease enzyme secreted by osteoclasts destroys the collagen, the organic matrix.

1. The collagen breakdown products have pyridinoline structures. One such important product is hydroxyproline.
2. Therefore, hydroxyprolinuria is an index of bone resorption.

**Balance Between Bone Formation and Resorption**

Normally, balance is maintained between the bone resorption and formation within its bone modeling units so that bone mass is maintained within the normal range.

1. Possibly, the balance is due to release of chemicals (some paracrine signals) from the osteoblasts or osteoclasts. For example, when osteoclasts cause destruction of bone matrix, the chemicals released from the osteoclasts attract osteoblasts to the site.
2. Osteoblasts then form bone matrix and fill the resorption cavity.
3. The flux of calcium into and out of bone reflects the remodeling, i.e. the turnover and formation of bone tissue.

**Factors that Control Bone Formation and Resorption**

Factors that stimulate bone formation: Growth hormone, growth factors, insulin, estrogen, testosterone, vitamin D, PDGF, transforming growth factors (TGF-β) and calcitonin.

Factors that inhibit bone formation: Cortisol.

Factors that stimulate bone resorption: Parathormone, cortisol, thyroxine, prostaglandins, interleukin 1 and 6, and TNF.

Factors that inhibit resorption: Estrogen, androgen, calcitonin, γ interferon, and TGF-β.

**Osteoporosis**

Osteoporosis is decrease in bone mass and density. All human beings gain bone early in life. The peak of total bone mass is attained between 25–35 years of age.

1. Usually, after the age of 40, bone mass gradually decreases. This is called involutional osteoporosis.
2. In females, the process of bone resorption is facilitated at the time of menopause due to cessation of estrogen secretion (postmenopausal osteoporosis).
3. In fact, the rate of bone remodeling increases with age. However, with increased osteoclastic activity, bone matrix is lost and more cavities are formed, which is not refilled by the osteoblastic activity. Also, the mineralization decreases. This causes reduction in bone density and bone mass. The process is known as osteoporosis.

**Causes**

Osteoporosis is commonly seen in following conditions:
1. Hyperparathyroidism,
2. Hyperthyroidism,
3. Cushing’s syndrome
4. Ovarian diseases reducing estrogen secretion,
5. Cigarette smoking
6. Alcoholism reducing calcium absorption
7. Deficiency of vitamin C (vitamin C causes collagen synthesis)
8. Inadequate dietary calcium.

**Features**

Osteoporosis increases the susceptibility of bones to fractures, especially in elderly. Osteoporosis is more common in vertebra, hip bones and in distal forearms, because these bones have more trabecular component. As trabecular bones are metabolically more active, they are lost rapidly.

**Treatment**

The treatment is by administration of calcium or vitamin D tablets, or in worst case estrogen therapy.

**Osteopetrosis**

Defective increase in bone formation is called osteopetrosis.
1. The bone resorption is defective due to decreased osteoclastic activity. Therefore, osteoblastic activity is unopposed. This results in unusual increase in bone formation.
2. Bone density is increased and bones are deformed.
3. Neurologic deficits occur due to bones compressing on nerves and hematologic abnormalities occur due to decreased marrow cavity.

**CHAPTER SUMMARY**

**Key Concepts**

1. Calcium, phosphate and magnesium are essential for bone formation and mineralization.
2. Calcium absorption from the intestine is inversely proportional to its intake, which prevents calcium overload when intake is high or maintains calcium concentration in low calcium intake.
3. Osteoblasts are meant for bone formation ad osteoclasts are for bone resorption. A balance between the two cells is essential for maintenance of bone mass.

**Important to Know (Must Read)**

1. In examination, Long Questions may not be asked from this chapter.
2. Osteoporosis, Functions of calcium and calcium metabolism, Mechanism of bone formation and bone resorption, Regulation of calcium and phosphate homeostasis, & Osteoblasts and osteoclasts, may be asked as Short Questions in exam.
3. In Viva, examiner may ask… List the functions of calcium, List the functions of phosphorous, How calcium and phosphorous are distributed in the body, How ECF calcium pool is exchanged with GIT, bone, kidneys and tissues, How ECF calcium pool is exchanged with GIT, bone, kidneys and tissues, How ECF phosphorous pool is exchanged with GIT, bone, kidneys and tissues, What are the types of bones, What is the composition of bone, What is the structure of bone, Functions of osteoblasts and osteocytes, Mechanism of bone formation, Mechanism of bone resorption, What are the factors that control bone formation and bone resorption, What are the cells in the bone, What are the functions of osteoblasts and osteoclasts, What are the stages of bone formation, Name the hormones that influence bone functions, Cause, features and treatment of osteoporosis, What is osteopetrosis and what are its symptoms, How epiphyseal closure determines age.
Parathyroid Gland, Calcitonin and Vitamin D

**Learning Objectives**

On completion of study of this chapter, the student **MUST** be able to:

1. Name the cell types in parathyroid gland and give their functions.
2. Describe the mechanism of action and functions of parathyroid hormone.
3. Describe the mechanism of action and functions of calcitonin.
4. Describe functions of vitamin D.
5. Understand the physiological basis of dysfunctions of PTH.

The student **MAY** also be able to:

1. Explain the role of calcitonin and vitamin D in calcium metabolism.
2. Describe the hormonal regulation of calcium metabolism.

Parathormone (PTH) secreted from parathyroid gland, calcitonin secreted from parafollicular cells of thyroid gland, and cholecalciferol (vitamin D₃) are the major hormones involved in the regulation of plasma calcium and phosphorous metabolisms and bone physiology. Therefore, usually functions of these three hormones are discussed together.

**Parathyroid Gland**

Parathyroid gland secretes hormone called **parathyroid hormone** or **parathormone** (PTH). PTH is the major regulator of calcium and phosphate metabolism. PTH **increases plasma calcium and decreases plasma phosphate level.**

**Scientist contributed**

Marcel Eugène Émile Gley (1857–1930) was a French physiologist and endocrinologist. Gley was the first to show the importance of the parathyroid glands for the maintenance of life in mammals. He had demonstrated tetany in animal models after removal of parathyroids. Because of his discovery, parathyroid glands have sometimes been referred to as ‘Gley’s glands’.

**Fig. 62.1:** Location parathyroid glands. Note that these are 4 small glands (2 superior parathyroids and 2 inferior parathyroids) located behind the thyroid gland. They receive blood supply from thyroid arteries.

**Physiological Anatomy**

In human beings, there are four parathyroid glands present in the anterior aspect of the neck embedded posteriorly at four poles in the thyroid tissue (Fig. 62.1). The glands develop at about 10th week of pregnancy from the 3rd and 4th branchial pouches. The total weight of the four glands is about 200 mg, each gland weighing approximately 50 mg. Parathyroid glands derive blood supply from the thyroid arteries.
Cell Types
Parathyroid glands contain two types of cells: chief cells and oxyphil cells (Figs. 62.2A and B).

Chief Cells
The chief cells are the predominant cells in the parathyroid gland that are present throughout life.
1. They have bigger Golgi apparatus in their cytoplasm.
2. They also contain numerous vesicles and endoplasmic reticulum.
3. Chief cells secrete PTH.

Oxyphil Cells
Oxyphil cells are less plentiful in parathyroid gland. They appear in the glands during puberty and then the number increases with the advancement of age.
1. They contain numerous oxyphil granules.
2. Normally, they do not secrete PTH. However, sometimes they secrete PTH and contribute to parathormone excess.

Parathyroid Hormone (PTH)

Structure
PTH is a single chain polypeptide containing 84 amino acids. The molecular weight is 9,500. The biological activity, especially the calcium regulating ability of PTH resides in the N-terminal portion of the molecule (within amino acids 1–27). The C-terminal portion is not related to its action on calcium metabolism.

Synthesis, Secretion and Metabolism
Like other peptide hormones, PTH is synthesized from the larger precursor molecule called prepro-PTH that contains 115 amino acids. The leader sequence containing 25 amino acids is removed from amino terminal in endoplasmic reticulum to form pro-PTH, which contains 90 amino acids. In the Golgi apparatus, another six amino acids are removed from the amino terminal to form PTH containing 86 amino acids. PTH is then packaged and stored in the granules of chief cells.

Regulation of Secretion
PTH secretion is regulated mainly by plasma calcium and vitamin D. Other factors also influence PTH secretion.

Plasma Calcium Level
The major regulator of PTH secretion is the plasma calcium level and they have inverse relationship.
1. With increased plasma concentration of ionized calcium, PTH secretion decreases. The relationship is linear between 3–6 mg% of the ionized plasma calcium (Fig. 62.3). It may be remembered that about 50% of the total plasma calcium is present in the ionized form. Conversely, with decreased plasma calcium, PTH secretion increases.
2. Recently, the receptors for sensing plasma calcium, the calcium sensing receptors (CaSR) have been identified on the membrane of chief cells. The CaSR is a G-protein coupled receptor attached to phospholipase C and on binding with calcium generates IP$_3$ and DAG.
3. IP$_3$ and DAG release calcium from cytosolic stores and activate protein kinase C that inhibits PTH secretion.
4. Another example of increase in calcium inhibiting hormone secretion is decreased renin release from JG cells of kidney.

Vitamin D
Vitamin D inhibits PTH secretion by decreasing the formation of preproPTH mRNA.

Other Factors
1. Plasma phosphate: Usually phosphate does not affect parathormone secretion. However, a rise in plasma...
phosphate stimulates PTH secretion by lowering plasma calcium and inhibiting formation of 1,25-dihydroxycholecalciferol.

2. **Cyclic AMP, β receptor agonists, dopamine, magnesium and histamine (H₂ receptor) stimulate PTH secretion.** Prostaglandins and α receptor agonists inhibit PTH secretion by decreasing cyclic AMP.

### Metabolism of PTH

The normal plasma PTH level is 10–60 pg/mL.

1. As PTH is rapidly degraded by Kupffer cells in liver to carboxy terminal and mid-region fragments, the half life of PTH is about 10 minutes.
2. The fragments are cleared by kidneys.
3. However, the synthetic PTH which has only 34 amino acid sequence of amino terminal of the natural PTH, possesses all the biological effects of the hormone.

### Mechanism of Action

**PTH Receptors**

There are three types of PTH receptors.

**Type 1 Receptor (PTH 1R)**

It binds to PTH and PTH related proteins (PTHrP). The action of PTH to regulate plasma calcium is mediated via PTH 1R.

**Type 2 Receptors (PTH 2R)**

It binds to PTH, but not to PTHrP. It is found in brain, pancreas and placenta.

**Type 3 Receptor (CPTH)**

It reacts only with carboxy terminal of PTH (therefore called CPTH) and not with the amino terminal of the hormone.

**G Proteins for PTH**

PTH 1R and 2R are coupled to two types of G proteins Gs and Gq. Therefore, the binding of PTH with the receptors activates both adenylate cyclase and phospholipase C in the membrane. This results in activation of two signaling systems (Fig. 62.4):

1. Activation of adenylate cyclase-cyclic AMP system that increases cAMP. cAMP activates protein kinase A that causes phosphorylation of intracellular proteins. Formation of phosphoproteins increases transport of calcium and other ions.
2. Activation of phospholipase C that increases IP₃ and DAG in the cell. IP₃ causes intracellular calcium mobilization and DAG causes activation of protein kinase C.

### Physiological Actions

The primary action of PTH is to increase the level of calcium and decrease the level of phosphate in the plasma. This is achieved by the action of PTH on three major target organs: kidney, bone, and GIT (Flowchart 62.1). PTH increases the influx of calcium into the plasma. By acting on bone and intestine, it increases phosphate influx but this is counter balanced by its action on kidney where it increases phosphate efflux. Therefore, finally plasma phosphate concentration decreases.

**Actions on Kidney**

PTH receptors are present on basolateral membrane of the epithelial cells of proximal and distal tubules. PTH has three major effects on kidney:

1. Increased reabsorption of calcium
2. Decreased reabsorption of phosphate
3. Stimulation of 1, 25-dihydroxycholecalciferol formation.
**Chapter 62: Parathyroid Gland, Calcitonin and Vitamin D**

**Increased reabsorption of calcium**

Normally, 65% of filtered calcium is reabsorbed in proximal tubule, 25% in thick ascending limb of loop of Henle and 5–10% in distal tubule. About 1–2% of filtered load is excreted in urine.

1. PTH increases calcium reabsorption from kidney by mainly acting on distal tubule and thick ascending limb of loop of Henle. Thus, PTH action on kidney decreases the calcium excretion to a very minimal quantity.
2. PTH action on kidney is relatively rapid that plasma calcium increases within minutes following its injections.
3. PTH also increases the formation of 1,25-dihydroxycholecalciferol, which in turn increases calcium absorption from distal tubule of kidney.

**Decreased reabsorption of phosphate**

PTH decreases reabsorption of phosphate by mainly acting on proximal tubule.

1. This causes phosphaturia.
2. By decreasing plasma phosphate concentration, PTH prevents calcium-phosphate salts to precipitate.

**Stimulation of 1, 25-dihydroxycholecalciferol formation**

In kidney, in the mitochondria of proximal tubule, PTH facilitates the hydroxylation of 25-hydroxycholecalciferol to 1,25-dihydroxycholecalciferol, the active form of vitamin D. 1,25-dihydroxycholecalciferol increases calcium reabsorption from kidney, calcium absorption from intestine and calcium mobilization from bone.

**Actions on Bone**

PTH acts on the receptors present in osteoblasts and osteoclasts. Thus, it activates both bone synthesis and resorption. However, the net effect is increased bone resorption and excess mobilization of calcium and phosphates from bones into plasma.

1. **Stimulation of osteoclastic activity**: This is a slower effect of PTH in which it stimulates osteoclasts to resorb mineralized bones. In this process both calcium and phosphate are transferred into the ECF. The bone matrix is hydrolyzed by the collagenase and lysosomal enzymes.
   - PTH increases the number and size of osteoclasts and also the synthesis of RNA in the osteoclast.
   - PTH also stimulates the differentiation of precursor cells (monocytes, macrophages, etc.) into the osteoclasts. Under the influence of PTH giant osteoclasts (osteoclasts of bigger size) are formed that cause resorption of the larger areas of bone (creates large resorption cavity).
   - The products of bone resorption are released into the plasma. These include calcium, phosphate, hydroxyproline and hydroxylysine.
   - Excretion of these substances in the urine especially that of hydroxyproline is an index of osteoclastic activity.
   - PTH acts on the osteoblasts: PTH inhibits the synthesis of collagen by osteoblasts at higher concentration.
   - This anabolic effect is mediated by increased secretion of insulin like growth factors and transforming growth factors.
   - Therefore, the net effect of PTH on bone is increase in the bone mass in lower concentration and decrease in bone mass in higher concentration.

**Applied Physiology**

**Hyperparathyroidism**

Hyperparathyroidism or PTH excess is divided into two main categories:

1. Primary hyperparathyroidism
2. Secondary hyperparathyroidism.

**Primary Hyperparathyroidism**

Primary hyperparathyroidism occurs due to the tumor of parathyroid gland such as parathyroid adenoma that secretes excess PTH. It may be part of multiple endocrine neoplasia (MEN) syndrome. It is common in MEN type 1.

1. The disease is characterized by hypercalcemia, hypophosphatemia, demineralization of bone, hypercalciuria, and renal stone formation.
2. The chronic effect of parathyroid excess results in the bone disease called osteitis fibrosa. There will be features of osteopenia and fracture.
3. In this disease, the bone marrow is fibrosed and bone resorption exceeds the bone formation.

**Secondary Hyperparathyroidism**

Secondary hyperparathyroidism occurs in chronic renal disease and rickets.

1. In these diseases, the chronic hypocalcemia causes increased secretion of PTH.
2. This results in secondary hypertrophy of parathyroid glands.
3. Neonatal primary hyperparathyroidism has also been described that occurs due to a genetic defect.

**In malignancy:** Hypercalcemia occurs in malignancies.

- This is mainly due to the bone metastases by the cancer cells that cause local osteolysis which results in hypercalcemia.
- Hypercalcemia also occurs in malignancy due to ectopic secretion of PTH or PTH related proteins (PTH rP).
- Usually, it is seen in the cancers of breast, kidney, lungs, ovary, and skin.

**Hypoparathyroidism**

Hypoparathyroidism is rarely seen as a disease of parathyroid gland. Rather, the common entity is pseudohypoparathyroidism in which symptoms of PTH deficiency are observed in the presence of normal PTH level in plasma.

There are two types of pseudohypoparathyroidism:

1. A congenital variety, in which the PTH does not increase the cyclic AMP and IP₃ formation in the target cells due to a receptor defect.
2. Another variety in which cyclic AMP synthesis by PTH remains normal but PTH fails to produce phosphaturic effect.

In hypoparathyroidism, hypocalcemia and increased bone density are main feature.

**Hypocalcemic Tetany**

Hyposecretion of parathyroid is rather common following thyroid surgery in which parathyroid glands are usually removed by mistake along with thyroid tissue. This causes severe hypoparathyroidism that leads to acute hypocalcemic tetany.

**Effects of Parathyroidectomy**

PTH is essential for maintenance of plasma calcium concentration. Acute deficiency of PTH results in neuromuscular defects that endanger life. Therefore, PTH is essential for survival. The symptoms of tetany following thyroid surgery develop in 24 to 48 hours postoperatively.

The features of tetany are:

1. **Chvostek’s sign:** This is the quick contraction of facial muscles of the same side by tapping over the facial nerve at the angle of jaw.
2. **Trousseau’s sign:** The spasm of the muscles of the upper extremity that causes flexion of the wrist and thumb (i.e. flexion at metacarpophalangeal joints) with extension of fingers (i.e. extension of interphalangeal joints).
3. **Carpopedal spasm:** There is also spasm of muscles at the wrist joints (carpal spasm) (Fig. 62.5). The changes at wrist and ankle joints together called as carpopedal spasm.
4. **Laryngospasm:** In hypocalcemic tetany, usually patients die due to hypoxia that occurs due to laryngospasm. The muscles of larynx contract and occlude the laryngeal passage.
5. **Hypocalcemia:** Significant hypocalcemia occurs.
6. Hyperphosphatemia.
The treatment of hypocalcemic tetany includes immediate administration of ionized calcium and parathormone.

Parathyroid Hormone-related Protein

The protein with PTH activity has been described recently. This is called parathyroid hormone related protein (PTHrP). This is produced by many tissues in the body. It is a polypeptide containing 140 amino acids. The gene that encodes the formation of PTHrP is present on the human chromosome 12 (it should be noted that the gene for PTH is present on chromosome 11). PTHrP has marked homology with the PTH.

Functions of PTHrP

1. PTHrP stimulates proliferation of chondrocytes in developing cartilage and inhibits their mineralization.
2. It also acts as a growth factor for the development of skin, hair follicle, and breast.
3. PTHrP is produced in large quantities in the lactating breast tissue and secreted in the milk. However, the plasma concentration of PTHrP does not increase during lactation.
4. PTHrP has also been described in kidney (renal glomeruli) and in the brain (cortex, hippocampus and cerebellum). But, functions of PTHrP in these organs are not clearly known.
5. It also causes calcium transport in placenta.
6. PTHrP is elevated in hypercalcemia of malignancy (Clinical Box 62.1)

Calcitonin

Source and Structure

Calcitonin is secreted from parafollicular cells (C cells) of the thyroid gland. The parafollicular cells develop from ultimobranchial bodies, a pair of glands that develop from 5th branchial pouch.

1. Calcitonin is a polypeptide hormone containing 32 amino acids. The molecular weight is 3,500.
2. The calcitonin gene that transcribes mRNA to form calcitonin is processed to form a different mRNA in the nervous system where it forms calcitonin gene-related peptides (CGRP).
3. CGRP is a potent vasodilator.

Synthesis, Secretion and Metabolism

Synthesis and Secretion

The major stimulus for calcitonin secretion is the rise in plasma calcium level. Above the concentration of 9 mg% of plasma calcium, the secretion of calcium becomes directly proportional to the calcium concentration. Other stimuli: The β receptor agonists, dopamine, GI hormones (CCK, glucagons, gastrin, secretin), and estrogen stimulate calcitonin secretion. Gastrin is a strong stimulus for calcitonin release (Clinical Box 62.2).

Metabolism

The concentration of calcitonin in plasma is 10–20 pg/mL. The concentration increases to almost 10 fold in hypercalcemia. The hormone is degraded in the liver. The half life of calcitonin is 5–10 minutes.

Mechanism of Action

Calcitonin acts on calcitonin receptors present on the target cells. The receptors are coupled to G proteins and therefore, stimulate the adenylate cyclase–cyclic AMP system.

Physiological Actions

Calcitonin decreases the plasma calcium level by acting mainly on bones and kidney (Flowchart 62.2).

Effects on Bone

Calcitonin mainly acts on the osteoclasts.

1. It decreases osteoclastic activity. Thus, it inhibits the bone resorption. The number and size of osteoclasts decrease.
2. The action of calcitonin on bone is just opposite to that of PTH with respect to the calcium turnover in the bone.
3. However, the effect on phosphate turnover is same as that of PTH. It decreases plasma phosphate level. This is due to inhibition of bone resorption, facilitation of phosphate entry into the bone, and also mild increase in urinary phosphate excretion.

4. Thus, the hypophosphatemic effect of calcitonin is independent of hypocalcemic effect.

**Effects on Kidney**

It decreases calcium reabsorption from kidney. Therefore, it produces calciuria. It also causes mild phosphaturia by acting on proximal tubule.

**Calcitonin Escape**

Injection of calcitonin produces hypocalcemia mainly by antiosteoclastic activity. However, this antiresorptive action of calcitonin begins to wane within hours.

1. The body escapes from hypocalcemic effects of calcitonin. This is called calcitonin escape, the exact mechanism of which is not known.

2. It is probably due to rapid down regulation of calcium receptors. Therefore, calcitonin is effective in decreasing plasma calcium only for a short duration.

3. This limits the use of calcitonin in long-term management of hypercalcemia.

**Physiological Significance**

1. Due to calcitonin escape phenomenon, the bones remain essentially normal in conditions of hypersecretion of calcitonin like medullary carcinoma of thyroid.

2. Calcitonin is secreted more in young individuals, which is believed to play some role in skeletal development.

3. **Feeding** increases calcitonin secretion by releasing GI hormones. Calcitonin escape protects against postprandial hypercalcemia.

4. During and after pregnancy, calcitonin protects bone loss in pregnant and lactating mother.

   – During pregnancy maternal calcium is used for bone formation in fetus and after pregnancy calcium is utilized for lactogenesis.

   – To maintain maternal plasma calcium, 1,25-dihydroxycholecalciferol level is increased, which causes bone resorption.

   – Therefore, increased calcitonin level in pregnancy prevents bone loss by opposing osteoclastic activity.

5. Calcitonin and CGRP are found together in some brain regions like pituitary, hypothalamus, limbic system etc, where they act as co-transmitters. They inhibit transmission of pain impulses in CNS independent of opioid system.

**Applied Aspects**

**Clinical Use of Calcitonin**

Calcitonin is used in the treatment of hypercalcemia, and in the diseases in which osteoclastic activity needs to be reduced, for example in Paget’s disease. Calcitonin is sometimes used as a central analgesic as it blocks the transmission of pain impulses in CNS.

**Paget’s Disease**

In this disease, increased osteoclastic activity of unknown etiology results in localized regions of bone resorption followed by a compensatory osteoblastic activity causing patchy new bone formations.

1. Thus, pagetic bones are structurally disorganized and susceptible to deformities.

2. X-ray pictures show areas of increased density and resorptive cavities.

3. Though most of the patients are initially asymptomatic, bone deformities resulting in compressive neuropathy and subsequent neuromuscular dysfunctions are not uncommon.

4. The disease is treated by daily injections of calcitonin.

5. Bisphosphonates like etidronate and pamidronate are more useful in preventing bone resorption.

**Vitamin D**

Vitamin D increases plasma calcium level by increasing calcium absorption from GI tract, calcium reabsorption from kidney and mobilization of calcium from bone. It has similar effects on phosphate.

**Synthesis of Vitamin D**

Vitamin D is acquired from two sources in human being.

1. Skin (ultraviolet irradiation of skin produces vitamin D), and
2. Diet.
**From Skin**

Vitamin D₃ is synthesized in keratinocytes present in the epidermis.

1. When the skin is exposed to sunlight (ultraviolet radiation at 290–315 nm), 7-dehydrocholesterol in the skin is converted to previtamin-D₃. For this conversion to occur, *minimum 20 mJ radiation energy* per sq-cm of skin is required.
2. Previtamin-D₃ is then automatically converted to vitamin D₃ in another 3 days.
3. However, continuous exposure to sunlight prevents this conversion.
4. Thus, mild to moderate exposure to sunlight induces, whereas excessive exposure prevents vitamin D synthesis.

**From Diet**

In food, minimum of 2.5 µg vitamin D should be ingested daily (*daily dietary recommendation* is 10 µg or 400 units). The important sources of vitamin D are liver, fish, cod liver oil and milk. Vitamin D ingested in the diet has very little biological activity.

1. It undergoes activation in the body to be converted into the active vitamin D. This requires successive hydroxylations in the liver and kidney.
2. In the liver, it is hydroxylated at position 25 by microsomal and mitochondrial enzyme, to form 25-hydroxycholecalciferol which undergoes further hydroxylation in the kidney at 1 position to form 1, 25-dihydroxycholecalciferol (Flowchart 62.3).
3. This occurs mainly in the mitochondria in epithelial cells of proximal and straight tubules.
4. The 1, 25-dihydroxycholecalciferol is the active metabolite of vitamin D.

**Mechanism of Action**

There are vitamin D receptors in the GIT, bone, and kidney. Mechanism of Vitamin D action is like that of steroid hormones.

1. It binds with the nuclear receptors and induces receptor phosphorylation, which targets DNA molecules.
2. The major product of vitamin D action is the increase in intracellular calcium binding protein called calbindin.

**Physiological Actions**

Vitamin D increases plasma calcium level by acting on intestine, kidney and bone (Flowchart 62.4).

On small intestine: Vitamin D mainly acts on the intestine to facilitate calcium absorption against the concentration gradient.

1. It combines with the receptors on the brush border of the intestinal epithelial cells and increases number of calcium pumps (Ca²⁺-H⁺ ATPase molecules) and calcium channels in the basolateral membrane and
calcibindin in the cytosol of the epithelial cells of the intestine.
2. Thus, calcium is actively pumped from the cell into the ECF.
3. It also facilitates the absorption of phosphate and magnesium across the intestinal epithelium.
4. PTH stimulates intestinal calcium absorption by promoting formation of 1,25-dihydroxycholecalciferol in kidney.

On kidney: It acts synergistically with PTH to increase calcium reabsorption from kidney.
1. It increases number of calcium pumps in the epithelial cells of proximal and distal tubules.
2. Unlike PTH that causes phosphate excretion, vitamin D increases phosphate reabsorption from kidney.
3. On bone: It acts on osteoblasts that have receptors for vitamin D.
   1. It increases synthetic activity of osteoblasts.
   2. It is also necessary for normal mineralization of bone. Mineralization of newly formed osteoid depends on vitamin D.
   3. It also stimulates bone resorption, not by directly increasing the osteoclastic activity, but by stimulating various paracrine signals that originate from the osteoblasts. Activated osteoclasts produce a number of proteins including phosphatase and collagenase that lyse bone matrix.
4. It promotes development of osteoclasts from their precursor cells.
5. It also increases osteocytic osteolysis.
6. Thus, the direct effect of vitamin D on bone is to mobilize calcium, which is contrary to its overall effect of promoting mineralization.

In summary, vitamin D increases calcium and phosphate absorption from intestine, and their reabsorption from kidney. Thus, vitamin D increases plasma level of calcium and phosphate (Flowchart 62.4), that increases bone mineralization. This overshadows the direct action of vitamin to mobilize these minerals from bone.

Other actions: Receptors for vitamin D are also found in skeletal and cardiac muscles, breast, anterior pituitary, skin, lymphocytes and monocytes.
1. It stimulates transport of calcium into skeletal and cardiac muscle. It has been observed that deficiency of vitamin D results in muscle weakness and cardiac dysfunction.
2. It promotes differentiation of immunological cells and keratinocytes in the skin. Therefore, skin infection is high in vitamin D deficiency.
3. It regulates growth of many tissues. It also stimulates production of tissue growth factors.
4. Anti-aging protein exerts effects partly via 1,25-dihydroxycholecalciferol (Application Box 62.1).

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**Vitamin D Excess**
Vitamin D excess usually occurs due to excess intake of the vitamin.

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**Vitamin D Deficiency**
(Rickets and Osteomalacia)
Deficiency of vitamin D causes rickets in children and osteomalacia in adults.

**Causes**
1. Inadequate intake
2. Lack of exposure to sunlight
3. Chronic liver disease (inadequate 25 hydroxylation)
4. Chronic renal disease (inadequate 1 hydroxylation)
5. Hypoparathyroidism
6. Anticonvulsant drugs like phenobarbital
7. Vit. D receptor deficiency
8. Vit. D dependent rickets (Hereditary deficiency of 1(alpha)-hydroxylase, an autosomal recessive disorder).

**Features**
The disease is characterized by inadequate mineralization of bone matrix. Both trabecular and cortical bones are involved. In both adult and children, excess unmineralized osteoid accumulates in the bone. The concentration of calcium and phosphate is significantly low. Bone strength is reduced, and therefore, they distort in response to mechanical load.

**In Children**
Decreased mineralization diminishes bone rigidity that results in bowing of the long bones of lower limbs and rickety chest. The epiphyseal growth centers are affected by defective mineralization of bone.

**In Adults**
As longitudinal growth of long bone is already completed in adults, bowing of bones does not occur. However, due to increased unmineralized osteoid content, bone pain, vertebral collapse, and fractures are common.

**Treatment**
Vitamin D supplement should start at the earliest.

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**Application Box 62.1**
\(\alpha\)-Klotho partly acts through Vit. D: The anti-aging protein \(\alpha\)-Klotho that plays a major role in calcium and phosphate metabolism has been observed to act partly through 1,25-dihydroxycholecalciferol.

- \(\alpha\)-Klotho facilitates membrane localization of proteins that are involved in calcium and phosphate reabsorption, such as TRPV5 and Na\(^{+}\)-K\(^{+}\) ATPase.
- It enhances the activity of FGF-23 (fibroblast growth factor) by acting at the receptor level.
- FGF-23 decreases the renal NaPi-IIc activity and inhibits synthesis of 1(alpha)-hydroxylase that reduces the level of 1,25-dihydroxycholecalciferol.
1. It causes hypercalcemia, hypercalciuria, and kidney stones.
2. Increased bone resorption is not uncommon.
3. Hyperphosphatemia is also present.

**Other Hormones Affecting Calcium and Bone Metabolism**

**Glucocorticoids**

Glucocorticoid decrease plasma calcium level by inhibiting osteoclastic activity and decreasing calcium absorption from intestine. Therefore, cortisol excess causes osteoporosis.

**Growth Hormone**

It increases calcium absorption from intestine, though it also promotes calcium excretion in urine. The net effect is mild increase in plasma calcium. Stimulates osteoblastic activity and bone formation.

**CHAPTER SUMMARY**

**Key Concepts**

1. The primary action of PTH is to increase the level of calcium and decrease the level of phosphate in the plasma. Though PTH acts on both osteoblasts and osteoclasts and activates both bone synthesis and resorption, the net effect is increased bone resorption and excess mobilization of calcium into plasma.
2. Calcitonin decreases the plasma calcium level by acting mainly on bones and kidney. It decreases osteoclastic activity and inhibits the bone resorption. The number and size of osteoclasts decrease.
3. Therefore, for normal bone health (balance between osteoblastic and osteoclastic activity), the normal concentration of PTH and calcitonin should be maintained.
4. Vit. D is also necessary for normal mineralization of bone. Mineralization of newly formed osteoid depends on vitamin D. It also increases synthetic activity of osteoblasts.

**Important to Know (Must Read)**

1. In examination, “Describe the hormones regulating calcium and bone metabolism,” “Describe the regulation of secretion and physiological actions of parathyroid hormone (PTH)” may be asked as Long Questions.
2. Hypoparathyroidism, Effects of Parathyroidectomy, Hypocalcemic tetany, Calcitonin, Physiological actions of vitamin D, Causes, features and treatment of rickets and osteomalacia are usual Short Questions in exam.
3. In Viva, examiner may ask— Name the three main hormones regulating calcium and bone metabolism, List the other hormones regulating calcium and bone metabolism, List the physiological actions of parathyroid hormone, List the physiological actions of calcitonin, List the physiological actions of vitamin D, Name the features of hypocalcemic tetany, What is treatment of hypocalcemic tetany, What are types and features of hyperparathyroidism, What is osteitis fibrosa, Why hypercalcemia occurs in malignancies, What is pseudohypoparathyroidism and what are the types, What are the causes, features and treatment of rickets and osteomalacia, How is the secretion of PTH regulated, Physiological anatomy and cell types of parathyroid gland, Name the PTH receptors, What is parathyroid hormone related protein (PTHrP) and what are its functions, What is the mechanism of action of PTH, What are the factors that stimulate calcitonin secretion, What is calcitonin escape, What are the physiological significances of calcitonin escape, What are the clinical uses of calcitonin, What are the causes, features and treatment of Paget’s disease, What are the sources of vitamin D, What are the steps of synthesis of vitamin D, What is the mechanism of action of vitamin D, What are the functions of Vit D, What is α-Klotho and what are its functions, What are the features of vitamin D excess, What is the difference between osteoporosis and osteomalacia, What is Gley’s glands.
Pineal gland is also known as epiphysis was initially described as the main controlling center in the brain that influences many CNS functions. In fact, for centuries, it has attracted attention of many researchers in physiology and medicine for its role in integral control of the being. However, till date functions of pineal gland are not clearly understood except its role in melatonin secretion and control of light-dark cycle. It is known to act as photoreceptor in fish, amphibian and reptiles, a biological clock in birds, and has become an endocrine gland in mammals. However, more research needs to be done in human beings to reveal the role of pineal in the evolutionary progression of the mankind.

**PHYSIOLOGICAL ANATOMY**

Pineal is embryologically derived from an evagination of the roof of third ventricle between the habenular and posterior commissures. Along with habenular and posterior commissures it makes up the epithalamus.
1. It is connected by a stalk to the posterior and habenular commissures (Fig. 63.1).
2. The stroma of the pineal gland consists of neuroglial cells, pinealocytes, and parenchymal tissue. Pinealocytes possess secretory function (Fig. 63.2).
3. It is larger in size during infancy and childhood. It regresses at the time of puberty and is small in size in adults.

4. The plasma level of melatonin, which is secreted from pineal gland, in children is about 250 pg/ml, which is much higher than the level in adults, i.e. about 70 pg/ml.

**Hormones of the Pineal Gland**

Pineal gland secretes the hormone melatonin. Recently, it has been detected that pineal gland secretes many other
peptides} that influence hypothalamic and other endocrine secretions. Peptides that have been identified in pineal gland in mammals are arginine vasotocin, arginine vasopressin, oxytocin, pro-opiomelanocortin derived peptides like ACTH, α-MSH and β-endorphin, angiotensin II, renin like activity, and TRH and GnRH like peptides. Thus, pineal gland might be involved in control of many endocrinal functions of the body.

**Melatonin**

Melatonin is N-acetyl-5-methoxytryptamine, which is synthesized from the amino acid tryptamine (Flowchart 63.1). It is synthesized by the parenchymal cells of the pineal gland and then released into circulation and CSF.

Melatonin secretion exhibits a remarkable diurnal variation. The secretion is maximum in the night, especially between 11 pm and 7 am (Fig. 63.3).

1. It is proposed that this diurnal variation is due to the alteration in sympathetic discharge to the pineal gland, mediated by norepinephrine via β receptors.
2. The pineal sympathetic output synchronized with light dark cycle is controlled by inputs from retinohypothalamic pathway that projects to suprachiasmatic nucleus.
3. Fibers from suprachiasmatic nucleus terminate in intermediolateral gray column of the thoracic spinal cord that in turn projects to superior cervical ganglion from where fibers originate to end in the pineal gland (Flowchart 63.2).
Melatonin Receptors

Two types of melatonin receptors have been described: ML 1 and ML 2.
1. Melatonin at ML 1 receptor subtype acts by increasing cAMP. ML 1 receptor has two subtypes: Mel 1a, and Mel 1b (For role of melatonin in sleep-wake cycle, refer, “Theories of sleep”).
2. ML 2 receptor subtype acts by increasing IP3 and DAG in the target cells.

Functions of Pineal Gland

Melatonin secreted from pineal has following functions:

1. Melatonin secreted from pineal has a definite diurnal pattern. This diurnal change in melatonin level is believed to coordinate the endocrinal responses of the body with the light-dark cycle in the environment.
2. Melatonin secreted from pineal inhibits gonadal function. It is believed that pineal secretion inhibits the onset of puberty. Therefore, regression of pineal stimulates the beginning of pubertal changes. It has been observed that precocious puberty and sexual precocity are associated with pineal abnormalities.
3. In lower animals, melatonin influences the activity of melanophores and thereby controls skin color. However, such function in human appears to be uncertain.

CHAPTER SUMMARY

Key Concepts

1. Though pineal gland is known to secrete melatonin, and control circadian rhythm, it might have many more functions.
2. The size of pineal gland decreases with the maturation of sex organs. May be melatonin is meant of inhibition for basic instincts, especially functions related to mating.

Important to Know (Must Read)

1. In examination, Long Questions are not asked from this chapter.
2. The Short Questions may be ‘Pineal gland’ and ‘Melatonin’.
3. In Viva, examiner may ask… List the functions of pineal gland, List the hormones of pineal gland, How the pathway from retina stimulates melatonin secretion, When the peak secretion of melatonin occurs.
Local Hormones

**Learning Objectives**

On completion of study of this chapter, the student **MUST** be able to:
1. Name the local hormones.
2. Name the receptors and describe the functions of histamine and serotonin
3. Classify prostaglandins and describe their functions.
4. Give the functions of other local hormones.

The student **MAY** also be able to:
1. Describe the function of all local hormones.
2. Explain the role of local hormones in various functions and dysfunctions of the body.

**Definition and Types**

**Definition:** Local hormones are chemicals that are secreted locally from an endocrine tissue that are primarily involved in control of local tissue functions where they are secreted.

**Types:** The known local hormones are: renin, erythropoietin, ANP, melatonin, thymosin, histamine, serotonin, bradykinin, and prostaglandins.

**Histamine**

**Source, Synthesis and Metabolism**

Histamine is secreted from **mast cells and basophils**:
1. It is widely distributed in mammalian tissues.
2. It has highest concentration in the skin, gastric mucosa, pituitary and lungs.
3. Histamine is synthesized from the amino acid **histidine** by the action of enzyme **histidine decarboxylase**.
4. Histamine is converted to **methylhistamine** by **histamine-N-methyltransferase**, which is further converted to **methyl-imidazole-acetic acid** (Flowchart 64.1).

**Histamine Receptors**

Three types of histamine receptors have been described so far. They are: **H₁**, **H₂** and **H₃** receptors. All are found in brain and peripheral tissues.

**H₁** receptors: They are attached to phospholipase C. On activation, they produce **IP₃** and **DAG** in the cell. These receptors mediate inflammation and allergy.

**H₂** receptors: Histamine at **H₂** receptors acts by **increasing intracellular cAMP**. They are found in gastric mucosa and mediate gastric acid secretion.

**H₃** receptors: They are found in CNS, especially in presynaptic membrane and are coupled to G proteins. They inhibit release of histamine and other neurotransmitters from presynaptic nerve terminals.
Physiological Actions
Histamine acts mainly on the cardiovascular system, smooth muscles, and exocrine secretions. It mediates inflammation and allergy.

On CVS
Histamine is a potent vasodilator. It decreases blood pressure (causes hypotension). It also decreases the cardiac output.

On Smooth Muscles
Histamine increases tone of most of the smooth muscles. It increases intestinal motility and causes bronchoconstriction.

On Exocrine Secretions
Histamine is a powerful stimulator of HCl secretion from parietal cells stomach. It also potentiates salivary, pancreatic, and intestinal secretions.

Role in Inflammation
It is an important mediator of inflammation. Histamine, by causing local vasodilatation increases blood flow at the site of inflammation and by increasing capillary permeability causes local swelling. It mediates responses (triple response) to local injury.

Role in Allergy
Allergic reactions are mostly mediated by histamine:
1. In acute systemic allergy (anaphylaxis), hypotension occurs due to release of histamine from mast cell that produces acute vasodilatation.
2. Local allergic reactions like urticaria, allergic rhinitis, hay fever, etc. are also due to the release of histamine at the local sites.
3. Bronchial asthma is precipitated or induced by histamine.
4. Histamine is released from mast cells in response to the antibody IgE (the reagin antibody). The antigen-antibody complex causes degranulation of mast cells and release histamine.
5. Histamine is released from mast cell along with slow reacting substance for anaphylaxis (SRS-A).
6. Histamine and SRS-A mediate allergic reactions. Hence, antihistaminics are mainstay of treatment for allergies.

Relation with Itch
Histamine acts as a neurotransmitter for itch sensation. Experimentally, when histamine is injected into the skin, it produces sever itch. Itching associated with skin allergy (urticaria) is cured by antihistaminics.

On CNS
Histamine is a neurotransmitter in many areas of the CNS. Histaminergic neurons project from tuberomamillary nucleus to all parts of the brain. These histaminergic fibers are involved in the control of blood pressure, sexual and ingestive behaviors, arousal and alertness, pain and secretion of anterior pituitary hormones.

Clinical Correlation
Antihistaminic Drugs
Antihistaminics are used frequently in clinical practice for the treatment of allergy and inflammations.

H1 receptor antagonists: H1 antagonists like mepyramine and promethazine are used to prevent histamine induced contractions of smooth muscles of intestine and bronchi. They are also used frequently in the treatment of allergies.

H2 receptor antagonists: H2 antagonists like cimetidine, ranitidine, and famotidine are used to inhibit histamine induced HCl secretion in the stomach. These drugs are frequently used in the treatment of peptic ulcer.

Serotonin

Source
Serotonin is 5-hydroxytryptamine (5-HT). It is distributed in various tissues. It is present in high concentration in GI tract, CNS, especially in the hypothalamus and brainstem, and skin. Due to its higher concentration in the intestine, it is also known as enteramine. It is also present in platelets and basophils.

Synthesis and Metabolism

Synthesis
Serotonin is synthesized from the amino acid tryptophan. Tryptophan is converted to 5-hydroxytryptophan by the action of enzyme tryptophan hydroxylase. 5-hydroxytryptophan is then decarboxylated to form 5-hydroxytryptamine (serotonin) catalyzed by the enzyme 5-hydroxytryptophan decarboxylase (Flowchart 64.2).

Metabolism
Serotonin is metabolized by the enzyme monoamine oxidase to form 5-hydroxyindole acetic acid (5-HIAA), which is excreted in urine. 5-HIAA is physiologically inactive. However, the level of it in urine is an index of serotonin secretion in the body.

Serotonin Receptors
Till date, seven types of serotonin receptors have been identified. They are 5-HT1, 5-HT2, 5-HT3, 5-HT4, 5-HT5, 5-HT6, 5-HT7. The 5-HT1 is further subdivided into 5-HT1A, 5-HT1B, 5-HT1D, 5-HT1F, and 5-HT1E. Also, 5-HT2 is further subdivided into 5-HT2A, 5-HT2B, and 5-HT2C. The 5-HT5 has two subtypes: 5-HT5A and 5-HT5B.
Physiological Actions
Serotonin mainly acts on cardiovascular system, respiratory system, kidney, smooth muscle, and nervous system.

Cardiovascular System
Serotonin, acting locally produces vasoconstriction. It facilitates platelet aggregation. These two functions of platelets are mediated via 5-HT_2A receptors, and are essential for temporary hemostasis. Serotonin also produces tachycardia.

Respiratory System
Serotonin increases bronchial tone (causes bronchoconstriction), which is mediated via 5-HT_2A receptors. In asthmatic patients, it induces the acute attack of asthma. It also stimulates rate of respiration.

Kidney
Serotonin has mild antidiuretic effect. It prevents diuresis by decreasing GFR, which occurs due to afferent glomerular arteriolar constriction. It also causes ureteric spasm and temporarily stops urination.

GI Tract
Serotonin stimulates GI secretions and motility. It promotes peristalsis and produce diarrhea. These effects are mediated by 5-HT_4 receptors.

Central Nervous System
It is released as a neurotransmitter in different parts of the brain. The most important serotonergic pathway in the brain is the raphespinal system that on stimulation produces analgesia. 5-HT_3 receptors are present in area postrema that on activation induces vomiting. 5-HT_6 and 5-HT_7 receptors are distributed throughout the limbic system, neocortex, cerebellum and spinal cord. It has following central functions:
1. Inhibits transmission of pain impulses in dorsal horn of spinal cord, and thus, it is an important component of endogenous analgesia system.
2. Stimulates prolactin secretion from anterior pituitary.
3. Regulates circadian rhythm (suprachiasmatic nucleus receives heavy serotonergic innervation). Serotonin is a strong antidepressant. Hallucinogens produce euphoria by releasing serotonin in the brain. Antidepressant drugs such as fluoxetine act by inhibiting serotonin reuptake in the brain.
4. Serotonin is a strong anorectic agent. It inhibits feeding by acting on hypothalamic and other feeding areas in the brain.
5. Serotonin suppresses sleep and serotonin antagonists enhance slow wave sleep.

Clinical Correlation
Increased serotonin activity is seen in some endocrine tumors, which is detected by estimation of urinary 5-HIAA. This is usually observed in carcinoid syndrome.

Bradykinin
Bradykinin is a peptide containing 9 amino acids (nonapeptide). It is synthesized in tissue and plasma.

Synthesis
Bradykinin is synthesized from high molecular weight kininogen (HMWK) by the action of plasma kallikrein in plasma and tissue kallikrein in tissue. Plasma kallikrein is formed from prekallikrein by activated clotting factor XII.

Physiological Actions
Bradykinin resembles histamine in its physiological functions:
1. It produces vasodilation and increases local blood flow.
2. It causes contraction of visceral smooth muscle.
3. It increases capillary permeability, attracts leucocytes to the site of injury and produces pain when injected into the skin.
4. It increases secretion from exocrine pancreas and salivary gland.
5. It also increases sweat secretion.

Clinical Importance
Bradykinin is secreted from abnormal endocrine tumors as seen in carcinoid syndrome.
Prostaglandins

Prostaglandin was first described by Von Euler in 1937. It was named prostaglandin for its enumeration in the secretion from prostate gland. Afterwards, prostaglandins were found to be synthesized in various tissues of the body. They are 20-carbon unsaturated fatty acids containing a cyclopentane ring. They act as local hormone in kidney, lungs, GI tract, uterus, skin and other tissues.

Scientist contributed

Ulf Svante von Euler (1905-1983) was a Swedish physiologist and pharmacologist. He shared the Nobel Prize in Physiology or Medicine in 1970 for his work on neurotransmitters. His short stay as a postdoctoral student in Dale’s laboratory was very fruitful, where in 1931 he worked with John H Gaddum and discovered substance P. After returning to Stockholm, he pursued his research, and discovered four other important endogenous active substances, prostaglandins, vesiglandin (1935), piperidine (1942) and noradrenaline (1946).

Types

There are different types of prostaglandins. The commonest prostaglandins are PGE, PGF, PGI, PFH and PGA. Further, each has subcategories like are PGA₁, PGA₂, PGE₁, PGE₂, PGF₁α and PGF₂α, etc.

Synthesis

Prostaglandins are synthesized from arachidonic acid, which is formed from membrane phospholipid by the enzyme phospholipase A₂:

1. Arachidonic acid is then converted to cyclic endoperoxides (PGH₂) by the action of enzyme cyclooxygenase (prostaglandin synthase).
2. PGH₂ is then converted to prostacyclin by the enzyme prostacyclin synthase or thromboxane A₂ by the enzyme thromboxane synthase (Flowchart 64.3).
3. PGH₂ is also converted to PGE₂, PGF₂α, etc. by various isomerases.

Physiological Actions

Prostaglandins are present in almost all the tissues of the body. They are mainly involved in the control of functions of various organ systems, hemostasis, and metabolisms and play an important role in inflammation.

Cardiovascular System

PGA₁ and PGA₂ cause peripheral arteriolar dilation. Prostacyclin produces vasodilation and thromboxane A₂ causes vasoconstriction.

Reproductive System

1. PGF₂α produces contraction of gravid uterus. The concentration of this chemical increases in the maternal blood during parturition. It is suggested that the increase in PGF₂α initiates labor in the presence of high concentration of oxytocin.
2. Prostaglandins facilitate luteolysis (regression of corpus luteum).
3. They decrease the secretion of progesterone.
4. Prostaglandins increase in uterine fluid and cause necrosis of blood vessels of the uterus just before the bleeding starts during menstrual cycle. It is proposed that prostaglandins initiate the process of bleeding.
5. It increases secretion of GnRH from hypothalamus.

Hemostasis

Prostacyclin inhibits platelet aggregation and produces vasodilation whereas thromboxane A₂ and endoperoxides promote platelet aggregation and cause vasoconstriction:

1. The balance between the prostacyclin and thromboxane A₂ determines the degree of platelet plug formation (refer to Fig. 20.9, Chapter 20).
2. Thus, prostaglandins greatly influence temporary hemostasis.

Respiratory System

Prostaglandin E causes bronchodilation whereas PGF₁α produces bronchoconstriction. PGF₂α is implicated in the genesis of bronchial asthma.

GI System

Prostaglandins E and F₂α inhibit absorption of sodium and water:
1. The **watery diarrhea** produced in cholera is mediated by prostaglandins.
2. They also stimulate **intestinal motility**.

### Central Nervous System
Prostaglandins act as neurotransmitters in various parts of the brain. PGE inhibits release of norepinephrine from the nerve endings.

### Inflammation
Prostaglandin E and A increase capillary permeability during inflammation. PGE sensitizes the nerve endings to bradykinin and produces pain.

### Metabolism
PGE inhibits lipolysis, induced by ACTH, GH, glucagons, and epinephrine.

### Clinical Correlation
Prostaglandin preparations are used in the treatment of different diseases.
1. **Steroidal anti-inflammatory drugs** such as cortisol inhibit prostaglandin production by inhibiting the enzyme phospholipase A$_2$.
2. **Nonsteroidal anti-inflammatory drugs** (NSAID) such as ibuprofen inhibit prostaglandin synthesis by inhibiting the enzyme cyclooxygenase (see Flowchart 64.3).

### Carcinoid Syndrome
These are tumors of enterochromaffin cells of the GI tract or bronchus or pancreas:
1. A characteristic feature of carcinoid syndrome is the episodic flushing of the skin, associated with hypertension, abdominal pain, diarrhea, and bronchoconstriction.
2. These episodic attacks occur due to secretion of various chemicals from the tumor enterochromaffin cells.
3. Usually, these tumors secrete excess of serotonin and histamine.
4. Therefore, the disease is diagnosed by the excretion of increased amount of 5-HIAA in the urine.

### Other Local Hormones

#### Renin
Renin is the hormone secreted from JG cells of kidney in response to hypovolemia and hypotension. It converts angiotensinogen to angiotensin I, which is further converted to angiotensin II by ACE (for details, refer renin-angiotensin system in Kidney).

#### Erythropoietin
Erythropoietin is the glycoprotein hormone containing 165 amino acids secreted mainly from interstitial cells in the peritubular capillary bed of kidney. It is the major regulator of erythropoiesis (for details, refer erythropoiesis in Section II "Blood").

### ANP
Atrial natriuretic peptide (ANP) is a peptide hormone synthesized by the atrial myocytes. It has profound natriuretic and diuretic effect. Therefore, it is known as a natriuretic peptide. ANP contains 28 amino acids. A similar peptide is isolated in the brain, called as brain natriuretic peptide (BNP), which contains 32 amino acids. A third natriuretic peptide is also described, the C type natriuretic peptide (CNP), which contains 22 amino acids. The CNP is present in the brain, kidney, and vascular endothelial cells. Actions of natriuretic peptides are almost similar (for details, see ANP in ‘cardiovascular system’).

The major functions of ANP are:
1. ANP increases sodium excretion (natriuresis). This is occurs due to increased GFR, which is produced by dilation of afferent arteriole and relaxation of mesangial cells in the glomerulus of kidney. Also, ANP acts on kidney tubule to inhibit sodium reabsorption.
2. It increases water excretion (diuresis). This occurs, secondary to natriuresis.
3. It causes vasodilation, leading to decrease in blood pressure.
4. It also increases capillary permeability that causes extravasation of fluid into interstitial tissue space. This decreases blood volume and pressure.
5. **It decreases the sensitivity of blood vessels to constrictors** like catecholamines and angiotensin II.
6. It decreases heart rate and cardiac output.

### Melatonin
Melatonin is secreted from the pineal gland. The secretion is more in children and less in adults. It inhibits gonadal function (for details, refer previous chapter).

### Thymosin
Thymosin is the hormone secreted from thymus. Thymus is present in the mediastinum posterior to sternum. At birth it weighs about 10 g, which increases in size to about 30 g during adolescence. Thereafter, it decreases to about 5 g in adults and 2 g in elderly:
1. Thymosin controls the development of lymphocytes and plays an important role in immunity. It is secreted by the reticular epithelial cells of thymus (for details, refer Chapter 18).
2. **Development of the T cells** occurs in thymus during childhood and thymosin plays a crucial role in this process.
3. Hence, thymus has the central position in the development of cellular immunity. For details of T cell development, refer ‘Immunity’.
CHAPTER SUMMARY

**Key Concepts**
1. Though local hormones act locally in the tissue where they are produced, some of them have systemic effects.
2. Many local hormones play an important role in many physiological functions such as platelet aggregation, hemostasis, GI secretion and motility, menstrual bleeding, CV functions, neurotransmission in brain, glomerular filtration, bronchial activity, respiratory functions. Therefore, they play a key role in many dysfunctions and diseases.

**Important to Know (Must Read)**
1. In examination, Long Questions are not asked from this chapter.
2. Physiological actions of histamine, Physiological actions of serotonin, Physiological actions of prostaglandins, Bradykinin, ANP, Carcinoid syndrome may be asked as Short Questions in exam.
3. In Viva, examiner may ask... List the physiological actions of histamine, List the physiological actions of serotonin, List the physiological actions of prostaglandins, List the physiological actions of bradykinin, List the physiological actions of ANP, What are the histamine receptors and where are they found, List some antihistaminic drugs and mention their uses, What is carcinoid syndrome and what are its features, What is the mechanism of action of anti-inflammatory drugs (both steroidal and nonsteroidal), List the local hormones, What is ANP and from where is it secreted, List the steps of synthesis and metabolism of histamine, List the steps of synthesis and metabolism of ANP, List the steps of synthesis and metabolism of prostaglandins, List the serotonin receptors and their subtypes, What is thymosin and what are its functions.
SECTION–7
Reproductive System

**Part A: General Reproductive Physiology**
65. Sex Differentiation and Development, Puberty and Menopause
66. Physiology of Puberty and Menopause

**Part B: Male Reproductive Physiology**
67. Male Reproductive System

**Part C: Female Reproductive Physiology**
68. Female Reproductive System: Functional Anatomy, Oogenesis and Follicular Development
69. Menstrual Cycle and Ovulation
70. Ovarian Hormones and Control of Ovarian Functions

**Part D: Physiology of Conception, Pregnancy, Lactation and Contraception**
71. Physiology of Copulation
72. Pregnancy and Parturition
73. Physiology of Breast Development and Lactation
74. Physiology of Contraception
“To live, to love are signs of infinite things,
Love is glory from eternity’s spheres.
Abased, disfigured, mocked by baser mights
That steal his name and shape and ecstasy,
He is still the Godhead by which all can change.”

Sri Aurobindo (in ‘SAVITRI’
Chapter 65
Sex Differentiation and Development, Puberty and Menopause

**Learning Objectives**

On completion of study of this chapter, the student **MUST** be able to:

1. Understand the physiology of sex determination and differentiation.
2. Describe the mechanism of sex differentiation and development in males and females.
3. List the abnormalities of sex differentiation and understand the physiological basis of their causation.
4. Apply the knowledge of sex determination and differentiation in understanding the physiology of reproductive system.

The student **MAY** also be able to:

1. Describe the mechanisms of dysfunctions of sex differentiation.

Reproduction serves a primary goal of the nature in preservation and perpetuation of the species. The creation of two sexually complete and different individuals in same species, known as **sexual dimorphism** is the central scheme of the nature to achieve its principal intention of continuation of species through reproduction. The complete sexual dimorphism is obtained through the attainment of **puberty**. The sexually matured adults then reproduce to maintain their progeny. Therefore, to appreciate the physiology of reproduction one should study the process of sex differentiation, development of gonads, gonadal functions, physiology of puberty and sexual maturation, the principles of functioning of the female and male reproductive systems, and the physiology of pregnancy, parturition and lactation.

**SEX DIFFERENTIATION**

**Sex Determination**

**Normal Chromosomal Pattern**

In a normal human being, there are 46 (23 pairs) chromosomes: 22 pairs are autosomes and one pair is sex chromosome (Fig. 65.1).

**In Males**

The pattern is 22 pairs of autosomes, and one X and one Y sex chromosomes (44 XY).

**In Females**

The pattern is 22 pairs of autosomes and two X chromosomes (44 XX).

**Sex Chromosomes**

Gender is determined by the genetic inheritance of two chromosomes, called sex chromosomes. The two sex chromosomes are the **X chromosome**, the larger one, and the **Y chromosome**, the smaller one.

1. Females possess two X chromosomes and males have one X and one Y chromosome. Thus, ovum always contributes only one X chromosome, whereas half of the sperms contribute X and another half Y.

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**Fig. 65.1:** Chromosomal pattern in males (44 XY) and females (44 XX).
2. Therefore, union of sperm and ovum (fertilization) usually results in half XX and half XY. Hence, ideally, births of male and female children should have been in equal proportion.

3. However, generally the male births are slightly more than the female births. This difference in sex ratio could be due to the fact that the sperms that contain Y chromosomes are lighter than the sperm that contain X chromosome as Y chromosome is smaller in size than X chromosome. Therefore, sperms with Y chromosomes swim faster in female genital tract and reach ovum earlier. Thus, the opportunity for these chromosomes to fertilize ovum is more than the sperm with X chromosomes.

**Genetic Male**

When a sperm containing Y chromosome fertilizes an ovum, the resultant zygote develops into a genetic male having XY pattern of sex chromosomes. The karyotype is 44 XY.

**Genetic Female**

When a sperm containing X chromosome fertilizes an ovum, the resultant zygote develops into a genetic female having XX pattern of sex chromosomes. The karyotype is 44 XX.

**Sex Determination**

**Karyotyping**

Sex determination is usually done by karyotyping, a technique used for determining sex chromosome composition by employing tissue culture visualization of all chromosomes. The differences in shape and size of chromosomes in males and females help in concrete determination of sex (Figs. 65.2A and B).

**Demonstration of Sex Chromatin**

This is a relatively easy method to demonstrate sex chromatin (Barr body) in leucocytes or mucosal cells of the oral cavity (refer to Fig. 5.7, Chapter 5).

1. Barr bodies are usually formed in females as they have two X chromosomes.
2. When two X chromosomes are present, one X chromosome is functional and other X chromosome which is nonfunctional condenses to form sex chromatin.
3. Thus, presence of Barr body generally indicates female sex.

**Sex Differentiation**

The process of sex differentiation includes the pattern of development of the gonads, genital ducts, and the external genitalia.

1. During pregnancy, no differences in the gonads of male and female fetuses occur during the first five weeks of gestation. This is called the stage of indifferent gonads.
2. The differentiation of gender starts from sixth week of intrauterine life and continues even after birth till the complete maturation of the gonad of either gender is achieved.
3. The process of development from the indifferent gonad to complete mature gonad of either gender is called the sex differentiation.

**Cell Lines of Development:** Following fertilization, two different cell lines develop in the indifferent gonad.

1. The one cell line forms the granulosa cells of the ovarian follicle and the Sertoli cells of the testicular seminiferous tubules. The cells of this cell line nurse the germ cells and promote their maturation, and finally guide their development into the genital duct system. These cells in females produce estrogen.
2. The other cell line of the indifferent gonad (the interstitial cells) forms theca cells in the ovary and Leydig cells in the testis. These cells mainly secrete androgen, which is essential for the development of male sexual characteristics and spermatogenesis. In females, they form the precursors for estrogen synthesis.

**Differentiation of the Genetic Sex, Gonadal Sex and Phenotypic Sex**

**Genetic Sex**

**Male Genetic Sex**

The male chromosomal pattern is 44 autosomes and a pair of sex chromosomes. The sex chromosomes in a normal male is X and Y. The presence of Y chromosomes determines the maleness of the individual, without which neither testis nor the male genital pattern develop.

1. The Y chromosome contains the SRY gene (sex-determining region of the Y) which is located on the distal part of its short arm (Fig. 65.3).
2. This gene encodes the testis determining factor (TDF). The TDF is a transcription factor that binds to DNA molecules.
3. A gene identical to SRY gene encodes a histocompatibility antigen known as H-Y antigen. These are glycoprotein antigens present on the surface of all male cells. Both these antigens are involved in rejection of male tissue by the female recipients. This H-Y antigen helps in development of male gonads, and masculinization.
4. Though SRY gene is essential for masculinization, it is not adequate for the complete maleness.
5. Virilization of the genital duct and external genitalia requires the presence of an androgen hormone receptor. This receptor is encoded by the gene present on the X chromosomes. Therefore, X chromosomal gene also contributes to the development of maleness.

**Female Genetic Sex**

The normal female chromosomal pattern consists of 44 autosomes and a pair of X chromosomes (XX).

1. In the absence of a Y chromosome, SRY gene is absent.
2. Therefore, TDF and H-Y antigen do not develop.
3. Hence, instead of male gonads ovaries develop when both the sex chromosomes are X.

**Gonadal Sex**

**Male Gonadal Sex**

In a normal genetic male, the seminiferous tubule starts to form at 6–7 weeks of gestation.

1. The Sertoli cells enclose the germ cells.
2. The Leydig cells appear at 8–9 weeks. The testis starts secreting testosterone during this period. This helps in development of male sex.
3. The SRY gene prevents the synthesis of estrogen and inhibits the enzyme aromatase, which inhibits the development of female systems.

**Female Gonadal Sex**

In a normal genetic female, the differentiation of indifferent gonad into the female gonads (ovaries) starts after nine weeks of gestation.

1. Both the X chromosomes in the germ cells are activated.
2. The germ cells then undergo divisions to form oogonia that continue to proliferate.
3. Immediately after the start of meiosis, oogonia are surrounded by granulosa cells.
4. Stroma appears inside it, which gives rise to interstitial cells. Now the germ cells are known as primary oocytes.
5. The female gonad remains in this stage (primary oocyte stage), i.e. in the stage of diplotene or late prophase of meiosis for many years until ovulation occurs.
6. Estrogen secreted from the granulosa cells help in the female development.

**Phenotypic (Genital) Sex**

The differentiation of genital duct and external genitalia requires hormones. The basic principle is that under the direction of a positive hormonal influence, the male gonadal system develops, and in the absence of any hormonal control, female genitals develop (Figs. 65.4 and 65.5). The development of brain is also linked to the phenotypic sex (Application Box 65.1).

**In Males**

In males, at about 9–10 weeks Wolffian or mesonephric ducts develop which gives rise to epididymis, vas deferens, seminal vesicles, and ejaculatory ducts.

1. Development of this system is presided by the appearance of Leydig cells in the testis that secrete testosterone.
2. Testosterone stimulates growth and differentiation of Wolffian duct into the male genital system (Flowchart 65.1).
Section 7: Reproductive System

Figs. 65.4A to C: Differentiation of the male and female internal genitalia. (A) Indifferent stage; (B) Male internal genitalia; (C) Female internal genitalia. Note, in male, Müllerian duct degenerates, and in female, Wolffian duct degenerates.

Figs. 65.5A to E: Differentiation of the male and female external genitalia. (A) Indifferent stage; (B) Male genitalia (7th to 8th week); (C) Male genitalia (at about 12th week); (D) Female genitalia (7th to 8th week); (E) Female genitalia (at about 12th week).
In Females
In females, the Wolffian duct degenerates at about 10–11 weeks as ovary does not secrete testosterone. From Wolffian ducts on each side, the Müllerian ducts arise, which develops into fallopian tubes and uterus.

Role of MIS:
In males, müllerian ducts regress at about 8 weeks of gestation, the time during which Sertoli cells appear in the testis. The Sertoli cells produce a hormone called müllerian inhibiting substance (MIS). This is also known as antimüllerian hormone or müllerian regression factor.

1. MIS causes degeneration of müllerian duct by facilitating its apoptosis.
2. The SRY gene promotes the synthesis of MIS.
3. In females who lack MIS, müllerian duct continues to grow and form fallopian tubes, uterus, cervix, and vagina (Flowchart 65.2). This differentiation is completed by 18–20 weeks of gestation.

Müllerian Inhibiting Substance (MIS)
Also known as antimüllerian hormone or müllerian regression factor, MIS is a polypeptide hormone containing 536 amino acids secreted by the Sertoli cells of testis. It belongs to the TGF β superfamily of growth factors that includes activin and inhibin.

1. In males, though the secretion of MIS starts at 8–10 weeks of gestation, the level in plasma reaches its peak, i.e., about 50 ng/mL at 1 to 2 years of age. The concentration then declines gradually to a low level during puberty and a lower concentration of about 2–5 ng/mL is maintained through rest of life.

Functions of MIS
1. During early embryonic life, MIS inhibits development of female gonads by promoting regression of müllerian duct. Therefore, it helps in male gonadal development.
2. Later during fetal life in males, it helps in testicular descent.
3. In both sexes, it helps in maturation of germ cells.

Abnormalities of Sex Differentiation
The abnormalities of sex differentiation can be broadly divided into two categories: chromosomal and developmental abnormalities.

Chromosomal Abnormalities
The common chromosomal abnormalities are Turner’s syndrome, Klinefelter’s syndrome, testicular feminization syndrome, superfemales and true hermaphroditism.
Section 7: Reproductive System

Turner’s Syndrome
This is otherwise known as gonadal or ovarian dysgenesis.
1. It is characterized by diminished sexual development, dwarfism, and webbing of the neck in patients with no gonadal tissue or rudimentary gonads (Fig. 65.6 A).
2. The chromosomal pattern of sex chromosomes is XO, which means there are 44 autosomes and one X chromosome (total 45 chromosomes).
3. It results from nondisjunction of one of the X chromosomes during oogenesis.
4. Usually, it presents with primary amenorrhea. No sexual maturation occurs at puberty.

Klinefelter’s Syndrome
This is the most common sex chromosome abnormality. The syndrome is otherwise called seminiferous tubule dysgenesis.
1. Typically, it is characterized by presence of feminine features in an apparent male with small testes (Figs. 65.6B).
2. The patient is genetically female, but the presence of an extra Y chromosome causes development of the testis. Therefore, the karyotype is 47 XXY (44 autosomes + XX sex chromosomes + one extra Y chromosome).
3. They have male genitalia and at puberty male characteristics develop due to adequate testosterone.
4. But, seminiferous tubules are not properly developed and therefore, infertility results.
5. Thus, the syndrome usually presents with primary hypogonadism and infertility in male. Mental retardation is common.

Superfemales
This is a common aberration of sexual differentiation. Usually, it is not associated with any characteristic abnormalities and therefore remains undetected. The abnormality is commonly diagnosed while performing chromosomal analysis for some other causes. The karyotype is XXX.

Testicular Feminization Syndrome
The patients appear normal females externally. At puberty though breasts develop normally, the growth of pubic and axillary hairs is scanty. It causes primary amenorrhea.
1. Though the external genitalia are of female type, there is no development of uterus. The vagina ends in a blind pouch.
2. The gonads are testis, with immature seminiferous tubules.
3. Therefore, it is called testicular feminization syndrome. Though testes are present, spermatogenesis does not occur. The patients are genetic males (XY karyotype).

True Hermaphroditism
This is a rare condition in which both testes and ovaries are present. Sometimes, ovary is present on one side and the testis on the opposite side. Both male and female sex differentiations occur with the development of combined

Figs. 65.6A and B: (A) Turner syndrome. Note the small breast, webbed neck and short stature in a female with this syndrome; (B) Klinefelter syndrome. Note the gynecomastia and small testis in a male with this syndrome.
female and male external and internal genitalia. Usually, the karyotype pattern is 46 XX-XY.

**Other Chromosomal Abnormalities**

Though other chromosomal abnormalities are not common, they do occur.

**Transposition of Chromosome**

Transposition of a part of one chromosome to another chromosome is possible. For example, genetic males may have XX chromosome pattern due to transposition of short arm of their father’s Y chromosome into the father’s X chromosome during meiosis. They receive X chromosome from their mother and transpositioned X chromosome from father.

**Deletion of a part of Chromosome**

Deletion of small portion of Y chromosome containing SRY gene occurs during meiosis. This results in females with XY karyotype.

**Nondisjunction of Chromosome**

Nondisjunction of chromosome 21 (an autosome) is not uncommon. This is called **trisomy 21**, which is associated with **Down’s syndrome or mongolism**. It is not an aberration of sex chromosome, rather an autosomal abnormality.

**Developmental Abnormalities**

The developmental abnormalities are mainly hormonal disorders. However, nonhormonal abnormalities are also encountered. The hormonal abnormalities are broadly divided into **pseudohermaphroditism** (both female and male patterns) and **enzyme deficiencies**.

**Pseudohermaphroditism**

A pseudohermaphrodite is an individual with genetic constitution and gonad of one sex, but the external genitalia of the other sex. There are male and female pseudohermaphroditism. In these conditions, the patients have normal gonadal development in accordance with their chromosomal sex, but afterward they develop heterosexual characteristics due to opposite hormonal excess.

**Female Pseudohermaphroditism**

Male external genital development occurs in **genetic females** exposed to androgen during 8th to 13th week of gestation.

1. Source of androgen is usually **congenital virilizing adrenal hyperplasia** of fetus or **virilizing ovarian tumor** of the mother.
2. Sometimes it may be **iatrogenically-induced** following treatment of mother with androgens or prophylactic drugs.
3. In a typical female pseudohermaphrodite, the individual possesses ovaries, oviducts, but there is varying degrees of **masculine differentiation of external genitalia**.
4. The chromosomal sex is female.

**Male Pseudohermaphroditism**

Development of female external genitalia in a **genetic male** is called male pseudohermaphroditism.

1. It is usually due to **defective testicular development**.
2. As MIS secreted from testis during early embryonic life prevents development of female gonads, in defective testicular development the internal genitalia are also of female pattern.
3. Male pseudohermaphroditism could also be due to **androgen resistance** that usually occurs in deficiency of 5α-reductase, the enzyme that forms dehydroandrosterone or due to defects in androgen receptors.
4. In **complete androgen resistance syndrome** (testicular feminizing syndrome), MIS is secreted as testes are normal; therefore, vagina ends blindly due to absence of internal genitalia.

**Enzyme Deficiencies**

Congenital **17α-hydroxylase deficiency** causes male pseudohermaphroditism. This also occurs in congenital adrenal hyperplasia in which enzyme defects block the formation of pregnenolone (for details, refer to Chapter 59, ‘The Adrenal Cortex’)

**CHAPTER SUMMARY**

**Key Concepts**

1. In males, the Y chromosome contains the **SRY gene** (sex-determining region of the Y), which encodes the **testis determining factor** (TDF). Another gene identical to SRY gene encodes a histocompatibility antigen known as **H-Y antigen**. Both these antigens are **involved in rejection of male tissue** by the female recipients. This H-Y antigen helps in **development of male gonads**, and masculinization.
2. Virilization of the genital duct and external genitalia requires the presence of an **androgen hormone receptor**. This receptor is encoded by the gene present on the X chromosomes. Therefore, X chromosomal gene also contributes to the development of maleness.
3. In the absence of a Y chromosome, SRY gene is absent. Therefore, TDF and H-Y antigen do not develop. Hence, instead of male gonads ovaries develop when both the sex chromosomes are X.

**Important to Know (Must Read)**

1. In examination, **Long Questions** are usually not asked from this chapter.
2. Mullerian inhibiting substance (MIS), Turner’s syndrome, Klinefelter’s syndrome, Pseudohermaphroditism, Testicular feminizing syndrome, Mechanism of sex differentiation in male, Mechanism of sex differentiation in female may be asked as **Short Questions** in exam.
3. In **Viva**, examiner may ask… How the genetic sex is determined in males and females, How the gonadal sex is determined in males and females, How the phenotypic (genital) sex is determined in males and females, What is called a genetic male and a genetic female? What are the methods for sex determination, What is MIS and what are its functions, What is the role of MIS in sex differentiation, What is SRY gene and what is its role, What is H-Y antigen and what is its role, List the chromosomal abnormalities, What are the features of Turner’s syndrome, What are the features of Klinefelter’s syndrome, What are the features of testicular feminizing syndrome, What is true hermaphroditism, What is pseudohermaphroditism and what are the types and features, What is superfemale, How the phenotypic sex development influences brain development.
Learning Objectives

On completion of study of this chapter, the student **MUST** be able to:
1. Understand the physiology of puberty in boys and girls.
2. Name the stages of puberty and the special features in each stage.
3. Understand the mechanism of onset of puberty.
4. Appreciate the physiological basis of causation of precocious puberty and delayed puberty.
5. Understand the mechanism of onset of menopause.

The student **MAY** also be able to:
1. Describe the mechanism of onset of puberty, and physiological basis of changes at puberty.
2. Explain the physiological basis of puberty abnormalities.

Puberty is the physiological phenomenon of attainment of sexual maturity. The ability to reproduce is attained with puberty and ceased with menopause. In females, reproducibility totally stops at menopause, whereas in males reproducibility continues.

**Puberty**

**Definition**

The period of transition from the non-reproductive state to the state of reproductive functions that allows procreation is defined as puberty. During this period, the hypothalamic-pituitary-gonadal axis is activated to bring the gametogenic functions of the gonads to their threshold of reproductive maturation.

1. Normally, gonads of both genders remain quiescent until the onset of puberty. Under the influence of gonadotropins secreted from pituitary, maturation of gonads occurs that in turn helps in maturation of the reproductive system.
2. This period of maturation is known as puberty or adolescence. During this period, the endocrine and gametogenic functions of the gonads first develop to the point where the reproduction becomes possible.

**Age and Initiating Stimulus**

**Age of Onset of Puberty**

The age of onset of puberty varies depending on various factors like socioeconomic and environmental conditions and genetic constitutions. In general, in developed countries, puberty occurs earlier than in the developing countries. In advanced nations, it occurs between the age of 8–13 in girls and 9–14 in boys. In developing nations, the age of onset of puberty is **11–16 years in girls** and **13–18 years in boys**.

**Initiating Stimulus**

The increased secretion of adrenal androgen, called adrenarche, occurs about 1 to 2 years before the onset of puberty.

1. This **increased adrenal androgen** is believed to stimulate the production of gonadal hormones that cause maturation of reproductive organs. As increase in adrenal androgen at this stage occurs without any alteration in ACTH or cortisol secretion, it is proposed that this is the primary and sole stimulus that heralds puberty.
2. One hypothesis suggests that increased secretion of adrenal androgen before puberty occurs due to secretion of **adrenal androgen stimulating hormone** (AASH) from pituitary. But actual nature and mechanism of it are not known.
**Stages of Puberty**

**In Boys**
The pubertal development in males occurs in five stages (by **Tanner method**, modified). Usually it is completed within 2 to 4 years from its onset.

**Stage 1**
This is the preadolescent stage. There is no enlargement of external genitalia (penis, scrotum and testes). No pubic hair is present. However, secretion of adrenal androgen is increased (**adrenarche**).

**Stage 2**
Testes enlarge to more than 2.5 cm, which occurs due to growth of seminiferous tubules.
1. Though this occurs due to the secretion of adrenal androgen (**adrenarche**), testicular androgen also contributes.
2. Testicular testosterone secretion increases significantly.
3. Pubic hairs appear in scanty at the root of the penis.

**Stage 3**
Penis enlarges in length. Scrotum and testes are further enlarged. **Pubic hairs become darker and coarser** above the pubis. **Sperm first appears** in the morning sample urine (**spermarchy**).

**Stage 4**
Penis enlarges in width and further in length. Scrotal and testicular enlargement continues. Pubic hair becomes adult type. **Ejaculation of sperm** occurs either in dreams, or on masturbation or by sexual act.

**Stage 5**
Full adult pattern of sexual features develops.

**In Girls**
The pubertal development in females also described in five stages (by **Tanner method**, modified). Usually, it is completed within 2 to 5 years from its onset.

**Stage 1**
This is the preadolescent stage. There is no breast development. No pubic hair is present. However, secretion of adrenal androgen is increased (**adrenarche**).

**Stage 2**
Breast development starts (**thelarche**). **Breast paillae is elevated** and the diameter of areola is increased. Secretion of estrogen from ovary increases significantly. Sparse hairs appear along the labia majora.

**Stage 3**
Breast enlarges with enlargement of areola. **Pubic hairs develop**, grow and become dark (**pubarche**). Hairs appear in axilla.

**Stage 4**
Breast further enlarges with areola and papillae projecting out of it. Pubic hair becomes adult type, but covers smaller area. **Menstrual cycle starts** (**menarche**), but irregular at the beginning.

**Stage 5**
Full adult pattern of breasts and pubic hairs develop. Menstrual cycle occurs regularly.

**Mechanism of Onset of Puberty**
The hypothalamic neurons gradually mature to **secrete more GnRH**. This maturational process is genetically preprogrammed.
1. Dehydroepiandrosterone secreted from adrenal gland plays some role in the maturation of hypothalamic neurons.
2. The adipose tissue via secretion of **leptin** also plays some role in the determination of the time of onset of puberty (see below).
3. Normally, the secretion of GnRH, LH and FSH is not pulsatile before the onset of puberty. It is clear that until puberty the release of GnRH is non-pulsatile, which prevents puberty to occur.
4. It is not known what mechanism inhibits the **GnRH pulse generator** till puberty. However, before puberty, the GnRH is secreted in a pulsatile pattern that stimulates pulsatile secretion of LH and FSH.
5. It is proposed that this pulsatile secretion of GnRH brings about the onset of puberty. This theory is supported by the fact that experimental pulsatile injection of GnRH in immature monkeys produces normal menstrual cycle and the cycles continue till the pulsatile injection continues.

**Role of Leptin**
It has been observed that **body weight increases to a critical level** before the onset of puberty, especially in females. It is also observed that the onset of puberty is **delayed in girls with lower body weight**.
1. Leptin, the hormone secreted from adipose tissue cell is believed to help in the **maturation of hypothalamic-gonadal axis**.
2. This is supported by the experimental evidence that injection of leptin in female mice results in precocious puberty.
3. But, the exact role of leptin in the control of puberty is not known.
Abnormalities of Puberty

Abnormalities of puberty can be broadly classified into precocious puberty and delayed puberty.

Precocious Puberty

Precocious puberty may be of two types: true precocious puberty and precocious pseudopuberty.

True Precocious Puberty

Early development of secondary sexual characteristics, may be associated with premature development of gonads is known as true precocious puberty. Precocious puberty can occur in both girls and boys. This occurs due to early pubertal pattern of secretion of gonadotropin from pituitary.

True precocious puberty occurs due to following causes:
1. **Hypothalamic precocity:** Usually occurs due to tumor or infection of hypothalamus that causes premature increased secretion of GnRH.
2. **Gonadotropin independent precocity:** Precocious gametogenesis without increase in gonadotropin secretion. Usually, it occurs due to increased sensitivity of LH receptors to gonadotropins.
3. **Constitutional precocity:** When the actual cause of precocity can not be ascertained.
4. **Tumor of pineal gland.**

Precocious Pseudopuberty

The development of secondary sexual characteristics without gametogenesis is called as precocious pseudopuberty. This occurs due to exposure of immature males to abnormal quantity of androgen, and in immature females the abnormal exposure to estrogen.

Precocious pseudopuberty occurs due to following causes:
1. **Adrenal causes:** Congenital virilizing adrenal hyperplasia can lead to precocious pseudopuberty. Androgen secreting or estrogen secreting tumors of adrenal gland resulting in precocious pseudopuberty are not uncommon.
2. **Gonadal causes:** Leydig cell tumor of testis in male or granulosa cell tumor of ovary in females can cause precocious pseudopuberty.

Delayed Puberty

When onset of puberty is delayed beyond the age of 17 in girls and 20 in boys, the condition is called delayed puberty.

1. It occurs usually due to panhypopituitarism that causes failure of maturation of gonads.
2. It is also associated with chromosomal abnormality of XO pattern or gonadal dysgenesis.
3. Puberty may be delayed in spite of presence of normal gonads, which is called eunuchoidism in males and primary amenorrhea in girls.

MENOPAUSE

With advancement of age, gonadal functions gradually decrease. In females, ovaries become unresponsive to the gonadotropins. The functions of gonads slowly decrease finally resulting in complete cessation of menstrual cycle. This is called menopause or climacteric.

Mechanism and Features

Mechanism of Menopause

The mechanism and purpose of menopause are not clear. The female gonads progressively become unresponsive to gonadotropins with advancing age. The mechanism by which ovarian responsiveness decreases is not known. Ovaries stop secreting progesterone and estrogen in appreciable amount. The **negative feedback effect** of ovarian hormones causes increased secretion of luteinizing hormone (LH) and FSH. The uterus and vagina become atrophic.

Age at Menopause

1. **In women,** onset of menopause occurs between the age of 45–55 years. In recent years, the age at menopause has gradually increased.
2. **In males,** though there is some decline in reproductive capacity from 5th decade of life, climacteric does not occur. Testicular functions and potency persist till 8th decade. Thus, in males, there is no andropause (male menopause).

Features of Menopause

Hot flushes (sensation of warmth spreading from trunk to the face) occur frequently. Night sweating, tachycardia, mental symptoms are also observed.

1. The mechanism of hot flushes is not exactly known but it coincides with the **surges of LH secretion.**
2. With the onset of menopause, LH secretion is increased, which occurs in episodes of 30–60 minutes. This is called circhoral secretion of LH.
3. Each hot flush coincides with an episode of LH surge. However, experimental evidences indicate that hot flushes are not due to episodic secretion of LH per se.
4. The decreased secretion of estrogen triggering some hypothalamic mechanism is proposed to cause both episodic secretion of LH and hot flushes.

Management of Menopause

The fear that the women will lose her womanhood may cause psychological depression.
1. It needs proper care, counseling and assurance of the spouse to make her understand and adjust to this physiological phenomenon of the nature.
2. In some cases, hot flushes and psychological symptoms become more problematic. In such women, hormonal supplementation of estrogen is usually helpful.
3. However, metabolic and other complications of estrogen should be kept in mind while continuing estrogen therapy for a longer period.

### CHAPTER SUMMARY

#### Key Concepts

1. Increased secretion of adrenal androgens (adrenarche) sensitizes hypothalamo-pituitary-gonadal axis for pubertal changes. Dehydroepiandrosterone secreted from adrenal gland plays some role in the maturation of hypothalamic neurons, and GnRH secretion becomes pulsatile. **Pulsatile secretion of GnRH** brings about the onset of puberty.
2. Reproducibility totally stops at menopause, whereas in males reproducibility continues. Therefore, the male menopause is not definitive.

#### Important to Know (Must Read)

1. In examination, **Long Questions** are usually not asked from this chapter.
2. Precocious puberty, Stages of puberty in boys and girls, Mechanism of onset of puberty, Mechanism, features and management of menopause may be asked as **Short Questions** in exam.
3. In **Viva**, examiner may ask… Define puberty, What is the age of onset of puberty in boys and girls, What are the stages of puberty in boys, What are the stages of puberty in girls, Explain the mechanism of onset of puberty, What is true precocious puberty and what are its causes, What is precocious pseudopuberty and what are its causes, What is delayed puberty, What is menopause, What is the mechanism of menopause, What is the age of menopause, What are the features of menopause, How menopause can be managed.
CHAPTER 67
Male Reproductive System

LEARNING OBJECTIVES
On completion of study of this chapter, the student MUST be able to:
1. Name the different parts of male reproductive system, and give the functions of each.
2. List the functions of Sertoli cells and Leydig cells.
3. Understand the importance of blood-testis barrier in testicular functions.
4. Name the steps of spermatogenesis and describe the mechanism and regulation of spermatogenesis.
5. Understand the importance of semen analysis.
6. List the testicular hormones and describe the functions of testosterone.
7. Name the secondary sex characteristics in males.
8. Understand the regulation of testicular functions.
9. Understand the physiological basis of testicular abnormalities.
The student MAY also be able to:
1. Describe the regulation of testicular functions and hypothalamo-pituitary-gonadal axis in males.
2. Describe the stages and mechanisms of spermatogenesis.
3. Explain the physiological basis of reproductive abnormalities in males.

The primary objective of the male reproductive system is to produce healthy sperms capable of fertilizing the ovum. The main specialty of male reproduction is that the male gametes are produced in millions and after puberty the process of production is a continuous phenomenon.

Scientist contributed
Enrico Sertoli (1842–1910) an Italian physiologist and histologist was a professor of anatomy and physiology at the Royal School of veterinary medicine in Milan, and after 1907, he worked only as a professor of physiology there. In Milan, he founded the laboratory of experimental physiology. He worked extensively on reproductive physiology. He is remembered for his 1865 discovery of the eponymous Sertoli cell that provide nourishment and support for developing sperm.

FUNCTIONAL ANATOMY
The male reproductive system consists of testes, epididymis, vas deferens, ejaculatory ducts, prostate, urethra, and penis. They have two important functions, spermatogenesis (formation of spermatozoa) and steroidogenesis (synthesis of testosterone). Spermatozoa produced by testes enter the epididymis from where they pass into the vas deferens (Fig. 67.1). Epididymis and proximal part of vas deferens store sperms. At the time of ejaculation, sperms enter into the urethra in the body of prostate through the ejaculatory duct and via urethra they come out of the genital tract.

The Testes
In human beings, testes are located in scrotum. During intrauterine life, testes are placed in the abdominal cavity beneath the posterior abdominal wall. Gradually they descend down to the inguinal canal during mid-pregnancy. During two months before term, they descend further through the inguinal canal into the scrotum (Application Box 67.1).

Application Box 67.1
Separate domicile for male gonad: Testes are the male gonads that are placed in separate sacs almost hanging away from the body. In female, the gonads (ovaries) are well preserved in the abdominal cavity. Then, why has the nature provided separate external compartments for male gonads? Scrotum is the sac, which keeps testis at about 2–3°C below the core body temperature and this cooler environment is highly favorable for spermatogenesis.
The scrotal temperature is cooler than core body temperature for the following reasons:

1. **Anatomical location:** Scrotum forms sacs that are like outpouching from the body, which has less direct transmission of the inner body temperature into it. Testes being placed in the scrotum avails scrotal temperature.

2. **Pampiniform plexus of the blood vessels:** Theseplexuses of blood vessels serve as counter-current exchanger between warm arterial blood entering the testes and cooler venous blood leaving the testes (Fig. 67.2).

3. **Role of cremasteric & darto muscles:** Cremaster muscle is a small band of skeletal muscle present in the spermatic cord that contracts or relaxes in response to change in environmental temperature.
   - It contracts on exposure to sever cold and elevates the testis and relaxes in warm environment and lowers the testis.
   - Also, a flap of smooth muscle is present in the fascial layer of scrotum, called darto muscle, which contracts on exposure to cold. This increases wrinkling of scrotal skin and facilitates lifting of the testis.
   - Thus, cremasteric and darto muscles on exposure to cold push testes close to the pelvic cavity where they can absorb body heat, and pull them away from the body in hot environment. Therefore, these muscles prevent temporary sterility in extreme weathers.

Weight of each testis is about 10–15 grams, length 5 cm and width 2.5 cm in adults. Testes receive blood supply from the spermatic arteries that originate directly from the aorta. Functional Histology of Testis

Testes are covered by thick connective tissue membrane called tunica albuginea. Each testis is made up of seminiferous tubules. Hundreds of tubules are tightly packed to from a mass of coiled loops (Fig. 67.3).

1. Each loop begins and ends in a single duct called tubulus rectus, which in turn drains into the epididymis.
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2. Tubules are arranged in lobules separated by septa formed by extensions of tunica albuginea. Each tubule is about 50 cm long and 250 µm in diameter.

3. **Myoid cells** are present surrounding the basal lamina of the seminiferous tubules and **interstitial cells of Leydig** are present in the space between the seminiferous tubules (Fig. 67.4).

4. Thus, testis consists of seminiferous tubules and interstitium that mainly contains Leydig cells, connective tissues and capillaries, and few myoid cells and fibroblasts.

Seminiferous Tubule

Each seminiferous tubule has a basement membrane that separates it from the surrounding Leydig cells, the peritubular cells (myoid cells) and the connective tissue.

1. Spermatogonia and Sertoli cells are located in the wall of the tubule just beneath the basement membrane (Fig. 67.5).

2. There are **two principal cell types** in seminiferous tubules: somatic cells (**Sertoli cells** and **germ cells**) (Fig. 67.6).

Sertoli Cells

Sertoli cells are the sustentacular cells in seminiferous tubules, which form the major cell mass in them.

Structure of Sertoli cells

They are irregularly shaped cells that are extended from the basement membrane into the lumen of seminiferous tubule. Sperms are attached to the apical membrane of...
the Sertoli cells. At their bases, Sertoli cells are attached to each other by tight junctions. Tight junctions divide tubules into two compartments: basal compartment and adluminal compartment (Fig. 67.7).

**Basal Compartment**
The basal compartment is the outer compartment that mainly consists of spermatogonium. It is exposed to circulating substances as the capillaries are in close contact with it and substances from blood can easily be exchanged.

**Adluminal Compartment**
Adluminal compartment is the inner compartment that consists of primary and secondary spermatocytes and spermatids. It is separated from blood-borne agents by the tight junctions between the Sertoli cells close to basal lamina.

**Blood-Testis Barrier**
Because, the tight junctions between Sertoli cells are too tight (Fig. 67.7), they do not allow substances from interstitial space and blood to pass through them except steroids and few proteins. Thus, **tight junction between Sertoli cells near the basal lamina** forms the blood-testis barrier (BTB).

1. The BTB prevents the large molecules to be transported from interstitial tissue and basal compartment to the interior of the tubule (adluminal compartment).
2. However, maturing germ cells pass through BTB to enter into adluminal compartment. If maturing spermatocytes can pass through the BTB one could easily doubt the veracity of the barrier.
3. The proposed explanation is that when spermatocytes attempt to penetrate the barrier, the tight junction in front of them dislocate and give way for them, and immediately after the spermatocytes pass through the tight junctions the new tight junctions are concomitantly formed behind them.
4. Thus, physiologically, maturing germ cells pass through BTB without actually disrupting it.

**Functions of BTB:**
1. BTB protects germ cells in the seminiferous tubules from harmful elements in blood.
2. Antigenic elements are produced by germ cells during their growth and multiplication, which are capable of inducing immunological reactions in the body. BTB prevents these antigenic products to escape into circulation from tubule and thus prevents autoimmune reactions.
3. BTB maintains the composition of the fluid in the lumen of seminiferous tubules. The tubular luminal fluid contains less protein and glucose as they do not pass from blood through the BTB. However, it is rich in androgens, estrogens, K⁺, inositol, glutamic and aspartic acids.
4. BTB maintains higher osmolality of luminal content of the seminiferous tubules, which favors the osmotic movement of fluid into the tubular lumen.

**Functions of Sertoli Cells**
Sertoli cells have multiple functions. Important among them are:

1. **Germ cell development:** Sertoli cells are critical to germ cell development. They provide structural support and nutrition to germ cells. Sertoli cells are rich in glycoproteins that nourish the germ cells.
2. **Phagocytosis:** Sertoli cells phagocytose residual bodies and damaged germ cells from the seminiferous tubules. Residual bodies are cytoplasmic fragments formed by excess cytoplasm resulting from transformation of spermatids to spermatozoa.
3. **Nourishment and growth of spermatids:** They nourish the developing spermatids. About 10 to 12 spermatids are attached to each Sertoli cells. Sertoli cells synthesize transferrin, an iron-transport protein that helps in development of sperms.
4. **Formation of tubular fluid:** They secrete fluid into the lumen of seminiferous tubule. This fluid later forms the major components of seminal fluid.
5. **Support spermiation:** This is the process of detachment of mature sperms from Sertoli cells into the lumen. In this process, plasmin, the proteolytic enzyme assists in release of sperms from Sertoli cells. Sertoli cells produce plasminogen activator that causes formation of plasmin.

6. **FSH and testosterone sensitivity:** Sertoli cells have receptors for FSH and testosterone. FSH receptors are present on the cell membrane and testosterone receptors are present within the nucleus. During puberty, receptors for these hormones on Sertoli cell increase. FSH increases cAMP in Sertoli cells and induces production of androgen-binding protein (see below) and plasminogen activator in Sertoli cells. These chemicals help in sperm development.

7. **Endocrine functions of Sertoli cells:** Sertoli cells secrete many hormones. These are:
   i. **Inhibin:** Inhibin suppresses FSH release from pituitary.
   ii. **Activin:** Activin stimulates release of FSH.
   iii. **Follistatin:** It decreases FSH secretion stimulated by activin.
   iv. **Müllerian regression substance:** During 8th week of gestation MIS is secreted from fetal Sertoli cells. MIS causes regression of müllerian duct in fetuses destined to become males.
   v. **Estrogens:** Sertoli cells contain the enzyme aromatase that converts androgen into estrogen.

8. **Secretory functions:** Sertoli cells secrete androgen binding proteins (ABP) and H-Y antigen. ABP maintains concentration of androgen in Sertoli cells, which is essential for maturation of spermatozoa. H-Y antigen is secreted by Sertoli cells during fetal life that controls development of testis.

**Androgen binding proteins (ABP):** Sertoli cells secrete and synthesize ABP, a protein having molecular weight 90,000 into the luminal fluid. ABP has high affinity for binding dihydrotestosterone and testosterone. It is present in high concentration in luminal fluid. A high concentration of androgen in luminal fluid is essential for production and maturation of spermatozoa. ABP maintains this concentration of androgen in luminal fluid. It helps in storage of androgen in seminiferous tubule and facilitates transport of testosterone from testis to epididymis.

9. **Blood-testis barrier:** Sertoli cells provide the blood-testis barrier (BTB) for the seminiferous tubules. Thus, they protect the developing sperms from harmful effects of blood-borne toxins. Other functions of BTB are discussed above.

**Germ Cells**

Germ cells are germinal epithelial cells of seminiferous tubules. Each germ cell is a spermatogonium that forms spermatocytes and spermatozoa in the process of its development, i.e., spermatogenesis (see below).

**Leydig Cells**

Leydig cells are primary cells of steroidogenesis. They produce testosterone.

**Scientist contributed**

Franz von Leydig, also known as Franz Leydig (1821–1908), a German zoologist and comparative anatomist discovered Leydig cells in 1850. Leydig cells are named after him.

**Location**

Leydig cells are present near the capillaries in the interstitial space between seminiferous tubules.

**Structure**

They are rich in mitochondria and endoplasmic reticulum. The activity of Leydig cells is different in different phases of life.

**Functions**

Leydig cells mainly secrete testosterone. However, dehydroepiandrosterone and androstenedione are also secreted to some extent.

**Leydig Cells Activity in Different Phases of Life**

1. **During 8-15 weeks of intrauterine life,** Leydig cells are active and prominent as steroidogenesis is essential for differentiation of male genital ducts. During this period, as hypothalamo-pituitary gonadal axis is not fully formed, steroidogenesis is controlled by hCG, not by LH.
2. **Thereafter,** Leydig cell activity decreases. In male infants, during 3–5 months of postnatal life, Leydig cell activity increases significantly that results in increased testosterone secretion. The physiological significance of this infantile testosterone surge is not known.
3. Leydig cells then remain dormant till puberty. At the **onset of puberty,** activity of Leydig cells in males attains maximum.
4. After puberty, Leydig cell activity decreases, but maintains at a basal rate till senescence.

**Regulation of Leydig Cell Activity**

Leydig cells have receptors for LH.

1. **LH controls Leydig cell steroidogenesis by cAMP-dependent mechanism.**
2. Leydig cells do not have receptors for FSH. However, FSH indirectly controls Leydig cells activity. FSH stimulates Sertoli cells to produce growth factors, which in turn promote the growth of Leydig cells.
3. **Leydig cell activity is stimulated by androgen and inhibited by estrogen.**

**Interaction of Leydig cells with Sertoli cells**

Sertoli cells do not produce testosterone, but have receptors for it. **Testosterone secreted from Leydig cell enters**
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**Sertoli cells** where it binds with the ABP. This testosterone is essential for three important functions:
1. **Spermatogenesis**
2. Functioning of Sertoli cells
3. Estrogen production in Sertoli cells. Estrogen produced in Sertoli cells diffuses back into the Leydig cells and regulates Leydig cell function (Fig. 67.8).

**Functions of Testis**
Testis serves three important functions.
1. **Spermatogenesis**, i.e., production of sperms (see below)
2. **Steroidogenesis**, i.e. synthesis of steroid hormones, mainly testosterone, which is later, converted to dihydrotestosterone and estradiol (see below).
3. Testis secretes other hormones like activin, inhibin and follistatin that influence many other reproductive functions.

**Epididymis**
Seminiferous tubules open into a network of tubules, called rete testis. Rete testis opens into epididymis via efferent ductules.

**Structure**
Epididymis is located on the posterior aspect of the testis. It contains a highly convoluted long duct, which is about 5 meter in length. Epididymis is divided into three parts: head (the part close to testis), body and tail (Fig. 67.9).

**Function**
The function of epididymis is the storage and maturation of spermatozoa (Application Box 67.2).
1. About 99% of testicular fluid is reabsorbed in the epididymis and rete testis. Spermatozoa from tubular fluid and rete testis are transferred to epididymis almost passively as they are essentially immotile.
2. In epididymis, they acquire the capacity for progressive forward movement and the ability to attach to zona pellucida of ovum and penetrate into it.
3. Thus, the sperms obtained directly from testis are functionally immature, whereas sperms obtained from body of the epididymis or further down in the male genital tract are fertile.

**Application Box 67.2**
Fate of stored spermatozoa: Spermatozoa are carried from epididymis via vas deferens and ejaculatory duct into the urethra in penis to be removed out of it at the time of ejaculation. The fate of sperms that are not voided through ejaculation is not clearly known. It has been suggested that these sperms are ultimately phagocytosed by macrophages in the epididymis or voided slowly in urine. However, in Indian spiritual scriptures, it has been noted that sperms in the body are converted into vital energy, which can be further transformed into spiritual energy if properly utilized with the help of higher spiritual forces. Therefore, in practice of yoga, it has been advised to conserve and properly utilize the energy of the semen.

**Vas Deferens**
Epididymis empties its content into the vas deferens (Fig. 67.9), which transfers sperms further down in the duct.
1. Proximal part of vas deferens like epididymis stores the sperms.
2. Vas deferens joins with the duct arising from seminal vesicle to from the ejaculatory duct.
3. The movement of sperm in vas deferens is active as they are capable of motility.
4. However, contraction of muscle in the wall of vas deferens facilitates the process of sperm movement.
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Seminal Vesicle

There are two seminal vesicles located close to the prostate. They secrete a viscid and alkaline fluid called seminal vesicular fluid.
1. Seminal vesicular fluid contributes to 70% of the total volume of the semen.
2. Rest 20% of the volume is contributed by epididymal fluid and fluid secreted from accessory sex glands, and 10% is contributed by sperms.
3. The seminal vesicles secrete vitamin C and fructose. The fluid appears yellowish as it contains flavins.
4. Most of the prostaglandins found in semen are contributed by seminal vesicle.

Accessory Sex Glands

Accessory male sex glands are prostate gland and bulbourethral glands of Cowper.

The Prostate Gland

The ejaculatory duct enters the prostatic portion of the urethra after passing through the prostate.
1. Prostate gland consists of 30–50 branched tubuloalveolar glands whose secretions empty into prostatic urethra.
2. The prostatic fluid contains fibrinolysin, fibrinogenase and large quantity of acid phosphatase. Fibrinolysin prevents sperm heads to clump.
3. Prostate gland also releases a factor, which contains sugar, sulfate and vitamin E derivative that also prevents sperm head to cluster.

Bulbourethral Glands

They secrete mucus and alkaline fluid. They also secrete the enzyme hyaluronidase that facilitates penetrability of oocytes by sperms.

SPERMATOGENESIS

Definition and Course

The process of development of male germ cells into spermatzoa is known as spermatogenesis. The primordial germ cells migrate into the gonad during embryogenesis. These cells later become immature germ cells, called as spermatogonia. Spermatogonia are located attached to the basement membrane of the semiferous tubule. From puberty onwards, these cells divide mitotically to continuously supply spermatocytes that form spermatooza.

Course of Spermatogenesis in Life: Spermatogenesis begins at puberty. The germ cells remain inactive throughout childhood.
1. At puberty, under the influence of increasing level of gonadotropins and testosterone, the germ cells are activated and spermatogenesis is initiated.
2. From puberty onwards, spermatogenesis continues throughout life, though the process declines at old age.

Steps of Spermatogenesis

The process of spermatogenesis can be divided into three distinct phases: Mitosis, meiosis and spermiogenesis (Flowchart 67.1). Spermatogonium undergoes mitosis to produce primary spermatocytes that undergo two meiotic divisions to form spermatids. Spermatids, by the process of spermiogenesis become mature spermaatoza.

Mitosis

The primitive germ cells (spermatogonia) that are present in the basal lamina of seminferous tubules undergo mitotic divisions to from primary spermatocytes. This is called spermatocytogenesis (Fig. 67.10). In fact, the spermatogonia undergo many mitotic divisions to produce two types of spermatogonia: spermatogonia A and spermatogonia B.

Spermatogonia A: Spermatogonia A formed by mitotic divisions resemble the original spermatogonia and are the source of subsequent spermatogonia in the testis. Thus, they form the spermatogonia reserve pool in the testis.

Spermatogonia B: Spermatogonia B grow and enter adluminal compartment where they develop into primary spermatocytes.

Meiosis

The primary spermatocytes (diploid 4N DNA) undergo two meiotic divisions.
1. The first meiotic division forms secondary spermatocytes (haploid 2N DNA) and the second meiotic division forms spermatids (haploid 1N DNA).
2. Spermatids contain half of the number of chromosomes (23 chromosomes, i.e., 22 autosomes and 1 sex chromosome, which is either an X or a Y chromosome).
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3. Of every four spermatids formed from primary spermatocytes, two of them contain X chromosome and two Y chromosomes.
4. Normally after undergoing several mitotic and two meiotic divisions in the process of spermatogenesis, each spermatogonium yields 512 spermatids.

Spermiogenesis

The process of development of spermatids into matured spermatozoa is called spermiogenesis. Spermatids are small, round and less characteristic cells. They undergo many structural changes to form spermatozoa (Fig. 67.10). The major changes are:

1. **Massive reduction in cytoplasm.** Cytoplasmic fragments are discarded as residual bodies.
2. **Elongation of the nucleus** to become head of spermatozoa.
3. Acquirement of an upper covering for the head called **acrosomal cap**.
4. Formation of a **middle piece and a tail piece** with the ability to move efficiently and swiftly.

All these changes occur in mature sperms to enable them to survive in a foreign and even hostile environment (**acidic vaginal pH**) in the female genital tract and to recognize and fertilize the ovum. These changes mainly help the sperm to move forward towards the ovum in the female genital tract, the movement known as **progressive motility** of the sperm.

Spermiation

Spermatozoa, after they are formed, remain in the lumen of the seminiferous tubule sticking to the apical membrane of the Sertoli cells. They **adhere tightly to the Sertoli cells.** The process of **detachment of head of spermatozoa and their free release** into the luminal fluid is called spermiation.

**Flowchart 67.1: Steps of spermatogenesis.** Note, till the stage of primary spermatocyte, each cell contains 46 chromosomes and from secondary spermatocyte to spermatozoa, each cell contains 23 chromosomes (number indicated against each category of cells as 46 chromosomes and 23 chromosomes in bracket).
Capacitation

When spermatozoa are ejaculated into the female genital tract, they undergo further maturation called capacitation. This includes increase in further motility of sperms and preparation for acrosomal reaction. However, capacitation is not so much essential for fertilization as fertilization can also occur in vitro.

Structure of Spermatozoa

A matured sperm is a motile cell rich in DNA, consisting of head, middle piece and tail (Fig. 67.11). A human sperm is about 65 µm long with average diameter of 2 µm.

Head

Head contains a prominent nucleus at the center, which is condensed with chromatin. The head is covered by a cap called acrosome, which is formed from Golgi apparatus. Acrosome is like a lysosome rich in proteolytic enzymes such as hyaluronidase, acrosin, neuraminidase and esterases that are activated during acrosomal reaction and help in sperm penetration of the ovum at the time of fertilization. The nucleus decondenses and becomes a pronucleus at the time of fertilization.

Middle Piece

The middle piece of sperm contains numerous mitochondria in the form of a spiral sheath surrounding a long axial filament made up of microtubules (9 + 2 arrangement; i.e., nine peripheral doublet surrounding a central pair) is typical that of cilia and flagella. Therefore, the tail portion is highly motile.

1. The tail of the sperm exhibits a twisting movement by which it propels the body of the sperm in forward direction. The twisting movement occurs due to the interaction between tubulin fibers and dynein side arms, which requires ATP.

2. The axoneme is surrounded by a fibrous sheath that provides strength to the tail. The membrane of the sheath contains germinal angiotensin converting enzyme. The exact function of this enzyme is not known but reduction of this enzyme concentration is suggested to be associated with decreased fertility.

Role of CatSper protein: The principal piece of tail contains a protein called CatSper protein, which is a calcium channel. This allows cAMP mediated calcium influx and facilitates sperm motility.

Duration of Spermatogenesis

In human beings, the process of formation of sperm from the spermatogonium takes 65–74 days. Sometimes, the stages of development of sperms are collectively called spermatogenic cycle. Each stage has a relatively constant duration:

1. Spermatogonia to primary spermatocytes is 16–20 days.
2. Primary spermatocytes to secondary spermatocytes is 23–25 days.
3. Secondary spermatocytes to spermatids is approximately 1 day.
4. Spermatids to spermatozoa is about 25 days.

Hormones like gonadotropins or androgen influence the number of spermatozoa produced, but not the duration of the cycle. Thus, the duration of spermatogenesis remains virtually constant. Normally, new cycles are initiated in every 2 to 4 weeks before the completion of old cycle. Therefore, in the tubules, cells of different stages are seen at any particular time. This ensures uninterrupted supply of spermatozoa throughout life.
Rate of Production of Sperms

A single spermatogonium forms 512 spermatids, if all of them remain alive.
1. Approximately, 200 millions of sperms are produced daily in an adult testis in humans. This is roughly same as the number of sperms in an ejaculate in a normal healthy adult. Expressed per unit weight of testicular tissue, about 6–7 million sperms are produced per gram per day.
2. The rate of production of sperms falls progressively in old age. After the age of 50, the rate of sperm production is 3.5–4 million per gram of tissue.
3. The decrease in production in elderly is due to degeneration of germ cells during meiotic prophase. However, appreciable spermatogenesis continues even at the age of 90.

Differences between Spermatogenesis and Oogenesis

There are few basic differences in the process of gametogenesis in females and males. These are:
1. In females, mitotic proliferation of germ cells completes before birth, whereas in males, spermatogonia grow only at the time of puberty and then continue to proliferate throughout life.
2. In female, the meiotic division of primary oocyte produces only one ovum, whereas in males one primary spermatocyte produces four spermatozoa.
3. In female, second meiotic division is completed only upon fertilization, whereas in males second meiotic division is completed during spermatogenesis.

Factors Controlling Spermatogenesis

Factors controlling spermatogenesis can be broadly divided into two categories: hormonal and environmental.

Hormonal Factors

Androgen, estrogen and gonadotropins mainly control spermatogenesis.
1. Androgen: Androgens stimulate spermatogenesis. LH creates an elevated local concentration of androgen in the testis by stimulating Leydig cells to secrete testosterone, and this high intertesticular testosterone is essential for spermatogenesis.
   - Testosterone secreted from Leydig cells diffuse into the seminiferous tubule. In tubule, only the Sertoli cells (not spermatogenic cells), have receptors for testosterone.
   - Testosterone binds with ABP in the Sertoli cells (Fig. 67.8), which maintains a high concentration of testosterone in the cell. However, it is not clearly known how testosterone promotes spermatogenesis.
   - It appears that, till the formation of spermatid does not depend on androgen, rather spermiogenesis (spermatids developing to spermatozoa) is androgen dependent.
2. Estrogen: Estrogen content of the fluid in the rete testis is high and there are estrogen receptors in the rete testis. Rete testis reabsorbs fluid and makes the spermatozoa concentrated, which is required for sperm maturation. The diluted volume of large fluid that enters rete testis and epididymis unless absorbed adequately results in infertility. Estrogen concentrates the fluid by facilitating fluid reabsorption.
3. LH and FSH: LH helps in spermatogenesis by producing a high local concentration of androgen. FSH maintains gametogenic functions of testis. It appears that FSH is required for initiation of spermatogenesis at the time of puberty, and then LH maintains it.

Environmental Factors

It is mainly the temperature that influences spermatogenesis. Lower environmental temperature facilitates spermatogenesis. Spermatogenesis best occurs at 30 to 35°C. However, very low temperature inhibits it. Conversely, increased temperature inhibits spermatogenesis. Therefore, in persons taking repeated hot bath or those who regularly use insulated athletic support for the scrotum, sperm count is invariably less. Generally, sperm count is more in comfortable winter and less in intense summer.

Semen Analysis

Semen analysis is one of the important tests for assessment of male fertility. It is performed to know if the sterility is related to sperm production. It is also performed after vasectomy, to check its completeness. It reflects the activity of the testes and accessory sex organs in males.

Analysis of freshly collected sample of semen gives the knowledge about the male fertility which is detected by examining the sample under microscope. Usually, the sample is collected after a period of sexual abstinence for 2 days.

Composition of Normal Semen

| Volume | 2 to 5 mL |
| Color | White, opalescent |
| Specific gravity | 1.028 |
| Motility | > 60% should be actively motile within 3 h of collection |
| Count | > 40 millions/mL is considered normal (usually, 100 millions/mL is seen normally) |
| Liquefaction | Should liquefy within half-an-hour |
| Morphology | > 80% should have normal morphology |
| pH | 7.3 to 7.5 |
| Fructose content | Fructose concentration is 2–7 mg/mL |
| Other biochemical constituents: | 1. Prostaglandins |
| | 2. Ascorbic acid |
| | 3. Flavins |
| | 4. Phosphorylcholine |
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5. Ergothioneine
6. Fibrinolysin, fibrinogenase
7. Acid phosphatase
8. Zinc
9. Phospholipids
10. Cholesterol
11. Spermin
12. Citric acid
13. Phosphate
14. Bicarbonate
15. Hyaluronidase

Fructose and other biochemical compositions as noted above from serial No. 1 to 5 are synthesized and secreted from seminal vesicles. The secretion from seminal vesicle constitutes 60% of the semen volume. Other biochemical compositions from serial No. 6 to 11 are released from prostate. Secretion from prostate contributes to 20% of the total semen volume. Bicarbonate and phosphates are buffers in the seminal fluid. Normally, sperm motility is facilitated by prostate specific antigen (PSA) (Application Box 67.3).

Application Box 67.3

**PSA is a marker of prostate cancer:** Prostate specific antigen (PSA) is 30 kDa serine protease formed and secreted from the prostate into the seminal fluid. Semenogelin, the sperm motility inhibitor is hydrolyzed by PSA. Thus, PSA facilitates sperm motility. The PSA gene has two androgen response elements. Though PSA is elevated in BHP (benign hyperplasia of prostate) and prostatitis, it is markedly increased in malignancy of prostate. Hence, elevated plasma PSA is used as a screening test for detection of prostate cancer.

Abnormalities

**Volume**

A low volume might suggest an anatomical or functional defect or an inflammatory condition of the genital tract. Volume decreases with advancing age.

**Motility**

In a normal sample, at least 60% of the sperms should exhibit forward motility within the first 3 hours of collection of the specimen. Motility less than 40% is associated with sterility. The speed of normal human sperm in female genital tract is 3 mm/min. Within one hour of ejaculation in the vagina, the sperms reach fallopian tube where they fertilize the ovum.

**Count**

Sperm count below 20 millions/ml results in sterility. A count between 20 to 40 millions/ml indicates borderline infertility.

**Liquefaction**

Delayed liquefaction of more than 2 hours suggests inflammation of accessory glands or enzyme defects in the secretory products of the glands.

**Morphology**

Normally 70% of the sperms should have normal morphology. Abnormalities more than 30% indicate serious pathology. The abnormalities include abnormal shapes, and poorly formed head or tail. The abnormal sperms may have bifurcated tail, bifid head, spirally coiled tail, or absence of head (Figs. 67.12A to D).

**pH**

pH below 7.0 indicates semen content is mainly prostatic fluid, which may be due to congenital absence of seminal vesicle.

**Fructose Content**

The normal fructose content of semen is 2–7 mg/mL. Its absence indicates obstruction or absence of the ejaculatory ducts or seminal vesicle.

Figs. 67.12A to D: Abnormal sperms. (A) Bifid tail; (B) Bifid head; (C) No head; (D) Curved tail.
**Effects of Vasectomy**

Vasectomy is the bilateral ligation of vas deferens. This is the permanent contraceptive procedure for males. This is a safe and convenient contraceptive procedure.

1. About 50% of vasectomized males develop antibodies against sperms. However, the incidence is more following restoration of patency of the vas deferens in males those who wish to restore their fertility.

2. As such, restoration of patency of the vas (recanalization) is a difficult procedure. The anti-sperm antibodies further reduce the fertility.

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**Testicular Hormones**

**Testosterone**

The principal steroid secreted from testis is testosterone. It is the essential hormone for male reproduction and its absence or decreased production leads to sterility. Primarily, it allows the development of male reproductive organs during fetal life, controls spermatogenesis, guides development of secondary sex characteristics and maintains male vigor.

**Source**

Testosterone is synthesized by the Leydig cells of testis. Testosterone is also secreted from adrenal cortex, ovaries and placenta. Thus, a small amount of testosterone is secreted in females.

**Structure, Synthesis and Secretion**

It is a C19 steroid hormone with an OH group at 17 position.

1. It is synthesized from cholesterol in Leydig cells of the testis. It is also formed from androstenedione secreted from adrenal cortex. Though, the biosynthetic pathways in the endocrine tissue that form steroid hormones are similar, minor differences exist among the enzymes that are involved in the process. For example, the 17α-hydroxylase is found in testis, whereas 11– and 21–β hydroxylases are found in adrenal cortex.

2. Therefore, in testis, pregnenolone is hydroxylated at 17 position.

3. Pregnenolone is converted to testosterone in two pathways: delta 5 and delta 4 pathways. Major steps of these pathways are summarized in Figure 67.13.

4. Pregnenolone also forms progesterone, which forms 17α-hydroxyprogesterone, which in turn forms androstenedione and testosterone by delta-4-pathway.

The normal secretion rate of testosterone from testes is 4–10 mg per day. The normal plasma concentration of testosterone is 300–1000 ng/dL in adult males and 30–70 ng/dL in females.

**Metabolism**

About 98% of testosterone binds with plasma proteins and only 2% is free in plasma. The free testosterone enters the target tissues in which it is converted to its active form dihydrotestosterone (DHT) by 5α-reductase and estradiol (17β-estradiol) by aromatase. Thus testosterone acts as prohormone and DHT is the biologically active hormone. DHT has 2–3 times more affinity than testosterone for binding with androgen receptors (Clinical Box 67.1). DHT causes hypertrophy of prostate (Clinical Box 67.2).

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**Clinical Box 67.1**

**Penis-at-14 syndrome:** Normally, DHT which is formed from testosterone by the enzyme 5α-reductase is essential for the development of male genitalia during early embryonic life. Therefore, deficiency of 5α-reductase in males results in confusing genitalia containing internal male and external female characters. In the absence of DHT, female genitalia predominate in genetic male (male pseudohermaphroditism) at birth. Undescended testis is a common feature in this condition and they are born as female children. At the time of puberty, testosterone secretion increases and they develop male figure with enlargement of clitoris to become a penis like structure. At this point in their life, they become boys and change their name and sex. They may even develop full male libido and do intercourse with women. As it occurs around the age of 14, syndrome is called penis-at-14-syndrome. It is common in Dominican Republic.

**17-ketosteroids:** About 65% binding of testosterone in plasma occurs with gonadal steroid binding globulin (GBG) and 33% with sex steroid binding globulin (SBG). Most of the circulating testosterone is converted into 17-ketosteroids by the enzyme 17β-dehydrogenase and a small amount into estrogen. 17-ketosteroids possess about 20% of androgen activity. The principal ketosteroids are androstenedione and etiocholanolone. Ketosteroids are excreted in urine. However, two-thirds of urinary ketosteroid is of adrenal origin and one-third of testicular origin.
Clinical Box 67.2

Treatment for prostate hypertrophy: DHT induces hypertrophy of prostate. Therefore drugs that inhibit 5α-reductase are currently used in the treatment of benign hypertrophy of prostate (BHP). If it is androgen dependent BHP or malignant neoplasia, GnRH antagonists are used that inhibit LH secretion which in turn inhibits estrogen secretion from Leydig cells.

Testicular Estrogen

Circulating estrogens are estradiol and estrone. Estradiol is the major estrogen. In men, the plasma estradiol level is 20 – 50 pg/ml. The production of estrogen increases with advancement of age in males, whereas estrogen production decreases with age in female. In males, only 15% of circulating estradiol and 5% of circulating estrone comes from testes (Clinical Box 67.3). Rest is produced by aromatization of estrogen from testosterone outside the testes and secreted from adrenal cortex.

Clinical Box 67.3

Gynecomastia in males: In adipose tissues, skin, liver and brain, testosterone is aromatized to estrogen, which is then released into circulation. Therefore in men, circulating level of estrogen (estradiol and estrone) is almost close to the level of estrogen in follicular phase in females. However, men are protected from feminization due to high level of androgens and high responsiveness of tissues to androgens. Therefore, use of anabolic steroids or testosterone analogues (as athletes use), decreased testosterone secretion, estrogen producing testicular tumors, and tissue insensitivity to androgens result in gynecomastia.

Mechanism of Action

Testosterone like other steroid hormones acts by binding with the cytoplasmic nuclear receptors. In the cell, two pathways are activated: DHT pathway, and testosterone pathway (Fig. 67.14).

DHT pathway

This pathway is mainly active in prostate, scrotum, penis, skin and bones. The testosterone is converted to DHT by 5α-reductase. DHT then binds with androgen receptors in the nucleus and induces RNA transcription to form various proteins. This causes sexual maturation at puberty and external virilization. Thus, DHT is mainly responsible for development of facial hair, skin changes, recession of hair line, and enlargement of prostate, scrotum, penis and bone.

Testosterone Pathway

Testosterone directly binds with testosterone receptors in the nucleus that induces m RNA activity. This pathway mainly regulates gonadotropin secretion, causes muscle development, controls spermatogenesis, and influences male sex drive and libido.

Physiological Actions

Testosterone is an anabolic hormone. It promotes growth of male reproductive system and causes development of secondary sex characters. Among various androgens, the potency in order of sequence is as follows:

- DHT > testosterone > androstenedione > DHEA
- DHEA is dehydroepiandrosterone.

Development of Secondary Sex Characteristics

Testosterone is primarily responsible for changes in males that occur at puberty. It develops and maintains male secondary sex characteristics. These features mainly include changes in external and internal genitalia, distribution of body hair, skin changes, mental growth, voice change, change in body configuration and musculoskeletal changes.

1. Changes in external genitalia: Penis enlarges in length and width. The scrotum becomes more rugose and pigmented.
2. Changes in internal genitalia: Seminal vesicles increase in size and start secreting fructose. Prostate gland enlarges and secretion increases from prostate and bulbourethral gland.
3. Growth and distribution of body hairs: Hair growth is classified into three groups depending on their sensitivity to androgens: nonsexual, ambisexual and sexual. Nonsexual hairs are hairs of eyebrows and extremities.
Ambisexual hairs are the hairs in the axilla, and sexual hairs are the hairs in the face, chest and upper pubic triangle. Androgen stimulates growth of all types of hairs, though the effects are more on sexual and ambisexual hairs. It promotes hair growth in axilla, on the chest and pubic triangle, and around the anus. In the face, hairs grow in the form of moustache and beard. Pubic hairs grow with male pattern (in the shape of a triangle of which the apex is upward). The hair line on the scalp recedes in males. Therefore, it is believed that baldness is a sign of maleness.

4. Skin changes: In general, skin becomes tough. Secretion of sebaceous gland increases and becomes thick. This leads to acne formation. Sometimes acne vulgaris appears. Skin derived from urogenital sinus, i.e., the prepuce, clitoris, labia majora and scrotum becomes more sensitive. Skin in and around genitalia becomes more pigmented.

5. Mental changes: The individual becomes more active and aggressive. He takes more interest in opposite sex.

6. Voice change: Enlargement of larynx and thickening of vocal cord occur. Therefore, voice becomes thick and deep.

7. Body configuration: General increase in body height and girth occurs (growth spurt). Especially, Broadening of the shoulder occurs with general increase in size of skeletal muscles. These changes impart masculine phenotypic expression.

8. Musculoskeletal changes: Growth of long bones, pectoral girdle and vertebral bones occurs at puberty. Testosterone causes closure of epiphysis of long bone, ultimately limiting increase in height of the individual. It causes muscle hypertrophy, increases muscle protein synthesis and increases muscle mass.

Effects on Spermatogenesis
Testosterone stimulates spermatogenesis, the exact mechanism of which is not known. It is essential for sperm production and maturation.

Effects during Embryonic Life
In male fetus, between 8th and 18th week testosterone causes the differentiation of male genitalia.

1. The development of Wolffian duct into epididymis, vas deferens and seminal vesicles depends directly on the effects of androgen.
2. Also, the differentiation of urogenital sinus and genital tubercle into penis, scrotum and testis is mediated by testosterone and DHT.
3. Further, descent of testis into scrotum is promoted by testosterone.

Anabolic Effects
Testosterone increases the synthesis of proteins and decreases its catabolism. This results in increased growth and development of bones and muscles.

1. It causes sodium, potassium, calcium, phosphate, and water retention from kidney.
2. It is used as an anabolic drug in patients suffering from wasting diseases. Athletes use it as anabolic steroids.

Effects on Brain
Many areas in the brain have androgen receptors. However, the receptors are densely located in limbic areas, especially in amygdala and septum, and in hypothalamus, pituitary and preoptic area.

1. In these areas, testosterone is aromatized to estrogen (Flowchart 67.2).
2. Sexual dimorphism of neurons in the brain with respect to their distribution, size, number and activity has been reported in preoptic area and amygdala.

Other Effects
Testosterone, by its negative feedback effects, inhibits GnRH secretion from hypothalamus and LH and FSH secretion from anterior pituitary. It stimulates erythropoiesis.
Other Testicular Hormones

Other testicular hormones are androgens other than testosterone, inhibin, activin and follistatin. Estrogen is also produced in testis by aromatization of androgen in Sertoli cells.

Androgens

Testis secretes androgens. Testicular androgens are testosterone (see above), androstenedione and dihydrotestosterone. Among them, testosterone is the major androgen (Table 67.1).

Inhibin

Source

Inhibin is secreted from Sertoli cells of testes in males and granulosa cells of ovaries in females.

Types and Structure

There are two types of inhibins: inhibin A and inhibin B. Inhibins are made up of two subunits: α and β. The β subunit is of two types βA and βB. The inhibin A is made up of αβA and inhibin B is made up of αβB. The α and β subunits are made up of polypeptides. Inhibin B is the FSH regulating peptide.

The homodimers and heterodimers of β subunits are formed. This results in formation of βAβB, βBβB, and βAβA. These are called activins. Activins and inhibins are found in the gonads, brain, and many other tissues. In bone marrow, activins stimulate leucopoiesis.

Functions

The main function of inhibin is to provide feedback signal to inhibit FSH secretion from anterior pituitary. Inhibin B mainly inhibits FSH secretion.

Activin

It is a polypeptide hormone with molecular weight of 30,000. Activin is produced by Sertoli cells.

1. It stimulates secretion of FSH.
2. Activin results from various combinations of βA and βB subunits of inhibin that forms βAβB, βBβB, and βAβA.
3. They stimulate WBC development in the bone marrow, formation of mesoderm during embryonic life and also gonadal development.

Follistatin

Follistatin is a single chain protein having molecular weight of about 40,000. It has various isoforms that binds and inactivates activin.

1. When activins bind with follistatin they loose their functions.
2. Thus, FSH secretion is reduced from pituitary cells.
3. Follistatin influences developing spermatogenic cells by a paracrine mechanism.

Regulation of Testicular Functions

The major function of testes is to secrete testicular hormones, especially testosterone that controls gametogenesis, sexual development and anabolic effects. Testicular functions are controlled by hypothalamic and pituitary inputs.

Hypothalamic Control

Hypothalamus secretes GnRH. GnRH stimulates LH and FSH secretion from anterior pituitary that in turn influences testicular steroidogenesis.

Pituitary Control

Anterior pituitary controls testicular functions by secreting LH and FSH. FSH stimulates Sertoli cells to produce inhibin that maintains the gametogenic functions of the testis. FSH also stimulates secretion of androgen binding protein. LH stimulates Leydig cells to secrete testosterone, which controls gametogenesis.

Steroid Feedback

Testosterone provides negative feedback signal to hypothalamus to inhibit the secretion of GnRH, and to gonadotrophs of anterior pituitary to inhibit secretion of LH (Fig. 67.15). Inhibin provides negative feedback effect on anterior pituitary to inhibit the secretion of FSH.

| Testosterone | 6.5 |
| Androstenedione | 1.5 |
| Dihydrotestosterone | 0.5 |

Table 67.1: Average plasma concentration (µg/lit) of androgens in adult male.

Fig. 67.15: Feedback regulation of gonadal hormone secretion. Minus sign indicates inhibition.
TESTICULAR ABNORMALITIES

Cryptorchidism
The failure of migration of testis from abdominal cavity into the scrotum during fetal development is called **undescended testis** or cryptorchidism. The usual site of undescended testis is inguinal rings.
1. From posterior wall of the abdomen testis first descends into the inguinal region and then from there into the scrotum.
2. The descent from abdomen into the inguinal region depends on MIS.
3. The descent from inguinal region to scrotum depends on testosterone and other factors.
4. Descent of testis normally completes in few days before the birth.

Treatment
Treatment with **gonadotropin hormones** facilitates the descent of testis. Surgical correction is required when the hormonal treatment fails.

Complications
Undescended testis decreases sperm production, as temperature is high in the abdomen. The incidence of **malignant tumors** is significantly more in undescended testis.

Male Hypogonadism
There are mainly **two types**: Hypergonadotrophic hypogonadism and hypogonadotrophic hypogonadism.

**Hypergonadotrophic Hypogonadism**
If this occurs due to testicular dysfunctions, plasma level of gonadotropin is increased.

**Hypogonadotrophic Hypogonadism**
This occurs mainly due to tumor of hypothalamus or pituitary. Gonadotropin level in plasma is depressed.
1. If hypogonadism occurs after puberty, the secondary sex characteristics regress slowly as androgenic maintenance of these features is less essential. However, loss of libido is common.
2. If loss of Leydig cells occurs from childhood, **eunuchoidism** results.
   - Enuchoids are usually tall, with narrow shoulders and less muscular development.
   - The genitalia are small.
   - Though pubic hairs are present they are usually sparse and exhibit female pattern, i.e. triangle with base up.

**Androgen Secreting Tumors**
Hypersecretion of testis is usually due to a tumor in the testis. Leydig cell tumors secrete androgen and result in precocious puberty in prepubertal boys.

CHAPTER SUMMARY

**Key Concepts**
1. In males, gametogenesis is a continuous process. Gametes (sperms) are produced in millions every day, and the process continues throughout life starting from puberty, though the rate of production decreases in old age.
2. Sertoli cells provide support to the germ cells in the process of spermatogenesis. The tight junctions between Sertoli cells from the blood-testis barrier that provides protection to developing sperms from harmful blood born toxins.
3. Leydig cells secrete testosterone that provides hormonal stimulation for spermatogenesis.

**Important to Know (Must Read)**
1. In examination, ‘Describe the steps of spermatogenesis and factors controlling spermatogenesis, may be asked as a Long Question.
2. Spermatogenesis, Factors controlling spermatogenesis, Functions of Sertoli cells, Leydig cells, Blood-testis barrier, Spermiogenesis, Semen analysis, Physiological actions of testosterone, Testicular abnormalities, are usual Short Questions in exam.
3. In Viva, examiner may ask…. List the steps of spermatogenesis, List the factors controlling spermatogenesis, What is the duration of spermatogenesis, What is the rate of production of sperms, List the functions of testis, Why the scrotal temperature is lower than core body temperature, What is blood-testis barrier and what are its functions, What is the structure of a spermatozoa, What is the fate of stored spermatozoa, What is the composition of normal semen, How do you assess male infertility, What is the source of testosterone, List the physiological actions of testosterone, What are the secondary sex characteristics in male, List the functions of Sertoli cells, What are the functions of Leydig cells, What is the fate of testosterone secreted from Leydig cells, What is cryptorchidism and what is its treatment and complications, What is spermiogenesis and what are the changes that occur during this period, What is spermiation, What is capacitation, What is acrosomal reaction, What are the function of prostate glands, Why is the prostate specific antigen (PSA) a marker of prostate cancer, What is the use of 5α-reductase inhibitors in prostate hypertrophy, What is penis-at-14 syndrome, What is the source of estrogen in males, What are the causes of gynecomasia in males, What are the types and causes of male hypogonadism, What is eunuchoidism, What are the other testicular hormones and what are their functions, How is the testicular functions regulated, What is the mechanism of action of testosterone, What is CatSper protein and what is its function, List the differences between spermatogenesis and oogenesis, What is the structure and function of epididymis, How do the Leydig cells and Sertoli cells interact, What are the function of vas deferens, What are the function of bulbourethral glands, What is the effect of androgen secreting tumors.
CHAPTER 68
Female Reproductive System: Functional Anatomy, Oogenesis and Follicular Development

LEARNING OBJECTIVES

On completion of study of this chapter, the student MUST be able to:
1. Correlate the functional organization of female reproductive tract with their functions.
2. Give the different parts of uterus and ovary, and list their functions.
3. Give the steps and regulation of oogenesis.
4. Understand the mechanism and regulation of different phases of ovarian follicle.
5. Describe the structure and function of corpus luteum.

The student MAY also be able to:
1. Describe the process of oogenesis.
2. Describe the ovarian follicular development.

The important event in female reproductive functions is the cyclical release of gamete during the reproductive cycles. The ovarian changes are mainly growth, maturation and release of ovum and secretion of hormones, and uterine changes are mainly endometrial alterations to nourish the implanted fertilized gamete, or shedding of endometrium associated with uterine bleeding in the absence of fertilization. The follicular development during each cycle is a detailed and organized process controlled by hypothalamo-pituitary-ovarian endocrine axis.

**Scientist contributed**

Regnier de Graaf (1641–1673), was a Dutch physician, anatomist and physiologist who made key discoveries in reproductive biology during his only 32 years of short life span. He was the first scientist who pioneered in the study of physiology of reproductive system in males and females. He had demonstrated ovulation in mammals. The ovarian follicles are named after him as Graafian follicles in recognition of his outstanding contribution in the field of reproductive physiology. He had also studied the pancreatic secretion.

**FUNCTIONAL ANATOMY**

**External Genitalia**

Female external genitalia include labia majora, labia minora, clitoris, vestibule of vagina and vestibular glands (Fig. 68.1). These structures are collectively called as vulva.
Labia Majora

Labia majora are analogous to scrotum in males. These are two major skin folds that form the outer lips of vaginal vestibule, which include vaginal and urethral openings.

Labia Minora

Labia minora are two minor skin folds present between labia majora and form inner lips of vaginal and urethral openings.

Clitoris

Clitoris is located at the anterior pole of vaginal vestibule. This is the female analogue of penis. It consists of erectile tissue. The tip of the clitoris is the glans clitoris similar to glans penis.

Vaginal Vestibule

Vaginal vestibule contains two openings: vaginal and urethral openings. Vaginal opening lies below and posterior to urethral opening. In virgins, vaginal opening is partially closed by hymen, a thin fold of mucous membrane. Vestibular glands empty their secretion into the vaginal vestibule.

Internal Genitalia

The female internal reproductive organs are present in the pelvis and consist of vagina, uterus, fallopian tubes and ovaries (Fig. 68.2).

Vagina

This is a tubular canal which is present anterior to rectum and posterior to the urethra and bladder. It connects vaginal opening in the vestibule to the uterine cervix.

1. The length of vagina in adult is about 8 cm, but it is highly stretchable as its wall is folded normally. It can elongate to about double of its length during sexual act.
2. Under the action of relaxin, it widens and stretches further during parturition.
3. Vaginal wall contains muscle and contraction of vagina during sexual act facilitates the transportation of sperm into the uterus.
4. Vaginal epithelium changes during different phases of menstrual cycle.
   − In proliferative phase, under the influence of estrogen, cornification (keratinization) of vaginal epithelium occurs that secretes thin mucus.
   − In the luteal phase, under the influence of progesterone, cornification of epithelium is reduced; rather, polymorphonuclear leucocytes infiltrate and epithelium proliferates to secrete thick mucus.
5. After menopause, vaginal epithelium becomes thin and secretion is scanty. Therefore, post-menopausal vagina is dry and susceptible to infections.

Uterus

This is hollow organ having thick muscular wall. It is placed between bladder anteriorly and rectum posteriorly. It has two parts: the corpus (or the body) and the cervix (Fig. 68.3).

Body of Uterus

Uterus consists mainly of two layers (myometrium and endometrium) and uterine cavity.

Myometrium

The myometrium consists of multiple layers of smooth muscle.
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Endometrium
The endometrium has two layers: a stromal layer close to myometrium and an epithelium.
1. The stroma is penetrated by spiral arteries and contains adequate connective tissue.
2. The epithelium contains uterine glands that are lined by columnar secretory cells. The glands also infiltrate into stroma. The arterial and glandular changes occur in different phases of menstrual cycle (see below).
3. Shedding of endometrial tissue at the end of luteal phase results in menstrual bleeding. Uterus enlarges during pregnancy to accommodate the growing fetus and expels the fetus at the time of parturition.

Uterine Cervix
The cervix is a narrow muscular tube that connects vagina with body of the uterus (Fig. 68.3). It has internal os, cervical canal and external os.
1. Cervical mucus changes from thick and viscous liquid to profuse, watery and highly elastic liquid, which forms the physiological basis of ‘spinnbarkeit test’ for ovulation (spinnbarkeit refers to a highly elastic substance).
2. Under the influence of estrogen, cervical mucus forms typical ‘fern pattern’, which disappears following ovulation.
3. Cervical dilation is done for uterine investigations (Clinical Box 68.1). Cervical dilation is the initial event in the initiation of parturition.

Clinical Box 68.1
D and C: Dilation of cervix is performed routinely in gynecological practice to obtain endometrial tissue from cervix or body of the uterus for diagnosis of various uterine pathologies. This is called dilatation and curettage (D and C). D and C are also performed therapeutically for treatment of few uterine diseases. Cervical dilation is done to evacuate the uterine content (conceptus) for abortion.

Fallopian Tubes
Two fallopian tubes (also called oviducts) arise from both sides of upper poles of uterus. Oviducts are 10–15 cm in length. They have three parts: isthmus, ampulla and infundibulum (Fig. 68.3).
1. Infundibulum opens into pelvic cavity close to ovaries. Infundibular openings have fimбриae (finger like projections lined by ciliated epithelium) that help in grasping ovum at the time of ovulation.
2. Normally, fertilization takes place in ampullary part of the oviduct and then fertilized ovum is transported to uterine cavity for implantation.
3. Thus, maldevelopment or diseases of fallopian tubes result in infertility (Clinical Box 68.2).

Clinical Box 68.2
BTL: Bilateral tubal ligation (BTL) is the commonest and permanent method of sterilization in parous women. Tubal ligation is preferred to tubectomy, when the parents keep the option for recanalization of the tubes if necessity arises in future.

Scientist contributed
Gabriele Falloppio (1523–1562) was Italian physician and anatomist of 15th century who pioneered in studying the structure and functions of the reproductive organs. He demonstrated the seminal vesicle, the uterine tubes and the details of the mammalian reproductive systems. For his immense contribution in the field of reproductive system during his life span of less than 40 years, the uterine tubes of female reproductive system are named after him as Fallopian tubes.

The Ovaries
There are two ovaries each weighing about 10 g in adults. Each one present on both sides of uterus and are attached by ovarian ligaments. Ovary consists of two zones: the cortex, and medulla.

Cortex of Ovary
The cortex is the outer and the major zone lined by the germinal epithelium. It is surrounded by a layer of fibrous tissue called tunica albuginea.
1. Cortex contains oocytes. Each oocyte is enclosed within a follicle, the ovarian follicle. Ovarian follicles are present in their various stages of development (Fig. 68.4).
2. The matured ovarian follicle is called Graafian follicle.
3. The stroma of the ovary is present in between the follicles, which consists of supporting connective tissue and interstitial cells.

Medulla of Ovary
The inner zone of the ovary forms the medulla that contains different types of interstitial and connective tissue cells. Blood vessels and lymphatics enter the ovary through its hilum.

Functions of Ovary:
Ovary like testis performs many functions:
1. Oogenesis during fetal life.
2. Maturation of oocyte.
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3. Ovulation

The primary function of ovary is to develop ovarian follicles and release ovum at the time of ovulation, and to secrete steroid hormones that control various reproductive and metabolic functions.

**Oogenesis**

Unlike spermatogenesis that starts at puberty and continues throughout life, the process of oogenesis starts in fetal life and ceases at menopause. Also, the process of development of each spermatocyte is completed in few days, whereas development of each oocyte that begins in intrauterine life is completed with ovulation that occurs during menstrual cycle.

Thus, many sperms are produced in few days whereas single ovum is produced during each cycle, the development of which occurs at different stages of life till ovulation. The stages of development of oocytes occur in three stages: Oogonium becoming primary oocyte, primary oocyte converted to secondary oocyte, and finally secondary oocyte developing to mature ovum.

**Oogonia Becoming Primary Oocyte**

**Oogonia**

The primordial germ cells (oogonia) migrate from the yolk sac of embryo to the genital ridge at about 6th week of gestation. The, oogonia undergo many mitotic divisions and the number of oogonia reaches to about 7 millions. When mitosis cease, the oogonia are called oocytes.

**Primary Oocytes**

The oocytes undergo two meiotic divisions (the meiotic cycle) at different stages of development to produce a haploid ovum. Oocytes in their preliminary stage of development are called primary oocytes.

**First Meiotic Division**

The first meiotic division starts in primary oocytes during fetal life, which occurs at about 8th week of pregnancy is arrested in prophase. The oocytes then grow in size.

1. However, the first meiotic division is not completed in fetal life, not even till puberty; in fact, it is completed just prior to ovulation.
2. Therefore, the life span of a primary oocyte can be up to 50 years, as ovulation can continue up to this age.
3. The suspension of oocyte division in prophase for such a long period depends on the internal hormonal environment provided by the surrounding supporting cells.

**Oocyte Degeneration**

The oocyte degeneration however, starts from the intrauterine life so that only about 1 million primary oocytes remain at the time of birth. By the time of puberty about 200,000 and by the age of 30 only about 26,000 oocytes remain in the ovary. At menopause, ovaries are virtually devoid of oocytes.

1. During a woman’s life, only about 400 oocytes are ovulated and the other oocytes degenerate. The process of degeneration of oocyte is called atresia.
2. A major difference in male and female gametogenesis is that the process of spermatogenesis is a continuous phenomenon and the production of sperm is unlimited, whereas primary oocytes degenerate with age (Application Box 68.1).
3. As new oogonia cannot be manufactured in ovary, the oocytes totally disappear at the time of menopause.

**Application Box 68.1**

*Age of oocyte contribute to health of children:* Because of the peculiar pattern of development of oocyte in females, the life span of an oocyte may be as old as 50 years. The oocyte that ovulates at about 40 years of age is about 25 years older than the oocyte that ovulates at the age of 15. This one of the important factors that contribute to the anomalies in children born to older woman as the aged-eggs oocyte have degenerative changes and therefore their fertilization may result in defective embryo.

**Primary Oocyte Converted to Secondary Oocyte**

In fetus, oogonia develop into primary oocyte, which undergo first meiotic division. However, the first meiotic division is not completed in fetus. This is called as meiotic arrest. Thus, all eggs present at birth are primary oocytes.
in meiotic arrest containing 46 chromosomes. This state continues until puberty.
1. The primary oocyte that is destined for ovulation completes the first meiotic division just before the ovulation.
2. This division results in production of two structures: one is the daughter cell, called secondary oocyte containing 23 chromosomes, and the other is the first polar body (Fig. 68.5).
3. However, the cytoplasmic division is grossly unequal in this process in which the secondary oocyte retains nearly all the cytoplasm with polar body containing very little of it. Thus, the polar body becomes completely nonfunctional.

Secondary Oocyte Forming Ovum
Second Meiotic Division
The second meiotic division occurs in the secondary oocyte after ovulation, and is arrested in metaphase.
1. The second meiotic division is completed when the egg is penetrated by a sperm. Thus, meiotic cycle is completed only on fertilization.
2. As a result of this meiotic division, the ovum containing 23 chromosomes and the second polar body are formed (Fig. 68.5). The polar body is extruded.
3. Thus, in the process of oogenesis, each primary oocyte produces only one ovum.

DEVELOPMENT OF OVARIAN FOLLICLE
The oocyte grows throughout their life in the ovarian follicle till ovulation when the ovum is released from the follicle (from the ovary). Along with development of oocyte in ovarian follicle, follicles also grow in different phases. This is called follicular growth or folliculogenesis.
1. It starts during intrauterine life and continues till ovulation that occurs during each menstrual cycle. Thus, a single ovarian follicle may develop from fetal life till menopause.
2. At the beginning of menstrual cycle, a number of follicles start growing. However, only the one dominant follicle finally matures and releases ovum, whereas rest others undergo degeneration (atresia).
3. About 99.9% of follicles present at birth undergo atresia during the reproductive life of a woman.

Stages of Follicular Development
Foliculogenesis occurs in four stages: Stages 1–4 (Figs. 68.6A to D).

Stage 1 (Primordial Follicular Stage)
The ovarian follicle, also called Graafian follicle begins as a primordial follicle (Fig. 68.6A).
1. The primordial follicle consists of a primary oocyte at the center surrounded by a layer of spindle cells (flattened pregranulosa cells) that form granulosa cells later. A basal lamina is formed outside the spindle cells. The size of oocyte in primordial follicle is about 25 μm.
2. Primordial follicles form the resting pool of follicles in the ovary.
3. However, they degenerate progressively from fetal life to menopause.
4. The oocyte enters into **first meiotic division** and the division is **arrested in prophase**. This prophase of oocyte is maintained till just prior to ovulation.

**Stage 2 (Primary Follicular Stage)**

The primordial follicles grow into the **primary follicles**.

1. During this process the flattened pregranulosa cells (spindle cells) become **cuboidal granulosa cells** that further proliferate to form a continuous cell layer surrounding the oocyte (Fig. 68.6B). The size of oocyte increases to about 80-140 µm.
2. A type of **glassy material** consisting of mucopolysaccharide is secreted from granulosa cells, which forms a thick layer between the oocyte and the granulosa cell layer, called as **zona pelucida**.
3. The primordial follicle becomes primary follicle at about 28th weeks of gestation.
4. The oocyte is maintained in prophase of its first meiotic division.

**Stage 3 (Secondary Follicular Stage)**

The primary follicle becomes **secondary follicle** in this stage during which the granulosa cells divide and form several layers of cells around the oocyte. Thus, the size of follicle increases enormously in this stage to about 500 µm.

1. At this stage, follicle is called **preantral follicle**. However, small antrum may develop (Fig. 68.6C).
2. A layer of spindle cells (**pre or early theca cells**) is formed at the periphery of the basal lamina, which forms the theca cell layer in the next stage.
3. All these development occur slowly in the prepubertal ovary.

**Stage 4 (Tertiary Follicular Stage)**

This is the final stage of follicular development. It occurs in two sub-stages: the early tertiary stage and the Graafian follicular stage.

**Early Tertiary or Antral Follicular Stage**

In this stage, the spindle cell layer surrounding the basement membrane proliferates and differentiates into inner **theca interna** and outer **theca externa**.

1. **Theca interna cells** multiply to form multiple cell layers and become steroidogenic.
2. **Theca externa cell** lie in a single layer and provide mechanical support to the follicle from outside.
3. Thca cells receive blood, lymphatic and nerve supply whereas **granulosa cells remain avascular** as blood vessel cannot penetrate the basement membrane.
4. Along with the expansion of theca cell layer, a fluid-filled space is created in the midst of granulosa cells, called as **antrum** (Fig. 68.6C). Therefore, the follicle in this stage is also called **early antral follicle** and the stage also as **early antral follicular stage**.
5. The granulosa cells increase in number and **acquire receptors for FSH** and start secreting estrogen. In fact, the progression of early tertiary stage to the next stage of follicular development is FSH dependent.

**Late Tertiary or Graafian Follicular Stage**

This is the most rapid stage of development. It occurs only in the post-pubertal ovary.
1. After 5–7 days of onset of menses, a single follicle becomes dominant follicle for that cycle. The follicle in this stage is called Graafian follicle (Fig. 68.6D) and the stage is called Graafian follicular stage. It occurs in one ovary during each cycle.

2. The size of the antrum and the amount of antral fluid are increased significantly. This pushes the oocyte to the periphery of the follicle.

3. The mucopolysaccharide, which is present in the antral fluid, is depolymerized to increase the osmotic pressure of the fluid. However, the total intrafollicular pressure remains within 20 mm Hg.

4. The antral fluid contains many hormones such as estrogen, progesterone, FSH, LH, prolactin, androsterone and growth factors, inhibin, activin, GnRH, CRH, opioid peptides and oxytocin.

5. It also contains plasminogen activator, mucopolysaccharide, proteins, electrolytes, glycosaminoglycans and proteoglycans.

6. The vascularity of theca increases.

7. The granulosa cells in this stage are anatomically divided into three compartments: antral, cumulus and mural granulosa cells (Fig. 68.7).

   - **Antral granulosa cells**: Granulosa cells lining the antral cavity are called antral granulosa cells (Discus proliferous). Antral granulosa cells are highly steroidogenic.

   - **Cumulus granulosa cells**: Granulosa cells surrounding the oocyte are cumulus granulosa cells (cumulus oophorius). Therefore, the granulosa cells that project into the antrum like a mound are collectively called as cumulus oophorus.

   - **Mural granulosa cells**: Granulosa cells that are attached to the basement membrane are called mural granulosa cells (Membrana granulosa). Mural granulosa cells are highly steroidogenic.

8. The diameter of the follicle in this stage is about 2.5 cm. The follicle is called pre-ovulatory follicle as it is ready for ovulation at this stage. The basement membrane close to the surface of the ovary undergoes proteolysis that slowly leads to rupture of the follicle resulting in release of oocyte from the follicle, the process called ovulation.

9. Just prior to ovulation, the first meiotic division is completed.

10. The oocyte is taken up by the fallopian tube.

11. If fertilization occurs, the penetration of ovum by the sperm completes the second meiotic division, which results in functional ovum (fertilized egg).

12. If fertilization does not occur, the oocyte begins to degenerate in 24 to 48 hours.

### Corpus Luteum Formation

#### Luteinization

After ovulation, the ruptured follicle is quickly filled with blood, and at this time, the follicle is called corpus hemorrhagicum. However, the cells lining the follicle rapidly proliferate to replace blood with luteal cells that are rich in lipid. Now, the follicle is called corpus luteum and its appearance heralds the beginning of luteal phase of the cycle.

1. The corpus luteum is a yellow body made up of endocrine tissue that consists of granulosa luteal cells, theca luteal cells and fibroblasts (Fig. 68.8). The number of mitochondria, lipid droplets and the endoplasmic reticulum increase in the granulosa cells of corpus luteum. These morphological changes are collectively known as luteinization, which is essential for synthesis of more steroid hormones.
2. The granulosa and theca cells of matured corpus luteum are respectively called as **granulosa lutein cells** and **theca lutein cells**.

3. Luteal granulosa cells are vascular unlike the follicular granulosa cells that are nonvascular.

4. Vascularity of luteal cells **facilitates the synthesis of steroid hormones** by promoting the supply of cholesterol from plasma to these cells.

### Regulation of Luteinization

Progesterone, estrogen and androgen are formed in corpus luteum.

1. **Progesterone secretion** reaches its peak in menstrual cycle at about 7 days after ovulation, which correlates with the full maturity of corpus luteum. These hormones (Progesterone, estrogen and androgen) may be playing some role in luteinization.

2. However, **LH is the major stimulator of luteinization.** LH maintains the functions of corpus luteum by promoting luteinization of its cells, and therefore, LH is referred to as luteinizing hormone.

3. **FSH** increases the number of LH receptors on the cells.

### Luteal Regression

If **fertilization does not occur**, corpus luteum degenerates in about 13 days after ovulation.

1. The endocrine cells of corpus luteum become necrotic, and are invaded by leucocytes and fibroblasts. This process is called **luteolysis** or luteal regression.

2. The exact mechanism of luteolysis is not known. However, it is proposed to be due to the action of **luteolysins** that are produced locally in the ovary. The proposed luteolysins are **oxytocin, prostaglandins and GnRH**.

3. **Luteolysins** promote luteal regression by preventing the action of LH on corpus luteum.

4. The degenerated corpus luteum is replaced by avascular and nonfunctional fibrous tissue, known as **corpus albicans**.

   If **fertilization occurs**, the implanted fertilized ovum secretes hCG from its embryonic trophoblast, which has LH-like activity. Therefore, **hCG maintains luteinization** and promotes the functions of the **corpus luteum of pregnancy**.

### Functions of Corpus Luteum:

1. The corpus luteum **secretes hormones**. During luteal phase, increased secretion of progesterone, estrogen and inhibin A occurs from corpus luteum. It also secretes androgen.

2. It provides endocrinial **environment for implantation of fertilized ovum**.

3. **It maintains the early part of pregnancy** by secreting progesterone in adequate concentration till the placenta becomes functional.

   Infertility occurs due to luteal deficiency (Clinical Box 68.3)

#### Clinical Box 68.3

**Luteal deficiency:** One of the common causes of infertility is the luteal deficiency, also called **luteal insufficiency**, in which either luteinization of granulosa cells of corpus luteum is deficient due to less number LH receptors on luteal cells, or due to ovulation of prematurely developed follicle that contains less number of luteinized granulosa cells. FSH also promotes expression of LH receptors in the follicular cells. Therefore, FSH deficiency can also cause luteal insufficiency. Due to **inadequate secretion of progesterone** from malfunctioning corpus luteum, pregnancy is terminated very early. Luteal insufficiency is diagnosed by demonstrating a **low progesterone level in the midluteal phase in successive cycles**. The condition is treated by administration of progesterone that maintain follicular phase and early part of pregnancy, or clomiphene citrate that induces follicular development, or hCG that facilitates luteinization of granulosa cells of corpus luteum.

### Atresia of Follicle

During the entire reproductive period of a woman, about 400 oocytes grow to culminate in ovulation. The follicles that do not become dominant undergo a **process of degeneration** called atresia. Atresia starts in fetal ovary soon after the appearance of primordial follicles. It occurs due to **apoptosis**, a process of programmed cell death.

### Regulation of Follicular Development

Factors affecting development of follicles are different in different phases of follicular growth.

#### Primordial Follicular Stage

The growth of primordial follicle is not affected by gonadotropins. It is purely a local phenomenon.

#### Primary Follicular Stage

The **chemical substances secreted by oocyte** stimulate the transformation of pregranulosa cells to granulosa cells. The **products of granulosa cells** stimulate the formation of spindle (pre-theca) cells. **FSH and LH** stimulate the development of follicle toward the later part of gestation. During early childhood, though LH and FSH are secreted in low concentration, they are essential to maintain follicular growth. Follicular growth is impaired without gonadotropins.

#### Secondary Follicular Stage

At the time of puberty, secondary follicles start developing. The granulosa cells of secondary follicles **acquire FSH receptors** and secrete estrogen in less concentration. This **follicular estrogen** promotes further follicular development.
**Early Tertiary Stage**

Immediately after menarche (the first menstrual bleeding), in the early luteal phase of the same first cycle, usually the secondary follicles enter the tertiary follicular stage of development. The granulosa cells respond to FSH in the follicular phase of the next cycle.

1. **The FSH stimulates the granulosa cell** and increases the aromatase activity, which increases the synthesis of estrogen from androgen. The follicular concentration of estrogen increases.

2. **Estrogen** along with FSH induces expression of LH receptors on granulosa cells. Estrogen also conditions the hypothalamo-anterior pituitary axis that maintains LH activity in the plasma.

3. LH synthesis is increased by estrogen at this point of time, which is essential for pre-ovulatory surge of LH.

4. Estrogen also increases the number and sensitivity of LH receptors in the theca cells. Therefore, LH stimulates androgen synthesis in the theca cells that becomes substrate for estrogen production in the granulosa cells (Fig. 68.9).

5. LH also stimulates granulosa cells to produce progesterone. Therefore, progesterone concentration also increases.

**Late Tertiary Stage**

Between 5th to 7th days of follicular phase, only one follicle grows sufficiently to become the dominant follicle.

1. The aromatase activity and number of FSH receptors increase in the granulosa cells of dominant follicle. This increases the synthesis of estrogen, and more concentration of estrogen allows the dominant follicles to prime the hypothalamo-pituitary axis to generate LH surge just prior to ovulation. Under normal conditions, estrogen inhibits LH secretion.

2. But, when the plasma concentration of estrogen reaches about 200 pg/ml and maintains that high concentration for about 36 hours, estrogen stimulates LH secretion. Thus, the positive feedback effect on LH increases secretion of LH. This leads to LH surge, which causes ovulation (see below). This positive feedback on LH secretion is due to the effect of estrogen both at the levels of hypothalamus and pituitary.

3. LH increases progesterone secretion, which increases the proteolytic enzyme activity and causes the distention of follicle due to rapid increase in follicular fluid volume.

4. LH also stimulates the activity of endoperoxide synthase that increases synthesis of prostaglandins and leukotrienes.

5. Prostaglandins cause lysis of follicular wall leading to rupture of follicle.

6. Breaking down of follicular wall is facilitated by the proteolytic enzyme plasmin. FSH, by stimulating the activity of plasminogen activator facilitates the formation of plasmin.

7. Oxytocin causes contraction of follicular wall and extrudes the oocyte. This is called ovulation. Immediately after LH surge, the number of LH receptors decreases. This desensitizes granulosa and theca cells to LH.
### Chapter Summary

#### Key Concepts

1. In females, the period of gametogenesis is restricted from puberty to menopause, which is less than 40 years. Gametes (sperms).
2. The process of oogenesis starts in fetal life. The development of each oocyte that begins in intrauterine life is completed with ovulation that occurs during menstrual cycle.
3. If fertilization occurs, corpus luteum provides hormonal support for implantation of fertilized egg and maintenance of early part of pregnancy.

#### Important to Know (Must Read)

1. In examination, Long questions are usually not asked from this chapter. However, ‘Describe the stages and regulation of ovarian follicle’ may come as a Long Question.
2. Oogenesis, Corpus luteum, Graafian follicle, Stages of follicular development, Regulation of follicular development, may be asked as Short Questions in exam.
3. In Viva, examiner may ask…… How the corpus luteum is formed, What are the functions of corpus luteum, How is the lutenization regulated, What is luteal regression, What is luteal deficiency and what are its features, What is D and C, What are the functions of ovary, What are the stages of oogenesis, How the age of oocyte contributes to the health of children, List the stages of follicular development, How the early tertiary stage is regulated, How is the late tertiary stage regulated, What are the changes that occur in primordial follicular stage, What are the changes that occur in primary follicular stage, What are the changes that occur in secondary follicular stage, What are the changes that occur in tertiary follicular stage.
The fundamental difference in female and male reproductive functions is the cyclical release of gametes in females during their reproductive life. The structural and functional changes of female reproductive system during each cycle are synchronized with the changes in hypothalamo-pituitary-ovarian axis. The ovarian changes are mainly growth, maturation and release of gamete and secretion of hormones, and uterine changes are mainly endometrial alterations to nourish the implanted fertilized gamete, or shedding of endometrium associated with uterine bleeding in the absence of fertilization. These cyclical changes in human beings are called menstrual cycles and the uterine bleeding is called menstruation.

Menstrual cycles start at puberty, temporarily stop during pregnancy and lactation, and finally cease at menopause. During different stages of menstrual cycle, ovarian hormones, especially estrogen and progesterone provide feedback effects on hypothalamo-pituitary secretions generating typical cyclic release of gonadotropins from anterior pituitary. As hypothalamo-pituitary axis is subjected to various influences, menstrual cycle can easily be affected by stress and psychosocial and environmental factors.

**MENSTRUAL CYCLE**

The reproductive functions in women exhibit cyclical changes that occur regularly over a period of about one month. This is called menstrual cycle, which is the periodic preparation of the reproductive system for fertilization and implantation of the fertilized ovum. In human beings and primates, the reproductive cycle externally manifests by periodic vaginal bleeding, called as menstruation. Therefore, the cycle is called menstrual cycle. The first menstrual cycle is called menarche. The usual age for menarche is 12–14 years.

**Duration and Phases of Menstrual Cycle**

**Duration of Menstrual Cycle**

The length of menstrual cycle usually averages at about 28 days. However, the duration of cycles is never fixed in all women. Also, the duration of all cycles is not same in one woman. This is because menstrual cycle is frequently influenced by psychological, environmental, nutritional, and social factors.
1. Normally, it ranges from 21 to 35 days.
2. The cycle is described by number of days, the first day being the day of beginning of menstrual bleeding.
3. The cycle becomes irregular toward menopause.
4. Nevertheless, irregularity in cycle length or in duration and quantity of bleeding in an apparently normal woman indicates dysfunctions of reproductive system, especially in the uterus or in the ovary.
5. Physiologically, menstrual cycle temporarily ceases during pregnancy and lactation.

**Phases of Menstrual Cycle**

Characteristically, the menstrual cycle is divided into two phases: follicular phase and luteal phase, separated by ovulation that occurs between these two phases.

**Follicular Phase**

This is the period from the onset of bleeding to the day of ovulation. This is called follicular phase as the dominant follicle in ovary matures in this phase to terminate in ovulation at the end of the phase.
1. This phase has two parts: the menstrual phase (the phase of menstrual bleeding), and the proliferative phase (phase of proliferation of the uterine endometrium).
2. Follicular phase is also called preovulatory phase.

**Luteal Phase**

This is the part of menstrual cycle between ovulation and the onset of the next menstrual bleeding.
1. This is called luteal phase as corpus luteum is formed in the ovary, which mainly controls activities of this phase.
2. This is also called the secretory phase as uterine endometrium is highly secretory during this phase.
3. Luteal phase is also called postovulatory phase.

**Length of Each Phase of Cycle**

In a 28 days cycle, the follicular phase is of 14 days (1–14 days) and the luteal phase is of 14 days (15–28 days).
1. However, alteration of the length of menstrual cycle occurs due to alteration in the duration of follicular phase. Once ovulation occurs, menstrual bleeding starts after 14 days of ovulation.
2. Thus, the length of the luteal phase remains constant irrespective of the cycle length.

Menstrual bleeding occurs in the early part of follicular phase, and usually, ovulation occurs in the mid-cycle or more appropriately, at the end of the proliferative phase and just prior to the beginning of the luteal phase. Therefore, menstrual cycle is sometimes loosely divided into four phases: bleeding phase, follicular phase, ovulatory phase, and luteal phase. However, it should be remembered that bleeding phase is part of the follicular phase and ovulation is not a separate phase, but an event that occurs at the junction between proliferative and secretory phases of the cycle.

**Changes in Reproductive Organs in Menstrual Cycle**

**Changes in the Follicular Phase (Menstrual and Proliferative Phase)**

**Ovarian Changes**

In the follicular phase, one follicle is selected to become the dominant follicle by 4th day. The mechanism of selection of a follicle to grow is not known.
1. The dominant follicle develops into mature follicle (for details of follicular development, see above).
2. The granulosa and theca interna cells proliferate and secrete estrogen.
3. The antrum increases in size and stromal fluid increases in volume.
4. The distended follicle ruptures at about 14th day resulting in ovulation.

**Uterine Changes**

In the early part of the follicular phase, along with the menstrual bleeding, layers of the uterine endometrium are sloughed. Under the influence of estrogen secreted from the developing follicle, thickness of endometrium rapidly increases from 5th to 14th day of the cycle. During this process, following uterine changes occur (Fig. 69.1C):
1. The uterine endometrium undergoes hyperplasia and hypertrophy and increases in size and thickness.
2. The endometrial glands lengthen and glands are drawn out. The glands are lined with columnar epithelium.
3. The endometrium is vascularized with formation of more number of spiral arteries that increase blood supply to the deeper layers. Increased blood supply further promotes growth of uterine mucosa.
4. Endometrial veins also grow in size.
5. Myometrial excitability increases due to the effect of estrogen.

As proliferation of endometrium is the major feature in this phase of the cycle, this is called proliferative phase. This is also called preovulatory phase as the changes occur before ovulation.

**Change in Uterine Cervix**

Under the effects of estrogen, volume of cervical mucous increases.
1. The alkalinity and elasticity of cervical mucous also increase.
2. This forms the physiological basis for spinnbarkeit.
3. The cervical epithelium becomes more secretory.

Sperm easily passes through the estrogen-dependent cervical mucous.
Section 7: Reproductive System

Figs. 69.1A to D: Changes in reproductive organs and secretion of hormones in menstrual cycle. (A) Changes in LH and FSH level; (B) Changes in estrogen and progesterone level; (C) Endometrial changes; (D) Follicular changes.
**Vaginal Changes**
During this phase, under the influence of estrogen, vaginal epithelial cells become cornified (keratinized). The cornification index increases from almost zero on day 1 to about 100 on 14th day.

**Changes in the Luteal Phase**  
**SECRETORY PHASE**

**Ovarian Changes**
Luteal phase starts after ovulation. Following ovulation, follicle is rapidly filled with blood. Therefore, the follicle is called corpus hemorrhagicum. The blood clot inside the follicle is replaced by proliferation of granulosa cells. The granulosa and theca cells of the follicle undergo luteinization to become granulosa lutein cells and theca lutein cells. Thus, the follicle develops into corpus luteum (Fig. 69.1D). The luteal cells secrete progesterone and estrogen. If pregnancy occurs, corpus luteum persists; otherwise it degenerates between 26th to 28th days, and forms corpus albicans.

**Uterine Changes**
During the luteal phase, progesterone secretion increases significantly. Estrogen secretion also increases to some extent. Therefore, following uterine changes occur under the combined effects of estrogen and progesterone. However, major changes are due to the effects of progesterone.
1. The uterine glands become coiled and tortuous.
2. The glandular cells store glycogen and secrete large quantity of carbohydrate-rich mucus and fluid. Therefore, this phase is called secretory phase.
3. The vascularity of endometrium increases further due to combined effects of estrogen and progesterone secreted from corpus luteum. Spiral arteries become tortuous (Fig. 69.1C).
4. Due to increased blood supply and increased secretion, the endometrium becomes edematous and thick.
5. Endometrial veins form venous lakes and anastomoses (Fig. 69.2).
6. Myometrial excitability decreases due to the effects of progesterone.
7. Toward later part, due to regression of corpus luteum, progesterone and estrogen secretions decrease. Coiled arteries constrict that reduces blood supply to endometrium. The foci of necrosis appear in the endometrium at many places.
8. Necrotic areas coalesce and endometrium starts sloughing at the end of secretory phase that heralds the onset of bleeding.

**Change in Cervical Mucous**
Under the effects of progesterone, the cervical mucus becomes thick and its elasticity decreases. Sperm cannot easily enter through the progesterone-dominated cervical mucus.

**Vaginal Changes**
Under the influence of progesterone, vaginal epithelium proliferates and secretes a thick mucus. The epithelium is infiltrated with leucocytes and cornification decreases.

**Mechanism of Menstrual Bleeding**
When corpus luteum degenerates, hormonal support of endometrium is withdrawn. This causes the endometrium to become necrotic.
1. The coiled arteries constrict and reduce blood supply to endometrium.
2. The foci of necrosis coalesce leading to confluent hemorrhage, which occurs along with sloughing of endometrium.
3. This results in menstrual bleeding.

**Mechanism**
The mechanism is initiated by decreased secretion of steroid hormones.
1. Reduction in steroids causes destabilization of lysosomal membranes in the endometrial cells, which leads to release of proteolytic enzymes and production of prostaglandins, especially PGF2α.
2. The proteolytic enzymes cause lysis of the endometrial tissue and PGF2α causes vasospasm producing endometrial ischemia (Application Box 69.1). The ischemic changes result in areas of local necrosis, which is facilitated by proteolytic damage.
3. The foci of necrosis join with each other that result in sloughing of the uterine endometrium.

**Application Box 69.1**
Prostaglandins cause menstrual cramps: Vasoconstriction and uterine contractions are mediated by prostaglandins formed in the endometrium in response to the decrease in plasma estrogen and progesterone. The main mechanism of dysmenorrhea (menstrual cramps) is overproduction of prostaglandins causing excessive uterine contractions. Prostaglandins also cause smooth muscle contraction in other parts of body that accounts for nausea, vomiting, and headache associated with dysmenorrhea.
Amount of Loss
During a normal menstruation, about 30–130 mL of blood is lost. The menstrual blood contains tissue debris, prostaglandins, and fibrinolysin. Increased menstrual loss is known as menorrhagia and decreased loss as hypomenorrhea.

Hormonal Changes in Menstrual Cycle

Estrogen
Estrogen has two peaks in the menstrual cycle.
1. The fist peak, which is the major peak, occurs about 48 hours before ovulation and the second peak, which the minor peak, occurs in the post-midluteal phase (Fig. 69.1B).
2. With the growth of ovarian follicle, estrogen concentration slowly increases in the early part of follicular phase. The concentration rises rapidly from 8th day of cycle to reach the peak about one or two days before ovulation. The concentration decreases thereafter and decreases further after ovulation.
3. About two days after ovulation (after the formation of corpus luteum), again estrogen concentration increases slowly to reach a smaller peak that occurs just after the midsecretory phase.

Progesterone
Progesterone concentration is minimal in the proliferative phase.
1. It starts rising following ovulation and reaches peak in 4 to 5 days after ovulation.
2. The peak is maintained till corpus luteum is active.
3. With regression of corpus luteum, progesterone concentration decreases toward the end of luteal phase.

LH
The concentration of Luteiuing hormone (LH) remains low almost throughout the proliferative phase. However, about a day before ovulation, the secretion of LH increases sharply to reach the peak about 8–10 hours before ovulation. This is called LH surge (see below). Then, it declines fast in 24–48 hours and remains low in rest of the secretory phase (Fig. 69.1A).

FSH
Follicle-stimulating hormone (FSH) secretion increases in the early part of the follicular phase (during menstrual bleeding), and then declines slowly. Synchronous with the rise in LH, secretion of FSH rises toward ovulation and thereafter the pattern of change in FSH is similar with LH pattern.

Inhibins
The pattern of change in concentration of inhibin B coincides with the change in concentration of FSH, whereas the concentration of inhibin A coincides with the pattern of progesterone.

OVULATION
Ovulation is the process of release of ovum (oocyte) from the ovary that results from rupture of Graafian follicle at the end of the follicular phase of the menstrual cycle. Usually, ovulation occurs on 14th day in a 28 days cycle. However, if the cycle length is altered, ovulation occurs 14 days before the onset of menstrual bleeding as the duration of luteal phase (which is 14 days) always remains constant.

Mechanism of Ovulation
Ovulation occurs due to midcycle LH surge.
1. LH surge occurs due to high rise in estrogen concentration toward the later part of follicular phase that provides a positive feedback effects on LH secretion from pituitary.
2. Normally, a mild to moderate increase in estrogen for a brief period is inhibitory on LH secretion.
3. Two-days prior to ovulation, estrogen concentration increases to a higher level and when this high concentration is maintained for about 36 hours, the process of ovulation is initiated.
4. A series of biochemical and morphological changes occur in ovarian follicle that culminates in follicular rupture.
5. Three chemical substances are involved in rupture of ovarian follicle: prostaglandins, cyclin D2 (a protein that regulates cell cycle), and C-EBP (CCAAT-enhancer binding protein). Decreased formation of these chemicals in ovary has been demonstrated to prevent ovulation in mice.

Steps of Follicular Rupture
1. LH surge increases release of prostaglandins and bradykinin that increase the ovarian blood flow. Thus, follicular blood flow increases.
2. The dominant follicle becomes highly vascularized and edematous. This increases the size of the follicle to about 25 mm.
3. Follicular blood flow also increases antral fluid volume, which in turn exerts pressure on the surrounding granulosa cells. The cumulus-oophorus complex gets detached from the follicular wall.
4. The basement membrane that separates granulosa cells and theca cells disintegrates due to intrafollicular pressure and the effects of proteolytic enzymes.
5. LH promotes synthesis of plasminogen activator by granulosa and theca cells, which causes conversion of plasminogen to plasmin. Plasmin causes proteolysis of follicular wall. Plasmin also activates the synthesis of collagenase that causes destruction of connective tissue matrix of the follicular wall and adjacent ovarian capsule.

6. Increased intrafollicular pressure and degeneration of the follicular wall facilitate the rupture of the follicle, which results in expulsion of oocyte from the follicle and the ovary.

7. FSH surge is not essential for ovulation (Application Box 69.2)

**Application Box 69.2**

FSH surge occurs simultaneously: It should be noted that though midcycle FSH surge also occurs almost simultaneously along with LH surge, FSH surge is not essential for ovulation. However, FSH primes the follicular cells to express adequate number of LH receptors.

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**Indicators of Ovulation**

It is important to know the day of ovulation for its physiological and clinical significance. The indicators of ovulation are as follows:

1. **Rise in of basal body temperature:** The basal body temperature (BBT) increases during ovulation by about 0.5°C. Accurate charting of this temperature can exactly detect the day of ovulation.
   a. It is recorded orally, early in the morning before getting up of the bed, and before taking any drink or washing the mouth.
   b. The increase in body temperature is due to the influence of progesterone that starts increasing with the beginning of secretory phase. Progesterone is thermogenic.

2. **Fleeting lower abdominal pain (mittelschmerz):** With ovulation, bleeding occurs into the antrum of the follicle.
   a. Small amount of blood also escapes into the abdominal cavity, which causes peritoneal irritation and produces fleeting (short-lived) lower abdominal pain.
   b. This is called as mittelschmerz.

3. **Vaginal discharge (spotting):** There may be transitory increase in vaginal discharge during ovulation.
   a. When rise in BBT is associated with mittelschmerz and spotting, they are collectively called as ovulation cascade.
   b. If all the three features are present, occurrence of ovulation is almost confirmed.

4. **Spinnbarkeit:** In the proliferative phase, estrogen makes the cervical mucous thin and alkaline.
   a. With the beginning of secretory phase, progesterone secreted from corpus luteum makes the cervical mucous thick and tenacious. Thus, uterine mucous is thinnest at the time of ovulation and its elasticity is maximal.
   b. Therefore, a drop of cervical mucous collected at the time of ovulation can be stretched to as long as 10 cm or more like a thread. This elastic nature of the mucous is called spinnbarkeit.
   c. Decreased elasticity indicates ovulation has already taken place.

5. **Fern test:** Under the effect of estrogen, the cervical mucous before ovulation forms an arborizing fern like pattern, when the mucous is spread on a slide.
   a. This is confirmed by microscopic examination of the mucous smear.
   b. Following ovulation, due to progesterone effect, the mucous become thick and fern pattern is not observed in smear of the mucous.

6. **Laparoscopic observation:** Demonstrating ovum in the abdominal cavity by laparoscopy confirms ovulation.

7. **Demonstration of LH peak:** LH surge occurs just prior to ovulation. Therefore, daily estimation of plasma LH in the periovulatory period will accurately detect the day of ovulation.

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**Physiological Importance**

Determination of day of ovulation helps in family planning.

1. The life span of ovum is about 72 hours.
2. Therefore, those who are desirous of a child, should have regular sexual act in the periovulatory period (day of ovulation, and two days before and after the ovulation).
3. Those who want to avoid pregnancy, should not have unprotected sexual act during the unsafe period (day of ovulation, four days before and after ovulation) of the cycle (Fig. 69.3).
4. Thus, it helps in planning both conception and contraception (for details, see “Female Contraceptives”).
Amenorrhea
Absence of menstrual cycle is referred to as amenorrhea. Amenorrhea is broadly classified into two categories: primary and secondary; each category subdivided into physiological and pathological.

Primary Amenorrhea
When menstruation has never occurred, the condition is called primary amenorrhea. There are physiological and pathological causes of primary amenorrhea.

Physiological Primary Amenorrhea
Amenorrhea occurs in many physiological conditions. Physiological amenorrhea is more common than pathological amenorrhea. The causes of physiological amenorrhea are:
1. Before puberty: Amenorrhea is normal in childhood and prepubertal age. Usually, menstruation starts at the age of 12–14 years.
2. Constitutional amenorrhea: Though menstrual cycle starts normally by the age of 16 years, sometimes it may not occur even at the age of 18 years or more, without there being any abnormality. This is called constitutional amenorrhea.

Pathological Primary Amenorrhea
When menstrual cycle does not start till the age of 18 years due to some prevailing disease, the condition is called pathological primary amenorrhea.
1. It usually occurs due to congenital and genetic defects. Examples are Turner syndrome, Kallman syndrome, etc.
2. Congenital malformations of reproductive tract like absence of uterus can also cause primary amenorrhea.

Secondary Amenorrhea
When menstrual cycle stops in a woman who had normal cycles before, the condition is called secondary amenorrhea. There are physiological and secondary causes.

Physiological Secondary Amenorrhea
Physiological causes of secondary amenorrhea are more common than pathological causes.
1. Peri-pubertal amenorrhea: In first one to two years of onset of menarche, menstrual cycles are often anovulatory and therefore irregular. Menstruation does not occur even for 2–4 consecutive cycles. This is called peripubertal amenorrhea. This very common till the age of 12–14 years.
2. Pregnancy: Pregnancy is the commonest cause of secondary amenorrhea. It is so common amongst all forms of amenorrhea that before investigating for the causes of amenorrhea, first, pregnancy should be ruled out.

Pathological Secondary Amenorrhea
Pathological secondary amenorrhea occurs due to a defect in hypothalamus, pituitary, ovary and uterus or due to a systemic disease or chronic use of some drugs.
1. Hypothalamic disorders: Hypothalamic diseases resulting in decreased secretion of GnRH leads to amenorrhea. The reduction in frequency of GnRH pulses rather than the absolute decrease in concentration of GnRH is the cause of hypothalamic amenorrhea. Recently, it has been observed that this occurs due to increased opioid activity and treatment with opioid blockers like naltrexone cures hypothalamic amenorrhea.
2. Pituitary disorders: Tumors and other diseases of pituitary (hypopituitarism) resulting in decreased secretion of gonadotropins leads to amenorrhea. Examples are Sheehan’s syndrome (post-partum ischemic necrosis of anterior pituitary due to severe hemorrhage during childbirth) and Simmond’s syndrome (complete destruction of anterior pituitary) etc.
3. Ovarian diseases: Diseases that decrease the production of estrogen and progesterone, frequently lead to amenorrhea. In Stein-Leventhal syndrome (polycystic ovarian disease), amenorrhea occurs due to abnormal production of hormone leading to high LH-FSH ratio and high androgen level.
4. Uterine pathology: Congenital absence of uterus, underdeveloped uterus and severe infective or non-infective endometritis produce amenorrhea.
5. Systemic illness: Amenorrhea occurs in chronic illnesses like chronic hypothyroidism, chronic renal failure and cirrhosis of liver. Any systemic illness that leads to cachexia may also cause amenorrhea.
6. Drugs: Phenothiazine derivatives, reserpine, ganglion blocking agents and estrogen-progesterone preparations (pill contraceptives) are common drugs that prevent menstrual cycles. They mainly act by inhibiting hypothalamic release of GnRH.
**Anovulation**

Absence of ovulation during a menstrual cycle is called anovulatory cycle.

1. **Following menarche**, menstrual cycles may be anovulatory for first 1 to 2 years.
2. Few cycles may also be anovulatory in lactating woman, and about six months before menopause.
3. Anovulation also occurs in severe strenuous exercise or severer job related stress.
4. Except these physiological situations, anovulation is abnormal and occurs mainly due to hormonal deficiencies.

**Treatment of Amenorrhea and Anovulation**

If amenorrhea is due to hypothalamo-pituitary defects, pulsatile administration of GnRH gives successful result. In hypopituitarism, sequential administration of FSH and hCG is useful. If amenorrhea is due to pituitary tumor, their surgical removal should be considered. Clomiphene, that binds with estrogen receptors and blocks estrogen action, induces ovulation. Clomiphene increases LH and FSH secretion by reducing the negative effects of estrogen. For uterine pathologies, corrective surgeries should be performed.

**Hypomenorrhea and Oligomenorrhea**

Decreased menstrual bleeding in duration or amount or both is called hypomenorrhea. It may be constitutional, or due to uterine pathology or due to hormonal disorders.

Decreased frequency (cycle more than 35 days) of menstrual cycle is called oligomenorrhea. Usually, oligomenorrhea occurs in ovarian diseases in which menstruation is irregular and infrequent.

**Dysmenorrhea**

Dysmenorrhea means painful menstrual bleeding. It is classified into two categories:

1. **Primary dysmenorrhea** in which there is no visible pelvic pathology, and
2. **Secondary dysmenorrhea** in which a uterine or pelvic pathology is associated with it. Usually, it occurs due to passage of clots in flow.

Dysmenorrhea occurs due to accumulation of prostaglandins in the uterine fluid. Therefore, use of prostaglandin inhibitors usually gives relief from dysmenorrhea.

**Menorrhagia, Metrorrhagia, and Polymenorrhea**

**Menorrhagia**

*Increased menstrual bleeding* in amount, duration or both is called menorrhagia.

1. It is generally caused by conditions that affect uterus and its vascular apparatus rather than any ovarian dysfunction.
2. Or, sometimes it may be a manifestation of coagulation disorder.

**Metrorrhagia**

Bleeding occurring between the periods (acyclical and irregular) is called metrorrhagia. It usually indicates a surface lesion in the genital tract, which may be benign or malignant.

**Polymenorrhea**

When menstrual cycle occurs frequently (less than 21 days), it is called polymenorrhea. Usually, the amount and duration of bleeding remain normal.

**Premenstrual Syndrome**

Few women develop some nonspecific features about a week before the onset of menstrual bleeding that are combinely called as premenstrual syndrome (PMS) or premenstrual dysphoric disorder (PMDD).

**Features:** The usual features are edema, painful or swollen breasts, depression, loss of concentration, irritability, headache, behavioral changes, and emotional disturbances. These features disappear within 1–3 days after the start of menstruation.

**Etiology:** Though the salt and water retention has mainly been attributed to PMS, the exact cause of it is not known. Recently, it has been proposed that PMS occurs due to an excess and complex interplay between the sex steroids and brain neurotransmitters.

**Treatment:** Treatment with drugs that prematurely terminate luteal phase of the cycle does not give any substantial relieve, which indicates that the PMS is not due to hormonal imbalance. Also, the plasma concentrations of hormones usually found to be normal in these patients. However, treatment with alprazolam (minor tranquilizer), prozac (serotonin uptake inhibitor) and GnRH agonist give some relief. This proves the hypothesis that PMS is due to the complex interplay between the gonadal steroids and neurotransmitters in the brain.

**Anorexia Nervosa**

It is a complex behavioral disorder in women which manifests as severe anorexia (loss of food intake) associated with functional abnormalities.

1. **Amenorrhea** is seen in 30–40% of cases.
2. Patient is grossly emaciated.
3. Emaciation (low body fat) and stress inhibit LH secretion through endorphins.
4. Pubic hair development and breast development usually remain unaffected.
CHAPTER SUMMARY

**Key Concepts**

1. The duration of menstrual cycle is not constant even in a healthy woman, as it affected by many psychosocial factors that alter hormones related to hypothalamo-pituitary-gonadal axis. The luteal phase remains constant and alteration in cycle occurs due to change in proliferative phase.
2. BBT is a good sign of ovulation. But BBT should be recorded properly to note the exact change in temperature.
3. LH surge is an example of positive feedback mechanism.

**Important to Know (Must Read)**

1. In examination, "With the help of a suitable diagram, describe the uterine and ovarian changes during different phases of menstrual cycle" is usually asked as a Long Question.
2. Indicators of ovulation, Uterine changes during different phases of menstrual cycle, Ovarian changes during different phases of menstrual cycle, Amenorrhea, Premenstrual syndrome (PMS), Mechanism of ovulation, Mechanism of menstrual bleeding, are asked as Short Questions in exam.
3. In Viva, examiner may ask… List the indicators of ovulation, What is mittelschmerz, What is spinnbarkeit, What is spotting, What is ovulation cascade, What is fern test, What are the phases of menstrual cycle, What are the uterine changes during different phases of menstrual cycle, What are the ovarian changes during different phases of menstrual cycle, What are the hormonal changes during different phases of menstrual cycle, What are the vaginal changes during different phases of menstrual cycle, What is the duration of menstrual cycle, How do you calculate the day of ovulation, How does the determination of the day of ovulation help in family planning, List the steps of follicular rupture, What is the mechanism of menstrual bleeding, What is the role of prostaglandins in menstruation, What is amenorrhea and what are its types, What is primary amenorrhea and what are its types, What is physiological primary amenorrhea and what are its causes, What is pathological primary amenorrhea and what are its causes, What is secondary amenorrhea and what are its types, What is physiological secondary amenorrhea and what are its causes, What is pathological secondary amenorrhea and what are its causes, What is the treatment of amenorrhea, What is anovulation and what is its treatment, What is hypomenorrhea and what are its causes, What is oligomenorrhea and what are its causes, What is dysmenorrhea and what are its causes, What is menorrhagia and what are its causes, What is metrorrhagia and what are its causes, What is polymenorrhea and what are its causes, What is premenstrual syndrome (PMS), What are the causes, features and treatment of PMS, What is anorexia nervosa and what are its features.
Ovarian Hormones and Control of Ovarian Functions

CHAPTER 70

Learning Objectives
On completion of study of this chapter, the student MUST be able to:
1. List the ovarian hormones.
2. Describe the functions of ovarian hormones.
3. List the secondary sexual characteristics in females.
4. Understand the mechanisms of control of ovarian functions.
5. Apply the knowledge of ovarian functions to understand the abnormalities of female reproduction.
The student MAY also be able to:
1. Describe the control of ovarian functions.

Ovarian Hormones

Ovary secretes estrogens, progesterone, Inhibins and relaxin.

Estrogens

The naturally synthesized estrogens are 17β-estradiol, estrone, and estriol (Fig. 70.1). Estradiol is 15 and 80 times more potent than estrone and estriol respectively.

Source

Estrogen is secreted from granulosa cells of ovarian follicles, corpus luteum, and placenta. Estrogen is not directly secreted from adrenal gland, but adrenal androgen is converted to estrogen by the enzyme aromatase.

Synthesis

Estrogen is a steroid hormone, synthesized from cholesterol (Flowchart 70.1). LH, acting on LH receptors located on theca interna cells converts cholesterol to androstenedione.

1. In theca cells, some androstenedione is converted into estradiol, but most of it is supplied to granulosa cells to be converted into estrogen in those cells.
2. FSH acting on the FSH receptors on the granulosa cells facilitates the conversion of androgen to estrogen by activating the activity of the enzyme aromatase.

Secretion

Estrogen secretion occurs in two peaks during the menstrual cycle. A larger peak occurs during proliferative phase just before ovulation and a smaller peak in the mid-secretory phase. The rate of secretion of estradiol is about 35 µg per day in the early follicular phase, 400 µg per day
just before ovulation and 250 µg per day in the midluteal phase. Secretion of estrone is similar to the secretion of estradiol. The secretion is very low after menopause.

**Metabolism**

Like other steroid hormones, about 98% of circulating estrogen is bound to proteins. 60% of the estrogen is bound to albumin and 38% to steroid-binding globulin. In liver, estrogens are metabolized through oxidation or are converted to their glucuronide and sulfate derivatives that are excreted in urine.

**Mechanism of Action**

Like other steroid hormones, estrogen binds with the receptor in the nucleus and increases the formation of mRNA. There are two types of estrogen receptors: ERα and ERβ. ERα are present mainly on uterus, liver, kidney and heart, and ERβ are present in ovaries, brain, GI tract, hemopoietic tissues, lungs and prostate. Estrogens have both genomic and nongenomic actions. Most of the actions are genomic that are mediated via production of mRNA. However, some of the effects are nongenomic that occur rapidly and are mediated via intracellular second messengers.

**Physiological Actions**

**Effects on Female Reproductive System**

Estrogen promotes growth of all components of female reproductive system. In general, it causes growth of smooth muscle and proliferation of epithelial lining of reproductive tract.

**On ovary**

Estrogen stimulates growth of ovary and ovarian follicles. Role of estrogen on follicular growth has been described in Chapter 70 ‘Follicular development’.

**On Fallopian Tubes**

Estrogen stimulates smooth muscle contraction of fallopian tubes and therefore increases fallopian tube motility. It also increases ciliary activity of the tubes.

**On Uterus**

Estrogen has wide ranging effects on uterus.

1. It increases uterine size. Therefore, in prepubertal girls or castrated females the uterus is small with thin myometrium.
2. During the follicular phase of menstrual cycle, estrogen causes proliferation of uterine endometrium.
3. During luteal phase, along with progesterone, it increases uterine fluid secretion. It also increases progesterone receptor on uterine endometrium. Thus estrogen prepares endometrium for progesterone action.
4. It increases uterine blood flow. Therefore, it increases myometrial size (Application Box 70.1).
5. It increases the content of contractile proteins in uterine smooth muscles. It increases the activity and excitability of uterine muscle. It also increases the sensitivity of uterus to oxytocin. Estrogen secretion increases towards term and all these uterine changes prepare the uterus for parturition.

**Effects on Cervical Mucus**

Under the influence of estrogen, cervical mucus becomes abundant, clear, and watery. These characteristics become more pronounced at the time of ovulation to allow sperm to easily pass through the cervical mucus barrier on their way to the uterus and fallopian tubes.

**Effects on Breast**

Estrogen causes breast enlargement during puberty. Therefore, it is known as “growth hormone of the breast”. It causes growth of ducts of the mammary gland. It also causes pigmentation of the areola.

**Effects on Secondary Sexual Characteristics**

At the time of puberty, secondary sexual characteristics develop in girls due to the effects of estrogen. Following are the secondary sexual characteristics in females:

1. Female body configuration: Narrow shoulders and broad hips.
2. Wide carrying angle: Thighs that converge and arms that diverge.
3. Female distribution of fat: Fat deposition in the hips and breast (breast enlargement).
4. Voice remains high pitched, as no change in larynx occurs.
5. Less hair on the body and more hair on the scalp.
6. **Pubic Hair:** Female escutcheon of pubic hair. Pubic hair distribution in females forms a triangle with **base upward and apex downward.**
7. **Internal Genitalia:** Vagina, uterus, ovary, fallopian tube grow in size and functions (as described above). It is believed that growth of pubic and axillary hairs occurs due to androgen in females rather than estrogen.

**Effects on Endocrine Organs**
Estrogen has wide ranging endocrine effects.
1. Estrogen **inhibits LH secretion** by its negative feedback effects directly on pituitary and by indirectly inhibiting GnRH secretion from hypothalamus. However, before ovulation, the feedback becomes positive that results in LH surge.
2. Estrogen **inhibits FSH secretion.**
3. It **stimulates prolactin secretion.** However, it inhibits the milk producing action of prolactin on breast.
4. It **promotes the synthesis of angiotensinogen** from liver. Thus, it **activates renin-angiotensin system.** By increasing the formation of angiotensin II, it **increases blood pressure,** especially when estrogen is taken for a longer period; for example as contraceptive pill.
5. Estrogen **increases the formation of thyroxine-binding globulin.** Therefore, the free thyroxine concentration decreases in estrogen therapy and also in later part of pregnancy when estrogen concentration is high.
6. It **stimulates androgen secretion** from adrenal cortex (Application Box 70.2).

**Application Box 70.2**

**Estrogen stimulates growth of animals:** In farm animals, commercially available estrogen injections are given to increase the body weight of the animal. This occurs due to the **protein anabolic effect** of estrogen, which is ** induced by increased androgen secretion** from adrenal cortex. Estrogen injection is known to promote growth of chickens and cattle.

**Effects on CNS**
By activating neurons in the limbic system, estrogen stimulates **libido.** Especially, acting on the suprachiasmatic area of hypothalamus, it produces **sexual behaviors.** Conversely, estrogen implantation into arcuate nucleus-ventral hypothalamus complex in experimental animals has been observed to produce ovarian atrophy.

**Effects on Musculoskeletal System**
Estrogen **stimulates bone growth.**
1. It **prevents osteoclastic activity.** Therefore, estrogen **prevents osteoporotic activity.**
2. Estrogen causes **epiphyseal closure.** Hence, it causes cessation of longitudinal growth of bone and therefore, **determines the height** of the female.
3. Estrogen has no direct effect on growth of skeletal muscle. However, by stimulating androgen secretion, it facilitates skeletal muscle development.

**Effects on Water and Electrolyte Metabolisms**
Estrogen promotes **salt and water retention** to some extent by directly acting on kidney tubules. This contributes to **ECF expansion and hypertension** in chronic estrogen therapy, in addition to its activation of renin-angiotensin system.

**Effects on Lipid Profile**
Estrogen is a strong **cholesterol lowering agent,** and therefore, it **prevents atherosclerosis.** Hence, coronary artery disease, especially myocardial infarction is less common in females during their reproductive life. Possibly, estrogen also increases HDL concentration in plasma.

**Effects on Sebaceous Secretion & Skin**
From sebaceous gland, estrogen promotes more fluid secretions. This antagonizes the thick sebaceous secretion of androgen. Thus, estrogen **inhibits the formation of acne.** Chronic high estrogen levels may produce cutaneous angiomas (Clinical Box 70.1)

**Clinical Box 70.1**

**Hyperestrogenemia in liver diseases:** Androstenedione is metabolized in liver. Therefore, in advanced liver diseases, circulating androstenedione concentration increases, that facilitates their conversion into estrogen. Hence, spider angiomas and breast enlargement are features of advanced stage of liver diseases.

**Estrogen Preparations**
Naturally occurring estrogens as extracted from plants have adverse effects when injected into farm animals. They may promote uterine and breast cancer. Therefore, **synthetic estrogens** that have no effects on uterine and breast tissue but have bone preserving and anabolic effects are used in humans and animals. This becomes possible due to the activation of selective estrogen receptor modulators in specific tissues. Examples of such estrogens are tamoxifen and raloxifene.

**Progesterone**

**Source, Synthesis and Metabolism**
Progesterone is secreted from **corpus luteum, placenta and ovarian follicle.** It is also an intermediate product in many steroid biosynthesis.
1. It is formed from pregnenolone. The plasma concentration of progesterone in follicular phase is about 0.9 ng/mL, and in luteal phase at its peak is 18 ng/mL.
2. Like other steroid hormones, progesterone is mainly bound to plasma proteins. Only 2% circulates freely in plasma, whereas 80% is bound to albumin and 18% is bound to corticosteroid-binding globulin.
3. In the liver, it is converted to **pregnanediol,** which forms **glucuronide conjugates** and excreted in urine (Flowchart 70.2).
Mechanism of Action
Like other steroid hormones, progesterone acts on the receptors located in the nucleus, and the biochemical mechanism of action is through increased gene transcription.

Physiological Actions

Effects on Reproductive Organs
Progesterone inhibits uterine myometrial contractions, by following mechanisms:
1. It opposes the stimulatory actions of estrogen and locally generated prostaglandins. This is very important for implantation of fertilized egg and continuation of pregnancy.
2. It increases the rate of conversion of 17β-estradiol to less active estrogens.
3. Progesterone also directly causes relaxation of uterine smooth muscles.
4. It also decreases excitability of myometrium and the sensitivity of myometrium to oxytocin. Progesterone increases the membrane potential and decreases the spontaneous electrical activity of myometrial cells.

Uterine quiescence is maintained throughout pregnancy by progesterone, which is crucial to prevent premature expulsion of the fetus (abortion).

Progesterone also has important effects on the secretion of mucus from the cervix. It causes the cervical mucus to become thick and sticky; in essence, this acts as a plug that prevents infective organisms from vagina to enter the uterus. This antibacterial blockage protects the growing embryo if conception has occurred.

Progesterone acts on estrogen-primed endometrium and increases secretions from endometrial glands. It inhibits proliferation of the cells lining the vagina.

Effects on Breast
It promotes growth of the breast, especially the growth of the glandular tissue (lobules and alveoli). It supports the differentiation of estrogen-prime ductal tissue. It also supports the secretory functions of breast during lactation.

Effects on Body Temperature
Immediately after ovulation, there is a small increase (approximately 0.5°C) in body temperature that occurs due to an action of progesterone on temperature regulatory centers in the brain. Progesterone is thermogenic.

Effects on Hypothalamo-Pituitary Axis
Progesterone has negative feedback effects on hypothalamo-pituitary axis. It decreases LH secretion. In large doses, it also potentiates the inhibitory effects of estrogen. Therefore, it prevents ovulation. Hence, it is used as a contraceptive agent.

Effects on Respiration
Progesterone stimulates respiration and produces tachypnea. Therefore, the PCO₂ decreases in pregnancy.

Effects on Blood Pressure
Progesterone causes vasodilation (relaxation of vascular smooth muscle). Therefore, diastolic pressure decreases in pregnancy. Thus, hypertension is never a feature of normal pregnancy. This also contributes to wide pulse pressure in pregnancy as systolic pressure increases due to ECF expansion.

On Electrolyte and Water Metabolisms
In large doses, progesterone produces natriuresis and diuresis by blocking the effects of aldosterone on kidney tubules.

Other Ovarian Hormones

Relaxin
Relaxin is a polypeptide hormone. Structurally it is similar to insulin and insulin like growth factors. The gene for relaxin is located on chromosome 9.

Source
Relaxin is secreted from corpus luteum, placenta, uterus and breast tissue in females, and prostate glands in males. The secretion of relaxin increases towards later part of pregnancy reaching a peak just before term.

Functions
1. It relaxes pubic symphysis and pelvic joints and ligaments.
2. It causes softening and dilation of uterine cervix. Thus it facilitates parturition.
3. It inhibits uterine contraction.
4. It promotes development of mammary gland.
5. In nonpregnant woman, it is secreted from corpus luteum in the secretory phase of menstrual cycle. However, its physiological significance is not known.
6. In males, it is secreted from prostate. It facilitates sperm motility and penetration of ovum by sperm.
Inhibins
Inhibins are secreted from granulosa cells of ovary. Inhibin inhibits the secretion of FSH from anterior pituitary (for details, refer ‘Male Reproductive System’).

Androgens
Androgens are secreted in women from adrenal glands and ovaries.
1. These androgens play some role in the female reproductive functions.
2. They stimulate the growth of pubic and axillary hairs and possibly they also promote growth of skeletal muscle.
3. They also help maintain sexual desire (libido).
4. However, excess production leads to male body hair distribution, abnormal skeletal muscle growth, enlargement of clitoris and reduction in breast size.

CONTROL OF OVARIAN FUNCTIONS
Ovaries have the following major functions:
2. Release of oocyte (ovulation) at the end of proliferative phase of the menstrual cycle.
3. Nourishment and development of resident oocytes.
4. Preparation of fallopian tube to facilitate fertilization.
5. Preparation of uterus for implantation of fertilized ovum.
6. Providing hormonal support to the embryo till placenta starts functioning.

The ovarian functions are controlled by hormones secreted from hypothalamus, anterior pituitary and ovary. They are GnRH, FSH and LH, and gonadal sex hormones. The major factor determining ovarian function is the pulsatile secretion of GnRH from hypothalamic neuroendocrine cells. The pattern of frequency and amplitude of GnRH pulses during a 24-hour period is different over the course of the menstrual cycle. Also, responsiveness of the pituitary to GnRH and of the ovaries to gonadotropins varies in different phases of the cycle.

Hypothalamic Control
Hypothalamus plays an important role in regulating ovarian functions by controlling the secretion of gonadotropins from anterior pituitary.
1. Hypothalamus secretes GnRH, which is released in episodic bursts. This episodic secretion of GnRH determines circannual peaks of LH secretion. It has been experimentally observed that maintaining a constant and steady release of GnRH inhibits (Clinical Box 70.2), whereas episodic and pulsatile injection of GnRH stimulates LH secretion.
2. Episodic secretion of GnRH not only stimulates LH secretion, but also regulates secretion of other hormones that control menstrual cycle.
3. The frequency of GnRH burst is important in bringing the LH surge.
4. Also, the sensitivity of gonadotrophs of anterior pituitary to the GnRH burst is important in bringing LH surge, which increases towards ovulation.
5. The higher concentration of estrogen stimulates this frequency towards the later part of follicular phase that finally culminates in LH surge.
6. However, the exact site of GnRH pulse generator in the hypothalamus is not known.
7. Progesterone, androgen, catecholamines, enkephalins and endorphins inhibit frequency of GnRH pulses.

Clinical Box 70.2
Long-acting GnRH analogs: As described above, a constantly elevated GnRH level in plasma inhibits LH secretion by down-regulating receptors on gonadotrophs. Therefore, long-acting GnRH analogs are used in treatment of precocious puberty as it inhibits LH secretion by chronically maintaining a GnRH level. It is also used in therapy of prostate cancer.

Pituitary Control
The anterior pituitary secretes LH and FSH. The major target organs of these hormones are gonads. Therefore they are called gonadotropins. Primarily, they control ovarian functions in both the phases of menstrual cycle. Ovarian control by gonadotropins has a similar analogy with testicular control (Clinical Box 70.3).

Clinical Box 70.3
Analogy with testis: The granulosa and theca cells of ovarian follicle are similar to the Sertoli and Leydig cells of testis. Sertoli cells provide microenvironment in testis in which the germ cells develop and mature, and Sertoli are stimulated by FSH. Granulosa cells resemble Sertoli cells in their function by providing environment for development of oocyte, and for their control by FSH. Like Sertoli cells granulosa cells also secrete inhibin-B that controls FSH secretion from pituitary. The theca cells are similar to the Leydig cells in that they produce androgens and are stimulated mainly by LH.

The Pattern of Secretion of Gonadotropins
The FSH level in plasma slowly increases in the early part of the follicular phase and then steadily decreases in the remainder of the phase and then increases rapidly towards the end of the phase to attain a mid-cycle peak (refer to Fig. 69.1, Chapter 69).
1. The LH in level plasma is low in most part of the cycle except in the periovulatory period. It starts increasing rapidly about a day before ovulation to reach a very large mid-cycle peak, called LH surge.
2. This is followed by a rapid decline of LH in the luteal phase.

Regulation by FSH
A number of primary and early antral follicles are always present in the ovary between puberty and menopause. Further development of these follicles beyond these stages requires stimulation by FSH.
1. **Before puberty**, the concentration of FSH in the plasma is too low to bring such development.

2. **After puberty**, during first half of each menstrual cycle, increase in FSH secretion stimulates follicular growth. FSH stimulates proliferation of granulosa cells to produce estrogen, and stimulates enlargement of the antrum.

3. FSH also primes the granulosa and theca cells to the effect of LH.

### Regulation by LH

LH acts on the theca and granulosa cells. These cells are converted to luteal cells as follicle becomes corpus luteum after ovulation. LH causes luteinization of these cells. LH surge is responsible for ovulation to occur (for details, refer “Mechanism of Ovulation” in previous chapter).

### The Ovarian Control

Ovary controls its own functions by secreting hormones that directly influence granulosa cell function or indirectly influence hypothalamic-pituitary-ovarian axis (feedback control).

### The Pattern of Secretion of Gonadal Hormones

#### Estrogen

After remaining low in the first week, estrogen first increases slowly and then rapidly in the second week as the dominant ovarian follicle grows and secretes more estrogen.

1. **Estrogen peak occurs about two days before ovulation** and then decreases rapidly in next two days.
2. The second but **slow and small peak** of estrogen occurs in luteal phase due to its secretion from corpus luteum.

#### Progesterone

Progesterone release is low during the follicular phase.

1. But, soon after ovulation, progesterone concentration **increases rapidly in luteal phase** due to its release in large amount from corpus luteum.
2. Inhibin-B secretion resembles the secretory pattern of FSH.

### Direct Regulation

Theca cells secrete androgen, which facilitates secretion of estrogen by the granulosa cells.

### Feedback Regulation

The dominant follicle secretes estrogen that inhibits gonadotropins (the **negative feedback effect**) from pituitary and GnRH secretion from hypothalamus (Fig. 70.2).

1. When plasma concentration of estrogen is very high the secretion of gonadotropin increases (the **positive feedback effect**).

### APPLIED ASPECTS

Reproductive abnormalities that either lead to or occur due to ovarian dysfunctions can broadly be classified into two categories: genetic and hormonal abnormalities.

### Genetic and Hormonal Abnormalities

Following genetic abnormalities are common in women, and are associated with hormonal dysfunctions:

#### GnRH Resistance

This occurs due to **decreased number and sensitivity of GnRH receptors** on gonadotrophs of pituitary.
FSH Resistance
This occurs due to decreased number and sensitivity of FSH receptors on granulosa cells of pituitary.

LH Resistance
This occurs due to decreased number and sensitivity of LH receptors on granulosa and theca cells of ovarian follicles.

Aromatase Deficiency
The enzyme aromatase is required for synthesis of gonadal hormones. Aromatase (CYP 19) converts testosterone to estradiol and androstenedione to estrone. Therefore, its deficiency leads to estrogen deficiency and amenorrhea.

McCune-Albright Syndrome
This is an example of receptor protein (G protein) abnormality in which $G_{sa}$ is active in some cells and defective in others. It causes endocrine dysfunctions associated with precocious puberty, amenorrhea and galactorrhea. Lina Medina of Peru was the commonly quoted example of Albright syndrome.

Kallmann’s Syndrome
This is a hypogonadal form of deficiency of LH and FSH neurons that occurs due to failure of migration of these neurons from the olfactory bulb. The GnRH secretion from hypothalamus is less and therefore deficiency occurs in secretion of LH and FSH. Pubertal maturation of testes in males and ovaries in females fail to occur (hypogonadotropic hypogonadism).

**CHAPTER SUMMARY**

**Key Concepts**

1. The major function of estrogen is development secondary sexual characters in women including the development of breast and reproductive structures. It causes proliferation of endometrium and follicular development in preovulatory phase of menstrual cycle. It protects from atherosclerosis and cardiovascular risks.

2. The major function of progesterone is relaxation of uterine smooth muscle and decreases excitability of the myometrium in early part of pregnancy. This favors implantation and prevents abortion. It causes uterine changes in the secretory phase of the cycle. It decreases BP.

3. Ovarian functions are controlled mainly by feedback regulation of hypothalamo-pituitary-gonadal axis driven by the circulating ovarian hormones.

**Important to Know (Must Read)**

1. In examination, ‘Describe the physiological actions and regulation of secretion of estrogen and progesterone’ may be asked as a Long Question.

2. Physiological actions of estrogen on female reproductive system, Secondary sexual characteristics in females, Physiological actions of progesterone, Hypothalamic control of ovarian function, Pituitary control of ovarian function, Ovarian control of ovarian function may be asked as Short Questions in exam.

3. In Viva, examiner may ask… Name the hormones secreted from ovary, What are the functions of estrogen, Name the secondary sexual characteristics in women, What are the physiological actions of estrogen on female reproductive system, on breast, on secondary sexual characteristics, on endocrine organs, on CNS on musculoskeletal system, on water and electrolyte metabolism, on lipid profile, Why is estrogen used in animal farms, Why does hyperestrogenemia occur in liver diseases, What are the estrogen preparations, List the functions of progesterone, What is the mechanism of action of estrogen and progesterone, What are the functions of relaxin, What is the function of inhibin released from ovary, What are the functions of androgens in women, List the functions of ovaries, How does the hypothalamus control the ovarian function, What is the use of long-acting GnRH analogs, How does the pituitary control the ovarian function, How does the ovary regulate its own function, List the genetic and hormonal abnormalities in women, What is McCune-Albright syndrome, What is Kallmann’s syndrome.
CHAPTER 71

Physiology of Copulation

LEARNING OBJECTIVES
On completion of study of this chapter, the student MUST be able to:
1. Understand the blood supply and innervation of the erectile tissues of the penis.
2. Learn the mechanism of erection of penis.
3. Understand the process of emission and ejaculation.
4. Describe the sexual responses in females.
5. Appreciate the mechanism of orgasm.
6. Define impotence and give its physiological basis.

The student MAY also be able to:
1. Describe the processes and mechanisms involved in male and female sexual act.

Copulation is the process of mating that occurs in human being by coitus, or the sexual act. Though the physiology of mating is similar in primates, the sexual responses of male and female are different in human being. It is important to understand the physiology of copulation to learn the pathophysiology of impotency, which is quite common in the present society.

MALE SEXUAL ACT
In sexual intercourse, male is the active partner. The major events of male sexual act include:

- Erection of penis,
- Penetration of penis into the vagina, and
- Ejaculation.

These are the series of reflexes that are integrated in the spinal cord and mediated via autonomic and somatic nerves.

1. Male genital tract receives both sympathetic and parasympathetic innervation, and in addition, penis receives the somatic innervation.
2. Though the sexual act per se is mainly a neural process, its overall integration is a behavioral phenomenon that includes hormonal, biochemical and psychological interactions.

Erection
Blood supply: Penis is supplied by internal pudendal arteries that enter the organ as dorsal artery of penis from its dorsal surface. Dorsal artery penetrates deeply into the erectile tissues of penis as deep artery (Fig. 71.1). Venous drainage from penis occurs by dorsal vein.

Mechanism
Erection occurs due to dilatation of the penile arterioles filling the erectile tissues of the penis by blood. The erectile tissues of penis are three in numbers: two corpora cavernosa and a corpus spongiosum (Fig. 71.2).

1. In normal state, penis is flaccid due to paucity of blood in the spaces of these erectile tissues.
2. Upon arousal, mainly the spaces in cavernous erectile tissue fill with blood and penis becomes turgid (Fig. 71.3).
3. Relaxation of the smooth muscles of corpora allows increased flow of blood into corporal interstices (cavernous spaces) that increases the volume and rigidity of the penis.
4. As veins are compressed by the erectile tissues filled with blood, engorgement of penis inhibits venous return and keeps the interstices filled with blood, which maintains erection.
3. **NO activates guanylyl cyclase that increases the production of cGMP,** which is a potent vasodilator. The drugs that inhibit the breakdown of cGMP such as tadalafil, vardenafil and sildenafil are very useful in the treatment of erectile dysfunctions.

**Impotency**

Impotency is the erectile dysfunction in males. It is the inability to keep the penis adequately erected to be penetrated into the female genital tract.

1. **Erectile dysfunction** (ED) or impotence is sexual dysfunction characterized by the inability to develop or maintain an erection of the penis during sexual activity in humans.
2. It can temporarily happen due to psychosocial disturbances or acute illness.
3. But it can regularly happen due to physical problems and neural deficits that causes deficit in sexual reflexes.
4. Now-a-days it can be treated by use of Viagra (Clinical Box 71.1)

Clinical Box 71.1

Use of Viagra: Spinal cord diseases, peripheral neuropathies, spinal cord injuries, psychological trauma, chronic physical stress and aging cause erectile dysfunction. Sildenafil (Viagra) is used for the treatment of erectile dysfunction. It prevents the breakdown of cGMP by inhibiting phosphodiesterase. Thus, it potentiates the action of NO. It is used worldwide for the treatment of impotence. It produces side effects like visual problems by inhibiting cGMP specific phosphodiesterase V1 (PDE V1) in the retina, transient inability to differentiate blue and green color, and hypotension (by aggravating vasodilation). Viagra should be taken one hour before planned intercourse. However, it should not be used regularly for its known side effects.

Ejaculation

Ejaculation is the emission of semen from the male genital tract and its propulsion out of the urethra at the time of orgasm. Thus, it is a two-part reflex response: the emission and the ejaculation per se.

Emission

Normally, sperms are stored in epididymis and proximal part of vas deferens.
1. Emission is the process in which sperms move into the urethra.
2. It is a sympathetic response that occurs due to contraction of smooth muscle of vas deferens and seminal vesicles.
3. The fibers travel in hypogastric nerve.

Ejaculation

This is the propulsion of semen out of urethra at the time of orgasm.
1. This occurs due to contraction of bulbocavernosus muscle, a skeletal muscle.
2. This is a spinal reflex integrated at lower lumbar and upper sacral segments of spinal cord (L5, S1-3). The efferent fibers traverse in pudendal nerve.
3. It is proposed that the carbon monoxide (CO) is the transmitter in the neural circuit of ejaculation as evidenced from the fact that the ejaculatory performance is diminished when the gene responsible for the enzyme HO2 (heme oxygenase) is knocked out. HO2 normally catalyzes the formation of CO. Retrograde ejaculation occurs in diabetes (Application Box 71.1).

Application Box 71.1

Retrograde ejaculation: The internal urethral sphincter constricts at the time of emission. When this sphincter fails to constrict, the sperms instead of passing down the urethra enter the urinary bladder. The quantity of ejaculate decreases and sperms appear in urine (more than 15 sperms per high power field). Usually it occurs in diabetic neuropathy, multiple sclerosis and use of sympatholytic drugs.

Premature Ejaculation

Premature ejaculation (PE) occurs when a man ejaculates sooner during sexual intercourse. Though the prevalence of PE vary, as many as 1 out of 3 men say they experience this problem at some time. As long as it happens infrequently, it is of no much concern. But if it occurs often, investigations should be done to detect its cause.

The diagnostic criteria for premature ejaculation are:
1. Always or nearly always ejaculate within one minute of penetration.
2. Are unable to delay ejaculation during intercourse all or nearly all of the time.
3. Feel distressed and frustrated, and tend to avoid sexual intimacy as a result.

Causes and Treatment

Both psychological and biological factors can play a role in premature ejaculation. Although many men feel embarrassed to talk about it, premature ejaculation is a common and treatable condition.
1. Regular counseling, learning the proper sexual techniques and few medications can delay ejaculation.
2. Physical, mental and psychosocial stress must be removed.
3. As penile erection is a parasympathetic and ejaculation is a sympathetic mechanism, the male partner has to learn to delay the sympathetic activation during sex to avoid early ejaculation.
4. Therefore, practice of Yoga helps a lot to improve sex life.

FEMALE SEXUAL ACT

Normally, during sexual intercourse, female is the passive partner. However, the responses to intercourse in females are not passive.
1. The female responses are characterized by marked increase in blood flow and muscular activities in many parts of the body.
2. Increased sexual excitement is accompanied by engorgement of the breasts and erection of the nipples, increased diameter and length of clitoris due to increased blood flow into these structures.
3. Nipple and clitoris have rich sensory innervation. Therefore, clitoral and nipple stimulations during sexual act add to sexual excitement.
4. Blood flow also increases to vagina due to release of VIP from vaginal nerves and increased fluid secretion from vagina lubricates vaginal epithelium.
5. Mucous secretion is also increased from vestibular glands.

Sexual desire in women is possibly dependent on androgens, because sex drive is maintained long after the menopause, when estrogen level in plasma is very low. Therefore, it is believed that androgens secreted by the adrenal glands maintain sexual appetite in females.
Chapter 71: Physiology of Copulation

**Vaginal Changes**

Vaginal secretion increases during sexual excitement and intercourse.

1. This occurs mostly due to release of VIP from vaginal nerves.
2. **Vestibular glands** secrete mucus that also helps in lubrication.
3. The stimuli for vaginal secretion are tactile stimulation of clitoris, labia minora, breast and sexual excitement. Visual, olfactory and auditory stimuli aid to the excitement.
4. The sexual gratification in females culminates in **orgasm** that manifests as rhythmic vaginal contractions mediated by autonomic influences (Application Box 71.2).

**ORGASM**

It is the climax of sexual excitement and pleasure that occurs with satisfactory intercourse. The natural sexual act culminates with orgasm.

**Orgasm** (climax) in female is associated with a pleasurable feeling accompanied by sudden increase in skeletal muscle activity, increased heart rate and blood pressure, and *rhythmic contraction of the vaginal wall*. Though, vaginal contraction during orgasm facilitates sperm transport, it is not essential for fertilization since conception can occur in the absence of orgasm.

Orgasm results in following psychological, physical and reflex responses.

1. Increase in heart rate, may be up to 150 beats/min
2. Increase in systemic arterial BP
3. Rapid breathing
4. Flushing of face
5. Sweating
6. Increased skeletal muscle activity

All these changes are due to increased epinephrine secretion into the circulation. There may be associated stimulation of the anterior pituitary, adrenal cortex and the thyroid gland.

**Fate of Sperms in Female Genital Tract**

Normally $10^8$ to $5 \times 10^8$ motile sperms are released into the female vagina on ejaculation. The sperms move at the rate of 1–3 mm/min and only about 100 sperms reach the oviduct within 30 minutes. Vaginal contraction during mating facilitates sperm movement.

1. The fluid medium in the uterus is favorable for sperms. **Uterine muscular movements** and ciliary activity may aid in migration of sperms within the uterus.
2. Some **spermatozoa can survive** in a viable state within the slightly alkaline medium of the cervical mucus for up to 48 hours.
3. Finally, only one sperm fertilizes the ovum.
   - The initial process is called **capacitation** which makes the sperm (spermatozoon) able to adhere to the ovum.
   - It is followed by the **acrosomal reaction** in which one spermatozoon releases enough lytic enzymes locally to penetrate the cumulus cells and the zona pellucida that surrounds the ovum.
4. The remaining sperms disintegrate and liquefy in the female genital tract.

**Application Box 71.2**

**Male orgasm should coincide with female orgasm**: For the best conjugal life and to derive maximal pleasure of sexual act, orgasms of male and female should appear concurrently during the sexual act. As female is the passive partner in the sexual act, orgasm in females comes usually later. Therefore, the male partner should learn to stimulate the female partner appropriately and adequately even before the initiation of intercourse so that orgasms in both arrive together. In males, orgasm occurs with ejaculation, following which the penis becomes flaccid. Unless female orgasm is attained along with the male orgasm, the female does not derive actual pleasure from the act, and repeated failure to attain orgasm in females is harmful both physically and mentally. Therefore, gradually the male should know the time of orgasm of his partner and should learn to maintain penile erection till then. Usually, after marriage it takes at least a year or more for both the partners to adjust to each other’s physical and emotional needs to bring about their orgasms. Thus, sexual act is more an art than science.

**CHAPTER SUMMARY**

**Key Concepts**

1. Penile erection is a parasympathetic and ejaculation is a sympathetic mechanism. Therefore, one must learn to delay the sympathetic activation to avoid early ejaculation.
2. The orgasms of male and female partner should occur simultaneously in an effective sex.
3. Yoga is helpful for healthier sex life.

**Important to Know (Must Read)**

1. In examination, there will be no Long Question from this chapter.
2. Male sexual responses, Female sexual responses, and Orgasm, may be asked as Short Questions in exam.
3. In Viva, examiner may ask… Speak the mechanism of male sexual responses, Mechanism of erection, emission, ejaculation, What is the role of NO, What is nerve erigentis, What is the role of parasympathetic and sympathetic nerves in male sexual act, Mechanism of male sexual responses, Mechanism of female sexual responses, Definition and mechanism of orgasm, Definition and causes of impotency, Definition and causes of premature ejaculation, Mechanism of action of Viagra, Fate of sperm in female genital tract.
Pregnancy and Parturition

CHAPTER 72

Learning Objectives
On completion of study of this chapter, the student MUST be able to:
1. Understand the importance of learning the physiology of pregnancy.
2. Understand the physiology at different steps of pregnancy starting from fertilization to fetal maturation.
3. List the placental hormones and mention their functions.
4. List the causes of female infertility.
5. Understand the concept, indication and steps of in-vitro fertilization.
6. Describe various systemic maternal changes in pregnancy.
7. Understand the mechanism of parturition.
The student MAY also be able to:
1. Describe the physiology of fertilization and fetal development.
2. Explain the feto-maternal changes in pregnancy.
3. Describe the physiological changes in different stages of labor.

Pregnancy is the most precious event in the life of a married woman as it proves her fertile capability, and brings completeness to her womanhood. Pregnancy occurs when a mature oocyte is fertilized by a sperm. The introduction of sperm into the female genital tract should ideally occur in the period of two days before and one day after ovulation. This is because the sperms following their ejaculation into the vagina remain capable of fertilizing an egg usually for 24 to 48 hours (though they may survive for 3 to 5 days in the female genital tract) and the fertilizable life of the ovulated egg is about 24 hours. Thus, if ovulation occurs on 14th day of the cycle, for conception to occur the coitus should take place on 12th, 13th, 14th, and 15th days (the fertile period) of the menstrual cycle).

Physiology of Pregnancy

Transport of Gametes

Transport of Egg
Following ovulation, the oocyte is extruded onto the surface of the ovary or into the abdominal cavity. The fimbriae located at the end of the fallopian tube actively pick up the oocyte.

1. The fimbriae contain smooth muscle and are lined with ciliated epithelium. Immediately after ovulation, smooth muscle of the fimbriae contract, which make the fimbrial end of the tube migrate close to the oocyte. The cilia of fimbriae beat in waves toward the interior of the duct.
2. These ciliary motions sweep the egg into the fallopian tube as the egg emerges from follicle to the ovarian surface. Inside the fallopian tube, egg movement is driven by fallopian-tube cilia, which is a normally a slow process.
3. Therefore, the egg takes about four days to reach the uterus. Hence, for fertilization to occur, it must naturally happen in the distal portion of fallopian tube (within a day of ovulation) because of the short viability of the unfertilized egg (Fig. 72.1).

Transport of Sperm
During the act of intercourse, some sperms are propelled from the vagina to the cervix, and many enter cervical canal after the sexual act. Though usual transit time of sperm to reach fallopian tube is 4–6 hours, within few minutes of ejaculation, sperms have been detected in the
uterus and tubes. However, many sperms die in the vagina due its acidic environment (vaginal pH is 5.5–6).

**Transport of Sperm in the Cervix**

Passage of sperms through cervix is favored by:

1. **Nature of cervical mucous**: Sperm transport is favored by estrogen-induced changes in consistency and orientation of the mucous. Estrogen makes the mucous thin and elastic.
2. **Orientation of the mucous**: Also, under the influence of estrogen, cervical mucous is oriented in parallel that favors the transport of sperm. Thus, sperms can easily swim through estrogen-dependent cervical mucous.

**Transport of Sperm in the Uterus**

Transport of the sperm through the entire length of the uterus and then into the fallopian tubes occurs due to sperm’s own motility and uterine contractions.

**Transport of Sperm in the Fallopian Tube**

In the fallopian tube, sperm motility is facilitated by three factors:

1. Ciliary movement in the tube
2. Peristaltic activity of the tube
3. Flow of fluid in the tube.

Though several millions of sperms are deposited in the vagina, only about 50–100 reach the fallopian tube. This is the major reasons that there must be many millions of motile sperms in the seminal fluid for fertilization to occur. Thus, decreased count and motility of sperms lead to infertility in males.

**Changes in Sperm in Female Genital Tract**

Sperms undergo changes in the female genital tract that make them able to fertilize the egg. These changes are collectively called **capacitation**, which occurs after sperms spend one to several hours in female genital tract. Capacitation includes **two major changes** in sperms:

1. The wavelike beating of the tail of the sperms is replaced by **whip-like movements** that propel the sperm forward in strong lurches.
2. **Alteration in the plasma membrane** (modification of surface proteins) of sperms that makes them capable of fusing with the surface of the egg. Freshly ejaculated sperm cannot immediately penetrate the ovum; only a capacitated sperm is capable of doing so.

**Fertilization**

Fertilization is the process of union of sperm with egg that results in zygote formation. Normally, fertilization occurs in the **ampullary portion of the fallopian tube**. Fertilization takes place in **four steps**: Fusion of sperm with egg, acrosome reaction and sperm penetration, polyspermy block, and formation of zygote.

**Fusion of Sperm with Egg**

It begins with the fusion of a sperm and egg. Many sperms move between the cumulus cells (granulosa cells still surrounding the egg) to bind to the **zona pellucida** of the egg.

1. The **zona pellucida contains glycoproteins** that serve as receptors for sperm surface proteins.
2. The sperm contains many such proteins and therefore binds simultaneously to many receptors on the zona pellucida.

**Acrosome Reaction**

The binding sperm to the zona pellucida triggers **acrosome reaction** in the bound sperm, which is required for
sperm penetration: The steps of acrosome reaction and penetration of the sperm are as follows:

1. **Change in membrane of sperm head:** Redistribution of constituents of the plasma membrane of the sperm head at the site of the attachment results in alteration in the membrane.

2. **Increased fluidity** of the membrane of the sperm head.

3. **Increased calcium permeability** of the sperm membrane.

4. **Digestion of a part of zona pellucida:** The membrane-bound acrosomal enzymes in the sperm head is exposed to the zona pellucida. These are proteolytic enzymes that include acrosin, a trypsin-like protease. They digest a portion of the zona pellucida at the site of binding.

5. **Penetration of sperm through perivitalline space:** As the zona pellucida gives way, sperm advances through this coating using its propulsive force of the tail. Then, sperm penetrates the **perivitalline space** (the space surrounding the egg membrane) to reach the egg’s plasma membrane.

6. **Fusion of sperm head with egg membrane:** The sperm head fuses with the egg’s membrane, which is facilitated by sperm surface protein, called **fertilin**. Then, sperm slowly enters the egg’s cytoplasm (ooplasm).

**Polyspermy Block**

Only one sperm penetrates the egg. This is because, as soon as the sperm penetration starts, immediately a series of changes occur in the egg that prevent the entry of additional sperms. This is called **polyspermy block**. There are two mechanisms of polyspermy block:

1. The initial fusion of the sperm with egg plasma membranes triggers a reaction that changes the membrane potential of the egg. The change in egg membrane potential prevents additional sperm binding.

2. The secretory vesicles that are located in the peripheral part of the egg cytoplasm release their contents into perivitalline space (the narrow space between the egg plasma membrane and the zona pellucida) by exocytosis. These vesicular content contains enzymes that enter the zona pellucida and cause inactivation of sperm-binding sites on zona pellucida and hardening of the entire zona pellucida. Thus, binding of additional sperms to the zona pellucida is prevented.

**Zygote Formation**

The sperm penetration of egg is called fertilization. The fertilized egg completes its second meiotic division in next few hours.

1. As a result of the second meiotic division, the second polar body is formed, which is extruded from ooplasm.

2. The remaining haploid nucleus containing 23 chromosomes is transformed into female pronucleus in two to three hours. The two sets of chromosomes (23 from the egg and 23 from the sperm) each surrounded by distinct membranes known as **pronuclei** (the male pronucleus and the female pronucleus) migrate to the center of the cell by contraction of microfilaments and microtubules.

3. In the mean-time, the DNA of chromosomes in both pro-nuclei is replicated and **pronuclei fuse**. Then the pro-nuclear membranes break down. Now, the cell is ready to undergo a mitotic division.

4. This completes the process of fertilization and the fertilized egg is called **zygote**.

**Cleavage**

The fertilized egg (zygote) remains in the fallopian tube for three to four days. While in the fallopian tube, a number of mitotic cell divisions take place. This process of mitotic cell divisions is known as **cleavage**. The cell divisions are unusual in that no cell growth takes place before each division. Therefore, the 16 to 32 cell conceptus that enters the uterus is essentially the same size as that of original fertilized egg.

The first cell division takes place in 24 hours to produce two unequal sized cells, the structure called **blastomere**. Then each cell divides to form a four cell conceptus, eight cell conceptus and **morula** (16 to 32 cell conceptus) in 48, 72, and 96 hours, respectively. Twin is formed if dividing cells grow into two independent cell masses (Application Box 72.1).

**Application Box 72.1**

Development of twins: Each of the cells of blastomere is a totipotent cell. They have the capacity to develop into an entire individual. Therefore, **identical (monozygotic) twins** result when, at the time of cleavage, the dividing cells are completely separated into two independently growing cell masses. And, **fraternal or dizygotic twins** result when, two eggs being ovulated and fertilized simultaneously.

After fertilization, the conceptus slowly moves from the ampulla of the fallopian tube into the uterus (Fig. 56.1). Till the morula stage, the conceptus is in the fallopian tube. Until implantation in the uterus, the conceptus is enclosed in the zona pellucida. The intact zona pellucida has three functions:

1. It prevents the adhesion of the conceptus to the inner wall of the fallopian tube.

2. It protects the conceptus from mechanical damage while being transported from ampulla of the tube into the uterus.

3. It also prevents the early immunological rejection of the conceptus.

**Blastocyst Formation and Implantation**

The morula enters uterus about four days of fertilization. After reaching the uterus, the conceptus floats free in the intrauterine fluid for approximately three days, during...
which it further undergoes cell divisions. Now, the conceptus is known as a **blastocyst**, the stage which the cells have lost their totipotentiality and have begun to differentiate. Blastocyst derives nourishment from the uterine fluid. The blastocyst consists of **three components** (Figs. 72.2A to D):

1. **Trophoblast**: An outer layer of cells of blastocyst is called trophoblast. The trophoblast consists of extraembryonic ectodermal cells present on embryonic membrane. It helps in implantation of the conceptus and contributes to placenta formation. Trophoblast and its membrane secrete human chorionic gonadotropin (hCG).
2. **Embryoblast**: An inner cell mass called embryoblast that gives rise to fetus.
3. **Blastocoele**: A central fluid-filled cavity of blastocyst is called blastocoele. It forms amniotic cavity.

In subsequent development, the inner cell mass gives rise to the fetus. The trophoblast surrounds the embryo or fetus throughout its development and is involved in its nutrition as well as in the secretion of several important hormones.

**Implantation and Early Development**

Implantation is the attachment of the blastocyst to the **endometrial surface** of the uterine wall.

1. It occurs about 7–8 days after fertilization. Implantation occurs in estrogen and progesterone primed uterus.
2. By the time the zygote develops into the blastocyst, the menstrual cycle reaches about day 21 of the normal menstrual cycle or 7th post-ovulatory day, during which the endometrium is being prepared by progesterone secreted from corpus luteum.
3. During this period, embedding of the blastocyst into the endometrium (implantation) begins. Progesterone prepares the endometrium for implantation of blastocyst.
4. The trophoblast cells are very sticky, especially at the region that overlie the inner cell mass and this part of blastocyst adheres to the uterine wall for implantation. Implantation outside uterus leads to ectopic pregnancy (Clinical Box 72.1).

**Mechanism of Implantation**

Implantation occurs due to almost simultaneous changes in blastocyst that is to be implanted and the endometrium that allows implantation to occur, and interaction of blastocyst with the endometrium.

**Changes in Blastocyst**

Enlargement of blastocyst (before implantation) results in rupture of zona pellucida. The trophoblast cells are denuded and become negatively charged. This facilitates the blastocyst attachment to endometrium via surface glycoprotein. The initial contact between blastocyst and endometrium induces rapid proliferation of the trophoblast.

**Interaction of Blastocyst with Endometrium**

The proteolytic enzymes secreted by the trophoblast allow the blastocyst to bury in the endometrium. Microvilli of trophoblast cells interdigitate with endometrial cells and form junctional complexes.

**Changes in Endometrium**

At the site of contact, endometrium also undergoes changes. By the influence of progesterone, endometrium undergoes decidualization. In this process, the endometrial cells are hypertrophied and their glycogen and lipid content increase. The endometrium at the site of implantation is called decidua (Fig. 72.3).
1. As soon as the implantation is completed, the nutrient-rich endometrial cells supply the nutrients required for early growth of the embryo, which is adequate for the embryo in the first few weeks.

2. Later, this function is taken over by the placenta.

**Clinical Box 72.1**

**Ectopic pregnancy:** The usual site of implantation is the dorsal wall in the body of uterus. Sometimes, a fertilized egg remains in the fallopian tube and gets implanted in the wall of the tube. This is called **tubal ectopic pregnancy.** Rarely, a fertilized egg may go backward from the fallopian tube into the abdominal cavity, where implantation may occur. Very rarely, fertilization may take place in the abdominal cavity. In both the cases, **abdominal ectopic pregnancy** occurs. Ectopic pregnancies usually do not continue for long as there is no uterine support for the developing embryo. However, early surgery should be performed to terminate ectopic pregnancy as there is risk of rupture and maternal hemorrhage.

**Placenta Formation**

Implantation is completed in about 10–12 days after ovulation. The trophoblast cells become **cytrophoblasts** (large polyhedral cells) that are surrounded by **syncytiotrophoblasts** (cells with no boundaries). The blood vessels in the endometrium dilate and form lacunae. **Chorionic villi,** the functional units of placenta begins to form at about 12th day from fertilization (Figs. 72.4A to C).

**Placenta** is the combination of interdigitating fetal and maternal tissues that serves as the organ of exchange between mother and fetus during pregnancy. The **fetal part of the placenta** is formed by the outermost layers of trophoblast cells, the **chorion,** and the **maternal part** is formed by the endometrium underlying the chorion.

**Chorionic villi** (fingerlike projections) originate from trophoblast cells and extend from the chorion into the endometrium. The chorionic villi contain a rich network of capillaries. Each villus is surrounded by a pool of maternal blood called **placental sinus,** supplied by maternal arterioles.

**Arrangement of Placental Circulation**

The maternal blood flows into placental sinuses via the uterine artery and come out via the uterine veins. Simultaneously, blood flows from the fetus into the capillaries of the chorionic villi via the umbilical arteries and come out of the villi back to the fetus via the umbilical vein. All these umbilical vessels are contained in the umbilical cord, a long, rope-like structure that connects the fetus to the placenta.

**Formation of Amnion**

Once placenta is well formed, the **fetal heart begins to pump blood at about 5th week of gestation.** A layer of epithelial cells in the villi and a layer of endothelial cells in the fetal capillaries separate the maternal and fetal blood. Exchange of materials between maternal and fetal blood occurs between these layers. A space is formed between the inner cell mass and the chorion, called the **amniotic cavity** (Fig. 72.5A).

1. The cavity enlarges and is slowly lined by epithelial cells layer derived from the inner cell mass called the **amnion,** or **amniotic sac.**

2. Amnion finally fuses with the chorion to form a single combined membrane that surrounds the fetus (Fig. 72.5B). The fluid in the amniotic cavity is the **amniotic fluid** (Clinical Box 72.2) that buffers mechanical stress and temperature variations. The fetus floats in the amniotic cavity. Fetus is attached to the placenta by the umbilical cord. Eventually, it is only the amniotic sac that separates the fetus from the uterine lumen.
Clinical Box 72.2

Amniocentesis: Aspiration of fluid from the amniotic sac is called amniocentesis. Amniocentesis can be done as early as the 16th week of pregnancy by inserting a needle into the amniotic cavity. Few genetic diseases can be diagnosed by demonstrating certain chemicals in the amniotic fluid or in sloughed fetal cells in the fluid. The chromosomes of these fetal cells can also be examined for diagnosis of certain disorders and determination of fetal sex. However, amniocentesis for diagnosis of fetal sex has been banned in India.

Techniques for Fetal Sex Diagnosis

In addition to amniocentesis, there are two other techniques of fetal diagnosis: chorionic villus sampling, and ultrasound.

Chorionic villus sampling: It involves obtaining tissue from chorionic villi of the placenta. The advantage is that it can be performed as early as 10th weeks of pregnancy. However, it carries higher risks including miscarriage.

Ultrasound: Ultrasound, especially with the help of 4D (four-dimensional) machine, that provides details of the picture of the fetus from all angles, fetal sex can be diagnosed as early 12th week.

Organogenesis and Fetal Nutrition

Once placenta is formed, fetus derives nutrition directly from the mother’s blood. Therefore, maternal nutrition is crucial for the fetal development. Malnutrition in mother causes fetal growth retardation.

1. Organogenesis starts as early as 5th to 8th weeks of pregnancy. Therefore, deficiency of specific nutrients from first-trimester onwards results in specific fetal organ deficiency. For example, deficiency of folic acid leads to fetal neural defects.

2. The developing fetus is also subjected to influences by many non-nutritional factors like noise, radiation, chemicals and infections to which the mother is exposed. Especially, drugs (like aspirin etc.), alcohol and cigarette smoke consumed by the mother can cause birth defects in the fetus. These chemicals are known teratogens.

Fetus as a Graft

Half of the fetal genes that are from the father differ from the genes that originate from the mother. Therefore, fetus and mother are two genetically different individuals. Hence, the fetus is in essence a graft (foreign transplant) in the mother. However, the fetal graft is not rejected by the mother, due to three mechanisms:

1. Trophoblast of placenta that separates mother and fetal tissues expresses HLA-G, a nonpolymorphic gene, instead of expressing HLA-I and II, the polymorphic genes. As antibodies are formed only against polymorphic genes, they do not develop against fetal tissues.

2. T cells that are activated against fetal proteins are destroyed (undergo apoptosis) by Fas, a placental surface ligand.

3. Placenta that forms barrier between fetal and maternal blood protects fetus from immunological insults by the mother, by preventing transfer of immunological materials.

Fetoplacental Unit

For steroidogenesis, fetus and placenta interact closely. This is called fetoplacental unit. Placenta forms pregnenolone from cholesterol, which in turn forms progesterone.

1. Placental pregnenolone enters fetal circulation and become substrate for synthesis of dehydroepiandrosterone (DHEA) and 16-hydroxydehydroepiandrosterone (16-OHDHEA) in fetal adrenal.
2. Formation of 16-OHDHEA also occurs in the fetal liver. DHEA and 16-OHDHEA formed in fetus are taken back into the placenta, where DHEA forms estradiol and 16-OHDHEA forms estriol.

3. The placental progesterone also enters fetal adrenal and forms cortisol, corticosterone and androgen in adrenal cortex of fetus (Fig. 72.6).

Clinical Significance
The major estrogen formed by placenta is estriol for which the substrate is fetal adrenal 16-OHDHEA. Therefore, excretion of estriol in maternal urine is a good index of health of the fetus.

Female Infertility
Infertility is defined as the inability of a couple to achieve conception after one year of unprotected sexual intercourse. In females, infertility may be due to following causes;

1. Ovarian factors: Anovulation (absence of release of ovum from the ovary) causes infertility. For details of anovulation, refer previous chapter.

2. Peritoneal factor: Pelvic adhesion or pelvic peritonitis prevents fallopian tube to pick up the ovum from pelvic cavity.

3. Tubal factors: Partial or complete bilateral tubal obstruction resulting from infective or noninfective salpingitis prevents fertilization to occur.

4. Uterine factors: Endometritis, intrauterine adhesions, or anatomical defects can prevent implantation of the fertilized ovum.

5. Cervical factors: Thick cervical mucus or presence of sperm antibodies in the mucus makes the sperm impenetrable.

6. Vaginal factors: Infections in the vagina or anatomical defects in the vagina prevent sperm to reach cervix.

In-Vitro Fertilization and Transfer (IVF-ET)
The field of reproductive medicine has changed forever with the birth of Louise Brown in 1978 by IVF-ET. Patrick Steptoe and Robert Edwards of England are remembered for their revolutionary work. The past decade has witnessed two more dramatic changes in the technique protocol of IVF-ET. One such change was from natural cycle to superovulation protocol and the other one was replacement of laparoscopy by vaginal sonography for ovum retrieval.

Patient Selection
- Age < 35 years
- Presence of ovarian reserve (D-3, serum FSH < 10 IU/L)
- Husband-normal semenogram
- Couple must be screened negative for HIV and hepatitis
- Normal uterine cavity as evaluated by hysteroscopy/sonohysterography.

Principal steps of an ART cycle
- Down regulation using GnRH agonist
- Controlled ovarian stimulation
- Monitoring of follicle growth
- Oocyte retrieval
- Fertilization in vitro
- Transfer of gametes of embryos
- Luteal support with progesterone.

Oocyte Retrieval
Oocyte retrieval is done aseptically through vaginal route under ultrasound guidance.

1. With the development of vaginal transducers, vaginal needle aspiration is done about 36 hours after hCG administration but before ovulation occurs. Intravenous analgesia and sedation (propofol) is adequate in most of the cases.

2. The oocyte is easily recognizable as a single cell surrounded by a mass cumulus cell. After recovery, the oocytes are maintained in culture in vitro for 4–6 hours.

Fertilization in Vitro
The sperm used for insemination in vitro is prepared by the wash and swim up or density gradient centrifugation (preferred) technique.

1. Approximately 50,000 to 100,000 capacitated sperm are placed in the culture media containing the oocyte within 4–6 hours of retrieval. The eggs may demonstrate signs of fertilization when examined 16–18 hours after insemination (presence of two pronuclei in the presence of a second polar body).
2. Sperm density and motility are two most important criteria for successful IVF. The semen is collected just prior to ovum retrieval.

**Embryo Transfer**

The fertilized ova at the 6–8 blastomere stage are placed into the uterine cavity close to the fundus about 3 days after fertilization through a fine flexible soft catheter transcervically. Not more than three embryo are transferred per cycle to minimize multiple pregnancy.

### PLACENTAL HORMONES

Placenta is an endocrine organ as it secretes many hormones (Table 72.1). When fertilization takes place, corpus luteum fails to regress. Corpus luteum secretes hormones that support pregnancy in its early part. Once placenta is fully formed and starts secreting hormones (usually after sixth weeks of pregnancy), functions of corpus luteum slowly decline. Corpus luteum remains vestigial after eighth week. Therefore, **ovariectomy after eighth week** of pregnancy does not result in abortion.

#### hCG

The human chorionic gonadotropin (hCG) is the gonadotropin secreted from syncytiotrophoblast of placenta. The syncytiotrophoblast cells start producing hCG between 6–8 days after fertilization.

### Functions

Human chorionic gonadotropin binds to LH receptors in corpus luteum. Therefore, it has mainly luteinizing and luteotropic activities. It has very less FSH activity.

1. **It stimulates progesterone production** from corpus luteum.
2. hCG plays a critical role in placental steroidogenesis.
3. hCG also helps in sexual differentiation in male fetus.
4. **hCG stimulates vomiting centers** in the brain. Therefore, morning sickness (sense of nausea and vomiting in the morning) is an early feature of pregnancy. Morning sickness is common in the first pregnancy, and usually disappears after first trimester.

### Clinical Significance

Human chorionic gonadotropin is detected in plasma as early as 6 days after conception. Therefore, demonstrating hCG in plasma by RIA is a **pregnancy diagnostic test**. hCG appears in urine from 14th day of conception onwards. Detection of hCG in urine is easier, as blood collection is
avoided. However, hCG is **not specific for pregnancy** as it is secreted from many gastrointestinal and chorionic tumors. It is also secreted in some amount from fetal kidney and liver.

**hCS**

The **human chorionic somatotropin** (hCS) is also called human chorionic somatomammotropin or human placental lactogen (hPL) or chorionic growth hormone.

**Source**

Human chorionic gonadotropin is secreted from syncytiotrophoblast of placenta.

**Structure and Secretion**

Structurally, it is similar to human growth hormone. hCS, GH and prolactin are formed from common precursor hormone. It contains 191 amino acids and has molecular weight 22,000. Secretion of hCS starts from **third week of pregnancy** and slowly rises till term. The peak concentration in plasma is achieved at term when it is about 15 mg/ml.

**Functions**

Human chorionic gonadotropin has functions similar to growth hormone and prolactin.

1. It promotes growth of mammary gland and **stimulates production of milk**. Therefore, it is also called **human placental lactogen**. However, hCS is less potent in milk production than prolactin secreted from anterior pituitary.

2. It **stimulates somatic growth of the fetus**. Therefore, it is also called growth hormone of pregnancy.

3. It **causes maternal lipolysis** and releases FFA from fat tissues.

4. It **decreases glucose utilization** in mother. It induces maternal insulin resistance and glucose intolerance.

5. It alters fuel availability for the fetus by antagonizing maternal glucose consumption and enhancing fat mobilization. This ensures adequate supply of fuel for the fetus.

**Clinical Significance**

The quantity of hCS secreted is proportional to size of the placenta. Therefore, a **low hCS level in third trimester indicates placental insufficiency.**

**Other Hormones**

1. **Relaxin** secreted from placenta causes **uterine relaxation** in the early part of pregnancy like progesterone to facilitate implantation and prevent expulsion of fetus. Its concentration in plasma is highest (1 ng/mL) in first trimester of pregnancy. Toward term, it causes relaxation of pubic symphysis and pelvic ligaments to facilitate delivery of fetus.

2. **Progesterone** causes myometrial quiescence and promotes continuation of pregnancy (see Fig. 72.7).

3. **Estrogen** secretion increases slowly in third trimester and reaches peak at term. The major estrogen secreted in pregnancy is **estriol**, which normally secreted in very less amount from the ovary of a nonpregnant woman. Estrogen facilitates parturition (discussed below).

**MATERNAL CHANGES DURING PREGNANCY**

Pregnancy is a state of altered physiology. Remarkable physiological changes occur in maternal systems during pregnancy. These changes are primarily meant to supply adequate oxygen and nutrients to fetus for its growth and development, and remove CO₂ and waste products from the fetal circulation. The major maternal changes are increase in blood volume and cardiac output, hyperventilation, increased renal blood flow and glomerular filtration, and considerable weight gain.

**Changes in Blood Volume**

There is rapid and significant increase in total blood volume in pregnancy. The increase is about 40% of the prepregnant stage, which occurs due to increase in both plasma and cell components (Fig. 72.8).

1. **The increase in plasma volume** is primarily due to the expansion of ECF volume. ECF volume increases by about 7 liters (about 40% above the prepregnant state), which accounts for about 70% increase in blood volume. The increase in ECF volume is due to sodium and water retention. The increase in plasma volume occurs at the earliest, as early as first month of gestation. The increase occurs by 400 mL at 8 weeks and 1 liter by 16 weeks of gestation.
In the early phase of pregnancy and is significantly high by the end of first trimester (Fig. 72.9). The increase in cardiac output reaches its maximum, i.e. about 40% increase from nonpregnant state, in mid-pregnancy and then remains elevated at that level till term. The increase in cardiac output is due to the increase in both stroke volume and heart rate.

**Stroke Volume**

Stoke volume increases by about 30%, which peaks at about 24 weeks of pregnancy. Increase in stroke volume is mainly due to increased end-diastolic volume (EDV), which occurs due to increased venous return as a consequence of increased blood volume.

**Heart Rate**

Heart rate increases by about 10–15%, reaching its peak at term.

**Systolic BP**

Systolic pressure increases in pregnancy, which is mainly due to increased cardiac output. The increase occurs more in the second and third trimester.

**Diastolic BP**

Diastolic pressure falls slowly, reaching a nadir at about 20th week, which then slowly increases to normal by term. Fall in diastolic pressure is due to the vasodilation effect of progesterone. Pulse pressure is wide due to increase in systolic and decrease in diastolic pressures.

**LVET and PEP**

Pre-ejection period (PEP) and left-ventricular ejection time (LVET) are reduced. However, cardiac output is high due to tachycardia and increased EDV.

**Regional Blood Flow**

In consequence to increased cardiac output and improved hemodynamics, blood flow increases in uterine, renal, mammary and cutaneous vascular bed.

**Changes in Respiratory System**

Respiratory changes aim to improve supply of oxygen to the fetus and removal of carbon dioxide from the fetus.

1. The most striking effect is the increase in minute ventilation. This increase begins in the early part of pregnancy. The increase is about 30% at the end of 8th week, which continues to increase to reach the peak of more than 50% of the nonpregnant value. The increase in ventilation is due to the stimulation of respiratory centers by estrogen.
2. Respiratory rate does not increase significantly. The increase in ventilation is mainly due to the increase in tidal volume (TV).
3. Residual volume (RV) decreases by 20%.
4. Expiratory reserve volume (ERV) decreases by 15%. Due to decrease in RV and ERV, functional residual capacity decreases.
5. Inspiratory reserve volume (IRV) increases. Increase in RV and ERV increases inspiratory capacity.
6. Diffusing capacity of alveolar membrane does not change significantly. In third trimester, inspite of elevation of diaphragm due to increase in size of uterus, ventilation is not impaired significantly.

**Changes in Kidney Functions**

1. Renal blood flow increases by 35% that parallels the increase in blood volume and cardiac output. Renal vasodilation occurs due to increased local production of prostaglandins that facilitates increased renal plasma flow.
2. There is remarkable increase in glomerular filtration rate (GFR). Significant increase in GFR occurs as early as 2nd week, which reaches to about 40% by mid-pregnancy and continues at that level till term.
3. The load of filtered glucose increases without increase in tubular capacity to reabsorb glucose. Therefore, glycosuria is common in pregnant woman. Aminoaciduria also occurs.
4. Inspite of increased GFR and sodium load, ability to excrete sodium and water remain normal in normal pregnancy. However, ECF expansion occurs due to increased reabsorption of water and sodium.

**Changes in GI System**

GI motility is decreased. As a result, gastric emptying time is increased.
1. Due to increased transit time for chyme to pass through intestinal lumen, more water is absorbed that leads to constipation.
2. Toward term, enlarged uterus presses on the stomach, which increases intragastric pressure.
3. This results in propulsion of acid-gastric content into the esophagus leading to reflux esophagitis.

**Changes in Hepatobiliary System**

1. Hepatic blood flow remains unchanged despite marked increase in cardiac output.
2. Total serum protein decreases due to decrease in serum albumin. This is due to expansion of plasma that occurs without increase in synthesis of plasma proteins by the liver, which causes dilutional hypalbuminemis. The γ-globulin concentration decreases. However, hepatic synthesis of fibrinogen increases.
3. Though SGPT and SGOT do not change much, alkaline phosphatase concentration increases 2–4 times.
4. Plasma lipid and cholesterol increase sharply in pregnancy to almost double the nonpregnant value. This may be due to inhibition of lipoprotein lipase activity by estrogen.
5. Gallbladder contraction induced by CCK is inhibited by progesterone. This leads to relaxation of biliary tract. Hence, bile flow is decreased. Gallbladder capacity increases. Also, residual volume of gallbladder increases.
6. The level of chenodeoxycholic acid in the bile, which increases the solubility of cholesterol, decreases due to the effect of estrogen. Also, there is increased cholesterol secretion into the bile. These factors predispose to cholesterol-stone formation.

**Changes in Endocrine System**

**Pituitary Secretions**

The size of anterior lobe increases two to three times during gestation, which is mainly due to increase in size and number of prolactin secreting cells.
1. Prolactin secretion starts increasing from end of first trimester and at term it is almost 10 times more than the pre-pregnant level.
2. However, due to negative feedback effects of estrogen, gonadotrophs decrease in size. The hypothalamo-pituitary-ovarian axis is suppressed by high level of sex steroids. This decreases LH and FSH secretion, and therefore ovulation does not occur during pregnancy.
3. Growth hormone secretion decreases and TSH secretion remains normal in pregnancy.
4. ACTH secretion is usually suppressed. However, ACTH secretion increases toward term due to its secretion from placenta.

**Thyroid Secretion**

Increase in GFR increases the renal clearance of iodine.
1. Depletion of iodine pool occurs due to increased renal excretion of iodine. This leads to moderate increase in size of the thyroid gland, unless dietary intake of iodine increases in pregnancy.
2. High level of circulating estrogen in the later part of pregnancy increases TBG (thyroxine binding globulin) production. Therefore, though secretion of T3 and T4 is increased, euthyroid state is maintained in pregnancy.

**Adrenocortical Secretion**

Secretion of glucocorticoid and mineralocorticoid is increased in pregnancy. The plasma free cortisol concentration increases due to its displacement from CBG (cortisol binding globulin). However, clinical hypercortisolism is not encountered.
Calcium Metabolism
The demand for calcium increases in pregnancy to facilitate fetal bone formation. This is achieved partly by increased absorption of calcium from GI tract by vitamin D and partly by the increase in parathyroid hormone secretion. However, total serum calcium decreases in the third trimester as fetal skeletal development is accelerated toward term. Therefore, calcium supplementation is invariably given in the later part of pregnancy.

Body Weight Gain
A major external change in the pregnant woman is the increase in body weight. In the first and second trimesters, it occurs mainly due to fat deposition induced by progesterone that stimulates food intake and diverts glucose for fat formation. In the third trimester, it is mainly due to increase in size of fetus and uterus. However, fluid retention significantly contributes to it. Body weight gain is usually more in eclampsia (Clinical Box 72.3).

Clinical Box 72.3
Toxemia of Pregnancy: About 5–10% of pregnant women develop edema, proteinuria and hypertension, known as pregnancy-induced hypertension (PIH). When all these features are present, the condition is called pre-eclampsia. If convulsion is associated, the condition is termed eclampsia. These two syndromes are collectively called toxemia of pregnancy. The maternal and fetal mortality is high in eclampsia. Exact mechanism of toxemia of pregnancy is not known. It is possibly due to the abnormal vasoconstriction of the maternal blood vessels and inadequate invasion of the endometrium by trophoblast cells that lead to poor blood perfusion of the placenta.

PARTURITION
Pregnancy terminates either in abortion or in parturition. When pregnancy terminates at or before 28th weeks of gestation (as per the Indian standard), the condition is called abortion; and termination after 28th week is called parturition. Normally, pregnancy terminates in parturition at term.

The duration of normal pregnancy in human is approximately 40 weeks from the first day of last menstrual cycle, or 38 weeks from the day of ovulation. When pregnancy completes its full duration, is called “term pregnancy”. Delivery of the fetus at term through vaginal route is called parturition (as fetus can also be delivered by cesarean section). When fetus is delivered after 28th week and before 37th complete week of gestation, is called premature infant.

1. The smooth muscle (myometrium) of uterus remains relaxed in most part of the pregnancy due to the effect of progesterone.
2. In last few weeks of pregnancy, due to increased level of estrogen, uterine muscle cells synthesize more connexin, the protein that form gap junctions. This facilitates coordinated contractions of myometrium.

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Stages of Labor
Presence of following signs indicates the onset of labor:
1. Painful uterine contractions (labor pain).
2. Slight blood-stained mucus discharge from vagina, called as “show”. This occurs due to minor hemorrhage from lower uterine segment and mucus secreted from cervix.
3. Commencement of dilatation of internal os.

Normal labor is divided into three stages:

Stage 1
This is the stage of dilatation of cervix. This is the preparatory stage during which cervix dilates to allow the expulsion of the fetus. First the formation of bag of membrane and forewaters occurs and then the membrane ruptures (Figs. 72.10A to C).
Stage 2
This is the stage of expulsion of the fetus. This stage begins after the completion of cervical dilation. Uterine muscles contract and increased abdominal pressure facilitates the expulsion (Fig. 72.11). The vagina dilates and the presenting part along with the fetus passes through the cervix and vagina (Figs. 72.12A and B).

Stage 3
This is the stage of expulsion of “after-birth”, which consists of delivery of umbilical cord, placenta and membranes (Figs. 72.13A and B).

Mechanism of Parturition
Parturition is produced by strong contractions of the myometrium. In fact, weak and infrequent uterine contractions begin to appear at about 25–30 weeks of gestation that gradually increase in magnitude and frequency as pregnancy advances. However, these are not the labor contractions. Labor contractions start with the onset of parturition. In the last month, the presenting part of the fetus, which is head in 90% of pregnancies (cephalic presentation) (Figs. 72.10A to C), descends toward cervix along entire uterine contents. At the onset of parturition, the amniotic sac ruptures, and the amniotic fluid flows through the vagina.

1. The uterine contractions become strong and appear every 10–15 min. Contraction waves originate in the upper part of the uterus and move downward toward cervix. Gradually, contractions increase in intensity and frequency.
2. The cervix progressively dilates to attain a maximum diameter of about 10–12 cm. Once cervical dilation is complete (Figs. 72.10A to C), every new contraction moves the fetus downward in the birth canal.
3. Finally, very strong uterine contractions push the fetus through the cervix and vagina (Fig. 72.11).
4. At this stage, the mother, by bearing down to increase abdominal pressure, adds to push the baby in the birth canal (Figs. 72.12A and B).
5. Within minutes of delivery of the baby, both the umbilical cord and the placenta are delivered (Figs. 72.13A and B). Placenta, which remains tightly adhered to uterine wall throughout pregnancy, is separated from it by a series of waves of uterine contractions and delivered after the delivery of the baby (hence, called after birth).

Parturition Reflex
Parturition is a reflex phenomenon. The inherent rhythmic contractions of smooth muscle cells of the myometrium are facilitated by stretch imparted by the growing fetus.
1. Toward term, the pregnant uterus synthesizes and secretes prostaglandins (PGE₂ and PGF₂α) that are potent stimulators of uterine muscle contraction (Fig. 72.14).
2. Oxytocin causes stronger uterine muscle contraction and also stimulates uterus to synthesize more prostaglandins.

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Fig. 72.11: Second stage of labor. Note the expulsion of the fetus by uterine contraction, of fetus facilitated by increased abdominal pressure.

Figs. 72.12A and B: Second stage of labor. Note the dilatation of vagina and delivery of the head of fetus (A) followed by the body (B).
3. **Estrogen** increases uterine muscle excitability and contractility. Under the effect of estrogen, the **number and sensitivity of oxytocin receptors in uterine muscle cells increase** during the last few weeks of pregnancy.

**Role of Oxytocin**

Oxytocin is reflexly **secreted from posterior pituitary** in response to inputs originating in hypothalamus.

1. When descending part of the fetus (which is usually head) presses on the lower part of the uterus and cervix, **stimulation of stretch receptors** increases impulses via ascending sensory fibers through spinal cord to thalamus.

2. These fibers from their pathway in brainstem give **collaterals to hypothalamus** which in turn activates posterior pituitary.

3. Oxytocin is released from posterior pituitary that increases uterine contractions. The uterine contractions exert **positive feedback effect** on oxytocin secretion (Flowchart 72.1).

4. As part of the efferent limb of the reflex pathway is hormonal as in milk ejection reflex, parturition is another example of **neurohumoral reflex**.

**Flowchart 72.1:** Changes in cardiac output, stroke volume, and diastolic pressure in pregnancy. Cardiac output increases by about 40%, and stroke volume increases by about 30%, whereas diastolic pressure decreases by about 10%.

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**Fig. 72.14:** Initiation of parturition.
CHAPTER SUMMARY

**Key Concepts**

1. Pregnancy starts with implantation of fertilized egg.
2. Placental hormones support fetal growth and continuation of pregnancy.
3. Pregnancy is an altered state of physiology, in which blood volume increases, C.O. and systolic BP increase, but diastolic BP decreases. All these changes facilitate blood and nutrition supply to fetus.
4. Parturition reflex is a neurohumoral reflex.

**Important to Know (Must Read)**

1. In examination, “Describe the maternal changes during pregnancy” may be asked as a **Long Question**.
2. Parturition reflex, Human chorionic gonadotropin (hCG), Human chorionic somatotropin (hCS), Cardiovascular changes during pregnancy, Respiratory changes during pregnancy, Hematological changes and changes in blood volume during pregnancy, Acrosome reaction, Polyspermy block, Mechanism of implantation, Fetoplacental unit, Female infertility may be asked as **Short Questions** in exam.
3. In **Viva**, examiner may ask… What is the mechanism of parturition reflex, List the placental hormones, What are the causes of female infertility, How does the transport of egg occur in female after ovulation, How does the transport of sperm occur in the cervix, uterus, How does the transport of sperm occur in fallopian tube, What are the changes occur in sperm in female genital tract, What is the usual site for fertilization, What are the steps of acrosome reaction and sperm penetration, What is polyspermy block and what are its mechanisms, How does the zygote formation occur, What is the mechanism of twin formation, What are the functions of intact zona pelucida, What is a blastocyst and what are its components, What is the mechanism of implantation, What is ectopic pregnancy and what are its sites, What is the mechanism of placenta formation, What is the mechanism of amnion formation, What is amniocentesis and what are its uses, Do you think fetus is a graft, if yes explain why, What is fetoplacental unit and what is its clinical significance, What is the source of hCG, What is the pattern of secretion of hCG, What are the functions of hCG, What is the clinical significance of hCG, What is the source of hCS, What is the pattern of secretion of hCS, What are the functions of hCS, What is the clinical significance of hCS, What are the functions of relaxin during pregnancy, What are the functions of estrogen during pregnancy, What are the functions of progesterone during pregnancy, What are the changes in blood volume during pregnancy, What are the hematological changes during pregnancy, What are the cardiovascular changes during pregnancy, What are the respiratory changes during pregnancy, What are the changes in kidney functions during pregnancy, What are the changes in GI system during pregnancy, What are the changes in hepatobiliary system during pregnancy, What are the changes in endocrine system during pregnancy, What are the changes in body weight during pregnancy, What is toxemia of pregnancy, What are stages of labor.
CHAPTER 73

Physiology of Breast Development and Lactation

Learning Objectives

On completion of study of this chapter, the student **MUST** be able to:
1. Understand the development of breast in different phases of life of women.
2. Understand the mechanism of lactogenesis.
3. List the composition of human milk.
4. Correlates the physiology of breast development and lactation with abnormalities of breast development and lactation.

The student **MAY** also be able to:
1. Describe the mechanisms and regulation of breast development.
2. Describe the mechanisms of lactogenesis and lactation.

Breast Development

The primary function of female breast is **synthesis and secretion of milk**. In mammals, especially in humans, milk secreted from breast (for breastfeeding) is the chief source of nutrition for the offspring. As breast milk contains all the ingredients necessary for complete development of the infant, deficient breastfeeding, especially in first six months during infancy results in retardation of growth. Therefore, in females, nature has designed the development of breast with the attainment of reproducibility and preparation of breast for lactogenesis and lactation during pregnancy, so that as soon as the baby is born, breast milk, the natural and the best nutrition for the newborn is available.

**Structure of Breast**

Breast is a mass of fibrofatty tissue containing **alveoli and ducts**.

1. Ducts in breast branch all through the mammary tissue and converge at the nipples.
2. Ducts begin in saclike glands called **alveoli**. Alveoli are the sites of milk secretion and they look like bunches of grapes with stems terminating in the ducts (Figs. 73.1A and B).
3. **Ductules** originate from alveoli. Ductules open to ducts that in turn drain to **lactiferous ducts**.
4. Lactiferous duct terminates in dilated **lactiferous sinus**.
5. Sinuses open to nipple through **nipple-pores**.
6. The alveoli and ducts are surrounded by specialized contractile cells called **myoepithelial cells**. **Contraction of myoepithelial cells** causes **milk ejection** into the lactiferous sinuses.

Breast Development in Different Phases of Life

Full development of breast requires many hormones.

1. The major hormones controlling development of mammary gland are estrogen, progesterone, prolactin, growth hormone (GH), thyroxine and cortisol.
2. Generally, **estrogen stimulates the development of duct system** and progesterone promotes growth of lobulo-alveolar system.
3. GH, prolactin and cortisol synergize the estrogen and progesterone effects.
4. **During pregnancy and lactation**, breast development and lactogenesis are dependent on prolactin and adrenal steroids.

**During Intrauterine Life**

Breast develops from **ectoderm**. When embryo is 7 mm in length, mammary tissue appears as a ridge called **mammary**
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**crest**, one on each side, extending along the ventrolateral body wall from axillary to the inguinal region.
1. The caudal part of the ridge regresses and the thoracic region condenses to form **primordial mammary bud** when the embryo is about 12 mm in length.
2. After 5th month of gestation, the primitive mammary bud forms about 20 **secondary buds** that form ductal system in the mature gland.
3. These ducts undergo proliferation in third trimester of pregnancy in response to various hormones.
4. **At birth**, mammary gland is rudimentary.
5. In some newborns, small quantity of milk is secreted due to high prolactin concentration in them at that time. This is called ‘**witch milk**’.

**During Childhood**
After birth, breast regresses due to lack of hormonal support and remains in a quiescent stage. The growth of breast occurs with the general growth of the body till puberty, which is proportionate to the somatic growth.

**During Puberty**
Before puberty, the breasts are small with less glandular structure. With the onset of puberty in girls, marked enlargement of breast occurs due to the effects of estrogen.
1. The breast development is mainly due to enhancement of duct growth and branching of ducts with relatively little growth of the alveoli.
2. The breast enlargement is significantly contributed by fat deposition.
3. When menstrual cycle starts at puberty, progesterone secretion commences in the luteal phase of each cycle, which contributes to breast enlargement by stimulating the growth of alveoli.
4. The areola enlarges and becomes more pigmented.

**During Each Menstrual Cycle**
During each menstrual cycle, temporary changes in breasts occur in different phases of cycle due to proliferation and regression of ductal and alveolar tissue in response to fluctuations in plasma level of estrogen and progesterone. Especially, in the late luteal phase, breast size increases due to alveolar growth in response to progesterone and ductal growth in response to estrogen.

**During Pregnancy**
Prominent changes occur in breast during pregnancy. Breast enlargement occurs due to the stimulatory effects of high plasma concentrations of estrogen, progesterone, prolactin, and hCS.
1. Appreciable breast enlargement occurs after second month of gestation.
2. The areola and nipple increase in size and become more pigmented.
3. **Montgomery tubercles** appear in the areola (Figs. 73.2A and B).
4. Alveoli, ducts and glands gradually increase in size.
5. In the last trimester, acini differentiate into secretory glands.
6. Parenchymal cells of alveoli undergo hypertrophy increasing the size of the breast. All these changes are meant to prepare breasts for lactogenesis and lactation.
Chapter 73: Physiology of Breast Development and Lactation

During Lactation

A lactating breast is large in size with nipple projecting about 2 cm beyond the areola. Breast changes during lactation occur mainly due to the action of prolactin secreted from anterior pituitary. Prolactin also stimulates the production of milk.

LACTOGENESIS AND LACTATION

Though prolactin level is high in later part of pregnancy, milk production does not occur due to the inhibitory effects of estrogen and progesterone at their high concentrations on lactogenesis.

1. Estrogen increases secretion of prolactin and along with prolactin promotes breast growth and differentiation. But, along with progesterone, it antagonizes prolactin action on milk production and secretion.
2. Following parturition, estrogen and progesterone concentration in plasma falls appreciably as placenta, which was secreting large amount of these hormones is no more present. Decreased level of these hormones facilitates unopposed milk production by prolactin.
3. The role of prolactin in lactogenesis has been described in the Chapter “Anterior Pituitary”.

Role of hypothalamus: The immediate fall in estrogen level following parturition decreases prolactin secretion from its peak pre-parturition level to new post-partum basal level. However, prolactin level still remains very high till the mother continues to nurse the breast milk.

1. Superimposed upon this high basal level, large secretory bursts of prolactin occur during each nursing period. This episodic burst of prolactin signals the breasts for maintenance of milk production. These prolactin pulses continue for several days after the mother completely stops nursing her infant.
2. The mechanism mediating prolactin pulses is initiated by stimulation of nipple receptors by suckling that sends afferent input to the hypothalamus.
3. This input inhibits hypothalamic release of dopamine. As normally dopamine inhibits prolactin secretion, decreased dopamine release stimulates prolactin secretion.
4. Prolactin also inhibits GnRH secretion from hypothalamus that prevents ovulation. Therefore, till mother continues to nurse the baby, normally pregnancy does not occur.
5. This is the natural procedure of contraception, which aims at spacing the subsequent pregnancies.

Suckling also initiates milk-ejection reflex (for details, refer Chapter “Posterior Pituitary”) that transfers milk from breast into the mouth of the baby.

Breast Milk

Immediately after delivery, a watery fluid is secreted from breasts called colostrum, which is rich in protein. Colostrum secretion is replaced by milk in about 24 to 48 hours.

1. Mother’s milk contains all nutrients in adequate quantity: carbohydrate in the form of lactose (milk sugar), protein (casein), fat, minerals, vitamins and water (Table 73.1).
3. Milk also contains growth factors and hormones that help in physical development and maturation, and large number of neuropeptides and endogenous opioids that facilitate the development of brain and behavior of infant.
4. Some of these substances are synthesized by the breast tissue itself, not just transported from blood to milk. It is virtually impossible to produce mother’s milk in a commercial formula.

5. The milk proteins are not destroyed in the GI tract as low gastric acidity in newborn fails to denature them.

6. Also, the intestinal epithelium in infant is more permeable to proteins than in the adult, which facilitates their absorption. Thus, mother’s milk proteins easily enter into blood of the infant. However, infectious agents like AIDS virus and various drugs can easily be transmitted through breast milk.

Abnormalities of Breast Development and Lactation

Small Breasts

Small breast due to poor breast development is common. This may be constitutional, genetic and familial. However, during pregnancy small breast becomes bigger and usually milk secretion becomes adequate.

Polythelia

Polythelia is more number of breasts. More number of breasts can develop in the milk line starting from axilla to the groin. Usually these multiple breasts are small in size. These are small and flat breast, and the nipples may be inverted or small that impede suckling and breast feeding.

Deficient Lactation

Deficiency of lactation can occur due to following causes.

1. Poor breast development, which may be constitutional, genetic and familial. A small, flat or inverted nipple may impede suckling.

2. Generalized illness: A chronic illness during pregnancy or lactation may decrease lactogenesis.

3. Hypothalamo-pituitary disorder: Decreased prolactin secretion due either to hypothalamic or to pituitary dysfunctions result in deficient lactation.

4. Failure of baby to suckle: Prematurity, congenital anomalies of mouth and neonatal illness can cause inefficient suckling.

Breast Engorgement

Failure of the baby to empty the breast sufficiently results in breast engorgement. Breast becomes hard, tense and painful. Treatment consists of drawing off milk using breast pump or expressing milk from the breast manually.

Galactorrhea

Galactorrhea is the continuous secretion of milk in the absence of recent pregnancy. Though it is common in woman, it can also occur in man.

1. In women, when it is associated with amenorrhea, the condition is called galactorrhea-amenorrhea syndrome. The tumor of pituitary gland causing excess secretion of prolactin can cause this syndrome (hyperprolactinemia-galactorrhea syndrome).

2. It is also seen in Chiari-Frommel syndrome, which is characterized by excess lactation, amenorrhea, and superinvolution of uterus dating from pregnancy.

3. It occurs due to persistent prolactin secretion without secretion of FSH and LH.

4. Galactorrhea may occur due to some drugs such as reserpine, phenothiazine derivatives, α-methyldopa and tricyclic antidepressants.

5. Galactorrhea may be seen in hypothyroidism in which increased TRH secretion (feedback effects of low thyroxine) stimulates prolactin secretion.

CHAPTER SUMMARY

Key Concepts

1. Estrogen stimulates the development of duct system and progesterone promotes growth of lobulo-alveolar system.

2. During pregnancy breast size increases under the influence of estrogen, progesterone, prolactin, and hCS. Prolactin increases synthesis and secretion of milk after delivery.

3. Breast milk is the best nutrition for the infant as it contains all the ingredients including minerals, vitamins and antibodies.

Important to Know (Must Read)

1. In examination, Long Questions are usually not asked from this chapter.

2. Development of breast, Breast milk, Mechanism of lactation, may be asked as Short Questions in exam.

3. In Viva, examiner may ask… What is the composition of breast milk, What are the abnormalities of lactation, What is Galactorrhea, what are its causes and features, When does breast engorgement occur, What is Polythelia, How does the breast develop during intrauterine life, What changes do occur in the breast during childhood, How does the breast develop during puberty, What changes do occur in the breast during each menstrual cycle, What changes do occur in the breast during pregnancy, What changes do occur in the breast during lactation, What is the mechanism of lactogenesis and lactation.
Learning Objectives

On completion of study of this chapter, the student **MUST** be able to:
1. Classify contraceptives for males and females.
2. Name the temporary and permanent methods of contraception.
4. Say which contraceptive will be better for which type of couple.

The student **MAY** also be able to:
1. Describe the mechanisms, merits and demerits of different types of contraceptives.

India is a highly populous nation. One of the major problems India facing in recent years is birth control. In April, 1976, India formulated its first ‘National Population Policy’, and ‘National Population Policy–2000’ is the latest in the series. All these policies primarily aim at reducing the birth rate.

When birth control procedures work prior to implantation of the fertilized egg, they are called **contraceptives**, and when work after implantation (cause death of the embryo), they are termed as **abortifacients**.

**Classification**

Contraceptive methods are classified into following categories:
1. Barrier methods
   - Physical methods
   - Chemical methods
2. Intrauterine devices
3. Hormonal methods
4. Post-conceptional methods
5. Permanent methods

They may also be classified as **temporary** and **permanent methods**.

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**Physical Methods**

**In Males**

**Condom** is the most widely used barrier device in male. It prevents sperm to be deposited in the vagina. The biggest advantage is that it provides **protection against sexually transmitted diseases**.

**In Females**

**Diaphragm** is the most commonly used vaginal barrier. A **spermicidal jelly** is usually used along with the diaphragm. Another female barrier device is **vaginal sponge**.

**Chemical Methods**

Various spermicidal agents like foams, creams and suppositories are inserted manually into vagina before intercourse. These act as ‘surface active agents’ that attach themselves to sperms and decrease their oxygen uptake and kill them. They are not usually used due to their high failure rate.

**Intrauterine Devices**

Intrauterine devices (IUDs) are most effective contraceptive devices for a parous lady (who has borne at least one child).

**Types**

The IUDs are of **three generations** (Figs. 74.1A to D):
1. **First generation IUD**
   - Lippes Loop
Section 7: Reproductive System

Figs. 74.1A to D: Commonly used IUDs.

2. **Second generation IUD**
   - Earlier devices: Copper–7; Copper T–200
   - Newer devices: Copper T variants (T–Cu 220C; T–Cu 380A; T–Cu 380Ag)

3. **Third generation IUD (with hormonal preparation)**
   - Progestasert: A T-shaped device filled with 38 mg of progesterone.

**Mechanism of Action**

IUD works by several mechanisms.

1. Usually, they work after fertilization has occurred but before implantation is completed. The presence of these small objects in the uterus brings about uterine changes that *interfere with the endometrial preparation* for acceptance of the blastocyst. Thus, implantation is prevented.

2. They also act as *foreign body* in the uterine cavity (Fig. 74.2) causing cellular and biochemical changes in the endometrium and the uterine fluid that impair the viability of the gamete. Therefore, the chance of fertilization is reduced.

3. **Copper facilitates cellular reaction** in endometrium, composition of cervical mucus, impairs sperm motility and impairs capacitation of sperm.

4. Hormone releasing devices increase the viscosity of cervical mucus by releasing progesterone. They **make mucus thick**, so that sperm cannot enter uterus. They also make the endometrium unfavorable for implantation.

**Merits and Demerits**

Merits and demerits of IUDs are summarized in Table 74.1.

**Hormonal Contraceptives**

1. **Oral contraceptive pills**
   - Combined pill
   - Progestogen only pill
   - Post-coital pill
   - Once-a-month pill
   - Male pill

2. **Depots** (slow releasing formulations)
   - Injectable preparations
   - Subcutaneous implants
   - Vaginal rings

**Oral Contraceptive Pills (OCPs)**

Presently, OCPs contain 30–35 mcg of estrogen and 0.5 to 1 mg of progesterone. The pill is given for 21 days from 5th day of the cycle. Oral contraceptives are based on the principle that estrogen and progesterone *inhibit pituitary*
gonadotropin release, thereby preventing ovulation. Progesterone only pill affects the composition of the cervical mucus, reducing the ability of the sperm to pass through the cervix, and inhibit the estrogen-induced proliferation of the endometrium, making it inhospitable for implantation.

**Side Effects of OCP**

Though OCPs are 100% effective in preventing pregnancy, there are risks of few side effects, especially when consumed for many years.

**Cardiovascular Side Effects**

Myocardial infarction, cerebral thrombosis, venous thrombosis and hypertension have been reported. These side effects are more seen in aged (more than 35 years) woman and in smokers. Hypertension occurs due to fluid retention and increased angiotensin level. Estrogen increases renin-angiotensin activity and thereby increases angiotensin II production which is a potent vasoconstrictor.

**Carcinogenesis**

Increased risks of cervical cancer and breast neoplasia have been reported. Hepatic tumors occurs rarely.

**Metabolic side Effects**

OCPs decrease HDL and alter blood coagulability. These two factors facilitate atherosclerosis and proneness to myocardial infarction and stroke. They also cause glucose intolerance and insulin resistance.

**Miscellaneous**

Other side effects include cholestatic jaundice, breast tenderness, weight gain and migraine.

**Depots**

Subcutaneous implants are contraceptive (progestogen) capsules (Norplant) implanted beneath the skin that releases hormone slowly and last for five years. Injectable forms (intra-muscular injection of progestogen substance like Depo-Provera every three months) are also available. Vaginal ring containing levonorgestril has been found to be effective.

**Post-Conceptional Pills**

Contraceptives can be used within 72 h after intercourse (post-coital contraception).

1. These pills interfere with ovulation, transport of the conceptus to the uterus, or implantation.
2. Usually, high dose of estrogen, or two large doses (12 h apart) of a combined estrogen-progestin oral preparations are prescribed.
3. Most effective with fewer side effects is the pill RU 486 (mifepristone), which antagonizes progesterone activity by binding competitively with progesterone receptors in the uterus.
4. This causes the endometrium to erode and the contractions of the fallopian tubes and myometrium to increase.

**Other Methods**

**The Rhythm (Safe Period) Method**

The rhythm method is the abstinence from sexual intercourse during the fertile period of the cycle (near the time of ovulation).

1. In 28 days regular cycles, normally ovulation occurs between 12th and 16th day (usually on 14th day). Functionally sperm can survive for two days and ovum for 3 days.
2. Therefore, unprotected intercourse should be avoided during the fertile period of the cycle, which will fall between 2 days before and 3 days after ovulation i.e. from 10th day to 19th day of the cycle (refer Fig. 69.4; Chapter 69). Rest of the period in the cycle is considered to be safe period.
3. However, the day of ovulation is not always fixed even in regular cycles and cycle length is also not always regular. Moreover, only the length of luteal phase is constant, which is 14 days from the day of ovulation, and practically it is difficult and tedious to know the day of ovulation.
4. Therefore, in practice, shortest cycle minus 18 days gives the first day of fertile period and longest cycle minus 10 days gives the last day of fertile period. For example, if the duration of shortest cycle is 25 days (25 – 18 = 7th day) and duration of longest cycle is 32 days (32 – 10 = 22nd day), the unprotected intercourse should be avoided between 7th and 22nd day of any cycle.
Table 74.2: Failure rate of contraceptive methods in first 12 months of use.

<table>
<thead>
<tr>
<th>Methods</th>
<th>Pregnancy rate per 100 women years (approx.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No method</td>
<td>85</td>
</tr>
<tr>
<td>Natural (calendar, temperature, mucus)</td>
<td>25</td>
</tr>
<tr>
<td>Withdrawal</td>
<td>27</td>
</tr>
<tr>
<td>Lactation amenorrhea</td>
<td>2</td>
</tr>
<tr>
<td>Condom (male)</td>
<td>15</td>
</tr>
<tr>
<td>Condom (female)</td>
<td>21</td>
</tr>
<tr>
<td>Diaphragm</td>
<td>16</td>
</tr>
<tr>
<td><strong>IUCD:</strong></td>
<td></td>
</tr>
<tr>
<td>Cu-T 380 A</td>
<td>0.8</td>
</tr>
<tr>
<td>LNG 20</td>
<td>0.1</td>
</tr>
<tr>
<td>Combined oral pill</td>
<td>0.1</td>
</tr>
<tr>
<td>Progestin only pill</td>
<td>1</td>
</tr>
<tr>
<td>DMPA and NET injectables</td>
<td>0.3</td>
</tr>
<tr>
<td>Norplant</td>
<td>0.05</td>
</tr>
<tr>
<td>Implanon</td>
<td>0.01</td>
</tr>
<tr>
<td>Vasectomy</td>
<td>0.15</td>
</tr>
<tr>
<td>Tubectomy</td>
<td>0.15</td>
</tr>
</tbody>
</table>

Failure rate is further less when methods are used correctly and consistently.

(IUCD: Intrauterine contraceptive devices; Cu-T: Copper-T; LNG: Levonorgestrel; DMPA: Depot medroxyprogesterone acetate; NET: Norethisterone).

5. However, pregnancy has been documented due to intercourse on any day of the cycle. Therefore, it is believed that no period in any cycle, even during the bleeding phase is absolutely safe.

**Coitus Interruptus**

In this method, during fertile period, the male partner withdraws penis from vagina before ejaculation. Thus, sperm is not deposited in the female genital tract in the fertile period.

**Breast-feeding**

Till the mother continues to nurse the baby with breast feeding, ovulation does not occur. This is because prolactin secreted during lactation produces lactational amenorrhea by inhibiting GnRH secretion.

**Family Planning Operations**

Government of India encourages people of India with different incentives to opt for family planning operation after a couple are blessed with a child (‘we two-ours one’ policy). It is done either by male or female sterilization.

**Male Sterilization**

Male sterilization is performed by vasectomy. In vasectomy, bilateral ligation of vas deferens is performed instead of sectioning the vas as recanalization can be taken up in future whenever needed. However, antibodies developed against spermatozoa following vasectomy causes infertility following restoration of patency of the vas.

**Female Sterilization**

Female sterilization is performed by bilateral tubal ligation. Tubal recanalization can also be performed later whenever needed.

**Failure Rates of Contraceptive Methods**

Failure rates of various contraceptive methods are summarized in Table 74.2.

**Effectiveness of contraceptive methods:** Summarized in Table 74.3.

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**CHAPTER SUMMARY**

**Key Concepts**

1. The best method in nulliparous woman is OCP and parous women are IUDs. However, there are failures in all methods.
2. The only dependable methods are sterilizations.

**Important to Know (Must Read)**

1. In examination, ‘Classify contraceptives, describe the mechanism, merits and demerits of various contraceptive methods used in females and males’ may be asked as Long Questions.
2. Intrauterine devices (IUDs), Oral contraceptive pills (OCPs) may be asked as Short Questions in exam.
3. In Viva, examiner may ask... List the contraceptive methods in female, List the contraceptive methods in male, What are the different types of IUDs, What is the mechanism of action of IUDs, List the hormonal contraceptives, What is the mechanism of action of OCPs, What are the side effects of OCPs, What is a post-conceptional pill, What is a Depot, How is the safe period calculated in the rhythm method, What is coitus interruptus, How does breast-feeding prevent conception, What is male sterilization, What is female sterilization, What is the physical contraceptive method in male, What is the physical contraceptive method in female, What is the chemical contraceptive method in male, What is the chemical contraceptive method in female.
SECTION–8
Renal System

75. General Introduction and Functional Anatomy of Kidney
76. Renal Blood Flow
77. Glomerular Filtration
78. Tubular Functions
79. Mechanisms of Urine Concentration and Dilution
80. Water Excretion, Diuresis, and Diuretics
81. Acidification of Urine
82. Kidney Function Tests and Pathophysiology of Renal Failure
83. Physiology of Micturition and Bladder Dysfunctions
“He is the godhead growing in human lives
And in the body of earth-being’s forms:
He is the soul of man climbing to God
In Nature’s surge out of earth’s ignorance.”

Sri Aurobindo (in ‘SAVITRI’)
CHAPTER 75
General Introduction and Functional Anatomy of Kidney

Learning Objectives
On completion of study of this chapter, the student MUST be able to:
1. List the functions of kidney.
2. Name the parts of the nephron and give their main functions.
3. Correlate histological modification at different segments of nephron with their specific functions.
4. List the differences between cortical and juxtamedullary nephrons.
5. Give the detail ultrastructure of glomerulo-capsular filtration barrier.
6. Describe the details of structure and functions of JG apparatus.
7. Explain the functions of renin-angiotensin system.
The student MAY also be able to:
1. Describe the structural and functional organization of different parts of the nephron.
2. Describe the role of JG apparatus in health and disease.

KIDNEY PLAYS HOMEOSTATIC ROLE
The kidneys play a vital role in homeostatic functions of the body. Therefore, impairment of kidney functions leads to many homeostatic abnormalities. Extracellular fluid (ECF) compartment is the interface between the external and the internal (cellular) milieus of living creatures. Water, minerals, nutrients and gases pass through the ECF before entering the cellular compartment, and cellular waste products pass through the ECF before being excreted from the body. Hence, maintenance of ECF volume and composition, the aim of many homeostatic mechanisms, is vital for organ functions. Kidneys play principal role in this homeostasis.

In clinical practice, biochemical investigations are important weapons of clinician’s diagnostic armamentarium. These routine and special investigations from medical or surgical wards and clinics are generally meant to reveal kidney functions and dysfunctions, directly or indirectly. Medical students, interns, residents and consultants need to learn more of kidney physiology to understand and interpret the reports of these investigations. Moreover, understanding renal physiology helps physicians learn the pathophysiology of renal diseases and plan the management strategies of these disorders.

Functions of Kidney
1. Urine formation and excretion of waste products: The primary function of the kidneys is to excrete waste products of metabolisms from the body such as urea, creatinine, uric acid, etc. dissolved in urine. Kidneys form urine and excrete many toxic waste products from the body dissolved in urine. Thus, kidneys prevent their accumulation to a dangerous level in the body.
2. Regulation of ECF volume: Kidneys play an important role in regulation of ECF volume of the body. Many hormones such as aldosterone, ADH, ANP, angiotensin etc. involved in regulation of ECF volume act on kidney tubules to achieve this function. These hormones modify reabsorption of sodium and water from kidneys to control ECF volume.
3. Regulation of blood pressure (BP): Kidneys contribute mainly to long-term regulation of arterial volume and pressure. Renin secreted from kidney activates renin-angiotensin-aldosterone axis that plays an important role in regulation of BP. Many hormones act on kidney to regulate blood volume and pressure. Kidneys have also their intrinsic mechanisms to alter sodium and water excretion to regulate BP.
4. **Regulation of electrolyte composition of body fluids:** Composition of electrolytes in ECF is mainly the function of kidney. *Excretion and reabsorption of electrolytes* from kidneys directly influence their concentration in ECF. Kidneys also contribute to vitamin D synthesis that controls plasma calcium concentration.

5. **Acid-base balance:** Kidneys contribute significantly to acid-base balance by controlling *bicarbonate excretion* and *H⁺ secretion*. Thus, it controls blood pH and pH of other body fluids. Therefore, kidney abnormalities may cause metabolic acidosis or alkalosis.

6. **Regulation of plasma osmolality:** By controlling *NaCl and water reabsorption*, kidneys control plasma osmolality. Change in plasma osmolality provides feedback signal for secretion of ADH that acts on kidney to regulate the osmolality.

7. **Regulation of erythropoiesis:** *Erythropoietin*, the major regulator of erythropoiesis is secreted from interstitial cells in the peritubular capillary bed of kidney. Therefore, kidney diseases result in anemia.

8. **Endocrine functions:** Kidney secretes *thromboxane A₂* and *prostaglandins*, in addition to the secretion of erythropoietin. Kidneys secrete *renin* that activates renin-angiotensin system. Kidneys also form *calcitriol* (1,25-dihydroxycholecalciferol), the active form of vitamin D₃ from 25-hydroxycholecalciferol.

9. **Gluconeogenesis:** Though kidneys are not the primary site of gluconeogenesis, in starvation, synthesis of glucose from noncarbohydrate sources, especially from glutamine occurs in these organs.

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**FUNCTIONAL ANATOMY**

Kidneys are primary organs of the urinary system that also includes ureters, urinary bladder, and urethra. Kidneys form urine from the blood, which is excreted through the ureters and stored in the bladder. Once volume of urine in the bladder attains a critical level, micturition reflex is activated that expels urine through the urethra.

**Gross Anatomy**

Kidneys are bean shaped paired *retroperitoneal organs* present in the posterior part of the abdomen just above the waist between the last thoracic and third lumbar vertebrae. The right kidney is slightly lower in position due to the presence of liver on the right side.

1. **Size of each kidney in adult** is roughly about the size of one’s fist (11 cm in length and 6 cm in width), and the weight is about 150 g.
2. **Kidneys are covered by a thick capsule** called *renal capsule*.
3. **The medial border of kidney is concaved and the center of the concavity is called *renal hilus*, from which the ureter comes out of the organ** (Fig. 75.1).

**Internal Structures of Kidney**

When the kidney is cut vertically into two halves, two distinct regions are seen: the outer cortex and the inner medulla.

**Cortex**

The cortex is *granular in appearance* and contains all glomeruli, convoluted tubules and cortical portion of collecting ducts.

**Medulla**

The medulla is *striated in appearance* due to presence of loop of Henle, medullary portion of collecting duct and blood vessels that are arranged in parallel.

The medulla is divided into 8–12 conical masses, called the *renal pyramids* that appear in a radiating pattern. Medullary pyramids originate at the corticomedullary junctions and terminate into a *papilla*, which is present within the *minor calyx*. Minor calyces unite to form *major calyx*. The major calyces open into *renal pelvis*, which is the upper expanded portion of the ureter (Fig. 75.2).

**Parts of the Nephron**

Nephron is the basic structural and functional unit of the kidney. There are about 1.2 millions of nephrons in each kidney in human beings. The nephrons are hollow tubes through which the blood is filtered and modified to finally form urine. A nephron consists of renal corpuscle, proximal convoluted tubule (PCT), loop of Henle, distal convoluted tubule (DCT), and collecting duct system (Fig. 75.3). Each segment of the nephron consists of a single layer of epithelial cells placed on the basement membrane that are modified to perform specific transport functions suitable for that segment. The total length of the nephron ranges from 45 to 65 mm.
The Renal Corpuscle

The renal corpuscle, also called Bowman’s capsule, contains the glomerulus, a tuft of capillaries. Bowman’s capsule filters blood from glomerular capillaries to form the ultrafiltrate, which is delivered into proximal tubule (details of renal corpuscle is described below).

The Proximal Tubule

This is the initial segment of the nephron, hence called proximal tubule (PT). As most part of it is coiled, proximal tubule is also called proximal convoluted tubule (PCT). It ends in a small straight segment that continues to form loop of Henle. The PT is about 15 mm long and 40 μm in diameter. PT recovers most of the filtrate for its considerable capacity of reabsorption. Hence the tubular cells of PT exhibit features that correlate with their functions (Fig. 75.4A).

1. The proximal tubular epithelial cells have extensive folding of the apical membrane. Also, apical membrane contains microvilli (brush border) that further increase the luminal surface area for transport of ions and water.
2. The basolateral membrane of epithelial cells has multiple infoldings.
3. The epithelial cells contain many mitochondria that are present toward the basal part of the cells.
4. The adjoining epithelial cells are bound to each other by tight junctions at their luminal ends creating paracellular and basolateral spaces that help in transport of solutes and water.

The PT is divided into two parts: The initial major convoluted portion (pars convoluta) and the distal smaller straight portion (pars recta) that opens into loop of Henle.

Pars convoluta constitutes about 70% of the PT. The number of infoldings of luminal border and the number of mitochondria in pars recta are less than in pars convoluta.

Loop of Henle

Loop of Henle (LOH) is the continuation of pars recta of PT. It consists of descending and ascending limbs. The descending limb is thin throughout in all nephrons, whereas the ascending limb has thin and thick portions in a typical juxtamedullary nephron.

1. Thus LOH in juxtamedullary nephrons has three parts: thin descending, thin ascending and thick ascending limbs.
2. In a cortical nephron, thin ascending part of LOH is almost absent.
3. The total length of LOH varies from 14 to 26 mm depending on the type of nephrons (see below). The thin limb is about 2 to 14 mm long and the thick limb is about 12 mm long.
4. About 15% of filtrate is reabsorbed in LOH. The LOH of juxtamedullary nephrons plays an important role in counter-current mechanism of urine concentration.
Thin Limb of LOH

The length of hairpin loop depends mainly on the length of thin part, which varies from 2 mm in superficial or cortical nephrons to 14 mm in juxtamedullary nephrons (see Fig. 75.3). In cortical nephrons, thin segment is very short and has no ascending portion and barely penetrates medulla. In juxtamedullary nephrons, the thin segment is long, has an ascending portion and penetrates deep into the medulla. The diameter of thin limb of LOH is about 15 μm. The structure of thin segment of LOH is simple suggesting the passive nature of exchange of solutes and water in this part.

1. The epithelial cells are flat with relatively smooth apical and basolateral membranes.
2. Cells contain few mitochondria (Fig. 75.4B).

Thick Limb of LOH

The transition between thin and thick parts of ascending limb is abrupt. The tubular diameter increases from 15 μm to 30 μm. Changes also occur in histology that suits for active transport of solutes and water.

1. Epithelial cells are small and cuboidal.
2. The basolateral membrane of cells has extensive infoldings.
3. The luminal surface of cells has less infoldings.
4. Cells contain numerous mitochondria that are mainly located toward basal part (Fig. 75.4C).

The thick ascending limb of LOH terminates in DCT. The junction between the thick ascending limb and DCT comes in contact with the afferent or efferent arteriole to form JG apparatus (described below). The tubular epithelial cells at this junction are histologically modified to form macula densa.

Connecting Segment

The DCT empties into collecting duct through the connecting segment or tubule. This is a small and relatively straight tubule with morphological and transport characteristics similar to that of collecting duct.

Collecting Duct

Several DCTs empty into collecting duct. The collecting duct is 20 mm long. It passes through the cortex and medulla to empty into the pelvis of the kidney at the apex of medullary pyramids. Thus, it is divided into two parts: cortical and the medullary portions. The medullary portion of the collecting duct is further divided into outer medullary and inner medullary parts. The epithelial cells of collecting duct are modified to participate in transport of ions and water. The water permeability is controlled mainly by ADH whereas Na⁺ transport is controlled by aldosterone. Histological modifications are as follows:

1. Epithelial cells are cuboidal with minimal infoldings of basolateral and apical membrane.
2. Mitochondria are few in number (Fig. 75.4E).
3. The epithelium of collecting duct contains two types of cells: principal cells (P cells) and intercalated cells (I cells). P cells are more in number than I cells.

P Cells

The P cells have moderate basolateral infoldings and few mitochondria. They are involved in sodium reabsorption and ADH-induced water reabsorption.

I Cells

The I cells contain more microvilli, more basolateral infoldings and more mitochondria. They are involved in acid
secretion and bicarbonate transport. Few I cells are also present in DCT.

**Secretory Cells of Kidney**

The secretory or endocrine cells in kidney are mainly two types:
1. **Juxtaglomerular (JG cells)**: JG cells secrete renin that activates renin-angiotensin system.
2. **Interstitial cells (IS cells)**: There are two types of interstitial cells: cortical and medullary.
   - **Cortical interstitial cells** are of two types: Phagocytic and fibroblast-like cells. **Fibroblast-like cells** (peritubular interstitial cells) secrete erythropoietin. **Medullary interstitial cells** are of two types: type-I and type-II. **Type-I medullary interstitial cells** secrete prostaglandins, especially PGE₂.

**Types of Nephron**

Anatomically, there are three types of nephrons:
1. Superficial nephrons
2. Mid-cortical nephrons
3. Juxtamedullary nephrons

Glomerular capsules of superficial nephrons are located in the superficial layer of the cortex, hence called superficial nephrons. Similarly, the renal corpuscles of mid-cortical nephrons are located in middle of the cortex. However, physiologically, superficial and mid-cortical nephrons are considered as cortical nephrons due to their similarity in histological morphologies and functions.

**Cortical Nephrons**

The renal corpuscle of cortical nephrons is located in superficial region of the cortex. The glomerulus is usually smaller. LOH of these nephrons is short. **LOH does not have a thin ascending limb** (Fig. 75.5).
1. However, the total length of the nephron is almost same as the juxtamedullary nephron, as the DCT is longer.
2. Efferent arteriole supplying cortical nephron branches into peritubular capillaries that surround the segments of the nephron of its own and of adjacent ones (Table 75.1).

**Juxtamedullary Nephrons**

The renal corpuscle of juxtamedullary nephrons is located in the juxtamedullary region of the cortex, i.e. the inner region of the cortex close to medulla.
1. The glomerulus is usually larger.
2. They have long loop of Henle that extends deep into the medulla. They also have a thin ascending part of LOH.
3. The efferent arterioles form peritubular capillaries and a series of vascular loops called **vasa recta** (for details, see next chapter).

---

**Table 75.1**: Differences between cortical and juxtamedullary nephrons.

<table>
<thead>
<tr>
<th></th>
<th>Cortical nephrons</th>
<th>Juxtamedullary nephrons</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Location of renal corpuscle</td>
<td>In the superficial region of cortex</td>
<td>In the juxtamedullary region of the cortex</td>
</tr>
<tr>
<td>2. Number</td>
<td>85% of total</td>
<td>15% of total</td>
</tr>
<tr>
<td>3. Length of LOH</td>
<td>Shorter</td>
<td>Longer</td>
</tr>
<tr>
<td>4. Length of thin limb of LOH</td>
<td>Shorter, and thin ascending is almost absent</td>
<td>Longer, and thin limb has ascending &amp; descending parts</td>
</tr>
<tr>
<td>5. Size of glomerulus</td>
<td>Usually small</td>
<td>Usually large</td>
</tr>
<tr>
<td>6. Rate of filtration</td>
<td>Slow</td>
<td>High</td>
</tr>
<tr>
<td>7. Efferent arteriole</td>
<td>Forms peritubular capillaries.</td>
<td>Forms peritubular capillaries and vasa recta.</td>
</tr>
<tr>
<td>8. Efficiency in concentrating urine</td>
<td>Less</td>
<td>More</td>
</tr>
<tr>
<td>9. Renin content</td>
<td>More</td>
<td>Less</td>
</tr>
<tr>
<td>10. Main function</td>
<td>Urine formation &amp; excretion of waste products</td>
<td>Urine concentration</td>
</tr>
</tbody>
</table>
Ultrastructure of Renal Corpuscle

Renal corpuscle consists of **Bowman's capsule** that contains **glomerulus** and **mesangium**. This executes the first step in urine formation. The formation of urine involves three major steps: Ultrafiltration, tubular reabsorption, and tubular secretion. **Ultrafiltration, the first step in urine formation** occurs in the renal corpuscle in which plasma is differentially filtered to form the tubular fluid. This is the initial and important step of urine formation as it considerably contributes to the volumes and composition of urine.

**Glomerulus**

Glomerulus, a tuft of capillaries located in the initial dilated and cup like portion of the tubule, called Bowman’s capsule. Glomerulus is formed by the capillaries that arise from **afferent arterioles**. The glomerular capillaries drain into the **efferent arteriole** (Fig. 75.6). The glomerular capillaries are the only capillaries in the body that start with arterioles and end in arterioles. The capillaries are covered by epithelial cells called podocytes.

**Bowman’s Capsule**

Bowman’s capsule is the initial cup like dilated portion of the nephron that accommodates glomerulus.

1. The capsule has two layers: **visceral layer** that remains in close contact with glomerulus and the **parietal layer** that forms the outer portion of the capsule.
2. The **podocytes** form the visceral layer of Bowman’s capsule.
3. The parietal layer of the Bowman’s capsule is formed by **parietal epithelial cells** (Fig. 75.7A). The space between visceral and parietal layers is called **Bowman’s space**.

**Filtration Barrier**

The endothelial cells of glomerular capillaries are covered by basement membrane. Podocytes surround this basement membrane. The capillary endothelial cells, basement membrane, and foot process of podocytes form the filtration barrier (Fig. 75.7B) through which blood from glomerular capillaries is filtered to form the tubular fluid.
Capillary Endothelium

The endothelium of glomerular capillaries is of fenestrated type as it contains fenestra (pores or windows).
1. These pores are large in diameter (50 to 100 nm in diameter) and therefore, they freely allow passage of large molecules through them. This allows water and small solutes like sodium, urea, and glucose to pass through the capillary endothelium.
2. The endothelial cells are negatively charged due to presence of anionic glycoproteins on their surface. The proteins in plasma are also anionic.
3. Therefore, protein molecules are not easily filtered through the filtration barrier. Hence, normally protein is absent in urine.

Basement Membrane

The basement membrane is made up of a meshwork of fine fibrils entrenched in a gel like matrix. It is an important barrier for the proteins as the matrix contains collagen, laminin, fibronectin, and other negatively charged proteins.

Foot Processes of Podocytes

The podocytes (foot cells) form the third or the inner layer of the filtration barrier.
1. Podocytes constitute the visceral layer of Bowman’s capsule. They are the epithelial cells.
2. Their extensions terminate as foot processes that lie on the outer layer of the basement membrane (see Fig. 75.7B). The foot processes of podocytes interdigitate to cover the basement membrane. The interdigitating processes are separated by gaps (the space between adjacent foot processes) called slit pores or filtration slits, having width of 20 nm (Fig. 75.8).
3. The filtration slits are bridged by a thin diaphragm called filtration slit diaphragm that contains small rectangular pores, which do not allow free passage of molecules.
4. The filtration slit diaphragm is made up of a key molecule called nephrin. The nephrin molecules form a zipper-like structure. Minute rectangular pores with dimensions of 40 x 140 Å are formed between the prongs of the zipper.
5. Thus, filtration, especially of protein molecules is greatly restricted by nephrins.

Mesangium

This is an important component of renal corpuscle. It consists of mesangial cells and mesangial matrix.

Mesangial Cells

Mesangial cells are derived from blood monocytes and are the components of mononuclear phagocyte system. Mesangial cells are present in the mesangium, close to the glomerular capillaries (Fig. 75.9). The function of mesangial cells are:
1. They provide structural support for the glomerular capillaries.
2. They secrete extracellular matrix (the glomerular or mesangial matrix).
3. They secrete prostaglandins and cytokines.
4. They are phagocytic in nature. They kill and remove the foreign organisms and debris in the kidney by phagocytosis.
Section 8: Renal System

5. The mesangial cells contain contractile units that are present in close contact with glomerular capillaries. Therefore, contraction or relaxation of mesangial cells influences alteration in GFR by changing blood flow through glomerular capillaries or by changing capillary surface area.

6. Mesangial cells that are present outside the glomerulus are called extraglomerular mesangial cells. They are also called lacis cells or Goormaghtigh cells. They are mainly located between the afferent and efferent arterioles. They secrete some quantity of renin and erythropoietin, and also possess phagocytic activity.

7. Mesangial cells are involved in the development of immune complex-mediated glomerular disease.

JUXTAGLOMERULAR APPARATUS

Structure

The thick ascending limb of loop of Henle when comes in contact with the glomerulus of the same renal corpuscle, structural modifications occur in the tubule and afferent and efferent arterioles (Fig. 75.10). The entire modified structure (tubular and vascular components and the cells between them) is called juxtaglomerular apparatus (JGA), which includes:

1. Macula densa of the thick ascending limb of LOH,
2. Juxtaglomerular cells (the modified muscle cells mainly of afferent arterioles), and
3. Lacis cells (extraglomerular mesangial cells).

Macula Densa

Macula densa cells are modified epithelial cells of the thick ascending limb of LOH when it comes in contact with the afferent and efferent arterioles.

Lacis Cells

Lacis cells are present in the triangular space formed by efferent and afferent arterioles and the macula densa. Lacis cells are the mesangial cells present outside the...
glomerulus; hence called extraglomerular mesangial cells. These are agranular cells that secrete some quantity of renin and erythropoietin.

**Functions of JG Apparatus**

1. JG cells secrete renin that activates renin-angiotensin system, which is involved primarily in the regulation of blood volume and pressure.
2. Macula densa cells act as sensor that detects the change in rate of flow and volume of flow in the tubule, and composition of tubular fluid. This provides feedback signal to the glomerulus to change the rate of filtration, which forms the physiological basis of tubuloglomerular feedback. Thus, they control glomerular filtration.
3. Lacis cells secrete renin and erythropoietin.

**RENIN-ANGIOTENSIN SYSTEM**

Renin secreted from JG cells of kidney activates angiotensinogen to angiotensin I, which on further enzymatic action gets converted into angiotensin II and III. Hence, this system is called renin-angiotensin system (RAS). RAS is primarily involved in the control of blood volume and blood pressure.

**Scientist Contributed**

Robert Adolf Armand Tigerstedt (1853–1923) Finnish-born medical scientist and physiologist who, with his student Per Bergman, discovered renin at the Karolinska Institute, Stockholm in 1898. He studied the nerve response to various kinds of mechanical stimulation. His important contribution was the discovery of a pressor substance (renin) formed in the kidney, which is discharged into circulation from renal veins, & regulates blood pressure, salt and water homeostasis and is an important therapeutic target.

**Renin**

Renin is an acid protease secreted from JG cells of kidney. This is a glycoprotein having the molecular weight of 37,326. Like other peptide hormones, it is synthesized as preprorenin (406 amino acids), prorenin (383 amino acids), that finally becomes renin (340 amino acids). Renin acts as an enzyme to convert angiotensinogen to angiotensin I.

**Regulation of Renin Secretion**

Renin activates the renin-angiotensin system, which is essential for regulation of ECF volume, blood volume and blood pressure. Control of ECF volume is closely related to control of plasma electrolyte concentration, especially Na⁺, Cl⁻ and K⁺. Therefore, change in plasma concentration of these solutes affects renin secretion. Blood volume and pressure are also affected by sympathetic activity, circulating catecholamine and ADH. Therefore, alteration in these factors alters renin-secretion.

**Factors that increase renin secretion:**

1. Decreased blood volume and/or pressure
2. Decreased plasma Na⁺ concentration, increased K⁺ concentration
3. Increased sympathetic activity (stimulation of JG cells via renal nerve)
4. Increased circulating catecholamines
5. Prostaglandins

**Factors that decrease renin secretion:**

1. Increased plasma Na⁺ concentration (increased sodium reabsorption via macula densa)
2. Increased blood pressure (increased afferent arteriolar pressure)
3. ADH
4. Angiotensin II

**Conditions in which secretion of renin is high:**

1. Hypotension
2. Hyponatremia
3. Hyperkalemia
4. Hypovolemia (like hemorrhage)
5. Dehydration
6. Constriction of renal artery (renal artery stenosis)
7. Cardiac failure
8. Standing
9. Cirrhosis of liver
10. Psychological depression

**Role of Sodium in Renin Secretion**

Sodium is an important electrolyte that controls renin secretion. Decreased plasma sodium decreases the filtration of sodium through the renal corpuscle (glomerular filtering membrane). This decreases Na⁺ concentration in the tubular fluid. The decreased Na⁺ load is sensed by the cells of macula densa, which immediately provides feedback information to the JG cells to secrete renin. Renin activates RAS that results in angiotensin II formation.

Angiotensin II increases sodium and water reabsorption that restores ECF volume. The opposite mechanism operates when sodium concentration increases in plasma.

**Angiotensinogen**

This is a glycoprotein synthesized in liver. It contains 453 amino acids. The concentration of angiotensinogen in the plasma increases by following hormones:

1. Glucocorticoids
2. Thyroid hormones
3. Estrogen
4. Cytokines
5. Angiotensin II

Angiotensin II provides a positive feedback for further synthesis of angiotensinogen. Angiotensinogen is physiologically inactive. It acts as precursor for the formation of angiotensin I. Angiotensin I is converted to angiotensin II by angiotensin converting enzyme.
Angiotensin Converting Enzyme

Angiotensin converting enzyme (ACE) is a carboxypeptidase that acts on angiotensin I to convert it to angiotensin II. Though ACE is synthesized and secreted by the endothelial cells of blood vessels throughout the body, it is more formed in blood vessels of lungs. Hence, major conversion of angiotensin I to angiotensin II occurs in the lungs. ACE exists in two forms: somatic form and general form. However, physiological significance of existence in two forms is not known. ACE also inactivates bradykinin.

Angiotensin II, III and IV

Angiotensin II is formed from angiotensin I by the action of ACE. Angiotensin II has short plasma half-life of 1–2 min as it is rapidly metabolized by various peptidases and is rapidly converted to form angiotensin III (Flowchart 75.1). Angiotensin III is converted to angiotensin IV. Physiologically, angiotensin II is the most active angiotensin. Angiotensin III and IV are also physiologically active. Plasma renin concentration is measured to determine the angiotensin activity in the body.

Physiological Actions of Angiotensins

Angiotensin I

It acts as precursor for angiotensin II. Otherwise, it is physiologically inactive.

Angiotensin II

This is the most important among all angiotensins. All of its actions are aimed at increasing the blood volume and pressure. The functions of angiotensin II can be divided into central and peripheral functions.

Peripheral Actions

1. It is one of the most potent angiotensin I vasoconstrictors. The vasoconstriction potency of is about 8 times more than that of norepinephrine. Thus, when injected, it increases blood pressure drastically. However, the vasoconstrictor activity of angiotensin II decreases in cirrhosis of liver and in patients with hyponatremia. A chronic increase in angiotensin II level in plasma in these conditions decreases the angiotensin II receptors on the smooth muscle of blood vessels. Therefore, the vasopressor activity of angiotensin II is less in hepatic cirrhosis and hyponatremic conditions.

2. It increases the synthesis and secretion of aldosterone from adrenal cortex. In fact, angiotensin II is the major regulator of aldosterone secretion. Therefore, the RAS is also called renin-angiotensin-aldosterone axis. Aldosterone increases sodium and water reabsorption from kidney that increases ECF volume.

3. Angiotensin II directly increases the release of norepinephrine (NE) from the post-ganglionic sympathetic neurons. Thus, it also potentiates vasoconstriction through NE release.

4. Angiotensin II causes contraction of mesangial cells that decreases the glomerular filtration. Therefore, angiotensin II decreases GFR.

5. Angiotensin II increases sodium, and water reabsorption from proximal convoluted tubules of kidney.
Central Actions

1. Angiotensin II acts on the brain centers to decrease the sensitivity of the baroreflex. This potentiates the pressure effect of angiotensin II.
2. It stimulates the thirst centers in the brain to increase water intake. It does not penetrate the blood brain barrier. However, it acts on the circumventricular organs (the structures present outside the blood brain barrier) that include subfornical organ (SFO), organum vasculosum of lamina terminalis (OVLT), area postrema (AP) and the posterior pituitary. SFO and OVLT are amongst the thirst centers in the brain.
3. Angiotensin II increases the secretion of ADH from posterior pituitary.
4. It increases the secretion of ACTH from anterior pituitary.
5. It acts as neurotransmitters in some areas of the brain.

Angiotensin III

Angiotensin III possesses about 40% vasopressor activity of angiotensin II. However, it has 100% aldosterone secreting activity of angiotensin II. Thus, angiotensin II and III are involved in aldosterone secretion, whereas angiotensin II in primarily involved in the regulation of blood volume and pressure.

Angiotensin IV

This is the recently discovered angiotensin. It has some biological activities in the brain. Probably, it stimulates vasomotor center.

Angiotensin II Receptors

There are two types of angiotensin II receptors: Angiotensin 1 (AT₁), and angiotensin 2 (AT₂).

AT₁ Receptors

These receptors are present in the arteries and adrenal cortex. Acting on AT₁ receptors, angiotensin increases intracellular calcium level via phospholipase C that forms diacylglycerol and IP₃. There are two sub-types of AT₁ receptors, AT₁A, and AT₁B. AT₁A receptors are present in blood vessels, and brain, and AT₁B receptors are present in adrenal cortex and anterior pituitary.

AT₂ Receptors

These receptors are attached to G proteins that increase the intracellular cGMP. These receptors are mainly present in fetuses and neonates. However, they are also present in brains in adults.

Clinical Importance of RAS

RAS is implicated in the genesis of hypertension. Renin increases angiotensin formation that causes intense vasoconstriction, which in turn increases blood pressure. Therefore, the conditions in which renin secretion is high, blood pressure is invariably high. This is the important physiological basis of renal hypertension.

1. In experimental animals, hypertension is produced by constricting the renal artery (producing renal ischemia). This is called Goldblatt hypertension.
2. Etiologically, hypertension is classified into three categories depending on the concentration of renin in the plasma: hyper-renin hypertension, normo-renin hypertension, and hypo-renin hypertension.
3. In the treatment of hypertension, the drugs that inhibit ACE (ACE blockers) are very effective in controlling blood pressure than other group of drugs.
4. The commonly administered ACE blockers are captopril and enalapril. Angiotensin II receptor blockers like losartan have been recently tried.

Tissue RAS

In many tissues, there is renin-angiotensin system (RAS) that generates A II independent of plasma A II. They are found in blood vessels, uterus, placenta, exocrine pancreas, heart, adipose tissue, anterior pituitary, pineal and brain. The exact function of tissue RAS is not known.

INNERVATION OF KIDNEY

Renal nerve controls renal blood flow, glomerular filtration rate, and electrolyte and water reabsorption from the kidney.

1. Renal nerve contains sympathetic noradrenergic fibers.
2. There is no parasympathetic innervation of kidney.
3. Sympathetic fibers also secrete dopamine.
4. Stimulation of sympathetic fibers causes constriction of both afferent and efferent arterioles. However, afferent arteriolar constriction is more. This decreases renal blood flow and glomerular filtration rate.
5. The JG cells are innervated by sympathetic fibers. Activation of sympathetic fibers results in increased renin secretion.

CHAPTER SUMMARY

Key Concepts

1. Kidney is an important structure for homeostasis of ECF volume and composition.
2. The structural medications of tubular epithelial cells in different parts of the nephrons are primarily meant for their participation in specific functions.
3. The loop of Henle of Juxtamedullary nephron is longer to facilitate its role in urine concentration.
4. JG apparatus is placed at a vital location in the tubule to sense and regulate blood volume and pressure through renin secretion.
Important to Know (Must Read)

1. In examination, Long questions are usually not asked from this chapter. However, ‘Describe the functional importance of structural modification of tubular epithelial cells in different parts of the kidney tubules’, and ‘Describe the structure and functions of JG apparatus in health and disease’, can come as Long Questions.

2. Juxtaglomerular (JG) apparatus, rennin, Angiotensin II (AT-II), Differences between cortical and juxtamedullary nephrons, Structure and functions of glomerulus, Functions of podocytes, Renal filtration barrier, are asked as Short Questions in exam.

3. In Viva, examiner may ask… List the functions of kidney, What is the structure of JG apparatus, What are the macula densa cells and what are their functions, What are the JG cells and what are their functions, What are the Lacis cells and what are their functions, Functions of different parts of nephron, How are epithelial cells modified in different parts of nephrons, Structure of the filtration barrier, How are filtration slits formed, What are the functions of podocytes, How is the renin secretion regulated, What are the factors that increase renin secretion, What are the factors that decrease renin secretion, What are the conditions in which renin secretion is high, How the AT-II is synthesized, List the central functions of AT-II, List the peripheral functions of AT-II, What is the clinical importance of renin-angiotensin system.
CHAPTER 76

Renal Blood Flow

**Learning Objectives**
On completion of study of this chapter, the student **MUST** be able to:
1. Appreciate the arrangement of arterial supply and venous drainage in renal circulation.
2. Understand why kidney is susceptible to early hypoxic damage in shock.
3. Understand the principles of measurement of renal blood flow (RBF).
4. Describe the mechanisms of regulation of RBF.
5. List the hormones that cause renal vasoconstriction and vasodilation.

The student **MAY** also be able to:
1. Describe the measurement and explain the regulation of RBF.

**Renal Circulation**

Kidneys receive about 23.5% of the cardiac output though they constitute less than 0.5% of the total body weight. The blood flow to kidneys is about 1260 mL/min or 420 mL/100 g of tissue/min. Thus, blood flow per unit weight of the kidney tissue is much more in comparison to other organs:

1. However, there is a difference in the regional distribution of renal blood flow, with flow being maximum in cortex and minimum in medulla. Average blood flow in the cortex is about 5 mL/min/g of tissue and in medulla, about 0.5 mL/min/g of tissue (Fig. 76.1).
2. The low blood flow in medulla helps to maintain hyperosmolality in the medullary interstitium that plays an important role in counter-current mechanism of urine concentration.

**Functional Anatomy**

**Arterial Supply**

Each kidney is supplied by a single **renal artery** that originates from aorta:

1. Renal artery branches to form **anterior and posterior divisions** that form total five **segmental arteries**.
2. Each segmental artery branches into **interlobar artery**, which in successive divisions forms **arcuate artery**, **interlobular artery** (cortical radial artery) and **afferent arteriole**.

**Fig. 76.1:** Difference in cortical and medullary blood flows in kidney.

3. Afferent arteriole gives rise to **glomerular capillary network** (glomerulus) that finally drains to **efferent arteriole**.
4. Efferent arterioles lead to a second capillary network, which is formed around the renal tubules (hence called **peritubular capillaries**) (Fig. 76.2).

**Venous Drainage**

The venous system of kidney parallels the arterial system:

1. Efferent arterioles form peritubular capillaries or vasa recta which drain into interlobular veins. The subsequent sequence of drainage is arcuate veins, interlobar veins, segmental veins and finally the renal vein (Flowchart 76.1).
In the medulla of kidney, blood supply is derived from efferent arteriole of juxtamedullary glomeruli. These efferent arterioles in juxtaglomerular nephrons, in addition to formation of peritubular capillaries, form an extra set of capillaries called vasa recta (Fig. 76.2). Vasa recta are straight and long capillaries that run deep into the medulla. Thus, there are descending and ascending limbs of vasa recta that remain in close contact with each other.

This arrangement of vasa recta helps it to function as the counter exchanger in urine concentrating mechanism by exchanging substances between blood flowing into and out of medulla. The blood from the inner medulla returns to the cortex in the ascending limb of vasa recta.

**Functions of Vasa Recta**
1. It provides oxygen and nutrients to the nephron segments.
2. It delivers substances to the nephron for secretion into the tubular lumen.
3. It serves as a pathway for the return of reabsorbed water and solutes to the circulatory system.
4. It participates in concentration (as counter current exchange) and dilution of urine.

**Importance of Renal Blood Flow (RBF)**
The flow of blood through kidneys serves following important functions:
1. Supplies oxygen, nutrients, and hormones that control kidney functions.
2. Delivers metabolites and waste products to the kidney for their excretion in the urine.
3. Controls concentration and dilution of urine.
4. Influences solute and water reabsorption from kidney.
5. Determines GFR (RBF is the main determinant of GFR).
As kidneys maintain blood volume, blood pressure and ionic composition of body fluids, alteration in RBF leads to serious consequences. Adequate renal blood flow is essential for these homeostatic functions. Therefore, in hemorrhagic shock, one of the primary objectives of treatment is to maintain renal blood flow so that azotemia does not develop and excretion of waste materials in urine continues to occur.

**Oxygen Consumption of Kidneys**
Kidneys are metabolically active organs. Therefore, the oxygen consumption by kidneys per unit tissue (6 mL per 100 g of tissue per min) is more than other metabolically active organs like liver (2 mL per 100 g of tissue per min) and brain (3.3 mL per 100 g of tissue per min). A greater blood flow to kidneys (23.5% of the cardiac output) ensures a higher oxygen supply to the organs.

1. However, oxygen supply to kidneys is more than they normally need. The oxygen consumption by kidneys as a whole is much less, which is about 18 mL per min, in comparison to 52 mL per min for liver, 50 mL per min for skeletal muscles, 45 mL per min for brain and 30 mL per min for heart.
2. Therefore, the arteriovenous oxygen difference across the kidney is considerably less than the other organs (Application Box 76.1). This ensures adequate oxygen reserve for kidneys in various physiological conditions.
3. Nevertheless, inspite of adequate oxygen supply to kidneys, damage to renal tissues occurs in hypoxic conditions as in shock (see below, in physiological significance).

**Physiological Significance**
The vascular arrangement in most part of kidney tissue permits shunting of a greater fraction of oxygen in the arterial blood to the venous blood before blood passes through the capillaries. Therefore, inspite of adequate oxygen supply to kidneys, the oxygenation of renal tissue is not as high as expected. Hence, kidney is susceptible to hypoxic damage in shock.

**Application Box 76.1**

**Venous blood of kidney is bright red:** The venous blood of kidney has a bright red color as the red cells in it have high oxyhemoglobin content. This is due to the low renal extraction of oxygen, because kidneys receive much more oxygen than they utilize. Thus, oxygen reserve for kidney is adequate in physiological conditions. Therefore, normally, venous content of oxygen is high (oxyhemoglobin content is more), which makes it bright red.

**MEASUREMENT OF RBF**

**Principle of Measurement**
RBF can be measured by Fick principle. Also, electromagnetic flow-meter and other flow-meters measure RBF. With the application of Fick principle, RBF is detected by measuring the amount of a given substance taken up by the organ per unit time and dividing this value with the arterio-venous (AV) difference of the substance across the organ (kidney).

Kidney filters blood to form urine. Therefore, renal blood flow equals the amount of a substance excreted per unit time divided by its renal AV difference. The substance used for measurement of RBF should have following properties:

1. The substance used should be measurable in the arterial and venous blood
2. Should not be metabolized
3. Should not be stored
4. Should not be produced by the kidney, and
5. By itself, should not affect the blood flow.

Usually the substance used for RBF measurement is para-aminohippuric acid (PAH), as it possesses all the above-mentioned properties. RBF is measured by using the appropriate formula that uses renal plasma flow (RPF) and hematocrit (see below). Renal blood flow is renal plasma flow (RPF) divided by 1 minus hematocrit (Hct).

\[
\text{Renal blood flow} = \text{RPF} \times \frac{1}{1 - \text{Hct}}
\]

Thus, first RPF and hematocrit are estimated. Hematocrit is determined by centrifuging a blood sample. RPF is measured by clearance of PAH infused intravenously.

**Measurement of Renal Plasma Flow**
RPF is measured by injecting PAH and then determining the concentration of PAH in urine and plasma. The extraction ratio (i.e. arterial concentration minus renal venous concentration divided by arterial concentration) for PAH is high, because PAH is filtered from the glomerulus and secreted by tubular cells:

1. Following its injection, 90% of PAH from the arterial blood is removed in the kidney through its single circulation.
2. Therefore, usually renal plasma flow is calculated by dividing the amount of PAH in urine by the plasma level of PAH (concentration of PAH in renal venous blood is ignored). Peripheral venous plasma is used as the concentration of PAH in peripheral plasma is essentially identical to that in the arterial plasma reaching the kidney.
3. The value obtained is called effective renal plasma flow (ERPF) to emphasize that the level in the renal venous plasma is not measured. In human being, ERPF is about 625 mL/min in average.

\[
\text{ERPF} = \frac{\text{U}_{\text{PAH}} \times \text{V}}{\text{P}_{\text{PAH}}} = \text{Clearance of PAH (C}_{\text{PAH}} \text{)}
\]

For example, when concentration of PAH in urine \((U_{\text{PAH}})\) is 12.4 mg/mL, urine flow (V) is 1 mL/min, and concentration of PAH in plasma \((P_{\text{PAH}})\) is 0.02 mg/mL, ERPF is calculated as follows:
ERPF = \frac{12.4 \times 1}{0.02} = 620 \text{ mL/min}

ERPF is converted to actual renal plasma flow by the following method:

As the average PAH extraction ratio is 0.9,

\text{Actual RPF} = \frac{\text{ERPF}}{\text{Extraction ratio}} = \frac{620}{0.9} = 689 \text{ mL/min}

If hematocrit (Hct) is 47%, then RBF will be:

\text{RBF} = \text{RPF} \times \frac{1}{1 - \text{Hct}} = 689 \times \frac{1}{1 - 0.47}

= 689 \times \frac{1}{0.53} = 1300 \text{ mL/min}

Thus, renal blood flow, as calculated from the above equations is 1300 mL/min.

**Regulation of RBF**

Renal blood flow is regulated by neural, hormonal, and local factors, and by autoregulatory mechanisms.

**Neural Factors**

**Sympathetic Control**

Stimulation of renal sympathetic fibers causes **vasoconstriction that decreases renal blood flow**:  
1. Therefore, conditions that activate renal sympathetic fibers such as hemorrhage, cold, pain, exercise, anesthesia, etc. decrease blood flow to kidney. Though sympathetic stimulation in these stressful situations aim at diverting blood from splanchnic circulation to vital organs, stimulation for a longer duration as occurs in hypovolemic shock causes **renal shutdown and azotemia**.  
2. Sympathetic stimulation also increases renin secretion from JG cells that activates renin-angiotensin system. Formation of angiotensin II causes renal vasoconstriction and further decreases blood flow.  
3. Fortunately, angiotensin II and sympathetic stimulation to kidneys stimulate the production of local prostaglandins (PG E\(_2\) and I\(_2\)) that produce vasodilation and oppose the vasoconstriction effects. Therefore, sympathetic stimulation unless intense and longstanding, can not ordinarily compromise renal functions.

**Hormonal Factors**

Circulating catecholamines and other vasoactive peptides like angiotensin II cause renal vasoconstriction (Table 76.1). However, dopamine in high dose causes renal vasodilation. Therefore, **dopamine is preferred for the treatment of cardiogenic shock**, as its systemic effect increases blood pressure and its renal vasodilation effect maintains renal perfusion.

**Local Factors**

Locally produced metabolites and chemicals like CO\(_2\), and **prostaglandins** cause renal vasodilation and maintain renal blood flow. Though adenosine is a potent vasodilator in coronary and cerebral circulations, it causes constriction in renal vascular bed.

**Autoregulation**

Blood flow to kidney is maintained at a relatively constant level despite the change in systemic pressure range between 80 to 180 mm Hg. This is called autoregulation of renal blood flow (Fig. 76.3).

**Myogenic Mechanism of Autoregulation**

1. The renal autoregulatory mechanism is an **intrinsic phenomenon** as it is also observed in isolated and denervated kidney.  
2. However, it is absent when renal vascular smooth muscle is paralyzed by drug administration. This indicates that the autoregulation is mainly due to direct contractile response of renal smooth muscle.

<table>
<thead>
<tr>
<th>Table 76.1: Hormones affecting renal blood flow.</th>
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<tr>
<td><strong>A. Vasoconstrictors</strong></td>
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<tr>
<td>Epinephrine</td>
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<tr>
<td>Norepinephrine</td>
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<tr>
<td>Angiotensin II</td>
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<tr>
<td>Adenosine (A(_1) receptor)</td>
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<td>Endothelin</td>
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<td>Vasopressin</td>
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<td>Thromboxane A(_2)</td>
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**Fig. 76.3:** Autoregulation of renal blood flow (RBF). Note, within the mean arterial pressure (MAP) range of 80–180 mm Hg, renal blood flow almost remains constant.
(of afferent arteriole) to stretch. This is called the myogenic mechanism of autoregulation. 

3. In this process, increased pressure in the blood vessel causes stretch of the vessel wall that opens cation channels resulting in depolarization. This leads to voltage-dependent opening of calcium channel causing calcium influx. Increased intracellular calcium causes vasoconstriction.

4. Opposite changes occur when blood pressure decreases.

**CHAPTER SUMMARY**

**Key Concepts**

1. RBF is 23.5% of cardiac output, which is much more compared to other organs. This adequate blood supply maintains RBF in conditions of hypovolemia and protects kidney functions.

2. The peritubular capillaries and vasa recta are in close contact with the tubules to facilitate the exchange between tubules and ECF.

3. RBF has well developed autoregulatory mechanisms that adjust blood flow to it despite sudden change in blood volume and pressure in systemic circulation.

**Important to Know (Must Read)**

1. In examination, Long Questions are usually not asked from this chapter.

2. Regulation of renal blood flow, Measurement of renal plasma flow, are asked as Short Questions in exam.

3. In Viva, examiner may ask...... How much is the RBF, How are vasa recta and peritubular capillary formed, List the sequence of arterial supply and venous drainage in kidney, What are the principles of measurement of renal blood flow, What is the method of measurement of renal plasma flow, What is effective renal plasma flow, List the factors regulating renal blood flow, List the neural factors affecting renal blood flow, List the hormones affecting renal blood flow, What is the autoregulation of renal blood flow, List the functions of vasa recta, What is the importance of renal blood flow, Why is kidney susceptible to hypoxia in shock, Why is the venous blood of kidney bright red.

**Metabolic Mechanism**

Local metabolic mechanism also contributes to autoregulation of blood flow, in which NO, prostaglandins, adenosine and other metabolites produced in the tissue influence blood flow.

**Glomerulotubular Feedback Mechanism**

Glomerulotubular feedback mechanism also plays role in autoregulation of blood flow, in which composition and flow of tubular fluid determine RBF and glomerular filtration.
Learning Objectives

On completion of study of this chapter, the student MUST be able to:

1. Define GFR and give its normal value.
2. Explain the mechanism of glomerular filtration.
3. List the factors that affect GFR.
4. List the chemicals that cause contraction, and chemicals that cause relaxation of mesangial cells of kidney.
5. Give the principles of measurement of GFR.
6. List the conditions that alter GFR.
7. Understand the mechanisms of regulation of GFR.

The student MAY also be able to:

1. Describe and explain the mechanisms regulation of glomerular filtration.

The glomerular filtration is the first step in urine formation. This involves ultrafiltration of plasma that takes place through the glomerulocapsular filtering membrane. The product of filtration is called filtrate (the ultrafiltrate of plasma) that flows down the tubular lumen. The composition of the filtrate is altered as it passes through different parts of the tubule to finally become the urine. As the rate of filtration is the major determinant of tubular load, the final output from tubule (the volume of urine) depends on glomerular filtration. In general, factors that impair filtration decrease urine formation, and the factors that facilitate filtration increase urine formation. Therefore, determination of glomerular filtration is an important test in the assessment of kidney functions.

MECHANISM OF GLOMERULAR FILTRATION

Glomerular filtration occurs through the glomerulocapsular filtration barrier that consists of fenestrated capillary endothelium, the basement membrane and podocytes of capsular epithelium (for details, refer “Filtration Barrier” in Chapter 75) This is governed by two major factors: pressure gradients or starling forces (hydrostatic and osmotic pressure gradients) across the glomerular capillary wall and filtration coefficient (size of the capillary bed and permeability of the capillaries).

Glomerular filtration = $K_f \left[ (P_{GC} - P_T) - (\Pi_{GC} - \Pi_T) \right]$

When, $K_f$ is filtration coefficient (the product of glomerular capillary wall permeability and the effective filtration surface area), $P_{GC}$ is the mean hydrostatic pressure in glomerular capillaries, $P_T$ is mean hydrostatic pressure in tubule, $\Pi_{GC}$ is glomerular capillary oncotic pressure, and $\Pi_T$ is osmotic pressure of the filtrate in the tubule.

Pressure Gradients

The pressure gradients across glomerular capillary wall are different from the pressure gradients along the capillaries of other vascular beds. Therefore, quality and degree of filtration are different through the filtration barrier. Like other capillary beds, the pressure gradients are due to operation of Starling forces that are hydrostatic and oncotic pressures.

Hydrostatic Pressure Gradient

The hydrostatic pressure in glomerular capillary is considerably higher than the capillary pressure in other parts of the body. This is because the afferent arterioles directly arise from interlobular arteries and they are short and straight:
Chapter 77: Glomerular Filtration

1. The glomerular capillary pressure at the afferent arteriolar end is same as at efferent arteriolar end, i.e. 45 mm Hg.

2. This pressure is opposed by the hydrostatic pressure in Bowman’s capsule, which is 10 mm Hg.

3. Therefore, the hydrostatic pressure gradient across the filtering membrane is 35 mm Hg (45–10 mm Hg) towards the capsule.

4. Thus, hydrostatic pressure strongly favors filtration across the entire glomerular capillary membrane from its afferent end to efferent end (Fig. 77.1).

Osmotic Pressure Gradient

The hydrostatic pressure gradient is opposed by the osmotic pressure gradient. Osmotic pressure gradient depends on the glomerular capillary oncotic pressure and the osmotic pressure of the filtrate in the tubular fluid:

1. Oncotic pressure at the afferent arteriolar end is 20 mm Hg and at the efferent arteriolar end is 35 mm Hg.

2. This pressure is opposed by tubular fluid osmotic pressure. However, the osmotic pressure of the tubular fluid is negligible.

3. Therefore, the osmotic pressure gradient is towards capillary lumen and the magnitude is more at efferent end than at afferent end.

The Net Filtration Pressure: The net filtration pressure (\(P_{\text{UF}}\)) across the glomerular membrane depends on the difference between the hydrostatic pressure gradient and the glomerular capillary oncotic pressure (as the oncotic pressure of tubular fluid is negligible).

\[ P_{\text{UF}} = (P_{\text{GC}} - P_{T}) - \Pi_{\text{GC}} \]

At the afferent end of glomerular capillary, the net filtration pressure is:

\[ Afferent \ P_{\text{UF}} = 35 \text{ mm Hg} - 20 \text{ mm Hg} = 15 \text{ mm Hg outward} \]

At efferent end of glomerular capillary, the net filtration pressure is:

\[ Efferent \ P_{\text{UF}} = 35 \text{ mm Hg} - 35 \text{ mm Hg} = 0 \text{ mm Hg} \]

Thus, the net filtration gradient is always from glomerular capillaries towards the tubule, which is high at afferent arteriolar end that slowly declines to almost nil at efferent arteriolar end.

Filtration Coefficient

Filtration coefficient (\(K_f\)) is the product of glomerular capillary wall permeability and the effective filtration surface area (size of the capillary bed).

Capillary Permeability

Glomerular capillaries are highly permeable, i.e. about 50 times that of capillaries in skeletal muscle. However, filtration depends on the size and shape of the molecules and the electrostatic charge they carry.

Molecular Size

Molecular size is an important determining factor of filterability of the substance. Neutral substances with molecular diameter of less than 4 nm are freely filtered and substances with diameter of more than 8 nm are not filtered.

Shape

The elongated particle with large molecular weight can pass easily, whereas globular particle of same molecular weight cannot pass through.

Electrostatic Charge

The particles with positive charge can easily pass through the glomerular filtering membrane than the negatively charged particles:

1. This is because the glomerular capillary wall is negatively charged due to the presence of sialoproteins.

2. Therefore, permeability of cationic molecules is more than neutral and anionic molecules.

3. The effective diameter of circulating albumin is less than 8 nm, but albumin is anionic. Therefore, normally albumin is not filtered through the glomerular capillaries.

4. However, in diseases in which the charge of glomerular capillaries is lost as occurs in glomerular nephritis, albumin appears in urine.

Clinical Significance

Normally, about less than 100 mg of protein is excreted in urine per day. This is not due to the filtration of proteins by the glomerular membrane, rather due to secretion by the tubular cells.
Table 77.1: Factors that alter mesangial cell activity.

A. Factors that produce contraction of mesangial cells
- Angiotensin II
- Norepinephrine
- Endothelins
- ADH
- PDGF (platelet derived growth factor)
- PAF (platelet activating factor)
- Histamine
- Thromboxane A₂
- PGF₂α
- Leukotrienes

B. Factors that produce relaxation of mesangial cells
- Dopamine
- ANP (atrial natriuretic peptide)
- cAMP
- PGE₂
- Nitric oxide
- Bradykinin

1. In fact, protein is not secreted, but it appears in tubular fluid due to shedding of tubular epithelial cells.
2. Albuminuria, which refers to excretion of significant amount of albumin in urine, is normally absent.
3. However, albuminuria is seen in diseases in which:
   - There is increase in the size of the pores in the glomerular membrane, or,
   - There is disruption of electrostatic force of glomerular membrane proteins.

Size of the Capillary Bed

The surface area for filtration of the capillary bed depends on the size of mesangial cells. The contraction of mesangial cells decreases the area available for filtration and relaxation of mesangial cells increases the area for filtration. Thus, mesangial cell contraction impairs and relaxation facilitates GFR. The factors that alter mesangial cell activity are listed in Table 77.1.

GLOMERULAR FILTRATION RATE

Definition and Normal Value: Glomerular filtration rate (GFR) is defined as the amount of filtrate formed by glomerular filtering membrane of both the kidneys in a unit time. Normally, it is 125 mL/min (7.5 L/h or 180 L/day).

Factors Affecting GFR

The GFR is influenced by factors that alter renal blood flow, pressure gradients, glomerular capillary permeability and surface area for filtration:
1. Change in renal blood flow: Increased blood flow to kidney increases the delivery of blood to glomerulus that promotes filtration and conversely decreased flow decreases filtration. Thus, renal vasodilation maintains GFR.
2. Glomerular capillary hydrostatic pressure: Hydrostatic pressure in glomerular capillary depends on the amount of blood delivered to and the amount of blood drained from the glomerulus:
   a. Afferent arteriolar dilation or efferent arteriolar constriction increases capillary hydrostatic pressure and therefore, increases GFR.
   b. Conversely, afferent arteriolar constriction or efferent arteriolar dilation decreases GFR.
3. Change in capsular hydrostatic pressure: Hydrostatic pressure in the Bowman’s capsule and tubule opposes filtration. Tubular obstruction increases tubular hydrostatic pressure and therefore decreases GFR.
4. Oncotic pressure: Osmotic pressure in glomerular capillaries due to plasma proteins opposes filtration. Therefore, hypoproteinemia results in more GFR. Conversely, dehydration decreases GFR and urine formation.
5. Glomerular capillary permeability: Integrity of glomerular capillary is an important determinant of GFR. Increased capillary permeability increases GFR as occurs in glomerulonephritis.
6. Effective filtration surface area: Size of filtration area depends on mesangial cells:
   a. Mesangial cell contraction distorts the capillary lumen and decreases the area available for filtration.
   b. Conversely, relaxation of mesangial cells increases filtration.
   c. Many hormones and chemicals control GFR by altering mesangial cell activity.
7. Size, shape and electrical charge of the macromolecules: Molecular size determines the filterability of the substance:
   a. Any substance having molecular weight less than 10,000 can be freely filtered by the glomerular filtration barrier and molecules with weight more than 10,000 have restricted filterability (Table 77.2).
   b. Most of the proteins in plasma are larger molecules and therefore can not be filtered. Also, the molecular shape influences filterability.
   c. Slender and supple molecules can easily pass through than the spherical and rigid molecules.
   d. Due to the presence of negatively charged particles in all the three layers of glomerular filtration barrier, molecules with negative charges can not easily be filtered, whereas neutral and cationic substances can do so.

Table 77.2: Filterability of some common substances through glomerular filtration barrier.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Mol. Wt.</th>
<th>Mol. Diameter</th>
<th>Filterability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>18</td>
<td>0.20</td>
<td>100</td>
</tr>
<tr>
<td>Glucose</td>
<td>180</td>
<td>0.70</td>
<td>100</td>
</tr>
<tr>
<td>Inulin</td>
<td>5,000</td>
<td>2.8</td>
<td>100</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>17,000</td>
<td>4.0</td>
<td>75</td>
</tr>
<tr>
<td>Hb</td>
<td>68,000</td>
<td>6.6</td>
<td>3</td>
</tr>
<tr>
<td>Albumin</td>
<td>69,000</td>
<td>7.2</td>
<td>0.01</td>
</tr>
</tbody>
</table>
Filtration Fraction

Filtration fraction (FF) is the ratio of the GFR to renal plasma flow:

1. Normal FF ranges between 0.16 to 0.20. This is a better index of glomerular activity. For example, decreased blood pressure increases FF without much change in GFR.
2. In hypotension, renal plasma flow decreases. However, GFR decrease is less than the decrease in renal plasma flow as efferent arteriolar constriction maintains GFR. Thus, FF remains elevated.

Measurement of GFR

Concept of Renal Clearance

Measurement of GFR and RBF is based on the principle of renal clearance. Renal clearance of a substance is defined as the volume of plasma from which that substance is completely cleared (removed) per unit time. When a substance is removed in urine, a certain volume of plasma is cleared (freed) of that substance. Therefore, clearance assesses an important aspect of kidney function as normally kidney is capable of clearing the substance from the plasma. The clearance of a substance can easily be assessed by determining the concentrations of the substance in plasma and urine, and by estimating the urine flow rate. The formula is as follows:

\[ C_x = \frac{U_x \times V}{P_x} \]

When, \( C_x \) is the clearance of the substance, \( U_x \) is the concentration of substance in urine, \( V \) is the urine flow in unit time, and \( P_x \) is the concentration of substance in the arterial plasma.

Inulin Clearance Test

Inulin clearance test is commonly used for the assessment of glomerular filtration. It can be measured by measuring the concentration of the substance in urine and the plasma concentration of the substance. The substance should be freely filtered through the glomeruli and should neither be secreted nor be reabsorbed by the tubules.

GFR is equal to the concentration of the substance (x) in urine times the urine flow per unit time divided by the arterial plasma concentration of the substance.

\[ GFR = \frac{U_x \times V}{P_x} \]

When, \( U_x \) is the concentration of substance in urine, \( V \) is the urine flow in unit time, and \( P_x \) is the concentration of substance in the arterial plasma. As the substance is not metabolized in the body the concentration in the venous plasma can be taken for the concentration in arterial plasma. This value of GFR is called the clearance of the substance (\( C_x \)).

Criteria of the substance used:
1. It should be freely filtered by the glomeruli.
2. It should be neither reabsorbed from nor secreted in the renal tubules.
3. It should not be synthesized or stored or altered in the kidney.
4. It should not be metabolized in the body.
5. It should be non-toxic to the body.
6. Its concentration in plasma and urine should be easily measured.

The substance usually used is inulin, a polymer of fructose, as it meets all the criteria of an ideal substance for measuring GFR.

It is injected intravenously initially as a bolus dose and then through the continuous infusion to maintain a constant concentration in the arterial plasma. Once, inulin equilibrates with body fluids, the urine and plasma sample are collected for its estimation.

For example, if urine concentration is 40 mg/mL, plasma concentration is 0.25 mg/mL and rate of urine flow is 0.8 mL/min. Then:

\[ C_{IN} = \frac{U_{IN} \times V}{P_{IN}} = \frac{40 \times 0.8}{0.25} \]

\[ C_{IN} = 128 \text{ mL/min} \]

As inulin is neither reabsorbed nor formed, altered and stored in kidney, the filtered load of inulin equals the rate of inulin excretion. Therefore, inulin clearance equals the GFR.

Creatinine Clearance Test

Endogenous creatinine clearance is used clinically to estimate GFR. Creatinine is the end product of creatine phosphate, a skeletal muscle derivative. It is produced continuously in the body and excreted continuously in urine. Therefore, the concentration of creatinine in plasma and urine are normally stable. Its concentrations are measured in plasma and urine and the urine flow rate is (volume of urine formed per unit time) determined. Then, creatinine clearance is calculated as:

\[ C_{Creatinine} = \frac{U_{Creatinine} \times V}{P_{Creatinine}} \]

The advantages are that no infusion of creatinine is required.

Conditions that Alter GFR

GFR changes in different physiological and non-physiological conditions. The changes are due to the change in renal blood flow, glomerular oncotic pressure, glomerular hydrostatic pressure, change in plasma protein concentration, etc.

Exercise: GFR decreases in exercise due to sympathetic stimulation that causes more afferent arteriolar constriction than constriction of efferent arteriole.
Pregnancy: In pregnancy, RBF and GFR increase. RBF increases due to increase in blood volume, cardiac output and decreased renal vascular resistance. GFR increases mostly secondary to increase in RBF. However, hormonal changes also play a role. About 40–50% increase of GFR occurs in second and third trimesters of pregnancy.

Posture: Maintaining body in standing position for a longer duration decreases GFR due to sympathetic stimulation and decreased effective blood volume (pooling of blood in veins of lower limbs and abdomen). Conversely, supine posture increases GFR.

Sleep: GFR is usually less during sleep due to decreased circulatory activity. However, supine position maintains GFR.

Weather: GFR is more in rainy season and less in summer. In summer, decreased ECF volume decreases GFR. In winter and rainy season, GFR is more due to decreased loss of water from the body and less humidity.

Gender: GFR is less in females than in males.

Age: GFR decreases in geriatric age group due to functional loss of nephrons. GFR is also less in children.

Food Intake: Diet rich in proteins increases GFR.

Regulation of GFR

Usually, factors that affect RBF also affect GFR. Thus, regulation of glomerular filtration involves neural mechanisms, hormonal mechanisms, myogenic mechanism and tubuloglomerular feedback.

Neural Mechanism

Both the afferent and efferent arterioles are innervated by sympathetic fibers:

1. The sympathetic activity in the renal nerve is less when the blood volume is normal. When blood volume decreases, increased renal sympathetic activity and increased circulating catecholamines cause renal vasoconstriction that in turn decreases RBF and GFR.
2. Activation of sympathetic fibers causes constriction of afferent arteriole that decreases blood flow to glomerulus and therefore, decreases GFR.
3. Thus, sympathetic stimulation as occurs in emotion, fear, pain, etc. decreases GFR.

Hormonal Mechanism

Various hormones affect GFR. These include angiotensin, histamine, dopamine, ANP, endothelin, bradykinin, nitric oxide, adenosine, glucocorticoids and prostaglandins.

Angiotensin

Angiotensin II causes constriction of afferent and efferent arterioles. However, the effect on afferent arteriole is more than that on the efferent arteriole. Therefore, it decreases GFR.

Histamine

The release of histamine locally plays an important role in the control of GFR. Though, histamine causes arterial dilation and increases renal blood flow. However, by causing contraction of mesangial cells it decreases GFR.

Dopamine

Dopamine causes renal vasodilation:

1. Thus, it increases renal blood flow and GFR.
2. Dopamine also relaxes mesangial cells and therefore, increases GFR.
3. Dopamine is produced by proximal tubular cells. Acting locally, it inhibits renin secretion from JG cells that decreases angiotensin II production.
4. This also contributes to increase in GFR.

ANP

Increased blood volume increases ANP secretion:

1. ANP causes dilation of the afferent arteriole and constriction of the efferent arteriole.
2. Therefore, it considerably increases GFR.
3. This contributes to decrease in blood volume by increasing urine formation (diuresis).

Endothelin

Endothelin is a potent vasoconstrictor:

1. It is secreted from the endothelial cells of the renal blood vessels, mesangial cells, and cells of the distal tubules.
2. It produces significant vasoconstriction of both afferent and efferent arterioles.
3. Therefore, it appreciably decreases GFR.

Nitric Oxide

The endothelial derived relaxing factor (EDRF) is the nitric oxide:

1. EDRF causes significant renal vasodilation and dilation of both afferent and efferent arterioles.
2. This also opposes the vasoconstriction effect of angiotensin II and catecholamines.
3. Thus, EDRF causes profound increase in GFR.

Bradykinin

Bradykinin is synthesized from kininogen:

1. It is a potent vasodilator.
2. It stimulates release of NO and prostaglandins.
3. Thus, it increases GFR.

Adenosine

Adenosine is produced by the kidney tissue:

1. It causes constriction of the afferent arteriole and therefore decreases GFR.
2. It also plays a role in tubuloglomerular feedback.
**Glucocorticoids**
Glucocorticoids increase RBF and GFR. But, the mechanism of alteration of GFR by glucocorticoids is not clearly known.

**Prostaglandins**
Prostaglandins are formed and secreted by kidney:
1. They increase RBF by decreasing the vasoconstrictor effects of catecholamines and angiotensin II.
2. PGE₃ causes relaxation of mesangial cells that contributes to increases in GFR.
3. In conditions like hypotension and shock, prostaglandins play important role in regulation of GFR.
4. In hypovolemic shock, sympathetic activation causes renal vasoconstriction, which deteriorates kidney functions.
5. However, renal vasodilation effect of prostaglandins in shock protects kidney by opposing the sympathetic constrictor activity and maintaining renal blood flow.

**Autoregulatory Mechanisms**
Similar to autoregulation of RBF, GFR is also autoregulated. The autoregulation of GFR maintains a constant rate of glomerular filtration despite change in systemic arterial pressure within the range of 80–180 mm Hg (Fig. 77.2). This is mainly due to the myogenic theory and theory of tubuloglomerular feedback.

**Myogenic Theory**
Myogenic mechanism controls renal blood flow and GFR. When arterial pressure increases, the afferent arteriole is stretched and stretch-induced contraction of the smooth muscles of afferent arteriole decreases GFR. Opposite mechanism operates in hypotension.

**Tubuloglomerular Feedback**
Increased renal arterial pressure increases pressure in the glomerular capillaries:

1. This increases GFR and flow of fluid in the tubule, which is sensed by macula densa cells.
2. Tubular cells provide signal to the glomerular apparatus that causes afferent arteriolar constriction to decrease GFR and bring it back to normal and maintain the constancy of load delivered to the tubule. This is called tubuloglomerular feedback.
3. The exact mechanism of macula densa cells sensing and then signaling the JGA is not clearly known. However, it is now clear that macula densa senses the flow dependent changes in NaCl reabsorption.
4. The likely mediators are adenosine and NO.
5. Though adenosine produces vasodilation in most of the vascular beds, it causes constriction of afferent arteriole.
6. The macula densa cells synthesize both vasoconstrictor (adenosine), and vasodilator (NO) metabolites. Thus, the change in afferent arteriolar activity is brought about depending on the need by alteration in the secretion of these vasoconstrictor and dilator agents.

**CHAPTER SUMMARY**

**Key Concepts**
1. Though GFR depends on hydrostatic and osmotic pressure gradients, normally hydrostatic pressure gradients plays a bigger role. However, integrity of filtering membrane is the most important.
2. Inulin clearance test is the best method to measure GFR.
3. Sympathetic regulation and tubuloglomerular feedback are major systems for regulating GFR.

**Important to Know (Must Read)**
1. In examination, ‘Describe the mechanism, measurement and factors affecting glomerular filtration rate (GFR)’ is usually asked as a Long Question.
2. Mechanism of GFR, Measurement of GFR, Regulation of GFR, Tubuloglomerular feedback, Filtration fraction, may be asked as Short Questions in exam.
3. In Viva, examiner may ask..... Define GFR, give its normal value, List the factors affecting GFR. How do the Starling forces (hydrostatic and osmotic pressure gradient) affect GFR, What is filtration coefficient, What are the factors that affect glomerular capillary permeability, List the factors that affect mesangial cell activity, Define filtration fraction, give its normal value, Define renal clearance, give its formula, List the criteria of the substance used to measure GFR, List the conditions that alter GFR, List the neural mechanisms regulating GFR, List the hormonal mechanisms regulating GFR, Explain the autoregulation of GFR, What is tubuloglomerular feedback and how it works.
CHAPTER 78

Tubular Functions

LEARNING OBJECTIVES

On completion of study of this chapter, the student MUST be able to:

1. Understand the importance of tubular functions in urine formation.
2. Appreciate the basic transport mechanisms that function across the tubular epithelium.
3. Understand the transport mechanisms of various solutes and water in different parts of renal tubule.
4. Describe the reabsorption of sodium and glucose from kidney tubules.
5. Define and explain transport maximum and renal splay.
6. Correlate tubular functions with its dysfunctions.
7. Understand the mechanisms of regulation of ions and water from kidney tubules.
8. Learn the importance of tubuloglomerular feedback and glomerulotubular balance.

The student MAY also be able to:

1. Explain the role of proximal tubule, loop of Henle, and distal tubule in urine formation.
2. Explain the mechanisms of sodium and water reabsorption from kidney tubules.
3. Explain the mechanism of tubuloglomerular feedback and glomerulotubular balance.

STEPS OF URINE FORMATION

Formation of urine occurs in three steps: Ultrafiltration of plasma through renal corpuscle, reabsorption of water and solutes from the tubular fluid, and secretion of selected solutes into the tubular fluid (Fig. 78.1).

1. Thus, after glomerular filtration, tubular handling of the filtrate is the most important step in the process of urine formation. Glomerular filtration is a nonspecific process in the sense that mechanism of filtration is not different for water and different solutes; whereas tubular transports are selective processes as solvent and different solutes are either reabsorbed or secreted by mechanisms specific to their transport.

2. Tubular exchange of water and electrolytes finally determines the volume and composition of urine.

3. Thus, tubular mechanisms are most important processes in determination in urine volume and composition. By modulating the reabsorption and secretion of substances of its luminal fluid, renal tubule plays an important role in the control of composition, osmolality, pH and volume of ECF.

Fig. 78.1: Major steps of urine formation. Note, glomerular filtration, tubular reabsorption and tubular secretion are three main processes in formation of urine. Number of arrows indicates the magnitude of the process.
Chapter 78: Tubular Functions

The glomerular filtration rate (GFR) is 180 liters/day, whereas quantity of urine formed is about 1.5 liters/day, which is less than 1% of the glomerular filtrate. Thus, the renal tubules have vast capacity to reabsorb water and solutes. Usually, glucose and HCO$_3^-$ are almost reabsorbed completely; water, Na$^+$ and Cl$^-$ are reabsorbed more than 99%; K$^+$ is reabsorbed more than 90% and urea is reabsorbed about 50% of the filtrate in the renal tubules (Table 78.1).

### COMMON PRINCIPLES OF TUBULAR FUNCTIONS

The major function of tubule is to reabsorb water and solutes from the tubular fluid, which is crucial for water and electrolyte homeostasis of the body. These transport mechanisms for various substances are different in different parts of the kidney tubule.

### Transport Mechanisms

Processes of transport across tubular epithelium can be broadly divided into two categories: passive and active. Transport of solutes involves both passive and active processes, whereas water reabsorption is a passive phenomenon.

#### Passive Transport Mechanisms

The passive transport mechanisms include diffusion, facilitated diffusion, solvent drag and osmosis.

**Diffusion**

The solutes are transported by means of diffusion from their area of higher concentration to the area of lower concentration. This is the transport along the chemical gradient. Especially, uncharged solutes like glucose are transported by this mechanism. However, though charged solutes especially ions are also transported by diffusion, their electrical gradient greatly influences this passive transport.

**Facilitated Diffusion**

In this transport mechanism, a specific carrier protein in the membrane facilitates the process of diffusion. Reabsorption of glucose via glucose transporter is an example of facilitated diffusion. Sodium and potassium ions are also reabsorbed from kidney tubule through the water, filled channels created by the carrier proteins. Transport of glucose, proteins and urea from the tubular fluid are other examples of facilitated diffusion.

**Coupled transport**, which is a form of facilitated diffusion, serves as major mechanism of transport of solutes in the tubules. There are two mechanisms of coupled transports: symport mechanism, and antiport mechanisms.

**Symport Mechanism**

The symport mechanism is the process of coupled transport of two or more solutes in same direction by a carrier protein. The examples are the transport of Na$^+$-glucose, Na$^+$-amino acid, etc.

**Antiport Mechanism**

Antiport mechanism is the process of coupled transport of two or more solutes in opposite direction by a carrier protein. An example is the Na$^+$–H$^+$ exchange in the proximal tubule that reabsorbs Na$^+$ from the tubular fluid in exchange for secretion of H$^+$ into it.

**Solvent Drag**

When bulk amount of water is reabsorbed, the solutes dissolved in water are also transported along with water across the tubular epithelium. This process is called solvent drag. This contributes to reabsorption of substantial amount of solutes in the proximal tubule.

**Osmosis**

When a considerable amount of osmotically active solute is transported, water is reabsorbed along with it to maintain the osmotic balance. This is the major mechanism for reabsorption of water from the tubular lumen. For example, water reabsorption follows reabsorption of Na$^+$ and Cl$^-$ from the tubular fluid. Conversely, increased osmolality of tubular fluid increases water excretion, known as osmotic diuresis (Application Box 78.1).

### Application Box 78.1

**Glycosuria causes osmotic diuresis:** Osmotic diuresis typically occurs in diabetes mellitus. In diabetes, when plasma glucose is more than renal threshold, glucose appears in urine (glycosuria). More glucose in tubular fluid causes water retention that leads to polyuria. This differentiates from water diuresis seen in diabetes insipidus that occurs due to ADH deficiency.
In this mechanism, solutes are transported from the area of lower concentration to the area of higher concentration. The best example of active transport mechanism is the Na⁺-K⁺ pump.

1. The Na⁺-K⁺ ATPase is mainly located in the basolateral membrane of the tubular epithelial cells. This pumps sodium out of the tubular epithelial cells and potassium into the cell.

2. The other examples include H⁺-ATPase, H⁺-K⁺ ATPase, Ca²⁺-ATPase, etc. Various transporters in different segment of tubule are listed in Table 78.2.

**Secondary Active Transport**

This is the major mechanism by which Na⁺, glucose and associated solutes are reabsorbed from kidney tubules. The active transport mechanism, i.e. Na⁺-K⁺ ATPase located in the basolateral membrane of epithelial cells pumps Na⁺ out of the cell. This creates a low concentration of Na⁺ in the tubular cells. Therefore, Na⁺ is reabsorbed from the tubular fluid along its concentration gradient into the tubular cells.

1. The carrier protein for Na⁺ facilitates reabsorption of Na⁺ into the tubular cells. Glucose is reabsorbed by the same carrier protein that reabsors Na⁺.

2. Therefore, reabsorption of glucose by this mechanism is an example of secondary active transport (for details, see blow).

3. The similar mechanism operates in the epithelial cells of intestine.

**Key Concepts in Transport Mechanisms**

**Paracellular Pathway of Transport**

Close to apical membrane, tubular epithelial cells have tight junctions between them. Immediately after the tight junctions between the epithelial cells, the lateral intercellular space starts.

**Active Transport Mechanisms**

Transport of solutes is considered to be active when ATP is utilized in the process. In this mechanism, solutes are transported from the area of lower concentration to the area of higher concentration. The best example of active transport mechanism is the Na⁺-K⁺ pump.

1. This is the rate at which the tubule maximally transports a particular solute. That means, the amount of a particular solute transported depends on the amount of the solute in tubular fluid present up to the Tm for the solute.

2. Tm is the amount of the substance delivered to the tubule per minute.

3. When the concentration of the solute in tubular fluid is more than the Tm concentration, the mechanism of transport is said to be saturated, and beyond this there will be no appreciable increase in transport of the solute. For example, the Tm for glucose is 375 mg/min. in males and 300 mg/min. in females.

**Table 78.2: Various transporters in apical membrane of epithelial cells in different segment of kidney tubule.**

<table>
<thead>
<tr>
<th>Site</th>
<th>Transporter</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCT</td>
<td>Na⁺-Glucose CT</td>
<td>Na⁺ and glucose uptake</td>
</tr>
<tr>
<td></td>
<td>Na⁺-Pi CT</td>
<td>Na⁺ and Pi uptake</td>
</tr>
<tr>
<td>Thick limb</td>
<td>Na⁺-AA CT</td>
<td>Na⁺ and AA uptake</td>
</tr>
<tr>
<td>Thick limb</td>
<td>Na⁺-lactate CT</td>
<td>Na⁺ and lactate uptake</td>
</tr>
<tr>
<td>Thick limb</td>
<td>Na⁺-H⁺ exchanger</td>
<td>Na⁺ uptake and H⁺ secretion</td>
</tr>
<tr>
<td>Thick limb</td>
<td>Na⁺,K⁺,Cl⁻ CT</td>
<td>Na⁺, K⁺, and Cl⁻ uptake</td>
</tr>
<tr>
<td>Thick limb</td>
<td>K⁺ channel</td>
<td>K⁺ secretion</td>
</tr>
<tr>
<td>DCT</td>
<td>NaCl CT</td>
<td>Na⁺ and Cl⁻ uptake</td>
</tr>
<tr>
<td>CD</td>
<td>ENaC</td>
<td>Na⁺ uptake</td>
</tr>
</tbody>
</table>

(Cr: Cotransporter; Pi: Inorganic phosphate; AA: Amino acids; PCT: Proximal tubule; DCT: Distal tubule; CD: Collecting duct.)

**Fig. 78.2:** Mechanisms of transcellular and paracellular transports across the renal tubular epithelium. Note that paracellular transport occurs through the tight junction between epithelial cells.

1. However, these tight junctions are not very tight and they have leaky channels. When transport of solutes and water occurs between the cells through tight junctions and lateral intercellular space, the process is called transport across the paracellular pathway.

2. A considerable quantity of Ca²⁺ and K⁺ are reabsorbed in proximal tubule via paracellular pathway.

3. Some amount of Na⁺ and water is also reabsorbed via this route.

4. This is to differentiate from the transcellular pathway of transport in which transport occurs through the cell.

5. Transport of sodium and glucose from tubular fluid into the tubular cells and from there into the ECF is the example of transport via transcellular pathway (Fig. 78.2).

**Transport Maximum**

The transport systems in the renal tubule like transport systems in other parts of the body have their maximal rate, which is called as the transport maximum (Tm).

1. This is the rate at which the tubule maximally transports a particular solute. That means, the amount of a particular solute transported depends on the amount of the solute in tubular fluid present up to the Tm for the solute.

2. Tm is the amount of the substance delivered to the tubule per minute.

3. When the concentration of the solute in tubular fluid is more than the Tm concentration, the mechanism of transport is said to be saturated, and beyond this there will be no appreciable increase in transport of the solute. For example, the Tm for glucose is 375 mg/min. in males and 300 mg/min. in females.
**Chapter 78: Tubular Functions**

**Tubular Load**
The quantity of a solute filtered by the glomerulo-capsular filtering barrier and presented to the tubular fluid is the tubular load.

1. Tubular load determines the amount of the substance to be reabsorbed from the tubule, as normally, a constant fraction of the load is reabsorbed by the kidney tubules, which is called glomerulotubular balance. Tubular load also determines the Tm for the substance.
2. The amount of the substance delivered to the tubular fluid per unit time (tubular load of the substance) greatly contributes to the maximum quantity of the substance that can be reabsorbed.
3. However, Tm depends on plasma concentration of the substance and the rate of filtration of the substance, i.e., plasma concentration \( \times \) GFR.
4. For example, Tm for glucose is 375 mg/min, which indicates that plasma concentration of glucose up to 300 gm%, tubule can transport glucose totally from the tubular fluid (300 mg/100 mL \( \times \) 125 mL/min).
5. However, normally, glucose appears in urine above 200 mg% (more accurately, above 180 mg% of venous blood) of plasma level. This is because of the mechanism of renal splay for glucose (for details, see “Glucose Reabsorption” below).

**Renal Threshold**
This is the concentration of the solute in the plasma at or above which the solute first appears in urine or appears in more amount than its normal concentration.

1. For example, normally glucose is not present in urine and its renal threshold is 180 mg% in venous plasma (200 mg% in arterial plasma).
2. Therefore, glucosuria occurs when plasma concentration of glucose is above 180 mg%.

**TUBULAR FUNCTIONS**

**Proximal Tubular Functions**
The proximal tubule is the most important part of the nephron as it reabsorbs about 67% of the filtered water, Na\(^+\), Cl\(^-\), K\(^+\), and HCO\(_3^-\) and almost all the filtered glucose and amino acids.

1. The proximal tubule has convoluted and straight portions. Though the convoluted part (PCT) comprises 70% of the tubule, functionally both the parts are similar with few minor differences.
2. The primary focus of reabsorption process in the proximal tubule is directed at the Na\(^+\) reabsorption, which is usually secondary to electrochemical gradient created by Na\(^+\)-K\(^+\) pump located on the basolateral membrane of the epithelial cells.
3. Reabsorption of water and most of the solutes is directly or indirectly linked with this pump. Therefore, abnormalities of this pump (i.e., Na\(^+\) reabsorption process in the tubule) results in many renal dysfunctions.

**Important Facts:** The fluid in the early part of proximal tubule is almost isosmotic to plasma.

1. Na\(^+\) and its two major anions Cl\(^-\) and HCO\(_3^-\) are the major solute in plasma and glomerular filtrate.
2. HCO\(_3^-\) is reabsorbed mainly in the convoluted portion of the proximal tubule leading to its drastic fall in rest of the tubule.
3. Cl\(^-\) is reabsorbed in the second half of the proximal tubule (later part of convoluted portion and straight portion) which creates a lumen positive transepithelial potential difference that favors passive reabsorption of Na\(^+\).
4. About of 67% of filtered K\(^+\) is reabsorbed along with 67% of water.
5. Glucose and amino acids are almost completely reabsorbed in proximal tubule resulting in their steep fall in rest of the tubule.
6. Thus, at the end of proximal tubule, only one-third of Na\(^+\), Cl\(^-\) and K\(^+\) remain with almost absence of glucose, amino acid and bicarbonate in the tubular fluid.
7. However, urea concentration is increased as it is not at all absorbed in this part of the tubule.

**Na\(^+\) Reabsorption**
In proximal tubule, reabsorption of Na\(^+\) is important among all transport processes as it generates the major driving force for reabsorption of water and other solutes. From tubular fluid, Na\(^+\) enters the tubular epithelial cells along the electrochemical gradient. Inside the tubular cells, concentration of Na\(^+\) is about 35 meq/L in comparison to about 140 meq/L in the tubular fluid.

1. The lower intracellular concentration of Na\(^+\) is due to the activity of Na\(^+\)-K\(^+\) pump located on the basolateral surface of the cells. Vigorous activity of Na\(^+\)-K\(^+\) ATPase constantly removes three Na\(^+\) out of the cell for bringing in two K\(^+\) for each cycle of the pump. This active transport mechanism constantly creates a low concentration of Na\(^+\) in the cell.
2. The Na\(^+\) removed from the cell into the lateral intercellular space enters interstitial fluid, and the K\(^+\) pumped into the cell diffuses out of it through basolateral membrane mostly via K\(^+\) channels.
3. Thus, the net effect is the decreased Na\(^+\) level in tubular cells. This causes Na\(^+\) from tubular fluid to enter the tubular cells (Fig. 78.3).
4. As the Na\(^+\) entry from the luminal surface into the cells utilizes the energy generated by Na\(^+\)-K\(^+\) pump on the basolateral surface, the process of Na\(^+\) reabsorption is an active transport mechanism. About 67% of filtered Na\(^+\) is reabsorbed in proximal tubule (Application Box 78.2).

**Cotransport and Antiport Mechanisms:** From tubular fluid, entry of Na\(^+\) into the tubular cells occurs via various cotransport and antiport mechanisms that are located on the apical cell membrane.
1. The carrier protein that transports Na⁺ also cotransports glucose, amino acids, phosphates, etc. Therefore, reabsorption of these solutes is considered as secondary active transport (for details, see below).

2. Na⁺ is also transported from tubular fluid by antiporter, especially by Na⁺-H⁺ exchanger which reabsorbs Na⁺ into the cell in exchange for secretion of H⁺ into the luminal fluid. Normally, Na⁺-H⁺ exchanger is the primary mechanism of entry of Na⁺ into the epithelial cells, which accounts for about 60% of the total Na⁺ entry.

**Associated Anion Reabsorption:** Na⁺ reabsorption is accompanied by reabsorption of anions, such as HCO₃⁻ and Cl⁻ to maintain electroneutrality. However, process of anion absorption along with Na⁺ is different in first and second half of proximal tubule.

**Application Box 78.2**

% of Na⁺ reabsorption in different parts of tubule: Normally, 67% of filtered Na⁺ is reabsorbed in proximal tubule which occurs mainly by Na⁺-H⁺ exchange. About 25% of Na⁺ is reabsorbed in thick ascending limb of loop of Henle that occurs via Na⁺-2Cl⁻-K⁺ cotransporter. In DCT, about 5% is reabsorbed by Na⁺-Cl⁻ cotransport mechanism. Remaining of about 3% is reabsorbed in collecting duct via ENaC (epithelial Na channels).

### In First Half of Proximal Tubule

In first half of proximal tubule, Na⁺ reabsorption is mainly associated with HCO₃⁻ and organic solutes like glucose, amino acids, phosphates, etc.

1. Thus, Na⁺ and HCO₃⁻ reabsorption in the first part of PCT is coupled to the transport of organic molecules.

2. Therefore, simultaneous reabsorption of Na⁺, bicarbonate, and organic solutes from the proximal tubular fluid establishes an osmotic gradient that results in reabsorption of water.

### In Second Half of Proximal Tubule

In second half of proximal tubule, Na⁺ reabsorption is mainly associated with Cl⁻ reabsorption via transcellular and paracellular pathway. In the later part of proximal tubule, Na⁺ reabsorption is coupled with the Cl⁻ rather than bicarbonate or organic solutes because of two reasons.

1. In distal part of proximal tubule, concentration of Cl⁻ is very high, whereas the concentration of glucose and amino acid is less in this region as Na⁺ and bicarbonate are preferentially reabsorbed in the first half of PCT.

2. Also, presence of more chloride-anion antiporter in the distal part of the proximal tubule facilitates transport of Cl⁻ into the cell. Na⁺ that enters cell is pumped out of it to enter ECF via Na⁺-K⁺ pump located on the basolateral membrane. The Cl⁻ leaves the cell by means of K⁺-Cl⁻ symporter located on the basolateral membrane. Thus, Na⁺ and Cl⁻ are reabsorbed from tubular fluid into the interstitial fluid via tubular cells. This is called transcellular pathway of reabsorption of solutes. Increased concentration Na⁺ in lateral-interstitial space creates an electrical gradient for Cl⁻ ions also to move through the paracellular pathway. This is because the tight junctions between the tubular cells at their apical margin contain leaky channels that transport Cl⁻ along its electrical concentration gradient from the tubular fluid into the interstitial space. This is called paracellular pathway of reabsorption solutes. This paracellular pathway of solute reabsorption constitutes about 25% of NaCl reabsorption in the proximal tubule.

Thus, Na⁺ reabsorption helps in reabsorption of Cl⁻, HCO₃⁻, other cations and anions and various organic solutes. Transfer of organic and inorganic solutes from tubular fluid into the interstitial space creates the osmotic gradient for the reabsorption of water in the proximal tubule.

### Water Reabsorption

Normally, 65% of the filtered water is reabsorbed in the PCT.

1. The driving force for water reabsorption is the transcellular osmotic gradient, which is established by absorption of Na⁺ and accompanying solutes.

2. Transcellular and paracellular reabsorption of NaCl and other solutes from tubular fluid into the lateral intercellular and interstitial spaces increases the osmolality of fluid in these spaces.

3. Permeability of epithelium of proximal tubule to water is extremely high. Water passes through the epithelial cells via water channels (aquaporin 1) present in the cell membranes and also through the water channels present in the paracellular route (in tight junctions between the cells).
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4. Therefore, even a smaller osmotic gradient (osmolality of tubular fluid of about 293 mosm/L against osmolality of interstitial fluid of about 285 mosm/L) result in adequate movement of water. Thus, water flows along the osmotic gradient via the transcellular and paracellular pathways.

5. Hence, reabsorption of water is coupled with the reabsorption of solutes, especially with that of Na\(^+\) and Cl\(^-\) (as NaCl is osmotically most active).

6. The transfer of large amount (bulk flow) of water helps in transport of ions like K\(^+\) and Ca\(^{2+}\) that are carried along with water. This process is called solvent drag.

7. Accumulation of water in the interstitial space increases the hydrostatic pressure that favors transfer of water from there into the ECF.

**Role of Peritubular Capillaries**

The peritubular capillaries play an important role in absorption of solutes and water from the tubule. Peritubular capillaries are derived from efferent arteriole and therefore receive blood from the glomerulus.

1. As the blood draining from glomerulus has already been filtered in the glomerular capillary and protein has not been filtered through the filtration barrier in the renal corpuscle, **blood in peritubular capillary has high oncotic pressure.**

2. Moreover, the hydrostatic pressure is also less in peritubular capillaries as blood has passed through the upstream resistance vessels before entering these capillaries.

3. Thus, high oncotic and low hydrostatic pressures favor uptake of water from the interstitial tissue space surrounding tubules.

4. This transfer of water from peritubular space into peritubular capillaries maintains the gradient for water reabsorption from tubular lumen.

**Glucose Reabsorption**

Glucose is reabsorbed completely from tubular fluid in the proximal tubule.

1. It is reabsorbed along with Na\(^+\) in the tubule. Na\(^+\) is pumped out of the tubular cells by Na\(^+\)-K\(^+\) pump located on the basolateral membrane. This decreases intracellular Na\(^+\) concentration and creates gradient for Na\(^+\) entry into the cell from the tubular fluid.

2. The carrier protein that transports Na\(^+\) also reabsorbs glucose (sodium-glucose cotransporter).

3. Thus, reabsorption of glucose from the luminal membrane is **secondary transport to the active process** located on the basolateral membrane of the cells and, therefore, is a typical example of secondary active transport (Fig. 78.5).
Glucose transport mechanism in the kidney is similar to the transport of glucose in the intestine. Glucose and Na⁺ bind to the SGLT 1 and 2 (sodium-glucose cotransporter, the common carrier protein that transports Na⁺, glucose and many other solutes like amino acids, lactate, phosphates, etc.). This helps in reabsorption of glucose along with Na⁺, when Na⁺ is reabsorbed into the tubular cell from the tubular fluid. From the cell, glucose is transported into the interstitial fluid by GLUT 2. Thus, glucose reabsorption is closely associated with Na⁺ reabsorption.

**TmG and Renal Splay**

Glucose is filtered at a rate of 100 mg/min. This is because the normal plasma glucose concentration is about 80 mg/dl and GFR is 125 mL/min (80 × 125). However, almost all the filtered glucose is reabsorbed in the proximal tubule, so that in normal condition, urine is essentially glucose free.

1. The rate of reabsorption of glucose is proportional to the amount of glucose filtered. Therefore, plasma glucose level determines the transport maximum for glucose (TmG).
2. The normal TmG is 375 mg/min in males and 300 mg/min in females. When TmG is exceeded, the glucose appears in urine. As the renal threshold is the TmG divided by GFR, the predicted value for renal threshold will be about 300 mg/dl, when TmG is 375 mg/min and the GFR is 125 mL/min (TmG divided by GFR should have been about 300 mg/dl).
3. However, the actual renal threshold is much below the predicted value, i.e. about 200 mg/dl in arterial plasma and 180 mg/dl in venous plasma.
4. Therefore, when the graph of TmG is plotted, the actual curve deviates from the ideal curve. This deviation is called renal splay (Fig. 78.6). The splay is due to two reasons:
   - TmG is not same in all the tubules, and
   - Amount of glucose removed from each tubule is not complete even when the amount filtered is below the TmG.

**Protein Reabsorption**

Normally proteins do not appear in the urine as they are almost totally reabsorbed in the proximal tubule. The glomerulus filters only a small quantity of proteins, which is about 7 g/day.

1. Amino acids from the tubular fluid are also reabsorbed by means of the carrier proteins that reabsorb Na⁺ and glucose (secondary active transport).
2. Also, enzymes present on the apical membrane of the epithelial cells of PCT partially degrade the proteins and endocytose them.
3. The proteins in the cell are degraded by the cellular enzymes into their respective amino acids.
4. Amino acids are then transported from the cell across the basolateral membrane into the interstitial tissue space and from there into the blood.
5. By these mechanisms almost all the filtered proteins and amino acids are reabsorbed in proximal tubule. Therefore, urine is practically protein free.
6. But, when amount of protein filtered is more or protein reabsorption is less, proteinuria occurs (Clinical Box 78.1). Usually proteinuria occurs due to the disruption of the glomerular filtering membrane as occurs in glomerulonephritis that increases the tubular load of proteins.

![Fig. 78.6: Mechanism of renal splay for glucose. Note, once the TmG for glucose is reached, glucose reabsorption saturates and glucose appears in urine. However, saturation of glucose reabsorption occurs earlier than the expected plasma level, which is called renal splay. Therefore, the actual curve for glucose transport deviates from the ideal curve.](image)

**Clinical Box 78.1**

**Proteinuria:** The protein that appears in urine comes from two sources. The primary source of protein in the urine is the excess filtration by the glomerular membrane or less reabsorption in the proximal tubule. The other source may be the protein synthesized by the cells of thick ascending limb of loop of Henle, which is called Tamm-Horsfall protein. This protein is usually excreted in urine as the mechanism for protein reabsorption is primarily located in the proximal tubule.

**Transport of Organic Solutes**

The proximal tubule secretes various organic cations and anions. Many of these ions are produced by metabolism, such as uric acid.

1. Many exogenous organic substances like PAH, drugs, etc. are also secreted by the proximal tubule. Usually, these organic compounds are bound to plasma proteins that prevent filtration of the substances via the glomerular membrane. However, the substances are transported from the peritubular capillaries into the tubular cells from where they are secreted into the tubular fluid.
2. Therefore, the excretion of this organic compound in urine via their secretion by the tubular cells (rather than through filtration), constitutes an important mechanism for their elimination from the body.
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**Physiological Significance**

Interference in renal transport of organic compounds help in treatment of many metabolic diseases. For example, **gout** occurs due to accumulation of uric acid in plasma. Therefore, aim of treatment of gout is to decrease the plasma uric acid level. Uric acid is both secreted and reabsorbed in proximal tubule. Thus, one mechanism to decrease uric acid level in gout is to promote its excretion by decreasing absorption in PCT.

**PAH Transport**

PAH is an organic anion secreted across the proximal tubular cells. The pathway for this secretion has low specificity, which means many other organic anions like bile salts, oxalates, urates, etc. are also secreted by this mechanism.

1. PAH enters the proximal tubular cells via basolateral membrane in exchange for α-ketoglutarate (αKG), i.e. via PAH-αKG antiport mechanism. Normally, αKG is produced in the cell by glutamate metabolism (Fig. 78.7).
2. The αKG that comes out of the cell into the interstitial tissue space is taken back in to the cells by Na⁺-α KG symport.
3. PAH is then taken into the tubular fluid along its concentration gradient. The mechanism that transports PAH into the tubular cell is non-specific.
4. Therefore, increased plasma concentration of one anion decreases the secretion of the other. For example, urinary excretion of PAH decreases in individual receiving penicillin, and, similarly, excretion of penicillin decreases in individual receiving PAH.
5. Therefore, during the World War II, when the availability of the penicillin was less, the penicillin was administered along with hippuric acid, which resulted in prolonged action of penicillin by decreasing its secretion through the tubular cells. Thus, penicillin in low dose could achieve the desirable therapeutic effects.

Defects in proximal tubular reabsorption causes Fanconi syndrome (Clinical Box 78.2).

**Clinical Box 78.2**

Fanconi syndrome: This is a generalized defect in proximal tubular reabsorption involving amino acids, glucose, phosphate, uric acid, sodium, potassium, bicarbonate and proteins. This renal disease occurs due to mutation of the carrier or transporter proteins that may be either inherited as autosomal or X-linked trait or acquired in diseases like multiple myeloma, amyloidosis, heavy metal toxicity and from chemotherapeutic drugs. Thus, impaired ability of the PCT to reabsorb these organic and inorganic compounds results in glucosuria with normal plasma glucose, hypophosphatemia, hypouricemia, hypokalemia, aminoaciduria, low molecular weight proteinuria and proximal renal tubular acidosis. Rickets and osteomalacia commonly occurs secondary to hypophosphatemia. Salt wasting and polyuria are also common.

**Chloride Reabsorption**

Chloride is mainly reabsorbed secondary to Na⁺ reabsorption in the proximal tubule to maintain electroneutrality. Recently, separate chloride channels have been identified in the kidney tubules. It has been observed that these Cl⁻ channels are linked with Ca²⁺ channels. But, how these two channels interact and operate is not clearly known. Defect in Cl⁻ channels causes Dent’s disease (Clinical Box 78.3).

**Clinical Box 78.3**

Dent’s disease: This is a disease of childhood that results from mutation of gene for chloride channels. Number of Cl⁻ channels decreases in kidney tubules in this dysfunction. As Cl⁻ channels are linked with Ca²⁺ channels, abnormality also occurs in calcium excretion. Therefore, hypercalciuria and kidney stones occur in this disease. The disorder presents with hypercalciuria, low molecular weight proteinuria, calcium nephrolithiasis and nephrocalcinosis in male children (female children are usually asymptomatic).

**Functions of Loop of Henle**

The loop of Henle (LOH) has descending and ascending limbs. LOH of juxtamedullary nephrons are longer than cortical nephrons and dip deep into the medullary pyramid.

1. In juxtamedullary nephrons, descending limb of LOH is totally thin, whereas ascending limb has thin and thick portions.
2. All the segments (thin descending, thin ascending and thick ascending segments) have different transport properties and permeability. Fluid from proximal tubule that enters LOH is almost isotonic, whereas fluid leaving LOH is considerably hypotonic having osmolality of 100 mosm/kg of H₂O (in comparison to 285 mosm/kg of H₂O of plasma and proximal tubular fluid).
3. This is because solutes are reabsorbed in ascending limb of LOH without reabsorption of water that makes the fluid more dilute.
4. Reabsorption of solutes into the interstitium from LOH plays a critical role in counter current mechanism of urine concentration.
5. Cortical nephrons are more in number and LOH of these nephrons help in urine formation, whereas juxtamedullary nephrons are less in number and LOH of these nephrons help in urine concentration.

**Transport of Solutes**

LOH reabsorbs about 25% of the filtered NaCl and K+, 30% of filtered Ca++, and 65% of filtered Mg++. The descending limb of LOH is permeable to water but not to the solutes, whereas the ascending limb is impermeable to water. Na+, K+, and Cl− are cotransported out of the thick segments of the ascending limb.

**Descending Limb**

Descending limb is permeable to water, but not to solutes. Therefore, fluid in terminal part of descending limb becomes hypertonic as water moves out of the tubule to enter the interstitial space.

**Thin Ascending Limb**

In the thin part of ascending limb of LOH, transport mechanism is predominantly a passive phenomenon, and permeability to water is less. Though passive transport of NaCl occurs to some extent, active transport of Na+ is almost nil.

**Thick Ascending Limb**

In the thick ascending limb of LOH, the fluid becomes more dilute (hypotonic) as Na+ and Cl− are taken out of the tubular fluid.
1. The active transport system for Na+ is well developed in this segment of LOH. Reabsorption of Na+ from tubular fluid is dependant on a carrier protein on the apical membrane that transports 1 Na+, 1 K+, and 2Cl− from the lumen into the cell. 1Na+-1K+-2Cl− cotransporter membrane protein is also present in airways, epithelial cells of GI tract and salivary gland.
2. From tubular cells, Na+ is transported into the interstitial fluid by Na+-K+ pump, which is quite efficient in thick ascending limb of LOH (Fig. 78.8).
3. K+ from cells diffuses back into the tubular fluid and also into the interstitium passively through ROMK and other membrane K+ channels.
4. Cl− that enters the cell is co-transported with K+ into the interstitial fluid.
5. Transepithelial transfer of these solutes creates a potential difference between tubular and interstitial fluid space, which drives transfer of solutes, especially transport of Na+, K+, Mg++ and Ca++ via paracellular pathway.
6. Thus, transport of solute occurs by both transcellular and paracellular routes.

7. However, the tubular epithelium of thick ascending limb of LOH is almost impermeable to water; therefore, solute transfer in this part of the tubule is not associated with transfer of water.
8. Defect in thick ascending limb transport process causes Batter syndrome (Clinical Box 78.4).

**Clinical Box 78.4**

**Batter syndrome:** This is a rare syndrome that occurs due to defective transport of solutes in thick ascending limb of LOH. It occurs due to mutation of gene for Barttin, an integral membrane protein essential for function of Cl− channels (CIC-Kb Cl− channels). Also, mutation of gene that causes synthesis of transporter proteins, such as 1Na+-1K+-2Cl− cotransporter and ROMK K+ channel occurs in this syndrome. The disease is characterized by chronic hyponatremia and hypovolemia that causes activation of renin-angiotensin system. The resultant increase in aldosterone secretion causes hyperkalemia and alkalosis. Hypertension does not occur. Patients of Batter syndrome with mutated Barttin are deaf because the defect in stria vascularis in inner ear, which maintains high concentration of K+ in scala media contains CIC-Kb Cl− channels.

**Reabsorption of Water**

The loop of Henle reabsorbs approximately 15% of the filtered water, which occurs exclusively in the descending limb, the ascending limb being impermeable to it.
1. The descending limb except at its terminal portion is highly permeable to water. In spite of active transport of solutes in thick ascending limb of LOH, water is not reabsorbed.
2. Both thin and thick portions of ascending limb of LOH are almost impermeable to water. Furosemide is a loop diuretic (Clinical Box 78.5).

**Clinical Box 78.5**
Loop Diuretics: The loop diuretics like furosemide inhibit the Na⁺-K⁺-2Cl⁻ symporter. This inhibits NaCl reabsorption from the thick ascending limb of loop of Henle. Thus, loop diuretics cause natriuresis and kaliuresis and increase osmolality of tubular fluid that lead to increase water excretion. However, K⁺ depletion may result from long use of loop diuretics. Hence, K⁺ supplement is usually given to patients under chronic furosemide therapy.

**Functions of Distal Convoluted Tubule**

**Reabsorption of Solutes and Water**
Distal convoluted tubule (DCT) is essentially the extension of the thick ascending limb of LOH. Hence, physiologically, it is close to thick ascending limb of LOH.
1. Therefore, DCT is relatively impermeable to water whereas solutes are transported. As solute is removed in excess of solvent, further dilution of the tubular fluid occurs in this part of the nephron.
2. In the initial segment of distal tubule, Na⁺ and Cl⁻ are reabsorbed from tubular fluid into the tubular cells by Na⁺-Cl⁻ symporter (Fig. 78.9).
3. Na⁺ is removed from tubular cells to interstitial space by Na⁺-K⁺ pump located on the basolateral membrane, and Cl⁻ is removed by Cl⁻ channels.
4. However, in spite of osmotic gradient across the tubular epithelium, water reabsorption is poor. Only about 5% of the filtered water is removed in DCT.
5. Thus, dilution of the tubular fluid that begins in the thick ascending limb of LOH continues in the DCT. Thiazide diuretics act on DCT (Clinical Box 78.6).

**Clinical Box 78.6**
Thiazide diuretics: Thiazide diuretics such as chlorthiazide, and metolazone diuretics, act by inhibiting Na⁺-Cl⁻ cotransporter in the early part of DCT. This causes natriuresis and diuresis.

**Functions of Collecting Duct**
Though collecting duct is considered as distal part of nephrons, strictly speaking, it is not the part of an individual nephron. However, as solute and water reabsorption continues in collecting duct system, physiologically, it is an essential component of a nephron.
1. As collecting duct is the terminal part of the nephron, its ability to alter transport of solutes and water contributes considerably to final osmolality, composition and volume of urine.
2. Most of the excreted K⁺ in urine comes from collecting duct as it secretes a significant quantity of this cation.

3. Transport processes in collecting duct are influenced and fine-tuned by hormones, such as aldosterone and ADH.

**Reabsorption of Solutes**
Epithelium of collecting duct contains two types of cells: principal cells and intercalated cells.
1. The principal cells reabsorb Na⁺ and secrete K⁺, whereas intercalated cells secrete either H⁺ or HCO₃⁻.
2. Thus, principal cells contribute to K⁺ metabolism and intercalated cells play an important role in acidification of urine.
3. Intercalated cells also reabsorb HCO₃⁻ and K⁺.

**Principal Cells**
Principal cells are present abundantly in the epithelium of collecting duct.
1. The reabsorption of Na⁺ and secretion of K⁺ in the principal cells depends on Na⁺-K⁺ pump in the basolateral membrane (Fig. 78.10). This pump provides chemical gradient for the movement of Na⁺ from the distal tubular fluid into the cells. K⁺ is secreted from blood via principal cells into the tubular fluid.
2. K⁺ secretion in the collecting duct mainly occurs in the cortical part.
3. In physiological conditions, most of K⁺ in urine comes from collecting duct secretion.
4. Though, in conditions of K⁺ excess like high K⁺ diet, collecting duct secretes more K⁺, in K⁺ depletion it reabsorbs K⁺.
5. K⁺ secretion is facilitated by aldosterone.

**Role of Aldosterone**

Na⁺ is reabsorbed from tubular fluid by principal cells by diffusion through Na⁺ channels (EnaC) that increases Na⁺ reabsorption, and also stimulates activity of Na⁺-K⁺ ATPase that helps in generating gradient for Na⁺ absorption and K⁺ secretion.

Clinical Box 78.7

K⁺-sparing diuretics: K⁺-sparing diuretics like amiloride and spironolactone inhibit Na⁺ reabsorption from collecting duct by antagonizing the effect of aldosterone. Amiloride directly inhibits the Na⁺ channel (EnaC) on the luminal membrane and spironolactone inhibits Na⁺-K⁺ pump on the basolateral membrane. Inhibition of Na⁺ reabsorption decreases the magnitude of negative charge in the lumen. This facilitates Cl⁻ reabsorption by paracellular pathway. Because it decreases the negative charge in the lumen, it also decreases K⁺ secretion into the tubular fluid. Thus, it decreases K⁺ excretion in the urine. Therefore, they are called K⁺-sparing diuretics. This is usually used in patients with increased K⁺ excretion in the urine.

**Intercalated Cells**

These cells are scattered among the major cells in the collecting duct.

1. On the luminal side, the membrane contains H⁺-K⁺ ATPase that pumps H⁺ into the cell and K⁺ into the tubular fluid (Fig. 78.10).
2. In the cell, H⁺ and HCO₃⁻ are formed from dissociation of H₂CO₃. H⁺ is secreted into the tubular lumen and HCO₃⁻ is reabsorbed into the interstitial space.
3. Thus, intercalated cells contribute to acidification of urine and conservation of K⁺.

**Reabsorption of Water**

Collecting duct has two parts: the cortical part and the medullary part. The reabsorption of solute and water from the collecting duct mainly depends on the efficacy of ADH acting on it.

**Cortical Part**

In the cortical part, ADH increases the permeability of collecting duct to water by inserting aquaporin-2 water channels, especially on the luminal membrane of the principal cells.
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1. ADH binds with V₂ receptor on the basolateral cell membrane, where it activates adenylate cyclase to increase the production of cAMP.
2. cAMP activates protein-kinase A, which promotes the insertion of vesicles containing aquaporin-2 on the luminal surface of the epithelial cells (Fig. 78.12) that increases water permeability.
3. cAMP also stimulates gene transcription that promotes formation of new aquaporin 2 in the cell.

In Medullary Part
In this portion of the collecting duct osmolality of interstitium is very high.
1. Water is reabsorbed from the tubular fluid into the hypertonic medullary interstitium by osmotic effect, which accounts for about 5% of water reabsorption.
2. This plays an important role in counter-current mechanism and makes the urine concentrated.

In human beings, in the presence of adequate ADH, osmolality of urine may reach 1400 mosm/kg of H₂O (99.7% of filtered water is reabsorbed). In the absence of ADH, the cells of collecting duct are relatively impermeable to water. Therefore, urine becomes hypotonic (osmolality as low as 30 mosm/kg of H₂O) in the absence of ADH (Clinical Box 78.8). However, collecting duct is not totally impermeable to water and, therefore, even in complete absence of ADH, about 2% filtered water is reabsorbed.

Clinical Box 78.8
Diabetes Insipidus: This is a condition of excess urine formation (polyuria) that occurs due to ADH deficiency (neurogenic DI) or failure of collecting duct to respond to the hormone (nephrogenic DI). The nephrogenic form, which is an X-linked recessive type, occurs due to mutation of the gene for V2 receptors.

Other Tubular Dysfunctions

Liddle’s Syndrome
This is a rare autosomal dominant disorder characterized by clinical features similar to hyperaldosteronism consisting of increase in ECF volume, hypertension, hypokalemia, and metabolic alkalosis. However, aldosterone and renin level in plasma are less.
1. In this disease, the amiloride-sensitive Na⁺ channels become hyperactive, which results in reabsorption of inappropriately large quantity of Na⁺ and increased K⁺ excretion from the tubular fluid.
2. This is accompanied by reabsorption of large quantity of water. Thus, ECF expansion occurs that causes hypertension.

Pseudohypoaldosteronism
There are two forms of pseudohypoaldosteronism: Type I and type II.

Pseudohypoaldosteronism Type I
This is a rare autosomal disease, which is just opposite to that of Liddle’s syndrome. The dominant form is due to the mutation of mineralocorticoid receptor gene and the recessive form is due to the mutation of amiloride-sensitive epithelial Na⁺ channels. The disease is characterized by significant reduction in ECF volume and, therefore, the condition is associated with hypotension. The decreased Na⁺ channels and decreased aldosterone activity in the tubule impair Na⁺ reabsorption. Consequently, increased Na⁺ excretion is accompanied by excretion of large amount of water. This causes contraction of ECF compartment and hypotension.

Pseudohypoaldosteronism Type II
In type II pseudohypoaldosteronism, (also called as Gordon syndrome), an autosomal dominant disease, hyperkalemia, hypertension and metabolic acidosis occur secondary to overactivity of thiazide-sensitive Na⁺-Cl⁻ cotransporter in DCT.
Gitelman’s Syndrome

This is an autosomal recessive disorder that has similarity with features of Bartter syndrome like hypokalemia, metabolic alkalosis, salt wasting, elevated renin and aldosterone level, and normal BP. However, hypomagnesemia and hypocalciuria differentiates it from Bartter syndrome.

1. The disease occurs due to mutation of thiazide-sensitive Na\(^+\)-Cl\(^-\) transporter.
2. Decreased Na\(^+\) reabsorption in DCT leads to volume depletion and hypokalemia.

Summary of Tubular Functions

From the above discussion on tubular functions, we learn that nephron is functionally divided into three parts: proximal segment, middle segment and distal segment.

Proximal Nephron

The proximal nephron is the proximal tubule that absorbs about 67% of the solutes and water (Fig. 78.13). Proximal nephron is nonselective and reabsors solute and solvent maximally. Thus, it greatly contributes to electrolyte and volume homeostasis.

1. In proximal nephron, Na\(^+\) and water reabsorption are closely coupled as the epithelial water permeability is very high and paracellular pathway of absorption of solute and water is well developed (as the tight junctions between the cells are leaky).
2. Inspite of a small osmotic gradient across tubular epithelium, the quantity of water reabsorbed is large due to greater permeability of PCT to water.
3. However, the tubular fluid is isosmotic.

Middle Nephron

Loop of Henle constitutes the middle segment of the nephron.

1. In this segment, reabsorption of solute in excess of water contributes to hyperosmolality of medullary interstitium and makes the tubular fluid hypotonic.
2. Thus, LOH plays an important role in generating hyperosmolar interstitium that is essential for counter current mechanism of urine concentration.

Distal Nephron

DCT and collecting duct form the distal nephron. Distal nephron is selective in reabsorption and absorbs smaller quantity of water and solutes. About 8% of Na\(^+\) and 18–20% of water is reabsorbed in this part of the tubule (Fig. 78.14).

1. Na\(^+\) and water reabsorption are not coupled as water permeability is very poor in distal nephron.
2. The tubular fluid is hypotonic.
3. Inspite of a steep osmotic gradient across the epithelium, reabsorption of water is extremely poor in the absence of aldosterone and ADH.
4. The paracellular route of transport is not well developed as the junctions between epithelial cells are really very tight.
5. Distal nephron contributes to concentration and acidification of urine.

REGULATION OF IONS AND WATER REABSORPTION

Regulation of NaCl and Water Reabsorption

Normally, of the total Na\(^+\) is filtered (26,000 meq) of in the glomerulus per day, 99.4% (25,850 meq/day) is reabsorbed in the tubule. Of the filtered Na\(^+\), about 67% is reabsorbed in the PCT, 25% in LOH, 5% in DCT and 3% in collecting duct (Fig. 78.15).
1. Reabsorption of $\text{Na}^+$ facilitates the reabsorption of many other solutes and establishes osmotic gradient for reabsorption of water.
2. The total urinary $\text{Na}^+$ output ranges between 1 meq and 400 meq (average about 150 meq) per day, depending on the dietary salt intake of the person.
3. Regulation of $\text{NaCl}$ and water reabsorption is primarily the function of various hormones acting on kidneys.
4. Neural factors, starling forces, tubuloglomerular feedback and glomerulotubular balance also play role in $\text{Na}^+$ regulatory mechanisms.

**Hormonal Factors**

The major hormones that influence $\text{NaCl}$ and water reabsorption form kidney are angiotensin II, aldosterone, ADH, dopamine, glucocorticoid, urodilatin and ANP.

**Angiotensin II**

Angiotensin II acts mainly on proximal tubule of the kidney. It increases $\text{NaCl}$ and water reabsorption and causes expansion of ECF volume. It is secreted in conditions of hypovolemia and hypotension.

**Clinical Correlation:** Captopril that inhibits angiotensin converting enzyme (ACE inhibitor) decreases ECF volume and blood pressure. This prevents formation of angiotensin I to angiotensin II. Therefore, decreased concentration of angiotensin II decreases $\text{NaCl}$ and water reabsorption from proximal tubule, and decreases vasoconstriction. This is used for the treatment of hypertension and in conditions of ECF volume expansion.

**Aldosterone**

Aldosterone stimulates reabsorption of $\text{NaCl}$ and water from collecting duct. It acts on $\text{Na}^+-\text{K}^+$ pump and ENaC.
facilitates K⁺ excretion in tubular fluid. Thus, it increases Na⁺ concentration in the plasma and decreases K⁺ concentration. Among the important regulator of aldosterone secretion are hyperkalemia and angiotensin II.

**ADH**

This is an important hormone for volume homeostasis. It is secreted from posterior pituitary in response to hyperosmolality of plasma and hypovolemia. It increases the permeability of collecting duct to water by incorporating water channels (aquaporins).

**Dopamine**

Dopamine inhibits NaCl and water reabsorption in the proximal tubule. Thus, it causes natriuresis and diuresis. Dopamine secretion increases in expansion of ECF volume.

**Glucocorticoids**

Glucocorticoids have mild mineralocorticoid (aldosterone) activity. They increase water and NaCl reabsorption.

**Urodilatin**

This is a polypeptide hormone containing 32 amino acids secreted by the distal tubule and collecting duct. It acts on the kidney tubule locally. It acts exclusively on collecting duct to inhibit NaCl and water reabsorption from the medullary part of the collecting duct. It is a potent natriuretic and diuretic hormone like that of ANP. However, recently the validity of this hormone has been questioned.

**ANP**

This is a polypeptide hormone containing 28 amino acids, secreted from myocytes of atria of the heart. It acts on distal tubule and collecting duct to inhibit NaCl and water reabsorption. Thus, it produces natriuresis and diuresis. It is secreted in response to ECF expansion and hypertension.

### Neural Factors

Neural regulation is mainly through sympathetic innervation of kidney that controls water and NaCl reabsorption.

1. Activation of sympathetic fiber results in increased NaCl and water reabsorption from the proximal tubule, thick ascending limb of LOH, distal tubule, and collecting duct.
2. Actions are mediated via release of norepinephrine, and epinephrine from the sympathetic nerve endings locally.

### Starling Forces

Starling forces regulate NaCl and water reabsorption across the proximal tubule. The peritubular capillary oncotic pressure and the hydrostatic pressure in the interstitial space favor this process. The interstitial space oncotic pressure and peritubular capillary hydrostatic pressure oppose this process. When solutes are reabsorbed from the tubular cells into the interstitial space, osmolality of the interstitium increases, this favors movement of water from tubular fluid into the interstitium via transcellular and paracellular pathways.

### Tubuloglomerular Feedback

Signals originating from renal tubules provide feedback for control of glomerular filtration, which is called as tubuloglomerular feedback.

1. In this process, the rate of flow of and the concentration of NaCl in the tubular fluid of thick ascending limb of LOH and initial part of distal tubule provides feedback information to the glomerulus to control the rate of filtration.
2. When the rate of flow through the tubule increases, rate of filtration decreases and when the rate of tubular flow decreases, rate of filtration increases in the same nephron.
3. Thus, the change in glomerular filtration maintains a constancy of tubular load, i.e. a constant volume of fluid delivered to the tubule.

### Role of Macula Densa

The macula densa in the terminal part of thick ascending limb of LOH act as sensor that detects the rate of flow of fluid through it.

1. Macula densa cells provide signal to the afferent arteriole. When tubular flow is high, concentration of Na⁺ and Cl⁻ in tubular fluid becomes high.
2. More quantity of Na⁺ and Cl⁻ enters the macula densa cells via 1Na⁺-1K⁺-2Cl⁻ cotransporter located in their apical membrane. Increased Na⁺ in the cell increases Na⁺-K⁺ ATPase activity.

3. Increased ATP hydrolysis results in formation of more adenosine. Adenosine acts on the A₁ adenosine receptors to increase the release of Ca²⁺ from macula densa cells that causes constriction of afferent arteriole (Flowchart 78.1).

4. Thus, glomerular filtration is decreased. Opposite mechanism operates when tubular flow decreases. It is also believed that a similar mechanism operates to control renin secretion from JG cells that influence glomerular function through angiotensin II. Also, change in diameter of the afferent arteriole is mediated by secretion of chemicals like thromboxane A₂ and prostaglandins locally. Alteration in glomerular filtration is also achieved by changing the surface area for filtration, which is brought about by constriction or relaxation of the mesangial cells.

**Glomerulotubular Balance**

Increase in GFR increases the load of solutes, especially of Na⁺ in the tubular fluid. This increases reabsorption of Na⁺ from the proximal tubule. Normally, a constant fraction of filtered solute is reabsorbed. That means, a constant percentage of the tubular load is reabsorbed by the proximal tubule. This is called glomerulotubular balance.

There are two possible mechanisms for glomerulotubular balance:

1. Change in oncotic and hydrostatic pressures between peritubular capillaries and lateral intercellular space: This mechanism is more effective for Na⁺. Increase in GFR increases protein concentration in the blood that leaves glomerular capillary, i.e. protein content becomes more in efferent arteriole. Efferent arteriolar blood enters the peritubular capillaries. Therefore, oncotic pressure in the peritubular capillaries increases, which causes increased Na⁺ reabsorption from tubular fluid. This is also accompanied by increased fluid movement into the interstitial space (lateral intercellular space) and then from there into the blood.

2. Increased load on the tubule: With increased GFR, filtered load of Na⁺, glucose and amino acids on the tubule increases. Increased Na⁺ reabsorption is associated with increase in glucose and amino acid reabsorption through the same carrier proteins. Thus, more solutes are reabsorbed.

**Regulation of K⁺ Excretion**

K⁺ is the primary intracellular cation. Its gradient across the cell membrane is mainly determined by the electrical potential across the membrane. As excitability of nerves and muscles depends on this electrical potential, precise control of K⁺ level in plasma is very essential, in which kidneys play an important role.

1. Normally 600 meq of K⁺ is filtered in 24 hours of which 99.3% (560 meq) is reabsorbed. The major reabsorption of K⁺ takes place in the proximal tubule, which removes about 65% of its filtered load.

- In thick ascending portion of LOH, about 25–30% of K⁺ reabsorption takes place.
- However, about 50 meq of K⁺ is secreted from the distal tubule. Thus, the net excretion of K⁺ in urine is about 90 meq/day.
- Minor reabsorption and secretion of K⁺ also take place in collecting duct, especially by the intercalated cell (Fig. 78.16).

2. The rate of secretion of K⁺ by distal tubular cells is proportionate to the rate of flow of fluid through it.
When the flow of fluid in the distal tubule is rapid, the secretion is more. This is because with rapid flow, the K⁺ in the tubular fluid gets less opportunity to prevent K⁺ secretion.

In the distal tubule, usually Na⁺ is reabsorbed and K⁺ is secreted. Though, the transport of K⁺ is mainly passive, movement of Na⁺ from the tubular fluid decreases the potential difference across the tubular cells; thus, K⁺ movement into the tubular lumen is favored.

Na⁺ is reabsorbed along with secretion of H⁺ into the lumen. Therefore, a competition between H⁺ and K⁺ exists for exchange with Na⁺. When K⁺ in the body increases H⁺ secretion decreases and when H⁺ secretion increases K⁺ secretion is inhibited. K⁺ secretion is mainly regulated by aldosterone.

Fig. 78.16: Transport of K⁺ in different parts of the nephron.

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**CHAPTER SUMMARY**

**Key Concepts**

1. Functionally nephron is divided into three parts: proximal segment, middle segment and distal segment.
2. The proximal nephron is the proximal tubule that absorbs about 67% of the solutes and water. Na⁺ and water reabsorption are closely coupled as the epithelial water permeability is very high and paracellular pathway of absorption of solute and water is well developed.
3. Loop of Henle constitutes the middle segment of the nephron. In this segment, reabsorption of solute in excess of water contributes to hyperosmolality of medullary interstitium and makes the tubular fluid hypotonic. It plays important role in generating hyperosmolar interstitium that is essential for counter current mechanism of urine concentration.
4. DCT and collecting duct form the distal nephron. In DCT, reabsorption of water is extremely poor in the absence of aldosterone and ADH.

**Important to Know (Must Read)**

1. In examination, ‘Describe the mechanism of Na⁺ and glucose reabsorption in proximal renal tubule’ may be asked as Long Questions.
2. Mechanism of glucose reabsorption in PCT, Mechanism of Na⁺ reabsorption in PCT, Functions of Loop of henle, Tubuloglomerular feedback, Glomerulotubular balance, Mechanism of water reabsorption in CD, Mechanism of solute transport in CD, Mechanism of transport of organic solutes PCT, Renal threshold, transport maximum, Renal splay, may be asked as Short Questions in exam.
3. In Viva, examiner may ask… List the ion transporters present in the PCT and list the function of each, List the ion transporters present in the thick limb of LOH and list the function of each, What is the ion transport present in the DCT and what is its function, What is the ion transporter present in the CD and what is its function, What is the percentage of Na⁺ reabsorption in different parts of renal tubule, List the hormones regulating NaCl and water reabsorption from kidney tubule, What is tubuloglomerular feedback and what is its mechanism, What is glomerulotubular balance and what is its mechanism, What is the mechanism of glucose reabsorption in PCT, What is T_m,c and what is its value, What is renal threshold for glucose and what is its value, What is renal splay and what are the reasons for it, List the cell types present in the collecting duct and give their functions, What is the mechanism of water reabsorption in CD, What is the mechanism of action of K⁺ sparing diuretics, give example, What is the mechanism of action of loop diuretics, give example, What is the mechanism of chloride reabsorption in PCT, What is the mechanism of K⁺ excretion from kidney tubule, What is the mechanism of protein reabsorption in PCT, What are the sources of protein that appears in urine in proteinuria, What are the mechanisms of reabsorption of organic solutes in PCT, What is Fanconi syndrome and what are its causes and features, What is Dent’s disease and what are its causes and features, What is Bartter syndrome and what are its causes and features, What is Liddle’s syndrome and what are its features, What is pseudohypoaldosteronism and what are its types and features, What is Gitelman’s syndrome and what are its features.
CHAPTER 79
Mechanisms of Urine Concentration and Dilution

LEARNING OBJECTIVES
On completion of study of this chapter, the student **MUST** be able to:
1. Acquire the concept of a counter current system.
2. Understand and describe the countercurrent mechanism of urine concentration.
3. Appreciate the importance of urine concentration.
Student **MAY** also be able to:
1. Explain the application of counter current mechanism in different situations.

GENERAL CONCEPT
In human beings, urine can be formed with an osmolality of as high as 1400 mosm/kg of H₂O and as low as 30 mosm/kg of H₂O. Such a capability of kidneys to pass urine with extremes of osmolality is a fundamental necessity for volume and electrolyte homeostasis. Though glomerular filtrate is about 180 liters a day, only about 1.5 liters of urine is excreted daily. Therefore, normally, kidney excretes a concentrated urine to prevent volume depletion from the body, in which **osmolality of urine is an index of its concentrating and diluting capacity**.

1. **Under physiological conditions**, urine osmolality is a function of solvent (water) excretion. That means, when kidney excretes osmotically concentrated urine, in effect, water output is reduced considerably, and when kidney excretes osmotically dilute urine, water excretion is increased significantly.
   - Thus, when kidney takes the responsibility of regulating the ECF volume of the body by changing its solvent output, the solute concentration automatically changes, which changes the urine osmolality.
   - For example, when the individual is exposed to a desert like condition or to a high environmental temperature, **kidney maintains effective blood volume by reducing the urine output**, and in the process it excretes concentrated urine. In fact, for excreting urine with osmolality of 1400 mosm/kg of H₂O, kidney excretes about 0.5 L of urine in a day. Thus, it prevents dehydration and hypovolemia by saving about 1 L of effective blood volume (as normal urine output is about 1.5 L per day).
   - By executing this regulatory process, kidney decreases the amount of water that we are obliged to drink (as water may not be available) in such extreme conditions. Hence, in a true sense, kidney is the best friend of human beings as it reduces the obligatory task of drinking water in adverse situations. Conversely, kidney also has the task of **excreting a water load** from the body by forming a large quantity of dilute urine.

2. **In pathological conditions**, increased urine osmolality is due to change in solute output, and **excretion of water is secondary to the change in osmolality** of tubular fluid.
   - For example, in diabetes mellitus, glycosuria results in osmotic diuresis. In such a condition, increased excretion of solute in urine is accompanied by increased volume output that results in dehydration.
   - Thus, in disease conditions, water loss in urine is a forced phenomenon in response to solute load in the urine, in which kidney fails to regulate the volume loss, and therefore dehydration sets in.
   - Thus, mechanisms and the consequences of increased urine osmolality in physiological and pathological situations are in effect, have different perspectives.
Diluting and Concentrating Ability of Kidney

Normally, the change in urine osmolality is brought about mainly by the action of ADH on collecting duct of kidney. In the presence of ADH, urine osmolality may be as high as 1400 mosm/kg of H₂O with volume of urine at about 0.5 liter per day (0.27% of glomerular filtrate) and in the absence of ADH, urine osmolality may fall to as low as 30 mosm/kg of H₂O with urine volume at about 23 liters per day (more than 12.7% of glomerular filtrate). In diabetes insipidus, which occurs due to ADH deficiency, urine output is more than 20 liters per day.

1. Thus, ADH, which is an external factor for kidney plays an important role in urine concentration and dilution. However, also kidney has its own intrinsic mechanism to concentrate and dilute urine.
2. To produce diluted urine to excrete a volume overload is a rare physiological phenomenon.
3. But, to form concentrated urine to prevent volume loss, which is encountered frequently in life, is a challenge to and responsibility of the kidneys.
4. As such, normally kidneys excrete concentrated urine, which is less than 1% of glomerular filtrate. Kidneys further concentrate urine in the presence of volume contraction.

Thus, kidney is well equipped with an inherent and well developed mechanism to concentrate urine in normal as well as abnormal conditions, the mechanism which is known for more than 50 years as countercurrent mechanism of urine concentration.

Requirements of a Counter Current Mechanism

The mechanism by which urine is concentrated is known as countercurrent mechanism. In medulla of the kidney, there is an increasing gradient of osmolality from outer region to inner region of medulla with highest osmolality at the tip of the renal papillae. This osmolality gradient, which is produced and maintained by the counter current mechanisms, is essential for urine concentration. Countercurrent means flow of fluid in opposite direction in adjacent structures. For any countercurrent mechanism to operate, it requires three conditions:

1. There should be two tubes (inflow and outflow tubes) that should run parallel to each other.
2. The movement of fluid in one tube should be in opposite direction to the other.
3. The tubes should be in close proximity to each other and should be selectively permeable.

The best example is the maintenance of air temperature in a furnace, in which the input duct carries cool air from outside into the furnace and output duct carries hot air from the inner furnace to outside. The temperature of the hot air in the output duct is exchanged with the cool air of the input duct as both of them are in close thermal contact with each other (Fig. 79.1). Thus, temperature of fresh air of the input pathway increases as it passes deep into the furnace and becomes maximum at the tip (deepest part) and the temperature in the output pathway decreases as air comes out of the furnace. As a result, inner temperature of the furnace circulates in the furnace without much loss to the environment. Consequently, a high temperature is maintained inside the furnace. Similar mechanism is also applied for preserving heat in heating the air of a building. Heat preservation of such systems becomes more effective when the passive diffusion of heat from the ascending limb is replaced by or added with active pumps that push heat forcefully out of the ascending pathway into the descending pathway.

In kidney, countercurrent mechanisms of similar nature operate. Two countercurrent processes work in the medulla of the kidney: countercurrent multiplication system and countercurrent exchange system. Loop of Henle (LOH) acts as countercurrent multiplier and vasa recta as countercurrent exchanger.

1. In LOH, fluid flows deep into the medulla along the descending limb and from medulla into the cortex along the ascending limb. The descending and ascending limbs for their selective permeabilities for water and solutes, establish an osmotic gradient in the medulla.
2. In vasa recta, blood flows in opposite direction in descending and ascending vessels during which water and solutes are exchanged passively between capillary blood vessels. This passive exchange in vasa recta maintains the osmotic gradient.
3. Collecting duct is juxtaposed to LOH and vasa recta (Fig. 79.2), and acts as an osmotic equilibrating device.

![Fig. 79.1: Mechanism of counter-current heat exchange between inflow (downward arrow) and outflow (upward arrow) tubes of a furnace. Note, cooler air of inflow tube exchanges heat with hot air of outflow tube, so that the heat is maintained in the inner furnace. Thick outer wall of inflow and outflow tubes are meant to insulate the tubes.](image)
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Counter current mechanism also operate in other vascular beds (Application Box 79.1).

**Application Box 79.1**

Counter current mechanism helps penguin to stand on ice for a long:

Ability of the kidney to concentrate urine is a critical adaptation of life on land. Counter current mechanisms not only operate in medulla of kidney, but also in many vascular beds where the blood vessels lie close to each other and allow exchange of solute, solvent and temperature between them. For example, exchange of heat between the inflowing and outflowing vessels of the feet of penguin helps penguin to maintain its body temperature when it stands on ice for a long.

**Countercurrent Multiplication System**

This is the process in which a small osmotic gradient established at any level of LOH is multiplied into a larger gradient. The osmotic gradient created at any level of LOH is called single effect, which is due to movement of solute out of the water impermeable ascending limb of LOH. Solute deposition in medulla is accompanied by removal of water from the descending limb.

As tubular fluid in the loop enters the deeper layers of medulla, it is more concentrated that creates increasing gradient of osmolality along the axis of the loop, called axial gradient. Magnitude of the axial gradient depends on three main factors:

1. The rate of fluid flow
2. Strength of single effect
3. The length of LOH.

Slower the fluid flow, larger the single effect and greater the loop length, the bigger is the axial gradient. Conversely, faster the fluid flow, smaller the single effect and shorter LOH reduces the axial gradient.

When the rate of flow is slower, time taken for transport of solutes in the ascending limb of LOH is more, and therefore, the strength of single effect and consequently the magnitude of axial gradient is enhanced. When LOH is longer, a greater axial gradient is generated as the chance for multiplication becomes higher.

**Countercurrent Exchange System**

The solute gradient created in the medulla by LOH is maintained by vasa recta.

1. The exchange of solutes and water takes place purely passively between descending and ascending limbs of vasa recta and this process of exchange decreases the dissipation of the solute gradient from medullary interstitium.
2. Water is removed from descending limb of vasa recta into the hyperosmolal interstitium, which is taken back by ascending limb of vasa recta (Fig. 79.3).
3. Thus, vasa recta prevents dilution of osmolality of interstitium.
4. Moreover, solutes come out of ascending limb and enter the descending limb of LOH, which ensures trapping of solutes in the medulla.
5. Thus, the process of exchange of water and solutes between descending and ascending limbs of vasa recta maintains the gradient of osmolality established by LOH.

**COUNTERCURRENT MECHANISM**

The countercurrent mechanism for urine concentration depends on generation and maintenance of an increasing osmolality gradient along the medullary pyramid from its outer layer to its deepest part. As discussed above, two systems operate to generate and maintain the high osmolality gradient in the medullary interstitium.

1. The osmolality gradient is generated by the loop of Henle, which acts as countercurrent multiplier, and maintained by vasa recta, which acts as countercurrent exchanger.
2. However, collecting duct contributes in this process by equilibrating the gradient (acts as equilibrating device). Thus, renal countercurrent mechanism depends on integrated functioning of loop of Henle, vasa recta and collecting duct.

**Role of Loop of Henle: The Counter-Current Multiplier**

The loop of Henle acts as counter current multiplier. About 30% of the volume originally filtered by the renal corpuscle enters LOH, which is isosmotic. In LOH, selective reabsorption of solutes and water occurs in its different segments that contribute to generate higher osmolality in medullary interstitium.
1. **Descending limb of LOH is highly permeable to water**, but *impermeable to solutes*. Therefore, fluid that flows down in the descending limb becomes increasingly hypertonic as water is selectively removed. Thus, fluid in the tip of the loop is highly concentrated and its osmolality is more than the interstitial osmolality.

2. **Thin ascending limb of LOH** is passively permeable to solutes. Due to high osmolality of fluid at the tip of the hairpin loop and in thin ascending limb of LOH, and the permeability of thin ascending limb to solutes, solutes are passively transported from the hypertonic tubular fluid in to the interstitium. However, the thin ascending limb of LOH is relatively impermeable to water. Therefore, every time the tubular fluid passes through the thin ascending limb of LOH, extra solutes enter the interstitium. This helps in multiplication of the interstitial osmolality.

3. Moreover, in **thick ascending limb of LOH**, Na\(^+\) and Cl\(^-\) are actively transported from the tubular fluid due to vigorous activity of Na\(^+\)-K\(^+\) ATPase in this part of the loop (Fig. 79.3). This actively removes solutes from the tubular fluid into the interstitium and considerably aids in building up of a higher interstitial osmolality. This makes the tubular fluid entering the distal tubule hypotonic.

The countercurrent multiplier role of LOH depends on its length. LOH of medullary nephrons are much longer than that of cortical nephrons. Therefore, though cortical nephrons are considerably more than medullary nephrons, higher osmolality is built only in the medullary interstitium. This is because more Na\(^+\) and Cl\(^-\) move down their concentration gradient along a longer thin ascending limb into the interstitium and also more are actively transported along a greater length of thick ascending limb of LOH.

### Role of Collecting Duct
Collecting duct acts as osmotic equilibrating device. It contributes to urinary concentration by **two ways**: first by its permeability to water and second for its permeability to urea.

1. **Collecting duct is permeable to water**. Therefore, *water moves out of tubular fluid* in the collecting duct into the hyperosmolar interstitium both in the cortical and medullary part. This *makes the tubular urine hypertonic*.

2. **Urea transported in collecting duct** also greatly helps in generating osmotic gradient in the medullary pyramid. In renal tubule, some quantity of urea is reabsorbed in PCT. However, except in the inner medullary portion of the collecting duct, the rest of tubular epithelium is relatively impermeable to urea. Some amount of urea is secreted into tubular fluid in the descending limb of LOH. Therefore, by the time tubular fluid reaches the medullary part of collecting duct, its urea concentration is very high. In the inner medulla, *urea moves from the tubular fluid into the interstitium passively*. When urea is removed from tubular fluid into the interstitium, *water moves out of tubular lumen* along with urea to maintain osmotic balance. Thus, *urine is*
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**Fig. 79.4:** Osmotic gradient across the medulla of kidney from its outer regions to the inner regions. Note, the tubular fluid and blood in vasa recta entering into medulla becomes gradually hyperosmolar and fluid and blood leaving medulla becomes gradually hypoosmolar. This occurs due to mechanisms as described in Figure 79.3. Thus, interstitial osmolality (mosm/kg of water) increases gradually from outer layers to inner layers of medulla reaching about 1200 mosm/kg of water in innermost part. This osmolal gradient transfers water from tubular fluid of collecting duct leaving the medulla that makes urine concentrated. Arrows indicate direction of movement. (VR: Vasa recta).

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more concentrated. Water that enters the interstitium is taken up by vasa recta (see below) and consequently osmolar dilution of interstitium is prevented.

**Urea Transporters:** The movement of urea in collecting duct of kidney is facilitated by urea transport protein A1 (UT-A1). There are two types of urea transporters that help urea transport by facilitated diffusion: urea transporter A (UT-A) and urea transporter B (UT-B). UT-A is found in kidney and UT-B in red cells. In kidney, UT-A has four subtypes: UT-A1 to UT-A1-4. The UT-A1 is influenced by ADH. Therefore, ADH facilitates urea reabsorption in kidney. The transport of urea and water in the collecting duct further helps in building up of hyperosmolality of the interstitium (Application Box 78.2).

Application Box 78.2

Protein content of diet controls concentration of urine: Urea content of tubular fluid depends mainly on the urea filtered in the glomerular filtering membrane, which depends on the protein content of the diet. Low-protein diet impairs the ability of kidney to maximally concentrate urine as this decreases the urea content in the medullary interstitium. Conversely, high protein diet makes the urine concentrated.

**Role of Vasa Recta: The Counter-Current Exchanger**

The concentration gradient generated by loop of Henle will not be long lasting if NaCl and urea accumulated in the interstitium are removed by circulation. However, the osmolal gradient is maintained by the vasa recta, which acts as counter current exchanger.

1. **In the descending limb** of vasa recta, **solute diffuses into the vessels** (that carries blood into the medullary pyramid), and in the ascending limb **solute diffuses out of the vessel** that carries blood toward the cortex.
2. Thus, solutes keep circulating in the interstitium. The movement of water is opposite to the movement of solutes.
3. **Water diffuses out of the descending limb and into the ascending limb** of the vasa recta (Fig. 79.3). Thus, while solutes recirculate in the medulla, water is removed from it. Therefore, hypertonicity of the interstitium is maintained.
4. Water in the vasa recta enters general circulation and helps in maintaining ECF volume.

**Net Effect**

Every time tubular fluid passes through LOH, water comes out of descending limb into interstitium (which is removed by ascending limb of VR) and solutes come out of ascending limb (NaCl diffuses passively into interstitium in thin ascending portion and pumped actively into interstitium in thick ascending limb).

1. A longer LOH accumulates more solutes across its length from outer layers into deeper layers of medulla. Thus, osmolality increases along LOH as it dips into the medulla (Fig. 79.4).
2. This causes a higher osmolality in the medullary interstitium, which is further enhanced by diffusion of urea from the collecting duct into it.
3. Water also moves out passively from collecting duct, but water is removed from interstitium by vasa recta.
4. Consequently, an osmolality of about 1200 to 1400 mosm/kg of H$_2$O is maintained in the deepest portion of medulla.
5. A maintained higher osmolality in medulla causes transfer of water from collecting duct and makes urine concentrated.

CHAPTER SUMMARY

**Key Concepts**

1. The counter-current system is meant mainly for concentration of the urine.
2. Loop of Henle acts as counter current multiplier, in which a small osmotic gradient established at any level of LOH is multiplied into a larger gradient. The osmotic gradient created in the medullary interstitium reabsorbs water from the tubule & makes urine concentrated.
3. Vasa recta is the counter-current exchanger. The solute gradient created in the medulla by LOH is maintained by vasa recta. It prevents dilution of osmolality of interstitium.

**Important to Know (Must Read)**

1. In examination, “Describe the countercurrent mechanism of urine concentration” is usually asked as Long Questions.
2. Explain the role of the loop of Henle as countercurrent multiplier, Explain the role of the vasa recta as countercurrent exchanger, Explain the role of collecting duct in countercurrent mechanism in kidney, may be asked as Short Questions in exam.
3. In Viva, examiner may ask... List the conditions required for the operation of any countercurrent mechanism, Give examples of countercurrent mechanisms that operates in different body systems, How does the loop of Henle act as countercurrent multiplier, What are the three factors on which the magnitude of the axial gradient depends, What is called single effect, What is the osmolality of the filtrate in the PCT and at the tip of the loop of Henle, How does the vasa recta act as countercurrent exchanger, How does the protein content of the diet control the concentration of urine, What is the role of collecting duct in countercurrent mechanism in kidney, What is the role of urea in countercurrent mechanism in kidney.
Learning Objectives

On completion of study of this chapter, the student **MUST** be able to:
1. Understand the mechanism of water reabsorption from kidney tubule.
2. Understand the concept of obligatory and facultative reabsorption of water.
3. List the difference between water and osmotic diuresis.
4. Describe the mechanism of water reabsorption in kidney tubules.
5. List the diuretics and give their mechanism of action.
6. Appreciate the functions of aquaporins

The student **MAY** also be able to:
1. Describe the physiological basis of use of diuretics.

Water Excretion

Controlled excretion of urine from the body is an important function of kidney. The volume of urine is determined by the quantity of water reabsorbed from renal tubule. Normally, kidney reabsorbs more than 99% of the filtered water. Therefore, though the GFR is about 180 liters per day, only 1 to 1.5 liters of urine is formed in 24 hours. Even when, more than 23 liters of urine is formed per day as occurs in ADH deficiency, kidney absorbs about 87% of the filtered water. Thus, the capacity of kidney to reabsorb water is enormous. This is the major mechanism of volume homeostasis of the body.

Types of Water Reabsorption

Water reabsorption in renal tubules is of two types: obligatory and facultative.

Obligatory Reabsorption

Obligatory reabsorption of water is the water reabsorption that occurs secondary to reabsorption of solutes. This accounts for about **85% of the total water reabsorption** in tubules and most part of it occurs in proximal tubule.

Facultative Reabsorption

Facultative reabsorption of water is the water reabsorption that occurs secondary to the effects of hormone, especially **ADH and aldosterone**. This accounts for about **15% of total water reabsorption** from kidney and occurs mostly in distal parts of nephron (in collecting duct and DCT).

Aquaporins

Transport of water across the membrane of epithelial cells depends on the presence of aquaporins, the water channels made up of proteins. Thirteen aquaporins have been identified till date. In human beings, four types of aquaporins have been characterized so far. These are aquaporin-1, aquaporin-2, aquaporin-5, and aquaporin-9. Aquaporins-1 and 2 are mainly involved in water reabsorption from kidney (Table 80.1).

Aquaporins in Kidney

Most of the aquaporins are found in kidney. Aquaporin-1 is found mainly in proximal tubule and loop of Henle, and...
Table 80.1: Distribution and functions of main 9 types of aquaporins.

<table>
<thead>
<tr>
<th>Types</th>
<th>Distribution</th>
<th>Functions</th>
<th>Expression in other tissues</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Renal aquaporins</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aquaporin 1</td>
<td>Apical and basolateral membrane of PCT and thin descending limb of LOH</td>
<td>67% of water reabsorption in PCT is due to AQP-1 Imparts free water permeability in thin descending limb of LOH</td>
<td>Multiple organs</td>
</tr>
<tr>
<td>Aquaporin 2</td>
<td>Apical membrane of late DCT and vesicles of principal cells of cortical part of CD</td>
<td>Mediate ADH effects</td>
<td>Testis</td>
</tr>
<tr>
<td>Aquaporin 3</td>
<td>Basolateral membrane of late DCT and cortical CD</td>
<td>Mediate ADH effects</td>
<td>Multiple organs</td>
</tr>
<tr>
<td>Aquaporin 4</td>
<td>Basolateral membrane of late DCT and cortical CD</td>
<td>Mediate ADH effects</td>
<td>Brain, other organs</td>
</tr>
<tr>
<td>Aquaporin 6</td>
<td>Vesicles of intercalated cells of CD</td>
<td>Acts as an internal ion channel of I cells</td>
<td></td>
</tr>
<tr>
<td>Aquaporin 7</td>
<td>Apical membrane of PCT</td>
<td>Water absorption in PCT</td>
<td>Testis and adipocytes</td>
</tr>
<tr>
<td>Aquaporin 8</td>
<td>Intracellular vesicles in cortex and medulla; in PCT</td>
<td>Exact function not known</td>
<td>Testis, epididymis, pancreas, liver, colon, heart, placenta</td>
</tr>
<tr>
<td><strong>B. Extrarenal aquaporins</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aquaporin 5</td>
<td>Salivary glands, lungs, eye</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aquaporin 9</td>
<td>Liver, leucocyte, brain, spleen, lungs, epididymis, testis</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Aquaporin-2** in the collecting duct. These aquaporins (aquaporins-1 and 2) control water reabsorption in renal tubules. Knock out of aquaporin 1 in PCT in experimental animals decreases water reabsorption by about 70%. These animals fail to maintain water balance in dehydration. This signifies the importance of aquaporin 1 in proximal tubule in water reabsorption (for details of aquaporins, refer page 594).

**Aquaporins in Extrarenal Tissues**

In addition, **aquaporin-5** is found in lacrimal gland and **aquaporin-9** is present in liver, spleen, lungs, and leucocytes. The role of aquaporins in extrarenal tissues is not clearly known.

**Structure and Functions of Aquaporins**

Aquaporins were discovered by Peter Agre and his team. Aquaporins have **six membrane spanning regions**, with intracellular membrane spanning both amino and carboxy terminals (Fig. 80.1). They form tetramers in the cell membrane, with each monomer acting as a water channel.

The distribution and function of main 9 types of aquaporins are listed in Table 80.1.

**Applied Aspects**

1. Nephrogenic diabetes insipidus is characterized by **decreased expression of aquaporin 2** in CD and DCT.
2. In chronic heart failure, there is increased expression of aquaporin-2.

**Mechanism of Water Reabsorption**

**In Proximal Tubule**

Normally, **65% of the filtered water** is reabsorbed in the proximal tubule.

1. The driving force for water reabsorption is the **transcellular osmotic gradient**, which is established by absorption of Na⁺ and accompanying solutes.
2. Permeability of epithelium of proximal tubule to water is extremely high, due to the presence of aquaporin-1 in the cell membranes.
3. Water also passes through the **water channels present in the paracellular route** (through tight junctions present between epithelial cells). Therefore, even a smaller osmotic gradient across the tubular cells results in adequate movement of water.
In LOH
The loop of Henle reabsorbs approximately 15% of the filtered water.
1. The ascending limb of LOH is impermeable to water and water reabsorption occurs mainly in the descending limb.
2. The water reabsorption in descending limb of LOH is a passive process that occurs secondary to higher osmolality of medullary interstitium.

In DCT
DCT is relatively impermeable to water. Inspite of osmotic gradient across the tubular epithelium, water reabsorption is poor. Only about 5% of the filtered water is removed in DCT, which is influenced by aldosterone and ADH. However, solute is removed actively in excess of solvent in this part of nephron.

In Collecting Duct
Collecting duct (CD) has two parts: cortical and medullary. Reabsorption of solute and water from the collecting duct mainly depends on the concentration of ADH acting on it. About 12–15% of water is reabsorbed in collecting duct.

Cortical CD
In the cortical part, ADH increases permeability of collecting duct to water by inserting aquaporin-2 water channels. ADH binds with V2 receptor on the basolateral membrane of tubular cell, where it activates adenylate cyclase to increase the production of cAMP. cAMP activates protein-kinase A, which promotes the formation and insertion of vesicle containing aquaporin-2 on luminal surface of the epithelial cells that increases water permeability. Therefore, in the presence of ADH, water moves out of relatively hypotonic tubular fluid into interstitium of the cortex, which accounts for a substantial reabsorption of water in CD in the presence of the hormone.

Medullary CD
In the medullary portion of the collecting duct, water is reabsorbed from the tubular fluid into the hypertonic medullary interstitium along the osmotic gradient, which accounts for 5–10% of water reabsorption. This plays an important role counter-current mechanism and makes the urine concentrated.

Diuresis
Diuresis is of broadly two categories: water diuresis and osmotic diuresis. Generally, diuresis occurs when osmolality of the tubular fluid is more or the reabsorptive capacity of the tubule for water is less. The former is called osmotic diuresis and the later is called water diuresis.

Water Diuresis
Water diuresis is the excretion of large volume of water without loss of excess of solute. This occurs when the capacity of the kidney tubule to reabsorb water is impaired.
1. Normally, the level of ADH determines the amount of water to be reabsorbed from the kidney.
2. Therefore, ADH deficiency, results in water diuresis. For example, in diabetes insipidus, urine is dilute and more in volume.
3. Water diuresis can be physiologically demonstrated by drinking a large amount of hypotonic fluid like water, in which polyuria occurs in 15 to 40 minutes of water ingestion.

Osmotic Diuresis
Increased concentration of osmotically active particles in the tubular fluid increases the excretion of water. This is called as osmotic diuresis.
1. This is typically seen in diabetes mellitus in which increased filtration of glucose (increased tubular glucose load) causes glycosuria, this causes osmotic diuresis. By osmotic effect, solutes hold water in the tubule (prevents reabsorption of water) that causes diuresis.
2. Osmotic diuresis is also produced by administration of mannitol. Mannitol is filtered in kidney but not reabsorbed and therefore, produces diuresis by its osmotic effect.
3. Osmotic diuresis can also be produced by infusion of large amount of NaCl or urea.

Diuretics
Diuretics are substances that promote diuresis (increased excretion of water in urine). In medical practice, diuretics are usually used to decrease the ECF volume. Frequently, administered diuretics are loop diuretics, aldosterone antagonists, thiazide diuretics, carbonic anhydrase inhibitors, xanthines, large quantities of osmotically active substances, ethanol, acidifying salts, vasopressin antagonists, and water.

Loop Diuretics
Furosemide (lasix) and ethacrynic acid are loop diuretics.
1. They act by inhibiting Na⁺-K⁺-2Cl⁻ cotransporter in the thick ascending limb of loop of Henle.
2. This inhibits Na⁺, K⁺, and Cl⁻ reabsorption causing diuresis with loss of these electrolytes.
Aldosterone Antagonists

Spironolactone is the commonly administered aldosterone antagonist.

1. It inhibits the action of aldosterone on the kidney tubules. Thus, it inhibits Na⁺-K⁺ exchange that leads to natriuresis and diuresis with retention of K⁺ in the body. Hence, it is called K⁺ sparing diuretic.

2. Amiloride and triamterene, other K⁺ sparing diuretics compete with sodium for a site in the ENaC (epithelial Na channel) and block sodium reabsorption and potassium secretion.

3. Thus, they produce diuresis with retention of K⁺.

Thiazide Diuretics

Usually administered thiazide diuretic is chlorothiazide. It inhibits Na⁺-Cl⁻ cotransporter in the early part of distal tubule. Thus, increased excretion of NaCl produces diuresis.

Carbonic Anhydrase Inhibitors

The usually administered diuretic is acetazolamide (diamox). As the name suggests, it inhibits carbonic anhydrase activity in renal tubular cells.

1. It decreases H⁺ secretion into tubular fluid.

2. Normally, H⁺ is exchanged with Na⁺ in luminal membrane of tubular epithelial cells by Na⁺-H⁺ antiport.

3. Therefore, decreased H⁺ secretion results in increase in Na⁺ (and also K⁺) excretion that leads to diuresis.

Xanthines

Xanthines such as caffeine and theophylline act as diuretics. They inhibit tubular reabsorption of Na⁺. They also increase GFR.

Osmotically Active Substances

Administration of large quantities of osmotically active substances like mannitol and glucose produce osmotic diuresis by increasing their concentration in tubular fluid.

Ethanol

Ethyl alcohol produces diuresis by inhibiting ADH secretion.

Acidifying Salts

Acidifying salts like calcium and ammonium chloride act as diuretics by increasing the tubular acid load. The H⁺ is buffered, but an anion is excreted with Na⁺ when the ability of kidney to replace Na⁺ with H⁺ is exceeded.

Vasopressin Antagonists

ADH antagonists inhibit the action of ADH on kidney tubules.

Water: Increased water consumption (hypervolemia) inhibits ADH secretion.

CHAPTER SUMMARY

Key Concepts

1. Diuresis is mainly for excreting excess water from the body when there is volume overload or ECF expansion.

2. In diabetes, osmotic diuresis occurs due to glycosuria.

3. Loop diuretics such as furosemide are commonly used in clinical practice.

Important to Know (Must Read)

1. In examination, no Long Question will be asked from this chapter.

2. Mechanism of water reabsorption in kidney, Types and mechanisms of diuresis, Aquaporins, may asked as Short Questions in exam.

3. In Viva, examiner may ask... What is obligatory reabsorption of water, What is facultative reabsorption of water, What is the mechanism of action of aquaporins, What is osmotic diuresis, give example, What is water diuresis, give example, List the important diuretics and give their mechanism of action.
Acidification of Urine

Learning Objectives

On completion of study of this chapter, the student MUST be able to:
1. Appreciate the purpose of acidification of urine.
2. Mention the factors that contribute to acidification of urine.
3. Learn the mechanisms of acidification of urine.

The student MAY also be able to:
1. Explain the mechanisms of acidification of urine.

General Concepts

Normally, pH of plasma is maintained within a narrow range of 7.35 to 7.45. To maintain this slender plasma pH range, major systems that work continuously and effectively are buffer systems, respiratory system and renal system. These three mechanisms work in concurrence. However, kidney plays an important role in long-term control of pH as most of the acid load from plasma is removed through renal system.

1. According to the increase or decrease of plasma pH, body excretes or retains acids and bicarbonates and urine becomes acidic or alkaline respectively.
2. Alkalization of urine is rare, which occurs mostly in pathological conditions.
3. However, during daily normal metabolism, acids are produced continuously. Therefore, unless acids are excreted, they accumulate in the body and produce acidosis.
4. In order to prevent acidosis, body excretes excess acids through kidney into urine, the process that leads to acidification of urine, which is a normal physiological phenomenon.

Excretion of acids through urine serves three important functions:
1. Eliminates extra acids that are harmful to our body. This prevents development of acidosis.
2. Contributes to normal acid-base balance of body.
3. Protects from urinary tract infection by preventing entry of pathogens by virtue of acidic pH of urine, which is especially important in females.

Factors Influencing Acidification of Urine

Four important factors contribute to acidification of urine:
1. Renal acid excretion
2. Renal bicarbonate reabsorption
3. Acid-base status of the body
4. Other factors (mainly hormonal).

Renal Acid Excretion

Kidney contributes to acid-base balance by excreting acid, which is proportionate to the production of non-volatile acids in the body. Kidney also prevents bicarbonate loss to achieve this purpose.

1. The major contribution to acid-base balance is by renal bicarbonate loss or gain as filtered load of HCO$_3^-$ (about 4300 meq/day) is much higher than the filtered load of non-volatile acid, which is about 80 meq/day.
2. Most of the filtered HCO$_3^-$ is reabsorbed. The reabsorption of filtered HCO$_3^-$ and excretion of acid are achieved through the process of H$^+$ secretion.
3. Most of the H$^+$ secreted into the tubular fluid, which is about 4400 meq/day, serves to reabsorb the filtered HCO$_3^-$, and only about 100 meq/day is excreted in urine. Therefore, normally urine is mildly acidic.
Renal Bicarbonate Reabsorption

Normally, the amount of bicarbonate reabsorbed from tubular fluid equals the amount filtered. Therefore, under normal conditions, HCO$_3^-$ is not excreted in urine.

1. About 80% of the total filtered bicarbonate is reabsorbed in PCT and 15% in thick ascending limb of LOH and 5% in the collecting duct (Fig. 81.1).
2. The mechanism of bicarbonate reabsorption in PCT involves Na$^+$-H$^+$ antiporter in the apical surface of the epithelial cells (for details, see below).
3. In the thick part of ascending limb of LOH, mechanism is similar to that in the PCT.
4. In DCT and collecting duct, reabsorption does not require Na$^+$-H$^+$ antiporter.
5. In intercalated cells in these parts of nephron, H$^+$ and HCO$_3^-$ are produced by hydration of CO$_2$, which is catalyzed by carbonic anhydrase. From the cell, H$^+$ is secreted into the tubular fluid by H$^+$-ATPase or K$^+$-H$^+$ ATPase and HCO$_3^-$ is added to interstitium by Cl$^-$-HCO$_3^-$ antiporter present in the basolateral membrane.

In PCT, permeability to H$^+$ and HCO$_3^-$ is more. Therefore, the pH of tubular fluid in the PCT is about 6.5. In collecting duct, permeability to H$^+$ is low. Therefore, tubular fluid in this part of the tubule is more acidic, having pH about 4.5. This ability of the collecting duct to reduce the tubular fluid pH is physiologically important for excretion of urinary buffers.

Formation of New Bicarbonate (Role of Ammonia)

Urinary ammonium excretion is intimately associated with acid-base balance.

1. Ammonium is important in HCO$_3^-$ formation. In the kidney, ammonium is produced by metabolism of glutamine.
2. Glutamine and Na$^+$ salts are the non-volatile acids. Kidney metabolizes glutamine, excrete NH$_4^+$ with acid-salts and add NaHCO$_3$ to the body.

3. In this process, new HCO$_3^-$ is formed which is dependent on the ability of the kidney to excrete the NH$_4^+$ in the urine (for details, see below).

Acid-Base Status of the Body

Systemic acid-base balance affects acid secretion and HCO$_3^-$ reabsorption and hence, acidification of urine. Therefore, acidification of urine cannot be addressed without mentioning acid-base balance.

1. In metabolic acidosis, acidification of intracellular fluid increases H$^+$ gradient between tubular cell and lumen. This results in increased H$^+$ secretion.
   - Acidification of intracellular fluid also increases the insertion of transporters into the apical membrane of the cells that increases the net H$^+$ ATPase activity.
   - Therefore, H$^+$ secretion is more, which in turn increases HCO$_3^-$ reabsorption, especially in PCT.
   - HCO$_3^-$ reabsorption is increased from the kidney to nullify the increased acidity of body fluids.
2. In metabolic alkalosis, HCO$_3^-$ reabsorption as well as acid secretion from kidney is decreased thereby causing a decrease in urine acidification rate.
   - In metabolic alkalosis, when plasma bicarbonate level goes beyond 28 mmol/L, renal threshold for bicarbonate reabsorption crosses its limits and the bicarbonate ions start appearing in the urine.
   - Hence, urine acidification drops down.
3. In respiratory acidosis, when pCO$_2$ increases, the H$^+$ formation increases by the action of carbonic anhydrase. This causes increased reabsorption of bicarbonate and acidification of urine.
4. The reverse happens in case of respiratory alkalosis.

Other Factors

Aldosterone, parathormone, angiotensin II and plasma K$^+$ contribute to acidification of urine.
1. **Aldosterone** increases H\(^+\) secretion from the tubular cells into the tubular fluid. Therefore, it contributes to urine acidification and alkali reabsorption in the kidney.

2. **Angiotensin II** secondarily affects Na\(^+\) concentration and Na\(^+\)-H\(^+\) exchange, hence affects urine acidification.

### MECHANISM OF URINE ACIDIFICATION

Acidification of urine is due to various mechanisms operating in different parts of the nephron, especially in PCT, DCT and collecting duct.

#### Acidification in Proximal Tubule

Increased CO\(_2\) in the body represents increased acidity in systemic circulation.

1. In proximal tubular cells, CO\(_2\) binds with H\(_2\)O to form carbonic acid. Dissociation of carbonic acid produces H\(^+\) and HCO\(_3^−\). Both the formation and dissociation of carbonic acid is catalyzed by the enzyme **carbonic anhydrase** at the brush border. This serves two purposes.
   - First, it helps to trap the acid in the form of CO\(_2\) which cannot be removed from kidney fully even after diffusion through tubular walls.
   - Second, it cleaves and extracts the acid and helps in its active secretion into the lumen while retaining the alkaline bicarbonate inside the cell.

2. **Secretion of H\(^+\) into tubular fluid** occurs via Na\(^+\)-H\(^+\) antiporter (Fig. 81.2).

3. The **bicarbonate ion diffuses into the interstitium** as K\(^+\) ion does.

4. The Na\(^+\) ion from tubular cells enters interstitium in exchange of a K\(^+\) by Na\(^+\)-K\(^+\) ATPase.

**Fig. 81.2:** Mechanism of acid secretion in PCT. H\(^+\) formed from dissociation of H\(_2\)CO\(_3\) is transferred into tubular fluid by Na\(^+\)-H\(^+\) antiport located on apical membrane of tubular epithelial cell and HCO\(_3^−\) diffuses into interstitial fluid. Thus, H\(^+\) secretion in PCT is Na\(^+\)-dependent.

5. Thus, cellular K\(^+\) concentration remains unaffected, while Na\(^+\) concentration decreases. The decreased Na\(^+\) concentration initiates entry of Na\(^+\) ions from luminal side into cell, which is coupled with secretion of H\(^+\). Thus, urine **acidification at PCT is dependent on Na\(^+\) concentration**. Parathormone inhibits Na\(^+\)-H\(^+\) exchanger in apical cell membrane of PCT and therefore, affects urine pH. It should be noted that as the carbonic anhydrase plays a vital role in PCT, drugs that inhibit its action (**carbonic anhydrase inhibitors**) decrease the process of acidification of urine.

#### Acidification in Distal Tubules and Collecting Duct

Unlike in PCT, the distal tubular cells acidify urine independent of their Na\(^+\) concentration. In DCT, secretion of H\(^+\) occurs by **active (ATP-driven) proton pumps**.

1. Aldosterone increases distal tubular acid secretion by acting on these pumps directly.

2. The **I cells of DCT** are equivalent to the parietal cells of stomach as they actively secrete acid. These cells are rich in carbonic anhydrase. The number of H\(^+\) pumps employed at the membrane increases for increased acid secretion.

3. **Band 3 protein**, an anion exchanger protein located at the basolateral membrane acts as **chloride-bicarbonate exchanger** (Fig. 81.3). It transports bicarbonate ion into interstitium, thereby it retains bicarbonate ion and acidifies urine.

4. Apart from this, **H\(^+\)-K\(^+\) ATPase** to some extent secretes H\(^+\) and absorbs K\(^+\) in the collecting tubules. pH changes of tubular fluid in DCT may be acute (Application Box 81.2)

**Fig. 81.3:** Mechanism of acid secretion in distal tubular cells (by intercalated (I) cells in DCT and CD). H\(^+\) formed from dissociation of H\(_2\)CO\(_3\) is transferred into tubular fluid by proton pumps (H\(^+\) ATPase or K\(^+\)-H\(^+\) ATPase) located on apical membrane of tubular epithelial cell and HCO\(_3^−\) diffuses into interstitial fluid by HCO\(_3^−\)-Cl\(^−\) antiport located on basolateral membrane. Thus, H\(^+\) secretion in DCT and CD is mostly Na\(^+\)-independent.
Maximum acidification: The minimum urinary pH at which maximal acidification can be achieved is 4.5. At this pH, $H^+$ concentration of urine is 1000 times more than that in plasma. This pH of tubular fluid is usually attained in the collecting ducts. Though acid secretion is more in PCT, it contributes to moderate change in urinary pH as the carbonic acid breaks down into carbon dioxide and water. On the other hand, urinary pH changes are acute in DCT though its acid secreting capacity is less.

Further Modifications in Acidification

After saturation of acid secreting mechanisms, there would be no further urine acidification. However, constant removal of $H^+$ by the action of urinary buffers from the tubules makes it possible for further acid secretion. It occurs via three different buffering mechanisms at different regions of tubules (Figs. 81.4A to C).

In PCT

Reaction with bicarbonate buffers occurs in the tubular fluid in PCT. Bicarbonate ion after filtration into tubular fluid reaches a concentration of about 24 millimoles. At this concentration, it is an effective buffer for the tubular acids. It forms carbonic acid that breaks down into carbon dioxide and water at the brush border of PCT by the action of carbonic anhydrase. The carbon dioxide diffuses into tubule cell and reacts with water molecule to resecrete acid into tubule and reclaim bicarbonate ion into interstitium (Fig. 81.4A).

In DCT and Collecting Duct

Secreted acid reacts with dibasic phosphate buffer whose $pK$ value is closer to urinary pH (Fig. 81.4B). The relative concentration of phosphate buffer here is more and effective, as it is not reabsorbed in PCT. The reaction is:

$$Na_2HPO_4 + H^+ \rightarrow Na^+ + NaH_2PO_4$$

The monobasic phosphate gets excreted while sodium gets reabsorbed. In DCT and collecting tubules, phosphate gets concentrated due to increased absorption of water. Hence, phosphate buffer is more active in these parts of nephron.

In PCT and DCT

In PCT and DCT, ammonia buffer also works. Soluble ammonia diffuses through cell membrane down its concentration gradient into tubular fluid. Here it reacts with acid to form ammonium ion, which subsequently gets excreted. Hence, it maintains the ammonia gradient for further ammonia diffusion. This is called nonionic diffusion. In case of acidosis, amount of ammonia diffusion into urine increases and hence the excretion of ammonium ions increases (Fig. 81.4C). The mechanism that increases ammonia diffusion into urine in acidosis is still not clear. However, the probable mechanism may be as follows.

In PCT

In proximal tubular cells, ammonium is produced from glutamine. Each glutamine molecule produces two molecules of $NH_4^+$ and a divalent anion.

1. Each anion is metabolized to produce two molecules of $HCO_3^-$ that enter the blood as new $HCO_3^-$ and $NH_4^+$ enters the tubular fluid.
2. Glutamine becomes glutamate by glutaminase, which in turn forms $\alpha$-ketoglutarate ($\alpha$-KG) by glutamic dehydrogenase.
3. $\alpha$-KG is converted to glucose by gluconeogenesis that enters interstitial fluid and from there into blood. $NH_4^+$ formed during this process is secreted into tubular fluid.
4. The mechanism of $NH_4^+$ secretion involves $Na^+\cdot H^+$ antiporter that substitutes $NH_4^+$ for $H^+$ (Fig. 81.5).
Chapter 81: Acidification of Urine

Ammonium ion is the major urinary acid when the pH of urine falls below 6, excretion of ammonium ions increases. When this imbalance occurs primarily due to alteration in $\text{HCO}_3^-$, the condition is called **metabolic acidosis** or **alkalosis**, and when it occurs primarily due to alteration in blood $\text{PCO}_2$, the condition is called **respiratory acidosis** or **alkalosis**.

The body has three general mechanisms to maintain pH produced by the acid-base disorder:
1. Extracellular and intracellular buffering.
2. Adjustment in blood $\text{PCO}_2$ by mainly changing the rate of ventilation
3. By adjusting the renal acid secretion.

Accordingly, the mechanisms are called extracellular and intracellular defenses, respiratory defense and renal defense.

**Extracellular and Intracellular Defenses**

The extracellular buffering occurs immediately whereas intracellular buffering occurs slowly. **Extracellular buffering** includes bicarbonate, phosphate, and protein buffers. The **intracellular buffers** are mainly phosphate, protein and hemoglobin buffers.

**Respiratory Defense**

This defense mechanism against acid-base disorder operates within few minutes, which is mainly mediated by the **rate of ventilation** that determines the level of $\text{PCO}_2$ in blood. Increased ventilation decreases $\text{PCO}_2$ and decreased ventilation increases $\text{PCO}_2$ (for details, refer chapter “Acid-Base Balance” in Section XIII). For example, in metabolic acidosis, increase in concentration of $\text{H}^+$ increases ventilation by stimulating central chemoreceptors. The typical example is **Kussmaul respiration** seen in diabetic ketoacidosis.

**Renal Defense**

The renal defense mechanism takes several days to be effectively operative. This is because kidney takes hours to days to synthesis the enzymes that are involved in $\text{NH}_4^+$ synthesis. **Ammonium ion is the major urinary acid.** When the pH of urine falls below 6, excretion of ammonium ions increases.

1. **In acidosis**, secretion of $\text{H}^+$ by the kidney is increased, and filtered bicarbonate is totally reabsorbed.
   - Synthesis and excretion of $\text{NH}_4^+$ are also increased. **This increases total acid excreted by the kidney.**
   - New $\text{HCO}_3^-$ generated during the acid excretion returns to blood that increases plasma bicarbonate.
   - In case of chronic acidosis like **diabetic ketoacidosis**, excretion of $\text{NH}_4^+$ can be increased 10 folds.
2. Conversely, **in alkalosis** secretion of $\text{H}^+$ by the kidney is inhibited. This **decreases bicarbonate reabsorption** and therefore, urine becomes alkaline (for details, refer chapter “Acid-Base Balance” in Section XIII).

**Clinical Box 81.3**

**Fanconi Syndrome:** Renal tubular acidosis (RTA) is the condition in which acidification of urine is decreased. In such a situation, kidneys are incapable of excreting a large amount of acid to balance the production of non-volatile acid. This results in **metabolic acidosis**. An example of RTA is Fanconi syndrome. When the defect is in the proximal tubule, the condition is called **proximal RTA** and when the defect is in distal part of the tubule, the condition is called **distal RTA**. Failure to produce and secrete $\text{NH}_4^+$ decreases the amount of net acid excreted by kidneys that results in metabolic acidosis.

**RESPONSE OF KIDNEY TO ACID-BASE DISORDER**

The pH of blood is maintained within a narrow range of 7.35 to 7.45. When pH falls acidosis occurs and when pH increases alkalosis occurs. When this imbalance occurs primarily due to alteration in $\text{HCO}_3^-$, the condition is called **metabolic acidosis** or **alkalosis**, and when it occurs primarily due to alteration in blood $\text{PCO}_2$, the condition is called **respiratory acidosis** or **alkalosis**.

The body has three general mechanisms to maintain pH produced by the acid-base disorder:
1. Extracellular and intracellular buffering.
2. Adjustment in blood $\text{PCO}_2$ by mainly changing the rate of ventilation
3. By adjusting the renal acid secretion.

Accordingly, the mechanisms are called extracellular and intracellular defenses, respiratory defense and renal defense.

**Extracellular and Intracellular Defenses**

The extracellular buffering occurs immediately whereas intracellular buffering occurs slowly. **Extracellular buffering** includes bicarbonate, phosphate, and protein buffers. The **intracellular buffers** are mainly phosphate, protein and hemoglobin buffers.

**Respiratory Defense**

This defense mechanism against acid-base disorder operates within few minutes, which is mainly mediated by the **rate of ventilation** that determines the level of $\text{PCO}_2$ in blood. Increased ventilation decreases $\text{PCO}_2$ and decreased ventilation increases $\text{PCO}_2$ (for details, refer chapter “Acid-Base Balance” in Section XIII). For example, in metabolic acidosis, increase in concentration of $\text{H}^+$ increases ventilation by stimulating central chemoreceptors. The typical example is **Kussmaul respiration** seen in diabetic ketoacidosis.

**Renal Defense**

The renal defense mechanism takes several days to be effectively operative. This is because kidney takes hours to days to synthesis the enzymes that are involved in $\text{NH}_4^+$ synthesis. **Ammonium ion is the major urinary acid.** When the pH of urine falls below 6, excretion of ammonium ions increases.

1. **In acidosis**, secretion of $\text{H}^+$ by the kidney is increased, and filtered bicarbonate is totally reabsorbed.
   - Synthesis and excretion of $\text{NH}_4^+$ are also increased. **This increases total acid excreted by the kidney.**
   - New $\text{HCO}_3^-$ generated during the acid excretion returns to blood that increases plasma bicarbonate.
   - In case of chronic acidosis like **diabetic ketoacidosis**, excretion of $\text{NH}_4^+$ can be increased 10 folds.
2. Conversely, **in alkalosis** secretion of $\text{H}^+$ by the kidney is inhibited. This **decreases bicarbonate reabsorption** and therefore, urine becomes alkaline (for details, refer chapter “Acid-Base Balance” in Section XIII).
CHAPTER SUMMARY

**Key Concepts**

1. Acids are continuously produced in the body by metabolism. Kidney eliminates extra acids & prevents development of acidosis, and therefore normally urine becomes acidic.
2. Acidic urine also prevents growth of bacteria in urinary tract and prevents the incidence of urinary tract infection.
3. Bicarbonate buffer and ammonia buffers are important buffers in the renal tubules.

**Important to Know (Must Read)**

1. In examination, ‘Describe the mechanism of acidification of urine’ is asked as a Long Question.
2. Mechanism of acidification of urine in PCT, Mechanism of acidification of urine in DCT and CD, Acid-base buffers in renal tubules, Handling of acid load by kidney, Response of kidney to acid base disorder, may be asked as Short Questions in exam.
3. In Viva, examiner may ask..... How is excretion of acids through urine helpful for our body, what are the factors that contribute to the acidification of urine, What do you mean by titratable acids, what are its components, List the buffer mechanisms for acidification of urine in PCT, DCT and CD, What is the mechanism of action of bicarbonate buffer, What is the mechanism of action of phosphate buffer, What is the mechanism of action of ammonia buffer, What is Fanconi syndrome and what are its features, What are the extracellular and intracellular defenses against the acid-base disorder, What are the respiratory defense mechanisms against the acid-base disorder, What are the renal defense mechanisms against the acid-base disorder.
LEARNING OBJECTIVES

On completion of study of this chapter, the student MUST be able to:
1. Classify kidney function tests (KFTs).
2. Understand the physiological basis of each KFT.
3. Learn the concept of clearance tests.
4. Understand the physiological basis of genesis and management of acute and chronic renal failures.
The student MAY also be able to:
1. Describe the procedure, application, merits and demerits of kidney function tests.

KIDNEY FUNCTION TESTS

As urine formation, the primary function of kidney is achieved by two principal mechanisms, the glomerular ultrafiltration, and tubular reabsorption & secretion, kidney function tests are physiologically grouped into two broad categories:
1. Tests that assess glomerular functions (glomerular function tests)
2. Tests that assess tubular functions (tubular function tests).

A. Glomerular function tests:
   1. Blood urea determination
   2. Blood creatinine estimation
   3. Inulin clearance test
   4. Creatinine clearance test
   5. Urea clearance test
   6. PAH clearance test
   7. Estimation of proteins in the urine

B. Tubular function tests:
   1. Urine concentration test
   2. Urine dilution test
   3. Detection of uric acid excretion
   4. Test for acidification of urine
   5. Test for alkalinisation of urine
   6. Test for amino acids in urine

However, clinically, kidney function tests are divided into four categories:
1. Routine tests: Complete urine analysis, measurement of BUN in blood, and measurement of serum electrolytes.
2. Tests for further assessments: Clearance test, determination of urine and plasma osmolality, and concentration and dilution tests.
3. Specific tests: Tests to assess renal acidification, tests to assess renal handling of sodium, measurement of renal plasma flow, and excretion of exogenous compound like PSP.
4. Special tests: Ultrasonography, CT scan, MRI, etc.

Routine Urine Analysis

In clinical laboratories, urine analysis is performed routinely for assessing the degree of kidney dysfunctions in kidney diseases and other diseases that affect kidney functions. Routine urine analysis includes analysis of physical and biochemical characteristics of urine.

Physical Characteristics

Volume

The normal volume of urine excreted is about 1–1.5 liters per day. Urine volume increases in excess water intake, high protein diet, diuretic therapy, diabetes mellitus, diabetes insipidus, and sometimes in chronic renal diseases. Urine volume decreases in excessive sweating, hot climate, dehydration, hypotension, hypovolemia, kidney diseases, acute renal failure, antidiuretic therapy, edema due to variety of causes, and shock.
Oliguria and Anuria
A person on normal diet can excrete the solute load at maximum concentration when the urine volume is between 300 to 500 ml/day.
1. Volume below this is termed as oliguria.
2. When urine flow is totally absent or less than 50 ml/day, the situation is called as anuria.
3. Oliguria and anuria could be due to decreased urine production (as seen in renal failure, renal infarction, rapidly progressive glomerulonephritis etc.) or due to obstruction to urine flow (as seen in urinary calculi, prostatic enlargement, atonic bladder, pelvic or retroperitoneal tumors, urethral strictures, urethral infection, urethral valves etc.).

Polyuria
When urine volume is inappropriately high (> 3 liters per day), the situation is called polyuria. Polyuria could be due to osmotic diuresis (increased urinary solute excretion) or due to water diuresis. The common causes of polyuria are:
1. Excessive fluid intake
2. Osmotic diuresis (due to hyperglycemia and hypercalcemia)
3. Neurogenic diabetes insipidus (decreased ADH secretion)
4. Nephrogenic diabetes insipidus (due to tubular defects, interstitial renal disease, hypokalemia, hypercalcemia, drugs such as lithium)
5. Diuretics
6. Psychogenic polydipsia
7. Increased frequency of micturition (as occurs in urethritis, cystitis, prosatitis) may cause polyuria.

Nocturia
Waking up in the night to void urine is called nocturia. It may occur due to polyuria, but often it is due to more fluid intake before going to bed in the night or use of diuretic in the night. It can also occur in chronic kidney diseases and prostatic enlargement, or in sleep disturbances/disorders without functional abnormalities of urinary tract.

Appearance
Normally, urine is clear. It becomes turbid when kept in a container for a long time as urea is converted to ammonium carbonate by the action of bacteria, which makes the urine alkaline and results in precipitation of calcium and magnesium phosphates. Urine may also be turbid if it contains more phosphates (as in alkaline urine) or pus as seen in infection of urinary tract, or chyle as occurs in obstruction of lymphatics of the urinary tract (for example, in filariasis).

Odor
Normally, the odor of urine is mildly aromatic due to presence of volatile organic acids. However, if kept for a long time, urine gives unpleasant ammoniacal smell due to conversion of urea into ammonium carbonate. Diabetic urine gives acidic-fruity odor due to the presence acetic acid in the urine. Excretion of different drugs in the urine also changes the smell of urine.

Color
Normal urine is straw colored or amber-yellow in color, which is due to the presence of the pigment urochrome in it.
1. Urine becomes yellow in bilirubinuria, as occurs in jaundice.
2. Urine becomes dark in alkaptonuria, melanuria (seen in malignant melanoma), and,
3. Red in hematuria, hemoglobinuria, myoglobinuria, porphyria or following intake of rifampicin (anti-tubercular antibiotic).

Specific Gravity
The normal specific gravity of urine is 1.005-1.030 (the theoretical range is 1.003 – 1.035). Specific gravity of urine of 1.010 normally corresponds to urine osmolality of 285 mosm/kg.
1. Specific gravity decreases when urine is diluted (as seen in diabetes insipidus) and increases when urine is concentrated (as occurs in dehydration).
2. Specific gravity also increases when urine contains glucose, protein and contrasts. In chronic renal failure, specific gravity is fixed at 1010.

Biochemical Characteristics
Reaction of Urine
Normally, urine is mildly acidic; the average pH being 6 (ranging between 4.5–7.5).
1. After a normal meal, urine becomes alkaline due to alkaline tide that occurs with secretion of acid in the stomach, which adds bicarbonate into the plasma.
2. However, if the meal is rich in protein, urine becomes acidic due to formation of sulfates and phosphates of amino acids in tubular fluid.
3. If the meal is rich in vegetables, then the urine becomes alkaline as organic acids like citric and tartraric acids extracted from vegetables are converted to bicarbonate in the body.
4. Alkaline urine is also feature of type II distal renal tubular acidosis, urinary tract infection by urease producing organisms, acetazolamide therapy and following ingestion of alkali.

Proteins in Urine
Normally, glomerulus is not permeable to substances with molecular weight more than 69000. Therefore, normally proteins are absent in urine.
1. Proteinuria occurs when glomerular filtering membrane is damaged in various glomerular diseases. Albumin being a smaller molecule passes easily through the damaged glomerulus than the heavier globulins.
Table 82.1: 24-hr urinary protein and protein-creatinine ratio in different conditions.

<table>
<thead>
<tr>
<th>24 h urinary protein (g)</th>
<th>Protein/Creatinine ratio</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 0.03</td>
<td>&lt; 2.5 in males &lt; 3.5 in females</td>
<td>Normal</td>
</tr>
<tr>
<td>0.03–0.3</td>
<td>3.5–15</td>
<td>Microalbuminuria</td>
</tr>
<tr>
<td>0.3–0.5</td>
<td>15–50</td>
<td>Dipstick positive</td>
</tr>
<tr>
<td>&gt; 2.5</td>
<td>&gt; 250</td>
<td>Glomerular disease likely</td>
</tr>
<tr>
<td>&gt; 4.0</td>
<td>&gt; 400</td>
<td>Glomerular disease</td>
</tr>
</tbody>
</table>

2. Therefore, in proteinuria, albumin predominates in urine. The molecular weight of hemoglobin is 67000; therefore, hemoglobinuria occurs in hemolysis. Though, normally proteins are not filtered by glomerulus, a very less amount (less than 150 mg per day) of it is excreted in urine, which is secreted by the tubular epithelial cells.

3. However, this trace quantity of protein is not detected by routine urine analysis test. The normal protein content of 24 hour urine is < 0.03 g.

**Protein-Creatinine Ratio**

The normal protein-creatinine ratio in a random urine sample is < 2.5 in males and < 3.5 in females (Table 82.1). Ratio > 3.5 indicates microalbuminuria and > 15 indicates proteinuria.

**Proteinuria**

Proteinuria is seen in nephritis, nephrosis, urinary tract infections (UTIs), eclampsia, heart failure and later part of pregnancy. strenuous exercise, Normally, protein filtered is reabsorbed by the tubule. Therefore, either in increased filtration or in diseases of tubule, proteinuria occurs. Protein in urine is detected by heat and acetic acid test, sulphosalicylic acid test, or by using Esbach’s albuminometer.

**Bence-Jones Proteinuria**

Bence-Jones protein is the B lymphocyte secreting immunoglobulin light chain protein. If it is present in blood it is freely secreted in urine. This occurs in multiple myeloma, amyloidosis and plasma cell dyscrasias. This is used as a marker in myeloma in which myeloma protein (Bence-Jones proteins) increases in plasma.

**Microalbuminuria**

Excretion of 24 hr urinary protein 30-300 mg (0.03 to 0.3 g) is described as microalbuminuria.

1. This is an early sign of glomerular dysfunction.
2. Patients with diabetes should be regularly screened for microalbuminuria to detect early development of diabetic nephropathy.
3. A persistent microalbuminuria has recently been found to be associated with increased risk of atherosclerosis and cardiovascular mortality.

**Blood in Urine**

Blood in urine is called hematuria. It may be microscopic hematuria or may be detected by patient (macroscopic hematuria). It indicates bleeding from anywhere in renal tract. Commonly, hematuria is seen in glomerulonephritis and injury to ureter, urinary bladder or urethra. It is detected by Benzidine test.

**Hemoglobinuria and Myoglobinuria**

Presence of hemoglobin (usually without red cells) in urine (hemoglobinuria) indicates intravascular hemolysis and presence of myoglobin in urine (myoglobinuria) indicates rhabdomyolysis.

**Reducing Sugars in Urine**

The presence of sugar in urine is called glycosuria (or more accurately, glucosuria). Normally, glucose does not appear in urine. Glucosuria occurs in conditions in which renal threshold (plasma glucose >180 mg%) for glucose is exceeded as in diabetes mellitus. Glucose in urine is detected by Benedict's test.

**Glycosuria occurs due to:**

1. Diabetes mellitus
2. Kidney diseases (renal glycosuria) due to decreased renal threshold
3. Alimentary glycosuria (lag storage glycosuria) in which transitory rise in blood glucose occurs following meals. It may be seen in normal people, after gastric surgery (rapid absorption of glucose into circulation due to rapid gastric emptying), hyperthyroidism, peptic ulcer and hepatic disease.
4. Gestational glycosuria: Glycosuria is common in normal pregnancy as renal threshold for glucose falls in pregnancy due to increased GFR. However, gestational diabetes should be ruled out.

**Other Biochemical Tests**

**Ketone Bodies in Urine**

The ketone bodies are acetoacetate, β-hydroxybutyrate and acetone. Excretion of ketone bodies in urine is called ketonuria. Normally, ketone bodies are not present in urine. Ketonuria occurs in severe diabetes mellitus, starvation, chronic vomiting, etc. Ketone bodies in urine are detected by Rothera’s test and Gerhardt’s test.

**Bile Salts in Urine**

Bile salts appear in urine in the early phase of obstructive jaundice. Bile salts are detected by Hay’s test and Peten-koffeister’s test.

**Bile Pigments in Urine**

Bile pigments (bilirubin and biliverdin) appear in urine in obstructive jaundice. Bilirubinuria is detected by modified van den Berg reaction or Fouchet’s test.

**Urobilinogen in Urine**

Normally, the main pigment in the urine is urochrome. However, small amounts of urobilinogen may also be present in urine. Urobilinogen excretion increases in persistent fevers, liver diseases, diseases of biliary tract and hemolysis. This is detected by Ehrlich test and Schlesinger’s test for urobilinogen.
Measurement of (NPN) in Urine

The non-protein nitrogen (NPN) in urine includes urea, creatinine and uric acid. These compounds are excreted from the body mainly through urine. Their concentration in urine increases in different physiological and pathological conditions.

1. Determination of creatinine is an important test for renal function.

2. Urea level is altered in many conditions. Even, increased intake of protein increases urea in urine. Hence, urea estimation is a non-specific kidney function test. However, blood urea concentration is a good index of renal functions.

3. Uric acid is increased in urine in conditions that are associated with increased purine catabolism.

Clearance Tests

Clearance tests mainly determine the glomerular function. GFR provides the most useful index for assessment of severity of the renal disease.

Clearance is defined as the quantity of blood or plasma cleared of a substance for unit time. This is expressed as ml per minute. It is the ml of plasma, which contains the amount of that substance excreted by the kidney within a minute. Actually, it estimates the amount of plasma that passes through the glomeruli per minute with complete removal of that substance (to account for the substance actually appears in the urine).

\[ \text{Clearance} = \frac{\text{mg of substance excreted per minute}}{\text{mg of substance per ml of plasma or serum}} \]

It is calculated by using the formula, \( C = \frac{U \times V}{P} \)

Where \( U = \) concentration of the substance in urine; \( P = \) concentration of the substance in plasma or serum and \( V = \) the ml of urine excreted per minute. The value is expressed as ml/minute.

Measurement of the clearance is predominantly a test of glomerular filtration rate (GFR). The relation between clearance value and GFR may be depicted as shown in Table 82.2.

The clearance of a substance, which is completely filtered but neither reabsorbed nor secreted by the tubule is ideally used to measure GFR. Therefore, inulin clearance test is the standard test for measurement of GFR. The PAH clearance determines the substance filtered and secreted but not reabsorbed. Therefore, it is best used for measurement of renal plasma flow. Normally, the RPF is 700 ml per minute and GFR is 120 ml per minute. Therefore, it is obvious that one fifth of the plasma that passes through the glomerulus becomes glomerular filtrate which is known as filtration fraction.

In normal practice, creatinine clearance test, urea clearance test, and inulin clearance test are used for determination of kidney functions.

### Table 82.2: Relationship between GFR and clearance value.

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Result</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substances filtered but neither reabsorbed nor excreted</td>
<td>GFR = clearance</td>
<td>Inulin</td>
</tr>
<tr>
<td>Substances filtered, reabsorbed &amp; excreted</td>
<td>GFR = clearance</td>
<td>Uric acid</td>
</tr>
<tr>
<td>Substances filtered &amp; partially reabsorbed</td>
<td>Clearance &lt; GFR</td>
<td>Urea, &amp; creatinine</td>
</tr>
<tr>
<td>Substances filtered &amp; secreted, but not reabsorbed</td>
<td>Clearance &gt; GFR</td>
<td>Diodrast &amp; PAH</td>
</tr>
</tbody>
</table>

Blood Investigations

Blood tests for assessing kidney functions are serum urea, creatinine, uric acid and electrolytes.

**Serum Urea**

Normal serum urea level is 15 – 40 mg%. Increased serum urea is seen in:

1. High intake (high protein diet, GI hemorrhage)
2. High production (trauma, burn, infections)
3. Reduced urinary excretion (renal failure, hypovolemia).

**Serum Creatinine**

Normal serum creatinine is 0.7–1.5 mg% in males and 0.5–1.2 mg% in females. It increases in:

1. High production (increased muscle mass, rhabdomyolysis)
2. Reduced excretion in urine (renal failure, drugs like trimethoprim, cimetidine).

**Serum Electrolytes**

Normal serum sodium value is 136 to 145 mM/L and normal serum potassium value is 3.5 to 5 mM/L. Hyperkalemia is an important feature of renal dysfunctions.

**Tubular Function Tests**

The primary function of tubule is to reabsorb solutes and water from the tubular fluid. Therefore, detecting concentration of solutes in urine gives the knowledge of tubular functions. The simplest tubular function test is the measurement of specific gravity of the urine.

**Determination of Specific Gravity**

The specific gravity depends on the concentration of solutes whereas the osmolality detects the presence of osmotically active particles in the urine. For example, in proteinuria specific gravity is significantly increased whereas osmolality is mildly elevated.

**Other Tests**

Tubular functions are determined by concentration and dilution tests. The earliest manifestation of the renal disease is the inability to concentrate urine. This is detected by concentration test.
Chapter 82: Kidney Function Tests and Pathophysiology of Renal Failure

Concentration Test
The patient is advised to eat normal food at 6 PM but fluid intake is restricted to 200 mL. He is further advised not to take anything throughout night. Next morning, at 7 AM, the bladder is emptied and this first specimen is discarded. At 8 AM, urine collected and this second specimen is obtained for measuring specific gravity.
1. If specific gravity is more than 1.022, the renal function is normal.
2. If specific gravity is below this value, a third sample is collected at 9 AM.
3. If this sample gives specific gravity less than 1.022, then the concentrating ability of the kidney is considered to be impaired.

Determination of Urine Volume
The measurement of the volume of urine passed in 24 hours is a simple test of tubular function. The urine volume is measured separately during night time and during day time.
1. Normally, the volume of urine in night is half the volume of urine excreted during day time.
2. An increased excretion of urine during night is an early indication of tubular dysfunctions.

Measurement of Osmolality
Osmolality of urine varies widely between 60–1200 mosm/kg. Urine osmolality is usually an index of plasma osmolality, which normally ranges between 285–295 mosm/kg. Therefore, the plasma and urine osmolarities are detected and the ratio of osmolality of urine to plasma is recorded. The normal ratio is 3–4.5. Urine osmolality is detected by ADH test, dilution tests, and free water clearance.

ADH Test
Normally, ADH reabsorbs water from the kidney tubule. With tubular dysfunction, effectiveness of tubular water reabsorption by ADH decreases, as seen in nephrogenic diabetes insipidus. Five units of ADH are injected and urine samples are collected for 24 hours for estimation of specific gravity and osmolality. Normally, at least one sample should have the specific gravity more than 1.020 and osmolality of 800 mosm/kg.

Dilution Test
The patient is not allowed to drink water or fluid after 10 PM. The bladder is emptied at 7AM in the next morning and patient is allowed to take a water load of 1.2 liter in half an-hour. Hourly urine samples are collected for four hours for determination of volume, specific gravity, and osmolality of each sample. Normally, a person excretes the total water load in four hours and the specific gravity of at least one sample should fall to 1.003 and osmolality to 50 mosm/kg.

Osmolal Clearance or Free Water Clearance
It actually detects the concentrating and diluting ability of the kidney. Therefore, it is a better test for tubular function. Osmolal clearance is defined as the volume of plasma water that is cleared of its solutes to excrete urine with same specific gravity as that of plasma.

\[
\text{Osmolar clearance (Cosm)} = \frac{\text{Uosm} \times V}{\text{Posm}}
\]

Where, Uosm is the urine osmolality, Posm is the plasma osmolality, and V is the urine excreted per minute.
1. When urine is hypertonic to plasma, more water is excreted. This is expressed as free water clearance (C water). C water is equal to V – Cosm ml/minute.
2. Usually this is used to differentiate polyuria of diabetes insipidus, diabetes mellitus, and excess water drinking.

Specific Tests

Tests for Urine Acidification
Usually, acid loading test is performed to detect the ability of the kidney to acidify urine. Ammonium chloride is given to the subject as an enteric coated tablet (0.1 g/kg b.w.).
1. Urine is collected every hour for 4–8 hours for determination of pH of urine and ammonia excretion of each sample.
2. At least, one sample should have pH of 5.3 or less and ammonia excretion should be 30–90 mmol per hour.

Tests for Renal Handling of Na⁺
The subject is given a load of 100 mmole of sodium as sodium chloride. Normally, 60–80 mmole of sodium is excreted per day. Then, the patient is given sodium free diet for 7 days (daily intake less than 10 mmole). After a week, urinary sodium output is estimated.
1. A normal person decreases Na⁺ excretion to 10 mmole per day after 7 days. When tubular function is impaired, ability to reabsorb sodium is lower.
2. In such conditions, natriuresis continues regardless of Na⁺ intake.

PSP Test
The PSP or phenolsulphalein (phenol red) test is used for determining the secretory capacity of the tubule. The subject is allowed to drink 600 mL of water following which PSP is given intravenously (6 mg in 1 mL solution). Bladder is emptied after 15 minutes, 30 minutes, 60 minutes and 120 minutes, and excretion of the phenol red is noted in each sample. Normally, the phenol content is 35% in the first sample and 70% of the dye should be eliminated in two hours. In tubular dysfunctions, the dye excreted in first sample is less and also the total amount excreted in two hours will be less. This is a useful test for detection of early stage of kidney disease.
Special Tests
Special tests to assess kidney functions include ultrasonography, intravenous pyelography, computed tomography and magnetic resonance imaging. Renal biopsy (percutaneous needle biopsy) is useful in some selective diseases.

RENAL FAILURE
Renal failure is the condition of decreased urine output due to loss of renal function that develops over a period of days to years. This is of two types: acute renal failure and chronic renal failure.

Acute Renal Failure
Definition and Causes
Acute renal failure (ARF) refers to a sudden, but usually reversible loss of renal function that develops over a period of days or weeks and is usually accompanied by a reduction in urine volume. The causes of ARF are listed in Table 82.3. The commonest pre-renal cause of ARF is hypovolemic shock and commonest renal cause is acute tubular necrosis (ATN). ATN usually occurs due to ischemia or nephrotoxicity caused by chemicals or bacterial toxins or both.

<table>
<thead>
<tr>
<th>Table 82.3: Causes of renal failure.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>I. Causes of acute renal failure</strong></td>
</tr>
<tr>
<td>A. Prerenal causes</td>
</tr>
<tr>
<td>1. Systemic causes</td>
</tr>
<tr>
<td>2. Local causes</td>
</tr>
<tr>
<td>3. Under-perfusion of kidney</td>
</tr>
<tr>
<td>B. Renal causes</td>
</tr>
<tr>
<td>1. Acute tubular necrosis/toxic/septic renal failure</td>
</tr>
<tr>
<td>2. Glomerular diseases</td>
</tr>
<tr>
<td>3. Interstitial kidney diseases</td>
</tr>
<tr>
<td>C. Postrenal causes</td>
</tr>
<tr>
<td>1. Obstruction to urine outflow</td>
</tr>
<tr>
<td>2. Renal artery stenosis</td>
</tr>
<tr>
<td><strong>II. Causes of chronic renal failure.</strong></td>
</tr>
<tr>
<td>1. Diabetes mellitus</td>
</tr>
<tr>
<td>2. Hypertension</td>
</tr>
<tr>
<td>3. Glomerular diseases (e. g. IgA nephropathy)</td>
</tr>
<tr>
<td>4. Renal interstitial diseases</td>
</tr>
<tr>
<td>5. Renal artery stenosis</td>
</tr>
<tr>
<td>6. Congenital and inherited (e. g. polycystic kidney disease, Alport syndrome)</td>
</tr>
<tr>
<td>7. Systemic inflammatory diseases (e.g. SLE, vasculitis)</td>
</tr>
</tbody>
</table>

Features
Patient is usually **oliguric** (24 hr urine volume < 500 mL).

1. **Hyperkalemia** is common. **Dilutional hyponatremia** and metabolic acidosis may be present. **Hypocalcemia** is common due to decreased production of 1,25 dihydroxycholecalciferol.
2. **Uremic features** include anorexia, nausea, vomiting, apathy, confusion, muscle twitching, hiccoughs, fits and coma.
3. **Pulmonary edema** may be present due to increased pulmonary capillary permeability.
4. **Anemia** is common.
5. **Bleeding** may occur due to platelet dysfunctions or disturbances in coagulation cascade. Spontaneous GI hemorrhage is not uncommon.

Treatment
Emergency resuscitation and follow up treatment include:

1. **Hyerkalemia is treated immediately** to prevent development of life threatening cardiac arrhythmias. Calcium gluconate (10 mL of 10% solution) is given i.v. to stabilize membrane potential. **Insulin and glucose solution** (5 units of insulin + 50 mL of 50% glucose) is injected i.v. along with sodium bicarbonate. Insulin facilitates entry of K⁺ into the cell and immediately decreases plasma K⁺ level. Glucose is given to combat insulin-induced hypoglycemia. Sodobicarb prevents acidosis. Dialysis may be needed in extreme cases.

2. **Circulating blood volume should be optimized.** For pre-renal ARF, hypovolemia is treated with rapid infusion of blood or plasma or isotonic saline and CVP should be monitored regularly.

3. **Metabolic acidosis is corrected** by isotonic sodium bicarbonate infusion.

4. **Fluid and electrolyte balance** is monitored after initial resuscitation. Fluid intake should equal the urine output.

5. **Treatment of underlying cause** include treatment for ATN or surgical removal of obstruction in post-renal ARF.

6. **Infection should be controlled** promptly.

7. **Renal replacement therapy** (intermittent hemodialysis, hemofiltration, intermittent hemodiafiltration and peritoneal dialysis) is considered if there is severe uremia, hyperkalemia and metabolic acidosis.

Chronic Renal Failure (CRF)
Definition
Chronic renal failure (CRF) refers to an irreversible deterioration in renal function that develops over a period of years. Initially, it manifests only as biochemical abnormality, but eventually loss of endocrine, metabolic and excretory functions of kidney leads to the development of signs and symptoms of renal failure, which are referred to as
uremia. When death is likely without renal replacement therapy, it is called **end-stage renal failure** (ESRF).

**Etiopathogenesis**

CRF is caused by any condition that destroys structure and functions of kidney. Presently, the commonest causes of CRF are diabetes mellitus and hypertension (Table 82.3). Disturbances in water, electrolyte and acid-base balance contribute to the clinical picture in CRF. Uremia is caused by accumulation of many intermediary products of metabolism, called as uremic toxins.

**Features**

CRF may be detected as raised blood urea and serum creatinine during routine investigation of patients of diabetes, hypertension, proteinuria and anemia. Unless GFR falls below 30 mL/min, patient remains asymptomatic. Nocturia occurs due to loss of concentric ability of kidney and increased osmotic load on nephron.

1. Tiredness and breathlessness are common.
2. ESRF patients appear ill and anemic and may show signs of sodium and water depletion. They present with anorexia, nausea, vomiting, hiccough, pruritus, muscular twitching, fits and drowsiness.
3. Features of metabolic acidosis such as **Kussmaul respiration** may be present.

**Treatment**

Consists of identifying the renal disease, identify the precipitating factors and preventing the further renal damage. Diabetes, hypertension and anemia are promptly treated. Fluid and electrolyte balance is maintained. Metabolic acidosis is corrected. Renal replacement therapy (dialysis/renal transplantation) is instituted.

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**CHAPTER SUMMARY**

**Key Concepts**

1. Physiologically, kidney function tests are done to assess glomerular filtration and tubular functions. Estimation of protein and glucose in urine, and pH of urine are routine tests.
2. Clearance test, ADH test and concentration/dilution tests are specific tubular function tests.
3. Hyperkalemia is an acute condition in ARF, and is immediately treated by IV insulin in glucose solution.

**Important to Know (Must Read)**

1. In examination, **Long Questions** are usually not asked from this chapter. However, ‘Describe the physiological basis and application of kidney function tests’ may come as a long question.
2. Tubular function tests, Glomerular function tests, Clearance tests, Urine analysis, Acute renal failure, Chronic renal failure may be asked as **Short Questions** in exam.
3. In **Viva**, examiner may ask...... List the glomerular function tests, List the tubular function tests, Physiologically, how do you classify kidney function tests, Clinically, how do you classify kidney function tests, What are the normal physical characteristics of urine, What is oliguria and anuria, What is polyuria and what are its causes, What is nocturia, What is protein-creatinine ratio in urine, What is proteinuria, What is Bence-Jones proteinuria, What is microalbuminuria, What is glycosuria and what are its causes, Define clearance, what is its formula and what area the substances used to calculate clearance, How does the urine concentration test is performed, What is ADH test, How does the urine dilution test is performed, What is osmolar clearance or free water clearance, What are the tests for urine acidification, What is PSP test and what is its use, What are the causes, features and treatment of acute renal failure, What are the causes, features and treatment of chronic renal failure.
CHAPTER 83

Physiology of Micturition and Bladder Dysfunctions

Learning Objectives

On completion of study of this chapter, the student MUST be able to:
1. Understand the relation of functional anatomy of urinary bladder with its functions.
2. Appreciate the innervation and arrangement of sphincters of urinary bladder.
3. Learn the mechanism and control of micturition reflex.
4. Explain the graph of cystometrogram.
5. Understand the physiological basis of bladder dysfunctions.

The student MAY also be able to:
1. Describe the abnormalities of bladder dysfunctions.

Urinary bladder stores urines. It accumulates urine without much rise in pressure in it and empties its content at appropriate time following suitable rise in pressure.
1. Urine from kidneys flows down to the bladder through ureters due to the action of gravity, which is aided by peristaltic movements of ureter.
2. Peristaltic waves in ureters originate by a pacemaker tissue located close to the calyces at a frequency of about one per minute.
3. Once in the bladder, regurgitation of urine back into the ureter is prevented passively by a valvular flap present at the entry point of the ureter at the base of the bladder. Ureteric pain occurs when ureters are distended by stones or by obstructions.

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3. Contraction of the detrusor causes emptying of the bladder.
4. The epithelium of the bladder is formed by a superficial layer of flat cells and a deep layer of cuboidal cells.

Innervation
Bladder is innervated by both sympathetic and parasympathetic fibers (Fig. 83.2).
1. Parasympathetic fibers originate from the sacral 2, 3 and 4 segments of the spinal cord and reach the bladder via pelvic nerves,
2. The sympathetic fibers originate from lumbar 1, 2 and 3 segments of the spinal cord and reach the bladder via hypogastric nerve (Fig. 83.2).

3. The somatic fibers originate from $S_2$, $S_3$, and $S_4$ and innervate bladder and external sphincter via pudendal nerve.

4. Filling of bladder, desire to pass urine and painful distension, all these sensations of bladder are mediated by afferents in the pelvic nerve.

**Urethral Sphincters**

There are two sphincters: Internal and external (Fig. 83.3).

1. The internal sphincter, which is located at the neck of the bladder, is made up of a bundle of smooth muscle (sphincter vesicae), and innervated by sympathetic (hypogastric) and parasympathetic (pelvic) nerves. Therefore, the internal sphinter is under autonomic control.

2. The external sphincter is made up of a flap of skeletal muscle, which is present around the urethra in its proximal part (sphincter urethrae). The external sphincter is innervated by somatic (pudendal) nerve and therefore under voluntary control.

**Functions of Urinary Bladder**

Urinary bladder serves two functions:

1. **Storage of urine** up to a critical volume.

2. **Emptying urine** into urethra when the critical volume is attained.

   The external sphincter is kept closed most of the time. The sensation of bladder filling is experienced at the bladder volume of about 150 mL and the sensation to pass urine is experienced at bladder volume of 150–250 mL. At volume of about 400 mL, the sensation to pass urine becomes uncomfortable and at about 700 mL, it becomes very painful leading to loss of control.
Micturition is the process of passing urine. This is primarily a reflex phenomenon, which is mostly integrated in the spinal cord.

1. This spinal reflex is influenced by activities of the higher centers.
2. Unless the bladder is filled, urine accumulates in urinary bladder without much increase in the intravesical pressure, as bladder wall is made up of smooth muscles that exhibit the property of plasticity.
3. Due to plasticity, tension produced by stretching is not maintained. This relationship between the bladder volume and pressure is best studied by cystometry.

**Cystometry:** Cystometry is the procedure to study the relationship between the bladder volume and pressure. For this purpose, a catheter is inserted into the bladder and bladder is completely emptied. Then, as bladder volume is gradually increased by slowly pushing water to fill bladder, intravesical pressure is recorded at different bladder volumes. This recording of pressure-volume relationship is called cystometrogram (Fig. 83.4).

1. The initial rise in intravesical pressure occurs when bladder is filled with 50 ml of water (at point A, Fig. 83.4) and thereafter no significant increase in pressure occurs with increase in volume to about 400 ml (between the points B and C, Fig. 83.4).
2. The intravesical pressure increases steeply when the intravesical volume exceeds 400 mL (between the points C and D, Fig. 83.4). This sharp rise in pressure initiates the reflex triggering of micturition.
3. The limb BC of cystometrogram is flat due to the application of Laplace law, which states that the pressure in a spherical viscus is equal to twice the wall tension divided by radius.
4. Urinary bladder being a spherical viscus, the tension increases as the organ fills. However, at the same time also the radius increases.
5. Therefore, increase in intravesical pressure is minimal unless the viscus is relatively full. However, above the volume of about 400 mL, pressure increases sharply as wall tension increases abruptly.

**Mechanism of Micturition**

The urge to pass urine is initiated with filling of the bladder, which is sensed by stretch receptors. The stretch receptors that are present in the wall of the bladder send impulses in the afferent nerve that initiate reflex contraction.

**Stimulus and Reflex Arc**

Filling of the bladder, causing stretch of bladder wall is the stimulus.

1. The stretch receptors that are present in the wall of the bladder send impulses in the afferent nerve that initiate reflex contraction.

**Control of Micturition**

The micturition reflex is controlled by centers in the brainstem.
1. The facilitatory area is present in pons and the inhibitory area is present in midbrain (Fig. 83.5). Therefore, section of neuraxis above pons promotes activity of micturition reflex in which less filling of bladder triggers its reflex evacuation, and section above midbrain does not affect it.

2. Posterior hypothalamus also contains a facilitatory area for micturition.

3. Cortex has voluntary inhibitory control on micturition. In children, below three years of age, cortical inhibition is not well developed; hence they often pass urine without their knowledge.

4. Control on urination starts to develop at about two years of age and completes by three years.

**BLADDER DYSFUNCTIONS**

**Abnormalities of Micturition**

The lesions at different segments of neuraxis result in bladder dysfunctions. There are three major neural defects that produce bladder dysfunctions:

1. Interruption of afferent fibers (deafferentation).
2. Interruption of both afferent and efferent fibers (denervation).
3. Interruption of influences from the facilitatory and inhibitory areas in the brain (spinal cord transection).

In these lesions, bladder may contract, but the contraction is not enough to empty the viscus. Therefore, residual urine is always left in the bladder.

**Deafferentation**

When, fibers originating from the sacral roots of the spinal cord are experimentally destroyed, reflex contraction of bladder is abolished.

1. The bladder is distended, thin-walled and hypotonic.
2. However, some contractions occur (due to intrinsic response of the smooth muscles to stretch).
3. This is typically seen in tabes dorsalis.

**Denervation**

When both the afferent and efferent fibers are cut, bladder becomes flaccid and distended at the beginning.

1. However, gradually the muscle of the bladder becomes active and the contraction of the bladder muscle removes urine in the form of dribbles.
2. Later, the bladder is shrunken and bladder wall becomes hypertrophied.
3. The hyperactive bladder suggests denervation hypersensitivity of bladder muscles.

**Spinal Cord Transection**

When the spinal cord is transected, typically three phases are observed: phase of shock, phase of recovery (increased reflex activity), and phase of failure.
1. During the phase of spinal shock, the bladder becomes flaccid and unresponsive. The overflow incontinence (urine dribbles through the sphincter when the bladder is overfilled) occurs.

2. In the phase of recovery, micturition reflex is the first reflex activity to return. However, voluntary control or control by the higher centers is abolished after transection.

   The spinal man (the human being following transection of the spinal cord) can train himself to initiate the micturition reflex by voluntarily activating the mass reflex (for details, see chapter “Spinal Regulation of Motor Control”).

   - The bladder capacity is decreased and the muscle of bladder is hypertrophied.
   - This type of bladder is known as spastic neurogenic bladder.

3. In the phase of failure, the infection of bladder makes the reflex activity worse.

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**CHAPTER SUMMARY**

**Key Concepts**

1. The initiation of micturition reflex occurs at 150 to 200 mL of bladder volume. At 400 mL urge to pass urine becomes urgent.
2. Micturition reflex is parasympathetic phenomenon. Cortical control has voluntary inhibitory effect.
3. Spastic neurogenic bladder is seen in spinal cord injury.

**Important to Know (Must Read)**

1. In examination, Long Questions are usually not asked from this chapter.
2. Micturition reflex, Cystometrogram, Mechanism of micturition, Abnormalities of micturition are asked as Short Questions in exam.
3. In Viva, examiner may ask... What are the functions of internal and external bladder sphincters, What are the innervations of urinary bladder, List the functions of urinary bladder, What are the phases of a cystometrogram, How is the micturition controlled voluntarily, How is the micturition controlled involuntarily, What is deafferentation, & what are its features, What is the effect of denervation on bladder and micturition, What is the effect of spinal cord transaction on bladder and micturition.
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Comprehensive Textbook of Medical Physiology
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Vol 2

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Comprehensive Textbook of Medical Physiology (Vol 2)

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Dedicated at the feet of
Sreema, the Divine Mother
and
Sri Aurobindo, the Divine Master

All Nature dumbly calls to her alone
To heal with her feet the aching throb of life
And break the seals on the dim soul of man
And kindle her fire in the closed heart of things.
All here shall be one day her sweetness’ home,
All contraries prepare her harmony;
Towards her our knowledge climbs, our passion gropes;
In her miraculous rapture we shall dwell,
Her clasp shall turn to ecstasy our pain.
Our self shall be one self with all through her.
In her confirmed because transformed in her,
Our life shall find in its fulfilled response
Above, the boundless hushed beatitudes,
Below, the wonder of the embrace divine.

Sri Aurobindo (in ‘SAVITRI’)
Oh India, land of light and spiritual knowledge!
Wake up to your true mission in the world,
Show the way to union and harmony.

The Mother (of Sri Aurobindo Ashram, Puducherry, India)

Physiology is the key subject in medicine. Starting from the knowledge of body functions, physiology provides the concept of dysfunctions, the basis of understanding the disease processes and the insight into disease management and prevention. Physiology is the core of medical wisdom. Due to its enormous contribution to the growth of medical knowledge, the Nobel Prize in health sector has been designated as Nobel Prize in Physiology and Medicine. Physiology as a subject in medical science has changed over the years from its nonclinical to preclinical and then to the current proclinical format with the incorporation of Applied and Clinical Physiology as the essential components in its core curriculum. Physiology is the foundation of medical practice. Many clinical investigations related to neurological disorders, autonomic dysfunctions, cardiovascular and respiratory diseases, endocrinal, renal, reproductive and metabolic problems are carried out in the well-equipped laboratories of physiology departments. Further, many research investigations are conducted in physiology laboratories. Sooner, the superspeciality course in Clinical Physiology will be a reality.

In India, Physiology as a subject in medical curriculum has changed immensely over decades. With the introduction of the new Medical Council of India (MCI) guidelines in 1997, the duration of first MBBS course was reduced from its original one-and-half years to one year. With subsequent modifications by MCI, directing physiology to become a more clinically oriented subject, a need aroused in reshaping the subject, integrating it with subjects of paraclinical and clinical medicine and orienting physiology knowledge for application-based learning. Therefore, in the present textbook, we have made all our sincere efforts without diluting the core concepts of physiology that includes regulation and integration of body functions, to amalgamate the knowledge in physiology with other subjects for its application in medicine.

After the publication of our Textbook and Practical Book of Physiology, the students and teachers in Physiology across the globe have been requesting to write a comprehensive book in Physiology that can offer a holistic concept of functions, integration, dysfunctions of body systems, and physiological basis of management and prevention of diseases. With all their wishes and blessings, finally this book has been made available to them. We hope this book will fulfill the aspiration of the readers in acquiring and applying the knowledge of physiology in clinics. Nevertheless, this is a project in evolution, and needs inputs, support and encouragement from our readers for its endless progression.

Gopal Krushna Pal
Pravati Pal
Nivedita Nanda
Let us work as we pray. For indeed work is the body's best prayer to the Divine.

The Mother (of Sri Aurobindo Ashram, Puducherry, India)

With pride and privilege, we acknowledge the contribution of all our past teachers, especially the professors of VSS Medical College, Burla, Odisha for educating us acquire the principle and practice of clinical medicine. We also gratefully acknowledge our past physiology teachers at Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry, India for having guided us learn the essentials of physiology, and notable among them are Dr DP Thombre, Dr V Srinivasan, and Dr (Late) DB Koner.

We sincerely acknowledge the contribution of Shri Jitendar P Vij, Group Chairman, Jaypee Brothers Medical Publishers Pvt Ltd, New Delhi for personally coming to Puducherry, and motivating and inspiring us to take up this special responsibility of writing such a wonderful book. For preparing the manuscript of the present book, we are especially thankful to Ms Chetna Malhotra Vohra (Associate Director - Content Strategy) and Ms Angima Shree (Senior Development Editor) for their constant support and timely help. The contribution of Ms Angima Shree is immense and praiseworthy. We also thank the other editors and designers of the Jaypee group who helped in the preparation of this book. We acknowledge Mr Narendra Singh Shekhawat (Delhi, India), Mr Venugopal (Bengaluru, Karnataka, India), and Mr Muralidharan (Puducherry, India) of the Jaypee group for their support. We are also thankful to Jaypee Brothers Medical Publishers for providing us many of the pictures and materials of their medical publications.

We are thankful to all our colleagues and students across the globe for reading our book and providing us their inputs for its further improvement. We thank all our colleagues and residents of JIPMER for their constant inspiration and support.

Auroprajna and Auroprakash, the divine children gifted to us, have been the constant support to us in all our endeavors. We shall fail in our duty if we do not appreciate the contribution of our sister Sabita Nanda, who has been constantly taking care of all our family requirements. We always keep in high esteem our parents Dr (Late) Artatran Nanda, Smt Anupama Nanda, Sri Mrutyunjay Pal and Srimati Malatimani Pal for showering on us their love and blessings and providing us everything to come to the greater heights in our life. We take this opportunity to express our heartfelt obeisance to Ms Kumud Ben of Sri Aurobindo Ashram, who is no more physically on this earth but lives in our hearts forever.
This *Comprehensive Textbook of Medical Physiology* has the following special features. These tips are meant for the readers to best use the book.

**Learning Objectives:** The topics start with *Learning Objectives*. By reading the learning objectives, a student will know the gross content of the topic, and how much he should acquire from it after reading the topic. The objectives have been divided into *Must Know* criteria that a student should minimum acquire, and *May Know* criteria that a student is desirable to acquire. These *Must Know* and *May Know* criteria will help a teacher prepare the content of his lecture class and to focus more on the major criteria.

**Scientists Contributed:** Invariably, important topics start with the contributions of great scientists in the concerned field, especially those who have received the Nobel Prize and/or are popular for their contributions in that field. Often, examiners ask to name the scientists who have invented/discovered the concepts or profoundly contributed to the development of the subject. This will not only give the information of the history of medicine, but will also inspire students and teachers to take up research in physiology and medicine. This part the readers should not miss!

**Application Box:** The concepts of Physiology have lot of applications in daily life and medical practice. Therefore, a major component of physiology is *Applied Physiology*. These important concepts and applied aspects of the topic are depicted in the *Application Box* and highlighted by green-colored boxes. If a student will miss to read these boxes, he/she will miss the core concepts in physiology.

**Clinical Physiology:** Presently, the learning in physiology is oriented to understand the etiology of the disease, and the physiological basis of management. Therefore, the major part of physiology is devoted for *Clinical Physiology*. The Clinical Physiology has been depicted in this book in the following formats:

- At the end of each topic, a description has been given for the common dysfunctions and disorders or diseases. A note has been given to explain the pathophysiology of the disease process and the physiological basis of the management. This is primarily to sensitize the 1st MBBS student for learning medicine, and to highlight the importance of physiology in learning medicine.
- Constructed pictures or original photos of the common diseases have been shown at the end of the topics. This is to create interest in the mind of the 1st MBBS student for clinical medicine. It also helps to understand and memorize Clinical Physiology.
- The core concepts related to diseases and patient management have been highlighted in *Clinical Box*. This provides the core concepts of understanding clinical medicine. The clinical boxes are highlighted with pink-colored bars.

The Clinical Physiology part is the uniqueness of this book, which is not given in any other textbook in this structured format. A student should never miss to read these clinical components in physiology.

**Important Note:** Some important and useful facts that are not covered in application or clinical boxes, are depicted as *Important Note*. These are useful information that may be asked in examinations, especially in viva voce.

**Structured Presentation:** Every chapter is divided into various parts by different headings and subheadings with different fonts and colors. Further, all important and complex mechanisms are structured and presented in a point-wise description. This structured presentation will help the student easily grasp the topic and memorize it. Further, this will ensure that a student does not miss any of the relevant points.
Flowcharts: All major concepts are simplified and summarized in ‘Flowcharts’. Not only it helps to memorize and recap the topic, but also, presenting the text along with flowcharts in examination helps the examiner easily assess the knowledge of the student. Usually, presentation with flowcharts in examination is more marks-fetching. The presentation of many flowcharts is a special feature of this book. A student must read and remember these flowcharts.

Schematic Diagrams and Graphs: All the relevant and significant mechanisms, theories and concepts are described in this book with the help of schematic diagrams and graphs. If a student is able to draw a labeled schematic diagram, it is always considered that a student has understood the topic. Especially in an examination, due to shortage of time, if a student draws a good schematic diagram and gives a brief answer with the help of flowcharts, even if he fails to give a descriptive answer, he gets good marks invariably. Therefore, the student should never miss to understand the diagrams and figures.

Tables: All important data, special concepts and lengthy information that a student needs to remember have been presented in structured tabular format. Reading the tables helps to revise and remember these facts quickly.

Histological Pictures: All mechanisms and manifestations of a disorder that require structural knowledge of a tissue or organ to comprehend the concept of the disease have been identified with appropriate histological pictures. For example, when a student sees the blood cells of an anemia in a blood smear, he understands and remembers better. A student must see these histological pictures.

Chapter Summary: All topics end with a ‘Chapter Summary’ that has been divided into two parts:
1. The first part is the ‘Key Concepts’, that depicts the central theme or the major take-home message of the topic. This is not the chapter summary, rather the summary of the main concepts.
2. The second part is the ‘Important to Know (Must Read)’ that provides all the probable long questions and short questions that usually come in theory examinations. Also, the questions that are usually asked in oral (viva) examination, are listed in this section. Students will definitely find it very useful. This will also help teachers to frame questions for the examination. A student should never miss this part.

Thus, this book is a comprehensive textbook that has incorporated all the requirements of a medical student for imparting the knowledge and skill of the subject, for acquiring all the ingredients needed to appear in the examination, and to complete the course with the best results.
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“A soul shall wake in the Inconscient’s house:
The mind shall be God-vision’s tabernacle,
The body intuition’s instrument,
And life a channel for God’s visible power.
....An unerring Hand shall shape event and act”

Sri Aurobindo (in ‘SAVITRI’
CHAPTER 84

Functional Organization of Cardiovascular System

**Learning Objectives**

On completion of study of this chapter, the student **MUST** be able to:
1. Name the different chambers of the heart and their valvular arrangement.
2. Correlate the functional histology of different layers in different blood vessels with their functions.
4. Name the different parts of vascular system and give their functions.
5. Appreciate the differences between systemic and pulmonary circulations.
6. Understand the importance of central and peripheral blood volumes.

The student **MAY** also be able to:
1. Describe the functional classification of blood vessels based on their histological specializations.

Cardiovascular system (CVS) or the circulatory system transports essential nutrients, oxygen, hormones, etc. to the tissues and removes the products of metabolism from the tissue and also contributes to the regulation of many homeostatic functions like fluid balance, temperature regulation of the body. These cardiovascular (CV) functions are carried out primarily by altering the amount of blood pumped by the heart and by changing the caliber of the vessels. It is important for circulatory system to perform efficiently to meet the usual challenges of day-to-day activities imposed by exercise, exposure to hot or cold environment, change in posture, emotional reactions, and other stressors. In recent years, there is an upsurge in the cardiovascular morbidity and mortality in Indian subcontinent due to increased prevalence of diabetes, hypertension, and ischemic heart disease. Therefore, a student of medicine should learn the fundamentals of cardiovascular physiology to appreciate the management of cardiovascular diseases.

**Components of CVS**

Cardiovascular system consists of a central pump, the heart that propels blood into circulation, and an extensive network of blood vessels that circulates blood throughout the body.

**Scientist contributed**

**William Harvey** (1578–1657), a student of Girolamo Fabriczi (1537–1619) at Padua, and greatly influenced by Aristotle, studied in detail his master’s work on venous valves. He analyzed the action of heart, blood is ejected by heart contraction, estimated amount of blood in the body, and demonstrated that blood continuously ejected from heart returns back to it. He extensively studied blood circulation through arteries and described the functions of venous valves and clarified circulation of blood through lungs. As a distinguished teacher and physician, his lecture notes on circulation in 1616 were highly appreciated by all.

**Central Pump: The Heart**

The primary function of the heart is to pump blood into circulation. The forceful ejection of blood creates energy for the blood to circulate in the blood vessels. The heart consists of a dual pump (the right and left pumps) that ejects blood into two serial circuits: the systemic and pulmonary circulations.

1. The right pump is the right ventricle that propels blood into the pulmonary circulation for exchange of gases in the lungs, and the left pump is the left ventricle that propels blood into the systemic circulation to supply oxygen and nutrients to the tissues (Fig. 84.1).
2. The output of each pump at rest is about 5 l/min. This accounts for about 400 million liters of blood pumped by the heart during the lifetime of a person who lives about 70 years, which is enough to fill a lake of 1 km long, 40 m wide and 10 m deep.

3. In addition to this resting output, pumping of blood is increased many-fold in daily routine works, exercise, emotion, etc.

Heart has four chambers: Two atria and two ventricles (Fig. 84.2). The right atrium and right ventricle constitute right side of the heart (sometimes, called as right heart, especially by clinicians), and the left atrium and left ventricle constitute the left side of the heart (or the left heart).

**Right Side of the Heart**

The right atrium receives blood from different parts of the body through superior and inferior vena cava and empties blood into the right ventricle (Fig. 84.3).

1. The right atrioventricular valve or the tricuspid valve guards the flow of blood from right atrium to right ventricle and prevents flow of blood in backward direction.

2. The right ventricle pumps blood into the pulmonary circulation through the pulmonary trunk (the pulmonary arteries) where gaseous exchange takes place.

3. Pulmonary valve prevents back flow of blood from pulmonary trunk into the right ventricle.

**Left side of the Heart**

The left atrium receives oxygenated blood from the pulmonary circulation via pulmonary veins and empties blood into left ventricle through left atrioventricular valve or mitral valve (Fig. 84.3).

1. The mitral valve ensures unidirectional flow of blood from the left atrium to the left ventricle.

2. **Left ventricle** pumps blood into the systemic circulation through the aorta.

3. The aortic or the semilunar valve, which is present at the base of aorta prevents back flow of blood into left ventricle from the aorta.

**Circulatory System: The Blood Vessels**

Blood is distributed to the different parts of the body by the systemic arteries. Blood from left ventricle is pumped to **systemic circulation** that delivers oxygenated blood to tissues (Fig. 84.4). The deoxygenated blood that returns from venous compartment is pumped by right ventricle to the **pulmonary circulation** for oxygenation.
Chapter 84: Functional Organization of Cardiovascular System

1. The systemic arteries are more extensively branched and thicker than the pulmonary arteries. Therefore, systemic circulation provides more resistance to flow of blood.

2. Hence, left ventricle pumps blood at a much higher pressure (to overcome the systemic resistance) than the right ventricle. The peak left ventricular pressure is about 120 mm Hg, whereas peak right ventricular pressure is only about 25 mm Hg.

3. This is the reason why left ventricular muscle mass (thickness of the left ventricular wall) is more than the muscle mass of right ventricle (Fig. 84.5).

The blood vessels form a close system of tubes (the vascular system) that transport blood from the heart to the tissues and return blood from the tissues to the heart (Fig. 84.4).

Functional Histology of Blood Vessels

Generally, the blood vessels have three layers: The tunica externa (adventitia) or the outer coat; tunica media (the muscle layer) or the middle coat; and the tunica interna (intima) or the inner layer (Fig. 84.6).

1. The tunica externa consists of elastic tissues and collagen fibers.

2. The tunica media consists of smooth muscles and elastic tissues. In tunica media, the smooth muscles are arranged circularly around the lumen of the blood vessels. Therefore, contraction of the smooth muscles as occurs in sympathetic stimulation leads to vasoconstriction, and relaxation of the smooth muscles as occurs in sympathetic inhibition leads to vasodilation.

3. The tunica intima is composed of an endothelial cell lining, which is a simple squamous epithelium. This is called the vascular endothelium.

Components of Vessel Wall

In accordance with three layers of blood vessels, four important components form the vascular wall: The endothelial cells, elastic tissue, smooth muscles and fibrous tissue (collagen fibers). Distribution of these components in various blood vessels determines their functional significance (Fig. 84.7). They also influence the ratio of wall thickness to the internal diameter that significantly contributes to their role in hemodynamics.
Endothelial Cells

Endothelial cells form the inner lining of all blood vessels, known as vascular endothelium.
1. They are present as a single continuous layer. Tight junctions and other intercellular connections keep the endothelial cells adhered to each other.
2. In capillaries, the vessel wall is formed only by a layer of endothelial cells present on the basal lamina. This makes them suitable for exchange of substances between blood and tissues.
3. However, the transport of substances across the capillary wall depends on how tightly the cells in the endothelium are adhered together. For example, tight junctions of endothelial cells of capillaries in brain are very tight, which contributes to the effectiveness of blood brain barrier, whereas in liver they are not so tight, which allows easy capillary filtration.
4. Endothelial cells also secrete many chemicals and hormones that control cardiovascular functions.

Elastic Tissue

The amount of elastic tissue present in the vessel wall determines the ability of the vessel to stretch. The percentage of elastic fibers in aorta and large arteries is more in comparison to their other components. Therefore, these vessels have more compliance. Elastic fibers are made of elastins and microfibrils.
1. Elastins are protein molecules formed by nonpolar amino acids such as glycine, alanine, valine and proline.
2. Microfibrils are minute fibril strands made up of glycoproteins. Elastin molecules assemble along with microfibrils to form a network of fibers that is highly capable of stretching. Elastic fibers are abundant in arteries and veins, less in arterioles, and absent in capillaries and venules.

Smooth Muscle

Smooth muscle is present in all vessels except capillaries and venules. The quantity of smooth muscles is more than elastic fibers in arterioles, metarterioles and small arteries. Therefore, these vessels serve as resistance vessels.

Fibrous Tissue

In blood vessels, fibrous tissue is primarily made up of collagen fibers, which are mainly type I and III collagens. Fibrous tissue is present in all parts of vascular tree except capillaries. It prevents distention of vessels and increases wall tension.

Physiological Classification of Blood Vessels

Functionally, blood vessels are classified into four categories: Windkessel vessels, resistance vessels, exchange vessels and capacitance vessels.

Windkessel Vessels

The aorta and large arteries are called Windkessel vessels. Windkessel, a German word, means an elastic reservoir. The aorta and large arteries have more elastic elements in their wall (Fig. 84.7). Therefore, these vessels have higher compliance.
1. During systole, their wall stretches to accommodate the blood ejected by ventricles, and during diastole, their wall recoils back to presystolic position. This recoiling effect is called Windkessel effect.
2. The Windkessel or the recoiling effect pushes blood in forward direction during diastole. Blood in blood vessels is pushed forward during systole by the force created by ventricular ejection and during diastole by the force created by arterial recoiling.
3. Thus, blood flow is continuous during both the phases of cardiac cycle.
Chapter 84: Functional Organization of Cardiovascular System

Resistance Vessels
Arterioles, metarterioles and smaller arteries are the resistance vessels.
1. They have thick muscle coat and less elastic fiber in their wall. Therefore, they provide resistance to flow of blood.
2. The maximum resistance to flow is offered by arterioles. Thus, arterioles form the major seat of peripheral resistance.
3. Hence, the pressure falls significantly when blood passes through the arterioles.

Exchange Vessels
Capillaries are thin vessels as their wall is formed only by a single layer of endothelial cells. The blood flow is sluggish in capillaries due to more total cross sectional area of these vessels. These two factors (thin wall and sluggish flow) favor exchange of gases and nutrients along the capillary wall (for details, see Chapter 95). Therefore, capillaries are called exchange vessels.

Capacitance Vessels
Normally, more than 60% of the total blood is present in the venous compartment. Veins can accommodate still larger amount of blood without increasing much pressure in them. Therefore, large and small veins are called capacitance vessels.

Divisions of Vascular System
The vascular tree consists of three systems: The arterial system, the system of capillary networks and the venous system.

Arterial System
The arterial system consists of the aorta, large arteries, smaller arteries, arterioles and metarterioles. Blood passes rapidly in the arterial system. The function of the arterial system is to deliver oxygenated blood from lungs and nutrients from intestine to the tissues.

Arteries
Arteries have thick tunica media.
1. The smooth muscles of arteries are extensively innervated by sympathetic fibers of the autonomic nervous system. Therefore, sympathetic stimulation results in vasoconstriction and sympathetic inhibition leads to vasodilation.
2. Vasoconstriction is the term generally used for constriction of the arteries, and vasodilation for dilation of the arteries.
3. The arteries are specifically susceptible to atherosclerosis, a process in which there is formation of atheromatous plaque on the lumen of the blood vessels.
4. Pathological atherosclerosis significantly reduces blood flow to the organs and causes myocardial infarction and stroke.

Arterioles
Arteries branch out to form the arterioles.
1. Arterioles regulate the resistance to flow through the various organs of the body. Thus, they control distribution and rate of blood flow to the organs.
2. Arterioles have thick muscle coat and few elastic fibers in their wall. The ratio of the thickness of the wall to the diameter of the vessel is high in arterioles.
3. Therefore, contraction or relaxation of the smooth muscles in arteriolar wall allows them to readily control the vessel caliber. Hence, arterioles are the main resistance vessels and they serve as the ‘stopcock’ of the circulation.
4. Atherosclerosis of arterioles is called arteriolosclerosis.

Metarterioles
Arterioles terminate in metarterioles.
1. Metarterioles are histologically same as that of arterioles except that the muscle coat is thin and the lumen is smaller.
2. A flap of smooth muscle called precapillary sphincter is present at the end of the metarterioles or arterioles. This sphincter regulates blood flow to the capillaries.

System of Capillary Network
The system of capillary network originates from arterioles and metarterioles. It consists of extensively branched, small and thin-walled vessels. The flow of blood is slow in the capillaries that favor exchange of materials between the blood and the tissues.
1. Capillaries connect the arterioles (or the metarterioles) to the venules. They are made up of only the tunica interna, a single layer of endothelial cells. Thus, their wall is thin, which makes them suitable for exchanging materials between the blood and the tissues. Capillaries are therefore called the exchange vessels.
2. The circulation of blood through the capillaries is called the microcirculation.
3. There is no smooth muscle in the capillaries. Therefore, constriction or dilation of capillaries occurs mostly passively by the amount of blood that passes through the capillary bed, which is regulated by the precapillary sphincter. However, capillaries do constrict directly due to the presence of pericytes and few myosin like filaments in their walls.
4. Due to their extensive branching networks, the total cross sectional area of capillaries is large. Hence, blood flow is very slow in this segment of circulation, which further facilitates the process of transcapillary exchange. The different types of capillaries and details of capillary circulation are discussed in Chapter 95.

Venous System
The venous system starts from capillaries. It consists of collecting venules, venules, and small and large veins. The
function of venous system is to collect, store and deliver blood into the atria. The collecting venules originate from capillaries and drain into venules that in turn drain into veins. The larger veins finally drain blood into the atria. The delivery of blood into atria is called venous return.

**Venules**

Many capillaries unite to form venules. The function of the venules is to collect blood from the capillaries and drain into the veins.

1. These small vessels have thinner muscular wall that form the low pressure collecting system. Like arterioles, venules are innervated by sympathetic fibers.
2. Contraction or relaxation of the venules contributes to the overall size of the venous compartment.

**Veins**

Venules unite to form veins that join together to form larger veins. Veins have thin muscle coat. About two-thirds of blood volume in the systemic circulation is present in the veins (Fig. 84.8). Therefore, veins serve as the blood reservoir.

1. The veins in the limbs, especially in the lower limbs contain valves within. The venous valves are made up of thin folds of tunica intima that forms a flap like cusps. These valves point toward the heart and prevent retrograde flow of blood. Thus, venous valves facilitate venous return.
2. Venous return is also assisted by contraction of the skeletal muscles of the limb in which the veins are located.

**SYSTEMIC vs PULMONARY CIRCULATION**

The circulatory system consists of two important parts: The systemic circulation and the pulmonary circulation.

# Table 84.1: The major differences between systemic and the pulmonary circulations.

<table>
<thead>
<tr>
<th></th>
<th>Systemic Circulation</th>
<th>Pulmonary Circulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Synonyms</td>
<td>Peripheral or greater circulation</td>
<td>Central or lesser circulation</td>
</tr>
<tr>
<td>2. Pressures</td>
<td>High pressure</td>
<td>Low pressure</td>
</tr>
<tr>
<td>− Systolic press</td>
<td>100–140 mm Hg</td>
<td>20–30 mm Hg</td>
</tr>
<tr>
<td>− Diastolic press</td>
<td>60–90 mm Hg</td>
<td>5–15 mm Hg</td>
</tr>
<tr>
<td>− Mean pressure</td>
<td>100 mm Hg</td>
<td>15 mm Hg</td>
</tr>
<tr>
<td>− Pulse pressure</td>
<td>20–50 mm Hg</td>
<td>10–15 mm Hg</td>
</tr>
<tr>
<td>3. Arteries</td>
<td>Thick wall and narrow lumen</td>
<td>Thin wall and large lumen</td>
</tr>
<tr>
<td>4. Arterioles</td>
<td>Thick wall and narrow lumen</td>
<td>Thin wall and large lumen</td>
</tr>
<tr>
<td>5. Capillaries</td>
<td>Long and narrow</td>
<td>Short and wide</td>
</tr>
<tr>
<td>6. Resting sympathetic tone</td>
<td>High vasoconstrictor tone at rest</td>
<td>Absent (lack vasoconstrictor tone)</td>
</tr>
<tr>
<td>7. Peripheral resistance</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>8. Capillary blood flow</td>
<td>Continuous</td>
<td>Pulsatile</td>
</tr>
</tbody>
</table>

**Systemic Circulation**

The circulation of blood through the systemic vascular bed is called systemic circulation. This is also called the peripheral circulation or the greater circulation.

1. This is the major circulation as it contains **80–85% of the total volume of blood**. It provides oxygenated blood to different parts of the body and drains venous blood back to the heart.
2. The left ventricle pumps blood into the systemic circulation. The pressure in the systemic circulation, especially in the arterial compartment, is high. The systolic pressure is 100 to 140 mm Hg.
3. The pressure drops progressively as the blood flows from the arteries to the veins. The arteries and arterioles have thicker walls and narrower lumen, and are richly innervated by the sympathetic fibers (Table 84.1).
4. They have **high resting vasoconstrictor tone**. Thus, they provide higher resistance to blood flow. The capillaries are longer and narrower. Therefore, systemic capillaries also provide resistance to blood flow.

**Pulmonary Circulation**

The circulation of blood through the pulmonary vascular bed is called pulmonary circulation. This is also called the central circulation or the lesser circulation. It is the minor circulation as it contains **only 10 to 12% of the total blood volume**. It provides a low resistance pathway for the entire output of the right ventricle to traverse through the lungs.

1. The primary function of pulmonary circulation is to exchange gases between the blood and the atmosphere. The arteries have thin wall and large lumen.
2. They have also rich sympathetic innervations, but unlike that of systemic arteries they lack resting vasoconstrictor tone.

3. The arterioles have thin wall and larger lumen. Therefore, pulmonary arterioles provide low resistance to flow. The capillaries are shorter and wider. Consequently, resistance is also less in capillaries.

4. The vessels in the pulmonary circulation are primarily designed to provide a low resistance circulation for the entire output of the right ventricle to pass through them.

5. The output of the right ventricle is same as the output of the left ventricle. The blood from the left ventricle is pumped into the systemic circulation, which is a much larger compartment that easily accommodates the left ventricular output. The equal amount of blood is ejected by the right ventricle, but into a much smaller compartment, the pulmonary circulation.

6. Therefore, the nature has provided a low resistance circuit for the pulmonary vascular bed to accommodate and quickly transfer the right ventricular output to left side of the heart.

VOLUME DISTRIBUTION IN VASCULAR COMPARTMENTS

Five to six liters of blood is present in an adult weighing 70 kg. Of the total blood volume, 10–12% is present in pulmonary circulation, 5–10% in heart and 80–85% in systemic vessels (Fig. 84.8).

1. In pulmonary circulation, the blood is almost equally distributed amongst arteries, capillaries and veins, whereas in systemic circulation, about three fourth of blood (75% of systemic circulation, or 60% of the total) is present in venous compartments.

2. Though, only 5% of the blood volume is present in capillaries, capillary blood is important for tissue oxygenation and nutrition.

Central vs Peripheral Blood Volume

Blood volume is divided into central or thoracic and peripheral or extrathoracic blood volume.

Central Blood Volume

The central blood volume is the volume of blood present in pulmonary circulation, heart, superior vena cava, intrathoracic portion of inferior vena cava and aorta.

1. It constitutes about 25% of the total blood volume.

2. Central blood volume is important as it determines the atrial filling and cardiac output.

3. Central venous pressure is a good indicator of central blood volume, as the compliance of intrathoracic vessels apparently remains same.

Peripheral Blood Volume

The peripheral blood volume is the volume of blood that is mainly present in veins of the extremities, abdominal cavities and head and neck.

1. It constitutes about 75% of the total blood volume.

2. Contribution of volume of blood in head and neck to the peripheral pool is less, as it is less in quantity. Therefore, mainly it is blood in limb and abdominal veins that constitute the peripheral blood volume.

3. Shift of blood from peripheral pool to central pool is physiologically important, as it finally determines the cardiac output (Clinical Box 84.1).

Clinical Box 84.1

Central and peripheral blood volumes are important: Alteration in total blood volume due to any cause affects both central and peripheral blood volumes. For example, acute hemorrhage that decreases effective blood volume decreases both central and peripheral volumes. However, a decrease in blood volume in central compartment is immediately compensated by shift of blood from the peripheral compartment. But, if adequate shift does not occur due to failure of the compensatory mechanisms, ventricular end-diastolic filling and cardiac output are greatly compromised.

CHAPTER SUMMARY

Key Concepts

1. Heart is the central pump that ejects blood and generates pressure for blood to circulate in blood vessels. Therefore, in cardiac arrest circulation stops.

2. Systemic circulation is the high resistance circulation and pulmonary circulation is the low resistance circulation.

3. Central blood volume (blood present in pulmonary circulation, heart, superior vena cava, intrathoracic portion of inferior vena cava and aorta) is important as it directly contributes to atrial filling, central venous pressure and cardiac output.

Important to Know (Must Read)

1. In examination, Long Questions are usually not asked from this chapter.

2. Types of blood vessels, Pulmonary circulation, Windkessel effect, Differences between systemic and pulmonary circulation, Vasoconstrictor tone, Central venous pressure, Central blood volume, may be asked as Short Questions in exam.

3. In Viva, examiner may ask… List the differences between systemic and pulmonary circulation, What are the layers of a blood vessel, What are the components of the vessel wall, How the blood vessels are classified physiologically, Which are the Windkessel vessels and what is Windkessel effect, Which are the resistance vessels and why, Which are the exchange vessels and why, Which are the capacitance vessels and why, What is resting vasoconstrictor tone, What is the percentage distribution of blood volume in different vascular compartments, What is central blood volume, What is central venous pressure, What is peripheral blood volume.
CHAPTER 85
Functional Anatomy of Heart, Cardiac Muscle, Conducting System, and Cardiac Innervation

Learning Objectives

On completion of study of this chapter, the student **MUST** be able to:
1. Give a note on functional anatomy of heart.
2. Apprehend the reflections of pericardial layers and understand the physiological consequence of pericardial effusion.
3. Understand the functional histology of cardiac muscles.
4. Appreciate the role of cardiac muscle as functional syncytium.
5. Understand the myocardial contractile system and arrangement of sarcotubular system in cardiac muscle.
6. Explain the length–tension relationship of cardiac muscle.
7. Define Frank-Starling law of the heart and explain its mechanisms.
8. Trace the conducting system of the heart.
9. Differentiate the effects of vagal and sympathetic stimulations of heart.
10. Appreciate the importance of cardiac vagal tone and sympathetic tone.

The student **MAY** also be able to:
1. Explain the myocardial contractile system.
2. Describe the properties of cardiac muscle.
3. Describe the innervation of the heart.

Heart is a very special organ as it beats automatically throughout life. Though, structurally cardiac muscle closely resembles skeletal muscle, it does not depend on external innervation for its contraction. Cardiac muscle contracts in response to the impulse generated intrinsically in pacemaker tissue, the SA node. Myocardial contraction results in pumping of blood, which is the primary function of the heart. Cardiac muscle is neither fatigued nor tetanized for its specialized histological, mechanical and electrical properties, excitation-contraction coupling, and length–tension relationship.

**FUNCTIONAL ANATOMY**

The size of the heart of an individual is approximately the size of his closed fist. The average weight of the heart is about 300 g in adults. The heart is covered and protected by pericardium. The heart consists of four–chambers: two atria (right and left) and two ventricles (right and left). The wall of each chamber of the heart has three layers of which most developed in the ventricles is the myocardium.

**Scientist contributed**

Ernest Henry Starling (1866–1927), was a great teacher and researcher in physiology, whose long career was marked by many contributions to the development in physiology. He studied the mechanism of lymph formation and described the mechanical factors for lymph production. He studied the osmotic effects of serum proteins on fluid movements along the capillaries, for which the mechanism of capillary filtration is popularly known as Starling hypothesis, and the factors are called Starling forces. He studied the chemical regulation of pancreatic secretion. He described the principles of cardiac functions, especially the **length–tension relationship of cardiac muscle**, which is popularly known as Starling’s law or Frank-Starling law of the heart. He had also studied the factors determining the growth of mammary gland.

**Pericardium**

The covering of heart is called pericardium. It is a double-layered sac that covers and protects the heart (Fig. 85.1).
Fibrous Pericardium
The outer layer is the fibrous pericardium, which is made up of dense irregular connective tissue. For its toughness and inelasticity, the fibrous pericardium prevents overdistension of the heart.

Serous Pericardium
The inner layer is the serous pericardium, which is further made up of two layers.
1. The outer layer of the serous pericardium is the parietal pericardium, which is in close contact with the fibrous pericardium.
2. The inner layer of the serous pericardium is the visceral pericardium, which is in close contact with the surface of the heart (Fig. 85.2).

Pericardial Cavity
The space between the two layers of the serous pericardium is called the pericardial cavity. The serous fluid present in the pericardial cavity is the pericardial fluid.
1. Normally, the amount of pericardial fluid is about 15 mL. The function of pericardial fluid is to reduce friction between the layers of the pericardium when heart expands or contracts.
2. When fluid accumulates in excess in the pericardial cavity as occurs in pericarditis, the condition is called pericardial effusion (Fig. 85.3) (Clinical Box 85.1).

Wall of Heart Chambers
The wall of heart chambers has three layers: epicardium, myocardium, and endocardium (Fig. 85.2).

Epicardium
This is the outermost layer. In fact, it is the visceral layer of the serous pericardium. It is made up of mesothelium and connective tissue.

Myocardium
The middle layer is the myocardium that consists of the cardiac muscle. Myocardium forms the bulk of the ventricles, which is primarily responsible for ventricular pump activity.
1. The wall of atria is thinner than the wall of ventricles. The wall thickness is proportionate to the pressure generated by the chambers to propel blood. Atrial wall is thinnest (2 mm thick) as the blood from atria enters ventricles mostly passively.

2. The left ventricular wall thickness is 10 mm as left ventricle generates maximum pressure to pump blood into the systemic circulation, and right ventricular wall thickness is 5 mm as right ventricle generates less pressure to pump blood into the pulmonary circulation (Fig. 85.4).

3. The muscles of the ventricles are arranged mostly spirally around the lumen of the chambers.

Endocardium

This is the inner wall of the heart. It consists of a layer of endothelium overlying a thin layer of connective tissue. It is continuous with the endothelial lining of large vessels that originate from the heart.

Cardiac Chambers

Heart is made up of four chambers: two atria (right and left) and two ventricles (right and left). The atria are thin-walled and low pressure chambers that serve as temporary reservoir to transmit blood into the respective ventricles. The ventricles are thick-walled chambers that pump blood into the circulation.

Right Atrium

Right atrium receives blood from different parts of the body via inferior and superior vena cava. This is called venous return. It pours blood into the right ventricle through the right atrioventricular valve or tricuspid valve. The emptying of blood from the right atrium into the right ventricle is largely passive. Only about 20% of the blood is transferred by atrial contraction (atrial systole).

Left Atrium

Left atrium receives blood from the pulmonary circulation via four pulmonary veins and empties blood into the left ventricle via left atrioventricular valve or the mitral valve.

Right Ventricle

The right ventricle pumps blood into pulmonary circulation through the pulmonary semilunar valves. The peak pressure generated by the right ventricular contraction is about 25 mm Hg.

Left Ventricle

The left ventricle pumps blood into the systemic circulation through the aortic semilunar valves. The peak pressure generated by the left ventricular contraction is about 120 mm Hg.

Cardiac Valves

The cardiac valves are present at the junction between the chambers and at the output orifices of the ventricles. They are made up of thin flaps (leaflets) of fibrous tissue covered with endothelium, which are firmly attached to the valve rings. The orientation of the valves allows unidirectional flow of blood through the heart and the movement of the valve leaflets opens or closes the valves. There are two atrioventricular valves and two semilunar valves.

Atrioventricular Valves

They are located between the atria and ventricles. The left atrioventricular valve is the mitral valve, which has two cusps (bicuspid valve). The right atrioventricular valve is the tricuspid valve (Figs. 85.5A and B).

1. The size of the cusps of the atrioventricular valves is more than the size of atrioventricular orifice. Therefore, when valves are closed there is adequate overlapping of the valve leaflets, which totally prevents leakage of blood back into the atrium.

2. The chordae tendineae (strong ligaments arising from the papillary muscles) are attached to the free edges of the valves, which prevents eversion of the valves during systole (Fig. 85.6).

Semilunar Valves

These are located at the outlet orifice of the ventricles. The pulmonary semilunar valve is located between the right ventricle and the pulmonary artery, and the aortic semilunar valve is located between the left ventricle and the aorta (Figs. 85.5A and B).

1. These valves consist of three cusps, which are attached to the valve rings.
2. At the beginning of ventricular systole, the cusps open and blood is propelled from ventricles and toward the end of systole when the blood flow reverses (from aorta toward the ventricle), the cusps snap and prevent regurgitation of blood into the ventricles.

**CARDIAC MUSCLE**

Heart muscle is classified as striated muscle. The myocardial fibers are separated from each other by sarcolemma. The nucleus of the muscle cell is centrally placed.

1. The muscle fibers are branched.
2. At the junctions of branching of fibers, there are specialized areas known as **intercalated discs** (Fig. 85.7). Intercalated discs contain many **gap junctions**.
3. Gap junctions are **electrical synapses** through which the wave of depolarization can easily pass through from cell to cell.
4. Therefore, when a cardiac muscle cell is stimulated, the electrical impulse spreads rapidly to the other muscle cells and heart contracts as a single unit. Thus, cardiac muscles act as a **functional syncytium**.

**Heart as a Functional Syncytium**

A syncytium is defined as a tissue containing cells with no boundaries (the nuclei are free in the protoplasm). Thus, syncytial tissue acts as a single unit. The example of a structural syncytium is the muscles in the body-wall of the earthworm.

1. In heart, the myocardial fibers are indeed separated from each other. Therefore, myocardial tissues are not anatomical syncytium. However, they are **physiological syncytium** as functionally they behave like a single unit due to presence of numerous gap junctions.
2. In fact, there are two syncytia in the heart: the atrial syncytium and the ventricular syncytium. This is because the atria are completely separated from the ventricles by a fibrous band of tissue known as atrioventricular ring.

3. Therefore, atria and ventricles are functionally two separate units.

Myocardial Contractile System

Though myocardial cells differ from skeletal muscle cells in few aspects, the contractile mechanisms in both the tissues are almost similar. The myocardial contractile unit consists of sarcomeres that contain thick and thin filaments. Thick filaments are myosin filaments and thin filaments are actin filaments. As in the skeletal muscle, shortening of cardiac muscle occurs by the sliding filament mechanism (for details, see chapter “Nerve and Muscle”). An important difference is that skeletal muscle cells contain few mitochondria whereas the cardiac muscle cells are rich in mitochondria, which allows them for rapid oxidation of substrates to produce ATP.

Sarcotubular System

The sarcotubular system is well developed in the myocardial cells.
1. It consists of transverse tubules (T tubules) and sarcoplasmic reticulum (cisterns).
2. The T tubules are present as deep invagination of the sarcolemma into the muscle fibers at the Z line (Fig. 85.8), which is present at A-I junction in the skeletal muscle (Table 85.1).
3. T tubules are almost continuous with the interstitial fluid for which they play important role in muscle contraction.
4. T tubules are more developed in ventricular myocytes than the atrial myocytes, and the diameter of T tubule in ventricular muscle cell is more than double the diameter of T tubules in skeletal muscle cell. The sarcoplasmic reticulum is present in close association with T tubular system forming a diad that contains one T tubule and one cistern (Fig. 85.8). This arrangement is in the form of a triad (one T tubule and two cisterns) in the skeletal muscle.

Length–Tension Relationship

The length–tension relationship of cardiac muscle is similar to that of skeletal muscle. The active force developed is maximum within the optimal length (Fig. 85.9).
1. The interaction of thick and thin filaments and the number of cross-bridge formation are maximal within this optimal length.
2. The increase in length of sarcomere increases the sensitivity of the myofilaments to calcium. It is also explained that stretch of myocardium increases the affinity of the troponin C to calcium.
3. When the sarcomeres are stretched beyond the optimal length, the force developed decreases due to decrease in the overlap and interaction between the thick and thin filaments. This decreases cross-bridge formation.
4. The length–tension relationship is implicated to explain the physiological activities of the heart.

**Frank-Starling Law of the heart**

This law states that the *force of contraction is directly proportional to the initial length of the muscle fiber, within the physiological limit*. This is also known as Starling’s Law of the heart. This explains how the increase in preload or end-diastolic volume increases cardiac output.

There are *four mechanisms* to explain Frank-Starling Law:
1. With increased end-diastolic volume, the initial length of the muscle fiber increases due to increase in chamber size that stretches the muscle fibers. With increased stretch of muscle fibers, *the interaction between thick and thin filament increases*, which increases the force of contraction.
2. The stretch of muscle fibers opens the *stretch-sensitive calcium channel* on the muscle cell membrane. This increases the calcium influx into the myocardial cell. Thus, force of contraction increases.
3. Increase in intracellular calcium ions due to calcium influx from ECF induced by stretch, increases the further *release of calcium from sarcoplasmic reticulum by activating the calcium-induced calcium channels* present on the membrane of this cell organelle. Increased intracellular calcium increases the force of contraction.
4. Stretch of myocardium enhances the affinity of troponin C for calcium. This increases the binding of calcium to troponin C, which enhances the force of contraction.

**Excitation-Contraction Coupling**

The mechanism of excitation-contraction coupling is similar to that of the mechanism that operates in skeletal muscle. However, the appropriate concentration of sodium and potassium in addition to the concentration of calcium is essential for excitation and contraction of cardiac muscle. These ions are needed for the generation of the resting membrane potential (RMP), the action potential and the pacemaker potential of cardiac tissues. K+ is mainly required for the RMP and therefore determines the excitability, Na+ and Ca++ are mainly required for action potential, K+ and Ca++ are mainly required for pacemaker potential, and concentration of Ca++ is required for the muscle contractility.

1. Na+ is required for action potential of cardiac muscle. Therefore, decreased extracellular Na+ concentration decreases the excitability of the heart.
2. Decreased extracellular K+ does not affect excitability significantly, whereas an increase in K+ concentration in the ECF causes depolarization and loss of excitation that finally results in cardiac arrest in diastole.
3. An *increase in extracellular Ca++* increases the force of contraction, and if the concentration is very high it causes cardiac arrest in systole (calcium rigor). The *decreased extracellular Ca++* concentration decreases the force of contraction and eventually cardiac arrest occurs in diastole. When excitation (the wave of depolarization) of the cardiac muscle spreads into the muscle cells through the T tubules, Ca++ is released from the sarcoplasmic reticulum. Calcium also enters the cytosol from the interstitial fluid. In fact, the Ca++ that enters the cell from the interstitial fluid triggers Ca++ release from the sarcoplasmic reticulum. The Ca++ then binds with the troponin C and the calcium-troponin complex interacts with the tropomyosin that uncovers the active site between the thick and thin filament. This allows the cross-bridge cycling, which causes muscle contraction. It is important to remember that alteration in cytosolic Ca++ alters the force of contraction. Therefore, mechanisms that increase the cytosolic Ca++ increase the force of contraction, and the mechanisms that decrease the cytosolic Ca++ decrease the force of contraction. For example, epinephrine enhances myocardial contractility by increasing the cytosolic Ca++.
4. Cytosolic Ca++ can be increased by *two mechanisms*: i. By increasing the Ca++ in the ECF. ii. By decreasing the Na+ gradient across the sarcolemma.

The Na+ gradient can be decreased either by increasing the intracellular Na+ or by decreasing the extracellular Na+. The increase in cytosolic Na+ retards the sodium-calcium exchange, which decreases the Ca++ removal from the cell so that concentration of Ca++ in the cell increases. The decreased ECF Na+ causes less Na+ to enter the cell, so that less Ca++ is exchanged for Na+, which results in accumulation of Ca++ in the cell. The cardiac glycosides like digoxin increase the force of contraction by inhibiting the Na+-K+ ATPase that results in accumulation of intracellular Na+. This decreases the sodium-calcium exchange and allows Ca++ to accumulate in the cell.

**Contractility**

This is defined as the *change in peak isovolumetric force at a given initial fiber length* (at a particular end-diastolic volume). It represents the performance of ventricle at a given preload or afterload. The increased contractility represents increase in developed force and velocity of contraction.

**Capillary Density**

Myocardium is highly rich in capillaries:
1. There is almost one capillary for one muscle fiber in cardiac muscle in comparison to one capillary for 100–500 muscle fibers in skeletal muscles.
2. Because of the anatomical closeness between the capillary and the muscle fiber, the diffusion distance (the distance between the capillary membrane and the sarcolemma) is very less, which facilitates delivery of oxygen and nutrients and removal of carbon dioxide and waste products.

Conducting System of the Heart

The conducting system of the heart consists of sinoatrial (SA) node, internodal pathways, atrioventricular (AV) node, His bundle, bundle branches and Purkinje fibers (Fig. 85.10).

SA Node

The SA node is the primary pacemaker in mammalian heart. It is situated in the right atrium close to the opening of superior vena cava. It is about 1.5 cm long and 0.5 cm wide in human beings.
1. It contains the pacemaker (P) cells and few myofilaments.
2. The pacemaker cells generate the impulse, which is transmitted in the conducting system for excitation and contraction of heart muscles.
3. The action potentials generated in SA node are of slow response type. The velocity of conduction of impulse in SA node is slow (0.05 m/s).

Internodal Pathways

There are three internodal pathways that connect SA node and AV node (Fig. 85.11).
1. The anterior internodal pathway is called the tract of Bachman, the middle internodal pathway is called the tract of Wenckebach, and the posterior internodal pathway is called the tract of Thorel.
2. There may be few other accessory internodal pathways.
3. The internodal pathways merge into the AV node. The velocity of conduction of impulse in internodal pathways is about 1 m/s. From SA node, a conducting tract arises and directly enters into the left atrium. This is called interatrial tract or Bachman’s bundle.

AV Node

AV node is situated in the lower part of the right atrium close to the interatrial septum and just above the atrioventricular ring. It is 22 mm in length, 10 mm in width, and 3 mm in thickness.
1. In AV node, the fiber diameter is small and there are multiple sub-branches. Therefore, the rate of impulse conduction is slow in AV node (0.05 m/s). Usually, a delay of about 0.1 s occurs for the impulse to be transmitted through AV node. This is called AV nodal delay.
2. This delay is shorted by sympathetic stimulation and lengthened by parasympathetic stimulation to the heart. The weak impulses may even die out in AV node.
3. The ability of the AV node to slow down the transmission of rapid impulses from SA node to the ventricle is also called decremental conduction (Application Box 85.1).
4. The action potentials generated in AV node are of slow response type.
5. Pacemaker (P) cells are also present in the AV node but normally AV node is not the pacemaker because the rate of impulse formation is lower in it than that of the SA node. The pacemaker cells of AV node are suppressed by the SA nodal impulses. However, when SA node stops producing impulses, AV node becomes the pacemaker of the heart.
Chapter 85: Functional Anatomy of Heart, Cardiac Muscle, Conducting System, and Cardiac Innervation

Application Box 85.1

Decremental conduction is useful: The property of AV node to slow down or block rapid impulses that arrive from SA node is the decremental conduction. It provides safety to the ventricles. When SA node discharges rapidly and all impulses are allowed to excite ventricles, extreme ventricular tachycardia interferes in ventricular performance. Therefore, nature has provided a safety factor in the form of decremental conduction to check the transmission of rapid impulses to ventricular muscles.

His Bundle

This is a small bundle of fibers that arises from AV node and terminates in the Purkinje system. This is situated below the AV node and passes toward the interventricular septum.
1. As the fibers are present in the form of a bundle, this is called bundle of His (not bundle of Her, as described by W His in 1893).
2. The length of the bundle of His is about 1 cm, which on entering the interventricular septum divides into right bundle branch and left bundle branch.
3. When SA node and AV node are defunct, the bundle of His generates impulses.

Bundle Branches

His bundle divides into right bundle branch that conducts impulse to the right ventricle and left bundle branch that conducts impulse to the left ventricle. The bundle branches enter the ventricular walls and then branch out into very small bundle of fibers in the inner walls of the ventricular muscle. These fibers are termed as Purkinje fibers. Bundle branches also have the potentiality to generate impulses.

Right Bundle Branch

Right bundle branch (RBB) is longer and thinner than the left bundle branch. It exclusively innervates right ventricle.

Left Bundle Branch

Left bundle branch (LBB) bifurcates into two divisions: the anterior division that supplies the anterior portion of the left ventricle, and the posterior division that supplies the posterolateral portion of the left ventricle.

Purkinje Fibers

This is a network of small bundles of conducting fibers that are present throughout the sub-endocardial regions of right and left ventricles.
1. The cells of the Purkinje system (are also called Purkinje cells) are the largest cells in the heart.
2. Numerous gap junctions (low impedance electrical synapses) are present between the cells.
3. Because of the larger diameters of the fiber and presence of low impedance cell-to-cell connections, the rate of impulse conduction is highest in the Purkinje fibers (Table 85.2).

4. The conduction rate is almost 4 m/s. The action potentials generated in the Purkinje fibers are of fast response type and resemble those produced in the ventricular muscles.

Table 85.2: Conduction velocity in cardiac tissues.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Conduction velocity (m/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SA node</td>
<td>0.05</td>
</tr>
<tr>
<td>Internodal pathways</td>
<td>1</td>
</tr>
<tr>
<td>AV node</td>
<td>0.05</td>
</tr>
<tr>
<td>His bundle</td>
<td>1</td>
</tr>
<tr>
<td>Purkinje fibers</td>
<td>4</td>
</tr>
<tr>
<td>Ventricular muscle</td>
<td>1</td>
</tr>
</tbody>
</table>

Scientist contributed

Johann Evangelista Purkinje (1787-1869) was a Czech Physiologist. In 1818, he graduated from Charles University in Prague with a degree in medicine, where he was appointed a Professor of Physiology. He discovered the Purkinje effect, the human eye’s much reduced sensitivity to dim red light compared to dim blue light. He created the world’s first Department of Physiology at the University of Breslau in Prussia (now Wroclaw, Poland) in 1839 and the world’s first official physiology laboratory in 1842. He is best known for his 1837 discovery of Purkinje cells, large neurons with many branching dendrites found in the cerebellum. He is also known for his discovery in 1839 of Purkinje fibers, the fibrous tissue that conducts electrical impulses from the atrioventricular node to all parts of the ventricles of the heart.

Cardiac Innervation

Both the sympathetic and parasympathetic divisions of the autonomic nervous system innervate the heart (Fig. 85.12). In general, stimulation of parasympathetic decreases and stimulation of sympathetic increases activities of the heart.
1. The cardiac pacemaker (SA node) activity is tonically influenced by both parasympathetic and sympathetic systems. However, in basal conditions, parasympathetic or vagal tone is more than the sympathetic tone. Therefore, normally the heart rate is the function of the vagal tone.
2. The ventricular muscle is predominantly supplied by the sympathetic fibers and the parasympathetic innervation of ventricle is sparse.
3. Therefore, stroke volume as the function of myocardial contractility is greatly influenced by the sympathetic tone.

Parasympathetic Innervation

The cardiac parasympathetic (Vagal) fibers originate from the three vagal nuclei present in the medulla oblongata. These are nucleus tractus solitarius, the dorsal motor
nucleus of vagus and the nucleus ambiguous. The preganglionic parasympathetic fibers traverse in the vagus nerve on both the sides. The ganglion cells are mostly located in the cardiac tissues and the postganglionic fibers are present in the heart itself.

There is a difference in the innervation of the heart by the right and left vagi (Fig. 85.12). The right vagus mainly innervates right atrium and SA node. Therefore, stimulation of right vagus strongly inhibits heart rate. The left vagus predominantly innervates the left atrium, AV node and the bundle of His. Therefore, stimulation of left vagus slows or blocks AV nodal conduction. However, stimulation of either of the nerves can inhibit functions of both the nodes, as there is sufficient overlap in nodal innervation by both the vagi. The innervation of ventricles by the vagi is sparse. Therefore, parasympathetic stimulation does not significantly affect the force of contraction.

Vagal Stimulation Results in:
1. Negative chronotropic effect (decreased heart rate)
2. Negative dromotropic effect (decreased rate of conduction)
3. Negative bathmotropic effect (decreased excitability of the heart)
4. Negative inotropic effect (decreased myocardial contractility).

Concept of Basal Heart Rate and Intrinsic Heart Rate
Normally, the SA nodal activity is primarily under the control of vagus nerve. Therefore, the basal heart rate depends on the vagal tone (Application Box 85.2).

1. The normal vagal tone is the rate of discharge in vagus nerve at rest.
2. It has been observed that denervation of the heart, i.e. abolition of influence of both sympathetic and parasympathetic fibers to heart by blocking noradrenergic and cholinergic systems, results in increase in the heart rate from its basal rate of about 70 per minute to about post-denervation rate of 110 per minute.
3. This is performed in experimental animals by cutting the vagal and sympathetic fibers supplying the heart. The increase in heart rate following denervation is the intrinsic heart rate, which indicates that normally vagal tone dominates over sympathetic tone for the control of heart rate.

Application Box 85.2
Basal heart rate is the function of vagal tone: The normal resting heart rate is an index of parasympathetic activity. Vagus nerve has profound influence on SA node at rest and dominates over the sympathetic influence. This is reflected on the basal heart rate, which is the heart rate at rest. As vagus nerve inhibits SA nodal discharge, the individual with high vagal tone has lower basal heart rate. Therefore, basal heart rate is considered as an important assessment of parasympathetic functions.

Sympathetic Innervation
The cardiac sympathetic fibers originate in the intermediolateral grey column of the spinal cord starting from lower two cervical segments to the upper five thoracic segments (Fig. 85.13).
1. The preganglionic fibers, after emerging from the spinal cord enter the paravertebral chain of ganglia.
2. The cell bodies of the postganglionic fibers are located in the superior, middle, and inferior cervical ganglia.
3. The fibers traverse along the adventitial surface of the blood vessels to reach the heart. The sympathetic fibers innervate the atria, nodal tissues and conducting pathways, and pierce ventricles to extensively supply the myocardium (Fig. 85.12).

Difference in Distribution of Left and Right Sympathetic Fibers
There is some difference in distribution of left and right sympathetic fibers supplying the heart.
1. The right side of the sympathetic division mainly supplies SA node, right atrium and right ventricle, whereas the left sympathetic fibers mainly supply the AV node, left atrium and the left ventricle.
2. Thus, stimulation of right sympathetic nerve predominantly affects the heart rate whereas stimulation of left sympathetic nerve mainly affects the myocardial contractility.
3. However, there is adequate overlap in innervation of heart by both sides of the sympathetic fibers. Therefore, stimulation of either side of sympathetic nerve can affect both rate as well as force of contraction.
CHAPTER SUMMARY

**Key Concepts**

1. Due to extensive branching of cardiac muscle fibers and presence of intercalated discs that contain numerous gap junctions, cardiac muscles are physiological syncytium.
2. Length-tension relationship of cardiac muscle follows the Frank-Starling law of the heart.
3. Vagal tone determines the basal heart rate.

**Important to Know (Must Read)**

1. In examination, Long Questions are usually not asked from this chapter.
2. Length-tension relationship in cardiac muscle, Frank-Starling law of the heart, Conducting system of the heart, Cardiac innervation are asked as Short Questions in exam.
3. In Viva, examiner may ask… Why the heart muscle is called a functional syncytium, List the differences between sarcotubular system of cardiac and skeletal muscle, What are the mechanisms to explain Frank-Starling law, What are the valves of the heart, What is the effect of increased ECF calcium on cardiac muscle, What is the mechanism of action of digitalis, What are the components of the conducting system of the heart in order of hierarchy, What is the conduction velocity of each component, What is AV nodal delay, and what are its causes, What is decremental conduction, and what are its uses, How does the vagus nerve innervate the heart, What is the effect of vagal stimulation on heart, Explain why basal heart rate is the function of vagal tone, How do the sympathetic nerves innervate the heart, What is the effect of sympathetic stimulation on heart.

**Sympathetic Stimulation Results in:**

1. Positive chronotropic effect (increased heart rate)
2. Positive inotropic effect (increased myocardial contractility)
3. Positive dromotropic effect (increased rate of conduction)
4. Positive bathmotropic effect (increased excitability of the heart).

At rest, there is adequate tonic discharge in the sympathetic nerve supplying the heart. This is called the sympathetic tone. However, as stated above, normally the influence of vagal tone on SA node predominates over the sympathetic tone. Therefore, the basal heart rate is the function of vagal tone rather than sympathetic tone.
Heart is a vital organ as cessation of its activity (cardiac arrest) for more than few minutes is detrimental to life. It pumps blood continuously throughout life due to many special properties inherent to its muscle. These special properties of cardiac muscle should be studied in details to understand cardiovascular functions and dysfunctions.

**IMPORTANT PROPERTIES**

The important properties of cardiac muscles are:

1. Automaticity
2. Rhythmicity
3. Conductivity
4. Contractility
5. Excitability
6. Distensibility
7. Long refractory period, for which cardiac muscle can not be tetanized.
8. Functional syncytium
9. Extrasystole and compensatory pause
10. All or none Law
11. Staircase phenomenon
12. Length–tension relationship
13. Frequency–force relationship
14. Load–velocity relationship

In experimental conditions, the properties from number 1 to 6 can be studied in a beating heart and number 7 to 12 can be studied in a quiescent heart (which is performed by placing Stannius ligatures).

**Automaticity**

Automaticity is the property of automatic beating of the heart. Heart continues to beat even when it is completely denervated or isolated (but artificially perfused) from the body.

1. The ability of the cardiac muscle to continue to contract regularly even in the absence of its nerve supply is referred to as automaticity. This is possible due to the spontaneous generation of impulses by the SA node, the primary pacemaker of the heart that displays pacemaker potential.
2. Automaticity is also the property of other potential pacemakers of the heart like AV node, His-Purkinje system, ventricular muscle, etc. (the mechanism of automaticity is described in Chapter 71; in Pacemaker Potential)

**Rhythmicity**

Rhythmicity refers to the rhythmic excitation of the heart. This is called autorhythmicity.

1. A normal heart beats in a perfectly regular rhythm as the interbeat intervals remain virtually constant. This occurs due to regularity in rhythmic discharge of the
pacemaker, which is normally the SA node. Thus, heart rate is the function of the rate at which the pacemaker generates the impulse.

2. SA node is the primary pacemaker of the heart as the excitation begins in the SA node. The rate of discharge of SA node is 60–100 per minute, the highest among all the pacemakers. Therefore, the normal heart rate is 60–100 per minute.

Pacemaking Tissues in the Heart

Normal heart has three intrinsic pacemaking tissues: SA node, AV node and His-Purkinje fibers. However, when the intrinsic pacemakers fail to discharge, ventricular muscle generates the pacemaking activity.

1. The term ‘pacemaking-activity’ refers to the spontaneous time-dependant depolarization that leads to action potential in an otherwise quiescent cell or tissue.

2. Any of the pacemakers can initiate the heartbeat. However, the pacemaker with highest frequency triggers the action potential that propagates throughout the heart. The SA node being the fastest pacemaker, overrides the activity of all other pacemakers. Therefore, SA node is the primary pacemaker.

3. Thus, till SA node is functioning normally, heart beats according to SA nodal rhythm. Cardiac pacemakers have a hierarchy among themselves (Table 86.1). Failure of SA nodal rhythm results in activation of AV node, the next in the hierarchy to take over the pacemaker activity. Thus, AV node and other cardiac pacemakers are called secondary pacemakers.

Demonstration of Hierarchy of Pacemakers

The hierarchy of pacemakers can be demonstrated experimentally, usually in frogs by placing Stannius ligatures. In this experiment, the normal cardiogram is recorded, which denotes rate of sinus venous, the natural pacemaker in frog’s heart.

1. The first Stannius ligature is placed between the sinus venous and atria, i.e. on the white crescentic line, and the cardiogram is recorded. This reflects the atrial rhythm, which is slower than the normal sinus rhythm as the impulses are generated in the atria.

2. The cardiogram is then recorded after placing second Stannius ligature, which is placed between the atria and the ventricles. This records ventricular rhythm (Fig. 86.1) as the impulses are generated in the ventricle, which is slower than the atrial rhythm.

| Table 86.1: The rate of discharge of potential pacemakers of heart. |
|---------------------------------|-----------------|
| Pacemaker tissue                | Rate/min        |
| SA node                         | 60–100          |
| AV node                         | 40–60           |
| His bundle                      | 25–40           |
| Purkinje fiber                  | 25–40           |
| Ventricular muscle              | 15–30           |

Conductivity

The cardiac muscles conduct impulses generated in the heart. Impulse produced in SA node is conducted by specialized conducting pathway to the ventricular muscle (for details, refer to previous chapter). The impulse then spreads rapidly in cardiac muscle to the different parts of the heart (mechanisms of propagation and conduction of cardiac impulse are described in the next chapter).

Contractility

Cardiac muscle contracts in response to a stimulus. In fact, heart contracts regularly and rhythmically in response to impulses generated by the SA node.

1. Ventricular contraction enables heart to pump blood into the circulation.

2. Thus increased contractility increases cardiac output and decreased contractility decreases cardiac output.

3. The property of contractility of the heart is well appreciated even in an isolated preparation.

Excitability

Cardiac muscle is an excitable tissue like nerve and other muscles.

1. Excitability is the ability of the cardiac muscle to respond to different stimuli.

2. Cardiac excitability can be altered by various factors like concentrations of ions and hormones, and most importantly the state of autonomic activity, i.e. the sympathetic and parasympathetic tones.

Distensibility

This is the ability to stretch. This occurs due to compliance of the cardiac muscle.

1. This property helps in filling of atrial and ventricular chambers.
2. Decreased ventricular distensibility decreases end-diastolic volume.

**Long Refractory Period**

Ventricular muscle has a long refractory period. Refractory period of the muscle is the period during which muscle does not evoke a response no matter how strong is the stimulus. The refractory period is divided into absolute and relative refractory periods.

1. The absolute refractory period of ventricular muscle is about 200 ms, and the relative refractory period is about 50 ms. An important difference exists in the duration of action potential of cardiac and skeletal muscles.

2. In cardiac muscle, the duration of action potential is almost same as the duration of its mechanical response. The refractory period extends to the most part of the mechanical response in ventricular muscle (Fig. 86.2A). Therefore, always a new action potential generates a fresh mechanical response. Consequently, a fresh contraction cannot occur before the completion of the previous mechanical response. Thus, mechanical responses of ventricular muscle cannot be merged, and therefore cardiac muscle cannot be tetanized (Application Box 86.1).

3. In skeletal muscle, mechanical responses can be merged and muscle can be tetanized, as the duration of action potential is much shorter than the duration of mechanical contraction (Fig. 86.2B).

**Application Box 86.1**

Long refractory period is very useful: Long refractory period is a unique property of the cardiac muscle that provides safety to the heart as it prevents tetanization of heart muscle (as described above).

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**Functional Syncytium**

Cardiac muscle is a functional syncytium due to the presence of numerous gap junctions. Therefore, all muscle fibers contract almost simultaneously (for details, see the previous chapter).

**Extrasystole and Compensatory Pause**

When a sufficiently stronger stimulus is applied in the relative refractory period in an experimental recording of normal cardiogram, an extra contractile response occurs. This is called extrasystole. This contraction occurs earlier than the normally expected one (Fig. 86.3).

1. The natural stimulus (impulse arising from the SA node) falls in the refractory period of the extrasystole and does not evoke a response; therefore the natural contraction is missed. This is why a long pause is seen following the extrasystole, which is called compensatory pause.

2. Extrasystole is not uncommon in real life, and it may not be always pathological. Usually, the extra-stimulus arises from an ectopic focus in the ventricle.

3. The magnitude of contraction following the compensatory pause is usually a higher one as the ventricle gets more time for filling during the pause. The increased end-diastolic volume increases the force of contraction by Frank-Starling mechanism. This is called post-extrasystolic potentiation (PEP).

4. PEP can also occur independent of ventricular filling. Therefore, it is believed that PEP may be an intrinsic mechanism of the heart that occurs due to accumulation of more amount of calcium ions in the myocardial cell during the compensatory pause.
5. However, in experimental set up for the demonstration of extrasystole, the magnitude of contraction following the compensatory pause may not be a larger one as the experiment is performed after placing the second Stannius ligature, which is placed between the atria and ventricles. Therefore, ventricular filling is not altered in such a situation.

### All or None Law

Heart muscle follows all or none law.

1. The all or none law states that the magnitude of response of a tissue to stimuli remains same irrespective of the strength of stimuli. That means, if the tissue responds, it responds optimally or it does not respond at all.

2. When the stimulus is of subthreshold strength, the muscle does not respond to it at all, and if the stimulus is of threshold intensity, the muscle exhibits the optimum response. The height of contraction does not increase following the application of suprathreshold stimulus (Fig. 86.4). This is called all or none law.

   All or none law occurs due to two reasons:
   i. All or none nature of the action potential
   ii. Syncytial nature of the heart muscle, which behaves as a single functional unit.

   The all or none law is applicable only when the conditions (both internal and external) remain same. The response may change if the condition is changed. For example, if heart is stimulated following the sympathetic activation, the magnitude of contraction increases. In this case, the excitability and contractility of the heart is changed by sympathetic stimulation and therefore the change in magnitude of contraction.

### Staircase Phenomenon

If a quiescent heart (following application of second Stannius ligature) is stimulated repeatedly keeping the interval between the stimuli just less than 10 s, the magnitude of first 3–4 contractions progressively increases (Fig. 86.5). This is called staircase phenomenon. It appears that the staircase phenomenon violates the principle of all or none law, but in fact it is not so, because each stimulus alters the condition for the next stimulus.

There are three mechanisms for staircase phenomenon:

1. The amount of calcium ion released during each contraction is not completely pumped back into the sarcoplasmic reticulum as the second stimulus is applied within 10 s. Therefore, some residual amount of calcium is left prior to the next stimulus. This increases the quantity of calcium available to the contractile machinery in the next stimulus (the calcium left in the previous contraction plus the calcium released by the present stimulus). Therefore, the second stimulus evokes a bigger response. Similarly, subsequent 2 to 3 stimuli progressively increase the magnitude of contraction.

2. During the first contraction, the temperature in the myocardial cells increases which enhances the enzymatic activity for the muscle contraction. Therefore, the second stimulus acts under the beneficial effect of increased enzymatic activity of the first one. Similarly, few subsequent stimuli progressively increase the height of contraction.

3. The first contraction decreases the viscosity of the sarcoplasm due to increased temperature. Thus, the resistance provided by the sarcoplasm for the second and subsequent contractions is significantly lower than the first one. This increases the magnitude of few subsequent contractions.
**Length-Tension Relationship**

The force of contraction is directly proportional to the initial length (the length of the muscle fiber prior to the contraction) of the muscle fiber, within the physiological limit, which is known as the Frank-Starlings Law of the heart.

1. With increase in length, the tension increases, reaches a peak and then decreases (for details, refer to previous chapter).
2. The length of muscle fiber of the heart chambers prior to contraction depends on the level of preload, which is the end diastolic volume.
3. Thus, as the ventricular filling increases (the increase in end-diastolic volume), the stroke volume increases. This is called heterometric autoregulation of cardiac output.

**Frequency-Force Relationship**

Increase in force of contraction due to increase in heart rate (frequency) is known as frequency–force relationship. This is known as Bowditch phenomenon as described by HP Bowditch (1840–1991). However, increase in myocardial performance is restricted to a limited increase in frequency (for details, refer ‘Regulation of Cardiac Output’ in Chapter 90).

**Load-Velocity Relationship**

The relationship of velocity of shortening to the load imparted on muscle with which the muscle contracts, is called the load-velocity relationship.

1. The velocity of contraction is inversely proportional with the load that acts against it. The velocity of muscle shortening or contraction decreases with increasing afterload. That means, shortening is faster with lower loads and slower with higher loads (Fig. 86.6).
2. It should be noted that the shortening velocity is maximum when there is no load. A student should not confuse the velocity of contraction with force of contraction.

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**CHAPTER SUMMARY**

**Key Concepts**

1. Cardiac muscles are endowed with special properties that provide the ability heart to pump blood without fatigue. Important among them are long refractory period, all or none law and force–frequency and length–tension relationship.
2. Cardiac muscle can not be tetanized due to the long refractory period of its action potential, during which the major part of the mechanical response of cardiac muscle ends.
3. The force of contraction increases with increase in frequency, within the physiological limit.

**Important to Know (Must Read)**

1. In examination, Long Questions are usually not asked from this chapter. However, ‘Describe the properties of cardiac muscle’ may come as a long question.
2. Refractory periods of cardiac muscle action potential, Extrasystole and compensatory pause, Staircase phenomenon, Length-tension relationship in cardiac muscle, Frequency-force relationship in cardiac muscle, Load-velocity relationship in cardiac muscle are asked as Short Questions in exam.
3. In Viva, examiner may ask… List the properties of cardiac muscle, Explain why cardiac muscle cannot be tetanized, What is the rate of discharge of potential pacemakers of the heart, How can the hierarchy of pacemakers be demonstrated in the cardiac tissue, How do the cardiac muscle act as functional syncytium, What is a Stannius ligature, What is an extrasystole, What is the cause of compensatory pause, What is the cause of postExtrasystolic potentiation, How is the staircase phenomenon demonstrated, What are the mechanisms for staircase phenomenon, What is the length–tension relationship in cardiac muscle, What is Bowditch phenomenon, What is the frequency–force relationship in cardiac muscle, What is the load-velocity relationship in cardiac muscle.
Learning Objectives

On completion of study of this chapter, the student **MUST** be able to:

1. Understand various ionic currents in cardiac tissues.
2. Draw the labeled diagrams of fast and slow response action potentials and give their ionic basis.
3. Give the ionic basis of pacemaker potential.
4. Explain the effects of sympathetic and vagal stimulation on pacemaker potential.
5. Appreciate and understand the propagation of electrical impulse in conducting system of heart.

The student **MAY** also be able to:

1. Describe the electrical properties of the cardiac muscle.
2. Describe the fast and slow response action potentials of cardiac tissues.
3. Explain the propagation of electrical impulse in conducting system of heart.

The resting membrane potential (RMP) of the cardiac muscle fiber is −90 mV and of Purkinje fibers is −95 mV. The RMP of pacemaker tissues is about −60 mV. SA node, the primary pacemaker of the heart generates impulses automatically due to its property of automaticity.

1. The action potential is generated in cardiac tissues when they are activated in response to the impulses arising from the SA node. The electrical activities in cardiac tissues are due to the operation of many ionic currents in their cell membranes.
2. There are **many ionic currents** that operate in different cardiac tissues. The ionic currents are due to the **presence of various ion channels** in the cell membrane.
3. Many ionic currents combine uniquely to produce action potentials in cardiac tissues.

Therefore, a student should learn basics of various ionic currents operating in cardiac tissues before understanding the electrophysiology of the heart.

### IONIC CURRENTS IN CARDIAC TISSUES

There are **four major types** of ionic currents in cardiac tissues: the sodium current, calcium current, potassium currents and pacemaker current (Table 87.1).

<table>
<thead>
<tr>
<th>Current</th>
<th>Type of channels involved</th>
<th>Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>I_Na</td>
<td>Voltage-gated Na(^+) channel</td>
<td>Produces Phase 0 of fast response action potential (FRAP)</td>
</tr>
<tr>
<td>I_CaL</td>
<td>L-type Ca(^{2+}) channel</td>
<td>Produces Phase 2 of FRAP, Produces Phase 0 of slow response action potential (SRAP)</td>
</tr>
<tr>
<td>I_CaT</td>
<td>T-type Ca(^{2+}) channel</td>
<td>Contributes to later part of pacemaker potential</td>
</tr>
<tr>
<td>I_Kr</td>
<td>Inward rectifying K(^+) channels</td>
<td>Maintains RMP (phase 4) of cardiac muscle cells</td>
</tr>
<tr>
<td>I_TO</td>
<td>Outward transient rectifying K(^+) channels</td>
<td>Contributes partially to phase 1 of FRAP</td>
</tr>
<tr>
<td>I_K1</td>
<td>Outward delayed rectifying K(^+) channels</td>
<td>Produces Phase 3 of FRAP, Produces Phase 3 of SRAP</td>
</tr>
<tr>
<td>I_KG</td>
<td>G protein activated K(^+) channels</td>
<td>Contributes to early part of prepotential by its closure</td>
</tr>
<tr>
<td>I_KATP</td>
<td>ATP sensitive K(^+) channels</td>
<td>Hyperpolarization of membrane in phase 4 of FRAP and SRAP</td>
</tr>
<tr>
<td>I_f</td>
<td>Mixed cation channel for both Na(^+) and K(^+)</td>
<td>Contributes to pacemaker potential</td>
</tr>
</tbody>
</table>

Note: FRAP occurs in atrial and ventricular muscles and Purkinje fibers, and SRAP occurs in SA and AV nodes.
**Na⁺ Current (I_{Na})**

The sodium current (I_{Na}) is the largest current in cardiac tissues, especially in ventricular muscles, atrial muscle and Purkinje fibers. There are about 200 Na⁺ channels per square micron of membrane in these tissues. However, I_{Na} current is not present in SA or AV nodal cells. The ion channel responsible for I_{Na} is a voltage-gated Na⁺ channel. Voltage-dependant Na⁺ channels are always fast sodium channels.

**Activation of Na⁺ Channels**

The Na⁺ channels are closed at RMP of the ventricular muscle cells. However, they are rapidly activated in response to stimulation by impulses (conducted action potentials) originating from the pacemaker that produce local depolarization.

1. The process of activation of Na⁺ channels occurs in about 0.1 to 0.2 ms. Activation of Na⁺ channels produces a massive inward flow of Na⁺ that results in rapid upstroke of the fast response action potential.
2. Voltage-gated Na⁺ channel in cardiac muscle has two gates: the outer gate (activation gate) and the inner gate (inactivation gate).
   - The outer gate opens at the beginning of depolarization that causes rapid Na⁺ influx (activation of Na⁺ channel).
   - The inner gate closes at the end of depolarization that stops Na⁺ influx (inactivation of Na⁺ channel).

**Inactivation of Na⁺ Channels**

If the membrane potential becomes positive, these channels close automatically, the process known as autoinactivation.

1. Though the process of inactivation is slower than activation, still it occurs quite rapidly in about 1 ms.
2. Inactivation of Na⁺ current is partly responsible for the rapid repolarization of the action potential in phase 1 (described below).

**Importance of I_{Na}**

The depolarization in fast response type of action potential is produced by the Na⁺ current. This Na⁺ current also activates other membrane currents like calcium (I_{Ca}) and potassium (I_{K}) currents. Antiarrhythmic drugs, such as lidocaine, exert their effect by partly blocking I_{Na}.

**Ca²⁺ Currents (I_{Ca})**

The calcium current (I_{Ca}) exists in all cardiac tissues. I_{Ca} is due to the presence of calcium channels. There are two types of voltage-gated calcium channels: L-type and T-type. Both the channels are voltage-gated Ca²⁺ channels. L-type is the dominant one in the heart muscle and T-type is present in smaller amount.

**L-type Ca²⁺ Channels (I_{CaL})**

This is the long lasting voltage-gated Ca²⁺ channel. It is present in all cardiac tissues including cardiac myocytes, SA node, AV node, and His-Purkinje system. It contributes to the action potential of pacemaker tissues, myocytes, and other tissues.

**In Pacemakers**

In both SA and AV nodes, I_{CaL} causes inward movement of positive charges produced by calcium influx and is responsible for the upstrokes or depolarization of the SA and AV nodal action potentials.

1. As I_{CaL} is not a fast channel like fast-sodium channel, the slope of upstroke of SA and AV nodal action potentials is less steep than the slope of depolarization of atrial and ventricular muscle.
2. The slower and less steep upstroke of action potential of nodal tissues contributes to the speed of the conducted action potential, which is much slower than that of any other cardiac tissues.

**In Ventricular and Atrial Muscles**

In ventricular and atrial muscles and the Purkinje fibers, I_{CaL} is responsible for state of sustained depolarization that results in the prolonged plateau phase. In atrial and ventricular myocytes, the Ca²⁺ that enters via L-type Ca²⁺ channels activates the release of Ca²⁺ from the sarcoplasmic reticulum by stimulating calcium-induced Ca²⁺ release. Clinical importance: Ca²⁺ channel blockers like verapamil, diltiazem, and nifedipine act by inhibiting L-type Ca²⁺ channels.

**T-type Ca²⁺ Channel (I_{CaT})**

This is the transient type voltage gated Ca²⁺ channel. This is present only in SA node and AV node. They are responsible for later part of the prepotential or pacemaker potential.

**Other Ca²⁺ Channels**

There are ligand-gated and stretch-sensitive Ca²⁺ channels.

1. Ligand-gated Ca²⁺ channels are present in less number in cardiac muscle, whereas stretch-sensitive Ca²⁺ channels are plentiful available.
2. Stretch-sensitive Ca²⁺ channels are sensitive to stretch (distension) of cardiac muscle. When diastolic filling of cardiac chamber increases, stretch-sensitive Ca²⁺ channels open and cause influx that increases myocardial contractility.

**K⁺ Currents (I_{K})**

There are two types of K⁺ channels in cardiac tissues: voltage-gated and ligand gated channels. K⁺ current is due to voltage-gated channels.
1. **The voltage-gated K⁺ channels** are of three types: Inward rectifying K⁺ channels, outward transient rectifying K⁺ channels, and outward delayed rectifying K⁺ channels.

2. **The ligand-gated K⁺ channels** are of five types: G-protein activated, calcium-activated, sodium-activated, arachidonic acid-activated, and ATP-sensitive K⁺ channels.

**Inward Rectifying K⁺ Channels**

Inward rectifying K⁺ channels (I_{K1}) are voltage-gated channels that maintain resting membrane potential (phase 4) of cardiac muscle by allowing outflux of K⁺ at highly negative membrane potential.

**Outward Transient Rectifying K⁺ Channels**

Outward transient rectifying K⁺ channels (I_{TO}) are voltage-gated channels. This is also called *early outward K⁺ current (A-Type Current)*.

1. It is present in atrial and ventricular muscle cells.
2. This current is activated by depolarization but it is rapidly inactivated.
3. It contributes to phase 1 repolarization by transiently permitting outflux of K⁺ at positive membrane potential.

**Outward Delayed Rectifying K⁺ Channels**

This is also called the *re-polarizing K⁺ current that turns on slowly (I_{Kr})*. This found plentily in atrial myocytes, Purkinje fibers, and ventricular myocytes. They open with a considerable delay.

1. They are responsible for repolarizing the membrane at the end of the action potential (phase 3) by permitting outflux of K⁺ after a delay when membrane repolarizes.
2. With repolarization, it slowly activates (after 20 to 100 msec).
3. In SA and AV nodal cells, it contributes to pacemaker activity by closing themselves early in phase 4.
4. Thus, they contribute to early part of prepotential.

**G Protein-activated K⁺ Channel**

This is a G-protein operated K⁺ channels produce G protein K⁺ current (I_{Kg}).

1. It is activated by acetylcholine (Ach) and adenosine.
2. Ach acting on muscarinic receptors stimulates βγ-subunits of G protein, which in turn activates the K⁺ channels.
3. This channel is prominent in SA and AV nodal cells, where it hyperpolarizes the membrane during phase 4 that slows the pacemaker potential.
4. In ventricular muscles and Purkinje fibers, this channel also hyperpolarizes the membrane during phase 4.

**K_ATP Channels**

There are numerous ATP-sensitive K⁺ channels (K_ATP). They play role in contractile functions of cardiac muscles that are regulated by electrical gradient.

**Pacemaker Current (I_f)**

The pacemaker current (I_f) is found in SA and AV nodal cells. The channel underlying this current is a nonspecific cation channel called HCN (Hyperpolarization-activated Cyclic Nucleotide-gated) channel.

1. HCN channels conduct both Na⁺ and K⁺. Therefore, the reversal potential of I_f is about −20 mV, between the Nernst potentials for K⁺ (about −90 mV) and Na⁺ (about +60 mV).
2. The HCN channels have the funny property (hence called “f” channel) such that they do not conduct at positive potentials, but their activation is slow (100 ms), and the current does not inactivate.
3. Thus, I_f produces an inward, depolarizing current as it is slowly activated at the end of phase 3. These channels are also known as “h” channels (I_h) as they are activated following hyperpolarization. I_h contributes to pacemaker potential.
4. However, in SA and AV nodal cells, I_{Ks} and I_{CaT} contribute significantly to the phase 4 depolarization, i.e., the prepotential or pacemaker potential.

**ACTION POTENTIALS IN CARDIAC TISSUES**

Normally, two types of action potentials are seen in the heart: the slow response action potential and the fast response action potential. The fast response action potential occurs in the atrial and ventricular muscles and Purkinje fibers, and the slow response action potential occurs in nodal tissues (SA and AV nodes). However, action potential vary from slow to fast type in different tissues of the heart (Fig. 87.1).

**Fast Response Action Potential**

Fast response type of action potential is recorded from atrial and ventricular muscles and Purkinje fibers.

**Phases and Ionic Basis**

Typically five phases (phases 0–4) are observed in a fast response action potential as recorded from ventricular muscle fibers (Figs. 87.2A and B).

**Phase 0**

This is the *phase of rapid depolarization and overshoot*. As soon as the membrane potential reaches threshold, rapid depolarization (a steep rise in the spike) occurs.

1. This is due to sudden increase in the permeability of the membrane to sodium ions, which occurs due to hundredfold opening of voltage-gated sodium channels.
2. The sodium ions enter rapidly into the myocardial cells.
Fig. 87.1: Action potentials in different tissues of the heart. Courtesy: Figure 27.12, page 511, Color Atlas of Cardiovascular Disease, by Glenn N Levine, 1st edition, 2015; Jaypee Brothers Medical Publishers (P) Ltd.

**Phase 1**
This is the phase of initial rapid repolarization.
1. This phase of partial repolarization is due to closure of sodium channels (cessation of sodium influx).
2. Opening of outward transient rectifying K⁺ channel that causes transient outflux of K⁺ also partly contributes.
3. This K⁺ current (Iₜₒ) is activated by depolarization and is rapidly inactivated.

**Phase 2**
This is known as plateau phase as the action potential in this phase remains in a state of sustained depolarization.
1. This phase is due to sustained increased permeability of the membrane to calcium ions (through slowly opening calcium channels) that results in slow calcium influx, which occurs due to slower but prolonged opening of voltage-gated calcium channels.
2. Efflux of potassium ions contributes to maintain sustained depolarization.
3. The plateau phase is of much lesser duration in atrial muscle than the ventricular muscles.

**Phase 3**
This is the phase of final repolarization.
1. This occurs due to cessation of calcium influx (closure of calcium channels) and increased membrane permeability to potassium (increased potassium efflux).
2. This phase is mainly due to opening of outward delayed rectifying K⁺ channels (I_{Ks}).

**Phase 4**
This is the **phase of restoration of membrane potential**.
1. The membrane permeability to K⁺ is restored towards resting value.
2. Opening of inward rectifying K⁺ channels (I_{Kr}) contributes to maintain the restored membrane potential.
3. The relative increase in permeability to K⁺ drives the membrane potential towards the equilibrium potential.

**Slow Response Action Potential**
This type of action potential is seen in nodal tissues (SA and AV nodes), AV junctional tissue, and ischemic or injured myocardium.

**Phases and Ionic Basis**
Slow response action potential consists mainly of three components (Fig. 87.3): the **phase 0** (depolarization or slow rising upstroke), **phase 3** (phase of repolarization), and **phase 4** (phase of slow depolarization). Phases 1 and 2 are usually absent in this type of action potential. In SA node in the phase 4, instead of restoration of the membrane potential, slow depolarization occurs.

**Phase 0**
It occurs due to the opening of calcium channels.
1. This phase is less steep in comparison to fast response action potential.
2. The depolarization is mainly due to **influx of calcium ions** through the long acting calcium channels (L-calcium channels).
3. Therefore, depolarization wave rises slowly.

**Phase 3**
This phase is due to **closure of calcium channels and opening of the potassium channels** (increased potassium efflux).

**Phase 4**
This is the phase of **slow diastolic depolarization**.
1. The **early part** of this phase is due to the **closure of potassium channels** (decreased potassium conductance).
2. The **later part** is due to **opening of transient calcium channels** (T-calcium channels). Calcium sparks (release of calcium locally from sarcoplasmic reticulum) also contributes.

**Pacemaker Potential**
The pacemaker of the heart (SA node) discharges rhythmically and automatically. The automaticity (the ability of the pacemaker to produce its own impulse) is possible due to **spontaneous diastolic depolarization** of the membrane potential following completion of each action potential. The resting (diastolic) membrane potential that depolarizes is called as the **prepotential** as it brings the membrane potential to the threshold level, which then triggers the action potential. The prepotential is known as **pacemaker potential** (Fig 87.4).

**Ionic Basis**
Ionic basis of pacemaker potential has two important parts: the ionic basis at initial part and at the later part of the potential.

**In the Initial Part**
The repolarization phase of the action potential in the nodal tissues is due to **efflux of potassium ions**.
1. At the peak of each action potential, potassium conductance \( I_{\text{K}} \) begins.
2. Toward the end of repolarization, the \( I_{\text{K}} \) declines, which is known as potassium decay.
3. At this moment, \( \text{"F" channels open} \) \( I_{\text{f}} \) or \( I_{\text{h}} \) current) and \( I_{\text{h}} \) increases.
4. These events allow membrane to depolarize.
5. Thus, \( K^+ \) decay and \( I_{\text{h}} \) contribute to the initial part of the pacemaker potential.

**In the Later Part**

The later part of pacemaker potential is due to the opening of transient calcium channels \( I_{\text{CaT}} \).
1. The entry of calcium through the T-channels completes the pacemaker potential and takes the membrane potential to the threshold level, which then fires to form the action potential.
2. The upstroke of action potential occurs due to opening of the long-lasting calcium channels \( I_{\text{CaL}} \).

**Other Ions**

Recently it has been suggested that the calcium sparks (release of calcium locally from sarcoplasmic reticulum) also contribute to the pacemaker potential.
1. Thus, the pacemaker potential is largely due to decay of the potassium efflux and \( I_{\text{h}} \) (the initial part), and influx of calcium (the later part).
2. Contribution of sodium ion is linked to the \( I_{\text{h}} \).

**Membrane potential is restless:** Actually, it is the resting membrane potential that automatically depolarizes to form the pacemaker potential. Thus, in pacemaking tissues there is no resting membrane potential, rather the pacemaker potential is the restless membrane potential.

Though the SA node is the primary pacemaker of the heart, the pacemaker potential can also be produced by AV node and other tissues of the heart (latent pacemakers) when SA node fails to generate impulse adequately. The rate of impulse generation depends on the slope of pacemaker potential. The sympathetic stimulation makes the slope steeper and increases the heart rate, whereas the parasympathetic stimulation makes the slope flat and decreases the heart rate.

**Effect of Vagal Stimulation**

The vagal stimulation to the heart decreases the heart rate. The heart rate decreases because the membrane is hyperpolarized and the slope of prepotential is decreased (becomes relatively flat). This occurs due to release of acetylcholine (Ach) from the parasympathetic nerve endings, which increases the potassium conductance and delays the potassium decay. Ach acts by two mechanisms:
1. Ach directly acts on \textit{G Protein-Activated K Channel} (as described above) that produces G protein \( K^+ \) current \( I_{\text{Ko}} \). Ach acting on \( M_2 \) muscarinic receptors stimulates \( \beta-\gamma \)-subunits of a G protein, which in turn activates the \( K^+ \) channels. It \textbf{counters the decay of} \( K^+ \) and hyperpolarizes the membrane during phase 4 that slows the pacemaker potential.
2. Ach, by acting on \( M_2 \) muscarinic receptors on the SA node \textbf{decreases the concentration of cyclic AMP} in the cells that in turn decreases the opening of calcium channels. This \textbf{decreases calcium influx} via T-type calcium channels that decreases the slope of prepotential and takes the membrane potential away from threshold level (Fig. 87.5). Therefore, \textbf{heart rate decreases}.
3. The decrease in intracellular calcium concentration also \textbf{decreases the force of contraction}. However, vagal stimulation mainly affects the rate rather than the force of contraction, because ventricles have sparse vagal innervation.

**Effect of Sympathetic Stimulation**

The sympathetic stimulation to the heart increases the heart rate and the force of contraction.
1. The \textbf{slope of prepotential becomes steeper}, so that the threshold level is reached earlier than the normal, which increases the heart rate.
2. This occurs due to release of norepinephrine at the sympathetic nerve endings. Norepinephrine binds to \( \beta_1 \) receptors, which \textbf{increases the intracellular cyclic AMP} that in turn increases the \textbf{opening of calcium channels}.
3. The membrane is \textbf{rapidly depolarized} to the firing level and the depolarization phase of the action potential becomes steeper (Fig. 87.5). Thus, \textbf{heart rate increases}. 

![Fig. 87.5: Effects of sympathetic and parasympathetic stimulations on pacemaker potential. These effects on heart rate are mainly due to their influence on the slope of pacemaker potential. Note that sympathetic stimulation increases heart rate by rapidly raising the pacemaker potential so that the slope of prepotential reaches threshold earlier. Parasympathetic stimulation decreases heart rate by slowly raising the pacemaker potential so that slope of prepotential reaches threshold later. Also, parasympathetic stimulation causes hyperpolarization (makes the membrane potential more negative) so that prepotential takes more time to reach threshold from a more negative value. Also note, in the same time scale, the normal discharge pattern of SA node had produced two action potentials, whereas sympathetic stimulation resulted in three and parasympathetic stimulation produced only one action potential.](image)
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2. The slow AV nodal conduction is also beneficial in pathological situations in which rate of atrial depolarization is very high as occurs in atrial fibrillation. In such conditions, all the electrical impulses from atria cannot reach the ventricles because of inherent AV nodal delay. Thus, ventricles contract at a lower rate than the atria. This low ventricular rate is helpful as diastolic filling is not severely impaired.

3. Many drugs such as digitalis, beta-blockers, etc. reduce heart rate partly by promoting AV nodal delay. Also vagal stimulation causes AV nodal block.

Conduction is Rapid in Ventricles

The impulse spreads rapidly into the ventricular muscles via His-Purkinje system (the His bundle, the bundle branches and the Purkinje fibers).

1. The Purkinje system is composed of fibers with large diameter. The slope of depolarization is also very steep (see Fig. 87.1). Therefore, the conduction velocity is maximum in Purkinje fibers (4 m/s) in the heart. The wave of depolarization takes about 0.08–0.1 s to spread rapidly from the top of the septum through the Purkinje fibers to all part of the ventricles (Fig. 87.7).

**INITIATION AND PROPAGATION OF CARDIAC ELECTRICAL ACTIVITY**

**Excitation is Initiated in the SA Node**

Electrical activity in the heart is normally initiated in SA node and spreads in an orderly fashion (Figs. 87.6A to E).

1. The wave of depolarization spreads in all directions, to right and left atria directly and to the AV node via internodal pathways.
2. However, impulse from atrial muscle cannot directly excite the ventricular muscle because the atrioventricular ring (a fibrous and non-conductive connective tissue ring) separates atria from the ventricles. Therefore, transmission of electrical activity from the atria to the ventricles occurs only from AV node through the bundle of His.

**Conduction Slows in AV Node**

For the impulse to excite all regions of the atria, it takes about 60 to 90 msec. Conduction of the impulse through the AV node occurs at a much slower velocity (0.05 m/sec). Thus, the transmission of impulse is delayed in the AV node for about 0.1 s. This is called the AV nodal delay.

The slower conduction velocity in AV node is due to three factors:

1. Small size of the nodal cells and their branching patterns.
2. Slow upstroke of the action potential, which occurs due to slow voltage-gated Ca\(^{2+}\) channels
3. Weak electrical coupling as a result of relatively few gap junctions.

**Importance of AV Nodal Delay**

There are many physiological and clinical importance of AV nodal delay, as given below:

1. Due to AV nodal delay, the atrial depolarization completes much before the beginning of ventricular depolarization. Therefore, when atrial systole occurs, ventricle is in diastole. This helps in ventricular filling to occur. Otherwise, atria and ventricles would have contracted simultaneously resulting in no ventricular filling. Thus, normally the slow AV nodal conduction allows the ventricular filling to occur before the ventricles are excited.

2. The slow AV nodal conduction is also beneficial in pathological situations in which rate of atrial depolarization is very high as occurs in atrial fibrillation. In such conditions, all the electrical impulses from atria cannot reach the ventricles because of inherent AV nodal delay. Thus, ventricles contract at a lower rate than the atria. This low ventricular rate is helpful as diastolic filling is not severely impaired.

**Conduction is Rapid in Ventricles**

The impulse spreads rapidly into the ventricular muscles via His-Purkinje system (the His bundle, the bundle branches and the Purkinje fibers).

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2. The depolarization of the ventricle starts at the left side of the interventricular septum and then crosses to the right side through the interventricular septum, and then spreads down to the apex of the heart (Figs. 87.6A to E).
3. Then it depolarizes the muscles in the wall of the ventricle from endocardial to the epicardial surface, proceeding from the apex toward the atrioventricular junction.
4. In ventricles, the endocardial surface depolarizes before the epicardial surface.
5. The portions to be depolarized last are the posterobasal portion of the left ventricle, the pulmonary conus, and the upper most portion of the septum.
6. The process of repolarization of ventricular muscle occurs almost in the reverse direction. That means, the epicardial surface repolarizes first and the septum and endocardial surface repolarize last. The conduction velocity through ventricular muscle is 1 m/sec. The complete excitation of both ventricles takes about 75 m/sec.
7. The rapid and synchronous excitation of the ventricles ensures almost simultaneous contraction of all ventricular muscles, which is essential for effective ventricular ejection of blood.

### CHAPTER SUMMARY

#### KEY CONCEPTS
1. Fast response action potentials are meant for immediate initiation of muscle contraction, hence seen in cardiac muscles.
2. Slow response action potentials are seen in nodal tissues, as faster conduction of impulse through these tissues may be harmful to the heart.
3. AV nodal delay ensures adequate ventricular filling.

#### IMPORTANT TO KNOW (MUST READ)
1. In examination, **Long Questions** are usually not asked from this chapter.
2. Ionic currents in cardiac tissues, Phases and ionic basis of ventricular muscle action potential, Phases and ionic basis of SA nodal action potential, Pacemaker potential, Prepotential, Initiation and propagation of cardiac electrical activity, are usually asked as **Short Questions** in exam.
3. In **Viva**, examiner may ask… Various ionic currents in cardiac tissues, What are the phases of ventricular muscle action potential, What is the ionic basis of ventricular muscle action potential, Phases of SA nodal action potential, Ionic basis of SA nodal action potential, What is the ionic basis of pacemaker potential, What are the effects of sympathetic stimulation on pacemaker potential, What is the ionic basis of pacemaker current, Why is it called “f” channel, Why is it called “h” channel, What is the cause of AV nodal delay, What is the importance of AV nodal delay.
CHAPTER 88

Electrocardiogram

Learning Objectives

On completion of study of this chapter, the student MUST be able to:
1. Define ECG and list the uses of ECG.
2. Classify ECG leads.
3. Draw a labeled diagram of Lead II ECG and identify the ECG waves, segments and intervals.
4. Define and give normal values and significance of various ECG waves, segments and intervals.
5. Understand the concept of a cardiac dipole and how the different ECG waveforms are produced.
6. Remember ECG tracings of normal 12 leads.
7. Interpret various aspects of ECG.
8. Determine mean QRS axis, and list the common causes of left and right axis deviations.
9. Understand the physiological basis of common ECG abnormalities, such as arrhythmias, heart blocks, myocardial infarction and electrolyte imbalance.
10. Learn the basics of His bundle electrogram.

The student MAY also be able to:
1. Describe the physiological basis of genesis of ECG waves.
2. Explain the mean cardiac vector and its application.
3. Interpret the abnormalities of ECG in common arrhythmias, electrolyte disturbances and AMI.

The electrocardiogram (ECG) is defined as the graphic record of electrical activities of the heart obtained by placing electrodes on the surface of the body that records the voltage differences generated by the heart. Electrocardiography is the method of recording of ECG. Electrocardiograph is the machine that records ECG, which amplifies the voltages and gives a plot of voltage as a function of time.

Body is a volume conductor, i.e. body fluids are a good conductor of electricity. Therefore, electrical changes occurring in the heart with each beat are conducted all over the body and can be picked up from the body surface. The record of these electrical fluctuations during the cardiac cycle is called electrocardiogram. Thus, ECG recorded at the body surface represents the algebraic sum of the action potential of the individual cardiac muscle fibers.

Uses of ECG: ECG is useful for diagnosis of heart diseases. However, ECG may be completely normal with a patient having organic heart disease or may show some nonspecific abnormalities in normal individuals. Therefore, ECG should be carefully interpreted with the clinical features of the disease and with the reports of other investigations.

EGC is useful for assessing:
1. Anatomical orientation of the heart.
2. Relative size of the chambers.
3. Disturbances of rhythm and conduction.
4. Ischemia of the myocardium, if present.
5. Location, extent and progress of myocardial infarction.

Scientist contributed

Willem Einthoven (1860–1927), the great cardiovascular physiologist from Netherlands in 1901, invented a series of string galvanometers, and used them for recording electrical activity of the heart. His assignment of the letters P, Q, R, S, and T waves to the various ECG deflections are the standards of ECG tracings. The term Einthoven’s triangle is named after him. He also described ECG features of a number of cardiovascular disorders. For his great discovery and contribution to the mechanism of the electrocardiogram and application of electrocardiography in medical diagnosis, the Nobel Prize in Physiology or Medicine in 1924 was awarded to him.
6. Effects of altered electrolyte concentration.
7. Influence of certain drugs like digitalis.
8. Efficiency of electronic pacemaker function.

**TECHNICAL ASPECTS**

In modern electrocardiography, two types of ECG machines are used: (1) the string galvanometer; and (2) the radio-amplifier. ECG is recorded on ECG paper fitted in the machine. ECG paper is a strip of graph paper that contains vertical and horizontal lines 1 mm apart. The horizontal axis represents time whereas the vertical axis denotes amplitude. There is a heavy line every 5 mm in both the planes. Thus, there are small squares of 1 mm x 1 mm, and big squares of 5 mm x 5 mm. After every 5 big squares, the heavy vertical line overshoots the margin. The ECG paper is a heat-sensitive plastic-coated paper. The ECG is inscribed on this paper by a hot stylus. Conventional ECG is taken at a speed of 25 mm/s. One small square (1 mm) corresponds to 0.04 second, while the big square (5 mm) is equivalent to 0.20 second.

**ECG Leads**

An ECG lead is a pair of electrodes (electrical conductors) used to detect the potential differences of the heart. The ECG leads are broadly classified into two categories, the direct, and the indirect leads.

1. **When a lead is directly applied to the surface of the heart, it is called direct lead.** These leads are used to record cardiac activities during cardiac surgery.
2. **When the leads are applied away from the heart (usually on the body surface) to record the cardiac activities, they are called indirect leads.** Conventionally, ECG is recorded using indirect leads. Indirect leads are limb leads, chest leads, and esophageal leads.

   Usually, a twelve-lead recording is performed for complete analysis of the ECG. The leads are connected in two planes that are perpendicular to each other. One is the frontal plane that defines six limb leads and the other is the transverse plane that defines six chest leads.

**Limb Leads**

Limb leads lie in the frontal plane. These are of two types: the bipolar and the unipolar limb leads.

1. A **bipolar lead** records the potential difference between two electrodes placed at different sites.
2. A **unipolar lead** is a pair of electrode giving the potential difference between an exploring and an indifferent electrode (a reference input).
3. The reference input comes from a combination of electrodes at different sites that roughly gives a zero potential.

**Bipolar Limb Leads**

Three bipolar standard limb leads (leads I, II, and III) are the original leads selected by Einthoven to record electrical potential on the frontal plane. In the method of recording by bipolar leads, two electrodes are placed on the body surface and the potential difference between these two electrodes is recorded. The electrodes are attached to the right arm, left arm, and left foot as indicated in the Einthoven triangle (Fig. 88.1). Another electrode is applied to the right leg, which acts as a ground wire to prevent external disturbances during recording.

**Einthoven Triangle**

Einthoven’s triangle is an equilateral triangle with each side representing the axis of one of the bipolar limb leads. It is an inverted triangle with apex pointing towards the groin and the base between two shoulders.

1. As body is a volume conductor, electrical attachment to an arm is similar to the connection at the corresponding shoulder joint, and attachment to either leg is similar to the connection at the groin. Einthoven proposed certain convention in analyzing the electrical activity of the heart.
2. The heart is considered to be present at the center of the Einthoven triangle. Each corner of the triangle serves as the location for an electrode for two leads to the ECG recorder. Thus, three limbs of the triangle represent three leads (Fig. 88.1).
3. The convention proposed by Einthoven was that one electrode causes an upward deflection on the recorder when it is under the influence of a positive dipole (the concept of dipole is discussed later in the chapter) relative to the other electrode.
4. Einthoven triangle is also used in calculation of mean QRS axis of the heart (discussed later in this chapter).
**Unipolar Limb Leads**

In the method of recording by unipolar leads, one electrode is the active or recording electrode and the other one is the indifferent electrode.

1. There are three unipolar limb leads: \( \text{aVR, aVL, and aVF} \). In this, ‘a’ stands for augmentation of the leads. The potential recorded in aVL is one-and-a-half times more than that recorded in VL, and similarly for aVR and aVF. Therefore, these leads are called augmented leads.

2. ‘V’ stands for unipolar, and R, L, and F indicate that the exploring or active electrode is on the right arm, left arm, and left foot respectively.

3. The other electrode, i.e. the indifferent electrode is connected to the remaining two leads through a resistance coil.

   For example, for recording of aVL, the active electrode is placed on the left arm and the indifferent electrode is connected through a high resistance to the other two electrodes placed on the left foot and left arm.

   \( \text{aVR: Between the right arm (positive electrode) and left arm + left leg (negative electrode).} \)

   \( \text{aVL: Between the left arm (positive electrode) and right arm + left leg (negative electrode).} \)

   \( \text{aVF: Between the left foot (positive electrode) and right arm + left arm (negative electrode).} \)

   Vector of augmented limb lead = \( \frac{3}{2} \) vector of unaugmented limb lead.

   \[ \text{aVR} = \text{VR} - (\text{VL} + \text{VF})/2 \]

   \[ 2\text{aVR} = 2\text{VR} - (\text{VL} + \text{VF}) \]

   Since \( \text{VR} + \text{VL} + \text{VF} = \text{zero} \) (Einthoven’s triangle),

   \[ \text{VR} = - (\text{VL} + \text{VF}) \]

   \[ 2\text{aVR} = 2\text{VR} + \text{VR} \]

   \[ \text{aVR} = \frac{3}{2}\text{VR} \]

**Chest Leads**

**Chest leads** or **precordial leads** lie in the transverse plane. These are of two types: the **unipolar** and the **bipolar chest leads**.

**Unipolar Chest Leads**

There are six precordial leads that are used routinely. These are \( \text{V}_1 \) to \( \text{V}_6 \) (‘V’ stands for unipolar). These leads employ an exploring electrode on the chest surface. The reference or the indifferent electrode is connected to the right arm, left arm and left leg through the high resistance, which is called \textbf{Wilson’s terminal} that is maintained at zero potential. The right leg is connected with a grounding electrode to avoid electrical interference. The position of the chest electrodes (positive electrodes) on chest surface for a different lead is as follows:

\( \text{V}_1 \) : In the right fourth intercostal space at the right border of the sternum.

\( \text{V}_2 \) : In the left fourth intercostal space at the left border of the sternum.

\( \text{V}_3 \) : At the midpoint between \( \text{V}_2 \) and \( \text{V}_4 \).

\( \text{V}_4 \) : In the left fifth intercostal space on the midclavicular line.

\( \text{V}_5 \) : In the left fifth intercostal space on the anterior axillary line.

\( \text{V}_6 \) : In the left fifth intercostal space on the midaxillary line.

There are other three chest leads \( \{\text{V}_7, \text{V}_8, \text{V}_9\} \) that are used on special occasions:

\( \text{V}_7 \) : In the left fifth intercostal space on the posterior axillary line.

\( \text{V}_8 \) : In the left fifth intercostal space on the posterior scapular line.

\( \text{V}_9 \) : In the left fifth intercostal space on the back just left to the spine.

**Bipolar Chest Leads**

These leads are used before the discovery of unipolar chest leads. These leads record differences of potential between any given position on the chest and on one extremity. These are not used now-a-days, because the potential in the extremity appreciably alter the pattern of the chest leads. \textbf{Lewis lead} is a special bipolar chest lead used for recording ECG in atrial arrhythmias. This lead amplifies the waves of atrial activity.

**Esophageal Leads**

In these leads, an electrode is fixed on the tip of the esophageal catheter, which is positioned in the esophagus close to the heart chambers. The leads are designated as \( \text{E}_{15–25} \), \( \text{E}_{25–35} \), etc. Here \( E \) stands for ‘esophageal’ and the number indicates the distance of the electrode from the incisor teeth expressed in centimeter.

\( \text{E}_{15–25} \) : Used for recording the activity of the right atrium.

\( \text{E}_{25–35} \) : Used for recording the activity from the AV groove region.

\( \text{E}_{40–50} \) : Used for recording the activity from the posterior surface of the left ventricle.

**NORMAL ECG**

The ECG tracing shows different waves, segments and intervals as depicted from a lead II tracing (Fig. 88.2A).

**ECG Waves**

Waves are positive or negative deflections from baseline. There are four waveforms: P wave, QRS complex, T and U waves.

**P Wave**

P wave is the first positive deflection in the ECG, produced by atrial depolarization.
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QRS Complex
This consists of Q, R, and S waves. The QRS complex consists of deflections produced by ventricular depolarization (Fig. 88.2B).

**Q wave:** Is the initial negative deflection in the QRS complex.

**R wave:** Is the positive deflection in the QRS complex.

**S wave:** Is the second negative deflection in the QRS complex.

T Wave
T wave is the positive deflection produced by ventricular repolarization.

U Wave
U wave is the final positive deflection in the ECG. Normally, this wave is not always present. It occurs due to slow repolarization of papillary muscle.

ECG Segments
Segments are isoelectric lines in ECG tracing. There are two segments: PR segment and ST segment.

**PR Segment**
This lies between the end of the P wave and the beginning of the QRS complex.

**ST Segment**
This lies between the end of the QRS complex and the beginning of the T wave. The point where the QRS complex ends and the ST segment begins is the J point. Elevation of J point suggests myocardial ischemia or infarction.

ECG Intervals
Intervals usually include waves and segments.

**PR Interval**
**Definition:** This is the interval between the beginning of the P wave and the beginning of the QRS complex.

**Normal duration:** The range of PR interval is from 0.12 to 0.20 second (average 0.18 s). PR interval shortens as the heart rate increases from the average of 0.18 s at the rate of 70 to 0.14 s at the rate of 130.

**Significance:** This represents atrial depolarization and conduction through AV node.

**QRS Interval (QRS Duration)**
**Definition:** This is the interval of the QRS complex. It is measured from the beginning of the Q wave (or R wave if Q wave is absent) to the J point.

**Normal duration:** The normal range is from 0.08 to 0.10 second.

**Significance:** This represents ventricular depolarization. The atrial repolarization also occurs in this period.

**QT Interval**
**Definition:** This is the interval for QRS complex, ST segment and T wave. It is measured from the beginning of the QRS complex to the end of the T wave.

**Normal duration:** The normal range is between 0.40 and 0.43 second.

**Significance:** This represents ventricular depolarization and ventricular repolarization. It corresponds to the duration of electrical systole.
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ST Interval
Definition: This is the interval between the J point and the end of T wave. It is calculated by deducting QRS interval from QT interval.
Normal duration: The average duration is 0.32 second.
Significance: This represents ventricular repolarization.

PP Interval
This is the interval measured between either the peak or the beginning of two successive P waves. PP interval is measured for calculation of the atrial rate.

RR Interval
This is the interval between two successive R waves. It is measured between the peaks of two successive R waves. RR interval is measured for calculating the heart rate (the ventricular rate).

PHYSIOLOGICAL BASIS OF ECG
What does ECC actually record? The ECG records the voltage between dipoles produced by electrical activities of the heart. ECG is the recording of the electrical activities generated by the heart. Before learning how heart creates a dipole, one should know what a dipole is.

Concept of a Dipole
Dipoles are combination of two poles (positive and negative poles) in an electrical field. An electrical field and a dipole in it can be created by connecting a solution containing charged ions to a source of volts like a battery.
1. When a negative wire (wire connected to the negative terminal of a battery) and a positive wire (wire connected to the positive terminal of the same battery) are placed in a container containing equal amount of charged ions, such as salt solution, the positively charged ions flow toward the tip of the negative wire (negative pole) and negatively charged ions flow toward the tip of the positive wire (positive pole).
2. Thus, two poles are created in the solution. If the tips of the wires are placed close to each other, the flow of ions is maximal between the two poles (Fig. 88.3). Though the two poles have opposite charges, they have the currents (flow of ions) equal in magnitude. The combination of these two poles that have equal and opposite charges is called a dipole.
3. The voltage associated with the dipole can be measured by using a voltmeter. If the electrodes of the voltmeter are placed opposite to each other in a horizontal plane between the two poles, the voltage recorded is maximum.
4. But, if the position of the electrodes is changed or the position of the dipole is changed, magnitude of vector is decreased and, therefore, the voltage is also decreased.

PHYSIOLOGICAL BASIS OF ECG
What does ECC actually record? The ECG records the voltage between dipoles produced by electrical activities of the heart. ECG is the recording of the electrical activities generated by the heart. Before learning how heart creates a dipole, one should know what a dipole is.

Heart as the Dipole
The above explanation helps us to understand the physiological basis of recording ECG. Our body is a volume conductor, and electrical activities of heart create many dipoles in the body. As heart creates many dipoles simultaneously, ECG records the voltage of the net dipole, which is average of all the dipoles.

How is the Dipole Created in the Heart?
Like any other excitable cell of the body, the muscle cell of the heart at rest is negatively charged inside and positively charged outside the cell membrane. When the cell is depolarized, the interior of the cell becomes positive and exterior becomes negative.
1. In the normal process of spread of excitation in the conducting pathway, the impulse spreads from SA node to the ventricular muscle as described in the previous chapter. In this process of spread of cardiac excitation, when the impulse excites a region of the
heart, that region is depolarized (outside of the region becomes negative) ahead of the other region of the heart. This generates the dipole.

2. The region of the myocardium depolarized constitutes the negative pole and the region ahead of it (yet to be depolarized) constitutes the positive pole.

3. Likewise, with natural spread of excitation, the dipole shifts from one region to the other.

4. So, heart contains the dipole, and heart is present in the body, which is the volume conductor.

5. The electrode located on the volume conductor (on the surface of the body) records the voltage resulting from the dipole, which is generated by electrical activity of the heart.

**Why Has ECG Various Waveforms?**

As it is stated above in the concept of a dipole, if the position of dipole changes in relation to electrodes, the voltage recorded from the volume conductor changes. In the myocardium, many dipoles are formed as the propagated impulse spreads to various parts of the ventricle (or of the heart) at a time by different branches of the conducting system. Thus, many dipoles are created simultaneously in the heart. The voltage of the net dipole (average of many individual dipoles at any given time) is recorded in the voltmeter (electrocardiographic recorder), which is connected to the two points of the volume conductor.

1. Let us consider the recording of lead II that records electrical activity between right arm (point B) and left leg (point A) (Fig. 88.4). According to the principle of electrophysiology, when point A is positive in relation to point B, the ECG deflection is upward, and when A is negative in relation to B, the deflection is downward.

2. The strength of voltage is indicated by the length of the net dipole. When the net dipole is parallel to the line AB, the magnitude (strength of voltage) is maximal and when it is perpendicular, magnitude is zero. Thus, the magnitude recorded by the electrocardiographic recorder depends on the direction of the net dipole, and the muscle mass of heart involved in generating the net dipole.

3. Accordingly, various positive and negative deflections of various magnitudes or isoelectric lines are produced in the ECG recordings for various leads depending on the position of electrodes on the body that records the potential difference at a given instant.

4. For lead II recording that records the voltage difference between point B (right arm) and point A (left leg), the following ECG waves are generated (Fig. 88.4).

**P Wave**

P wave represents atrial depolarization. SA nodal discharge spreads over the atria and causes atrial excitation.

1. The magnitude of the net dipole is proportionate to the atrial muscle mass and direction of depolarization.

2. Point A is positive in relation to point B that results in an upward or positive deflection of the ECG (Fig. 88.4I).

3. Thus, the voltage change due to atrial depolarization appears on the ECG as the P wave, which is positive with less height and dome shape.

4. The height of P wave is less because of less atrial muscle mass and dome shape is due to the slow nature of depolarization of atrial muscle. When atria are completely depolarized, the ECG tracing returns to zero.

**PR Interval**

The PR interval includes the P wave and PR segment. It represents atrial depolarization (P wave) and atrioventricular conduction.

1. The PR segment is an isoelectric line.

2. During PR segment, the wave of depolarization passes slowly through the AV node, and then through the His bundle.

3. The net dipole created by depolarization of these structures is too small in magnitude to produce any deflection on the ECG recording.

**QRS Complex**

The QRS complex represents ventricular depolarization. It consists of three waves: Q wave, R wave, and S wave.

**Q Wave**

The wave of depolarization passes along the bundle branches, and Purkinje fibers to first excite the interventricular septum, which depolarizes from left to right.

1. Thus, Point A becomes negative in relation to point B that results in a negative wave (Fig. 88.4II).

2. Therefore, the net dipole of initial depolarization manifests in ECG as Q wave, which is a small downward sharp wave.

3. The small wave is due to less quantity of septal muscle and less time of depolarization and sharpness is due to the rapidity of depolarization.

4. However, normally the Q wave is so small that it does not often appear in ECG.

**R Wave**

The wave of depolarization then spreads from the subendocardial muscle layer to the subepicardial muscle layer.

1. The ventricular muscle mass is more and the left ventricle is much thicker than the right ventricle. Therefore, the direction and amplitude of the net dipole during this phase is such that point A becomes positive in relation to point B (Fig. 88.4III; note the bigger length of the arrow, which indicates the magnitude of the dipole).

2. The deflection in the ECG appears as R wave, which is an upward, bigger and sharp wave.
3. R is largest wave in ECG because of the greater muscle mass of ventricles.
4. The sharpness of the wave represents the rapidity of depolarization.
5. R wave returns to baseline when the whole ventricle is depolarized and there exists no potential difference between two points of dipole created in ventricle.

**S Wave**

The last parts to be depolarized are the posterobasal portion of the left ventricle and the pulmonary conus.

1. The direction and duration of depolarization are such that the net dipole has direction with point A negative in comparison to point B (Fig. 88.4IV).
2. This appears in ECG as a small downward wave.
3. Thus, the S wave is a small, negative and sharp wave. The Q, R, and S waves together form the QRS complex, which represents ventricular depolarization. The duration, magnitude and sharpness of the QRS complex indicate the greater muscle mass of the ventricles depolarized and the rapidness of ventricular excitation.
ST Segment

As soon as the ventricular depolarization is completed, all dipoles associated with it disappear and the ECG tracing returns to baseline.

1. The ST segment starts from the end of the S wave and ends with the beginning of the T wave. The ST segment is an isoelectric line.
2. This indicates that in this phase, dipoles are not adequate enough to create any wave in ECG. This is the phase of beginning of ventricular repolarization.

T Wave

T wave represents ventricular repolarization. Electrophysiologically, repolarization is an opposite process of depolarization and repolarization also creates dipoles like that of depolarization. Therefore, repolarization should have produced a downward deflection in ECG.

1. However, the process of repolarization occurs in the opposite direction to that of depolarization, i.e. from epicardial to endocardial surface of the ventricular muscle.
2. Thus, subepicardial cells repolarize first, and the subepicardium becomes positive relative to the subendocardium.
3. Therefore, the polarity of the net dipole of repolarization is the same as the polarity of the dipole of depolarization. This results in an upward deflection in ECG, point A being positive with respect to point B (Fig. 88.4V).
4. This appears in ECG as T wave, which is a dome-shaped positive wave of longer duration. This indicates that repolarization is slower than depolarization. If ventricular repolarization is delayed, the QT interval is prolonged.

As we saw in the above discussion, the waveform of lead II ECG depends on the direction and magnitude of dipole in relation to the recording electrodes. For recording ECG of other leads, the placement and connections of electrodes change, whereas the direction and magnitude of dipole remain the same. Therefore, net magnitude and direction of dipole change in relation to electrodes, that creates different patterns of waveforms in different leads.

Cardiac Dipoles as Vectors

As we have seen in our preceding discussion, cardiac dipoles act as vectors in both direction and magnitude. The net vector (average vector of all cardiac dipoles) can be determined from the ECG recording. The direction of the vectors is determined from the ECG recorded from leads placed in frontal and horizontal planes of the body.

1. Three bipolar limb leads (leads I, II, and III) and three augmented limb leads (aVR, aVL, and aVF) give information in the frontal plane of the body. The lead I records the potential differences between the left and right arms and records the electrical vector that is parallel to its axis. The axis of lead I passes as a horizontal line through the center of the chest from right to the left arm (Fig. 88.5).
2. Similarly, the axis of lead II passes as a 60° line with negative end from right arm to the positive end towards left leg, and lead III as a 120° line from left arm to left leg (positive end).
3. The axes of augmented limb leads are interspersed between the bipolar limb leads as shown in the figure. Thus, the picture of the axis of leads in the frontal plane is called the hexaxial reference system.
4. When the net cardiac dipole with its positive charge is directed towards the positive end of the axis of a lead, upward deflection occurs in the recording.
5. If the net cardiac dipole with its positive charge is directed towards the negative end of the axis of a lead, downward deflection occurs.
6. No deflection occurs if positive charge of net cardiac dipole is directed at right angle to the axis of a lead. This helps us to understand ECG changes in various leads in health and diseases.

Normal 12 Lead ECG

Normally, recording of 12 leads (Fig. 88.6) is considered for complete analysis and interpretation of ECG. The deflection of waves in a particular lead is governed by the basic law of dipole, as described above.

1. The positive or upward deflection is seen in any lead, if depolarization spreads towards the positive pole of that lead, and a negative or a downward deflection is seen in any lead, if depolarization spreads towards the negative pole of that lead.
2. An isoelectric or biphasic deflection is seen when the depolarization starts in SA node and spreads downward to the subject’s left (toward the positive pole of lead I and away from positive pole of lead aVR).
3. The P wave is always positive in lead II and negative in lead aVR.
4. Ventricular septum depolarizes from left to right (towards lead V₁ and away from lead V₆). This produces small “q” wave (septal q wave) in V₆ and small “r” wave (septal r wave) in lead V₁.
5. During ventricular depolarization, as the left ventricular mass is more than right ventricular mass, the net direction of depolarization is towards the left chest leads (Fig. 88.7).
6. This produces tall “R” wave in leads V₅ and V₆, and a deep S wave in leads V₁ and V₂. Chest leads between these two positions show a transitional pattern.
7. In extremity lead, the QRS complex varies depending on whether the heart is more horizontal or vertical. When the heart is more vertical, leads II, III, and aVF show a “qR” pattern and when the heart is more horizontal, the leads I and aVL show a “qr” pattern.
8. The T wave normally follows the direction of the QRS complex deflections. In chest leads, the T wave is positive in the left-sided leads (and also in V₂). In V₁, T wave may be positive or negative.

**Systematic Interpretation of ECG**

A routine screening of the ECG needs stepwise examination of the ECG.

1. What is the heart rate? What is the atrial rate and what is the ventricular rate?
2. Is the rhythm regular or irregular?
3. What is the mean cardiac vector?
4. Are the P waves normal? Do the P waves have a fixed relation to the QRS complexes?
5. What is the duration of PR interval? What are the duration, amplitude and configuration of the QRS complex?
6. Is the ST segment isoelectric?
7. Are the T waves normal?
8. What is the duration of QT interval? Is the QTc appropriate for the heart rate (QTc is the QT interval corrected for the rate)?

**Heart Rate**

In interpretation of ECG, heart rate should be calculated first. The comment should be made on both atrial and ventricular rates. Usually, the heart rate means the ventricular rate. At a paper speed of 25 mm/s, the atrial rate per minute is calculated by dividing 1500 with PP interval (in mm). Similarly, ventricular rate per minute is calculated by dividing 1500 with RR interval (in mm). Normally, the RR interval is equal to the PP interval. But, sometimes ventricular rate may be different from the atrial rate.

**Calculation of the ventricular rate when RR interval is irregular:** When RR interval is irregular as seen in atrial fibrillation, the number of QRS complexes is counted over 5 seconds (125 mm) in the rhythm strip and this number is multiplied by 12 to provide the number of QRS complexes in 60 seconds (1 minute). This enables the measurement of the average ventricular rate.

The normal heart rate is **60 to 100 per minute**.

**Abnormalities of Heart Rate**

**Bradycardia**

Heart rate < 60/min is called **bradycardia**.
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1. Sinus bradycardia
   - Athletes
   - Sick sinus syndrome
   - Drugs (e.g. beta blockers)
   - Obstructive jaundice
   - Raised intracranial pressure
   - Myxedema
2. Junctional (nodal) rhythm
3. Complete heart block

**Tachycardia**
Heart rate > 100/min is called **tachycardia**.

1. Sinus tachycardia
   - Anxiety
   - Fever
   - Hypoxemia
   - Thyrotoxicosis
   - Cardiac failure
   - Acute carditis
2. Ectopic (reentrant) tachycardia
3. Atrial premature beats
   - Anxiety
   - Excess tea or coffee intake
   - Viral infections
   - Rheumatic heart disease
   - Digitalis toxicity
   - Cardiomyopathies
4. Paroxysmal supraventricular tachycardia
5. Atrial fibrillation
   - Rheumatic heart disease with mitral stenosis
   - Coronary artery disease
   - Cardiomyopathies
   - Thyrotoxicosis
6. Atrial flutter
   - Rheumatic heart disease
   - Coronary artery disease
7. Ventricular premature beats
8. Ventricular tachycardia

Cardiac Rhythm
Normally the rhythm is regular. That means heart normally beats at regular intervals. It is observed by calculating the successive cycle length (RR intervals). Sometimes, there may be minor variation of the rhythm in normal conditions. A variation of maximum up to 10% in adjacent cycle length is considered to be normal. Abnormalities of cardiac rhythm are called **arrhythmia** (described here with). An example of physiological arrhythmia is sinus arrhythmia.

Waves and Intervals

**P Wave**
Duration and Amplitude: Normal P wave duration does not exceed 0.10 s and P waves are not more than 2.5 mm tall.
Configuration: Usually, P waves are upright in lead I to aVF and V₃–V₆; inverted in aVR; and upright, inverted or biphasic in lead III, aVL, and V₁ and V₆. P wave morphology is best studied in lead II and V₁.

P Wave Abnormalities: P wave may be abnormal due to atrial enlargement and intra-atrial conduction abnormalities. Atrial enlargement results in tall and peaked P waves.

**PR Interval**
Normal PR interval is 0.12 to 0.20 second, i.e. 3 to 5 small squares. Normally, there should not be any variation in PR intervals.

Short PR Interval:
1. WPW syndrome
2. Nodal rhythm
3. Atrial premature beats

Long PR Interval: (First-degree AV block; discussed below)
1. Rheumatic carditis
2. Digitalis effect
3. Coronary artery disease

**QRS Complex**
Amplitude: In limb leads (in lead I, II, III, aVR, aVL, and aVF), the total amplitude of QRS should be 5 mm or more. In chest leads, the amplitude of QRS complex should be 10 mm or more.
Duration: Normal duration of QRS complex does not exceed 0.11 s.
Configuration: Normally, R wave is dominant in leads I, II, V₄–V₆ and S wave is dominant in aVR, V₁ and V₂. Either R or S wave may be dominant in lead III, aVL, aVF, and V₃ depending on the position of the heart.
Main QRS abnormalities manifest in the amplitude.

Low amplitude:
1. Marked emphysema
2. Myxedema
3. Pericardial effusion
4. Cardiomyopathy

High amplitude: Seen in ventricular hypertrophy

**Q Wave**
Normally, Q waves are small in lead I, aVL, V₅ and V₆. A QS complex is commonly found in aVF. There may be deep Q waves in lead III alone in normal individuals and may become less prominent on deep inspiration. Occasionally a deep Q wave is found in V₁ and V₂ normally. Depth of Q wave is less than 25% of the height of the ensuing R wave in most leads and may be up to 50% in aVF. Any Q wave with amplitude more than this is considered to be pathological.

Pathological Q Waves
When the depth of Q wave is more than 25% of the height of the ensuing R wave, or more than 0.04 s in duration is considered pathological. Common causes are:
1. Acute or old myocardial infarction
2. Unstable angina
3. Dilated cardiomyopathy
4. Hypertrophic cardiomyopathy

**ST Segment**

The normal ST segment is isoelectric. ST depression less than 0.5 mm is not abnormal. ST elevation up to 1 mm in limb leads and V₅ and V₆ and 2 mm in V₁–V₄ may be normal.

**ST Elevation:** Commonly seen in acute myocardial infarction and sometimes in acute pericarditis.

**ST Depression:** Commonly seen in myocardial ischemia.

**T Wave**

T waves are upright in lead I, II, V₄–V₆; inverted in aVR; and upright, inverted or biphasic in lead III, aVL, aVF, and V₁–V₃.

T wave abnormalities involve either a tall or an inverted T wave.

**Tall T wave:**
1. Hyperkalemia
2. Acute myocardial infarction

**Inverted T wave:**
A. *Physiological*
   1. Young children
   2. Deep inspiration (sometimes)
   3. After heavy meal (sometimes)
B. *Pathological*
   1. Ventricular hypertrophy (strain)
   2. Bundle branch block
   3. Digitalis effect
   4. Myocardial ischemia

**QT Interval**

The upper limit of normal QT interval is 0.42 s in males and 0.43 s in females. QT intervals should be measured in the lead where the end of the T wave is best discernible. QT interval varies with heart rate. Therefore, corrected QT interval (QTc) is measured by using Bazett’s formula.

\[
\text{QTc} = \frac{\text{QT}}{\sqrt{\text{RR}}} \quad \text{(QT is QT interval and RR is RR interval)}
\]

**Abnormal QT Interval**

QT interval may be either prolonged or shortened.

**Prolonged QT interval:**
1. Hereditary
2. Antiarrhythmic drugs, e.g. quinidine
3. Hypokalemia
4. Acute myocardial infarction

**Shortened QT interval:** This is of less clinical significance and may be seen in hypercalcemia.

**Mean QRS Axis**

Mean QRS axis is the magnitude and direction of the mean cardiac dipole (the mean cardiac vector).
**Accurate Estimation**

Accurate estimation of mean cardiac vector can be done by two methods: one, by using Einthoven’s triangle, and other, by using hexaxial reference system.

**Using Einthoven’s Triangle:** Mean cardiac vector can be measured by using Einthoven’s triangle. The net magnitude of the QRS complex of any two of the three standard limb leads is measured and plotted on the appropriate axis in the Einthoven’s triangle.

1. In each lead, distances equal to the height of the R wave minus the height of the largest negative deflection in the QRS complex are measured. These distances are drawn from the midpoint to the positive or negative side of the triangle representing that lead. Perpendicular lines are drawn from the midpoint of the arms of triangle to the center. Perpendicular lines are also drawn from the end of the QRS magnitudes drawn on the triangle.
2. An arrow is drawn from the center of triangle to the point of intersection of perpendiculars extended from the distances measured on the sides. This arrow represents the magnitude and direction of the mean QRS vector.
3. For example, as demonstrated in Figure 88.9, the net magnitudes of the QRS complexes of leads I and II are 8 mm and 10 mm respectively. Accordingly, the distances are plotted and perpendicular lines are drawn on Einthoven’s triangle. The mean cardiac vector was found to be 40°.

**Using Hexaxial Reference System:** In this method, first the six limb leads are examined to find the one in which the net deflection QRS complex is close to zero or R wave has equal upward or downward deflections.

1. As we know that when the cardiac dipole is perpendicular to a particular lead, the net deflection of that lead is zero. Thus, it means that the mean cardiac vector is perpendicular to that lead in which the net QRS deflection is close to zero.
2. Now, the hexaxial reference system is plotted and consulted to estimate the angle of that axis.
3. For example, the net QRS deflection is close to zero in lead III. Lead aVR is perpendicular to the axis of lead III in the hexaxial reference system. Thus, the mean QRS axis is estimated to be about +30° (see Fig. 88.6).

**Abnormal Axis Deviations**

**Right axis deviation:**
1. Right ventricular hypertrophy
2. Left posterior hemiblock
3. WPW syndrome
4. Dextrocardia

**Left axis deviation:**
1. Left ventricular hypertrophy
2. Left anterior hemiblock
3. WPW syndrome

**Abnormal ECG**

ECG is useful in detecting four types of abnormalities:
1. Abnormal pattern of cardiac excitation resulting in different types of arrhythmias.
2. Abnormalities of myocardium.
3. Cardiac abnormalities due to alteration in plasma electrolytes.
4. Cardiac involvement secondary to other diseases.

**Cardiac Arrhythmias**

Disorder of the property of rhythmicity of the heart is called arrhythmia. Abnormalities of the rhythm should be better termed as dysrhythmia rather than arrhythmia. Clinically, cardiac dysrythmias can be broadly divided into two categories: bradyarrhythmias (arrhythmias in which cardiac rate is decreased) and tachyarrhythmias (type of arrhythmias in which cardiac rate is increased). However, physiologically cardiac dysrythmias can be divided into four categories depending on the functional site affected: Disorders of SA node, Atrial arrhythmias, Ventricular arrhythmias, and Conduction disorders.
Disorders of SA Node

The common disorders of SA node are sinus arrhythmia, sick sinus syndrome, sinus tachycardia and sinus bradycardia.

Sinus Arrhythmia

Sinus arrhythmia is a normal physiological phenomenon referred to the alteration in heart rate in respiratory cycles. Heart rate increases in inspiration and decreases in expiration. This is also called respiratory sinus arrhythmia. It is explained by four mechanisms:

1. Alteration in autonomic activity: During inspiration, sympathetic discharge increases, and during expiration, vagal activity increases.

2. Activation of Bainbridge reflex: During inspiration, increased venous return to the right atrium increases heart rate. The decrease in intrathoracic pressure during inspiration increases right atrial filling and stretches the right atrium. Thus, atrial tachycardia producing receptors are activated that produces tachycardia. Right atrial stretching also stretches SA node, which causes tachycardia.

3. Irradiation from inspiratory center: Increased irradiation from inspiratory center to the vasomotor center during inspiration increases the heart rate.

4. Activation of atrial stretch reflex: Increased venous return during inspiration stimulates type B atrial stretch receptors. This increases heart rate.

Sick Sinus Syndrome

Decrease in heart rate due to disease of SA node is called sick sinus syndrome.

Sinus Tachycardia

When heart rate is more than 100/min in adult, the condition is called sinus tachycardia. Rarely sinus tachycardia is more than 200/min. It is actually not a primary arrhythmia. Causes of sinus tachycardia are:

- Anxiety
- Fever
- Hypoxemia
- Thyrotoxicosis
- Cardiac failure
- Acute carditis
- Drugs like atropine

Sinus Bradycardia

When heart rate is less than 60/min, the condition is called sinus bradycardia. This occurs due to suppression of SA node. Causes of sinus bradycardia are:

- Strong athletes
- Sick sinus syndrome
- Drugs (e.g. beta blockers)
- Obstructive jaundice
- Raised intracranial pressure
- Myxedema

Atrial Arrhythmias

The common atrial arrhythmias are atrial premature beats, paroxysmal supraventricular tachycardia, atrial flutter and atrial fibrillation.

Atrial Premature Beats

Atrial premature beats occur due to premature discharge from an ectopic atrial focus. The P wave of premature atrial ectopic beat appears before the next sinus P wave.

1. The configuration of the ectopic P wave is abnormal. This is also called atrial extrasystole (Fig. 88.10A).

2. Atrial ectopics are seen in physiological conditions, like anxiety, consumption of excess tea or coffee, or in heart diseases, like rheumatic heart disease, coronary artery disease, cardiomyopathies or digitalis toxicity.

Paroxysmal Supraventricular Tachycardia

There are two types of paroxysmal supraventricular tachycardia (PST): Nodal PST and Atrial PST.

Nodal PST

In this condition, tachycardia (heart rate is usually from 170 to 250/min) occurs due to an AV nodal reentry, which occurs due to the presence of dual AV nodal pathways.

1. It usually occurs in paroxysm that lasts for few minutes to hours. Identification of P wave becomes difficult as atria and ventricles depolarize almost simultaneously.

2. Sometimes abnormal P wave may follow QRS complex. The morphology of QRS complex remains normal and the rhythm is absolutely normal (constant RR intervals).

3. It is commonly seen in normal individuals.

4. However, it may be associated with Wolff-Parkinson-White Syndrome, Lown-Ganong-Levine Syndrome and hyperthyroidism. It is usually precipitated by atrial premature beats.

Atrial Tachycardia (Atrial PST)

This occurs when an atrial focus discharges regularly. The atrial rate is usually above 200/min (Fig. 88.10B). It is seen in patients receiving digitalis. Atrial tachycardia may be one of the causes of paroxysmal ventricular tachycardia.

Atrial Flutter

In this condition flutter wave (F wave) appears in ECG, which is characterized by undulating baseline resulting in corrugated or saw tooth appearance. Usually, rhythm is regular.

1. All impulses from atria fail to be transmitted to the ventricle. Usually, two flutter waves precede one QRS complex (2:1 AV block).

2. However, 1:1, 4:1 or rarely 3:1 AV block may occur.

3. Heart rate varies between 250 and 350 per min.

4. Atrial flutter is usually seen in coronary artery disease, mitral valve disease, rheumatic heart disease and thyrotoxicosis. Usually, it occurs due to intra-atrial reentry.
Atrial Fibrillation

In atrial fibrillation, atria beat rapidly but irregularly in a totally disorganized way. Atria beat at a rate of 300 to 500/min and ventricle beats at 100–180/min.
1. In ECG, the baseline is irregular and wavy due to the appearance of fibrillatory waves (Fig. 88.10C).
2. QRS complexes are normal.
3. It is usually seen in rheumatic heart disease, mitral valvular defects, coronary artery disease, cardiomyopathies, and thyrotoxicosis.
4. It occurs due to the presence of multiple reentrant excitation waves in the atria.

Ventricular Arrhythmias

The common ventricular arrhythmias are ventricular extrasystole, paroxysmal ventricular tachycardia, and ventricular fibrillation.

Ventricular Extrasystole

This occurs due to premature discharge from a ventricular ectopic focus.
1. The QRS complex appears early than anticipated and looks wide, bizarre and slurred or notched.
2. The P wave is not seen as it is buried in the QRS of the extrasystole.
3. The ventricular premature beats are followed by compensatory pause (Fig. 88.10D).
4. It may occur in normal individuals or patients suffering from heart diseases.

Paroxysmal Ventricular Tachycardia

Paroxysmal ventricular tachycardia (PVT) occurs due to circus movement (propagation of wave of excitation continuously within a closed circuit) within the ventricle.
1. PVT is distinguished from supraventricular paroxysmal tachycardia (PST) by recording His bundle electrogram (HBE), in which H deflection (His bundle deflection) appears in PST but not in PVT.
2. PST is usually benign, whereas PVT is usually serious as it may lead to ventricular fibrillation.

Ventricular Fibrillation

Ventricular fibrillation occurs due to discharge from multiple ventricular ectopic foci or due to the presence of circus movement in the ventricle.
1. Ventricular rate is very high and irregular (Fig. 88.10E).
2. Ventricular contraction is totally disorganized and ineffective due to rapid discharge. Ventricles look like a bag of worms. Pumping of blood by fibrillating ventricle is grossly inadequate.
3. Therefore, circulation of blood stops. Thus, ventricular fibrillation is a medical emergency.
4. In the absence of emergency treatment, death ensues within few minutes.
5. Ventricular fibrillation occurs usually in patients with acute myocardial infarction that leads to sudden death. Ventricular fibrillation may lead to flutter.
6. Ventricular flutter appears as a sine wave in ECG with a rate 150 to 300/min from.

Conduction Disorders

Conduction disorder may be conduction block or conduction acceleration.

Conduction Block (Heart Block)

Defect in transmission of impulses from atria to ventricles is called atrioventricular block (AV block) or heart block. There are three types of AV blocks: First-degree, second-degree and third-degree AV blocks. In first and second-degree heart blocks, conduction of impulses between atria and ventricles is not completely interrupted. Therefore, they are called incomplete heart blocks. In third degree heart block, atrioventricular conduction of impulse is completely stopped (complete heart block).

First-Degree Heart Block

All the atrial impulses reach the ventricle, but the conduction of impulse is slowed due to increased delay in AV node. Therefore, PR interval is abnormally prolonged (Fig. 88.11A).
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Figs. 88.11A to D: Heart Blocks. (A) First-degree heart block (note, prolongation of PR interval); (B) Second degree heart block of 2:1 variety (also called Mobitz Type II block in which for every two P waves one QRS complex is formed); (C) Second-degree heart block or Mobitz type I in which PR interval progressively lengthens in each cardiac cycle till one QRS complex is dropped, and again the new cycle of progressive prolongation of PR interval starts, this is also called Wenckebach phenomenon; (D) Third-degree or complete heart block in which atrial impulses do not reach ventricle at all, therefore, atria and ventricles beat separately.

**Second-Degree Heart Block**

This is further subdivided into Mobitz type I, and Mobitz type II blocks.

**Mobitz Type I:** In this type of heart block, PR interval is prolonged progressively in repeated sequence of beats till a QRS complex (ventricular beat) is dropped. PR interval following the dropped beat is usually normal, but in subsequent beats PR interval progressively prolongs till the next beat is dropped. This is called *Wenckebach Phenomenon* (Fig. 88.11C). This is usually seen in acute inferior myocardial infarction, digitalis toxicity and acute carditis.

**Mobitz Type II:** In this type of heart block, all atrial impulses are not conducted to the ventricles. A ventricular beat occurs in every two or three atrial beats. Accordingly, it is called **2:1 or 3:1 block** and so on (Fig. 88.11B). This is usually seen in acute anterior myocardial infarction, digitalis toxicity and acute carditis.

**Third-Degree Heart Block**

This is known as **complete heart block** as conduction of impulses from atria to ventricles is completely interrupted. Atria and ventricles beat separately (atrioventricular dissociation).

1. Therefore, in ECG, P waves that appear regularly bear no relationship with QRS complexes (Fig. 88.11D).
2. Complete heart block occurs due to disease of the AV node (**AV nodal block**) or disease of the conducting system below the AV node (**infranodal block**).
3. In these conditions, especially in infranodal block, a portion of ventricular muscle becomes the pacemaker.

This is called **idioventricular rhythm**, the rate of which is 15–40 beats/min.

4. When the heart rate is as low as 15/min, blood circulation decreases that results in cerebral ischemia and causes fainting. This is called **Stokes-Adams syndrome**.

5. Common causes of complete heart block are septal myocardial infarction, His bundle injury during surgical procedure for repair of ventricular septal defect, digitalis toxicity and degenerative diseases of the conductive system.

6. Now a days, implantation of electronic **pacemaker** is the usual treatment of complete heart block.

**Bundle Branch Block**

Block of either of the bundle branch results in prolongation of QRS complex. In right bundle branch block (RBBB), depression of ST segment and inversion of T wave occurs (Fig. 88.12). In left bundle branch block (LBBB), R wave widens with two peaks.

**Acceleration of Conduction**

There are two important defects of conduction accelerations: WPW syndrome and LGL syndrome.

**Wolff-Parkinson-White (WPW) Syndrome**

In WPW syndrome, in addition to three normal internodal pathways, an aberrant pathway (**bundle of Kent**) exists between atria and ventricle.

1. This abnormal pathway transmits impulse faster than the usual transmission of impulse through the AV node. Therefore, ventricle is **excited early**.
2. The QRS complex of this premature activation merges with the normal QRS so that the PR interval is shortened and QRS complex is widened and slurred in its upstroke (Fig. 88.13A).
3. However, PJ interval is normal. WPW syndrome predisposes to paroxysmal atrial tachycardia.
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Lown-Ganong-Levine (LGL) Syndrome
In LGL syndrome, impulses from SA node bypass the AV node and merge with the conducting pathway distal to the AV node via aberrant pathways. Therefore, PR interval shortens in the presence of normal QRS complex (Fig. 88.13B). PJ interval shortens due to decreased PR interval.

Myocardial Abnormalities

Myocardial Ischemia
Myocardial ischemia occurs due to decreased blood supply to the ventricular tissue. In the ischemic region, myocardial cells partially depolarize to a lower resting membrane potential. This occurs due to decreased gradient of potassium ion concentration (though they still produce action potentials).

1. Normally, during TP interval (interval between completion of T wave and onset of P wave), the myocardial cells remain in their resting membrane potential.

2. Due to partial depolarization, a dipole is present during the TP interval in injured myocardium due to the voltage difference between polarized (normal) and partially polarized (ischemic) tissue.

3. However, no dipole is present during the ST segment as depolarization is complete and uniform in both normal and ischemic tissues.

4. Thus, ST segment may be recorded as either positive or negative waves. The ST segment changes provide major clue for the diagnosis of cardiac ischemic injury.

Myocardial Infarction
Acute interruption of blood supply to myocardium results in myocardial ischemia, and if interruption continues, acute myocardial infarction (AMI) occurs. ECG changes are very useful in the diagnosis of myocardial infarction and also to assess the location and extent of infarction. During the stage of myocardial ischemia, T wave inversion is seen in ECG. The usual changes in AMI are ST segment elevation in the leads overlying the infarction and ST segment depression in the leads located opposite to infarction (Fig. 88.14).

Physiological Basis of ECG Changes in AMI
The hallmark of ECG change in AMI is ST segment elevation. This occurs due to three mechanisms that operate in sequence:

1. Rapid repolarization of the infarcted tissue: Within a few seconds of infarction, the infarcted tissue rapidly repolarizes due to fast opening of K⁺ channels. Therefore, the membrane potential of the infarcted area is higher than the membrane potential of the normal surrounding area during the later part of repolarization. This makes the normal region negative to the infarcted region. As normally current flows from positive to negative region, in AMI, the current flows from infarcted area to the surrounding areas and also towards the electrode placed on the infarcted area. This causes increased positivity between the S and T waves in ECG and results in ST elevation.

2. Decreased RMP of infarcted muscle fibers: Loss of intracellular K⁺ decreases resting membrane potential in the infarcted muscle cell. This causes current flow into the infarcted tissue during diastole, which causes TQ segment depression in ECG that manifests as ST elevation.

3. Delayed depolarization of infarcted cells: Infarcted muscle fibers depolarize very slowly in comparison to the surrounding normal fibers. This makes the infarcted tissue to be relatively positive to the normal tissue, especially in early part of repolarization, and causes ST segment elevation. In chronic case, the dead infarcted tissue forms scar tissue and becomes electrically silent. Therefore, the infarcted tissue becomes negative relative to the normal tissue.
tissue. The ECG manifestations of this negativity are many, though the common feature is the appearance of Q wave, which was not present previously.

**Ventricular Hypertrophy**

Ventricular hypertrophies are commonly detected by assessing the deviation of mean QRS axis of the heart and by observing the amplitude of R wave in ECG (Fig. 88.15). In right ventricular hypertrophy, mean QRS vector deviates to right, and R wave is high in amplitude (about 25 mm more than normal) in V1 lead. In left ventricular hypertrophy, mean QRS vector deviates to left, and R wave is high in amplitude (about 5 mm more than normal) in V5 and V6 leads.

**Myocarditis and Cardiomyopathies**

ECG changes are nonspecific in myocarditis and cardiomyopathies.

**Effects of Electrolyte Disturbances**

Alteration in plasma concentration of K⁺, Ca++, and Na⁺ usually affects cardiac functions.
Section 9: Cardiovascular System

Alteration in Plasma K$^+$
The ECG findings depend upon the concentration of plasma K$^+$.

**Hyperkalemia**
Plasma K$^+$ at about 7 meq/L: Tall and peaked T wave (Fig. 88.16A).
Plasma K$^+$ at about 8.5 meq/L: Broadening and slurring of QRS complex, and Tall and slender T wave.
Plasma K$^+$ more than 9 meq/L: Ventricular tachycardia and ventricular fibrillation.

**Hypokalemia**
Plasma K$^+$ at about 3 meq/L: ST segment depression and appearance of prominent U wave immediately following the T wave (Fig. 88.16B).
Plasma K$^+$ at about 2 meq/L: Prolongation of PR interval, ST segment depression, T wave inversion and prominent U wave.

**Alteration in Plasma Ca$^{++}$**
**Hypercalcemia:** ECG usually remains normal, though hypercalcemia increases the force of contraction and in excess causes calcium rigor. Rarely, QT interval is shortened.

**Hypocalcemia:** Prolongation of ST segment that causes lengthening of QT interval.

**Alteration in Plasma Na$^+$**
**Hypernatremia:** ECG changes are usually normal except that high-voltage ECG complexes appear.
**Hyponatremia:** Low-voltage ECG complexes appear.

**HIS BUNDLE ELECTROGRAM**
Recording of electrical activities of His bundle is called **His bundle electrogram (HBE)**. HBE is compared with ECG of standard limb leads, especially of lead II to determine few electrocardiographic intervals that help in better diagnosis of heart blocks. For this purpose, a catheter containing an electrode at its tip is inserted through an arm vein into the right atrium close to the tricuspid valve. The electrode records HBE.

HBE has the following deflections:
1. **A deflection**: Represents activation of AV node.
2. **H deflection**: Represents transmission of impulse through His bundle.
3. **V deflection**: Represents ventricular depolarization.
**HBE Intervals**

Simultaneously, lead II ECG is recorded on the same graph below the HBE on the same time scale. The following three intervals are obtained (Fig. 88.17).

**PA Interval**

This is the interval starting from the beginning of P wave in lead II ECG to the downward deflection of A wave of HBE. This represents the time of conduction from SA node to AV node. Normally, PA interval is 27 ms.

**AH Interval**

This is the interval starting from A wave to the H spike of HBE. This represents the time of conduction of impulse through AV node. Normally, AH interval is 92 ms.

**HV Interval**

This is the interval starting from H spike of HBE to the start of QRS complex in lead II ECG. This should better be designated as HQ interval. This represents the time of conduction of impulse through His bundle and bundle branches. Normally, HV interval is 43 ms.

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**CHAPTER SUMMARY**

**Key Concepts**

1. The waves and intervals of ECG represent sum of various electrical activities in the heart at the particular moment.
2. ECG is very useful in the diagnosis of arrhythmias, myocardial ischemia, heart blocks, and electrolyte imbalance.
3. Being a noninvasive procedure that does not require much expense, ECG is a widely practiced investigation.

**Important to Know (Must Read)**

1. In examination, ‘With the help of a labeled diagram of lead II ECG, describe the various waves and intervals of ECG and their physiological basis’ may be asked as a Long Question.
2. Mean QRS axis, Conduction blocks, Sinus arrhythmia, Ventricular arrhythmias, His bundle electrogram are asked as Short Questions in exam.
3. In Viva, examiner may ask… Classify ECG leads, What is Einthoven triangle, What are the connections of different bipolar limb leads, What are the connections of different unipolar limb leads, What is an augmented lead, What are the connections of different unipolar chest leads, What are the esophageal leads, What are the waves of ECG and what do they signify, What are the intervals of ECG and what do they signify, What are the segments of ECG and what do they signify, What are the causes of tachycardia, What are the causes of bradycardia, Define mean QRS axis, give its normal value, What are the factors that affect mean QRS axis, What are the causes of right axis deviation, What are the causes of left axis deviation, What are the types of conduction blocks, What is the feature of first-degree heart block, What are the types of second-degree heart block, What are the features of Mobitz type I, What are the features of Mobitz type II, What are the feature of third-degree heart block, What is Stokes-Adams syndrome, What are the mechanisms of sinus arrhythmia, What is sick sinus syndrome, What is an atrial premature beat, What are the types of paroxysmal supraventricular tachycardia (PST), What is nodal PST, in what condition is it seen, What is atrial flutter, What is atrial fibrillation, What is ventricular extrasystole, What is paroxysmal ventricular tachycardia (PVT), What is ventricular fibrillation, What is ventricular flutter, What are the features of Wolff-Parkinson-White syndrome, What are the features of Lown-Ganong-Levine syndrome, What are the ECG changes in myocardial ischemia, What are the ECG changes in myocardial infarction, What are the physiological bases of ECG changes in myocardial infarction, What are the ECG changes in ventricular hypertrophy, What are the ECG changes in hyperkalemia, What are the ECG changes in hypokalemia, What are the ECG changes in hypertrophy, What are the ECG changes in hypercalcaemia, What are the ECG changes in hypocalcaemia, What are the ECG changes in hypernatremia, What are the ECG changes in hyponatremia, What are the deflections and intervals of His bundle electrocardiogram.
Cardiac cycle is defined as the sequence of electrical and mechanical events occurring in heart during a single beat. The resulting changes in volume, pressure, and flow in different chambers and the electrical activities that are recorded in the form of ECG are precisely repeated in each beat. The similar electromechanical events occur almost simultaneously in both left and right sides of the heart. However, the major difference is the pressure, which is significantly high on the left side, especially in the left ventricle and the aorta. Though the nature of changes is similar on both sides, usually for better understanding and presentation, discussion on cardiac cycle is performed for the activities in left side of the heart that includes left atrium, left ventricle, aorta and left jugular vein.

### PHASES OF CARDIAC CYCLE

Cardiac cycle consists of the atrial systole and diastole, and the ventricular systole and diastole. The atrial diastole is merged in ventricular systole. Therefore, events of cardiac cycle are described in three phases: atrial systole, ventricular systole, and ventricular diastole.

### Atrial Systole

The onset of atrial systole coincides with the peak of P wave in the ECG. During this period, blood from atria enters the ventricle due to atrial contraction that completes ventricular filling:

1. However, at rest, atrial systole is not essential for effective ventricular filling as it contributes to less than 20% of the filling of the ventricle. Thus, in normal conditions, more than 80% of ventricular filling occurs passively. Therefore, ventricular volume curve rises slightly during this phase (Fig. 89.1).
2. However, when there is significant tachycardia, the ventricular filling contributed by atrial contraction assumes great importance as the duration of ventricular diastole is reduced (Note: In tachycardia, decreased cardiac cycle length occurs mainly at the reduction of the duration of diastole).
3. As passive ventricular filling is reduced due to decreased duration of diastole, atrial systole contributes significantly to the filling in such conditions.
4. Also, when increased cardiac output is required to meet the increased need for blood supply to the tissues of the
body as happens during exercise, contribution by atrial systole assumes importance. In exercise, increased sympathetic activity boosts atrial pump activity, and increases the contribution by atrial systole to ventricular filling by 30–40%. In such conditions, patients with atrial fibrillation fail to increase their cardiac output adequately to meet the demand of the body.

Major events in atrial systole are:
1. Atrial systole begins from the peak of P wave and ends with the peak of QRS complex as noted in the ECG tracing.
2. Ventricular volume increases mildly.
3. Atrial pressure rises due to atrial contraction that causes genesis of ‘a’ wave in the atrial or jugular-venous pressure (JVP) curve.
4. Atrial pressure exceeds the ventricular pressure.
5. Fourth heart sound is recorded in phonocardiogram. This is produced by inrush of blood into ventricle.
6. Mitral valve closes at the end of atrial systole when ventricular pressure exceeds atrial pressure.

Atrial Diastole
Atrial diastole follows atrial systole during which atrial filling occurs.
1. Atrial diastole occurs during ventricular systole.
2. At the beginning of ventricular systole, bulging of atrioventricular valve into the atrium results in increase in atrial pressure that produces ‘c’ wave in JVP.
3. Venous return increases atrial volume and increases atrial pressure. This produces ‘v’ wave in JVP (discussed below).

Ventricular Systole
The phase of ventricular systole is divided into two parts: the phase of isovolumetric contraction, and the phase of ejection.

Phase of Isovolumetric Contraction
Appearance of QRS complex in ECG heralds the onset of systole. This phase starts with the closure of mitral valve and ends with the opening of aortic valve (Fig. 89.2). Thus, ventricular volume the remains same throughout this phase. Inspite of forceful contraction, ventricular size (the muscle length) apparently remains the same (isometric type of contraction). Therefore, this phase is called isovolumetric contraction phase.

Major events in this phase are:
1. This phase starts from the peak of QRS complex in ECG.
2. First heart sound appears in PCG (Fig. 89.1).
3. The ventricular pressure rises abruptly from 0 to 80 mm Hg in left ventricle and to 8 mm Hg in right ventricle. The valves of the heart remain closed; therefore, rise in ventricular pressure occurs rapidly.
4. Ventricular volume does not change and aortic blood flow is nil.
5. This phase ends with the opening of the semilunar valves when ventricular pressure exceeds aortic pressure.

Phase of Ventricular Ejection
Opening of the aortic valve marks the onset of the phase of ejection. This phase is further divided into two parts:
the phase of rapid ejection (lesser duration), and the phase of reduced ejection (longer duration). The phase of rapid ejection is differentiated from the phase of reduced ejection by two important features:

1. Quick increase in ventricular and aortic pressures, and
2. Greater decrease in ventricular volume that occurs due to abrupt increase in aortic blood flow (ventricular output).

**Rapid Ejection Phase**

During isovolumetric contraction phase, enough tension builds up in ventricular wall. Therefore, with opening of aortic valve, aortic blood flow increases abruptly and the blood is ejected forcefully into the aorta from the left ventricle. Similarly, blood ejected from right ventricle sharply increases the flow in the pulmonary trunk.

1. The ventricular pressure continues to rise sharply.
2. Aortic pressure also increases sharply, but it remains below the ventricular pressure almost throughout rapid ejection phase.
3. A large amount (about two-thirds) of ventricular volume is emptied rapidly in this phase (hence the name rapid ejection phase) that causes a steep fall in ventricular volume curve.
4. The atrial pressure decreases sharply at the beginning of rapid ejection phase due to descent of the base of the heart and stretch of the atria that occur due to strong ventricular contraction. However, atrial pressure rises slowly after the initial fall due to venous return.
5. The ‘c’ wave is produced in the jugular venous pressure curve in the early part of rapid ejection due to impact of common carotid artery with the adjacent jugular vein and also due to bulging of closed tricuspid valve into atrial chamber.

**Important events in this phase are:**

1. This phase starts with opening of aortic valve.
2. Steep increase in aortic blood flow.
3. Steep fall in ventricular volume.
4. Ventricular pressure increases further.
5. Aortic pressure also increases but remains just below the ventricular pressure.
6. Atrial pressure falls in the early part, but slowly increases thereafter.
7. The ‘c’ wave is produced in JVP.
8. This phase corresponds to ST segment in the ECG.

**Reduced Ejection Phase**

This is the longer phase of ventricular systole during which both ventricular and aortic pressures decrease.

1. Aortic pressure declines due to run-off of blood from the aorta to the periphery that exceeds ventricular output. The aortic pressure exceeds the left ventricular pressure in this phase.
2. In fact, reduced ejection phase begins at about the time when the aortic pressure slowly exceeds the left ventricular pressure. Stored potential energy in the stretched arterial wall contributes to the alteration in ventricular-aortic pressure gradient.
3. Inspite of this change in pressure gradient, blood flow continues from ventricle into the aorta due to the momentum of forward flow of blood caused during the rapid ejection phase. This keeps the aortic valve open.
4. However, the aortic flow is significantly reduced in this phase (hence, the name reduced ejection phase).
5. Ventricular volume continues to decrease and reaches the end-systolic volume at the end of this phase.

**Important events in this phase are:**
1. Ventricular and aortic pressures decrease, but aortic pressure exceeds ventricular pressure.
2. Aortic blood flow is greatly decreased.
3. Ventricular volume is further decreased.
4. Atrial pressure continues to increase slowly due to blood returning to atria (atria relax throughout the ventricular systole).
5. ‘T’ wave in ECG appears in this phase.
6. The phase ends with closure of aortic valve.

### Ventricular Diastole

Ventricular diastole has two phases: the phase of isovolumetric relaxation and the phase of ventricular filling. Ventricular filling phase is subdivided into the phases of rapid filling and reduced filling.

#### Phase of Isovolumetric Relaxation

Ventricle starts relaxing in this phase that results in steep fall in left ventricular pressure. During this phase, no change occurs in ventricular volume as this period is placed between the closure of aortic valve and the opening of mitral valves (see Fig. 89.2). Therefore, this phase is known as isovolumic relaxation phase.
1. This is also called isovolumetric relaxation phase, as apparently there is no change in ventricular muscle length.
2. Due to closure of the aortic valve, a characteristic notch is produced on the descending limb of the aortic pressure curve at the beginning of this phase.
3. Vibrations set up by the closure of aortic valve produce the second heart sounds in the phonocardiogram tracing. It terminates with the opening of mitral valve.

**Important events in this phase are:**
1. Rapid fall in ventricular pressure.
2. No change in ventricular volume.
3. Aortic blood flow is almost nil.
4. Second heart sound appears in the first half of this phase.
5. Atrial pressure continues to rise, and the peak of ‘v’ wave of JVP appears towards the end of this phase.
6. This phase ends with the opening of mitral valve.

#### Phase of Ventricular Filling

This phase is further divided into rapid filling phase and reduced filling phase (diastasis).

### Rapid Filling Phase

As soon as the left ventricular pressure falls below the left atrial pressure, mitral valve opens. The blood that accumulates in atria during the ventricular systole rapidly enters into the ventricles as soon as the atrioventricular valves open.

1. The major part of the ventricular filling occurs rapidly in this phase (hence the name rapid filling phase) that increases ventricular volume to a greater extent.
2. Inspite of rapid filling, ventricular pressure continues to fall due to ventricular relaxation.
3. The rapid flow of blood from left atrium into the left ventricle decreases left atrial pressure (Fig. 89.1).
4. Vibrations set up due to this rapid inrush of blood into the ventricle produces third heart sound, which is recorded in the early part of this phase in the phonocardiogram.

**Important events in this phase are:**
1. This phase starts with the opening of mitral valve, which corresponds to the peak of ‘v’ wave in JVP.
2. Rapid increase in ventricular volume.
3. Decrease in ventricular and atrial pressure (atrial pressure is just above the ventricular pressure).
4. Third heart sound appears in PCG in the initial part.

### Diastasis

This is the phase of slow filling of the ventricle. During this phase, as lesser filling occurs and filling occurs slowly, ventricular volume curve rises slowly.

1. Ventricular pressure and atrial pressure almost remain unchanged.
2. It should be noted that (as discussed above) normally the greater part of ventricular filling occurs passively in both rapid and slow filling phase, and about 20% filling occurs actively (by atrial contraction during atrial systole).
3. Thus, atrial systole only tops up the ventricular volume.

**Main events in this phase are:**
1. Slow rise in ventricular volume.
2. No change in left atrial, left ventricular and aortic pressures.
3. In ECG, ‘P’ wave begins to appear towards the end of this phase.

### Pressure–Volume Relationship

The pressure-volume relationship of the left ventricle plotted in a graphical form (Fig. 89.3) forms the pressure-volume loop.

1. Diastolic filling of ventricle starts at ‘A’ as mitral valve opens and terminates at ‘B’ as mitral valve closes. During this period, ventricular volume increases greatly without much change in pressure as this occurs during the relaxation phase of the ventricle (A–B).
2. With the onset of isovolumetric contraction (B–C), ventricular pressure rises steeply without change in volume.
3. Aortic valve opens at point C that marks the onset of ejection of the ventricle.
4. The ventricular volume decreases, but ventricular pressure continues to rise, which reaches a peak at point D (the rapid ejection phase ends) and then decreases till point E (the end of slow ejection phase).
5. Then starts the isovolumetric relaxation phase, during which pressure falls abruptly (E–A) to meet the point A, which marks the opening of the mitral valve.
6. This completes one cardiac cycle. Note the position of diastolic pressure-volume and isovolumic pressure-volume curve in the normal condition.

**Effects of Ventricular Dysfunctions on Pressure-Volume Loop**

Ventricular dysfunction may be **systolic** or **diastolic**.

**Systolic Dysfunction**

Systolic dysfunction is the inability of the ventricle to contract. In this condition, ventricular output is decreased due to impaired myocardial contractility. The isovolumetric pressure-volume curve shifts to right that decreases the stroke volume from C–E to c–e (Fig. 89.4).

**Diastolic Dysfunction**

Diastolic dysfunction is the inability of the ventricle to relax. In this condition, ventricular output is decreased due to impaired end-diastolic volume. The diastolic pressure-volume curve shifts upward and to the left from A–B to a’–b’ (Fig. 89.5). This decreases the end-diastolic volume that results in decreased stroke volume.

**Duration of Cardiac Cycle**

The duration of cardiac cycle is 0.8 s when heart rate is 75/min. The duration of systole is 0.3 s and diastole is about 0.5 s (Fig. 89.6).
1. When **heart rate is increased** to about 200/min, duration of cardiac cycle is 0.3 s with the systole about 0.16 s and diastole about 0.14 s. The shortening of duration of systole is proportionately very less than the duration of diastole (in fact, systole is more than diastole).
2. When **heart rate decreases** to about 40/min, duration of cardiac cycle is 1.5 s with the systole about 0.37 s and diastole about 1.13 s. The prolongation of systole is proportionately very less than the prolongation of diastole (in fact, systole is less than one-fourth of diastole).
3. This indicates that the change in length of cardiac cycle occurs mainly by changing the duration of diastole.
4. Therefore, in tachycardia, ventricular filling is greatly compromised due to decreased duration of diastole.
Chapter 89: Cardiac Cycle

JUGULAR VENOUS PULSE

Pressure change in atrium is directly reflected in internal jugular veins (Jugular Venous Pulse; JVP). Therefore, JVP in the right side of the neck is examined clinically to assess the atrial pressure changes.

Waves of JVP

Jugular venous pulse (JVP) has five waves: three positive waves and two negative waves (descents). The positive waves are a, c, and v waves, and two descents are x and y descents (Fig. 89.7).

- **a wave:** This is due to atrial contraction.
- **c wave:** This wave coincides with the onset of ventricular systole and results from the bulging of tricuspid valve ring into the right atrium as the right ventricular pressure rises.
- **v wave:** It indicates the passive rise in pressure in the right atrium as venous return continues while the tricuspid valve remains closed.
- **x descent:** It is caused by a fall of right atrial pressure due to relaxation of the right atrium.
- **y descent:** It is due to fall in right atrial pressure when blood enters into the right ventricle as tricuspid valve opens.

Conditions that raise JVP

1. Right-side heart failure
2. Obstruction of superior vena cava
3. Increase in circulating blood volume:
   - Pregnancy
   - Acute nephritis
   - Overenthusiastic treatment with IV fluids
4. Congestive heart failure
5. Constrictive pericarditis
6. Tricuspid incompetence

Note: Persistent elevation of JVP is one of the earliest signs of cardiac failure and it is probably the most reliable sign of heart failure.

Prominent 'a' Wave

1. Pulmonary stenosis
2. Pulmonary hypertension
3. Tricuspid stenosis (if atrial fibrillation is associated with it, a wave may not be seen).
4. Myxoma of right atrium
5. Distended right atrium in atrial septal defect
6. Cardiomyopathy

Physiological basis: Prominent ‘a’ wave occurs due to increased force of right atrial contraction associated with right atrial hypertrophy or hypertrophy of right ventricle. When right atrium contracts against increased resistance, prominent ‘a’ wave occurs.

Cannon Wave

When amplitude of ‘a’ wave is abnormally big, it is called giant ‘a’ wave or cannon wave (Fig. 89.8). It occurs when right atrium contracts against a closed tricuspid valve, which is seen in:
1. Complete heart block when atrial and ventricular systoles coincide.
2. Nodal rhythm when the atrium and ventricle are activated simultaneously.

Absence of ‘a’ Wave

The ‘a’ wave disappears in atrial fibrillation.

Prominent ‘v’ Wave

It is seen in tricuspid regurgitation because when ventricle contracts during systole, blood enters into right atrium through the incompetent tricuspid valve.
Section 9: Cardiovascular System

Scientist contributed

James Mackenzie (1853–1925), British cardiologist, who had worked extensively on cardiac arrhythmias, had also pioneered in the study of circulation. For the first time, Mackenzie gave a clear idea of the cardiac cycle and correlated arterial and venous pulses with the events of cardiac cycle.

HEART SOUNDS

Four heart sounds have been described. These are first heart sound (S1), second heart sound (S2), third heart sound (S3), and fourth heart sound (S4). S1 and S2 are heard normally.

First Heart Sound

First heart sound represents the beginning of systole.

Causes

S1 occurs due to vibration set up by:
1. Sudden closure of the AV valves.
2. Rapid increase in tension in the ventricular muscles during isometric contraction acting on filled ventricles.
3. Turbulence created in the blood due to ventricular contraction.

Characters

Duration: about 0.15 second
Frequency: 25 to 45 Hz
It is a soft sound heard as ‘lub’.

Significance

It signifies the beginning of ventricular systole and AV valve closure.

a. Accentuation of first heart sound occurs in:
   – Exercise
   – Hyperkinetic circulatory states like anemia, beriberi
   – Hypertension
b. Diminution of first heart sound occurs in:
   – Shock
   – Acute myocardial infarction
   – Constrictive pericarditis
   – Pericardial effusion
   – Cardiomyopathy (in the advanced stage)
   – Obesity
   – Emphysema

Splitting of S1

First heart sound has two components: the mitral and the tricuspid components.
1. The mitral valve closes slightly before the tricuspid valve. This gives rise to splitting of the first heart sound.
2. But this splitting cannot be detected by auscultation, because both the components are very low-pitched and merge into each other.
3. Therefore, splitting of the first heart sound is always considered as pathological.

Second Heart Sound

Causes

1. This occurs primarily due to closure of semilunar valves.
2. Rush of blood into the ventricles due to opening of the AV valves contributes.

Character

Duration: about 0.12 second
Frequency: 50 Hz
This is heard as ‘dup’.

Significance

Second heart sound signifies the end of clinical systole and closure of semilunar valves.

a. Loud A2 (aortic component) occurs in:
   – Systemic hypertension
   – Aortic dilatation
b. Diminished A2 occurs in:
   – Aortic stenosis
   – Aortic incompetence
c. Loud P2 (pulmonary component) occurs in:
   – Pulmonary hypertension
   – Pulmonary artery dilation
d. Diminished P2 occurs in:
   – Pulmonary stenosis

Splitting of S2

Splitting of the second sound is due to the gap between the aortic and pulmonary components. It is easy to detect because sounds of aortic and pulmonary valve closure
are high-pitched and can be separated. Splitting is most easily heard in children and may not be audible in elderly subjects.

**Mechanism of Splitting**
The splitting of the second heart sound is due to the separation between the closure of aortic and pulmonary valves.

1. The closure of pulmonary valve always follows the closure of aortic valve (aortic valve closes first). The splitting is distinctly heard during inspiration.

2. During inspiration, more blood is drawn into the thorax. Therefore, venous return to right atrium increases and right ventricular stroke volume increases. This increases the duration of right ventricular systole. Thus, P2 is slightly delayed.

3. Also, during inspiration, left ventricular stroke volume decreases, because blood is pooled in the dilated pulmonary vessels and dilated left atrium, which occurs due to increased negative intrathoracic pressure. Therefore, left ventricular systole is shortened and A2 arrives earlier.

4. Thus, during inspiration, A2 occurs earlier and P2 occurs later. Hence, splitting of the second sound widens during inspiration.

5. Opposite mechanisms operate during expiration and splitting narrows.

**Reverse Splitting**
When pulmonary valve closes earlier to aortic valve closure, the condition is called reverse splitting. This occurs when the left ventricle takes more time to empty than the right ventricle. It is seen in left bundle branch block (LBBB) and in left ventricular failure.

**Third Heart Sound**
Third heart sound is usually not heard, though it is always prominently detected in phonocardiogram. Sometimes, it may be heard in children and in young adults. It is usually heard in conditions in which the circulation becomes hyperkinetic. A third sound can arise from either side of the heart, but usually, it arises in the left ventricle.

**Causes**
1. It is caused by the vibration set-up in the ventricle during the early period of rapid ventricular filling.

2. Rebound fencing of the cusp of the valve and chordae of the respective valve due to vigorous elongation of the ventricle caused by rapid inflow of blood.

**Character**
- Duration: 0.1 second
- Pitch: low-pitched
- It is best heard in the mitral area.

Significance
1. It is attributed to rapid ventricular filling and is found in relatively hyperkinetic circulation in young persons. It is heard in diseases in which the mitral diastolic flow is increased as occurs in mitral regurgitation and ventricular septal defect.

2. It is an important sign of heart failure due to any cause. In heart failure, the atrial pressure is increased and the early filling of the ventricle is rapid.

3. It may be heard shortly after myocardial infarction or in diseases where the distensibility of the ventricular muscle is altered. The sound arises from vibrations in the atroventricular valve structures and in the ventricular muscle.

**Fourth Heart Sound**
This is also called atrial sound, because it is produced during atrial contraction. It is never heard in normal individuals. Presence of the fourth heart sound is always considered as abnormal.

**Causes**
1. It is caused by atrial contraction.

2. It is produced by the vibration set up within the ventricle due to inflow of blood produced by atrial systole.

**Character**
1. Low-pitched

2. Occurs just before the first sound, i.e. late in the diastole.

Significance
1. It always indicates an increased stiffness or non-compliance of the ventricles. Therefore, when the bolus of blood is delivered into the ventricle by atrial contraction, it facilitates a sudden increase in ventricular pressure.

2. It is seen in left ventricular hypertrophy due to hypertension, myocardial infarction, pulmonary embolism, and pulmonary hypertension.

**Triple Heart Sound**
This consists of three heart sounds: the first and second heart sounds, and the third sound can be either the third or fourth heart sound.

1. The triple rhythm associated with the normal heart rate may not be a serious one, but if it is present with a definite cardiac pathology, it may signify the seriousness of the condition.

2. When the heart rate increases to more than 100 per minute, the triple rhythm is called gallop rhythm, because it produces a typical cadence of the gallop of a horse.
3. The individual sounds cannot be identified separately. If the gallop is due to the third heart sound, it is called protodiastolic gallop; or if it is due to the fourth heart sound, it is called presystolic gallop.

**Murmurs**

Murmur occurs due to turbulence in the blood flow at or near a valve, or an abnormal communication within the heart. Murmurs differ from the heart sounds in the sense that these are of longer duration and higher frequency, whereas heart sounds have shorter duration and lower frequency. When a murmur is present, the following points are carefully noted.

**Site of Origin:** The area over which murmur is maximally heard is noted. The point of maximal intensity usually (but not always) indicates its site of origin.

**Timing and duration:** Depending on the timing of murmur, murmurs are classified into systolic, diastolic, or continuous. Depending on the duration, it may be early diastolic, mid-diastolic, early systolic, pan-systolic, etc.

**Character:** The murmur may be soft-blowing to harsh, rough, and rumbling. Loud and rough murmurs are usually associated with organic valvular and congenital lesions.

**Radiation (Conduction):** From the site of maximum intensity, auscultation is performed in different directions to detect whether the murmur is localized or conducted to other parts. Conduction is characteristic of some murmurs, e.g., the murmur of mitral stenosis is usually localized whereas the murmur of mitral incompetence selectively propagates towards the axilla.

**Relation with respiration:** During inspiration, the stroke volume of the right ventricle increases while that of the left ventricle decreases. Therefore, any murmur becoming louder during inspiration is considered to originate from the right ventricle, and any murmur increasing during expiration is attributed to originate from the left side of the heart.

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**CHAPTER SUMMARY**

**Key Concepts**

1. In alteration in heart rate, cardiac cycle length alters at the expense of diastole. Therefore, in tachycardia, C.O. decreased due to decrease in ventricular filling.

2. Atrial filling occurs mostly passively. But in tachycardia, atrial systole contributes significantly.

3. In diastolic dysfunction, ventricular P–V curve shifts to left, indicating decrease in ventricular volume and pressure.

**Important to Know (Must Read)**

1. In examination, ‘Depicting the aortic pressure, left ventricular pressure, left atrial pressure, left ventricular volume, JVP, ECG and phonocardiogram on the same time scale, describe various elecromechanical events in the different phases of cardiac cycle’ is usually asked as Long Question in exam.

2. Atrial systole, ventricular systole, Ventricular diastole, Jugular venous pulse, Heart sounds, Pressure-volume relationship of the left ventricle are asked as Short Questions in exam.

3. In Viva, examiner may ask… Define cardiac cycle, Name the phases of cardiac cycle, What are the major events of atrial systole, List the phases of ventricular systole, List the major events of isovolumetric ventricular contraction, What are the major events of rapid ventricular ejection, What are the major events of reduced ventricular ejection, What are the phases of ventricular diastole, What are the major events of isovolumetric ventricular relaxation, What are the major events of rapid ventricular filling phase, What are the major events of slow ventricular filling phase, What are the phases of the pressure-volume loop of the left ventricle, What is the effect of systolic dysfunction on pressure-volume loop of the left ventricle, What are the waves of JVP and what is their significance, Name the conditions of raised JVP, Name the conditions of prominent ‘a’ wave, In which condition is the ‘a’ wave absent, In which condition is the ‘v’ wave prominent, What are the heart sounds, What are the causes of first heart sound, What are the characters of first heart sound, In which conditions is the first heart sound accentuated, In which conditions is the first heart sound diminished, What is splitting of the first heart sound, What are the causes of second heart sound, What are the characters of second heart sound, In which conditions is the second heart sound accentuated, In which conditions is the second heart sound diminished, What is splitting of the second heart sound, What are the causes of third heart sound, What are the characters of third heart sound, What are the significances of third heart sound, What are the causes of fourth heart sound, What are the characters of fourth heart sound, What are the significances of fourth heart sound, What is a triple heart sound, What is a gallop rhythm, What is the cause of a murmur, What are the points should be noted for a murmur.
The primary function of heart is to pump blood. The output of the heart maintains circulation, which is essential for supplying oxygen and nutrients to the tissues of the body. Cardiac output, the output of each ventricle of heart per minute provides vital information about the condition of the heart of the patient to the physician. Therefore, cardiac output assessment is a routine procedure in the assessment of cardiac health of a patient in medicine and cardiology clinic and in intensive care units. Now-a-days cardiac output measurement is performed routinely by echocardiography.

**Scientist contributed**

Adolph Eugen Fick (1829–1901), German physiologist and biophysicist, in 1855, introduced Fick's law of diffusion, which governs the diffusion of a gas across a fluid membrane. In 1870, he was the first to measure cardiac output, using what is now called the Fick principle. Fick investigated the application of physical principles to physiology and devised methods for cardiac output measurement and blood flow to many organs. His work led to the development of the direct Fick method for measuring cardiac output.

**Normal value:** Normal stroke volume is about 70 mL. So, if heart rate is 70 per minute, the cardiac output is 70 mL $\times$ 70 = 4900 mL (5 lit/min approximately).

**Cardiac Index (CI)**

Definition: Cardiac output divided by body surface area in square meter is the cardiac index.

\[
CI = \frac{\text{Cardiac output at rest}}{\text{Body surface area}}
\]

(Considering body surface area about 1.7 sq. meter, and cardiac output as 5 L/min of an adult male)

\[
= 3 \text{ L/min/m}^2 \text{ (approximately)}
\]
Normal Value: The normal average cardiac index is 3.2 L. Body surface area is calculated by using Dubois formula. Cardiac index is used to standardize cardiac output among individuals of different body sizes.

End-diastolic Volume
Definition: The volume of blood remaining in each ventricle at the end of diastole is end-diastolic volume (EDV).
Normal value: 130 mL

Ejection Fraction
Definition: The percentage of end-diastolic volume ejected with each beat is the ejection fraction (EF). It is the percentage-ratio of stroke volume to end-diastolic volume, i.e. \( \text{EF} = \frac{SV}{EDV} \times 100 \).
Normal value: Normally EF is about 65%. EF is a good index of myocardial performance.

End-systolic Volume
Definition: The volume of blood remaining in each ventricle at the end of systole is end-systolic volume (ESV).
Normal value: It is about 50 mL.

Cardiac Reserve
Definition: It is the amount of blood that can be pumped by each ventricle in excess of normal cardiac output.
Normal value: 15–25 lit/min in non-athletes and 20 to 40 lit/min in trained athletes.

Physiological Conditions that Alter Cardiac Output
A. Conditions that increase cardiac output
1. Exercise
2. Anxiety
3. Emotion and excitement
4. Increased environmental temperature
5. After eating
6. Pregnancy
B. Conditions that decrease cardiac output
1. Standing from lying posture
2. Excessive sweating
C. Conditions that do not change cardiac output
1. Sleep
2. Mild to moderate change in environmental temperature

Age
Cardiac output is less in elderly. This occurs due to decrease in heart rate and stroke volume in aged individuals. In children, though the heart rate is more, cardiac output is less due to less stroke volume.

Gender
Cardiac output is about 10% less in females.

Exercise
Cardiac output increases in exercise.
1. In exercise, sympathetic stimulation increases heart rate and myocardial contractility that increase cardiac output.
2. Also, venoconstriction due to sympathetic stimulation increases venous return and EDV, which in turn increase cardiac output.
3. In exercise, cardiac output can be increased up to 700%.

Excitement
Cardiac output increases in emotional excitement due to sympathetic stimulation.

Pregnancy
Cardiac output increases in pregnancy due to increase in blood volume that mainly occurs due to expansion of ECF volume.

After Eating
Cardiac output increases after eating due to increased metabolism.

Posture
Assuming standing posture from lying position decreases cardiac output due to accumulation of blood in veins of lower extremities caused by effect of gravity.

Environmental Temperature
Increased environmental temperature increases cardiac output by two mechanisms:
1. First, by activating sympathetic system, and
2. Second, by increasing body temperature.
3. Decreased temperature has the opposite effect.
   However, mild to moderate change in temperature does not affect cardiac output. Cardiac output changes only in extreme temperatures.

MEASUREMENT OF CARDIAC OUTPUT
Cardiac output can be measured by direct and indirect methods.

Direct Methods
Cardiac output can be measured directly by placing an electromagnetic flow meter in the ascending aorta or by using a cardiometer. These are accurate methods of measuring cardiac output. However, these methods are applicable only in experimental animals or in humans (in patients) undergoing open thoracic surgery. In humans, cardiac output is usually determined by using Doppler combined with echocardiography.
Indirect Methods

1. Fick method
2. Indicator dilution method
3. Thermodilution method
4. Ballistocardiography
5. Echocardiography
6. X-ray method
7. Pulse-pressure method

Fick Method

Definition

Fick principle is defined as the amount of a substance taken up by an organ or by the whole body per unit of time is equal to the arteriovenous difference of the substance times blood flow.

Procedure

Cardiac output can be measured by measuring the amount of oxygen consumed by the body in a given period and dividing this value by the arteriovenous difference of oxygen across the lungs.

1. The oxygen consumption of the body is measured by spirometry.
2. As the arterial content of oxygen is same in all parts of the body, for measuring oxygen content of the arterial blood, the blood is obtained from any peripheral artery.
3. The venous blood is collected from the pulmonary artery by placing a catheter into it through the heart.

The cardiac output is calculated as:

\[
\text{Output of left ventricle} = \frac{O_2 \text{ consumption (mL/min)}}{A_0 \text{ }_2 - V_0 \text{ }_2}
\]

\[A_0 \text{ }_2: \text{ Oxygen content of arterial blood, which is 200 mL/L}\]
\[V_0 \text{ }_2: \text{ Oxygen content of venous blood, which is 150 mL/L}\]
\[= \frac{250 \text{ mL/min}}{200 \text{ mL} - 150 \text{ mL}}\]
\[= \frac{250 \text{ mL/min}}{50 \text{ mL}}\]
\[= 5 \text{ L/min}\]

Advantages

1. Result is accurate.
2. No chemical is injected.

Disadvantages

1. Catheterization should be done by expert hand.
2. Hospitalization is required for catheterization.
3. Patient may be apprehensive of catheterization that increases cardiac output.
4. Simultaneous measurement of oxygen consumption makes the process difficult.

5. It is difficult to measure cardiac output by this method in ambulatory patients and during exercise.

Indicator Dilution Method

Principle

In this method, a known amount of an indicator (a dye or a radioactive isotope) is injected into circulation usually through an arm vein and the concentration of the indicator is measured in serial samples of the arterial blood. The output of the heart is equal to the amount of indicator injected divided by its average concentration in arterial blood after a single circulation through the heart.

Procedure

This method is popularly known as Hamilton's dye dilution method (as described by Hamilton).

1. The dye injected is usually the Evans' blue or indocyanine green.
2. Before injection of the dye, 10 ml of peripheral venous blood is withdrawn and divided equally into two samples. In one sample of 5 ml, enough quantity of the dye is injected to give a concentration of 0.5 mg/100 mL (used as standard).
3. The other sample is used as blank.
4. One ml of the dye solution containing 5 mg is injected rapidly into the basilic vein.
5. From a limb artery, the blood samples are collected at an interval of 2s in serial tubes.
6. The tubes are then centrifuged together with the standard and blank tubes, following which the concentration of dye is determined photo colorimetrically.
7. The concentration of the successive samples is plotted on a semi-log paper. The resulting concentration of dye in the arterial blood changes with time.
   - First, the concentration rises as the indicator carried by the fast moving blood reaches the arterial sampling point;
   - Second, reaches a peak as majority of indicator substance arrives at the sampling point; and
   - Finally, the concentration falls as indicator carried by slow moving blood arrives at the point.
8. Thus, the result obtained gives a curve with an ascending limb, a peak, and a descending limb. But, the descending limb ends with a rise (Fig. 90.1).
9. The slope of descending limb is extrapolated to the abscissa. The point on the time scale at which it touches the abscissa, gives the time of first passage of the dye through the artery (t).

Let us say the time of first passage of the dye is 36s.

Cardiac output is then calculated from the following formula:

\[
F = \frac{1}{c \times t}
\]
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Advantages
1. Saline is harmless
2. Cold is dissipated, so recirculation is not a problem
3. Can be repeated many times, if needed
4. Usually preferred for children as saline is nontoxic
5. Useful in severely sick patients (serious patients in intensive care units).

Disadvantages
Cardiac catheterization is required.

Other Methods

**Ballistocardiography**
In this method, the vibrations generated by each heart-beat are received and converted into waveforms by a transducer that records the cardiac activities on a ink recorder. From the recording, cardiac output is calculated by using a special formula by analyzing the recorded waves. However, cardiac output measured by this method is not an accurate one.

**Echocardiography**
This is a noninvasive technique in which ultrasonic waves emitted from a transducer detects waves reflected from various parts of the heart. When it is combined with Doppler technique it determines the velocity and volume of flow of blood through various cardiac valves. Thus, it measures cardiac output. It is useful in detecting valvular lesions and size of the chambers.

**X-ray Method**
In this method, a radio-opaque dye is injected intravenously and then the size of the heart is detected by serial x-rays in systole and diastole from which cardiac output is measured using computer programmes.

**Pulse-Pressure Method**
Pulse pressure (difference between systolic and diastolic pressures) provides a rough idea of cardiac output.

**FACTORS AFFECTING CARDIAC OUTPUT**
Cardiac output is the product of stroke volume and heart rate (CO = SV × HR). Therefore, factors that affect stroke volume and heart rate, affect cardiac output.

**Factors Affecting Stroke Volume**
Stroke volume depends on three major factors: The preload (degree of ventricular filling or the end diastolic volume), the inotropic state (myocardial contractility), and the afterload (resistance offered to the ventricular output).
Preload

The end-diastolic volume (EDV) is considered as the preload.

1. Increase in EDV increases stroke volume and decrease in EDV decreases stroke volume. This is explained by Frank-Starling’s law of the heart, which states that within the physiological limit, the force of contraction is directly proportional to the initial length of the muscle fiber.

2. The ‘initial length’ of the muscle fiber means the fiber length prior to ventricular contraction (i.e. just before the onset of systole). The muscle length prior to contraction depends on the level of stretch of ventricular muscle at the end of diastole. The extent to which ventricle is distended (stretched) depends on the degree of end-diastolic filling of the ventricle.

3. Increased ventricular filling increases the fiber length that increases the force of contraction (for details, refer Chapter 86). This reflects the relationship between end-diastolic fiber length and the force of contraction (Fig. 90.2). This is also known as heterometric autoregulation of cardiac output, as the stroke volume varies at various ventricular muscle lengths.

The end diastolic filling of ventricle depends on three major factors: venous return, atrial pump activity, and ventricular compliance.

Venous Return

Venous return is the amount of blood that returns to the right atrium from systemic venous circulation. It depends on the factors like skeletal muscle pump, thoracic pump, abdominal pump, ECF volume and sympathetic activity.

Skeletal Muscle Pump

In lower limbs, majority of veins are surrounded by skeletal muscles.

1. The limb veins contain valves that open only toward the heart. Therefore, contraction of skeletal muscle compresses the veins and pushes blood toward the heart. This increases venous return. The back flow of blood in veins is prevented by closed distal valves (Fig. 90.3).

2. Thus, cardiac output increases by increased skeletal muscle activities, as for example, during walking.

3. On the contrary, during quiet standing for a longer period, pooling of blood occurs in the leg veins due to absence of muscle activity that decreases venous return, which in turn decreases cardiac output.

4. Sometimes, quiet standing for a longer period may decrease cardiac output to a greater extent that causes fainting. Therefore, the traffic police personnel are usually advised to walk around the traffic or make their limb movements frequently while performing their duties for a longer period.

Thoracic Pump

Venous return increases during inspiration. This occurs due to the thoracic pump activity.

1. During inspiration, the intrathoracic pressure becomes more negative due to expansion of thoracic cage, which is transmitted to the great veins.

2. Therefore, the central venous pressure falls. The fall in venous pressure during inspiration facilitates venous return.

Abdominal Pump

During inspiration, intra-abdominal pressure rises due to descent of diaphragm.

1. The increased abdominal pressure compresses the intra-abdominal blood vessels so that the blood is pushed toward the heart.

2. Blood is not pushed toward the leg veins as they are guarded by venous valves. Thus, venous return increases.
ECF Volume
Venous return greatly depends on the extracellular fluid volume, especially the plasma volume. Decrease in ECF volume as occurs in diarrhea, vomiting, etc., decreases cardiac output and increase in ECF volume as occurs in pregnancy increases cardiac output.

Sympathetic Activity
Veins are innervated by sympathetic fibers.
1. Stimulation of sympathetic system causes vasoconstriction, which in turn increases venous return. This is one of the mechanisms of increased cardiac output in exercise.
2. Decreased sympathetic stimulation causes venodilation that decreases cardiac output due to increased venous pooling of blood.

Atrial Pump Activity
The ventricular filling occurs mostly passively. About 15 to 20% of ventricular filling at rest occurs due to atrial contraction (the atrial pump activity).
1. Therefore, normally, atrial pump does not contribute significantly to the stroke volume.
2. However, in conditions of increased demand for cardiac output as occurs in exercise, the atrial systole contributes significantly to the end diastolic filling of the ventricle.
3. The atrial contraction increases due to sympathetic stimulation.

Ventricular Compliance
Normally, the ventricular muscle is compliant (stretchable) enough to accommodate adequate blood during diastole. The normal EDV is 130 mL. In pathological conditions like cardiomyopathies or infiltrative diseases of the heart, ventricular filling decreases due to decreased compliance of ventricular muscle (the ability to stretch decreases). Thus, cardiac output decreases in such conditions.
1. Sometimes, the ventricular muscle may be compliant, but if there is more restriction to the distention of ventricles due to pressure from outside as occurs in pericardial effusion, the end diastolic volume decreases.
2. In pericardial effusion, the fluid accumulates in excess in the pericardial cavity, which increases intrapericardial pressure that prevents ventricular expansion.
3. In cardiac tamponade (massive pericardial effusion), the end diastolic filling may be reduced severely, so that stroke volume is decreased to a dangerously low level.

Myocardial Contractility
Myocardial contractility exerts a major influence on the cardiac output. Factors that increase myocardial contractility are said to be positively inotropic, and factors that decrease myocardial contractility are said to be negatively inotropic. Myocardial contractility depends mainly on the ventricular muscle mass, state of autonomic activity, concentration of various hormones and chemicals in the blood, drugs, and heart rate. Factors that stimulate contractility shift the curve to the left and factors that inhibit contractility shift the curve to the right (Fig. 90.4).

Ventricular Muscle Mass
Myocardial mass is important in determining stroke volume.
1. Ventricular muscle mass decreases either due to loss of myocardium as occurs in myocardial infarction or due to muscle atrophy as seen in some form of cardiomyopathy. In such conditions, cardiac output decreases significantly.
2. Increase in myocardial mass increases cardiac output. The common physiological condition of increased ventricular mass is the regular practice of physical exercises.
3. In strong athletes, the heart size increases due to physiological hypertrophy of the myocardium. In these individuals, cardiac output increases during exercise by mainly increasing the stroke volume rather than the heart rate (Application Box 90.1).

Application Box 90.1
Advantage of Training: In fact, the trained athletes have lower basal heart rate and greater stroke volume. Therefore, they achieve a given increase in cardiac output by increasing their stroke volume without much increase in heart rate, than the untrained individuals.

Autonomic Activity
Ventricles are supplied by sympathetic fibers. Sympathetic stimulation increases ventricular contractility and sympathetic inhibition decreases contractility. Ventricles are sparsely innervated by vagal fibers. Therefore, vagal
stimulation has less effect on myocardial contractility. However, vagal stimulation decreases cardiac output mainly by decreasing the heart rate.

**Hormonal Factors**

**Catecholamines**
Catecholamines are positively inotropic.
1. They act on β1 adrenergic receptors in the ventricle.
2. The increased adenylate cyclase activity increases cyclic AMP formation in the muscle cell.
3. Cyclic AMP increases the intracellular calcium concentration, which in turn increases myocardial contractility.

**Acetylcholine**
Acetylcholine acts on muscarinic receptors and decreases intracellular cyclic AMP concentration. Thus, acetylcholine decreases myocardial contractility.

**Glucagon**
Glucagon increases the intracellular cyclic AMP. Glucagon is a potent inotropic agent and sometimes used clinically to enhance the myocardial performance.

**Insulin**
Insulin is positively inotropic.

**Thyroxine**
Thyroxine increases cardiac output by three mechanisms.
1. It increases the number of β1 receptors in the nodal tissues and ventricular muscles.
2. It increases the sensitivity of these receptors to catecholamines. Thus, thyroxine produces tachycardia and increases myocardial contractility.
3. Thyroxine also increases myosin ATPase activity in the ventricular muscles, which significantly increases myocardial contractility.

**Chemical Factors**

**Xanthines**
Xanthines such as caffeine and theophylline are positively inotropic. They inhibit intracellular break down of cyclic AMP so that a high concentration of cyclic AMP is maintained in the ventricular muscle cell.

**Inhibiting Factors**
The following chemical agents suppress myocardial contractility.
- Hypercapnia
- Hypoxia
- Acidosis (details given below)
- General anesthetics
- Toxins

**Drugs**

**Digitalis**
Digitalis increases myocardial contractility by inhibiting the Na⁺ - K⁺ ATPase activity on the membrane of myocardial cells. This increases intracellular concentration of sodium that opposes the sodium gradient across the membrane from outside to inside the cell. Decreased sodium gradient decreases the activity of sodium-calcium exchanger on the membrane. As normally, sodium is taken in and calcium is taken out through this exchanger, decreased exchange of these ions leads to intracellular accumulation of calcium. Thus, increased cytosolic calcium increases myocardial contractility.

**Other Drugs**
Quinidine, procainamide and barbiturates inhibit myocardial contractility.

**Afterload**
Afterload is the force against which ventricular muscle fibers shorten. Essentially, it is the resistance offered against ejection of blood from ventricles.
1. Peripheral resistance is considered as the afterload.
2. Cardiac output is inversely proportional to the afterload.
3. Thus, increase in peripheral resistance decreases cardiac output and decrease in peripheral resistance increases cardiac output.
4. Cardiac output changes without change in ventricular muscle length. Therefore, this type of regulation of cardiac output is called homometric autoregulation. This is also known as Anrep effect (as described by Anrep in 1974).

Afterload (the peripheral resistance) depends primarily on two factors: vessel diameter (vascular hindrance) and viscosity of blood (hematological hindrance).

**Vessel Diameter**
The decrease in vessel diameter (vasoconstriction) increases peripheral resistance that decreases stroke volume. Stroke volume increases in vasodilation due to decreased afterload.

**Viscosity of Blood**
Increased viscosity as occurs in polycythemia, increases peripheral resistance and therefore decreases cardiac output. Cardiac output increases in anemia due to decreased viscosity.

**Factors Affecting Heart Rate**
Heart rate is influenced mainly by autonomic activity.
1. The vagal stimulation decreases heart rate and sympathetic stimulation increases heart rate. Normally, the heart rate depends on the balance between the sympathetic and parasympathetic influences.
2. Normally, increase in heart rate should result in increased cardiac output, as cardiac output is the product of heart rate and stroke volume. However, unless associated with increased venous return (end diastolic volume) increase in heart rate may not increase cardiac output proportionately.
3. In severe tachycardia, the duration of diastole shortens more than the duration of systole. Therefore, ventricle gets less time to be filled. Consequently, end diastolic volume decreases. Thus, cardiac output does not increase proportionately with increased heart rate.

4. Similarly, when heart rate decreases (diastole prolongs than systole), diastolic filling of the ventricle increases, which may compensate to maintain cardiac output.

**Heart-Lung Preparation**

The effect of preload and afterload on cardiac output can be demonstrated and verified in heart-lung preparation, which is usually carried out in a dog.

1. For this experiment (Fig. 90.5), the dog is anesthetized and the heart and lungs are cannulated. The cannulations are performed in such a way that the blood ejected from ventricles into aorta flows through a system of tubing and reservoirs to the right atrium. From right atrium, blood flows through the heart and lung back to the aorta.

2. Heart rate does not change as it is denervated physiologically.

3. When the caliber of the outflow tube decreases the peripheral resistance increases. This in turn decreases stroke volume. The decrease in stroke volume leads to accumulation of blood in the ventricle so that in the next beat stroke volume increases by Frank-Starling mechanism.

4. The effect of preload can also be directly demonstrated in this preparation. When, the reservoir emptying into the right atrium is raised to a higher level, the venous pressure increases.

5. This increases the preload (end-diastolic volume) that in turn increases the stroke volume. The stroke volume decreases when the reservoir is lowered.

**Applied aspect**: Heart-lung machine is used in cardiac O.T. during open cardiac surgery.

**REGULATION OF CARDIAC OUTPUT**

Mechanisms regulating cardiac output are broadly divided into two categories: (a) intrinsic mechanisms and (b) extrinsic mechanisms.

**Intrinsic Regulation**

The intrinsic regulation is also called as *autoregulation of cardiac output*. Two principal intrinsic mechanisms regulate cardiac output. These are Frank-Starling mechanism, and rate-induced regulation.

**Frank-Starling’s Mechanism**

The German Physiologist *Otto Frank* and the English Physiologist *Ernest Starling* independently described the effect of preload on myocardial performance. This law is therefore known as *Frank-Starling Law* (for details, see above). The increased ventricular volume (end diastolic volume) increases ventricular contraction (stroke volume), and decreased end-diastolic volume decreases stroke volume. This effect can be demonstrated even in denervated heart. Therefore, *regulation by change in preload is purely an intrinsic mechanism*. Such type of regulation of cardiac output by change in ventricular muscle length at various ventricular volumes is known as *heterometric autoregulation of the heart*.

**Scientists contributed**

- **Otto Frank** (1865–1944) was a German born doctor and physiologist who made several important contributions to cardiac physiology and cardiology. The *Frank-Starling law of the heart* is named after him and Ernest Starling. Frank also explained the Windkessel effects of larger arteries.

- **Ernest Henry Starling** (1866–1927) was an English physiologist. He worked mainly at University College London, although he also worked for many years in Germany and France. His main collaborator in London was his brother-in-law, Sir William Maddock Bayliss. Starling is most famous for developing the “Frank-Starling law of the heart”, presented in 1915 and modified in 1919.

**Rate-induced Regulation**

Myocardial performance is also regulated by change in frequency at which heart muscles contract. With increased frequency the force of contraction increases (*force–frequency relationship*). This is due to accumulation of intracellular calcium concentration, which occurs due to:
1. **Increase in number of depolarization per minute**: Calcium ions enter the myocardial cells during the plateau phase (phase 2) of the action potential. Thus, with increase in the number of action potentials (increase in the number of plateau) calcium entry into the cell increases inspite of decrease in the duration of the action potential.

2. **Increase in the inner calcium current per depolarization**: As the interval between the beats is diminished due to increased frequency, the inner calcium current progressively increases with each successive beat, which increases the force of contraction.

### Extrinsic Regulation

Extrinsic regulation depends on three major factors: Afterload, neural influences and humoral influences.

#### Afterload

Change in peripheral resistance (afterload) alters cardiac output. The increase in afterload decreases cardiac output and decrease in afterload increases cardiac output. This type of regulation is known as **homometric regulation of the heart**, as cardiac output is altered without change in muscle length (Application Box 90.2).

#### Neural Control

Neural control is primarily by autonomic influences.

#### Sympathetic Influence

Stimulation of sympathetic nerve to the heart **increases heart rate and myocardial contractility** that increase cardiac output. Sympathetic stimulation also causes **venoconstriction** and increases the venous return, which also contributes to increase in cardiac output.

#### Parasympathetic Influence

Parasympathetic stimulation decreases cardiac output mainly by **decreasing heart rate**. Ventricular contractility is marginally decreased, as vagal innervation of ventricle is sparse (Flowchart 90.1).

### Humoral Control

Humoral control includes regulation by hormones and chemicals.

#### Hormonal Regulation

Hormonal regulation is mainly by catecholamines, thyroid hormones, insulin, glucagon and growth hormone.

#### Adrenomedullary Hormones

The hormones secreted by adrenal medulla (epinephrine, norepinephrine and dopamine) increase myocardial contractility and heart rate (details, described above). Thus, cardiac output increases.

#### Thyroid Hormones

Thyroxine increases cardiac output by following mechanisms:

1. **By increasing the myosin ATPase activity**, it increases the excitation-contraction coupling.
2. **It increases the number of β receptors and sensitivity of the receptors to catecholamines** in the nodal tissues and ventricular muscles. Thus, heart rate and myocardial contractility increases.
3. **It increases protein synthesis** in the myocardial cells that causes ventricular hypertrophy. Increased ventricular mass increases myocardial contractility.
4. **It increases body metabolism.** The increased body temperature causes vasodilation that in turn **decreases peripheral resistance.** Decreased afterload increases cardiac output.

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**Flowchart 90.1**: Schematic presentation of major factors regulating cardiac output.
**Insulin**: Insulin has **direct positive inotropic effect** on the heart.

**Glucagon**: Glucagon has both positive inotropic and chronotropic effects. It acts by **increasing intracellular cyclic AMP**. Cyclic AMP increases calcium concentration in the myocardial cell by two mechanisms. **Firstly**, it increases calcium influx by opening the calcium channels on the cell membranes, and **secondly**, it increases calcium release from the sarcoplasmic reticulum.

**Growth Hormone**: Growth hormone increases cardiac output by **increasing the myocardial contractility**.

**Chemical Regulation**: Chemical regulation is mainly by blood gases and pH.

**Oxygen**: Mild hypoxia causes tachycardia. Moderate to severe hypoxia decreases cardiac output by directly **suppressing myocardial contractility** and also by stimulating the peripheral chemoreceptors.

**Carbon Dioxide**: Carbon dioxide has direct and indirect effects on myocardium. **Directly it depresses the myocardium**. The indirect effect is neurally mediated through its action on central and peripheral chemoreceptors.

**Acidosis**: Acidosis **depresses myocardium**. The reduced intracellular pH diminishes the amount of calcium released from the sarcoplasmic reticulum and also decreases the sensitivity of the myofilaments to the calcium. Increase in intracellular pH has the opposite effect.

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### CHAPTER SUMMARY

#### Key Concepts

1. Cardiac output is a vital cardiac parameter that indicates the efficiency of cardiac pumping.
2. Though it is controlled by sympathetic and parasympathetic systems, it has its own well-developed autoregulatory mechanisms.
3. Effects of preload (EDV) is a major mechanism of C.O. regulation. This is called heterometric autoregulation (Frank-Starling mechanism).
4. Effects of afterload (peripheral resistance) is an another important mechanism of C.O. regulation. This is called homometric autoregulation (Anrep effect).

#### Important to Know (Must Read)

1. In examination, ‘Describe the methods of measurement of cardiac output (CO)’; ‘Describe the factors affecting CO’; ‘Describe the regulation of CO’; are usually asked as **Long Questions**.
2. Preload, Afterload, Frank-Starling mechanism, Measurement of CO, Fick principle, Hamilton’s indicator dilution method, Fick method, Ventricular function curve, Anrep effect, may be asked as **Short Questions** in exam.
3. In **Viva**, examiner may ask… Define CO and give its normal value, Define stroke volume and give its normal value, Define end-diastolic volume and give its normal value, Define end-systolic volume and give its normal value, Define ejection fraction and give its normal value, Define cardiac reserve and give its normal value. Define cardiac index and give its normal value, List the physiological factors that increase CO, List the physiological factors that decrease CO, List the physiological factors that do not alter CO, List the direct methods of measurement of CO, List the indirect methods of measurement of CO, What is the principle of Fick method, What are the advantages and disadvantages of Fick method, What is the principle of indicator dilution method, What are the advantages and disadvantages of indicator dilution method, What is the principle of thermodilution method, What are the advantages and disadvantages of thermodilution method, What is the principle of ballistocardiography, What is the principle of echocardiography, What is the principle of X-ray method, What is the principle of pulse-pressure method, List the factors affecting CO, List the factors affecting preload, How does the skeletal muscle pump contributes to venous return, How does the thoracic pump contributes to venous return, How does the abdominal pump contributes to venous return, How does the ECF volume contributes to venous return, How does the sympathetic activity contributes to venous return, How does the atrial pump activity contributes to venous return, How does the ventricular compliance contributes to venous return, List the factors affecting myocardial contractility, How does the ventricular muscle mass contributes to myocardial contractility, How does the autonomic activity contributes to myocardial contractility, List the hormonal factors that contributes to myocardial contractility and mention the effect of each, List the chemical factors that contributes to myocardial contractility and mention the effect of each, List the drugs that contributes to myocardial contractility, How does digitalis affect myocardial contractility, List the factors affecting afterload, How does vessel diameter affect afterload, How does viscosity of blood affect afterload, List the intrinsic mechanisms of regulation of CO, List the extrinsic mechanisms of regulation of CO, What is Anrep effect, What is heart-lung preparation and what is its application.
Heart Rate and Arterial Pulse

**Learning Objectives**

On completion of study of this chapter, the student **MUST** be able to:

1. Give the normal range of heart rate and physiological variation of heart rate.
2. Understand the mechanisms of regulation of heart rate.
3. Appreciate the tracing of arterial pulse record.
4. List the causes of bradycardia and tachycardia.
5. Understand the physiological basis of common abnormal pulses.

The student **MAY** also be able to:

1. Explain the physiological basis and mechanism of alterations of common abnormal pulses.

In normal person, pulse rate represents heart rate. With each stroke output, blood ejected into circulation produces arterial pulsation. Thus, arterial pulse rate coincides with ventricular ejection rate.

**Heart Rate**

The normal heart rate is **60–100 per minute** in adults.

1. Heart rate less than 60/min is called **bradycardia** and above 100/min is called **tachycardia**.
2. Normally, heart rate is more in infants and children and less in geriatric age group.
3. Heart rate reflects the rate of discharge of the cardiac pacemaker. As SA node is the natural primary pacemaker, **heart rate is the rate of discharge of SA node**.

**Physiological Variations**

Heart rate is easily influenced by various physiological factors. Some of the common and important factors are described below.

**Age**

Heart rate is more in infants and children. After the age of sixty, heart rate decreases.

**Gender**

Heart rate is comparatively less in females due to their high parasympathetic tone and less basal metabolism.

**Diurnal Variation**

Heart rate is more in the day, especially in the afternoon, and less in the night, especially during sleep. This difference is due to the less physical activity and sympathetic discharge in the night, and due to the low level of stress in sleep than in awakened state.

**Respiration**

Heart rate is more during inspiration and less during expiration (**sinus arrhythmia**).

**Body Temperature**

Increase in body temperature increases heart rate and decrease in temperature decreases heart rate.

**Environmental Temperature**

Heart rate is more in summer and less in winter.

**Food Intake**

Food intake increases heart rate by increasing body metabolism.
Posture
Change in posture from supine to standing increases heart rate due to decreased stimulation of baroreceptors (for details, see Baroreceptor Reflex).

Exercise
Heart rate increases in exercise due to sympathetic stimulation. Heart rate may even increase before starting the exercise due to psychological effects that acts through limbic system.

Regulation of Heart Rate
Heart rate is one of the physiological parameters of the body, which is influenced easily by external and internal factors.
1. Heart rate is primarily controlled by autonomic nervous system.
2. Vagus (parasympathetic) nerve inhibits and sympathetic nerves stimulate heart rate.
3. However, HR is primarily a vagal function.
   Mechanisms regulating heart rate can be divided broadly into two categories: (i) Neural mechanisms and (ii) Humoral mechanisms.

Neural Control Mechanisms
Neural regulating mechanisms are divided into three categories:
1. Autonomic regulation
2. Reflex regulation
3. Regulation by higher centers

Autonomic Control
Both parasympathetic and sympathetic divisions of autonomic nervous system influence heart rate.
1. Normally, parasympathetic control of SA node dominates the sympathetic control. Therefore, the basal heart rate is less than the intrinsic heart rate.
2. The intrinsic heart rate is the rate of discharge of SA node when the heart is completely denervated. It is about 110 per minute. This indicates that the heart rate is more in the absence of neural influences.
3. With intact autonomic innervation, due to more vagal influence (as vagus is inhibitory) on SA node, the normal heart rate is less than the intrinsic heart rate.

Parasympathetic Control
The parasympathetic fibers supplying the heart originate in the nucleus tractus solitarius, dorsal motor nucleus of vagus, and the nucleus ambiguous. The fibers travel in the vagus nerve to supply the SA node.
1. The right vagus nerve predominantly supplies SA node and the left vagus nerve predominantly supplies AV node.
2. Stimulation of vagus nerve results in decrease in heart rate. This occurs due to the secretion of acetylcholine at vagal nerve endings that suppresses SA node.

Sympathetic Control
The sympathetic fibers supplying the pacemaker tissue originate from the lower two cervical and upper six thoracic segments of spinal cord.
1. The stimulation of sympathetic fibers results in increase in heart rate.
2. In contrast to the effect of parasympathetic stimulation on SA node, the effect of sympathetic stimulation persists longer as there is no specific enzyme to degrade norepinephrine, which is released at the sympathetic nerve endings.
3. Norepinephrine is mostly taken up by the nerve terminals, and the remaining amount is slowly absorbed into circulation. Moreover, the effect of norepinephrine is mediated by adenylyl-cyclase system, which takes longer time to exert its effect.
4. Thus, the effect of sympathetic stimulation on heart rate is longer than the vagal stimulation.

Reflex Control
Cardiovascular reflexes that regulate blood pressure also control heart rate, which is part of the integrated control mechanisms. Details of these reflexes are described in the regulation of blood pressure (for details, refer Chapter 96).

Baroreceptor Reflex
Baroreceptors located in the carotid sinus and aortic arch are stimulated when blood pressure rises, which in turn stimulates the nucleus tractus solitarius (NTS) in medulla via 9th and 10th cranial nerves. NTS inhibits the vasomotor center (VMC).
1. Inhibition of VMC decreases sympathetic activity via bulbo-spinal pathway. Decreased sympathetic discharge decreases heart rate.
2. Stimulation of NTS also directly stimulates the vagus nerve that causes of bradycardia.
3. Heart rate increases in conditions in which baroreceptors are less stimulated as occurs in hypotension.

Chemoreceptor Reflex
Chemoreceptors are stimulated by change in chemical composition of blood as occurs in hypoxia, hypercapnia and acidosis. Activation of chemoreceptors primarily produces bradycardia, but heart rate may remain unchanged or even slightly increased by secondary effects.

Bainbridge Reflex
Bainbridge, in 1915, demonstrated that rapid infusion of saline or blood in dogs increases heart rate, if the initial
heart rate is less. This is known as Bainbridge reflex. The receptors are present in the atria at the veno-atrial junction, and are known as tachycardia producing receptors (TPR). This reflex accounts for tachycardia produced following saline infusion or blood transfusion. The effect of Bainbridge reflex on heart rate is more observable if the initial heart rate is less. This reflex competes with baroreceptors reflex and tries to increase heart rate.

**Cushing's Reflex**
Cushing’s reflex is activated in gross hypotension that decreases blood flow to the VMC. Direct stimulation of VMC produces vasoconstriction and tachycardia. But, the consequent increase in pressure stimulates the baroreceptors that finally result in bradycardia.

**Control by Higher Centers**
Stimulation of motor cortex, frontal lobe, and thalamus increases heart rate. Increase in heart rate in emotional states, anxiety, and excitement is due to stimulation of limbic system.

**Humoral Control Mechanisms**

**Hormonal Control**
Thyroxine and catecholamines increase heart rate (for details, refer previous chapter).

**Chemical Control**
Hypoxia increases heart rate, which is partly mediated by release of catecholamines from adrenal medulla. Hypercapnea and acidosis decrease heart rate.

### ARTERIAL PULSE

**Physiological Aspects**

**Definition**
Arterial pulse is defined as the rhythmic expansion of the arterial wall due to transmission of pressure waves along the walls of the arteries that are produced during each systole of the heart.

**Clinical Importance**
Clinically, radial pulse is examined for the assessment of arterial pulse. It is an important and essential part of the clinical examination of a patient as pulse is one of the vital signs of a living being (other vital signs are BP, respiration and temperature). Examination of arterial pulse also provides valuable information regarding the functioning of the heart, condition of hemodynamics like blood pressure, and the condition of blood vessels.

**Normal Pulse Rate**
Normally, pulse rate is same as heart rate. Thus, the normal pulse rate is 60 to 100 per minute.

1. The deficit between pulse rate and heart rate is called pulse deficit (Clinical Box 91.1).
2. Here is no pulse deficit in normal conditions. That means the pulse rate exactly coincides with the rate of heart beat.
3. Pulse rate more than 100 is called tachycardia, and less than 60, is called bradycardia. Normally, the heart rate is more in children and less in elderly people.

**Clinical Box 91.1**

**Pulse deficit:** When the cardiac rhythm is irregular due to some organic heart disease, few contractions of the ventricle may not be sufficient enough to generate pressure waves in the walls of the arteries. Therefore, a deficit occurs between the rate of heart contraction and the pulse rate. Pulse deficit is usually seen in atrial fibrillation in which the deficit is more than 10. Pulse deficit seen in other arrhythmias and diseases causing heart blocks is usually less than 10.

**Normal Pulse Tracing**
Arterial pulse tracing shows two waves and one notch. The waves are percussion (p) wave, also called as tidal wave, and dicrotic (d) wave (Fig. 91.1), and the notch is dicrotic notch (n).

1. **Percussion wave** occurs due to ejection of blood during ventricular systole.
2. **Dicrotic wave** occurs due to rebound of blood against the closed aortic valve during diastole.
3. **Dicrotic notch** represents closure of aortic valve.

**Causes of Tachycardia**

**Physiological:**
1. Exercise
2. After eating
3. Anger
4. Emotion and excitement
5. Infants and children
6. Pregnancy
7. High environmental temperature
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Pathological:
1. Fever
2. Anemia
3. Thyrotoxicosis
4. Beriberi
5. Paget’s disease
6. Arterio-venous fistula
7. Heart failure
8. Paroxysmal atrial tachycardia
9. Ventricular or supra ventricular tachycardia
10. Other tachyarrhythmias

Causes of Bradycardia

Physiological:
1. Athletes
2. Fear
3. Grief
4. Very old age
5. Yogis

Pathological:
1. Myxedema
2. Increased intracranial pressure, e.g. brain tumors
3. Obstructive jaundice
4. Different types of heart block
5. Drugs, e.g. digitalis

Common Abnormal Pulses

The character of a pulse is described as normal when no abnormalities are detected. Different types of abnormal pulses are described in the Text-books of Clinical Medicine. Some of these common abnormal pulses are anacrotic pulse, dicrotic pulse, water-hammer pulse, pulsus bisferiens, pulsus paradoxus, and pulsus alternans.

Anacrotic Pulse

This is also called as anadicrotic pulse, which means two up beats. A secondary wave occurs in the upstroke of the pulse. It is commonly found in aortic stenosis. The upstroke is slow and sloping (Fig. 91.2A).

Dicrotic Pulse

It is also called as twice-beating pulse. The dicrotic wave is prominent and gives the feeling of two beats (Fig. 91.2B). It is commonly seen in febrile states, especially in typhoid fever.

Water-Hammer Pulse

This is also called as collapsing pulse or Corrigan’s pulse. It is typically seen in aortic regurgitation.
1. The collapsing pulse is characterized by a rapid upstroke (ascent) and a rapid down stroke (descent) of the pulse wave (Fig. 91.2C).
2. The rapid upstroke is due to greatly increased stroke volume and the rapid descent is due to the collapse of the pulse. The collapse of the pulse occurs due to two factors:
   - The diastolic run-off of blood into the left ventricle, and
   - Rapid run-off of blood into the periphery because of decreased systemic vascular resistance.
3. The pulse pressure is therefore very high, may be as high as 100 mm Hg.

Causes

1. Aortic incompetence
2. Patent ductus arteriosus
3. Arteriovenous fistula
4. Ventricular septal defect (VSD)
5. Hyperkinetic circulatory states, e.g. thyrotoxicosis

Physiological Basis

The collapsing pulse is a steep rising, forceful, high amplitude percussion wave which gives a sharp tap to the palpating hand that suddenly disappears. The steep rise of the ascending limb of the pulse wave is due to:

- Increased end diastolic volume (EDV) of the left ventricle, as during diastole in addition to the ventricular filling from the left atrium, the filling also occurs from the incompetent aortic valve.
- This increases the total EDV, which increases the force of contraction of the left ventricle during systole by Frank-Starling mechanism.
- Thus, steep rise occurs in the percussion wave during systole. The steep fall of the descending limb of the pulse wave is due to two factors.
   1. The diastolic run-off of blood into the left ventricle.
   2. Rapid run-off of blood into the periphery because of decreased systemic vascular resistance.

Pulsus Bisferiens

Pulsus bisferiens is a combination of the low rising pulse (anacrotic pulse) and the collapsing pulse. This is typically seen in aortic stenosis associated with aortic incompetence.

Pulsus Paradoxus

This is a misnomer. There is nothing paradoxical in this pulse.
1. Actually, this is an accentuation of the normal phenomenon.
2. Normally, the amplitude of the pulse decreases in inspiration and increases in expiration.
3. In pulsus paradoxus, in inspiration the volume of the pulse is grossly decreased, or may be absent in severe cases (Fig. 91.2E).
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Causes

Common Causes
1. Constrictive pericarditis
2. Pericardial effusion

Less Common Causes
1. Emphysema
2. Asthma
3. Massive pleural effusion
4. A mass in the thorax
5. Advanced right ventricular failure

Mechanisms
1. During inspiration, the intrathoracic pressure becomes more negative. Pooling of blood occurs in the pulmonary vascular bed, which decreases venous return to the left atrium. Thus, left atrial filling decreases that in turn decreases left ventricular stroke volume. Therefore, volume of the pulse decreases in inspiration. This is more accentuated in the above-mentioned diseased conditions.

2. During inspiration, the intrapericardial pressure increases due to the traction from the attachments put on the pericardium. This decreases venous return to the heart and results in low stroke volume. This is more accentuated in pericardial effusion and constrictive pericarditis.

3. In constrictive pericarditis and pericardial effusion, the filling of the atria and ventricles decreases due to restriction to the expansion of the heart chambers. The limitation in the diastolic filling of the right atrium and the right ventricle during inspiration results in lowering of left ventricular stroke volume.

4. In advanced stage of ventricular failure, increase in lung volume in inspiration accommodates more blood than normal due to much decreased pulmonary vascular resistance. As such, there is decreased right ventricular output. These two factors decrease venous return to left atrium that in turn decreases left ventricular stroke volume.

5. In bronchial asthma, the increased respiratory effort makes intrathoracic pressure more negative during inspiration. Hence, there is more pooling of blood in the pulmonary veins that results in decreased ventricular stroke volume.

Pulsus Alternans

The pulse is regular, but the alternating beats are strong and weak (Fig. 91.2D). It is difficult to appreciate pulsus alternans by palpating the artery. Diagnosis is confirmed while measuring blood pressure. A difference of 5 to 20 mm Hg in the systolic pressure is marked between two alternate beats. When the column of mercury in the manometer is being lowered, the stronger beats are heard first and, on further lowering, the weaker beats also become audible. Thus, suddenly the number of audible beats is doubled.

Causes
1. Left ventricular failure (commonest cause)
2. Toxic carditis

Physiological Basis

In left ventricular failure, due to decreased myocardial contractility the stroke volume is decreased. This results in low volume pulse.

1. Due to inadequate ejection of blood during systole, the end-systolic volume of the left ventricle increases.
2. Therefore, EDV increases before the onset of next ventricular contraction.
3. This increases the force of contraction of the ventricle in the next beat by Frank-Starling mechanism.
4. Consequently, the second beat becomes stronger. Likewise, the strong and weak beats alternate.

Figs. 91.2A to E: The abnormal arterial pulses. (A) Anacrotic pulse, note that anacrotic wave (a) is abnormally prominent, which is normally not visible; (B) Dicrotic pulse in which dicrotic wave (d) is abnormally large; (C) Water-hammer pulse (note the rapid up-stroke and rapid down-stroke without a dicrotic notch); D: Pulsus alternans; E: Pulsus paradoxus (note the gross decrease in amplitude during inspiration).
**CHAPTER SUMMARY**

**Key Concepts**

1. Heart rate is primarily a parasympathetic function. Tachycardia reflects decreased vagal tone and bradycardia indicated increased vagal tone.

2. Pulsus alternans is observed in heart failure and water-hammer pulse seen in aortic regurgitation.

**Important to Know (Must Read)**

1. In examination, **Long Questions** are usually not asked from this chapter.

2. Heart rate, Arterial pulse, Water-Hammer pulse, Pulsus paradoxus, Pulsus alternans are asked as **Short Questions** in exam.

3. In **Viva**, examiner may ask... What is the normal HR, What are the physiological variations of HR (tachycardia and bradycardia), What are the factors regulating HR, What are the neural control mechanisms of HR, What are the reflex control mechanisms of HR, What are the humoral control mechanisms of HR, Define pulse rate and what is its normal value, What is the clinical importance of pulse rate, What are the waves of a normal pulse tracing, List the factors causing tachycardia, List the factors causing bradycardia, List the common abnormal pulses, What is anacrotic pulse, What is dicrotic pulse, What is Water-Hammer pulse, What are the causes of water-hammer pulse, What is the physiological basis of Water-Hammer pulse, What is pulsus paradoxus, What are the causes of pulsus paradoxus, What are the mechanisms of pulsus paradoxus, What is pulsus alternans, What are the causes of pulsus alternans, What is the physiological basis of pulsus alternans, What is pulsus bisferiens.
CHAPTER 92

Principles of Hemodynamics

Learning Objectives

On completion of study of this chapter, the student MUST be able to:
1. Apply the knowledge of various physical principles of hemodynamics in understanding cardiovascular functions.
2. Understand velocity-flow-pressure relation of blood vessels.
3. List the differences between laminar and turbulent flow.
4. Understand the factors governing peripheral resistance.
5. Understand the application of law of Laplace in determining various cardiovascular functions.

The student MAY also be able to:
1. Explain the application of various hemodynamic principle in health and disease.

Application of Physical Principles

Hemodynamics is the interaction of various physical principles that govern circulation of blood. Cardiovascular system is a complex system of heart and blood vessels. The heart is the mechanical pump, which continuously pumps blood into circulation that creates the pressure (force) needed for forward movement of blood in blood vessels. Blood vessels transport blood between heart and tissues.

1. Nonetheless, blood vessels in different parts of circulatory system have different dimensions, thickness and elasticity that greatly influence flow of blood. Blood flow is also influenced by composition and rheological properties of blood. Thus, blood circulation is influenced by many physicochemical factors.
2. However, blood flow primarily depends on two important factors:
   i. The properties of blood and
   ii. The diameter of blood vessels.
3. Blood vessels are always under the influence of various neural and humoral factors, and vessel-diameter is the outcome of the integrated effect of these factors on blood vessels.
4. Blood is not a simple liquid, rather a suspension of cells and fats that are dispersed in the colloidal solution of proteins.
   In spite of the complexity of blood as a fluid, and susceptibility of vessels for abrupt change in their diameter, the circulation of blood occurs harmoniously due to the operation of various principles of hemodynamics. Therefore, before studying the physiology of circulation, it is essential to learn the basics of hemodynamics.

Velocity-Flow-Pressure Relationship

Velocity vs Blood Flow

Velocity is defined as the rate of displacement of fluid with respect to time. This is expressed as distance per unit time, for example in cm/s. Flow is defined as the volume displacement per unit time (cm³/s). The velocity of fluid in a system depends on the area and the flow. Flow in turn depends on the pressure gradient, properties of the fluid and dimensions of the hydraulic system of the tube. Velocity is the flow divided by the area of the conduit.

\[ V = \frac{Q}{A} \]

V is the velocity, Q is the flow and A is the area.

The average velocity of fluid movement in a system is directly proportional to the flow and inversely proportional to the total cross sectional area of the system. Therefore, in vascular system, velocity gradually decreases as blood flows from the aorta through arteries and arterioles into capillaries due to progressive increase in the cross sectional area (Fig. 92.1). Velocity slowly increases as blood passes from venules through veins into the vena
Velocity vs Pressure

Change in pressure in a hydraulic system changes the velocity of fluid movement.

1. The pressure in a hydraulic system has two components: The lateral (or the static) pressure component and the dynamic pressure component.

2. Lateral pressure is the side pressure that is exerted constantly on the wall of the tube.

3. Dynamic pressure is the component of pressure, which is affected by the kinetic energy of flow; for example, increase in flow increases the dynamic component.

4. The total pressure (the static plus the dynamic pressure) in a closed system remains always constant (Bernoulli’s Principle). As the total pressure does not change, the alteration in any component of pressure occurs at the cost of the other. Especially, change in dynamic pressure component that occurs frequently in a hydraulic system changes the lateral pressure.

5. In circulatory system, at the sites of narrowing of blood vessel the velocity of flow increases, which in turn increases the dynamic component of the pressure. The increase in dynamic pressure decreases the lateral pressure (Fig. 92.2).

6. Lateral pressure in the blood vessel determines the degree of perfusion of tissues as it decides the amount of blood that enters into the branches from the main vessel. Thus, at the site of constriction the perfusion of tissue decreases (Clinical Box 92.1).

Clinical Box 92.1
AMI is common in aortic stenosis: In aortic stenosis, narrowed aortic valve increases the velocity of aortic flow. This increases the dynamic pressure and decreases the lateral pressure. Coronary arteries that originate almost at ninety degree angle from the root of the aorta receive less blood as reduced lateral pressure retards perfusion. This induces myocardial ischemia, which aids to already hypertrophied ventricle pumping blood against a higher resistance. Hence, acute myocardial infarction is common in aortic stenosis.

Flow, Pressure and Resistance

Pressure is one of the principal determinants of the rate of flow. Increased pressure increases the flow and decreased pressure decreases flow. The relationship between flow, pressure, and resistance in a hydraulic system is well compared with the relationship between current, electromotive force, and resistance in an electrical circuit. According to Ohm’s Law:

\[ \text{Current (I)} = \frac{\text{Electromotive force (E)}}{\text{Resistance (R)}} \]

Similarly, Flow (F) = \frac{\text{Pressure (P)}}{\text{Resistance (R)}}

In vascular system, pressure is the effective perfusion pressure (the mean intraluminal pressure at the arterial end minus the mean pressure at the venous end) and
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resistance is the peripheral resistance. Flow is directly proportional to the pressure and inversely proportional to the resistance. Increase in resistance decreases flow.

Types of Blood Flow

Blood flow is of two types: laminar (streamline) flow and turbulent flow.

Laminar Flow

Normally, flow of blood in the vessels is laminar in nature (laminar means ‘in layers’). That means, the normal blood flow occurs in layers.

1. Laminar flow is also called streamline flow. The layer of the blood, which is in close contact with the wall of the vessel, does not move at all due to the frictional resistance with the vessel wall, the next layer moves with lesser velocity. The velocity slowly increases toward the center of the vessel.

2. The velocity of flow is maximal in the central layer of blood in the vessel (Fig. 92.3).

3. However, laminar flow occurs up to a certain velocity beyond which the flow becomes turbulent. The velocity above which the flow is turbulent is known as critical velocity.

Turbulent Flow

The turbulent flow occurs when velocity is above critical velocity. Turbulence of flow depends on the diameter of the vessel and the viscosity of the blood. The probability of turbulence is expressed in terms of Reynolds number, which is calculated as:

\[ Re = \frac{\rho D V}{\eta} \]

Where, ‘Re’ is the Reynolds number, ‘\(\rho\)’ is the density of the fluid, ‘D’ is the diameter of the tube, ‘V’ is the velocity of flow, and ‘\(\eta\)’ is the viscosity of the fluid.

- The probability of turbulence increases when the value of ‘Re’ is greater. Usually, when the ‘Re’ is less than 2000 the flow is laminar and when the ‘Re’ is more than 3000 the flow is turbulent.

- In cardiovascular system, at the site of constriction, increase in the velocity of blood flow makes the flow turbulent. Turbulence of flow produces sound (Table 92.1). Therefore, a murmurish sound is heard on auscultation at the site of constriction of the vessel or cardiac orifice.

- For example, a bruit is auscultated over an arterial constriction, or a murmur is heard over a stenotic cardiac valve. A better example is the auscultation of Korotkoff sounds on the brachial artery in the cubital fossa due to constriction of the artery by inflated BP cuff, during measurement of blood pressure by auscultatory method.

Poiseuille-Hagen Formula

Poiseuille-Hagen formula denotes the relationship between viscosity of the fluid with the radius and length of the tube.

\[ F = (P_a - P_v) \times \left(\frac{\pi}{8}\right) \times \left(\frac{1}{\eta}\right) \times \left(\frac{r^4}{L}\right) \]

Where,

- \(F\) = Flow
- \(P_a - P_v\) = Pressure difference between the both ends of the tube
- \(\eta\) = Viscosity of fluid
- \(r\) = Radius of tube
- \(L\) = Length of tube

As discussed above, flow is equal to the pressure difference divided by resistance. Therefore, resistance (R) is calculated from the following formula:

\[ R = \frac{8\eta L}{\pi r^4} \]

Flow varies directly with the fourth power of the radius. Blood flow is markedly affected by a small change in the diameter of the blood vessel. The flow becomes double in a vessel by just increasing the radius to about 20% Consequently, blood flow is significantly altered by mildly changing the diameter of vessels. Flow affects pressure. Thus, change in diameter changes flow and pressure. In fact, blood pressure is markedly increased by vasoconstriction.

![Fig. 92.3: Laminar or streamline flow of blood that occurs in layers. Arrows represent the direction of flow. The length of arrows indicates the velocity of flow in that layer. The velocities have a parabolic distribution with maximum velocity at the center of the stream.](image)

![Table 92.1: Differences between laminar flow and turbulent flow.](table)
and decreased by vasodilation. On the contrary, resistance is inversely proportional to the radius (discussed below).

**PERIPHERAL RESISTANCE**

Resistance offered to the flow of blood in the peripheral circulation is the peripheral resistance.

**Factors Affecting Peripheral Resistance**

Peripheral resistance is determined by two main factors: Caliber of blood vessel and viscosity of blood. Vessel diameter is the most important determinant of peripheral resistance.

**Radius of Blood Vessel**

The radius of the blood vessel significantly affects peripheral resistance. This is called *vascular hindrance*.

1. Vasoconstriction increases and vasodilation decreases peripheral resistance.
2. *Decrease in radius of vessels to half increases peripheral resistance by 16 times.*
3. This decreases blood flow to organs by 16 times.
4. Conversely, when radius is doubled, resistance is reduced to 6% of its previous value.

**Viscosity of Blood**

Viscosity of blood also affects peripheral resistance. This is called *hematological hindrance*. Viscosity mainly depends on the factors like hematocrit, composition of plasma, resistance of red cells to deformation and temperature.

**Hematocrit**

Hematocrit is the single most factor that greatly affects viscosity of blood.

1. Hematocrit is the packed cell volume, which depends mainly on the number of red cells in the blood.
2. The effect of change in viscosity on resistance is less in smaller vessels than in the larger vessels. This is due to the difference in nature of flow through the small vessels.
3. Viscosity increases in polycythemia and decreases in anemia.
4. In anemia, circulation is hyperdynamic due to decreased peripheral resistance.

**Composition of Plasma**

In plasma, it is mainly the concentration of plasma proteins that affects viscosity. Viscosity increases in conditions in which concentration of plasma protein is more, for example, in paraproteinemia and multiple myeloma (increase in myeloma protein). Viscosity decreases in hypoproteinemia.

**Resistance of Red Cells to Deformation**

When the red cells become rigid as seen in hereditary spherocytosis, viscosity increases.

**Temperature**

Increase in body temperature decreases viscosity and decrease in temperature increases viscosity.

**Plasma Skimming**

In blood vessels, cells mostly accumulate at the center of the flowing column of blood. Therefore, the portion of blood, which is available at the periphery of blood vessel (close to the vessel wall) has low cell content.

1. Blood entering into smaller branches arising from a large vessel mainly comes from the peripheral part of the column of the blood.
2. Thus, *smaller branches receive blood containing less red cell* (Fig. 92.4). This phenomenon is called plasma skimming.
3. This explains why the hematocrit of capillary blood is about 25% less than the whole-body hematocrit.

**Critical Closing Pressure**

In a rigid tube, a linear relationship exists between flow and pressure (Fig. 92.5A). With increased flow, the pressure increases. But in blood vessels this relationship is different.

1. Especially in capillaries, flow ceases when pressure is reduced beyond a point (but pressure is not zero). The *pressure at which the flow stops is called critical closing pressure* (Fig. 92.5B).
2. This is explained by two factors: (i) some pressure is required to force the red cells to pass through the capillaries which have the smaller diameter than the red cells, and (ii) blood vessels are surrounded by tissues that exert pressure (tissue pressure) on them.
3. Thus, intraluminal pressure in capillaries should be more than the tissue pressure for flow to resume.

**LAW OF LAPLACE**

This law explains the relationship between the distending pressure ($P$), the tension ($T$) in the wall of a structure and its radius ($r$).
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\[ P = T \left( \frac{1}{r_1} + \frac{1}{r_2} \right) \]

Where, \( r_1 \) and \( r_2 \) are its two principal radii of curvature.

In a sphere, \( r_1 = r_2 \), so \[ P = \frac{2T}{r} \]

In a cylindrical structure such as a thin-walled blood vessel, the curvature occurs in only one dimension. The other radius is infinite, so,

\[ P = \frac{T}{r} \quad \text{or} \quad T = P \times r \]

So, in a small diameter blood vessel, less wall tension is required to balance the distending pressure. This protects small vessels from rupture (Application Box 92.1).

**Application Box 92.1**

**Capillaries are less prone to rupture:** Smaller the radius of the blood vessel, lesser the tension in the wall needed to balance the distending pressure. Therefore, though the capillaries are thin they are less prone to rupture.

If the thickness of the vessel wall (\( w \)) is taken into consideration, like in an artery,

\[ \text{Wall tension (T)} = P \times \frac{r}{w} \]

**Application of Laplace Law**

The Laplace law helps us to understand the physiological mechanisms in altered situations and pathological conditions affecting the functioning of many organs.

1. In **hollow viscus** like bladder, ventricle or the alveoli of the lungs, the wall tension depends on the distending pressure and its radius. That means the wall tension increases when the organ gets filled (distending pressure rises) or the cavity size (radius) increases.

2. Also the wall tension decreases when the wall thickness is more and the wall tension increases when the wall thickness decreases.

3. So, in **ventricular hypertrophy** as the wall is thick, wall tension is less.

4. On the other hand, in a **dilated heart** as seen in heart failure, more energy is required to pump blood as the wall tension is more. Therefore, a **dilated or distended heart pumps blood less efficiently** (Clinical Box 92.2).

**Clinical Box 92.2**

**Dilated heart fails faster:** A dilated heart has to do more work than a non-dilated heart, as with increased radius of the cardiac chamber greater tension must be developed in the myocardium to produce any given pressure (Fig. 92.6).
CHAPTER SUMMARY

**Key Concepts**

1. Dynamics of blood flow depends mostly on vessel diameter.
2. Lateral pressure in a vessel determines the rate of perfusion. At the area of constriction, lateral pressure decreases and therefore perfusion decreases.
3. Viscosity of blood opposes blood flow.

**Important to Know (Must Read)**

1. In examination, **Long Questions** are usually not asked from this chapter.
2. Plasma skimming, Critical closing pressure, Relationship between dynamic and lateral pressure in vascular system, Laminar and turbulent flow, Application of Laplace law, are asked as **Short Questions** in exam.
3. In **Viva**, examiner may ask...... What is laminar flow, What is turbulent flow, What is critical velocity, What are the differences between laminar flow and turbulent flow, What is Reynolds number, What is Poiseuille-Hagen formula, What is vascular hindrance, What is hematological hindrance, List the factors and their contribution to hematological hindrance, Why acute myocardial infarction is common in aortic stenosis, What is plasma skimming, What is critical closing pressure, What is the law of Laplace, Why are capillaries less prone to rupture, Why does the dilated heart fail faster.
Chapters 93: Arterial System

Learning Objectives

On completion of study of this chapter, the student MUST be able to:

1. Understand the importance of sympathetic innervation of arterial system for maintaining arterial volume and pressure.
2. Appreciate the importance of Windkessel effect of larger arteries.
3. Trace the arterial pulse pressure waves.
4. Learn the principles of direct and indirect methods of BP measurement.

The student MAY also be able to:

1. Describe the principle of arterial hemodynamics in in various physiological conditions.

Functional Organization

The arterial system carries blood from heart to the tissues. It consists of aorta, large and small arteries, arterioles and metarterioles. These vessels have all the three layers, namely tunica intima, tunica media and tunica adventitia. The tunica media containing smooth muscle is thicker in the arterial compartment than the other compartments of circulatory system. However, the amount of smooth muscle present varies in different parts of arterial system (for details, refer to Fig. 84.7, Chapter 84).

Innervation

Arterial system is richly supplied by sympathetic fibers. Sympathetic fibers originate from thoracic and lumbar segment of spinal cord.

1. Normally at rest, there is a tonic discharge of sympathetic fibers. This is called sympathetic tone. Stimulation of sympathetic fibers results in vasoconstriction. Therefore, sympathetic tone is also known as vasoconstrictor tone.
2. Vasoconstrictor tone is essential for maintaining normal blood pressure. Increase in vasoconstrictor tone increases and decrease in vasoconstrictor tone decreases blood pressure.
3. There is no parasympathetic innervation of blood vessel in general circulation.

Types of Vessels

Types of vessels in arterial system.

Elastic Vessels

Elastic vessels are aorta and large arteries. In these vessels, the quantity of elastic component is more than the muscle component.

1. Therefore, these arteries are more compliant (stretchable).
2. These are called Windkessel vessels (elastic reservoir).

Muscular Vessels

These include small arteries, arterioles and metarterioles. In these vessels, quantity of smooth muscle is more than elastic tissues. Therefore, these vessels provide maximum resistance to blood flow.

1. These are called resistance vessels. Arterioles are the primary seat of peripheral resistance.
2. In fact, a significant fall in blood pressure occurs when blood passes through arterioles.

Functions of Arteries

1. Arteries transport blood from heart to the tissues. Thus, they supply oxygen and nutrients to the different parts of the body.
2. The aorta and the large arteries due to their elastic recoil property, maintain forward movement of blood during diastole (details, described below).
3. Small arteries, arterioles and metarterioles are richly innervated by sympathetic fibers and offer maximum resistance to blood flow. Therefore, these vessels mainly control blood pressure.

Functional Aspects

Arterial Elasticity

Aorta and large arteries have more compliance due to the presence of more elastic elements in their wall. When blood is ejected forcefully into the aorta and its major branches during ventricular systole, these vessels are distended.

1. During diastole, the aortic wall immediately recoils back to its previous position. This property of recoiling is known as Windkessel effect (Figs. 93.1A and B). Due to the Windkessel effect, the vessel wall that recoils back on the blood column pushes the blood to move in forward direction during diastole.

2. During systole, forward movement of blood is due to the energy created by forceful ejection of blood that occurs due to ventricular contraction.
3. Had the aorta and large arteries been stiff (no recoiling effect), flow of blood during diastole would have stopped and that would have resulted in intermittent blood flow only during systole.
4. Thus, blood moves continuously during systole and diastole due to the Windkessel effect of elastic arteries.
5. Continuous arterial flow is essential for adequate tissue perfusion.

Arterial Pressure Pulse

Arterial pressure pulse is the pressure wave that travels along the wall of the arteries created by forceful ejection of blood into the arterial system during ventricular systole. These pressure waves are felt as arterial pulses when clinically examined by the physician.

1. The velocity of transmission of pulse wave in the wall of the artery is fifteen times the velocity of flow of blood in the lumen of the artery. For example, in the aorta, the velocity of flow is 0.33 m/s, whereas the velocity of transmission of pressure wave is about 4 m/s.
2. The velocity of transmission of pressure wave increases toward the periphery. In large arteries, it is about 8 m/s and in small arteries about 20 m/s. Thus, the radial pulse in wrist is felt in 0.1 s from the peak of ventricular ejection.
3. The amplitude and the pattern of arterial pulse also change from central arteries to peripheral arteries. Arterial pulses are recorded (pulse tracing) by a sphygmograph or a physiograph.
4. The central arterial pulse has higher amplitude, steep ascending limb, less sharp peak and incissura in the upper part of the descending limb, which is less steep.
5. The peripheral arterial pulse has steep ascending limb, sharp peak, steep descending limbs, and the dicrotic notch (instead of incissura) present toward the lower part in the descending limb (Figs. 93.2A and B).

In arterial pulse, there are two waves: Percussion wave and dicrotic wave (Figs. 93.2A and B). The percussion wave or tidal wave occurs due to ejection of blood during ventricular systole. This corresponds to the maximum ejection phase. The dicrotic wave occurs due to rebound of blood against the closed aortic valve during diastole. The dicrotic notch represents closure of aortic valve (Fig. 91.1, Chapter 91).

Arterial Pressure

Arterial pressure is defined as the lateral pressure exerted by the column of blood on the walls of the arteries. Blood pressure usually means the arterial pressure. The pressure in the arteries fluctuates during systole and diastole of the cardiac cycle.
1. The maximum pressure is recorded during systole (systolic blood pressure) and the minimum pressure is recorded during diastole (diastolic blood pressure).

2. In adults, the systolic pressure ranges between 100–140 mm of Hg and diastolic pressure ranges between 60–90 mm Hg (for details of blood pressure, refer Chapter 96).

**Pulse Pressure**

Pulse pressure (PP) is the difference between the systolic and diastolic pressures. Normally it ranges between 20–50 mm Hg. The change in pressure during systole and diastole of a cardiac cycle produces pulse pressure. The pulse pressure is high in the aorta and large artery. It grossly decreases across the arterioles, almost negligible in capillaries and nil in veins (Fig. 93.3).

**Mean Arterial Pressure**

Mean arterial pressure (MAP) is the average pressure recorded during the cardiac cycle. It is calculated as:

\[ \text{MAP} = \text{Diastolic pressure} + \frac{1}{3} \text{PP} \]

Because the duration of systole is less than the duration of diastole, MAP is slightly less than the value halfway between systolic and diastolic pressure (for details, see Chapter 96). Normal MAP ranges between 80–105 mm Hg.

**MEASUREMENT OF BLOOD PRESSURE**

Methods of measurement of blood pressure are broadly divided into two categories: (1) direct methods and (2) indirect methods.

**Direct Methods**

The blood pressure is measured directly by placing a cannula in the artery and connecting the cannula to a mercury manometer or a pressure transducer. This is the method used for recording blood pressure in experimental animals. In human beings, blood pressure is usually measured by indirect method.

**Indirect Methods**

Blood pressure is usually measured with the help of a sphygmomanometer. The procedure is called sphygmomanometry.

1. In this method, the cuff of the sphygmomanometer is wrapped around the arm of the subject. The cuff is then inflated until the air pressure in the cuff overcomes the arterial pressure and obliterates the arterial lumen. This is confirmed by palpating the radial pulse that disappears when the cuff pressure is raised above the arterial pressure. Pressure is then raised further by about 20 mm Hg and then slowly reduced.

2. When pressure in the cuff reaches just below the arterial pressure, blood escapes beyond the occlusion into the peripheral part of the artery and pulse starts reappearing.

3. This is detected by the appearance of sounds in the stethoscope, which is taken as the systolic pressure. Then, the quality of the sound changes and finally disappears.

4. The level where sound disappears is noted as the diastolic pressure. Sound disappears because the flow in the blood vessel becomes laminar.

The blood pressure can be measured by three methods: (1) palpatory, (2) auscultatory, and (3) oscillatory method. Ideally, blood pressure should be measured first by the palpatory and then by the auscultatory method.

**Palpatory Method**

In palpatory method, pressure in the cuff is progressively raised and radial artery pulse is palpated simultaneously. The point where the pulsation disappears is the systolic pressure. Diastolic pressure cannot be measured by palpatory method.
CHAPTER SUMMARY

**Key Concepts**

1. The Windkessel effect is due to arterial elasticity, which facilitates the movement of blood in arteries in forward direction during diastole.
2. The more quantity of smooth muscle in comparison to the vessel wall in arterioles and metarterioles offers resistance to distension. Therefore, these vessels are resistance vessels. Dense sympathetic innervation also contributes to this.

**Important to Know (Must Read)**

1. In examination, Long Questions are usually not asked from this chapter.
2. Windkessel effect, Korotkow sound, Sympathetic tone/Vasoconstrictor tone, may be asked as Short Questions in exam.
3. In Viva, examiner may ask…. What is Sympathetic/vasoconstrictor tone, What are the functions of arteries, What are the types of vessels in arterial system, What is Windkessel effect, How does the blood move continuously during systole and diastole, Define arterial blood pressure, Define pulse pressure, Define mean arterial pressure, List the methods of measurement of BP, What is palpatory method of measuring BP, What is auscultatory method of measuring BP, What are the phases of Korotkow sound, What is oscillatory method of measuring BP.

**Auscultatory Method**

In auscultatory method, pressure in the cuff is raised by about 20 mm Hg above palpatory level and then progressively lowered during which brachial artery is auscultated for sounds by placing the diaphragm of a stethoscope on it (Fig. 93.4). The sounds undergo a series of changes in their quality and intensity. These sounds are known as Korotkow sounds (described by the Russian scientist Korotkow in 1905). The sounds are heard in five different phases.

- **Phase I**: Sudden appearance of faint tapping sound which becomes gradually louder and clearer during the succeeding 10 mm Hg fall in pressure.
- **Phase II**: The sound becomes murmurous in the next 10 mm Hg fall in pressure.
- **Phase III**: The sound changes little in quality but becomes clearer and louder in next 15 mm Hg fall in pressure.
- **Phase IV**: Sounds become muffling in character during next 5 mm Hg fall.
- **Phase V**: Sounds completely disappear.

Appearance of the sound is recorded as systolic blood pressure and disappearance of sound is recorded as diastolic blood pressure. In persons having severe hypertension, muffling rather than the disappearance of sound is taken as diastolic pressure. In children, muffling is also noted as diastolic pressure.

**Oscillatory Method**

In oscillatory method, the procedure is same as that of palpatory method. But, instead of palpating the artery, oscillations of the mercury column in the sphygmomanometer is noted to record BP. The pressure in the cuff is raised and the appearance and the disappearance of the oscillations of the mercury column are noted. The point of appearance of the oscillation gives systolic pressure and the point of disappearance of the oscillations gives diastolic pressure.
CHAPTER 94

Venous System

Learning Objectives

On completion of study of this chapter, the student MUST be able to:
1. Understand the difference in pressure volume relation between arteries and veins.
2. Learn the importance of venous circulation in determining cardiac output.
3. Give the normal values of CVP, and peripheral venous pressure at different levels in standing position.
4. Appreciate the clinical importance of venous pressure, especially during neurosurgical procedures.

FUNCTIONAL ASPECTS

Components

The venous system starts from the capillaries. It consists of venules, small veins, large veins and vena cava. Vena cava drains into right atrium.

Wall of Veins

Veins contain all the three layers of the blood vessels. However, veins are significantly thinner due to the thin muscle coat.

Functions of Veins

1. Venous system transports deoxygenated blood from tissues to the heart. From heart, blood is pumped into the lungs where carbon dioxide is removed from it.
2. Veins are capacitance vessels as they can accommodate a large volume of blood. They act as reservoir of blood. In fact, more than 60% of the total blood is present in the venous compartment.

Pressure Volume Relationship of Veins

The veins are thin walled and have less quantity of smooth muscle. Therefore, they are easily distensible and collapsible.
1. The increase in volume of blood in veins does not affect significant change in pressure up to a point beyond which the pressure rises steeply (Fig. 94.1B).
2. Normally, veins are partially collapsed. Thus, they easily accommodate a large amount of blood without any change in pressure till they are fully distended.
3. However, once the lumen of veins becomes spherical pressure rises steeply, whereas in arteries, the pressure-volume relationship is almost linear (Fig. 94.1A).

Innervation

Veins are innervated by sympathetic fibers.
1. The density of innervation is less in comparison to arteries.
2. However, veins are more sensitive to sympathetic discharge than arteries due to a greater nerve fiber to muscle fiber ratio.
3. Therefore, a significant venoconstriction occurs even at mild sympathetic stimulation that increases venous return adequately.

Venous Return

The amount of blood that drains into the right atrium from the venous compartment is called venous return. It is one of the major determinants of cardiac output.
1. Change in venous return changes cardiac output by changing end-diastolic volume (the preload) of the ventricle by Frank-Starling mechanism.
2. Venous return is influenced by total blood volume, sympathetic stimulation, venous tone, skeletal muscle pumping, thoracic pumping and abdominal pumping (for details, refer to Chapter 90).
Venous Valves

Veins are provided with valves that allow forward flow of blood. Valves are well developed especially in limb veins. When skeletal muscles contract (skeletal muscle pumping), blood is squeezed in veins towards heart that increases venous return and cardiac output, the back flow of blood is prevented by venous valves (refer Fig. 90.3, Chapter 90) (Clinical Box 94.1).

Clinical Box 94.1
Varicose veins: Venous valves are incompetent in varicose veins that decrease venous return and causes venous stasis. Therefore, ankle edema occurs in patients with varicose veins. Cardiac output is also decreased in such conditions.

VENOUS PRESSURES

Central Venous Pressure

The pressure in the great veins at their entrance into the right atrium is called central venous pressure (CVP).
1. CVP is normally about 0 to 5 mm Hg. But, it can be –5 mm Hg when rate and force of contraction of heart increases.
2. CVP is measured directly by inserting a catheter into the great veins in the thorax.
3. The right atrial pressure is also measured to determine CVP.
4. Jugular venous pressure (JVP) reflects the right atrial activity (for JVP, refer Chapter 89).

Peripheral Venous Pressure

Pressure in the venules is about 15 mm Hg. Venous pressure decreases as blood flows in to larger veins. The pressure in great veins outside the thorax is 5.5 mm Hg.

Effect of Gravity on Venous Pressure

In standing posture, the venous pressure decreases by 0.77 mm Hg for each centimeter above the heart. Thus, venous pressure is –10 mm Hg in superior sagittal sinus (Fig. 94.2). Venous pressure increases by 0.77 mm Hg
per cm below the right atrium so that it is as high as +85 mm Hg at feet.

Clinical importance: In upright position, venous pressure above heart level decreases due to effect of gravity so that the venous pressure in the neck and head is negative. These veins are therefore normally collapsed. The pressure in collapsed vein is zero, rather than subatmospheric. However, the pressure in dural sinuses in upright or sitting posture is subatmospheric, as sinuses do not collapse due to their rigid walls (Clinical Box 94.2).

Clinical Box 94.2

Air embolism in neurosurgical procedures: Sometimes, neurosurgical procedures are performed in sitting posture, during which if a sinus is opened accidentally or as part of the surgical procedure, air embolism results as air is sucked into the sinus. A neurosurgeon should be cautious about this possible complication before performing the surgery.

CHAPTER SUMMARY

**Key Concepts**

1. Veins have less smooth muscle in their wall and less density sympathetic innervation, which make them the capacitance vessels for accommodating large amount of blood. They accommodate more blood with less rise in venous pressure.
2. Due to effect of gravity venous pressure above heart level in standing posture is negative.

**Important to Know (Must Read)**

1. In examination, Long Questions are not asked from this chapter.
2. Central venous pressure, Effect of gravity on venous pressure, may be asked as Short Questions in exam.
3. In Viva, examiner may ask… What is the pressure volume relationship in arteries and veins, What are the functions of veins, What is varicose vein, What is central venous pressure, What is peripheral venous pressure, What is the effect of gravity on venous pressure, what is its clinical importance, Why air embolism can occur in brain during neurosurgery in sitting posture.
Capillary Circulation

Learning Objectives

On completion of study of this chapter, the student MUST be able to:
1. Classify capillaries and understand importance of histological difference in capillaries.
2. List the functional specialities of capillaries.
3. Understand the forces acting across the capillary membrane that contribute to capillary filtration.
4. Describe the mechanism of capillary filtration.
5. Define edema and understand the physiological mechanisms of edema formation.

The student MAY also be able to:
1. Explain the various mechanisms of capillary exchange.
2. Explain the physiological mechanisms of edema formation.

Concept of Microcirculation

Though only about 5% of blood volume circulates in capillaries, physiologically this is most significant as exchange of nutrients, gases, water and waste products between the blood and tissues occurs in capillaries. Hence, capillaries are known as exchange vessels. The supply of oxygen and nutrients is essential for survival of tissues. The circulation of blood from arterioles to venules through capillaries is called the microcirculation.

1. Capillaries are present close to all the metabolically active cells so that substrates for metabolism and products of metabolism can easily be exchanged between cells and blood through the capillary wall.
2. Except lens and cornea, all cells in the body are in direct contact with the microvessels.
3. Cornea and lens derive their nutrients from the fluids present in the eye.

Functional Morphology

Capillaries form extensive branching networks that increase the surface area for rapid exchange of materials. Capillaries are made up of a single layer of endothelial cells surrounded by the basement membrane. Endothelial cells are joined together by inter-endothelial junctions. Tunica media is completely absent. Absence of tunica media and the gap in the inter-endothelial junctions contribute to the permeability of the capillaries. True capillaries emerge from the arteriole or metarteriole and form anastomosing network that drain into the venules (Fig. 95.1). In many vascular beds, metarteriole is connected directly with venule by a thoroughfare vessel. Capillary networks anastomose with the side branches of thoroughfare vessels.

Types of Capillaries

There are three types of capillaries: continuous, fenestrated, and sinusoidal capillaries. This classification is based on the size of gaps between the endothelial cells of the capillaries.

Continuous Capillaries

Most capillaries in the body are of this type.
1. Endothelial cells form a continuous ring around the lumen of capillary except at the gaps (intercellular clefts) between endothelial cells.
2. The clefts (gaps at inter-endothelial junctions) are usually 4 to 10 nm (less than 15 nm) in diameter. There is no discontinuity in endothelium of the capillaries (Figs. 95.2A and B).
3. This type of capillary is seen in skeletal and smooth muscles, connective tissues, and lungs.
Chapter 95: Capillary Circulation

4. The clefts are absent in capillaries of cerebral blood vessels (blood-brain barrier) as the inter-endothelial junctions in these vessels are tight junctions.

**Fenestrated Capillaries**

These capillaries differ from continuous capillaries as their endothelial cells are perforated by many fenestrations (pores), which are 20 to 100 nm in diameter.

1. The cytoplasm of endothelial cells is attenuated to form gaps, which are called fenestrations (Figs. 95.3A and B).
2. They allow passage of molecules having the molecular weight of up to 69,000.
3. These capillaries usually surround the epithelia as in intestinal villi, choroid plexuses of ventricles in brain, ciliary processes of eyes, exocrine glands and parts of kidneys.

**Sinusoidal or Discontinuous Capillaries**

These capillaries have larger discontinuities between the endothelial cells.

1. The gaps between the endothelial cells are more than 400 nm in diameter (Figs. 95.4A and B).
2. Some gaps are 600 to 3000 nm in diameter.
3. Also, they have multiple large fenestrae in their cytoplasm. Therefore, large molecules can easily pass through these capillaries.
4. Such capillaries are present in liver, spleen and bone marrow.

Capillary Flow
Capillary pressure differs from tissue to tissue, and therefore the capillary blood flow differs. It is about 37 mm Hg at the arteriolar end and 17 mm Hg at the venular end of the capillaries of the skeletal muscle. Though capillaries are short, blood flow is very slow due to large total cross-sectional area. The flow rate is about 0.07 cm/sec. The pulse pressure is also less in capillaries, which is about 5 mm Hg at the arteriolar end and almost zero at the venular end. Therefore, capillary blood flow is less pulsatile.

Functional Specialties of Capillaries
1. The capillary density (the ratio of the number of capillaries to the number of cells in the tissue) determines the degree of exchange across the capillary. Capillary density is high in the metabolically active tissues like cardiac muscle and glands. It is low in less active tissues like subcutaneous tissue and cartilage.
2. Capillary diameter is different in different tissues. However, diameter of a true capillary at arterial end is about 5 µm and at venular end is about 8 µm. Most capillaries have diameter less than the diameter of red cells. Therefore, red cells just squeeze through the lumen of the capillaries in single file. Red cells assume the shape of a parachute during this process. Moreover, the contact between red cell membrane and capillary membrane becomes maximal that facilitate capillary exchange.
3. Capillaries do not contain smooth muscle, therefore they do not directly respond to vasoconstrictors or vasodilators. The flow of blood through capillaries is largely regulated by change in the capacity of precapillary vessels (arterioles and metarterioles).
4. At the origin of capillaries from metarterioles, a flap of smooth muscle forms a cuff, called precapillary sphincter. This sphincter is usually not innervated, but responds to various vasoconstrictor and dilator chemicals. The capillary circulation largely depends on the contraction or relaxation of the precapillary sphincter.
5. Though capillaries do not dilate or constrict actively due to lack of smooth muscle, change in their caliber occurs passively due to alteration in flow of blood through their lumen. It is also proposed that few contractile elements like myosin and actin filaments are present in the capillary walls. Moreover, endothelial cells of capillaries are surrounded by pericytes or Rouget cells (see Fig. 95.2). Rouget cells are believed to be the primitive form of vascular smooth muscle or myoepithelial cells that respond to vasoconstrictor agents. They themselves also release vasoactive substances. Therefore, active capillary constriction and dilation do occur to some extent.
6. Capillaries are thin vessels that are made up of single layer of endothelial cells and contain no muscle. The flow of blood is sluggish in capillaries. Therefore, capillaries are best suited for exchange of materials between the blood and the tissue (exchange vessels).
7. The capillaries are least innervated by the sympathetic fibers and they do not have smooth muscles. Therefore, sympathetic stimulation usually does not directly change the caliber of capillaries.
8. The capillaries are weak vessels and surrounded by tissues. Therefore, if the capillary pressure decreases below the tissue pressure, capillaries collapse and the flow in capillaries ceases. The capillary pressure, below which the flow stops, is called critical closing pressure.
9. There are few vessels that directly connect the arterioles and venules, bypassing the capillaries. These are called thoroughfare vessels. The flow of blood mainly occurs through thoroughfare vessels during resting conditions (resting tissue). However, flow through true capillaries increases during exercise (active tissue).
10. The flow of blood through capillaries is usually intermittent (not continuous). This is because intermittent contraction and relaxation of the arterioles and metarterioles, which regulate flow through the capillaries. Such intermittent flow (which usually occurs 5–10 times per minute) is called vasomotion. Vasomotion is partly contributed by the chemicals released by the endothelium of the blood vessels.
11. Diameter of capillaries in most tissues is less than the diameter of RBCs. Therefore, red cells while passing through the lumen of capillaries come in very close contact with the capillary membrane. As such, the flow of blood in capillaries is very slow. These two factors, in addition to the thin capillary wall facilitate the exchange of gasses between capillaries and tissues.
12. Though individual capillaries have small diameter and they provide high vascular resistance, the total capillary resistance is much less due to their parallel arrangement.
13. Endothelial cells of capillaries contain many endocytic vesicles that contribute to transcytosis (described below) of water and water-soluble substances across the capillary wall. Some endothelial cells have fenestrations that run completely through the cells from capillary-interior to the interstitial space.

Active vs Inactive Capillaries
At rest, most of the capillaries are closed (inactive capillaries) and blood flow occurs mainly by thoroughfare vessels. In active tissues, arterioles and precapillary sphincters
dilate that result in flow of blood through the capillaries. This opens up many capillaries and improves capillary circulation (active capillaries).

**Capillary Exchange**

Substances are transported across the capillaries by three ways: diffusion, vesicular transport that includes endocytosis and exocytosis, and filtration.

**Diffusion**

This is the common method of exchange across the capillary bed in most of the tissues.
1. The gases (oxygen and carbon dioxide), the nutrients (glucose, and amino acids), hormones, and other substances are exchanged by means of diffusion.
2. In liver, because of sinusoidal nature of the capillaries, large molecules like proteins diffuse through capillaries easily.
3. This is the important way of transport of synthesized proteins like fibrinogen and albumin to enter circulation.

**Vesicular Transport**

The transport of substances by means of endocytosis and exocytosis is called the vesicular transport.
1. In this process, substances like dissolved proteins from plasma are taken up by endocytosis, transported across the endothelial cells and then discharged outside by exocytosis.
2. As the substances are actually transported in the form of vesicles, the process is called vesicular transport.
3. As transport occurs across endothelial cells, the process is also called transcytosis.
4. This process is important mainly for transport of large lipid-insoluble substances that cannot pass through the capillary wall by means of other mechanisms.

**Capillary Filtration**

This is the major route of transport of fluid between the blood and the interstitial tissue space. Filtration occurs because of the difference in various pressures of the intravascular fluid (blood) and the extravascular fluid (fluid in interstitial tissue space). Two pressures promote filtration and two pressures oppose filtration.

**Pressures promoting filtration are:**
1. Hydrostatic pressure of the blood, and
2. Osmotic pressure of the interstitial fluid.

**Pressures that oppose filtration are:**
1. Oncotic pressure (the osmotic pressure of blood due to the plasma proteins), and
2. The tissue hydrostatic pressure.

The balance between these pressures determines the net filtration. The forces that control capillary filtration are named as Starling's forces (as described by EH Starling).

**Scientist contributed**

**Ernest Henry Starling** (1866–1927) was an English physiologist. As professor of physiology at University College, London (1899–1923), Starling began a highly profitable collaboration with the British physiologist William Bayliss that immediately saw their demonstration (1899) of the nervous control of the peristaltic wave. In 1902 they isolated a substance that they called secretin, released from duodenum. Two years later, Starling coined the term hormone to denote secretion from endocrine glands. Starling demonstrated the forces causing filtration in capillaries, known as Starling forces.

**Hydrostatic Pressure Gradient**

This is the difference between the hydrostatic pressure of vascular compartment (i.e. of blood) and the interstitial tissues compartment (i.e. of tissue fluid).
1. The hydrostatic pressure of any fluid compartment always pushes fluid out of the compartment.
2. In skeletal muscle for example, the hydrostatic pressure of blood at the arteriolar end and at the venular end of capillaries is about 37 and 17 mm Hg respectively, and in the interstitial tissues space, it is negligible, which is about 1 mm Hg (Fig. 95.5).
3. Therefore, the hydrostatic pressure gradient favors filtration both at arteriolar and venular end of the capillaries.
4. However, at arteriolar end, the gradient is more, i.e. 36 \((37-1)\) mm Hg than at the venular end of the capillaries, which is 16 \((17-1)\) mm Hg.

**Osmotic Pressure Gradient**

This is the difference between the oncotic pressure (the osmotic pressure of blood), which is 25 mm Hg, and the osmotic pressure in the interstitial tissue space, which is very negligible (almost zero).

1. Osmotic pressure of a fluid compartment witheld fluid (prevents escape of fluid) in the compartment. Therefore, osmotic pressure opposes filtration.

2. As, osmotic pressure of tissue space is nil, the osmotic pressure gradient along the capillary wall is always inward, which favors absorption of fluid from the interstitial tissue space into the capillary.

**Net Filtration**

The net filtration of fluid along the capillary wall depends on the difference between the hydrostatic pressure gradient and the oncotic pressure gradient at arteriolar and venular end of capillaries.

**At the arteriolar end of the capillary:**

- The net filtration pressure is 11 mm Hg \(\left[ (37-1) - 25 \right] \) in outward direction.

**At the venular end of the capillary:**

- The net filtration pressure is 9 mm Hg \(\left[ 25 - (17-1) \right] \) in the inward direction.

Thus, at the arteriolar end, fluid moves out of the capillaries and at the venular end, fluid moves into the capillaries. About two units of fluid are left in the interstitial tissue space as the outward filtration at the arteriolar end is 2 mm Hg more than the inward filtration at the venular end. This amount of fluid is usually taken up by the lymphatics in the interstitial space, which is finally brought back to the circulation as lymphatics at last drain into the veins.

**Capillary Permeability**

In addition to the pressure gradients, filtration also depends on permeability of the capillary membrane. Capillary permeability depends on the integrity of the capillary endothelial membrane. Capillary permeability is increased especially in inflammatory conditions.

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**EDEMA**

**Definition**

The accumulation of free fluid in excess in the interstitial tissues space is called edema.

**Mechanisms of Edema Formation**

Edema can occur due two important mechanisms:

1. Increased filtration of fluid into the interstitial tissues space.

2. Decreased removal of fluid from the interstitial tissues space.

**Increased Filtration of Fluid into the Interstitial Tissues Space**

This can occur by three mechanisms: increased hydrostatic pressure of capillary blood, decreased oncotic pressure and increased capillary permeability.

**Increased Hydrostatic Pressure of Capillaries**

Capillary hydrostatic pressure increases in following conditions:

1. Increased venous pressure that increases capillary pressure, e.g. congestive cardiac failure.

2. Venular constriction, e.g. a tumor pressing on a vein causes edema in its territory of drainage.

3. Increased extracellular fluid volume, e.g. fluid retention.

4. Arteriolar dilation, e.g. local inflammation.

**Decreased Oncotic Pressure**

The oncotic pressure decreases due to hypoproteinemia that occurs in liver diseases (decreased production of plasma proteins), kidney diseases (increased excretion of plasma proteins), malnutrition (decreased intake of proteins), and burns (exudation of protein rich fluid from the burn surface).

**Increased Capillary Permeability**

The capillary permeability increases by the action of chemical substances like histamine, bradykinin, substance P and bacterial toxins. Edema that occurs in inflammation is due to increased capillary permeability.

**Scientist contributed**

**Thomas Lewis** (1881–1945)

Lewis analyzed the mechanism of the heart beat and promoted many clinical application of cardiology. He made careful studies on capillary function and pioneered in examining histamine effects on capillaries. A special chest lead used for recording ECG in atrial arrhythmias is named after him as Lewis lead.

**Decreased Removal of Fluid into the Interstitial Tissues Space**

From interstitial space water is removed by lymphatics. Thus, decreased lymphatic drainage causes edema formation. This can occur either due to diseases of the lymphatics (lymphangitis), surgery (radical mastectomy that removes lymphatic ducts), or by infection (filariasis). The decreased lymph flow decreases the removal of excess fluid from the interstitial tissues space that results in edema formation. Edema due to lymphatic obstruction is usually non-pitting type.
Treatment of Edema

Treatment for edema depends on the type of edema and the cause of edema.

1. If it is **generalized edema**, as occurs in heart failure, the treatment includes administration of **diuretics and salt restriction** in the diet in addition to the specific treatment for heart failure.
2. If it is **localized edema** as occurs in localized inflammation, **anti-inflammatory drugs** are prescribed.
3. **Special form of edema** like cerebral edema or pulmonary edema is treated judiciously by diuretics or other drugs depending on the cause of the edema. For example, pulmonary edema developed at high altitude in acute mountain sickness is treated by **glucocorticoids**.

Arteriovenous Anastomoses

There are short vascular channels that directly connect arterioles to venules bypassing capillaries (Fig. 95.6).

1. These are called arteriovenous anastomoses or *arteriovenous shunts*.
2. They have thick muscular wall and are densely innervated by sympathetic nerves.
3. AV anastomoses are especially present in the fingers, palms and ear lobes.

**CHAPTER SUMMARY**

**Key Concepts**

1. Capillaries are exchange vessels. The gap or fenestration between the endothelial cells determines the degree of filtration and exchange.
2. Capillary filtration depends on hydrostatic pressure gradient that pushes fluid out of vascular compartment and the osmotic pressure gradient that opposes the filtration.
3. Therefore, decreased oncotic pressure or increased hydrostatic pressure causes edema formation.

**Important to Know (Must Read)**

1. In examination, ‘Explain the mechanism of capillary filtration across the skeletal muscle capillary bed’ may be asked as a Long Question.
2. Types of capillaries, Functional specialties of capillaries, Mechanism of capillary filtration, Starling forces, Mechanism of edema formation, are asked as Short Questions in exam.
3. In Viva, examiner may ask… What are the types of capillaries, What is a continuous capillary and where is it found, What is a fenestrated capillary and where is it found, What is a sinusoidal capillary and where is it found, What is the nature of capillary flow, List the functional specialties of capillaries, What is a Rouget cell and what is its function, What is a precapillary sphincter and what is its function, What do you understand by active and inactive capillaries, What is vesicular transport, What are the factors promoting capillary filtration, What is the net filtration pressure at the arterial end of capillary, What is the net filtration pressure at the venular end of capillary, Define edema, What are the mechanisms of edema formation, What are the factors that increase the filtration of fluid into the interstitial tissue spaces, What are the conditions that increase hydrostatic pressure of capillaries, What are the conditions that decrease oncotic pressure of capillaries, What are the conditions that increase capillary permeability, What is a non-pitting edema, and in which conditions is it seen, What is localized and generalized edema, How is edema treated, What is arteriovenous anastomosis/shunts.
Chapter 96

Regulation of Blood Pressure

Learning Objectives

On completion of study of this chapter, the student **MUST** be able to:
1. Define systolic and diastolic blood pressure, mean arterial pressure and pulse pressure.
2. Give the normal values and significance of systolic and diastolic pressure, mean pressure and pulse pressure.
3. List the factors affecting BP.
4. Appreciate the physiological variations of BP.
5. Describe the mechanisms of regulation of BP.
6. Draw a schematic diagram of baroreceptor reflex.
7. Understand the importance of baroreceptor reflex in regulation of BP.
8. Understand the importance of other reflexes in regulation of BP.
9. Appreciate the importance of sympathetic tone in regulation of BP.

The student **MAY** also be able to:
1. Describe the role of vascular smooth muscle in the regulation of BP.
2. Explain the role of vascular endothelium in the regulation of BP.
3. Describe the role of medulla in the regulation of BP.

Regulation of blood pressure (BP) is a fundamental physiological process for survival. Therefore, BP is among the vitals signs of clinical examination of a subject or a patient. Acute hypotension is dangerous and chronic hypertension is detrimental to health. Mild rise in BP for a longer period (prehypertension) has recently been identified as universal silent killer. Therefore, understanding the mechanism of BP regulation, which helps in learning the methods to prevent hypertension, is important in clinical physiology and medicine.

**Vascular Tone**

Tone of the blood vessel plays a crucial role in the regulation of blood pressure. Tone of vascular smooth muscle depends on many neural and humoral actors. Therefore, in introduction to this chapter, we provide the concept on vascular tone and the basic mechanism of contraction and relaxation of vascular smooth muscle.

1. Smooth muscles of blood vessel like other smooth muscles normally exhibit tone, a state of prolonged partial contraction.

2. Blood pressure is mostly influenced by the tone of blood vessels, especially of the resistance vessels (arterioles and small arteries).
3. Vascular tone increases by vasoconstriction that increases blood pressure and decreases by vasodilation that decreases blood pressure.
4. Tone of vascular smooth muscle is influenced profoundly by the state of sympathetic discharge and

**Scientist contributed**

Carl Friedrich Wilhelm Ludwig (1816–1895) was an outstanding German physiologist. Ludwig exercised enormous influence on the progress of physiology. There is indeed scarcely any branch of physiology, except the physiology of the senses, to which Ludwig did not make important contributions. In his recognition, at University of Leipzig, the Physiology Institute is designated as Carl Ludwig Institute of Physiology. He contributed enormously to cardiovascular physiology and BP regulation. He discovered vasomotor reflexes and proposed renal secretion theory for regulation of BP. For his outstanding contributions, since 1932, the Carl Ludwig Honorary Medal is awarded by the German Society for Cardiology to outstanding investigators in the area of cardiovascular research.
the concentration of various circulating neurohormonal factors.
5. Vasoconstrictor and vasodilator agents alter blood pressure by altering the activity of vascular smooth muscle.
6. Many of these chemicals affect vascular tone by mainly altering the intracellular calcium concentration in vascular smooth muscle.

**Vascular Smooth Muscle**

Cell membrane of vascular smooth muscles contains K\(^+\), Ca\(^{++}\) and Cl\(^-\) channels. Concentration of intracellular Ca\(^{++}\) ions and the activity of myosin light-chain kinase (MLCK) influence the contraction of vascular smooth muscles. The vascular tone that occurs due to prolonged contraction of vascular smooth muscle depends mainly on latch-bridge mechanism, though other factors also contribute to it.

1. **Influx of calcium** into the muscle due to opening of voltage-gated calcium channels (VGCC) increases cytosolic calcium.
2. **Calcium activates myosin light chain kinase** that phosphorlyates myosin light chain, which in turn causes muscle contraction.
3. Increased cytoplasmic calcium also causes opening of ryanodine receptor-mediated calcium channels in the sarcoplasmic reticulum (calcium mediated calcium release) that increases release of calcium from this organell.
4. Cytosolic calcium concentration increases to a greater extent that opens up the calcium-activated K\(^+\) channels on cell membrane and causes K\(^+\) efflux (Fig. 96.1).
5. These potassium channels are BK channels (Big-K\(^+\) channels) as K\(^+\) efflux occurs at a very high rate through these channels. Calcium acts on \(\beta_1\) subunits of BK channels. 
6. K\(^+\) efflux increases the membrane potential that closes the calcium channel and produces relaxation.

**PHYSIOLOGICAL ASPECTS**

**Definition, Normal Values and Significance**

**Blood Pressure**

Blood pressure is defined as the lateral pressure exerted by the column of blood on the wall of arteries. Blood pressure means the arterial pressure. The arterial pressure fluctuates during systole and diastole of the heart.

**Systolic Blood Pressure**

**Definition**

Systolic BP is defined as the maximum pressure recorded during the cardiac cycle. As maximum pressure is recorded during systole, the pressure is called systolic pressure.

**Diastolic Blood Pressure**

**Definition**

Diastolic blood pressure is defined as the minimum pressure recorded during the cardiac cycle. As the minimum pressure is recorded during diastole, the pressure is called diastolic pressure.

**Significance**

**Normal Value**

Normal systolic BP is **100 to 119 mm Hg** in adults. Systolic BP 120 to 139 mm Hg is classified as prehypertension and 140 mm Hg or above is considered as hypertension.

**Significance**

Systolic pressure depends mainly on the cardiac output. Thus, systolic pressure increases when cardiac output increases and decreases when cardiac output decreases.

1. However, systolic pressure is also affected by the compliance of arteries. When arteries become stiff as occurs in advanced age, systolic pressure increases.
2. In a stiff vessel, the ability to accommodate a particular amount of cardiac output decreases in comparison to a normal vessel.

**PHYSIOLOGICAL ASPECTS**

**Definition, Normal Values and Significance**

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**Systolic Blood Pressure**

**Definition**

Systolic BP is defined as the maximum pressure recorded during the cardiac cycle. As maximum pressure is recorded during systole, the pressure is called systolic pressure.
alteration in peripheral resistance. Peripheral resistance depends mainly on the diameter of the blood vessels and viscosity of the blood. Vasoconstriction increases diastolic pressure and vasodilation decreases diastolic pressure.

**Normal Value**
Normal diastolic BP is **60 to 79 mm Hg** in adults. Diastolic BP 80 to 89 mm Hg is classified as prehypertension and 90 mm Hg or above is considered as hypertension.

**Pulse Pressure**
**Definition:** Pulse pressure (PP) is the difference between systolic and diastolic blood pressure.
**Normal value:** 20 to 50 mm Hg
**Significance:** This is the pressure that maintains the normal pulsatile nature of flow of blood in the vascular compartment. The pulsatile nature of flow is required for the perfusion of the tissues.

**Mean Arterial Pressure**
**Definition:** Mean arterial pressure (MAP) is the average pressure recorded during the cardiac cycle.
As the duration of systole is less than the duration of diastole, the MAP is less than the value halfway between systolic and diastolic pressures (Fig. 96.2A).
It is calculated as:
\[
\text{MAP} = \text{diastolic pressure} + \frac{1}{3} \text{PP}
\]
**Significance:** The MAP is the pressure that helps in forward movement of blood in the lumen of blood vessels. It also maintains perfusion of tissues.
**Normal value:** 75 to 105 mm Hg.

**Casual Blood Pressure**
Blood pressure measured at any time of the day or night is known as the casual pressure.

**Basal Blood Pressure**
Blood pressure recorded in the basal state is called basal blood pressure. Basal condition means the subject is in full physical and mental rest following 12 hours of fasting. Basal blood pressure recording following 12 hours of fasting is not normally practiced. Usually in clinical practice, basal blood pressure is recorded following **physical and mental rest for 10–20 minutes** in supine position (resting basal BP).

**FACTORS AFFECTING BLOOD PRESSURE**
Blood pressure = cardiac output × peripheral resistance
Therefore, factors that influence cardiac output or peripheral resistance affect the blood pressure. Alteration in cardiac output mainly affects systolic pressure and alteration in peripheral resistance mainly affects diastolic pressure.

**Factors Affecting Cardiac Output**
Cardiac output = stroke volume × heart rate
As cardiac output is the product of stroke volume and heart rate, any factor that affects either of these two parameters, affects cardiac output. The stroke volume is affected by preload, afterload and myocardial contractility, and the heart rate is mainly affected by autonomic influences (refer Fig. 90.6, Chapter 90).

**Preload**
Preload is the end-diastolic volume (EDV). Increase in EDV increases cardiac output and decrease in EDV decreases cardiac output. This occurs due to the operation of Frank-Starling mechanism. EDV depends on venous return, atrial pump activity and ventricular compliance (for details, see Chapter 90).
Factors that increase preload:
1. Increased total blood volume
2. Increased venous tone, e.g. sympathetic discharge
3. Increased pumping action of skeletal muscle as occurs in isotonic exercise
4. Increased thoracic pump activity (increased negative intrathoracic pressure) as happens in increased respiration
5. Increased abdominal pumping, e.g. increased abdominal respiration
6. Increased atrial contraction, as occurs in exercise

Factors that decrease preload:
1. Decreased blood volume, e.g. dehydration
2. Venodilation, e.g. decreased sympathetic discharge
3. Increased intrapericardial pressure, e.g. pericardial effusion
4. Decreased ventricular compliance, e.g. cardiomyopathy
5. Decreased respiratory activity.

Afterload
Afterload is the peripheral resistance, which is inversely proportional to cardiac output. Increase in peripheral resistance as occurs in vasoconstriction decreases cardiac output and decrease in peripheral resistance as occurs in vasodilation increases cardiac output.

Myocardial Contractility
The contractility of myocardium exerts a major influence on the cardiac output. The myocardial contractility is called inotropic state of the heart. The factors that increase contractility are said to be positively inotropic and the factors that decrease contractility are said to be negatively inotropic.

Factors that are positively inotropic:
1. Sympathetic stimulation
2. Digitalis
3. Glucagon
4. Caffeine and theophylline

Factors that are negatively inotropic:
1. Parasympathetic stimulation
2. Hypoxia, hypercapnea and acidosis
3. Loss of myocardium
4. Drugs like quinidine, barbiturate, etc.

Heart Rate
Increase in heart rate increases cardiac output, and decrease in heart rate decreases cardiac output. However, change in heart rate cannot significantly alter cardiac output unless associated with proportionate change in ventricular filling. Heart rate is mainly controlled by autonomic influences. Stimulation of parasympathetic (vagus nerve) system inhibits and sympathetic system stimulates heart rate.

Factors Affecting Peripheral Resistance
Peripheral resistance depends mainly on diameter of blood vessel and viscosity of blood.

Diameter of Blood Vessels
Vasoconstriction increases peripheral resistance and thereby increases blood pressure and vasodilation decreases blood pressure by decreasing peripheral resistance. Diameter of blood vessels depends primarily on the vasomotor tone, which is the sympathetic vasoconstrictor tone. Also, when blood vessels become stiff (less compliant), peripheral resistance increases that increases blood pressure.

Viscosity
Viscosity depends on composition of plasma, total number of cells in the blood, resistance of the cells to deformation and temperature.

Factors that increase viscosity:
1. Polycythemia
2. Hyperproteinemia
3. Hereditary spherocytosis
4. Decreased temperature

Factors that decrease viscosity:
1. Anemia
2. Hypoproteinemia
3. Increased temperature

Physiological Variations

Age
Blood pressure increases with age.
In children: Systolic pressure ranges from 90 to 120 mm Hg and diastolic pressure from 50 to 80 mm Hg.
In adults: Systolic pressure ranges from 100 to 119 mm Hg and diastolic pressure from 60 to 89 mm Hg. As per JNC report on hypertension, systolic BP between 120 and 139 mm Hg and diastolic BP between 80 and 89 mm Hg are classified as prehypertension.
In elderly: The upper limit of systolic is considered to be 140 mm Hg. However, diastolic above 90 mm Hg is always considered to be abnormal.

Gender
Blood pressure is comparatively less in females during their reproductive life (Fig. 96.2B).
1. This difference disappears after menopause.
2. Blood pressure is less in females due to the effect of progesterone that relaxes the smooth muscles of the blood vessels and estrogen that prevents atherosclerosis.

Eating
Blood pressure increases after a meal. This is due to increased body metabolism that increases circulation and cardiac output following food intake.
Sleep
Blood pressure is less during sleep than in the awakened state. When human being is awake, he is under the influence of stress and strain of life. Acute stress activates sympathetic activity that increases blood pressure. The stress is apparently absent in sleep. In addition, absence of physical activity during sleep aids to decrease in blood pressure.

Emotion and Excitement
In emotion and excitement, increased sympathetic discharge increases blood pressure.

Exercise
During exercise, the systolic pressure always rises due to increased cardiac output.
1. Diastolic pressure depends on the degree of exercise, which increases in mild exercise due to vasoconstriction, but decreases in moderate to severe exercise due to metabolic vasodilation and increased body temperature.
2. Thus, pulse pressure is more in severe exercise.

Posture
On immediate standing from supine posture, blood pressure decreases due to venous pooling of blood in the lower limbs. However, blood pressure returns to normal or may mildly increase due to the immediate corrections initiated by baroreceptor reflex. Baroreceptor reflex controls blood pressure in 15 to 30 seconds.

Temperature
Blood pressure decreases in hot environment due to cutaneous vasodilation and increases in cold environment due to vasoconstriction.

Pregnancy
Cardiac output increases in pregnancy due to increased blood volume that increases systolic pressure.
1. Diastolic pressure falls due to decreased peripheral resistance, which occurs due to the effect of progesterone on blood vessels.
2. Progesterone relaxes smooth muscle and therefore causes vasodilation.
3. Thus, pulse pressure increases in pregnancy.

REGULATION OF BLOOD PRESSURE
The mechanisms involved in regulation of blood pressure can be divided broadly into two categories: short-term regulation and long-term regulation.
1. Short-term regulation is mainly neural.
2. Long-term regulation is mainly hormonal and renal.

SHORT-TERM REGULATION OF BP
Short-term regulation means the regulation of blood pressure within seconds or minutes to hours. Short-term regulation occurs mainly by the neural mechanisms, though the vascular and hormonal mechanisms also contribute to it.

Mechanisms of short-term regulation of blood pressure are as follows:
1. Neural mechanisms
   - Autonomic regulation
     - Sympathetic control
     - Parasympathetic control
   - Medullary control
   - Hypothalamic control
   - Cortical control
   - Reflex regulation
     - Baroreceptor reflex
     - Chemoreceptor reflex
     - Cushing reflex
     - Somatosympathetic reflex
     - Atrial stretch reflex
     - Ventricular and pulmonary stretch reflexes
     - Nonphysiological chemoreflexes (coronary and pulmonary chemoreflexes)
     - Bainbridge reflex
2. Vascular mechanisms
   - Capillary fluid-shift
   - Stress relaxation
3. Hormonal mechanisms
   - Catecholamines
   - Renin-angiotensin system
   - Antidiuretic hormone (ADH)
   - Atrial natriuretic peptide (ANP)
   - Kinin-kallikrein system
   - Histamine
   - Endothelins
   - EDRFs
   - Adrenomedullin

Neural Mechanisms

Autonomic Regulation
Autonomic control of blood pressure is mainly by sympathetic control, as systemic blood vessels are innervated by sympathetic fibers and they lack parasympathetic innervation.

Sympathetic Control
Sympathetic regulation is the main control mechanism for blood pressure as the sympathetic fibers are the final pathways of all neural inputs to the blood vessels. There are two types of sympathetic systems for blood vessels: Sympathetic vasoconstrictor system and sympathetic vasodilator system.
Sympathetic Vasoconstrictor System
Noradrenergic fibers supplying blood vessels are **vasoconstrictor in nature**. Therefore, stimulation of these fibers increases blood pressure.

1. Especially, the sympathetic innervation of resistance vessels like arterioles, small arteries and metarterioles is important for the regulation of blood pressure.
2. The sympathetic vasoconstrictor fibers originate from the intermediolateral horn of the spinal cord and innervate blood vessels, adrenal medulla and heart (Fig. 96.3).
3. Stimulation of these fibers produces vasoconstriction and therefore they are called vasoconstrictor fibers.
4. It is important to note that the sympathetic vasoconstrictor fibers have the **basal rate of tonic discharge**, which is essential for maintaining the normal vascular tone. Thus, the vascular sympathetic tone is also known as **sympathetic vasoconstrictor tone**.
5. Simultaneously, sympathetic stimulation also produces **venoconstriction**. Venoconstriction increases venous return and cardiac output and shifts blood from the venous compartment to the arterial compartment that aids in increasing blood pressure.
6. Sympathetic stimulation also increases blood pressure by its cardiostimulatory effects and effects on catecholamine secretion from adrenal medulla.

**In summary**, sympathetic stimulation increases BP by following mechanisms:
1. Vasoconstriction, especially constriction of resistance vessels.
2. Venoconstriction that increases venous return.
3. Cardiac stimulation (increased heart rate and myocardial contractility).
4. Increased secretion of catecholamines from adrenal medulla.

Sympathetic regulation of blood pressure is the crucial component of blood pressure regulation.

**Decrease in the rate of tonic discharge of sympathetic vasoconstrictor nerves produces vasodilation** that results in fall in blood pressure. For example, hypotension following spinal transection occurs due to decreased sympathetic outflow (see below).

Sympathetic Vasodilator System
Stimulation of sympathetic fibers supplying arteries and arterioles of skeletal muscles produces vasodilation as they are innervated by **sympathetic cholinergic fibers**.

1. Therefore, this system of sympathetic innervation of blood vessel is called as sympathetic vasodilator system. These fibers originate from frontal cortex and relay in hypothalamus and midbrain (Fig. 96.4).
2. However, they pass through medulla without any relay in medullary cardiovascular centers and terminate in intermediolateral gray column of spinal cord.
3. It should be noted that there is no **basal tonic discharge** of sympathetic vasodilator fibers.

Parasympathetic Control
Systemic blood vessels are not supplied by parasympathetic fibers. However, vagal stimulation decreases blood pressure by decreasing the heart rate and cardiac output.
Medullary Control

Cardiovascular centers are mainly located in the medulla. These centers primarily control the autonomic output on heart and blood vessels, which is the major cardiovascular regulatory pathway. In addition, medullary centers are major integrative centers for cardiovascular regulation as they coordinate the cortical, limbic, hypothalamic and mesencephalic influences on sympathovagal output. They also coordinate the cardiovascular interaction with respiratory centers and the ascending neural influences.

Medullary cardiovascular centers are broadly divided into two: vasomotor center and cardioinhibitory centers.

Vasomotor Center

This center is present in the rostral ventrolateral medulla (RVLM).

1. This area has caudal, intermediate and rostral parts; and accordingly they are called caudal ventrolateral medulla (CVLM), intermediate ventrolateral medulla (IVLM) and rostral ventrolateral medulla (RVLM).

2. Though RVLM is the major area in these group of neurons, these neuronal groups are collectively known as vasomotor centers (VMC), which directly projects to the intermediolateral gray column of the spinal cord (via bulbospinal pathway) from where the sympathetic vasoconstrictor fibers originate (Fig. 96.5).

3. Neurons in VMC are always active and provide tonic excitatory drive to the spinal cord neurons. The basal discharge in sympathetic vasoconstrictor nerves is due to the drive of preganglionic sympathetic neurons by the tonic discharge from the neurons of the VMC.

4. This medullary spinal cord cardiovascular axis is the most important pathway for control of blood pressure. As vasomotor center regulates activity of this pathway, control of blood pressure by it is called vasomotor control.

5. Stimulation of VMC causes intense vasoconstriction and cardioacceleration that increase blood pressure, and inhibition of VMC causes vasodilation and cardioinhibition that decrease blood pressure.

6. Influence of VMC on control of blood pressure is so crucial that loss of this influence as occurs following transection of spinal cord results in gross hypotension in the acute phase of spinal shock.

Vasomotor center receives inputs from the following structure of the body:

1. Aortic and carotid baroreceptors (inhibitory)
2. Cardiopulmonary baroreceptors (inhibitory)
3. Carotid and aortic chemoreceptors (excitatory)
4. Ascending pain pathways (excitatory)
5. Ascending pathways carrying proprioceptive information (excitatory)
6. Lungs via vagus nerves (inhibitory)
7. Limbic cortex via hypothalamus (usually excitatory, but may be inhibitory)

Fibers from limbic cortex to vasomotor center mediate change in blood pressure during emotion and excitement. Lung inflation inhibits vasomotor center that produces vasodilation and decreases blood pressure. Pain and proprioceptive stimuli increase blood pressure by stimulating vasomotor center. Carotid and aortic chemoreceptors stimulate whereas baroreceptors inhibit vasomotor center. Hypoxia and carbon dioxide directly stimulate vasomotor center.

Some authors have described pressor and depressor areas for sympathetic centers in the medulla. However, as
complexities exist for locations and interaction between these areas, and the nature of their output is confusing, we describe only the vasomotor center for sympathetic neurons in the medulla.

**Cardioinhibitory Centers**
Cardioinhibitory center in the medulla is formed by **nucleus tractus solitarius** (NTS), **nucleus ambiguous** and **dorsal motor nucleus of vagus**. Stimulation of these areas results in **bradycardia and decreased cardiac output** by two mechanisms.
1. Firstly, vagus nerve originates from cardioinhibitory areas (mainly from NTS), which on stimulation inhibits heart functions (Fig. 96.6).
2. Secondly, NTS inhibits vasomotor center via local inhibitory interneurons.
Thus, stimulation of NTS decreases sympathetic activity and increases vagal activity that in turn results in bradycardia, decreased cardiac output, vasodilation and decreased blood pressure.

**Hypothalamic Control**
Stimulation of **anterior hypothalamus** produces hypotension and bradycardia and stimulation of **posterolateral part of hypothalamus** produces hypertension and tachycardia.
1. Hypothalamus controls **cutaneous blood flow** that affects blood pressure.
2. Hypothalamus also mediates the effects of cardiovascular reflexes such as **atrial stretch reflex**.
3. A reciprocal connection exists between hypothalamus and **vasomotor center**.

**Cortical Control**
Cortex does not directly influence blood pressure. However, stimulation of motor and premotor cortices usually increases blood pressure. **Sympathetic vasodilator system originates** from frontal cortex.

**Reflex Regulation of Blood Pressure**
Regulation of blood pressure by various cardiovascular reflexes is the most important short-term mechanism as reflexes operate swiftly and effectively in response to change in blood pressure. The important reflexes are baroreceptor reflex, chemoreceptor reflex and Cushing’s reflex (the list of other reflexes is given above).

**Baroreceptor Reflex**
Among all the cardiovascular reflexes, most important is the baroreceptor reflex as it regulates blood pressure within seconds. Baroreceptor reflex is therefore a lifesaving reflex. This is also called baroreflex or **sino-aortic reflex**.

**Receptors and Stimulus**
The receptors for baroreceptor reflex are **baroreceptors**. They detect change in pressure in the blood vessels and chambers of the heart. Usually, baroreceptors are classified into two categories: high pressure and low pressure receptors. **High-pressure baroreceptors** are located in the ventricle and arterial side of circulation, and the low-pressure baroreceptors are mainly present in the atria and pulmonary circulation (cardiopulmonary baroreceptors).
1. Receptors for baroreceptor reflex are high-pressure baroreceptors that are present in the **wall of the carotid sinus and aortic arch**.
2. **Carotid sinus** is the initial dilated portion of the internal carotid artery at its origin from common carotid artery (Fig. 96.7).
3. These receptors are branched, knobby and intertwined terminals of myelinated nerve fibers.
4. Baroreceptors detect change in pressure in blood vessels in the wall of which they are located.
5. The increase in blood pressure causes distension of carotid sinus and aortic arch and stimulates receptors as they respond to stretch of the organ.
6. Conversely, decreased pressure decreases the receptor stimulation.

**Afferent Pathways**

Ninth cranial (glossopharyngeal) nerve is the afferent from carotid sinus and tenth cranial (vagus) nerve is the afferent from aortic arch (Fig. 96.8).
1. The fibers from carotid sinus in the glossopharyngeal nerve form a distinct branch called carotid sinus nerve. This is also called as buffer nerve as it buffers blood pressure when blood pressure changes.
2. The fibers in the vagus nerve that carry sensation from aortic arch form the aortic nerve. However, aortic nerve forms a distinct branch of vagus nerve only in rabbit.
3. Distension of carotid sinus and aortic arch causes stretching of the baroreceptors and increases the firing (nerve traffic) in IX and X cranial nerves respectively.

**Centers**

Centers for baroreceptor reflex are medullary cardiovascular centers.

1. These include vasomotor center and cardioinhibitory centers (described above).
2. Through bulbo-spinal pathway, vasomotor center projects to the intermediolateral gray column of the spinal cord from where sympathetic fibers originate.
3. Vagus nerve originates from NTS (the major cardioinhibitory center).
4. Normally, NTS inhibits vasomotor center via interneurons. Thus, excitation of cardioinhibitory center stimulates vagus nerve and inhibits sympathetic fibers.

**Efferent Pathways and Effector Organs**

Efferent fibers for baroreceptors are sympathetic fibers and vagus nerve.
1. Sympathetic fibers originate from intermediolateral gray column of the spinal cord, which is controlled by vasomotor center.
2. Vagus nerve originates from NTS. Vagus nerve innervates heart and sympathetic fibers innervate heart and blood vessels.

**Responses**

Responses depend on the nature of change (increase or decrease) in blood pressure. Responses also depend on degree and rate of change in blood pressure.

**When Blood Pressure Increases:** Increase in blood pressure stimulates baroreceptors in carotid sinus and aortic arch that increases the nerve traffic in IX and X cranial nerves.
1. This leads to the excitation of the NTS in the medulla, which in turn inhibits vasomotor center via interneurons.
2. Inhibition of vasomotor center decreases sympathetic output and causes vasodilation, bradycardia, decrease in cardiac output, and fall in blood pressure.
3. Excitation of NTS also directly inhibits heart by stimulating vagus nerve (Fig. 96.9).

**When Blood Pressure Decreases:** Fall in blood pressure causes less distension of carotid sinus and aortic arch that decrease receptor activity and discharge rate in theafferent nerves.
1. This inhibits nucleus tractus solitarius that in turn causes disinhibition of vasomotor center (removal of inhibition of VMC).
2. Thus, vasomotor center is stimulated that increases sympathetic discharge and causes vasoconstriction, tachycardia, increased cardiac output, and increase in blood pressure.
3. Inhibition of NTS also stimulates heart by inhibiting vagus nerve (Fig. 96.10).
4. Sympathetic activation also promotes release of catecholamines from adrenal medulla that stimulate heart and cause vasoconstriction.

**Pressure Range for Responses**
Baroreceptors regulate blood pressure in the pressure range of 50 to 200 mm Hg. However, a linear relationship is observed for the change in blood pressure and the baroreceptor discharge between pressure range of 70 to 140 mm Hg. No response is detected when pressure is less than 50 mm Hg and no further increase in response occurs when pressure is more than 200 mm Hg (Fig. 96.11).

**Types of Responses**
Baroreceptors respond to change in pulse pressure and change in mean arterial pressure.
1. Response to change in pulse pressure is called phasic or dynamic response and response to change in sustained pressure is called tonic or static response.
2. Decrease in pulse pressure without change in mean arterial pressure decreases carotid sinus discharge
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(Figs. 96.12A and B), and decrease in mean arterial pressure without change in pulse pressure also decreases sinus nerve discharge (Figs. 96.13A and B).

3. However, changes in pulse pressure and mean arterial pressure usually occur simultaneously.

**Physiological Significance of Baroreceptor Reflex**

1. When blood pressure falls, baroreceptor reflex operates within few seconds to correct the pressure, which is essential and life saving in acute hypotension and hemorrhage. This is also life saving in day-to-day activities (Application Box 96.1). For example, blood pressure falls by about 30% on standing from lying posture, which is immediately corrected by baroreceptor reflex. Thus, baroreceptor reflex is the first and foremost reflex for regulation of blood pressure.

2. Baroreceptor reflex regulates blood pressure when pressure change is within the range of 50–200 mm Hg. Thus, baroreceptors and their reflex pathway constitute a feedback mechanism to stabilize blood pressure over a wide range of fluctuation in pressure.

3. Baroreceptor reflex explains the physiological basis for **Marey’s law**, which states that heart rate is inversely proportional to blood pressure (but not the vice versa).

4. Baroreceptor resetting occurs in chronic hypertension (discussed below).

(Figs. 96.12A and B), and decrease in mean arterial pressure without change in pulse pressure also decreases sinus nerve discharge (Figs. 96.13A and B).

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Chapter 96: Regulation of Blood Pressure

**Application Box 96.1**

Baroreflex is life-saving in daily life: Change in posture, especially getting up from supine posture decreases blood pressure due to peripheral pooling of blood that decreases effective blood volume, which may be equivalent to about 15% acute hemorrhage. Such an acute hemorrhage usually leads to shock. However, baroreflex adjusts BP so efficiently and promptly that we never realize we had a situation akin to acute severe hemorrhage.

**Chemoreceptor Reflex**

Chemoreceptors are located in the aortic and carotid bodies. They respond to change in chemical composition of blood that occurs in conditions like hypoxia, hypercapnia and acidosis.

1. Afferent from carotid body is IX and from aortic body is X cranial nerve, that project to the medullary respiratory centers.
2. Afferent fibers also project to the cardiovascular centers, especially to both the vasomotor and cardio-inhibitory centers (Fig. 96.14).
3. Stimulation of chemoreceptors has two phase effects: the primary effects and the secondary effects.
4. The primary effects of chemoreceptor stimulation are bradycardia and vasoconstriction. Stimulation of cardio-inhibitory center causes bradycardia and stimulation of vasomotor center causes vasoconstriction.
5. However, hypoxia that stimulates chemoreceptors also causes pulmonary hyperventilation and increases catecholamine secretion from adrenal medulla that in turn increases heart rate (secondary effects).
6. Therefore, the net effect of stimulation of chemoreceptors is no change in heart rate or mild tachycardia and vasoconstriction that increases blood pressure.
7. The range of regulation of blood pressure by chemoreceptor reflex is 40 to 70 mm Hg.

**CNS Ischemic Response (Cushing’s Reflex)**

In conditions of gross hypotension as seen in acute severe hemorrhage, blood flow is compromised to the vital organs of the body. Decreased cerebral blood flow produces hypoxia and hypercapnia of vasomotor center.

1. Direct hypoxia and hypercapnia stimulates vasomotor center to its maximum, which causes intense vasoconstriction so that raised pressure maintains minimum blood flow to important organs. The reflex response is called as CNS ischemic response.
2. This is activated when blood pressure falls below 40 mm Hg. This is the last physiological reflex to correct blood pressure, failure of which leads to irreversible shock. Effects of raised ICP: When intracranial pressure (ICP) is high, as occurs in brain tumor (Clinical Box 96.1), the blood flow to the vasomotor center decreases due to compression of cerebral blood vessels. This causes hypoxic stimulation of vasomotor center and causes intense vasoconstriction that in turn increases blood pressure. This is known as Cushing’s reflex (Flowchart 96.1). The increase in blood pressure in carotid sinus activates baroreceptor reflex and causes reflex bradycardia.

**Clinical Box 96.1**

Bradycardia occurs in brain tumors: Increased ICP causes reflex bradycardia by activation of Cushing and baroreceptor reflexes. Therefore, bradycardia is a prominent feature of brain tumors that increase intracranial pressure.
**Somatosympathetic Reflex**

The increase in blood pressure in response to stimulation of somatic afferent nerves is called somatosympathetic reflex.

1. It is activated usually following **stimulation of ascending pain fibers**. Stimulation of proprioceptive pathway also increases blood pressure.
2. The pressor response is mediated via **vasomotor center** that receives excitatory input from ascending sensory pathway in the brainstem (Fig. 96.15).

**Atrial Stretch Reflex**

Two types of stretch receptors are present in atria: type A and type B receptors.

1. **Type A receptors** are stimulated during atrial systole and **type B receptors** are stimulated during peak atrial filling.
2. Increased venous return as occurs in fluid retention or increased blood volume increases atrial filling that stimulates type B receptors.
3. The **responses** to increased atrial filling are **vasodilation, decrease in blood pressure and tachycardia**.
4. Conversely, decreased ECF volume decreases the activity of atrial stretch receptors that **increase ADH release**. ADH in turn causes water retention and increases blood volume and pressure (Flowchart 96.2).
5. Decreased atrial stretch also stimulates sympathetic system that increases renin release, which activates renin-angiotensin-aldosterone system (see below).

**Cardiopulmonary Stretch Reflexes**

Cardiopulmonary baroreceptors are distributed in the atria (discussed above), ventricles and pulmonary vascular bed.

**Ventricular Stretch Reflex**

Increased distention of ventricle due to **excess ventricular filling** as occurs in increased blood volume (volume overload) results in **bradycardia, vasodilation and hypotension**. Thus, rise in blood pressure is checked. Ventricular stretch reflex also plays a role in maintaining **vagal tone** that checks basal heart rate.
Pulmonary Stretch Reflex
Pulmonary baroreceptors are located in pulmonary arteries. They are stimulated when pulmonary arterial pressure is increased as occurs in pulmonary hypertension. The responses observed are bradycardia and hypotension.

Nonphysiological Chemoreflexes

Coronary Chemoreflex
Chemoreceptors present in coronary arteries and ventricles are C-fiber endings.
1. The afferents from these chemoreceptors are vagal fibers.
2. Injection of chemicals like capsaicin, veratridine, phenylbiguanide and serotonin into left coronary artery produces hyperventilation, bradycardia and hypotension.
3. This is called coronary chemoreflex or Bezold-Jarisch reflex.
4. In myocardial infarction, chemical substances released from the infarcted tissue stimulate ventricular chemoreceptors and produce bradycardia and hypotension.

Pulmonary Chemoreflex
Injection of above-mentioned chemical substances into pulmonary arteries produce similar features (hyperventilation, bradycardia and hypotension). The responses are blocked by vagotomy. Such responses are observed in pulmonary embolism that produces pulmonary microinfarction.

Bainbridge Reflex
Infusion of saline or transfusion of blood produces tachycardia if the initial heart rate is low. This is called Bainbridge reflex (described by Bainbridge in 1915).
1. The receptors are tachycardia producing atrial receptors (TPAR) located in the atrial wall where vena cava open into atria (at venoatrial junction).
2. The reflex is abolished following vagotomy as the responses are mediated by vagus nerves (Flowchart 96.3).
3. The tachycardia produced by this reflex competes with the bradycardia produced by baroreceptor reflex in response to volume expansion.

Scientist contributed
Francis Arthur Bainbridge (1874–1921), British Physiologist in 1915 demonstrated acceleration of the heart rate resulting from increased blood pressure, or increased distension of the large systemic veins and the right chamber of the heart. This reflex was named after him as Bainbridge reflex.

Vascular Mechanisms
The vascular mechanisms operate within seconds to minutes of alteration in blood pressure. These are capillary fluid shift and stress relaxation.

Capillary Fluid Shift
When blood pressure decreases significantly as in acute hemorrhagic shock, the hydrostatic pressure in the capillaries decreases.
1. This causes shift of fluid from interstitial tissue space (extravascular compartment) into the intravascular compartment through the capillary membrane.
2. As a result, circulating blood volume increases and blood pressure returns to normal. In this mechanism, about 300 mL of interstitial fluid is added to the blood.
3. Reverse mechanism operates when rise in blood pressure increases capillary pressure and facilitates capillary filtration. This decreases blood volume and tends to lower blood pressure.

Stress Relaxation
When blood pressure increases abruptly, blood vessels distend in response to high pressure.
1. This imparts acute stretch on the vessel wall. The smooth muscles of the blood vessels relax in response to sudden stretch.
2. This decreases vascular tone and lowers pressure.
3. Reverse stress relaxation occurs when blood pressure decreases. Acute fall in blood pressure reduces the normal stretch of the vascular smooth muscle. This in turn causes contraction of smooth muscle and increases vascular tone, which increases blood pressure.

Hormonal Mechanisms
There are many hormones and chemicals that change blood pressure by causing either vasodilation or vasoconstriction (Table 96.1).
Catecholamines

In acute hypotension, stimulation of sympathetic fibers to adrenal medulla releases catecholamines. Catecholamines (especially, norepinephrine) are vasoconstrictors. They also increase heart rate and cardiac output. Thus, catecholamines increase blood pressure (for details, see chapter 58 “Adrenal Medulla”).

Renin-Angiotensin System

Fall in blood pressure stimulates release of renin from the JG cells of the kidney. Renin converts angiotensinogen to angiotensin I, which is further converted to angiotensin II by angiotensin-converting enzymes.
1. Angiotensin II is a potent vasoconstrictor that increases blood pressure.
2. Angiotensin II increases synthesis and secretion of aldosterone, which increases sodium and water reabsorption from kidney.
3. Angiotensin II stimulates thirst to increase water intake. It also increases water reabsorption from kidney by its direct action on proximal convoluted tubule (for details, see Chapter 75).

Vasopressin

Decrease in blood pressure and blood volume increases the release of vasopressin or antiuretic hormone (ADH) from posterior pituitary. ADH increases water reabsorption from kidney that restores blood volume and pressure. ADH also causes vasoconstriction in supraphysiological concentration (for details, see chapter 56 “Posterior Pituitary”).

Atrial Natriuretic Peptide

Atrial natriuretic peptide (ANP) is secreted from atrial myocytes in response to stretch. Increased atrial filling due to increased venous return stimulates secretion of ANP.
1. ANP produces diuresis and natriuresis that decreases blood volume (for details, see chapter 64 “Local Hormones”).
2. ANP also causes vasodilation that decreases blood pressure.
3. Another natriuretic hormone has been detected in plasma, the Na⁺-K⁺ ATPase inhibiting factor, which is proposed to be the endogenous ouabain that increases blood pressure.

Kallikrein-Kinin System

Kinins that cause vasodilation are bradykinin and lysylbradykinin. Physiologically, kinins resemble histamine.

Bradykinin

Bradykinin is formed from high molecular weight kininogen by the action of plasma kallikrein, which is formed from prekallikrein. Activated factor XII (Hageman factor) of clotting system acts on prekallikrein to convert it into plasma kallikrein.
1. Bradykinin is a vasodilator and decreases blood pressure.
2. It relaxes smooth muscle of blood vessels via nitric oxide.
3. Bradykinin also increases capillary permeability and facilitates chemotaxis (for details, see chapter 64 “Local Hormones”).

Lysylbradykinin

Lysylbradykinin is formed from low molecular weight kininogen by the action of tissue kallikrein. Tissue kallikrein is present in pancreas, kidney, intestine, salivary glands, and prostate, and in many other tissues.
1. Tissue kallikrein is located in the apical membrane of cells in these tissues and is involved primarily in transcellular electrolyte transport.
2. Lysylbradykinin increases tissue blood flow and mediates local inflammatory response.

Histamine

Histamine is a potent vasodilator and, therefore, decreases blood pressure. It is secreted from mast cells. During anaphylactic reactions, histamine is released by degranulation of mast cells that causes hypotension (for details, see chapter 64 “Local Hormones”).

Endothelins

There are 3 different types of endothelins: endothelin type 1, type 2 and type 3. Endothelin 1 is formed from big-endothelin 1, which also possesses endothelin activity.
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1. **Endothelin 1** is the most potent vasoconstrictor known so far. Veins are more sensitive to endothelin 1 than arteries.
2. It is also a potent positive chronotropic and inotropic agent (increases heart rate and myocardial contractility).
3. Thus, it increases blood pressure by causing vasoconstriction and increasing cardiac output (for details, see chapter 64 “Local Hormones”).

**Endothelium-derived Relaxing Factor**

Endothelium-derived relaxing factor (EDRF) is the nitric oxide synthesized from arginine in the endothelial cells of blood vessels.
1. It produces relaxation of vascular smooth muscle and decreases blood pressure.
2. In fact, EDRF mediates the action of many vasodilator substances.
3. Histamine (at H1 receptors), acetylcholine, bradykinin, VIP and substance P depend on EDRF for producing vasodilation.
4. However, ANP and adenosine do not depend on EDRF for their vasorelaxation effect (for details of EDRF, see chapter 64 “Local Hormones”).

**Scientists contributed**

Robert F Furchgott
Louis J Ignarro
Ferid Murad

The Nobel Prize in Physiology or Medicine 1998 was awarded jointly to three American cardiovascular physiologists Robert F Furchgott, Louis J Ignarro and Ferid Murad “for their discoveries concerning nitric oxide as a signalling molecule in the cardiovascular system”.

**Adrenomedullin**

Adrenomedullin is a polypeptide hormone secreted from adrenal medulla. It was first discovered in pheochromocytoma cells. It causes vasodilation by increasing the synthesis of nitric oxide. Adrenomedullin decreases blood pressure and inhibits aldosterone synthesis. Adrenomedullin is synthesized from proadrenomedullin, which also causes vasodilation by decreasing peripheral sympathetic activity.

**Long-term Regulation of BP**

Long-term regulation of blood pressure occurs by renal and hormonal mechanisms that primarily involve change in fluid volume of the body. Kidneys play an important role in this regulation. Resetting of baroreceptor reflex occurs in long-term elevation of blood pressure.

**Renal Mechanism**

Kidney controls blood volume by controlling urinary excretion of salt and water. As suggested by Guyton, alteration in blood pressure changes the rate of excretion of sodium and water by kidneys that tend to restore blood pressure over days and weeks (Fig. 96.16). It was suggested that this is an intrinsic property of the kidney to control blood volume and pressure.

**Hormonal Mechanisms**

Hormones that are involved in long-term control of blood pressure are aldosterone, ADH and ANP. These hormones act on kidney to regulate water and sodium excretion.

**Renin-Angiotensin-Aldosterone System**

Fall in blood pressure increases release of renin from the JG cells of the kidney. Renin converts angiotensinogen to angiotensin I, which is further converted to angiotensin II. Angiotensin II causes vasoconstriction and increases blood pressure by short-term mechanism. Angiotensin II increases synthesis and secretion of aldosterone that increases sodium and water reabsorption from kidney and increases blood pressure by long-term mechanism.

**ADH**

When blood volume decreases, ADH secretion from posterior pituitary increases. ADH increases water reabsorption from kidney and increases blood volume and pressure. It also causes vasoconstriction.

**ANP**

Increased blood volume increases venous return that increases synthesis of atrial natriuretic peptide in atrial myocytes. ANP causes natriuresis and diuresis that decreases blood pressure.

**Baroreceptor Resetting and Central Adaptation**

In chronic hypertension, baroreceptors are reset to regulate the elevated blood pressure. The firing rate of baroreceptors decreases in response to chronic hypertension. Medullary cardiovascular centers also adapt to elevated pressure.
CHAPTER SUMMARY

**Key Concepts**
1. Short-term regulation of BP is mainly neural mechanism, activated by stimulation of VMC in the medulla that results in sympathetic activation.
2. Baroreceptor reflex works within a wide range and is the first reflex to be activated by rise in BP (deactivated by fall in BP). This is the most important reflex for BP control.
3. Cushing reflex is the last reflex to increase BP in severe hypotension and hemorrhage.
4. Long-term regulation of BP is mainly by hormonal, renal and baroreceptor resetting mechanisms.

**Important to Know (Must Read)**
1. In examination, ‘Describe the short-term regulation of blood pressure’ or ‘Describe the reflex regulation of blood pressure’ or ‘Describe the regulation of BP’ is invariably asked as a Long Question.
2. Baroreceptor reflex, Chemoreceptor reflex, Cushing’s reflex, Somatosympathetic reflex, Bainbridge reflex, Long-term regulation of BP, Capillary fluid shift, Arterial baroreceptors, Medullary CV centers, Medullary control of BP, ANP, EDRF, Renin-angiotensin system, Kinins, Vascular tone, Sympathetic vasodilator system, are asked as Short Questions in exam.
3. In Viva, examiner may ask… Define BP, Define systolic BP and give its normal value, What is the significance of systolic BP, Define diastolic BP and give its normal value, What is the significance of diastolic BP, Define mean arterial pressure and give its normal value, What is the significance of mean arterial pressure, Define pulse pressure and give its normal value, What is the significance of pulse pressure, Define casual BP, What is basal BP, What is vascular tone, What is the mechanism of smooth muscle contraction and relaxation, List the factors affecting BP, Name the physiological conditions that alter BP, Why the BP is less in females, Why the BP is less during sleep, What happens to the BP after eating and why, What happens to BP following emotion and excitement and why, What is the effect of exercise on BP, What is the effect of posture on BP, What is the effect of temperature on BP, What is the effect of pregnancy on BP, What is sympathetic vasoconstrictor system, What are the mechanisms by which sympathetic stimulation increases BP, What is sympathetic vasodilator system, What is the role of vasomotor center in the regulation of BP, What is the role of cardioinhibitory center in the regulation of BP, How does the hypothalamus regulate BP, How does the cortex affect BP, Name the short-term mechanisms for regulation of BP, Name the neural mechanisms for regulation of BP, Name the reflexes for regulation of BP, What is the response of baroreceptor reflex to increase in BP, What is the response of baroreceptor reflex to decrease in BP, What is the dynamic response of baroreceptor, What is the static response of baroreceptor, What are the physiological significances of baroreceptor reflex, What is the effect of stimulation of chemoreceptors, What is Cushing’s reflex, What is somatosympathetic reflex, What is atrial stretch reflex, What is Bainbridge reflex, List the cardiopulmonary stretch reflexes, What is ventricular stretch reflex, What is pulmonary stretch reflex, List the nonphysiological chemoreflexes, What is coronary chemoreflex, What is pulmonary chemoreflex, List the vascular mechanisms for regulation of BP, How does capillary fluid shift help to regulate BP, How does stress relaxation help to regulate BP, List the hormonal mechanisms for regulation of BP, Name the chemicals or factors that cause vasodilation, Name the chemicals or factors that cause vasoconstriction, Name the long-term mechanisms for regulation of BP, What is the meaning of baroreceptor resetting and central adaptation to high BP.
Learning Objectives

On completion of study of this chapter, the student MUST be able to:
1. Understand the importance of integrated regulation of cardiovascular functions (CVF).
2. Appreciate the principles of neural and hormonal control of CVF.
3. Learn the local control systems of peripheral blood flow.
4. Explain the mechanism and importance of autoregulation of blood flow.
5. Understand how integrated regulation of CVF is essential for stable body functions in different situations, such as during exercise, fight-and-flight response, etc.

The student MAY also be able to:
1. Describe the integrated mechanisms of regulation of cardiovascular functions.

General Concept

Major functions of cardiovascular system are generation and control of heartbeat (pulse rate), pumping of blood by ventricles (stroke volume or cardiac output) that maintains circulation and blood flow to different parts of the body, and control of blood pressure that maintains tissue perfusion. The details of regulation of heart rate, cardiac output, and blood pressure have been discussed in previous chapters. However, regulations of various cardiovascular parameters and mechanisms occur simultaneously and are interdependent on each other. Integrated mechanisms play a critical role in regulation of cardiovascular function in exercise, fight-or-flight reactions, emotion, syncope and so on.

The mechanisms controlling cardiovascular (CV) functions can be divided into three systems:
1. Neural control systems
2. Hormonal control systems
3. Local control systems

Integrated regulation of cardiovascular functions is the result of integration of activity by all the three-control systems.

1. Blood volume and blood pressure are regulated by neural and hormonal mechanisms. A satisfactory blood volume is required to maintain adequate cardiac output and blood pressure, and a normal blood pressure is necessary to maintain adequate tissue perfusion.
2. Blood volume is monitored by stretch receptors located in the low-pressure compartments of circulation (veins and atria) whereas blood pressure is monitored by stretch receptors in the high-pressure compartments of circulation (arteries and ventricles).
3. Afferent impulses from these receptors integrate in cardiovascular centers in the medulla oblongata that adjusts cardiac output, vessel diameter, and absorption of fluid from kidney to maintain blood volume and arterial pressure.
4. This is mainly achieved by altering sympathetic activity and by adjusting secretion of various hormones, such as ADH, angiotensin II, aldosterone, and ANP (for details, see the previous chapter). Neural mechanisms play a major role in short-term regulation, whereas hormonal mechanisms play role in both the short-term and long-term regulations of cardiovascular functions.
5. The mechanisms that regulate blood volume and pressure also regulate heart rate, cardiac output, vessel diameter, blood flow and peripheral resistance.
Neural regulation of the cardiovascular functions involves autonomic regulation, spinal regulation, medullary control, reflex regulation and supramedullary (hypothalamic and cortical control).

Autonomic Regulation

Autonomic control consists of sympathetic and parasympathetic controls.

Control of Heart Functions

The heart is innervated by parasympathetic and sympathetic fibers. The two divisions of the autonomic nervous system tend to oppose each other but work in a reciprocal and balanced fashion.

Parasympathetic Control

Parasympathetic fibers (vagus nerve) are cholinergic and mainly innervate SA node, AV node, conducting tissues up to His bundle and atrial muscles (ventricular innervation is sparse). Acetylcholine released from the nerve ending acts on muscarinic receptors present in these tissues. Stimulation of vagal fibers slows heart rate significantly. Under normal conditions, heart rate is the function of vagal activity (vagal tone). It also decreases conduction velocity and cardiac output. The myocardial contractility is less affected as ventricles are sparsely innervated by parasympathetic fibers. However, parasympathetic fibers also terminate on sympathetic nerves, and stimulation of vagal fibers inhibits the release of norepinephrine from sympathetic nerve terminals. Therefore, in the presence of sympathetic activity, parasympathetic activation reduces cardiac contractility.

Sympathetic Control

Sympathetic fibers to the heart innervate SA node, AV node, conducting tissues and atrial and ventricular muscles. They release norepinephrine, which binds to \( \alpha_1 \)-adrenergic receptors. Sympathetic stimulation increases heart rate, conduction velocity, myocardial contractility and cardiac output. For details refer Chapter 96.

Control of Vascular Functions

Generally, blood vessels in the systemic circulation receive sympathetic innervation only. They release norepinephrine, which binds to \( \alpha_2 \)-adrenergic receptors. Stimulation of sympathetic fibers causes vasoconstriction. Basal sympathetic activity (sympathetic tone) maintains vascular tone. Epinephrine released from the adrenal medulla binds to \( \beta_2 \)-adrenergic receptors and produce vasodilation. Postganglionic parasympathetic fibers to blood vessels in the external genitalia release acetylcholine and nitric oxide that produce vasodilation.

Spinal Control of CV Functions

Sympathetic neurons originate from thoracolumbar segments of the spinal cord. The sympathetic tone produces a background level of vasoconstriction, which contributes to the maintenance of normal blood pressure. This tonic sympathetic activity is generated by signals arising from the medulla oblongata. In spinal cord transection, the loss of excitatory signals from medulla produces significant fall in blood pressure in the stage of spinal shock.

Medullary Integration of CV Functions

Four major cardiovascular functions are performed by medulla:

1. The vasomotor center of medulla generates tonic discharge that directly determines spinal sympathetic output.
2. Medulla receives inputs from various receptors located in different parts of circulatory and respiratory systems and accordingly integrates various cardiovascular reflexes.
3. Medulla receives signals from supramedullary neural networks, especially from cortex and limbic system, and therefore mediates the cardiovascular responses in emotion, exercise, etc.
4. Circulating hormones and drugs also act on medullary centers to modulate cardiovascular responses.

For details of medullary control, refer Chapter 96.

Reflex Control

Reflexes that control cardiovascular functions are baroreceptor reflex, chemoreceptor reflex, Cushing’s reflex, somatosympathetic reflex, Bainbridge reflex, and other cardiopulmonary baro- and chemoreflexes. Reflexes play an important role in short-term regulation of cardiovascular functions, especially for control of blood pressure. For details of each cardiovascular reflex, refer Chapter 96.

Effect of Blood Volume on Cardiopulmonary Baroreceptors

Cardiopulmonary baroreceptors located in the atria, at the venoatrial junction (junction of the great veins and atria), in the ventricular myocardium, and in thoracic vessels are involved in the control of blood volume and pressure. The afferent fibers from these receptors project to medullary and supramedullary areas, especially to hypothalamus. Decreased blood volume decreases firing of cardiopulmonary baroreceptors that in turn increases sympathetic activity and decreases parasympathetic nerve activity. Increased blood volume on the other hand enhances the activity of cardiopulmonary baroreceptors and inhibits sympathetic activity. However, alteration in blood volume affects cardiovascular functions mainly by altering secretion of various hormones.
Reflex Responses Induced by Pain Receptors

Stimulation of pain receptors causes increased sympathetic activity via somatosympathetic responses. These events increase heart rate, cardiac output and arterial pressure. The example of reflex action is the cold pressor response in which the blood pressure and heart rate increase when a limb is placed in ice water. However, a second type of response is produced by induction of deep pain. For example, the stimulation of deep pain fibers associated with distension of the abdominal viscera or crush injury results in diminished sympathetic activity and enhanced parasympathetic activity that decrease cardiac output and blood pressure. These responses contribute to development of traumatic shock (for details, refer Chapter 101).

Integrated Supramedullary CV Control

The higher centers of organization for autonomic, spinal and medullary controls are supramedullary networks of neurons located in the limbic cortex, amygdala, and hypothalamus. These supramedullary centers are not important for regulation of cardiovascular functions under normal conditions. However, they play significant role in cardiovascular control mechanisms in specific emotional and behavioral changes. The influences are mediated mainly via their projections to sympathetic system. Unlike medullary vasomotor center, supramedullary networks do not contribute to the tonic maintenance of vascular tone and blood pressure.

CV Changes in Fight-or-Flight Reaction

The initial responses in fight-or-flight reaction include increased skeletal muscle tone and general alertness. The sympathetic neural activity to blood vessels and the heart is increased. This results in increase in heart rate, cardiac output, and blood pressure. These responses are mediated via limbic system-hypothalamo-sympathetic axis. In humans, emotional situations often provoke the fight-or-flight response.

CV Changes in Vasovagal Syncope

Vasovagal syncope is a type of fainting in which somatic and cardiovascular responses occur in response to certain emotional experiences. Stimulation of certain limbic cortical areas results in relaxation of skeletal muscles, depression of respiration, and loss of consciousness. The cardiovascular changes include profound bradycardia and hypotension induced by vagal stimulation and withdrawal of resting sympathetic vasoconstrictor tone. The immediate decrease in mean arterial pressure lowers cerebral blood flow that results in unconsciousness.

CV Changes in Exercise

In exercise, in the initial part, withdrawal of vagal tone to the heart results in increase in heart rate and cardiac output. The increased cardiac output supplies blood to exercising muscle. In the later stage as intensity of exercise increases, sympathetic tone increases that increases heart rate and contractility. It activates sympathetic vasodilator system to skeletal muscle that increases blood flow to the exercising muscle. It also stimulates sympathetic vasoconstriction fibers that redistribute blood from splanchnic vascular bed to skeletal muscles. Finally, afferent impulses from exercising skeletal muscle terminate in the medulla that further augment sympathetic tone. Vagal withdrawal and sympathetic stimulation in exercise are mainly due to central commands initiated in cortex. For details of cardiovascular changes in exercise refer Chapter 157 (Physiological changes in exercise).

Though, normally medullary cardiovascular control mechanisms predominate over other control mechanisms, sometimes in special circumstances supramedullary control mechanisms override medullary cardiovascular reflex activities. For example, during fight-or-flight reaction, the heart rate increases despite a simultaneous rise in arterial pressure. In these conditions, the hypothalamic-medullary projection inhibits medullary centers that in turn inhibit the baroreceptor reflex activity. The corticohypothalamic response predominates over the medullary response. Another example is exercise, during which the inputs from supramedullary regions inhibit the baroreceptor reflex, leading to increase in sympathetic tone and decrease in parasympathetic tone despite an increase in arterial pressure.

HORMONAL CONTROL SYSTEMS

Various hormones play an important role in the control of cardiovascular functions. Hormones are secreted by neural stimulation, by reflex activation or by the direct hemodynamic effects on the blood vessels or heart. The important organs that secrete hormones for cardiovascular control are adrenal medulla, posterior pituitary, kidney, atrial muscle and vascular endothelium. Catecholamines, ADH, angiotensin, ANP, EDRF, endothelins, histamines and kinins are among important hormones. For details of hormonal mechanisms refer Chapter 96 (Hormonal control of blood pressure).

LOCAL CONTROL SYSTEMS

(Regulation of Peripheral Blood Flow)

The local control systems mainly regulate the peripheral blood flow to various organs. The amount of blood flowing through an organ depends mainly on the diameter of artery and arterioles supplying the organ. If the vessels are dilated, blood flow increases and if the vessels are constricted, blood flow decreases. Diameter of vessels supplying an organ depends on the state of sympathetic and parasympathetic activity to that organ. However, many organs regulate their blood flow according to their need.
via autoregulatory mechanisms. Regulation of peripheral blood flow is broadly divided into two categories: intrinsic and extrinsic regulations.

**Intrinsic Regulation**

Intrinsic regulation of blood flow is due to operation of two mechanisms: autoregulation and endothelium-mediated regulation.

**Autoregulation of Blood Flow**

Autoregulation is defined as *capacity of tissues to regulate their own blood flow without any external influence*. Autoregulatory mechanisms for blood flow are well developed in many organs like kidney, heart, liver, brain and skeletal muscle. The possible theories that have been advocated to explain the autoregulation of blood flow are myogenic theory, metabolic theory and tissue pressure theory.

**Myogenic Theory**

According to myogenic theory, vascular smooth muscles contract in response to increased transmural pressure and relax in response to decreased transmural pressure.

1. When perfusion pressure increases abruptly, the *initial blood flow to the organ increases*.
2. However, increased pressure distends the vessel that causes *contraction of vascular smooth muscles*.
3. *Vasoconstriction decreases the blood flow* and returns blood flow to the previous levels. Degree of constriction is proportional to the degree of distending pressure.

**Metabolic Theory**

According to metabolic theory, blood flow to an organ or tissue is controlled by the metabolic activity of the tissue.

1. When blood flow decreases to the tissue, decreased oxygen supply results in formation of *vasodilator metabolites* that dilate the blood vessels and restore the flow.
2. The metabolites formed in the tissue act locally to dilate the resistance vessel.
3. Also, when metabolic activity increases, the metabolites accumulated in the tissue cause vasodilation that increases blood flow. This helps to wash the metabolites from the tissue.

   The known metabolic vasodilators are:
   - Lactic acid
   - CO₂
   - Hypoxia
   - Hydrogen ion
   - Potassium ion
   - Inorganic phosphate
   - Interstitial fluid osmolality
   - Adenosine
   - Local temperature

**Endothelium-mediated Regulation of Blood Flow**

Endothelium of blood vessels secretes vasoactive substances like endothelins and vasorelaxing substances like EDRF (endothelium-derived relaxing factor). These chemicals are secreted in various situations to alter blood flow locally according to the need of the organ or tissue.

**Extrinsic Regulation**

Extrinsic control of local blood flow is mainly neural and hormonal in nature.

**Neural Regulation**

Neural control is divided into sympathetic and parasympathetic control and reflex regulation.

**Sympathetic Control**

Stimulation of sympathetic fibers supplying the organ or tissue causes vasoconstriction and decreases blood flow.

**Parasympathetic Control**

Stimulation of parasympathetic fibers supplying the organ or tissue causes vasodilation and increases blood flow.

**Vascular Reflexes**

Activation of various cardiovascular reflexes like baroreceptor reflex or chemoreceptor reflex control blood flow by altering the diameter of resistance vessels (for details, refer Chapter 96).

**Hormonal Regulation**

Hormones that act on β-receptors like epinephrine cause vasodilation and increase local blood flow, and hormones that act on α-receptors like norepinephrine cause vasoconstriction and decrease local blood flow. Simulation of cholinergic and histaminergic receptors also causes vasodilation and increases blood flow.
### KEY CONCEPTS

1. Regulation of cardiovascular function is usually integrated. For example, the system activated to control HR, also affects cardiac output and BP.

2. Effective integrated regulation usually more widespread and effective in stressful and emergency situation.

### Important to Know (Must Read)

1. In examination, Long Questions are usually not asked from this chapter.
2. ‘Autoregulation of blood flow’ may be asked as a Short Question in exam.
3. In Viva, examiner may ask… What are the neural control systems for regulation of cardiovascular functions, How does autonomic control regulate heart functions, How does autonomic control regulate vascular functions, How does spinal cord control cardiovascular functions, How does medulla control cardiovascular functions, List the reflexes that regulate cardiovascular functions, What is the effect of blood volume on cardiopulmonary baroreceptors, What are the reflex responses induced by pain receptors, What is the role of supramedullary centers in the regulation of cardiovascular functions, What are the cardiovascular changes in fight-or-flight reaction, What are the cardiovascular changes in vasovagal syncope, What are the cardiovascular changes in exercise, List the hormones that regulate cardiovascular functions, List the regulating systems for peripheral blood flow, List the mechanisms of intrinsic regulation of peripheral blood flow, List the theories for autoregulation of blood flow, What is myogenic theory, What is metabolic theory, What is tissue pressure theory, What is endothelium-mediated regulation of blood flow, List the mechanisms of extrinsic regulation of peripheral blood flow, How is the neural regulation of local blood flow mediated, How is the hormonal regulation of local blood flow mediated.
Regional Circulations

LEARNING OBJECTIVES
On completion of study of this chapter, the student **MUST** be able to:

1. Understand the importance of regional circulations.
2. Give the normal values of blood flow (expressed in mL/100 gm/min) to important visceral organs.
3. List the special features of cerebral, coronary, cutaneous, splanchnic and skeletal muscle circulations.
4. Understand the regulatory mechanisms of cerebral, coronary, cutaneous, splanchnic and skeletal muscle circulations.
5. Understand the pathophysiology of stroke and AMI, and give the physiological basis of their treatment.
6. Define triple response and explain its mechanism.

The student **MAY** also be able to:

1. Describe the factors controlling various regional circulations and explain their mechanisms of regulation.

INTRODUCTION
Cardiac output is distributed to different parts and organs of the body according their metabolic need and participation in carrying out various functions of the body.

1. The blood flow expressed in unit time per unit weight of the tissue is maximum to the carotid body, which is 2000 mL/100 gm/min, and is minimum to the skeletal muscle, i.e. 2.7 mL/100 gm/min.

2. Among the organs, flow is maximum to kidney (420 mL/100 gm/min), followed by heart (84 mL/100 gm/min), liver (58 mL/100 gm/min) and brain (54 mL/100 gm/min) (Table 98.1).

3. There are special arrangements of blood vessels and special regulatory mechanisms in these organs to meet their metabolic requirements.

CEREBRAL CIRCULATION
Brain is the most essential organ as the controlling centers for all vital functions, such as pulse, blood pressure, respiration and temperature of the body are located in it.

| Table 98.1: Blood flow and oxygen consumption of different visceral organs. |
|---------------------------------|---|---|---|---|---|---|---|---|
|                  | Weight (Kg) | BF mL/min | BF (mL/100g/min) | % of CO | OC (mL/min) | OC (mL/100 g/min) | % of total OC | AV-OD (mL/L) |
| Kidneys          | 0.3          | 1260      | 420              | 23.5    | 18          | 6                | 7.2           | 14          |
| Heart            | 0.3          | 250       | 84               | 4.7     | 29          | 9.7              | 11.6          | 114         |
| Liver            | 2.6          | 1500      | 58               | 27.8    | 51          | 2                | 20.5          | 34          |
| Brain            | 1.4          | 750       | 54               | 13.9    | 46          | 3.3              | 18.4          | 62          |
| Skin             | 3.5          | 460       | 12.8             | 8.6     | 12          | 0.3              | 4.8           | 25          |
| Skeletal muscle  | 31           | 840       | 2.7              | 15.6    | 50          | 0.2              | 20            | 60          |
| Rest of body     | 24           | 340       | 1.4              | 6.2     | 44          | 0.2              | 17.6          | 129         |
| Whole body       | 63           | 5400      | 8.6              | 100     | 250         | 0.4              | 100           | 46          |

(BF: Blood flow; CO: Cardiac output; OC: Oxygen consumption; AV-OD: Arteriovenous oxygen difference).
Brainstem contains cardiovascular and respiratory centers. Hypothalamus controls visceral functions including regulation of body temperature. Cortex is the seat of all higher cognitive functions including language and speech. Sensory processing, motor activities and behavioral functions are integrated in different parts of the brain. Thus, intact and adequate cerebral blood flow is essential to carry out these important functions, which is one of the primary objectives of cardiovascular system. At the time of shock, cardiovascular regulatory mechanisms operate to maintain at least minimum cerebral blood flow so that visceral and vital centers remain alive. Viability of the brain is so important that in medicolegal cases, brain death is ensured (absence of EEG tracing) before declaring the death of an individual.

**Cerebral Metabolism**

1. Brain tissue is **highly sensitive to hypoxia**. Therefore, adequate blood supply to brain tissue should be continuously maintained. The stoppage of blood flow for more than 15–30 seconds results in unconsciousness, and for more than 5 minutes causes irreparable damage (leads to coma).

2. Brain utilizes **glucose as the main fuel**. Therefore, prolonged hypoglycemia results in cerebral dysfunction. However, in conditions of chronic scarcity, the brain can utilize ketone bodies. It should be noted that utilization of glucose by tissues of the brain (except ventromedial hypothalamus) is independent of insulin.

3. The **metabolic requirements** of brain remain fairly constant irrespective of activities of the brain tissue and cerebral blood flow (CBF).

**Special Features of CBF**

1. Cerebral arteries are **end arteries**.

2. Brain is present in the cranium, the rigid cage. The intracranial contents are incompressible. Therefore, a unique feature of cerebral circulation is that increase in cerebral blood flow (e.g. arteriolar dilation) is usually associated with comparable increase in venous outflow.

3. In the brain, volume of blood and extravascular fluid remains relatively constant. The change in one component is associated with opposite alteration in the other.

4. The capillaries in brain are mostly **non-fenestrated**.

5. The cerebral blood vessels (especially capillaries) are surrounded by the **end-feet processes of astrocytes**. This provides the anatomical basis for the formation of **blood-brain barrier** (Fig. 98.1).

   - Due to the presence of BBB, substances from brain tissue like neurotransmitters cannot easily enter general circulation, and substances from blood also cannot easily enter the brain tissue.

   - BBB provides protection to the brain from the toxic and harmful substances circulating in the blood.

   - As BBB is not well developed in infants and children, **kernicterus** (a neurological complication that occurs due to deposition of bilirubin in the basal ganglia) occurs in them in hemolytic jaundice.

   - BBB is also disrupted by inflammation of brain tissue, and brain tumors.

6. The mechanism for **vesicular transport** from blood into brain tissue via endothelial wall of the cerebral blood vessels is less developed.

7. The **tight junctions** between the endothelial cells of capillaries are very tight and, therefore, do not permit the passage of substances through them. The **basement membrane of capillaries is also thick**. These anatomical modifications contribute to the formation of BBB.

**Cerebral Blood Vessels**

**Arterial Supply**

The brain receives blood supply from two major sources:

1. The **vertebrobasilar system**: Two vertebral arteries join to from the basilar artery, which finally merges into the circle of Willis.

2. The **internal carotid arteries**: Two internal carotid arteries along with basilar artery form the circle of Willis (Fig. 98.2).

   - The **circle of Willis**, which is formed by basilar artery and two internal carotids, gives rise to three pairs of large vessels supplying the brain. These are anterior cerebral, middle cerebral and posterior cerebral arteries. There is
hardly any overlapping of the territories of brain tissues supplied by these cerebral arteries. Thus, functionally, they are *end arteries*. Therefore, block in any of the artery results in ischemic damage to the part supplied by the artery.

**Venous Drainage**

The venous drainage occurs by the deep veins and dural sinuses. They drain into the internal jugular veins.

### Innervation of Cerebral Vessels

Cerebral blood vessels are innervated by sympathetic, parasympathetic and sensory fibers.

#### Sympathetic Supply

Sympathetic fibers are *vasoconstrictors*. Cell bodies of sympathetic neurons are located in superior cervical ganglia. They release norepinephrine and neuropeptide Y at their nerve endings. However, vasoconstriction effect of sympathetic stimulation on cerebral blood vessels is less marked.

#### Parasympathetic Supply

The parasympathetic innervation is not well developed. These fibers secrete acetylcholine, VIP and PHM–27. Cell bodies of cholinergic neurons are present in sphenopalatine ganglia. Stimulation of parasympathetic fibers results in vasodilation.

**Sensory Neurons**

The sensory nerves contain substance P, CGRP, neuropeptide A and VIP. These neurotransmitters *cause vasodilation*. Cell bodies of sensory neurons are located in trigeminal ganglia. The cerebral blood vessels are *highly sensitive to pain*. Pressure on cerebral blood vessels causes pain. But interestingly, the brain tissues do not receive pain fibers.

### Normal Values of CBF

The brain weighs about 1.4 kg. The total cerebral blood flow is 750 mL per minute, which is about 14% of cardiac output. This accounts for about *54 mL of blood per 100 gm of brain tissue per minute*. Oxygen consumption of brain is about 45 mL per minute, which is about 3.3 mL per 100 gm per minute.

**Measurement of CBF**

Cerebral blood flow (CBF) can be measured by various methods. The important methods are:

1. Kety method
2. By using radioactive substances
3. By using flow meters
4. Positron emission tomography (PET)
5. Single photon emission computerized tomography (SPECT)

#### Kety Method

This method uses *Fick’s principle*. In this method, nitrous oxide is used. The subject inhales *15% of nitrous oxide* for 10 minutes. The arterial sample and the venous sample (from internal jugular bulb) are collected and the arteriovenous difference is measured.

\[
\text{CBF} = \frac{\text{Amount of nitrous oxide taken by the brain}}{\text{Arteriovenous difference of nitrous oxide across the brain}}
\]

#### Using Radioactive Substances

Radioactive substance usually used is radioactive Xenon (\(^{133}\text{Xe}\)). The substance is injected into the carotid artery and the radioactivity of different areas of the brain is measured by placing scintillation counters around the skull. This is a useful method for studying the regional distribution of CBF in different parts of the brain.

#### Using Flowmeters

The flowmeters can be directly placed in the cerebral arteries in experimental animals or in patients undergoing neurosurgery and CBF can be measured accurately.

**Positron Emission Tomography (PET)**

This is mainly used for monitoring regional blood flow of various parts of the brain. In this method, a *short-lived radioisotope* is used to label a substance, which is injected. Scintillation detectors placed on the head to monitor the
appearance and clearance of the tracer. The information from detectors is processed in a computer that quantifies the flow in a particular region of the brain. Single photon emission computerized tomography (SPECT) is also used for the purpose.

*Magnetic Resonance Imaging (MRI)*

This technique is based on detecting resonant signals from different tissues in magnetic field. The resolution of MRI is better than the PET. Recently, developed fMRI (functional magnetic resonance imaging), measures blood supply to a specific area of the brain.

**Regulation of CBF**

CBF is affected by alteration in intracranial pressure, neural and metabolic factors, and autoregulatory mechanisms.

**Role of Intracranial Pressure**

The brain tissue and cerebrospinal fluid (CSF) are essentially incompressible. Thus, at any given time, the total volume of blood, CSF volume and brain tissue in the cranial cavity remains constant. This is called Monro-Kellie doctrine.

1. Any increase in venous pressure causes similar increase in intracranial pressure (ICP). Rise in intracranial pressure results in compression of cerebral arteries that decreases blood flow.
2. This compensates for changes in arterial blood pressure at the level of head. Increase in arterial pressure as occurs during downward acceleration, increases arterial pressure in the head.
3. However, ICP also rises simultaneously. Increased ICP prevents rupture of blood vessels by supporting them.
4. Thus, increased ICP as occurs in downward acceleration or straining (e.g., strenuous defecation during constipation) helps in protecting cerebral vessels.

In brain tumors, compression of cerebral vessels decreases blood flow. Decreased blood flow causes hypoxia at vasomotor center. This activates Cushing’s reflex, which tries to restore cerebral blood flow.

**Neural Regulation**

Stimulation of sympathetic fibers causes vasoconstriction, but it is not important, as the vasoconstrictor system is not well developed in cerebral vascular bed. Parasympathetic stimulation causes vasodilation.

**Metabolic Regulation**

CBF is significantly altered by production of local vasodilator metabolites. The vasodilator substances are usually potassium, hydrogen, and adenosine. The cerebral vessels also respond to change in carbon dioxide and oxygen. Acidosis, hypoxia, and hypercapnea in the brain tissue produce potent vasodilation. A fall in pCO₂ results in cerebral vasoconstriction as seen during hyperventilation.

**Autoregulation**

CBF remains relatively constant within a pressure range of 60–140 mm Hg (Fig. 98.3). The autoregulation may be due to neural and metabolic factors.

**Clinical Importance**

1. Disruption of BBB occurs at the sight of tumor or inflammation. Therefore, radioactive study helps in identifying the site of such lesions.
2. As the cerebral blood vessels do not allow all the substances to enter into the brain tissue, the physician must know the penetrability of a drug into the brain tissue and accordingly prescribe the drug in proper concentration.
3. In cerebral infections or inflammations, due to disruption of BBB the organisms get access into the general circulation and spread to the other parts of the body.
4. Stroke: Interruption of blood supply to a part of the brain causes ischemic damage to that part. This is called stroke.

**Stroke**

*Types of Strokes*

There are two types of strokes: hemorrhagic and ischemic strokes. Hemorrhagic stroke usually occurs due to rupture of a branch of cerebral artery at the site of aneurysm. Rupture of Charcot’s artery (the artery of cerebral hemorrhage), i.e., the lenticulostriate branch of middle cerebral artery is the common cause of stroke that produces contralateral hemiplegia. Ischemic stroke occurs due to blockage of an artery by thromboembolism.
Treatment of Stroke

The treatment of stroke includes antiexcitotoxic and fibrinolytic drugs.

- **Fibrinolytic drugs:** Fibrinolytic (clot lysing) drugs like t-PA given early in the course of stroke is very useful. Therefore, it is important to diagnose and initiate the treatment of stroke at the earliest possible.

- **Antiexcitotoxic drugs:** Normally, brain cells take up and utilize glutamate. Ischemia of brain tissue decreases glutamate uptake that increases local glutamate concentration. Glutamate causes excitotoxic neuronal damage of the brain tissue (excitotoxic lesion). Therefore, antiexcitotoxic drugs are very helpful in the treatment of stroke.

**CORONARY CIRCULATION**

Coronary circulation is important as it supplies blood to the heart, a key vital organ of the body. Cessation of heart functions for more than a couple of minutes is life threatening. Therefore, it is essential to maintain an uninterrupted and adequate blood supply to the heart.

**Blood Supply**

**Arterial Supply**

The heart is supplied by right and left coronary arteries that originate from the root of the aorta behind right and left cusps of aortic valve respectively.

1. The right coronary artery principally supplies the right ventricle and the right atrium, and the left coronary artery supplies the left ventricle and the left atrium. Physiologically, there are no anastomoses between the right and left coronary arteries (Fig. 98.4).
2. Thus, the coronary arteries are end arteries in human beings as the territories of blood supply by these arteries do not overlap.
3. The sinuses of Valsalva (small outpouches of the aorta) are present behind the semilunar valves where the eddy currents develop which keep the valve leaflets away from the wall of the vessels during systole. In fact, the cusps are not totally pushed to the walls of the aorta during systole; rather they float in the blood in between the wall in their closing position.
4. This allows blood to enter into the coronary arteries (these arteries arise from aorta at their origin, behind the cusps of the valve) to some extent, as the coronary ostia are not blocked by the valve cusps.

**Venous Drainage**

The heart has multiple venous drainage systems. The major system consists of the coronary sinus and the anterior cardiac veins that drain into the right atrium. The minor systems consist of arteriosinusoidal vessels that drain from arterioles to cardiac chambers, and arterioluminal vessels that directly drain from coronary arteries to heart chambers.

**Innervation of Coronary Blood Vessels**

The coronary blood vessels are supplied by sympathetic and parasympathetic fibers.

- **Sympathetic innervation:** Sympathetic stimulation to the heart causes coronary vasodilation as coronary arteries are rich in α receptors.
- **Parasympathetic innervation:** Parasympathetic stimulation to the heart causes vasodilation, which is mediated via cholinergic receptors.

**Special Features of Coronary Circulation**

1. Heart receives its major blood supply during diastole. Coronary blood flow decreases during systole and increases during diastole. The cardiac blood vessels are mechanically compressed during systole due to contraction of the ventricular muscle. Therefore, blood flow during systole decreases to the heart muscle. This vasocompression effect is maximally observed in the deeper layers (sub-endocardial layers of the myocardium). The vasocompression during systole is more marked in the left ventricle than right ventricle, as the pressure generated by left ventricle is significantly more. During diastole, relaxation of cardiac muscle increases coronary blood flow.
2. The coronary arteries are end-arteries. Therefore, blockage of the coronary arteries results in ischemia and infarction of the cardiac muscles supplied by the artery.
3. The metabolic regulation of coronary circulation is well developed. Therefore, coronary blood flow is adjusted according to the metabolic need of the myocardium.
4. Unlike cerebral circulation, significant alteration occurs in the coronary blood flow depending on the cardiac activities. Coronary blood flow can be increased about four to five folds to meet the heart’s more oxygen need during exercise. Thus, there is adequate coronary blood flow reserve.

5. The heart utilizes wide varieties of substrates for its metabolism. These include free fatty acids, glucose, pyruvate, lactate, ketone bodies, and amino acids. But the major source (about 1/3) of energy supply is free fatty acids.

6. Heart muscle extracts about 80% of the oxygen from the arterial blood. Thus, arteriovenous oxygen difference is very high in heart even at rest. Therefore, major method to increase the oxygen supply to the heart as required during exercise is to increase the coronary blood flow.

**Normal Values**

Heart weighs about 300 g. The blood flow to the heart is approximately 250 mL per minute, which is about 85 mL per 100 gm of the cardiac tissue per minute. The rate of coronary blood flow is the second highest in the body, next to the renal blood flow, which is 420 mL per 100 g of tissue per minute. The coronary blood flow constitutes about 4.7% of the total cardiac output. However, the rate of oxygen consumption of heart is highest of all organs of the body, which is 9.7 mL per 100 g of tissue per minute.

**Measurement of Coronary Blood Flow**

Coronary blood flow is measured by direct method or indirect methods. Indirect methods are Fick method or radionuclide method.

**Direct Method**

Coronary blood flow can be measured directly by placing an electromagnetic flow meter in the coronary artery. This is used in experimental animals and in humans undergoing open-heart surgery.

**Using Fick Principle**

In this method, subject inhales a mixture of air and an inert gas (like nitrous oxide) till the gas is distributed in the tissues according to its partition co-efficient. Then the arterial blood (from any peripheral artery) and venous samples (from coronary sinus) are collected. The coronary blood flow is calculated as the ratio of the amount of the inert gas passing through the coronary arteries in unit time to the average arteriovenous gas concentration difference.

\[
\text{Blood flow} = \frac{\text{Amount of inert gas taken up/min}}{\text{Arteriovenous difference of the gas}}
\]

**Radionuclide Methods**

A radioactive substance like thallium (\(^{201}\text{TI}\)) is injected intravenously. The gamma camera is placed on the chest to monitor the thallium uptake by the heart. Radioactive xenon (\(^{133}\text{Xe}\)) can also be used for the purpose. By this method, the difference in blood supply through individual arteries to different parts of the heart can also be measured.

**Regulation of Coronary Blood Flow**

Coronary blood flow is regulated by neural factors, metabolic factors, physical factors and autoregulatory mechanisms.

**Neural Regulation**

Neural regulation is by either sympathetic or parasympathetic fibers.

**Sympathetic Control**

Stimulation of sympathetic fibers produces vasodilation. This is due to the predominance of beta-receptors in the coronary vessels (stimulation of beta receptors results in vasodilation). Vasodilation also occurs partly due to the deposition of metabolites as sympathetic stimulation accelerates cardiac activity that increases myocardial metabolism.

**Parasympathetic Control**

Parasympathetic (vagal) stimulation causes vasodilation, but there is sparse parasympathetic innervation of the coronary blood vessel.

**Metabolic Regulation**

Metabolic regulation of blood flow is well developed in the heart. A close relationship exists between the coronary blood flow and the oxygen consumption of myocardium. The metabolic factors that cause coronary vasodilation are hypoxia, increased local concentration of carbon dioxide, increased hydrogen and potassium ion concentration, accumulation of lactate, adenosine, and adenine nucleotide, and increased release of prostaglandins locally. Adenosine is believed to play an important role in regulation of coronary blood flow. Adenosine mediates reactive hyperemia of ventricular muscle (increased blood flow to the area supplied by a coronary artery when the flow is re-established following temporary occlusion of the artery).

**Autoregulation**

An autoregulatory mechanism exists for the coronary blood flow, which maintains a normal blood flow within the pressure range of 70–110 mm Hg. However, the autoregulation of blood flow in the heart is not so well developed like that in brain.

**Physical Factors**

The blood flow to the cardiac muscle is dependant on the myocardial tension, the pressure that builds up in the
muscle during contraction. This is the primary factor in determining coronary perfusion. The vasocompression during myocardial contraction decreases the coronary blood flow (Clinical Box 98.1). Coronary blood flow increases during diastole (Figs. 98.5A and B).

Clinical Box 98.1

Subendocardial portion is prone to ischemic damage: The left ventricular muscle tension (the pressure within the ventricular muscle) during systole is maximum in the endocardial portion and minimum in the epicardial portion, as the pressure is more from epicardium to endocardium. The high pressure in the endocardial region decreases blood flow significantly to this part of the ventricle during systole. However in normal conditions, increased blood flow to the endocardium during diastole compensates for the decreased blood supply during systole. Conditions in which the coronary blood flow is compromised, the compensation does not occur. Therefore, the subendocardial portion of the left ventricular muscle is prone for ischemic damage and myocardial infarction.

Clinical Importance

Coronary Artery Disease

Decreased blood flow to the heart leads to myocardial ischemia (angina pectoris) and, if severe and prolonged, results in myocardial infarction (the heart attack).

1. This is one of the commonest cause of sudden death, especially in the developed countries. Recently, the incidence of coronary disease has also increased in developing nations.
2. It usually occurs due to coronary atherosclerosis. Usually, the disease starts with angina, and infarction occurs when the obstruction occupies at least 75% of the lumen of the coronary artery.
3. The known risk factors for CAD are age (> 40 years), gender (males are more susceptible), family history, smoking, hypertension, hypercholesterolemia, diabetes, hemostatic factors (platelet activation, fibrinogen, antiphospholipid antibody), physical inactivity, obesity, alcohol intake, hyperhomocysteinemia and stress.

Angina Pectoris

Angina literally means chest pain. Angina pectoris refers to the chest pain due to ischemia of the cardiac muscle. The pain is usually felt in the chest below the sternum toward the left side. The cause of pain is myocardial ischemia due to decreased blood supply, which results in accumulation of “P factor” in the ischemic tissue. The pain typically radiates to the ulnar border of the left hand, but it can also radiate to the back or even to the neck or abdomen.

Myocardial Infarction

Infarction (ischemic cell death) occurs when the ischemia is prolonged, following more than 75% obstruction of the coronary arteries.

1. The factors that precipitate acute myocardial infarction (AMI) are spasm of the coronary artery at the site of atherosclerosis, platelet aggregation at the site of obstruction, and hemorrhage into the atherosclerotic plaque of the coronary artery.
2. Stress is known to induce coronary artery spasm.
3. Increased circulating level of lipoprotein-a (Lp-a), ultra-sensitive C-reactive protein (usCRP), oxidized LDL, anti-phospholipid antibody, troponin I and homocysteine are strongly correlated with AMI.
4. AMI causes severe chest pain that radiates along the ulnar border of the upper arm, arm and hand. The chest pain is usually associated with excessive sweating.

Diagnosis

Diagnosis of AMI is based on specific electrocardiographic changes, elevation of enzymes specific for myocardial damage, and typical clinical presentation of the patient.

1. Typical ECG changes: The ECG is very useful for diagnosing and locating areas of infarction. In acute infarction, the first change to occur is the ST segment elevation in the ECG leads recorded with electrodes placed over chest representing the infarcted area (Fig. 98.6). The leads placed on the opposite side of the infarction record ST segment depression (for details, refer Chapter 88; Abnormal ECG).
2. **Change in enzymes in the plasma:** The damaged myocardium releases enzymes into the circulation. Detection of elevated level of these enzymes plays an important role in the diagnosis. The enzymes most commonly measured are **creatine kinase** (CK) and **lactate dehydrogenase** (LDH), but the most specific is the increased concentration of **CK-MB isoenzyme** of CK, and fraction 1 of LDH.

3. **Typical clinical presentation:** The severe chest pain associated with excessive sweating with (or without) radiation to the ulnar border of the left hand in a person above 40 years is highly suggestive of AMI. If such patients get prompt relief by nitrate tablets (or sublingual sorbitrates), diagnosis is almost confirmed. However, it should be remembered that **chronic diabetic patient** may have painless AMI.

4. **Coronary angiography:** Coronary angiography will show the details of contour of the coronaries and the site and extent of obstruction in the arteries (Figs. 98.7A and B).

**Physiological Basis of Treatment**

The treatment of angina pectoris and acute myocardial infarction consists of both medical and surgical interventions.

**Medical Treatment**

Treatment for AMI should start at the earliest possible.

1. **Vasodilators:** Nitrates like nitroglycerin produce prompt improvement, as they are potent vasodilators. They cause arterial dilation that **decreases the afterload**, and cause venodilation that **decreases the preload** (the venous return). Thus, preload and afterload on ventricle are reduced that decreases myocardial oxygen consumption. This improves heart function.

2. **Streptokinase:** Streptokinase causes **lysis of the intra-coronary clot** when injected intravenously. It facilitates conversion of plasminogen to **plasmin that causes fibrinolysis**. If streptokinase is injected in the early part of onset of infarction, it removes obstruction (lyses clot) and prevents further progress of infarction. **TPA (tissue plasminogen activator)** is another clot lytic agent, frequently used clinically. But, this is highly expensive as it is produced in the laboratory by recombinant DNA technology. It also activates plasmin and causes fibrinolysis. The advantage of using TPA is that it **preferentially causes lysis of clot at the site of thrombosis**, as it activates plasminogen bound to the fibrin clot. However, due to its high cost, TPA is less used in developing countries.

3. **Coronary angioplasty:** The mainstay of treatment of myocardial infarction is the removal of obstruction in the coronary artery at the earliest possible. Therefore (if facilities are available), immediately following the confirmation of diagnosis, a **catheter containing a balloon is inserted into the coronary artery** and then the **balloon is inflated at the site of obstruction** to dilate the constricted artery. This procedure is called...
coronary angioplasty. Though angioplasty is very useful for immediate treatment of AMI, unless it is assisted by specific medical or surgical intervention in following few days, the chances of reocclusion of the artery is not uncommon.

4. **Calcium channel blockers**: Calcium channel blockers like verapamil are useful as they produce coronary vasodilation.

5. **Antiplatelet aggregating agents**: The commonly used drug to prevent platelet aggregation is low dose of aspirin. Aspirin inhibits cyclo-oxygenase, which normally helps in thromboxane A$_2$ (TxA$_2$) formation. TxA$_2$ potentiates vasoconstriction and platelet aggregation. Thus, aspirin inhibits platelet aggregation (refer to Flowchart 20.3, Chapter 20).

6. **Folic acid and vitamin B12**: Increased plasma level of homocysteine is strongly correlated with myocardial infarction. Homocysteine produces damage to the endothelial cells of blood vessels that becomes the site for platelet aggregation and facilitates atherosclerosis. Folic acid and vitamin B12 convert homocysteine to methionine, a nontoxic compound.

**Surgical Treatment**

The definitive treatment of myocardial infarction is to bypass the block in the artery by implanting a vessel in the heart, taken from other parts of the body (*bypass surgery*). This is called **coronary artery bypass graft (CABG)**. The grafted artery bypasses the blocked coronary artery. Usually, the artery is directly connected from aorta to the ventricular muscle. Therefore, it is called **aortic CABG**.

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**CUTANEOUS CIRCULATION**

Circulation of blood through skin is called cutaneous circulation. Skin is an important structure of the body as it covers and protects the whole body. The health of the skin is the beauty of the body. Cutaneous blood flow plays an important role in temperature regulation.

**Functional Anatomy**

**Blood Supply**

The blood supply of the skin of **apical regions** (fingers, toes, palm, feet, nose, ear lobes, lips, etc.) is different from the **non-apical regions** (the body torso) of the body.

**Apical Areas**

In Apical areas, an **arteriolar arcade** (network) exists at the boundary of dermis and the subcutaneous tissue. From this arcade, arterioles ascend from deep dermis to the superficial layer of the dermis, where they form a **second network**. Capillary loops originate from the superficial dermal network and perfuse the dermal papilla and epidermis (Fig. 98.8). The dermal arteriolar arcade also provides vessels that supply hair follicles, sebaceous glands and sweat glands.

**Nonapical Areas**

In nonapical areas, vascular pattern is modified. The direct vascular connection between arterioles and venules (known as **arteriovenous anastomoses or glomerulus**), mainly occur in the superficial dermal tissue. Arteriovenous anastomoses are absent or very few in nonapical areas.

**Normal Values**

Blood flow to the skin varies from 1 to 150 mL per 100 g of tissue (skin) per min.

**Innervation**

The skin blood vessels are supplied by sympathetic fibers. There is **no parasympathetic innervation** of cutaneous vessels. **Activation of sympathetic fibers results in vasodilation**. Vasodilation occurs by decreasing the sympathetic activity.

**Regulation of Cutaneous Blood Flow**

Cutaneous blood flow is regulated by neural, thermal and metabolic factors.

**Neural Regulation**

The cutaneous blood vessels are supplied by sympathetic fibers. There is **no vasodilator system** supplying the skin blood vessels.

**Thermal Regulation**

Cutaneous blood flow is mainly regulated by body temperature. **Increased body temperature causes vasodilation** and decreased temperature causes vasoconstriction.
Metabolic Regulation

Metabolic regulation is not important for cutaneous circulation. However, local production of bradykinin in the sweat causes cutaneous vasodilation.

Applied Physiology

Physiological and applied significance of cutaneous circulation lies in the vascular responses to injury and temporary occlusion.

Vascular Responses to Injury

Two types of responses are observed to injury: white reaction in response to light stroke and triple response in reaction to firm stroke.

White Reaction

When the skin is stroked lightly with a pointed object, the stroke line becomes pale. This is called white reaction. This occurs due to decreased blood flow in the capillaries due to contraction of precapillary sphincter in response to injury. The response is observed in about 15 seconds.

Triple Response

When the skin is stroked firmly with a pointed object, the response to the injury manifests as triple response. This is called triple response as it has three components: red reaction, wheal, and flare (Figs. 98.9A and B).

Red Reaction

The skin becomes red in about 10 seconds. Redness occurs due to capillary dilation that increases capillary blood flow. Capillary dilation occurs due to direct response of capillaries to pressure.

Wheal

The swelling (local edema) is called wheal. This occurs within few minutes following red reaction.

1. Wheal occurs due to increased permeability of the capillaries and post capillary venules (Figs. 98.9A and B).

2. The histamine released from local mast cells causes vasodilation and increases capillary permeability that results in extravasation of fluid.

Flare

Spreading out of redness from the site of injury to the surrounding area is called flare.

1. This occurs due to arteriolar dilation. Arteriolar dilation occurs by activation of axon reflex (Fig. 98.10).

2. From the site of injury, the impulse is conducted in the afferent fibers.
3. These sensory neurons give branches to the blood vessels.
4. The impulse, in addition to its conduction to the spinal cord orthodromically, is also relayed antidromically to the blood vessels.
5. Axon reflex is an example of antidromic conduction of the impulse.
6. The endings of sensory fibers on the blood vessels release substance P and CGRP that produce arteriolar dilation.
7. Thus, redness spreads out from the injury to the surrounding skin in the form of flare.

**Vascular Responses to Temporary Occlusion**

Reactive hyperemia occurs in response to temporary vascular occlusion.

**Reactive Hyperemia**

This is defined as *increased blood flow to an area, when blood supply to the area is reestablished following a brief period of occlusion.*

1. The blood flow to the skin increases when the circulation is reestablished after a short period of occlusion. The reactive hyperemia also occurs in visceral organs. It occurs due to vasodilation produced by hypoxia during occlusion.
2. When circulation is reestablished, blood flow increases through dilated vessels and the skin becomes red.
3. A better example is the *redness of the forearm* of a person immediately following his blood pressure measurement by sphygmomanometry. During blood pressure recording, BP cuff is tied around the arm and pressure in the cuff is raised that occludes the forearm arteries. When pressure in the BP cuff is released, the limb becomes red due to reactive hyperemia.

**Splanchnic Circulation**

Splanchnic circulation means circulation of blood through the abdominal viscera.

It has three components as follows:

1. Circulation through the gastrointestinal tract proper and the mesenteric attachments (intestinal circulation).
2. Circulation through the liver (hepatic circulation), and
3. Circulation through the spleen (the splenic circulation).

**Intestinal Circulation**

The major function of the intestine is digestion and absorption of nutrients. The normal intestinal blood flow is about 20% of cardiac output at rest, which increases to about 50% following a large meal. Without this increase in blood flow, proper digestion and absorption do not occur.

**Blood Supply**

**Arterial Supply**

The gastrointestinal tract is supplied by three main arteries: celiac, superior mesenteric, and inferior mesenteric arteries (Fig. 98.11).

1. The superior mesenteric artery is the largest branch of the aorta that carries more than 10% of the cardiac output. This artery supply to many parts of small and large intestine (Fig. 98.12).
2. The branches of the mesenteric arteries (that are called small mesenteric arteries) form an extensive vascular network in the submucosa of the gastrointestinal tract.
3. The branches from these arteries penetrate deep into the muscle layers and form the arterioles. The arterioles supply the tip of the villi.

4. Intestinal mucosa receives about 60–70% of total intestinal blood flow.

**Venous Drainage**

The capillaries in the villi drain into the venules that finally drain into the mucosal venules and then into the veins. The direction of blood flow in the arterioles and venules in a villus is opposite to each other, which forms a typical counter current exchange system. This permits diffusion of oxygen from arterioles to venules directly (Application Box 98.1). Therefore, supply of oxygen to the mucosal cells at the tip of the villus (villus capillaries) is reduced.

**Normal Blood Flow**

The blood flow of gastrointestinal tract can be divided mainly into three parts as follows:

1. Gastric blood flow: 40 mL/100 gm of tissue/minute
2. Intestinal blood flow: 60 mL/100 gm of tissue/minute
3. Pancreatic blood flow: 80 mL/100 gm of tissue/minute

**Application Box 98.1**

**Intestinal necrosis is common in shock:** In GI tract, oxygen directly diffuses from arterioles to venules in the mucosa bypassing the capillary. Therefore, when intestinal blood flow is reduced as occurs in shock, the shunting of oxygen from the arterioles into the venules is enhanced that causes extensive necrosis of the intestinal villi. This facilitates entry of toxins and bacteria from intestine into general circulation.

**Regulation of Intestinal Circulation**

**Neural Regulation**

The neural control of intestinal and mesenteric circulation is mainly achieved by the sympathetic system.

1. Stimulation of the sympathetic fibers results in constriction of the mesenteric arteries and arterioles and greatly reduces the blood flow.
2. These effects are mediated via alpha receptors. During exercise, the sympathetic vasoconstriction shifts blood from the mesenteric vascular bed to the skeletal muscle.

**Autoregulation**

Autoregulatory mechanism is not well developed in intestinal circulation. The autoregulation of blood flow is mainly due to metabolic and myogenic mechanisms.

**Metabolic Regulation**

Metabolic regulation occurs mainly by adenosine, osmolality and potassium.

- **Adenosine:** It plays an important role in regulation of intestinal circulation. It causes vasodilation and increases blood flow.
- **Osmolality:** Increased osmolality of the blood passing through the intestine as occurs following ingestion, causes vasodilation.
- **Potassium:** \( K^+ \) causes vasodilation.

**Regulation by GI Activity**

GI blood flow increases with increase in GI activity as occurs during digestion of food. Increased absorption of food also affects intestinal blood flow. Several products of digestion, like chyme, glucose and fatty acids, are potent vasodilators.

**Physiological Importance**

Food intake increases gastrointestinal blood flow. This is known as functional hyperemia. This is partly due to secretion of gastrointestinal hormones like gastrin and cholecystokinin and partly to products of digestion. Increased blood flow helps in digestion and absorption of food (Application Box 98.2).

**Application Box 98.2**

**Do not perform strenuous exercise after meals:** Immediately after a meal, one should not perform strenuous physical exercise. During exercise, the sympathetic stimulation causes splanchnic vasoconstriction that diverts blood from GI tract to the exercising muscle. Therefore, the blood will not be adequately available to carry out GI secretion and motility and for assisting in digestion and absorption of food.

**Hepatic Circulation**

Liver is an important vital organ as it performs major metabolic activities of the body. Liver requires adequate blood supply to carry out its routine functions. Hepatic blood flow constitutes about 28% of the cardiac output.

**Blood Supply**

Blood flow to the liver is derived from two sources: (1) the portal vein and (2) the hepatic artery.
1. **The portal vein:** It provides about 75% of the blood flow to the liver. This blood mainly comes from the gastrointestinal tract (Fig. 98.13). As the blood has already passed through the capillary bed of the GI tract (oxygen has already been extracted once), the oxygen concentration is less.

2. **The hepatic artery:** It provides 25% of the blood to the liver. It is fully saturated with oxygen. Therefore, the oxygen utilized by the liver is primarily derived from the hepatic arterial blood.

### Normal Blood Flow

Liver weighs about 2.6 kg. The blood flow is 1500 mL/min (58 mL/100 gm/min). This is about 28% of the cardiac output.

### Regulation of Hepatic Blood Flow

An interactive relationship exists between the regulation of portal venous and hepatic arterial blood flow. Normally, alteration in hepatic arterial blood flow occurs reciprocally with portal venous blood flow. This is called as hepatic arterial buffer response. It compensates for change in portal blood flow.

- **Neural regulation:** Both hepatic artery and portal vein are innervated by sympathetic vasoconstrictor fibers. These fibers control the hepatic blood flow.

- **Metabolic regulation:** With increased hepatic activity, the accumulated metabolites like carbon dioxide and hydrogen ions cause vasodilation and increase hepatic arterial flow. Adenosine also causes hepatic vasodilation.

- **Autoregulation:** The portal venous system does not autoregulate its blood flow. However, autoregulation exists for hepatic arterial blood flow.

- **Regulation by intestinal activity:** The activities of the GI tract (increased intestinal movement, splenic contraction, etc.) increase blood flow to the liver via portal system.

### Clinical Importance

1. The blood coming out of the GI tract before entering into the systemic circulation passes through the liver. Liver therefore, filters the blood before allowing it into the general circulation. Toxic substances when absorbed from the GI tract as occurs in poisoning via oral route, are detoxified in the liver.

2. The nutrients absorbed from the GI tract pass through the liver and are stored or metabolized according to the need of the body.

3. When central venous pressure is elevated as occurs in congestive heart failure, the hydrostatic pressure in the vessels of the liver increases. This results in exudation of fluid into the liver tissue and causes tender hepatomegaly. This is an important feature of heart failure.

4. Extensive fibrosis of the liver as occurs in hepatic cirrhosis causes increased hepatic vascular resistance. This increases portal venous pressure, which consequently increases capillary hydrostatic pressure in the splanchnic vascular bed. This results in transudation of fluid into the abdominal cavity that causes ascites.

5. In portal hypertension (increased portal venous pressure), enlargement of the esophageal veins (esophageal varices) occur. These varices may rupture and bleed to cause hematemesis (blood vomiting). To prevent this, portacaval shunt (anastomosis between portal vein and inferior venacava) is performed surgically to decrease the portal venous pressure.

6. Liver stores about 15% of the total blood volume of the body. Therefore, at the time of need as required in hemorrhagic shock, about 60% of the blood from the liver can be rapidly expelled into the systemic circulation to increase the blood volume. This is achieved by constriction of the capacitance vessels of the liver by the sympathetic stimulation. Hence, liver is an important reservoir of blood in humans. In animals, spleen (not the liver) acts as an important blood reservoir.

### Splenic Circulation

#### Blood Supply

The main source of blood flow to the spleen is the splenic artery.
Regulation of Splenic Blood Flow

Splenic artery is supplied by the sympathetic vasocostric- tor fibers. Sympathetic stimulation diverts blood from the splenic circulation to the hepatic or systemic circulation.

Clinical Importance

Spleen functions as a reservoir of blood, especially in animals. Stimulation of sympathetic fibers not only causes vasoconstriction but also produces contraction of smooth muscles present in the capsule and trabeculae of the spleen. The spleen contracts as a whole. Splenic contraction releases adequate amount of blood into the general circulation. This serves as a protective mechanism in exercise and shock. However, spleen is not considered an important reservoir of blood in human being.

SKELETAL MUSCLE CIRCULATION

Functional Aspects

Skeletal muscles constitute the largest mass of the body. In an adult weighing 70 kg, the weight of the skeletal muscle is about 30 kg (40% of the body weight), whereas the blood supply is only about 15% of the cardiac output. However, resistance offered by blood vessels of skeletal muscle accounts for 25% of systemic vascular resistance. Therefore, caliber of skeletal muscle blood vessels contributes significantly to the control of blood pressure. Moreover, circulation of blood through the skeletal muscles depends greatly on the activity of the muscle.

Normal Blood Flow

At rest, blood flow through the skeletal muscle is very low (2–6 mL/100 gm/min). During muscular exercise, blood flow increases 20–50 times the resting level (about 100 mL/100 gm/min).

Blood Supply

The blood supply to skeletal muscle is derived from the skeletal muscle arteries. One of the important features of this circulation is the presence of large number of arteriovenous thoroughfare channels. These blood vessels short-circuit the blood from the arterial side to the venous side by bypassing the capillaries (without exchange of fluid or metabolites at the tissue level).

Innervation

Skeletal muscle blood vessels are richly innervated by the sympathetic fibers, which are of two types:

1. Sympathetic vasodilator system: The sympathetic fibers supplying the blood vessels of the skeletal muscle are cholinergic. Therefore, stimulation of these fibers results in vasodilation (this is important especially during exercise).

2. Sympathetic vasoconstrictor system: There are also sympathetic noradrenergic fibers that secrete noradrenaline and cause vasoconstriction. These fibers restrict muscle blood flow only during resting conditions.

Regulation of Blood Flow

Neural Regulation

At rest, the resistance vessels exhibit a basal tone that occurs due to continuous low frequency activity of the sympathetic vasoconstrictor system. This tonic activity is greatly influenced by activity of the baroreceptor reflex. Stimulation of baroreceptors (increased carotid sinus pressure) results in dilation of blood vessels of the skeletal muscle, and decrease in carotid sinus pressure causes vasoconstriction. The muscle resistance vessels contribute significantly to maintenance of the blood pressure, as blood vessels in the skeletal muscle constitute the largest vascular bed in the body.

At the beginning of the exercise, the stimulation of sympathetic cholinergic fibers increases blood flow to the skeletal muscle due to vasodilation. These vasodilator fibers supply mainly the thoroughfare (arteriovenous) channels, not the precapillary sphincters. Therefore, this does not contribute significantly to the increase in oxygen and nutrients supply to the skeletal muscle.

But once exercise continues, the metabolites accumulate in the exercising muscle and cause vasodilation. The metabolites and local hypoxia cause dilation of the precapillary sphincters and increase blood flow through the capillaries. This increases oxygen and nutrient supply to the skeletal muscle.

Local Regulation

In active muscles, blood flow is regulated by metabolic factors. ADP, adenosine, hydrogen ion, carbon dioxide, lactic acid, and hypoxia cause vasodilation and increase blood flow. Increased temperature in the active muscles during exercise also contributes to vasodilation.

Regulation by Physical Factors

During exercise, the contraction of skeletal muscle mechanically compresses the blood vessels, therefore, decreases the blood flow. Thus, during active muscle contraction, blood flow is minimal. During strong sustained contraction, blood flow almost ceases temporarily. However, in intermittent contractions, blood flow occurs during the relaxation period and venous valves prevent back flow of blood in the veins between the contractions.

Clinical Importance

1. Skeletal vascular bed is the largest vascular bed in the body. Therefore, tone of the resistance vessels of the skeletal muscle contributes significantly to the maintenance of systemic blood pressure.
2. The blood present in the skeletal vascular bed contributes significantly to the venous return (the skeletal muscle pump activity). When the skeletal muscle pump activity increases, venous return increases and, therefore, cardiac output increases.
3. When the venous valves become incompetent, especially of the superficial leg veins, as seen in pregnancy or in old age, the veins become dilated and tortuous. These are called varicose veins. Cardiac output decreases in such conditions due to stasis of blood in dilated and incompetent veins. These conditions are treated by injection of sclerosing agents or by using elastic stockings.

### CHAPTER SUMMARY

**Key Concepts**

1. Regional circulations have their own importance of circulation and special arrangement of circulation.
2. The regulation of circulation in special regions is different and autoregulation aims at their special requirements.

**Important to Know (Must Read)**

1. In examination, **Long Questions** are usually not asked from this chapter.
2. Measurement of cerebral blood flow, Regulation of cerebral blood flow, Special features of cerebral blood flow, Vascular responses to injury/Triple response/ Axon reflex, Regulation of intestinal blood flow, Regulation and clinical importance of skeletal blood flow, Regulation of hepatic blood flow and its clinical importance, may be asked as **Short Questions** in exam.
3. In **Viva**, examiner may ask… What is the normal blood flow and oxygen consumption of kidney, What is the normal blood flow and oxygen consumption of brain, List the special features of cerebral blood flow, How is the circle of Willis formed, How are the cerebral blood vessels innervated, Name the methods of measurement of cerebral blood flow, What is Kety method, What are the radioactive substances used to measure cerebral blood flow, What is the clinical importance of measurement of cerebral blood flow, What is stroke and its types, What are the drugs used for the treatment of stroke, What are the regulatory mechanisms for cerebral blood flow, What is the normal blood flow and oxygen consumption of heart, List the special features of coronary blood flow, What is the vascular supply to the heart, Why is the subendocardial portion prone to ischemic damage, What is the innervations of coronary vessels, List the methods of measurement of coronary blood flow, What is Fick method of measurement of coronary blood flow, What are the radioactive substances used to measure coronary blood flow, What are the regulatory mechanisms for coronary blood flow, What is angina pectoris and what are its symptoms, What is acute myocardial infarction and what are its symptoms, How is AMI diagnosed, What are the physiological basis of treatment of AMI, What is the normal blood flow to the skin, How is the cutaneous blood flow regulated, What are the vascular responses to injury, What is white reaction, What is triple response and what are its components, What is red reaction, What is wheal, What is flare, What is axon reflex, What is reactive hyperemia, How much is the normal gastric, intestinal and pancreatic blood flow, How is the intestinal blood flow regulated, What is functional hyperemia, Why is strenuous exercise not advised after meals, Why is intestinal necrosis common in shock, What is the normal hepatic blood flow, How is the hepatic blood flow regulated, List the clinical importance of hepatic blood flow, What is the regulation and clinical importance of splenic circulation, What is the normal skeletal blood flow at rest and during muscular exercise, What is the function of arteriovenous thoroughfare channels in skeletal muscle circulation, What is the innervations of skeletal muscle blood vessels, How is the skeletal muscle blood flow regulated at rest, at the beginning of the exercise and during the exercise, How does the local regulation skeletal muscle blood flow take place, How do the physical factors regulate skeletal muscle blood flow, List the clinical importance of skeletal muscle blood flow.
Fetal Circulation

**Learning Objectives**

On completion of study of this chapter, the student **MUST** be able to:

1. Appreciate the design of fetal circulation.
2. List the special features of fetal circulation.
3. List the changes in fetal circulation that occur at birth.
4. Correlate the circulatory changes that occur in fetuses due to developmental abnormalities.

**Functional Aspects**

Circulation in fetus (before birth) differs significantly from circulation in postnatal life. The main difference is that the fetal lungs are functionally inactive and fetus derives oxygen and nutrients from the placenta.

**Design of Fetal Circulation**

Fetus receives blood supply from the placenta through umbilical veins (Fig. 99.1).

1. This blood is only **80% saturated** with oxygen as placenta has extracted some of its oxygen. The blood from the umbilical veins enters the liver, and some amount bypasses the liver and enters the inferior vena cava through the ductus venosus. Blood also comes from other sources.
2. The inferior vena cava drains into right atrium. About 50% of blood from the right atrium enters the left atrium through the foramen ovale, which is located in the interatrial septum. Another 50% of blood from right atrium enters into the right ventricle, from where it is pumped into the pulmonary artery.
3. As the **pulmonary vascular resistance is very high** (because fetal lungs are collapsed), only a small quantity of blood reaches left atrium through the pulmonary veins.
4. The main bulk of the blood from the right ventricle **directly enters the aorta** through ductus arteriosus.
5. The blood in the aorta is then distributed to the whole body.

![Fig. 99.1: Schematic picture of fetal circulation. Direction of arrows indicates the route of circulation. The oxygenation of blood is roughly indicated by the redness of the fluid in different parts of the fetal vascular tree (more the redness more is the oxygenation). Umbilical vein supplies more oxygenated blood from placenta to fetus and umbilical artery drains less oxygenated blood from fetus to the placenta.](image-url)
6. However, the umbilical artery, which arises from the aorta, transports blood to the placenta, where it is oxygenated and sent back to the fetus via fetal veins. Thus, placenta functions like lungs during fetal life.

**Special Features**
1. Right ventricle pumps blood into pulmonary artery against higher resistance as the pulmonary vascular resistance is very high.
2. Most part of the right ventricular output enters directly into the aorta through the ductus arteriosus.
3. The right ventricle receives blood from the placenta and pumps into the aorta from where about 60% of the blood goes to the placenta for oxygenation and only 40% is distributed to the different parts of the body. This occurs because peripheral resistance of the fetal vessels is high. Whereas resistance of the placenta is relatively low.
4. About 50% of the blood from the right atrium directly enters into the left atrium via foramen ovale.
5. Oxygen saturation of the fetal arterial blood is much lower than that of the adult. However, fetal tissues are highly resistant to the effect of hypoxia. The fetal hemoglobin also has higher affinity to the oxygen.
6. In fetus, the cardiovascular regulatory mechanisms operate mainly by the local factors. The baroreceptor and chemoreceptor reflexes develop at about 30th week of intrauterine life.

**Changes Occurring at Birth**
1. Closure of the umbilical vessel ceases blood flow through the umbilical veins. This results in closure of ductus venosus.
2. The clamping of umbilical vessels immediately after birth causes asphyxia that activates respiratory center. Respiratory signal is generated. This results in expansion of the lungs. As the lung fills with air, the pulmonary vascular resistance decreases significantly.
3. Closure of the umbilical vessels increases the total peripheral resistance and blood pressure. The left atrial reserve is raised above the volume of blood present in inferior vena cava and right atrium. This occurs because:
   i. The decreased pulmonary resistance increases blood flow through the lungs to the left atrium,
   ii. The decreased flow of blood to the right atrium occurs due to closure of umbilical vein, and,
   iii. Occlusion of umbilical artery increases the resistance to the left ventricular output.

The change in pressure gradient across the atria abruptly closes the valve of the foramen ovale, and gradually the interatrial septum closes.
4. The pulmonary arterial pressure decreases significantly due to decreased pulmonary vascular resistance. This along with increased aortic pressure reverses the flow of blood through the ductus arteriosus. However, constriction of ductus arteriosus begins within few minutes and is completely closed within 1–2 days after birth.
5. Before birth, the thickness of both ventricles is equal. After birth, thickness of the right ventricular wall decreases, whereas left ventricular thickness increases.

**Clinical Importance**
1. If the opening between the two atria (foramen ovale) does not close after birth, the condition is called atrial septal defect (patent foramen ovale). This results in mixing of oxygenated blood from the left atrium with that of deoxygenated blood in the right atrium. Thus, oxygen supply decreases to the tissues due to left to right shunt.
2. The failure of the closure of ductus arteriosus results in flow of blood from the aorta to the pulmonary artery, as the pressure in the aorta is more. This condition is called patent ductus arteriosus. This is also an example of left to right shunt. Oxygenation of the tissues of the body decreases in this condition.
3. A condition known as tetralogy of Fallot occurs rarely in which there are four cardiovascular defects: ventricular septal defect, pulmonary stenosis, right ventricular hypertrophy, and overriding of the aorta. Due to ventricular septal defect and pulmonary stenosis, the oxygenation of blood is grossly reduced. Therefore, tissues suffer from severe hypoxia, and cyanosis develops.

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**CHAPTER SUMMARY**

**Key Concepts**
1. Fetal circulation is designed to provide adequate oxygen and nutrient to the fetus.
2. Also, to prevent the entry of harmful substances into fetal blood.

**Important to Know (Must Read)**
1. In examination, Long Questions are usually not asked from this chapter.
2. Changes occurring at birth in fetal circulation may be asked as a Short Question in exam.
3. In Viva, examiner may ask… What are the special features of fetal circulation, What are the changes occurring at birth in fetal circulation, What are the clinical importance of fetal circulation.
HYPERTENSION
Physiological Aspects

Definition
Hypertension is defined as sustained elevation of systemic arterial pressure. Usually, hypertension means rise in diastolic pressure. Systolic pressure depends on cardiac output which is the product of stroke volume and heart rate.

Physiologically, heart rate is highly variable, and heart rate and stroke volume change frequently in daily life. Therefore, change in systolic pressure unless persistent for a longer period, is not considered as hypertension. Such a hypertension is prequalified as systolic hypertension. However, a highly elevated diastolic pressure is also associated with increase in systolic pressure. Also, chronic rise in systolic pressure is equally harmful like that of rise in diastolic pressure.

Degrees of hypertension (in adults) (based on the level of diastolic BP):
- 60 to 79 mm Hg : Normal
- 80 to 89 mm Hg : Prehypertension
- 90 to 104 mm Hg : Mild hypertension
- 105 to 115 mm Hg : Moderate hypertension
- More than 115 mm Hg : Severe hypertension

According to recent report Joint National Committee (JNC-7) on hypertension, systolic blood pressure < 120 mm Hg and Diastolic blood pressure < 80 mm Hg are considered normal (Table 100.1).

Types of Hypertension
Hypertension is broadly classified into two categories: Essential and secondary.

Essential Hypertension
Essential hypertension is also called primary hypertension. Essential hypertension is a misnomer as it is not essential for
health; rather it is nonessential for a healthy life. It constitutes about 90% of all types of hypertension. The causes of essential hypertension are not exactly known. However, sympathetic hyperactivity, increased renin secretion, high blood volume, excess salt intake, increased production of salt and water retaining hormones, increased synthesis of vasoconstrictors and decreased synthesis of vasodilators have been implicated in the genesis of essential hypertension.

Risk Factors
Followings are the known risk factors for essential hypertension.

Heredity
Hypertension is inherited as Mendelian dominant. If both parents are hypertensives, chance of children developing hypertension is more than 50%, and when one of the parents is hypertensive, the chance is 25%.

Age
Blood pressure increases with age. Age related rise in blood pressure is due to increased arterial wall thickness and degenerative changes (decreased compliance) in the arteries and arterioles.

Gender
Hypertension is less common in females before menopause due to the effects of gonadal hormones estrogen and progesterone. Estrogen decreases blood cholesterol and therefore, slows the process of atherosclerosis. Progesterone causes relaxation of smooth muscles and therefore produces vasodilation. However, after menopause, the incidence of hypertension in females is equal with males.

Race
Black Africans are more prone to develop hypertension.

Stress
Stress causes vasospasm by inducing sympathetic activity. Stress also causes hyperlipidemia. Thus, stress predisposes to hypertension, ischemic heart disease and other cardiovascular diseases.

Smoking
Chronic smoking increases nicotine and carbon monoxide concentration in plasma. Nicotine and carbon monoxide are potent vasoconstrictors. Nicotine also increases secretion of catecholamines.

Obesity
Obesity with or without hyperlipidemia increases the susceptibility to hypertension. Reduction in body weight by one kg reduces blood pressure by about 2 mm Hg.

Hyperlipidemia
Hyperlipidemia facilitates the process of atherosclerosis.

Sedentary Life
Sedentary life is the commonest cause of high blood pressure and coronary artery disease. Lack of exercise is known to produces obesity and hyperlipidemia.

Personality
Type A personality (sympathetic overactivity) is more prone to hypertension.

Environmental Factors
Occupation, family size and crowding influence blood pressure.

Salt Sensitivity
Salt intake increases blood pressure in salt-sensitive individuals.

Alcohol
Chronic and excess intake of alcohol increases blood pressure.

Secondary Hypertension
Hypertension that occurs due to various diseases is called secondary hypertension. Secondary hypertension is classified into following categories.

A. Renal hypertension
   - Glomerulonephritis
   - Pyelonephritis
   - Polycystic renal disease
   - Renin producing tumors (of JG cells)
   - Renal artery stenosis

B. Endocrinal hypertension
   1. Adrenocortical diseases
      ▪ Hyperaldosteronism
      ▪ Cushings syndrome
      ▪ Hypertensive form of congenital virilizing tumor
   2. Pheochromocytoma
   3. Acromegaly
   4. Myxedema

C. Neurogenic hypertension
   1. Acute increase in intracranial pressure
   2. Polyneuritis (acute porphyria, lead poisoning)
   3. Diencephalic syndrome

D. Miscellaneous forms of hypertension
   1. Pill hypertension (chronic use of oral contraceptives)
      Oral contraceptive pills (estrogen-progesterone combination) produce hypertension if taken for many years. Oral contraceptives are known to cause sodium and water retention from the kidney (expand ECF volume), and also increase the release of renin (activate renin-angiotensin system).
   2. Severe polycythemia
   3. Pregnancy induced hypertension (toxemia of pregnancy)
      Hypertension is not a feature of normal pregnancy, as progesterone produces vasodilation. Pregnancy induced hypertension is seen in complicated and risk pregnancies. In such conditions, the placenta produces pressor peptides (that cause vasoconstriction) or the blood vessels become hypersensitive to the circulating vasoconstrictors.
4. Coarctation of aorta
5. Hypercalcemia
6. Increased blood volume (e.g. excess transfusion, or administration of more i.v. fluid)
7. Glucocorticoid therapy

**Experimental Hypertension**

Hypertension produced in animals models by various experimental techniques, is called experimental hypertension.

1. Renal hypertension
   - Constriction of renal artery by applying clips produces hypertension (Goldblatt hypertension). This type of hypertension is cured by removing the constriction from renal artery. When one renal artery is clipped with other kidney intact (one-clip, two kidney Goldblatt hypertension), the hypertension is due to high renin secretion that resembles the renal hypertension in human beings. However, renin concentration remains normal in one-clip one-kidney Goldblatt hypertension.

2. Neurogenic hypertension
   - Denervation of carotid sinus and aortic arch
   - Bilateral lesion of NTS

3. Steroid-induced hypertension
4. Partial adrenalectomy
5. Salt-induced hypertension (in salt-sensitive rats)

**Effects of Hypertension**

Chronic hypertension results in following physiological alterations.

**Effects on CVS**

**On Heart**

Peripheral resistance increases in hypertension that increases afterload on the heart.

1. Compensation for increased excessive workload on heart occurs first by concentric left ventricular hypertrophy that increases the wall thickness.
2. Afterwards, dilatation of the cavity occurs.
3. Ultimately, cardiac function deteriorates and heart failure sets in. Myocardial oxygen requirement increases due to increased ventricular mass.
4. This causes angina pectoris and ischemic heart disease.

**On Blood Vessels**

Hypertension produces stiffness of the vessel wall and decreases vascular compliance. Hypertension promotes atherosclerosis.

**Decreased vascular compliance:** The blood vessels become stiff (less compliant) due to increased smooth muscles in their wall in response to chronic wall stress. This further aggravates hypertension.

**Increased atherosclerosis:** Accelerated atherosclerosis occurs commonly in hypertensive patients. Atherosclerosis and decreased compliance of the vessel wall result in narrowing of the vessel lumen. Atherosclerosis in the coronary and cerebral arteries is harmful. Hypertensive patients are predisposed to cerebral thromboses and hemorrhage.

**Effects on CNS**

Increased cerebral atherosclerosis causes cerebral infarction. Hypertension causes the development of cerebral vascular microaneurysm (Charcot-Bouchard aneurysm). Increased arterial pressure and vascular microaneurysm result in cerebral hemorrhage. Severe chronic hypertension causes hypertensive encephalopathy and retinopathy.

**Effects on Kidney**

Arteriosclerosis occurs in afferent and efferent arterioles and glomerular capillaries. This decreases GFR and tubular function. Proteinuria and hematuria are not uncommon.

**Physiological Basis of Management**

Primary hypertension, in its early phase can be treated without drugs. The treatment includes weight reduction, dietary alteration, physical exercise and mental relaxation. However, for secondary hypertension, and in chronic or advanced stage of hypertension, antihypertensive drugs are required for reducing the blood pressure. These include vasodilators, anti-adrenergic drugs, calcium channel blockers, diuretics, ACE inhibitors and angiotensin receptor antagonist.

**Weight Reduction**

As hypertension is common in obese persons, reduction of body weight should be an integral part of treatment of all obese hypertensives. It has been observed that with reduction of 1 kg of body weight the blood pressure usually decreases by about 2 mm Hg.

**Dietary Modification**

As salt accumulation causes water retention and increases blood volume, restriction of salt in the diet decreases blood pressure in hypertensive patients.

1. Experimentally, it has been observed that salt restriction decreases blood pressure in salt sensitive animals. In human beings, it is difficult to know the sensitivity to salt. Therefore, currently salt restriction is advised to all hypertensive patients. Salt restriction also prevents development of hypertension in risk groups. Thus, salt restriction is also advisable in normotensives.
2. **Restriction of fat**, especially animal fat in the diet decreases the process of atherosclerosis and therefore, reduces blood pressure.
3. Increased fiber content of the diet controls obesity and hyperlipidemia. Fibers in the diet decrease the absorption of fat from the intestine and therefore, prevent hyperlipidemia.

**Regular Physical Exercise**

Regular practice of isotonic exercise is very helpful in reducing blood pressure and body weight. Hypertension and other cardiovascular diseases are seen in sedentary individuals. The best form of exercise for reducing blood pressure is the brisk morning walk (at a speed of 25% more than the normal walk for about 30–60 minutes). Regular exercise controls blood pressure by decreasing the sympathetic tone and increasing vagal tone.

**Mental Relaxation**

Change in temperament (to remain cool or to become less aggressive), relaxation exercises, and yoga therapy are known to decrease blood pressure in hypertensives. Stress is amongst the common causes of hypertension. Mental relaxation through practice of meditation, yogasana or by listening soothing music, etc. is known to improve the condition of the patients. Happiness of the mind is the prerequisite for a healthy life and for maintaining normal blood pressure.

**Stoppage of Alcohol and Smoking**

Smoking must be stopped completely and alcohol intake should be drastically reduced, as smoking and alcoholism are known risk factors for hypertension.

**Antihypertensive Drugs**

If hypertension persist inspite of above-mentioned treatments, drug therapy should be instituted. However, above-mentioned modalities of treatment should also continue. Drugs include vasodilators, anti-adrenergic drugs, calcium-channel blockers, diuretics, ACE inhibitors and angiotensin receptor antagonists.

**Vasodilators**

Vasodilators like hydralazine decrease blood pressure by directly causing relaxation of vascular smooth muscle.

**Anti-adrenergic Drugs**

These are α receptor blockers and β receptor blockers.

- **α receptor blockers:** α receptor blockers like phentolamine decrease blood pressure by blocking the action of norepinephrine on blood vessels. They also decrease the release of norepinephrine at sympathetic nerve endings.

- **β receptor blockers:** β receptor blockers like propranolol and atenolol decrease blood pressure by blocking sympathetic effects on heart that decreases heart rate and cardiac output. They also reduce adrenergic-nerve mediated release of renin from JG cells, which is an important component of lowering blood pressure.

**Calcium Channel Blockers**

Calcium channel blockers like verapamil (a phenylalkylamine derivative) and diltiazem (a benzothiazepine) inhibit calcium entry into the cells by interacting with specific binding sites on the α₁-subunit of the L-type of voltage-dependent calcium channel. This decreases vascular tone that in turn decreases blood pressure. Verapamil and diltiazem also slow atrioventricular conduction that decreases heart rate.

**Diuretics**

Thiazide diuretics are frequently used in the treatment of hypertension. They produce sodium diuresis and volume depletion. They also reduce peripheral vascular resistance.

- **Furosemide,** a loop-diuretic also decreases blood pressure.

- **Spironolactone** decreases the effect of mineralocorticoid on kidney and causes renal sodium loss (it is effective, especially in hypertension due to hyperaldosteronism).

**ACE Inhibitors**

ACE inhibitors like captopril, enalapril, etc. inhibit the action of angiotensin converting enzyme (ACE). Thus they inhibit the generation of angiotensin II. They also retard the degradation of bradykinin, a potent vasodilator and also reduce the activity of adrenergic nervous system. These drugs are especially useful in the treatment of renal or renovascular hypertension, malignant and accelerated hypertension.

**Angiotensin Receptor Antagonist**

Angiotensin receptor antagonists like losartan, valsartan etc. block the action of angiotensin II on AT₁ receptor subtype.

**HYPOTENSION**

Usually hypotension refers to decrease in systolic blood pressure. When systolic pressure is less than 90 mm Hg in adults, the condition is known as hypotension (90 to 99 mm Hg is considered borderline hypotension). However, diastolic pressure is also low in condition when systolic pressure is significantly low. Clinically, there are three types of hypotension: (1) chronic hypotension, (2) acute hypotension, and (3) postural hypotension.

**Chronic Hypotension**

Chronic hypotension is characterized by persistent low blood pressure. Though the patients are usually asymptomatic, they complain lethargy, weakness and giddiness.

**Causes of chronic hypotension:**

1. Primary adrenocortical insufficiency
2. Hypopituitarism
3. Malnutrition
4. Chronic diarrhea
5. Prolonged bed rest
Chapter 100: Pathophysiology of Hypertension and Hypotension

**Acute Hypotension**
When blood pressure falls suddenly, the condition is called acute hypotension. If severe, it is associated with fainting.

**Causes of acute hypotension:**
1. Acute hemorrhage
2. Acute diarrhea
3. Severe vomiting
4. Acute myocardial infarction
5. Excessive diuresis
6. Acute vasodilatation

**Postural Hypotension**
If systolic blood pressure falls by 20 mm Hg or more, when a subject assumes erect posture from the supine posture, the condition is called postural or orthostatic hypotension. This usually results from autonomic imbalance. It may be acute or chronic type.

**Acute Postural Hypotension**
This is due to temporary fall of blood pressure that may result in transient fainting. It is usually seen in following conditions.

1. Prolonged standing.
2. Getting up from bed after a prolonged illness.
3. Strenuous physical exercise.

**Chronic Postural Hypotension**
Chronic postural hypotension can be primary or secondary variety.

**Chronic primary postural hypotension:** This is called idiopathic postural hypotension. The cause of this hypotension is not known. It usually occurs in elderly and may be due to degeneration of peripheral autonomic nerves.

**Chronic secondary postural hypotension:** This occurs in following conditions.
1. **Polyneuropathy** as seen in diabetes, amyloidosis and beriberi
2. **Autonomic neuropathy** as seen in syringomyelia, tabes dorsalis, and subacute combined degeneration of spinal cord.
3. Patients receiving sympatholytic drugs.
4. Surgical sympathectomy.

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**CHAPTER SUMMARY**

**Key Concepts**
1. Primary hypertension is usually due to chronically increased sympathetic tone due to any case. Therefore, methods to decrease sympathetic stimulation such as relaxation and yoga are very helpful. Restriction of salt intake and reduction in body weight help a lot.
2. Orthostatic hypotension is the commonest form of hypotension. It happens due to sympathetic insufficiency.

**Important to Know (Must Read)**
1. In examination, **Long Questions** are usually not asked from this chapter.
2. Orthostatic or Postural hypotension, Experimental hypertension, Cardiac changes in hypertension, Physiological basis of management of hypertension may be asked as **Short Questions** in exam.
3. In **Viva**, examiner may ask… Define hypertension, Classify diastolic hypertension, Classify systolic hypertension, What is essential hypertension, List the risk factors for essential hypertension, List the causes of secondary hypertension, What is the effect of hypertension on CVS, What is the effect of hypertension on CNS, What is the effect of hypertension on kidney, Say the physiological basis of management of hypertension, How does weight reduction reduce BP, How does dietary modification reduce BP, How does regular physical exercise reduce BP, How does mental relaxation reduce BP, How does stoppage of alcohol and smoking reduce BP, List the antihypertensive drugs, Name the vasodilators, Name the anti-adrenergic drugs, List the calcium channel blockers, List the diuretics, Name the ACE inhibitors, Name the Angiotensin receptor antagonists, Define hypertension, What is chronic hypertension, what are its causes, What is acute hypertension, what are its causes, What is postural hypertension, what are its types, What is acute postural hypotension and in which condition it occurs, What are the types of chronic postural hypotension, What is the cause of chronic primary postural hypotension, What are the conditions in which chronic secondary postural hypotension occurs.
CHAPTER 101

Pathophysiology of Shock

LEARNING OBJECTIVES

On completion of study of this chapter, the student MUST be able to:
1. Define and classify shock, and give example for each type of shock.
2. Describe the compensatory mechanisms of hypovolemic shock.
3. Understand the mechanisms of refractory shock.
4. Briefly describe other types of shock.
5. Appreciate physiological basis of treatment of shock.

The student MAY also be able to:
1. Explain the compensatory mechanisms of reversible shock.
2. Describe the causes of irreversible shock.
3. Explain physiological basis of treatment of different types of shock.

DEFINITION AND TYPES

Definition

Shock is a syndrome in which there is inadequate tissue perfusion associated with a relative or absolute decrease in cardiac output.

Types

Shock is classified broadly into four categories based on the mechanisms that decrease cardiac output.

A. Hypovolemic Shock

In this type of shock, the inadequate cardiac output is due to decreased amount of fluid in the vascular system. This occurs due to:
1. Acute hemorrhage (hemorrhagic shock)
2. Injury (traumatic shock)
3. Blood loss during surgery (surgical shock)
4. Burn (burn shock)
5. Metabolic diseases like adrenal insufficiency, diabetic ketoacidosis, etc.
6. Severe diarrhea or vomiting.

B. Distributive Shock

In this type of shock, blood volume is normal but blood pressure decreases due to sudden increase in the capacity of the vascular compartment due to acute marked vasodilation. Cardiac output also decreases in response to decreased venous return due to venodilation. This may be of following types:
1. Neurogenic shock (as seen in fainting)
2. Anaphylactic shock (due to acute systemic allergy or anaphylaxis)
3. Septic shock (following septicemia)

C. Cardiogenic Shock

In this type of shock, cardiac output decreases due to decreased ability of the heart to pump blood. It is seen in:
1. Acute myocardial infarction
2. Heart failure
3. Cardiac arrhythmias

D. Obstructive Shock

In this type of shock, cardiac output is decreased because of obstruction to the ejection of blood from the ventricle. It is seen in:
1. Cardiac tamponade
2. Constrictive pericarditis
3. Cardiac tumor
4. Tension pneumothorax

**HYPOVOLUMIC SHOCK**

Hypovolemic shock is the commonest of all shocks. Common forms of hypovolemic shock are hemorrhagic, traumatic, surgical and burn shocks.

**Hemorrhagic Shock**

This is the common hypovolemic shock and the commonest of all shocks.

**Cause**

Acute hemorrhage of more than 15 mL/kg body weight results in shock.

**Features**

1. Hypotension
2. Tachycardia
3. Rapid and thready pulse
4. Cold and pale skin (cold shock)
5. Tachypnea
6. Intense thirst
7. Restlessness
8. Usually, patient is conscious

**Stages of Shock**

Shock has two stages: Reversible stage and irreversible stage.

**Reversible Stage**

In this stage, with activation of compensatory mechanisms the condition of the patient improves. The compensatory reactions are divided into short-term and long-term mechanisms.

**A. Rapid Compensatory Mechanisms**

The rapid compensatory mechanisms are primarily neural and vascular, though the hormonal mechanisms also play role. Rapid compensatory mechanisms are mostly due to the stimulation of sympathetic system. Sympathetic activation occurs mainly due to the decreased stimulation of baroreceptors and hypoxia. But in severe shock, chemoreflex and Cushing’s reflexes are also activated.

The neurohormonal mechanisms compensate hypovolemia by following processes:

1. **Tachycardia**: Loss of blood decreases blood volume and blood pressure that decrease stimulation of baroreceptors (due to decreased stretch), which results in sympathetic activation. This increases the heart rate.
2. **Vasoconstriction**: This also occurs due to decreased stimulation of baroreceptors that activates sympathetic system. Vasoconstriction occurs in almost all parts of the body except in the cerebral and the coronary arteries. Thus, coronary and cerebral blood flow is maintained in shock. The skin becomes pale and cold due to intense cutaneous vasoconstriction. Therefore, acute hemorrhagic shock is called cold shock.

In kidney, the renal vasoconstriction decreases renal blood flow and GFR. But, efferent arterioles are constricted more than the afferent arterioles. Urine formation is grossly reduced. The nitrogenous waste products are retained in the body in prolonged shock that results in uremia (azotemia).

3. **Increased venous return**: Venous return increases due to vasoconstriction, which occurs due to sympathetic stimulation.
4. **Increased thoracic pumping**: The patients in hemorrhagic shock usually hyperventilate (exhibit rapid respiration). This increases the thoracic pump and contributes to increased venous return.
5. **Increased skeletal muscle pumping**: The subject is usually restless. The skeletal muscle activity is increased due to active movements of different body parts. This in turn increases skeletal muscle pumping that contributes significantly to increased venous return.
6. **Capillary fluid shift**: This is the shift of blood from interstitial fluid compartment into capillaries. In hemorrhagic shock, due to acute blood loss, the hydrostatic pressure inside the vessel decreases. This reverses the net filtration pressure gradient, and fluid from the interstitial tissue space enters into the capillaries. This also contributes to increase in blood volume that increases venous return.
7. **Activation of chemoreceptor reflex**: When blood loss is severe enough to cause general hypoxia, the chemoreceptors are stimulated. Chemoreceptors stimulation results in intense vasoconstriction (that restores blood pressure) and stimulates respiratory center (that causes hyperventilation).
8. **Activation of Cushing’s reflex**: When hypotension is severe and persistent due to gross hemorrhage, cerebral hypoxia directly stimulates vasomotor center. This results in intense vasoconstriction and tries to maintain blood pressure and flow to the vital organs of the body.
9. **Increased secretion of catecholamines**: Hemorrhage is a potent stimulus for secretion of catecholamines from the adrenal medulla. This occurs mainly due to sympathetic stimulation. Increased catecholamines in circulation stimulate heart (maintain cardiac output) and also cause vasoconstriction (maintain blood pressure). Catecholamines also stimulate the reticular activating system, for which patient is often conscious and restless.
10. **Increased secretion of vasopressin**: Decreased blood volume stimulates secretion of ADH from the posterior pituitary. ADH increases reabsorption of water from
the collecting duct of the kidney that restores blood volume. In higher concentration, it also causes vasoconstriction that restores blood pressure.

11. Increased secretion of cortisol: Glucocorticoid secretion increases due to increased ACTH secretion. Glucocorticoid plays a crucial role by increasing the responsivenes of the blood vessels to catecholamines through its permissive and vasoreactivity effects.

12. Activation of rennin-angiotensin system: Hypovolemia and sympathetic stimulation increase the secretion of rennin from JG cells of the kidney. Rennin converts angiotensinogen to angiotensin-I, which is further converted to angiotensin-II by the action of angiotensin converting enzyme. Angiotensin-II restores the blood volume and blood pressure by the following actions:

   I. Peripheral actions
   - It is a potent vasoconstrictor. Hence, it increases blood pressure.
   - It increases aldosterone secretion from adrenal cortex. Aldosterone increases water reabsorption from kidney.
   - It directly increases sodium and water reabsorption from kidney by acting on proximal convoluted tubule.
   - It increases the release of norepinephrine from sympathetic nerve endings.

   II. Central actions
   - It stimulates dipsogenesis (increases drinking) by stimulating thirst centers.
   - It stimulates vasomotor center and increases blood pressure.
   - It increases ADH secretion.
   - It also increases ACTH secretion.

13. Increased aldosterone secretion: Aldosterone secretion from adrenal cortex increases due to the action of angiotensin-II, ACTH, and hypovolemia. Aldosterone increases water and sodium reabsorption from the collecting duct and the distal convoluted tubule of the kidney.

B. Long-term Compensatory Mechanisms
The long-term compensatory mechanisms are mainly intended to increase the red cell mass of the body so that the oxygen carrying capacity of the blood increases. These are increased synthesis of erythropoietin and plasma proteins.

1. Increased synthesis of erythropoietin: Erythropoietin secretion from kidney increases within 48 hours. This increases red cell production, which (red cells count) returns to normal within 2–6 weeks.

2. Increased plasma protein synthesis: The synthesis of protein by the liver increases within 2–4 days. This increases the hemoglobin synthesis and therefore, the RBC production. Increased plasma protein also restores the oncotic pressure of the plasma.

Irreversible Stage
In this stage, the compensatory mechanisms fail to improve body functions. Inspite of compensatory mechanism, shock progresses to a stage in which the cardiovascular responses fail. Cardiac output and blood pressure decrease despite the normal blood volume. This is also called refractory shock or irreversible shock. Usually, patients die irrespective of judicious treatment to increase the circulatory status.

Refractory Shock
Refractory shock occurs due to the following mechanisms:

1. Initially, precapillary sphincters constrict due to unknown mechanisms. This decreases blood flow to the viscera and results in hypoxic tissue damage. Free oxygen radicals (released from damaged tissue) and granulocytes adhered to the injured vessel wall facilitate further vessel damage.

2. Later, in advanced refractory shock, the precapillary sphincters dilate, but the venules constrict. This increases capillary hydrostatic pressure, which causes further injury to the vessel wall and increases transudation of fluid into the interstitial tissue space. This promotes additional decrease in blood volume and pressure.

3. The hypoxic damage to GI tract disrupts the mucosa of the intestine that allows entry of bacteria into circulation. This results in bacteremia and septicemia. Release of endotoxins from bacteria and toxic materials from septic tissues inhibit cardiorespiratory centers.

4. The hypoxia of the brain for a longer duration depresses medullary cardio-respiratory centers, especially the vasomotor center, which further decreases blood pressure and suppresses heart functions.

5. Due to hypotension and tachycardia, coronary blood flow decreases. This further decreases myocardial function.

6. Damaged blood vessels and damaged tissue release various coagulants that trigger disseminated intravascular coagulation. This results in microembolism in different vascular beds.

7. Severe pulmonary damage occurs due to pulmonary microembolism. This finally leads to acute respiratory distress syndrome (shock lung).

Other Hypovolemic Shocks

Traumatic Shock
This occurs due to severe injury, especially when muscles and bones are extensively damaged. Usually, it occurs in road accidents or in battle casualties.

Mechanisms of Traumatic Shock

1. Bleeding into the injured muscles is the main cause of shock. As most part of bleeding is internal (occurs in
the injured tissue), the degree of external blood loss or injury does not reflect the actual extent of hemorrhage. Big muscles like rectus femoris or gluteus maximus can accommodate about one liter of blood without significant increase in their size (Clinical Box 101.1).

2. **Rhabdomyolysis** (break down of the skeletal muscle) due to muscle crushing is another cause of shock. Myoglobin released from damaged muscles blocks the renal tubules and causes acute renal failure.

3. In the reperfused areas, **increased accumulation of calcium** (due to exchange of excess sodium for intracellular calcium) also causes tissue damage.

### Clinical Box 101.1

**Reperfusion-induced injury:** In muscle trauma, blood accumulating in large amount in the muscle compresses the muscle blood vessels. This decreases blood supply to injured muscle. Free radicals are generated from hypoxic and injured muscles. Therefore, in traumatic shock, blood accumulated in the muscle is collected and then transfused to the patient (autotransfusion). However, autotransfusion further complicates the condition. The removal of blood from the injured muscle relieves the pressure on blood vessel. This, plus autotransfusion reestablish the blood supply to the tissue (reperfusion). When blood supply is reestablished, the free radicals are generated by conversion of tissue xanthine dehydrogenase to xanthine oxidase. The xanthine oxidase generates free oxygen radicals and hydrogen peroxides that further damage the tissue. This is called reperfusion-induced injury. It has been observed that treatment with allopurinol, a xanthine oxidase inhibitor, reduces the severity of reperfusion-induced injury.

### Surgical Shock

This is seen in surgical procedures with improper hemostasis or in prolonged surgeries. It may be due to internal (into the tissue) or external hemorrhage or both.

### Burn Shock

In burns, **plasma is lost from the burned surface as exudate.** The exudate is rich in plasma proteins and fluid, and does not contain red cells. This results in hemoconcentration. Therefore, **hematocrit is high in burn shock.** Infection leading to septic shock complicates the hypovolemic shock in burns. **Hemolytic anemia** also complicates the condition in burns.

### Other Types of Shock

#### Distributive Shock

In this condition, capacity of the vascular bed is increased suddenly by **marked acute vasodilation.** This is also called **warm shock**, as the skin blood flow increases due to cutaneous vasodilation (this is in contrast to the cold shock that occurs in hemorrhagic shock). The examples of distributive shock are anaphylactic shock, septic shock, endotoxic shock and neurogenic shock.

### Anaphylactic Shock

This is seen in acute systemic allergy. The antigen-antibody complex **releases histamine** from the mast cells that causes severe hypotension due to vasodilation and **acute hypovolemia due to increased capillary permeability.** Thus, anaphylactic shock is both distributive and hypovolemic in nature.

### Septic Shock

This is seen in **systemic sepsis** (bacterial infection). The bacterial toxins cause vasodilation, suppress myocardial contractility and increase capillary permeability. Therefore, septic shock is a combination of distributive, cardiogenic and hypovolemic shock.

### Endotoxic Shock

This is a special variety of septic shock that occurs due to infection by **gram-negative bacteria.** These bacteria release endotoxins that produce vasodilation. The cell wall of these organisms contains lipopolysaccharides that stimulate macrophages to secrete more cytokines.

### Neurogenic Shock

In this shock, **sudden vasodilation occurs due to activation of autonomic responses.** This causes peripheral pooling of blood due to vasodilation. This is frequently seen in emotional outburst, overexcitement and severe fear or grief. Fainting or **syncope** (sudden and transient loss of consciousness) occurs in neurogenic shock.

### Cardiogenic Shock

Shock occurs due to **decreased myocardial contractility.** This may occur due to extensive myocardial damage as seen in acute myocardial infarction, or myocardial malfunction as seen in heart failure. Cardiac output decreases due to decreased pumping of the blood by the heart.

### Obstructive Shock

The shock occurs either due to **obstruction to the outflow** of blood from the heart as seen in aortic stenosis or due to the **obstruction to expansion of the heart** as seen in cardiac tamponade (bleeding into the pericardial cavity). In cardiac tamponade, ventricular muscles cannot relax in diastole due to external pressure that decreases end-diastolic volume; therefore, cardiac output decreases.

### TREATMENT OF SHOCK

The aim of shock is to restore adequate tissue perfusion. Restoring blood volume in hypovolemic shock, ensuing vasoconstriction in distributive shock and increasing cardiac output in cardiogenic shock achieve this goal.
The common modes of treatment are as follows:

1. **Blood transfusion** is required in hemorrhagic shock to increase blood volume.
2. **Plasma transfusion** is needed in burn shock to increase plasma volume. Administering concentrated human serum albumin that improves plasma volume by drawing fluid from extravascular space can also increase plasma volume. Injection of plasma expanders (sugars of high molecular weight like dextran) is also helpful.
3. **Epinephrine** is injected in anaphylactic shock that increases blood pressure by causing vasoconstriction and by increasing cardiac output.
4. **Dopamine** is the drug of choice in traumatic and cardiogenic shock for three reasons:
   i. It produces renal vasodilation that maintains kidney function.
   ii. It has positive inotropic effect that increases cardiac output.
   iii. It causes vasoconstriction in the systemic blood vessels that increases blood pressure.
5. **Sedatives should not be used** in shock as they depress central nervous system.
6. **Over-warming of the body should be prevented** as it causes cutaneous vasodilation and precipitates shock (Clinical Box 101.2).

**Clinical Box 101.2**

**Body should not be covered in shock**: In hemorrhagic shock, the skin is usually cold. There is a general perception to cover the body by blankets so that skin remains warm. But, it should never be done, as increased skin temperature causes cutaneous vasodilation and further precipitates shock. Rather, the body should be kept exposed.

### CHAPTER SUMMARY

#### Key Concepts

1. In shock, glucocorticoid must be administered along with vasoconstrictors. Cortisol by permissive action initiates and potentiates vasoconstriction effects.
2. When a shock patient is transported to hospital, the boy should not be covered with blankets. It caused cutaneous vasodilation and further decreases BP.
3. Dopamine is usually preferred in cardiogenic shock, as it maintain renal perfusion.
4. **Important to Know (Must Read)**
   1. In examination, ‘Describe the rapid compensatory mechanisms activated by hemorrhagic shock’ may be asked as a Long Question.
   2. Refractory shock, Compensatory mechanisms of shock, Reversible shock, irreversible shock, Refractory shock, Reperfusion-induced injury, Traumatic shock, Distributive shock, Physiological basis of management of shock may be asked as Short Questions in exam.
   3. In viva, examiner may ask… Define shock, Classify shock, What is hypovolemic shock, what are its causes, What are the stages of shock, What are the rapid compensatory mechanisms activated by hemorrhagic shock, List the features of hemorrhagic shock, What is the cause of tachycardia, What is the cause of rapid and thready pulse, What is the cause of cold and pale skin, What is the cause of tachypnea, What is the cause of intense thirst, What is the cause of restlessness, What are the long-term compensatory mechanisms activated by hemorrhagic shock, What is refractory shock, What are the mechanisms of refractory shock, What is traumatic shock, What are the mechanisms of traumatic shock, What is reperfusion-induced injury, what is its mechanism, What is surgical shock, What is burn shock, What is distributive shock, what are its types, What is anaphylactic shock, What is septic shock, What is endotoxic shock, What is neurogenic shock, what are its causes, What is cardiogenic shock, what are its causes, What is obstructive shock, what are its causes, What are the common modes of treatment of shock, Why is the body should not be covered in shock, Why is plasma transfusion needed in burn shock, Why is dopamine the drug of choice in cardiogenic and traumatic shock, Why sedatives should not be used in shock.
Pathophysiology of Heart Failure

LEARNING OBJECTIVES
On completion of study of this chapter, the student **MUST** be able to:
1. Define and classify heart failure.
2. Understand the physiological basis of cardiac changes due to pressure and volume overload in heart failure.
3. List the features of heart failure.
4. Give the physiological basis of orthopnea, paroxysmal nocturnal dyspnea and dependent edema in heart failure.
5. Understand the physiological basis of treatment of heart failure.

The student **MAY** also be able to:
1. Explain the physiological basis of changes in CVS in heart failure due to volume overload and pressure load.

DEFINITION AND TYPES
In general, laymen have a different concept on heart failure. Many think heart failure means fainting, and some think it as the stage just before death. However, heart failure is a specific entity of cardiac abnormality that results in decreased pumping of the heart.

**Definition**
This is a pathophysiologic state in which an abnormality of cardiac function results in inability of the heart to pump blood at a rate adequate for the requirements of the tissues of the body.

It occurs either due to decreased myocardial contractility or due to an increased pressure or volume overload. The usual physiological alterations are a **decreased stroke volume** (in forward failure) and **daming of blood in the venous compartment** (in backward failure). Both these pathophysiologic mechanisms coexist and both are due to a single dysfunction, i.e. inability of the heart to pump blood.

**Types of Heart Failure**
Heart failure has been classified in various ways.

**Acute vs Chronic Failure**
Acute heart failure is seen following acute myocardial infarction or rupture of a heart valve.
1. In such conditions, **sudden decrease in cardiac output** causes severe hypotension (therefore, **edema is not a feature**).
2. In chronic failure, which occurs due to a **slowly progressive disease** like cardiomyopathy, or chronic valvular disease, blood pressure may be maintained (**edema in the dependent parts is a feature**).

**Left-sided vs Right-sided Failure**
1. In left ventricular failure, the **congestion occurs in the pulmonary circulation**; therefore, dyspnea and orthopnea are common features.
2. In right-sided failure, **congestion occurs in the systemic circulation**, therefore, increased JVP, hepatomegaly, and edema in the dependent parts are usual features.

**High Output vs Low Output Failure**
In high output failure, heart **pumps abnormally large quantities of blood to deliver adequate oxygen** to the tissues. This occurs in following conditions:
- Severe anemia
- Hyperthyroidism
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- Beriberi
- Arteriovenous fistula
- Paget’s disease

Low output failure occurs due to failure of the heart to pump blood (due to decreased myocardial contractility).

Forward vs Backward Failure
1. In backward heart failure, the pressure and volume in the atrium and venous compartment behind the failed ventricle are more, therefore, edema occurs.
2. In forward heart failure, inability of the ventricle to pump blood causes tissue hypoxia that increases renin secretion (due to renal hypoxia). This activates rennin-angiotensin-aldosterone axis, therefore, edema (due to water retention) may be seen. However, usually both forward as well as backward failures coexist.

Systolic vs Diastolic Failure
The systolic heart failure occurs due to the systolic ventricular dysfunction (decreased myocardial contractility). It is commonly seen in ischemic heart disease or due to dilated cardiomyopathy.
1. The systolic failure decreases blood supply to the tissues.
2. The diastolic failure occurs due to the failure of relaxation of the ventricle. This is usually seen in restrictive cardiomyopathy as seen in amyloidosis in which the heart muscle is infiltrated by amyloid proteins. Due to inability of the ventricles to relax, the end-diastolic volume and therefore, cardiac output decreases.

Cardiac Changes in Heart Failure
Cardiac changes in heart failure depend mainly on whether the failure is predominantly due to pressure overload, which finally causes ventricular systolic dysfunction or due to volume overload, which may finally lead to diastolic dysfunction.

Pressure Overload
In pressure overload, increased blood pressure increases the afterload. Heart pumps blood against increased resistance.
1. This results in concentric hypertrophy of the ventricles (increased ventricular wall thickening in comparison to the increase in ventricular cavity size (Fig. 102.1A). The functional capacity of ventricle in such a situation depends on the stress exerted on the ventricle.
2. According to Laplace law, stress in the ventricular wall is the product of pressure and radius of ventricular cavity divided by ventricular wall thickness.
3. In the compensatory stage (stage of concentric hypertrophy) of pressure overload, wall stress normalizes due to increase in wall thickness and decrease in cavity radius of ventricle (Figs. 102.2A to C). Therefore, ventricular function is maintained.
4. But in the stage of hypertrophy and dilatation, ventricular stress increases due to proportionate increases in radius due to chamber dilation, which leads to failure of the myocardium to pump blood, causing systolic failure. This shifts the pressure-volume curve of left ventricle to right (refer Fig. 89.4, Chapter 89) that decreases ventricular ejection.

Volume Overload
In volume overload, due to increased venous return, diastolic pressure increases.
1. This causes chamber enlargement that mainly results in eccentric hypertrophy (Figs. 102.3A to D). In volume overload, dilation of chamber occurs first to accommodate larger end-diastolic volume.
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2. In the early phase (stage of dilation), wall stress increases due to increased radius of the ventricular chamber.

3. Subsequently, in the stage of eccentric hypertrophy, wall stress is normalized due to increased wall thickness and decreased radius of the chamber. Thus, ventricular function is maintained in this stage.

4. Later, in the stage of further dilation (Fig. 102.1B), wall stress becomes more due to increased radius of ventricular chamber that finally causes heart failure.

5. A chronically dilated heart may develop muscle fibrosis and fail to relax properly causing diastolic dysfunction that shifts the pressure-volume curve of left ventricle to left (refer Fig. 89.5, Chapter 89).

In ventricular failure due to either pressure overload or volume overload, initially there will be ventricular hypertrophy and compensation; but, later dilatation of chambers occurs that causes heart failure. A dilated ventricle works more to pump the same amount of blood than the normal heart, as explained by Laplace law (for details, see Chapter 92). Therefore, heart fails to pump adequate blood.

Clinical Features

Dyspnea

Dyspnea means breathlessness.

1. This occurs due to failure of the left ventricle to pump blood effectively that produces tissue hypoxia.

2. In heart failure, in the early stage, dyspnea occurs during exercise (dyspnea on exertion) due to failure of left ventricular output to meet oxygen demand during exercise.

3. In advanced stage of heart failure, dyspnea occurs even at rest due to increased pulmonary venous pressure, or due to gross inadequacy of ventricular pumping.

Figs. 102.2A to C: Cardiac changes in heart failure due to pressure overload. (A) Normal heart: Stress in the ventricular wall is the product of pressure (P) and radius of the ventricular cavity (R) divided by thickness of ventricular wall (T). In early phase of pressure overload, ventricular pressure increases that increases wall stress. (B) In the stage of concentric hypertrophy (the stage of compensation), wall stress normalizes due to greater increase in wall thickness that decreases cavity radius. (C) In the stage of hypertrophy and dilation, stress increases due to disproportionate increase in pressure, thickness and radius. Thus, dilation of heart that increases wall stress heralds heart failure.

Figs. 102.3A to D: Cardiac changes in heart failure due to volume overload. (A) Normal heart: Stress in the ventricular wall is the product of pressure (P) and radius of the ventricular cavity (R) divided by thickness of ventricular wall (T). (B) In early phase of volume overload, ventricle dilates (phase of dilation) to accommodate more blood, which increases wall stress due to increased cavity diameter. (C) In the stage of dilation and hypertrophy, eccentric hypertrophy of ventricular muscle (moderate increase in wall thickness) normalizes wall stress due to proportionate increase in radius and thickness. (D) In stage of failure (stage of further dilation), wall stress increases due to greater increase in ventricular cavity size that occurs due to dilation of the heart. Thus, dilation of heart that increases wall stress heralds heart failure.
Orthopnea
Orthopnea means dyspnea in recumbent position. It is usually relieved by sitting upright. The difficulty in breathing in supine posture occurs due to:

i. **Redistribution of blood** from abdomen and lower extremities into the chest during recumbency causes an increase in pulmonary hydrostatic pressure. Pooling of blood in the pulmonary vascular bed adds to the already congested lungs.

ii. Reduction in vital capacity occurs as **diaphragm is pushed toward lungs** in supine position. This is aggravated by hepatomegaly or ascites.

Paroxysmal Nocturnal Dyspnea
This is defined as episodes of dyspnea and cough of sudden onset in nights that usually awaken the patient from sleep.

1. This occurs partly due to the **depression of respiratory centers during sleep** and partly to the **accumulation of excess fluid in the lungs** in recumbent posture in sleep.

2. During sleep, pulmonary venous pressure and pulmonary capillary pressure increase. This results in **transudation of fluid into the air spaces**.

3. Typically, the patient wakes up from sleep frightened with the feeling of breathlessness and choking, and usually sits upright or walks around. He gets immediate relief as the pulmonary vascular congestion decreases in upright posture. He feels comfortable and then sleeps.

4. However, after some time the same events are repeated and throughout night many such episodes occur in paroxysms. Therefore, it is called paroxysmal dyspnea. As it occurs in the night, it is also called paroxysmal nocturnal dyspnea.

Fatigue (Weakness and Exercise Intolerance)
This occurs due to decreased cardiac output that causes tissue hypoxia. The exercise intolerance occurs due to decreased perfusion of skeletal muscles and decreased oxygen supply to meet the need of the body.

Edema in the Dependent Parts
This is an important feature of congestive cardiac failure. Edema occurs in the lower parts of legs (ankle or pedal edema) in ambulatory patients (Fig. 102.4), and in the sacral region (sacral edema) in bed-ridden patients. Edema occurs due to the following **three mechanisms** (Flowchart 102.1):


2. Decreased cardiac output also **increases sympathetic activity** that causes renal vasoconstriction. This results in decreased GFR that decreases urinary excretion of sodium and water. The retention of water causes edema formation.

3. Decreased ability of the heart to pump blood results in venous congestion (due to damming of blood in the venous compartment) and increases venous pressure. This increases capillary hydrostatic pressure that increases capillary filtration. Thus, edema results from increased capillary permeability. In the dependent parts of the body, the capillary pressure increases further due to pooling of blood in the parts. Therefore, dependant edema is a common feature of congestive cardiac failure.

Hepatomegaly
This occurs due to hepatic congestion. **Tender hepatomegaly** is an important feature of right ventricular failure (and also of congestive cardiac failure).

Increased JVP
Jugular venous pressure is elevated due to increased right atrial pressure (Figs. 102.5A and B). Raised JVP is the most reliable sign of congestive cardiac failure.

Ascites
Accumulation of excess of free fluid in peritoneal cavity is called ascites. This occurs in advanced stage of heart failure.

**Physiological Basis of Management**

1. **Rest**: Decreased physical activity decreases the demand on the heart. Adequate rest decreases venous pressure and congestion.
2. **Diet:** A salt restricted, but a normal caloric diet is prescribed for heart failure patients. Salt restriction decreases water retention.

3. **Digitalis:** Digitalis improves heart function by its positive inotropic effect. It increases myocardial contractility and therefore, cardiac output. Digitalis acts by inhibiting sodium potassium pump activity on the myocardial cells. Therefore, intracellular sodium increases, which is exchanged with extracellular calcium. This results in increased calcium concentration in the cell that increases myocardial contractility.

4. **Diuretics:** Diuretics promote water excretion. By decreasing ECF volume, diuretics decrease venous return to the heart. This decreases load on the heart.

5. **Vasodilators:** Vasodilators decrease peripheral resistance (afterload). Therefore, cardiac output increases.

6. **ACE inhibitors:** ACE inhibitors prevent the formation of angiotensin II.

7. **Angiotensin receptor antagonists:** Angiotensin antagonist such as losartan prevents the action of angiotensin on blood vessel.

8. **Treatment for the primary cause:** Removal of precipitating factors and correction of underlying cause of heart failure should be initiated along with other modalities.
### CHAPTER SUMMARY

#### Key Concepts
1. In heart failure due to pressure overload such as hypertension results in concentric ventricular hypertrophy, which is beneficial at the beginning. Dilatation of heart occurs late in the uncompensated stage.
2. In heart failure due to volume overload such as increased ECF volume results in dilation (eccentric hypertrophy) of ventricles from early stage, which is detrimental to the functioning of heart.

#### Important to Know (Must Read)
1. In examination, Long Questions are usually not asked from this chapter.
2. Cardiac changes in heart failure, Physiological basis of management of heart failure, Paroxysmal nocturnal dyspnea, Mechanism of edema formation in heart failure may be asked as Short Questions in exam.
3. In Viva, examiner may ask... Define heart failure, What are the types of HF, What is acute HF, What is chronic HF, What is high output failure, What is low output failure, What is forward failure, What is backward failure, What is systolic failure, What is diastolic failure, What are the changes in the heart due to pressure overload, What are the changes in the heart due to volume overload, What is dyspnea, what are its causes, What is orthopnea, what are its causes, What is paroxysmal nocturnal dyspnea, what are its causes, What is the cause of fatigue in HF, What is the cause of edema in HF, What is the cause of hepatomegaly in HF, What is the cause of increased JVP in HF, What is the cause of ascites in HF, What is the physiological basis of management of heart failure.
103. Functional Organization of Respiratory System
104. Mechanics of Breathing
105. Alveolar Ventilation and Gas Exchange in Lungs
106. Pulmonary Circulation and Ventilation-Perfusion Ratio
107. Transport of Gases in Blood
108. Regulation of Respiration
109. Physiological Changes at High Altitude
110. Hypoxia and Oxygen Therapy
111. Hazards of Deep Sea Diving and Effects of Increased Barometric Pressure
112. Respiration in Abnormal Conditions and Abnormal Respirations
113. Artificial Ventilation and Cardiopulmonary Resuscitation
114. Pulmonary Function Tests
“The soul is a figure of the Unmenifest,
The mind labours to think the Unthinkable,
The life to call the Immortal into birth,
The body to enshrine the Illimitable.
The world is not cut off from Truth and God”

......But first high Truth must set her feet on earth
And man aspire to the Eternal’s light
And all his members feel the Spirit’s touch
And all his life obey an inner Force,
This too shall be,................

Sri Aurobindo (in ‘SAVITRI’)
GENERAL CONCEPT

Respiration is defined as the process by which oxygen from atmosphere is delivered to the tissue and carbon dioxide from the tissue is removed into the atmosphere. Respiration takes place in four stages: ventilation stage, transport stage, exchange stage and tissue stage (Fig. 103.1).
1. The first stage is the ventilation stage in which exchange of gases between the atmosphere and pulmonary capillary blood occurs due to pulmonary ventilation.
2. The second stage is the transport stage during which gases are transported between the lungs and the tissue.
3. The third stage is the exchange stage in which gases are exchanged between the systemic blood and the tissue.
4. In the tissue stage, oxygen delivered to the tissue is utilized by the mitochondrial enzymes of the cells for the metabolism of foodstuffs for the production of energy during which carbon dioxide is produced (this is also called cellular respiration).

Oxygen and carbon dioxide move in and out of cells by simple diffusion. In unicellular organisms, the process of gas exchange does not require special respiratory mechanisms as it occurs by simple diffusion. In complex multicellular organisms, special respiratory system has evolved as cells deep in the body cannot exchange gasses by simple diffusion between them and the external environment. These include tracheal tubes in insects, gills in fish, and lungs in air-breathing animals.

COMPONENTS OF RESPIRATORY SYSTEMS

The respiratory system begins with nose and ends in the most terminal alveoli that are present in lung parenchyma (Fig. 103.2). Practically, respiratory system is divided into two parts: the upper respiratory tract and the lower respiratory tract.
1. The upper respiratory tract consists of structures from nose to the vocal cords that include sinuses, glottis, pharynx and larynx.
2. The lower respiratory tract consists of trachea, airways and alveoli.

The respiratory apparatus also consists of thoracic cavity and associated skeletal muscle, and the muscles of respiration.

Upper Respiratory Tract

The main function of the upper respiratory tract (URT) is to process the inspired air so that it gets humidified and it
Section 10: Respiratory System

attains body temperature by the time it reaches trachea. Components of URT are nose, paranasal sinuses, pharynx and larynx.

**Nose**

Nose contains **olfactory epithelium** that receives smell sensation. Nose **filters particle greater than 10 µm**. Nasal secretion contains **immunoglobulins and interferons** that kill the organisms. Though the volume of nose is only 20 ml, the cross-sectional area is greatly increased by nasal turbinates. Nose **offers about 50% of resistance** to airflow in the respiratory system. Resistance to airflow in the nose **increases during viral infections** like common cold.

**Paranasal Sinuses**

Paranasal sinuses are present almost around the nasal passage. They are mainly maxillary sinus, sphenoid sinus, ethmoid sinus and frontal sinus. The sinuses open into nasal turbinates that makes them vulnerable to nasal infections (Clinical Box 103.1). They have **three major functions**:

1. They **offer resonance** to the voice.
2. They **lighten the skull**, for which upright posture becomes easier.
3. They provide **protection to brain** during facial trauma.

**Clinical Box 103.1**

**Sinusitis:** The sinuses open into the nasal turbinates through their ostia. During inflammation and edema of nasal mucosa, the ostia of sinuses are readily blocked. This results in retention of secretions in the sinuses that in turn causes sinusitis.

**Pharynx**

Pharynx is divided into nasopharynx, oropharynx and laryngopharynx.

1. **Nasopharynx** is located behind the nose and extends from posterior nares to the level of soft palate. Eustachian tubes and posterior nares open into nasopharynx.
2. **Oropharynx** is located behind the mouth from soft palate above to the level of hyoid bone below.
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Larynx and Glottis
Larynx consists of epiglottis, arytenoids and vocal cords.

1. **Epiglottis** and **arytenoids** cover the vocal cord during deglutition that prevents aspiration of food particles into the respiratory tract.

2. **Arytenoids** enlarge during infections, especially in children that increase resistance to air flow significantly. The muscles of larynx are innervated by vagal fibers. The abductor muscles in the larynx contract in the early part of inspiration that pulls vocal cord wide apart and open the glottis.

3. During swallowing, reflex contraction of adductor muscles close the glottis, which prevents entry of food materials into the respiratory tract.

4. In unconscious and anesthetized patients, incomplete closure of glottis allows fluid and food particles to enter into the lungs that causes aspiration pneumonia.

5. Paralysis of adductors of larynx also produces aspiration pneumonia. Paralysis of adductors produces stridor during inspiration (inspiratory stridor).

6. Cervical vagotomy in animals produces severe pulmonary congestion and edema, which could partly be due to aspiration.

Clinical Box 103.2

URTI: Upper respiratory tract infections (URTI) are probably the common of all infections. The commonest of all is the common cold that manifests mainly in the form of acute nasopharyngitis with the features of increased nasal secretions, sneezing, mild and temporary nasal obstruction due to nasopharyngeal mucosal edema, and mild increase in body temperature. It is usually a self limiting viral infection. However, secondary bacterial infection may supervene and infection may spread to larynx (laryngitis) and sinuses (sinusitis), which warrants antibiotic treatment.

**Lower Respiratory Tract**
The lower respiratory tract consists of airway tree and lungs. Airway tree consists of trachea, which bifurcates into mainstem bronchi that enter the lungs.

**Airway Tree**
A series of branching tubes constitute airway tree. The tubes decrease in diameter at each branching. Trachea, the main airway branches into two bronchi. Each bronchus enters a lung and branches many times into progressively smaller bronchi, which in turn form bronchioles. This system of tubes is called tracheobronchial tree.

1. Trachea and bronchi contain cartilages and their smooth muscle content is relatively less. Trachea and bronchi are lined by a ciliated epithelium that contains numerous mucous and serous glands. In respiratory tract, cilia are present till respiratory bronchioles, but glands are absent in bronchioles and terminal bronchioles.

2. However, bronchioles and terminal bronchioles have adequate smooth muscles and the quantity of smooth muscle as % of wall thickness in respiratory tract is maximum in terminal bronchiole. Cartilages are absent from bronchioles downward in the airway.

**Bronchial Tone**
Tone of smooth muscles of bronchi is called bronchial tone. This contributes to patency of bronchi and aids in respiration.

1. Though bronchial tone is mainly due to neural influence, it is considerably influenced by chemicals and hormones, such as inflammatory modulators, cytokines, adenosine, substance P, histamine and VIP.

2. Bronchodilation is produced by sympathetic stimulation and bronchoconstriction by vagal stimulation. Irritation of airways by allergens and chemicals produce reflex bronchoconstriction, which is usually mediated by vagal cholinergic fibers.

3. Sudden entry of cool air into respiratory tract produces bronchoconstriction. Sometimes, exercise induces bronchoconstriction, which could be due to entry of cool air into bronchial tree due to hypoventilation that occurs during exercise.

4. Interestingly, there is a circadian rhythm for bronchial tone with maximum dilation occurring at 6 PM and maximum constriction occurring at 6 AM.

The progressively bifurcating airways are designated by their generation numbers. Trachea is the zero generation. The left and right main bronchi are the first generation. As the generation number increases, the following changes occur:

1. The airways become smaller, narrower and shorter.
2. The amount of cilia decreases.
3. The number of mucus secreting cells decreases.
4. The quantity of submucosal glands becomes less.
5. The amount of cartilage in the airway wall decreases.
6. The quantity of smooth muscle increases.

**Functions of mucus:** Mucus lines the inner wall of airway like carpet. It traps small foreign particles including bacteria.

**Functions of cilia:** Cilia beat toward pharynx. Thus, it prevents entry of small organisms into the lungs. Cilia also sweep the carpet of mucus. When mucus secretion is excessive, cilia remove mucus into the pharynx from where mucus is swallowed into the stomach or spit outside.

**Functions of cartilage:** Cartilage prevents collapse of airway. Cartilage is present up to the 10th generation (bronchi).

**Branching of Airways**
Airways progressively branch 23 times (23 generations of airways). They are broadly divided into two categories: conducting airways that move air by convection into the...
gas exchange areas of lungs (conducting zone) and respiratory airways that participate in gas exchange (respiratory zone).

**Conducting Zone**
The trachea and first 16 generations of airway form the conducting zone (Fig. 103.3).

**Arrangement and Functions**
The trachea, bronchi, and bronchioles have following functions:
1. They warm and humidify inspired air.
2. They distribute air evenly to the deeper parts of the lungs.
3. They also serve as part of nonspecific defense system of the body by removing dust, bacteria, and harmful gases from the respiratory tract.

**Structural Specialization**
1. The considerable amount of cartilage is present in first four generations of the conducting zone. This prevents collapse of these airways as they are subjected to changes in thoracic pressures. Some amount of cartilage is present up to 10th generation, up to which the airways are referred to as bronchi (Clinical Box 103.3).
2. The cartilage is absent totally in the bronchioles. The smallest airways in the conducting zone are the terminal bronchioles. Though bronchioles lack cartilage, elasticity of the lung tissue maintains their patency as they are suspended by elastic tissue of the lung parenchyma. However, bronchioles are susceptible to collapse especially during expiration.
3. As there is no alveoli up to 16th generation, gas exchange does not occur in the conducting zone. Therefore, the volume of respiratory tract starting from nose to the generation-16 airways is called anatomic dead space (details given below).

**Blood Supply**
The conducting zone receives blood supply from the bronchial circulation. The bronchial circulation is fed by bronchial artery that originates from the descending aorta and drained by pulmonary veins.

**Nerve Supply**
The bronchi and bronchioles are innervated by ANS.
1. Vagal stimulation causes bronchoconstriction mediated by muscarinic receptors that are plentifully available in bronchial tree.
2. Sympathetic stimulation causes bronchodilation, mediated by $\beta_2$ receptors that are present in bronchial epithelium and smooth muscle (For details, refer ‘Airway Resistance’ in Chapter 104). Activation of $\beta_2$ receptors increases bronchial secretion and $\alpha_2$ receptors inhibits secretion.
3. Also, there is noncholinergic-nonadrenergic innervation of bronchiole that causes bronchodilation, which is most likely mediated by VIP.

**Clinical Box 103.3**

Infection of right lung is common: The right side of the main bronchus has more diameter than the left side. Moreover, it is present in parallel with trachea. Therefore, inhaled foreign bodies lodge easily in the right lungs than the left. Thus, infection of right lung is common than that of left lung.

**Respiratory Zone**

The last seven generations of airway form the respiratory zone. Respiratory zone is the site for gas exchange.

**Arrangement and Functions**

The respiratory zone consists of respiratory bronchioles, alveolar ducts and alveoli. Alveoli start budding off bronchioles from 17th generations. Bronchioles from 17th to 19th generations are called respiratory bronchioles. With each descending generation, number of alveoli increases. Generations 20–22 are the alveolar ducts that finally terminate in the alveolar sac (generation 23).

**Blood Supply**

The respiratory zone receives blood supply from pulmonary circulation. The lungs have extensive capillary network that occupies about 80% of the alveolar surface area. Capillaries surrounding each alveolus bring blood into close proximity with the air inside alveolus. This helps in easy diffusion of gasses along the alveolocapillary membrane.

**Alveolus**

Alveolus is the functional unit of gas exchange. There are about 300 millions of alveoli present in both the lungs in adults. Diameter of alveoli ranges from 75 to 300 µm. The total surface area of all alveoli of both lungs is between 50 to 100 m², which is roughly the size of a tennis court. Thus, alveoli are among the largest biological membranes in the body (Application Box 103.1).
1. The alveoli are surrounded by capillaries that remain in close contact with each other forming alveolar-capillary membrane through which gas exchange takes place.
2. Alveolar epithelium is lined by epithelial cells that are of two types: Type I and Type II.
3. Type I epithelial cells are present in more numbers and cover 95% of epithelial surface area of alveoli.
4. Type II cells secrete surfactant (for details, refer ‘Pulmonary surfactant’) and play a important role in alveolar repair.
5. Alveoli also contain pulmonary alveolar macrophages (PAMs) that cause phagocytosis, and neuroendocrine cells, mast cells, lymphocyte and plasma cells that participate in allergic reactions. Especially, mast cells contain histamine, heparin and lipids that contribute significantly to local allergy.

![Fig. 103.4: Layers of pleura, and pleural cavity.](image)

**Lungs**

The gas exchange organs consist of two lungs that are divided into many lobes. The lungs are covered by pleura.

**Pleura**

Pleura is the covering of lungs. There are two layers of pleura: the parietal pleura and visceral pleura (Fig. 103.4).

1. The parietal pleura is the outer layer of pleural sac that contains blood vessels.
2. The visceral pleura lies directly on the lung.
3. It is proposed that the parietal pleura produces the pleural fluid, which is the ultrafiltrate of plasma. Normally, about 10–20 ml of this fluid is present in the pleural cavity (Clinical Box 103.4), the space between the parietal pleura and visceral pleura.
4. The viscous pleural fluid forms a lining of about 10 µm thick between the two layers of pleura, which functions as a lubricant so that lungs can slide against the chest wall.
   - Thus, pleural fluid facilitates the change in size and shape of lungs during respiration.
   - It also protects lungs from external damage.

**Clinical Box 103.4**

Pleural effusion: Accumulation of pleural fluid in excess in the pleural cavity is called as pleural effusion. Significant pleural effusion limits lung expansion and decreases gas exchange. Entry of air into the pleural cavity that occurs either due to trauma or rupture of alveoli results in pneumothorax, and entry of blood is called hemothorax.
Lobs of Lungs

Lungs are divided into lobes. There are three lobes in right lung and two lobes in left lung. The right lung constitutes about 55% of the total lung mass and function. Lungs consist of vascular tree and airway tree that are embedded in elastic connective tissue, the lung parenchyma. The vascular tree consists of arteries, veins and capillaries.

Bronchopulmonary Segments

Bronchopulmonary segment is the part of the lungs supplied by a segmental bronchus.

1. There are 10 bronchopulmonary segments in right lung and nine segments in left lung. These are apical, posterior, anterior, lateral, medial (inferior), superior, medial-basal, anterior-basal, lateral-basal, and posterior-basal.

2. Each bronchopulmonary segment is anatomically a single functional unit. Therefore, normally a disease process involves a bronchopulmonary segment at a time.

FUNCTIONS OF RESPIRATORY SYSTEM

Functions of respiratory system including lungs can be broadly divided into respiratory and nonrespiratory functions.

Respiratory Functions

1. Gas exchange: Lungs are the central structures in respiration. Inhalation of oxygen into the body and removal of carbon dioxide from the body take place through lungs.

2. Regulation of blood pH: By controlling carbon dioxide output from the body, lungs control plasma bicarbonate concentration. Therefore, lungs play an important role in acid-base balance.

Non-respiratory Functions

1. Left ventricular reservoir: The entire cardiac output from right ventricle is pumped into pulmonary circulation. Due to their high compliance, pulmonary vessels normally accommodate about 0.5 liter of blood at any given time. This serves as reservoir for left ventricular filling.

2. Filtering small emboli from blood: Venous blood normally contains microemboli of blood clots, fats or air bubbles. If these emboli escape into systemic arterial circulation, tissue damage may occur. Pulmonary vasculature traps and removes these emboli before they get the chance to enter into systemic circulation. However, larger emboli cause damage to the lung tissue.

3. Biochemical functions: Many chemical substances are removed in the lungs. Lungs decide which chemical substance should reach systemic circulation. Substances that are removed by lungs are: PGE_1, PGE_2, PGF_2α, leukotrienes, serotonin and bradykinin.

4. Olfactory function: Breathing is essential for delivering odorants from the environment to the olfactory epithelium. Olfaction is required for many physiological activities including limbic functions.

5. Processing of inhaled air: Filtration of inhaled air in the conducting airways is a respiratory function. This prevents entry of toxic substances and infective organisms into the body. Conducting airways filter these particles and expel them in expired air. Warming and moisturizing of inhaled air by the conducting airways also help to prevent alveolar damage.

6. Endocrine Functions: Angiotensin converting enzyme is mainly secreted by endothelium of pulmonary blood vessels that converts angiotensin I to angiotensin II, which occurs in pulmonary circulation. Few local hormones, such as prostaglandins and histamine are also synthesized by lungs.

7. Defence functions: Respiratory system is involved in defence functions by following mechanisms:
   i. Organisms that enter the lungs are phagocytosed by pulmonary alveolar macrophages (dust cells) or interstitial macrophages in the lung. Thus lungs play role in nonspecific defence of the body. Particles less than 2 µm in diameter reach alveoli where they are phagocytosed by alveolar macrophages. Particles with 2–10 µm in diameter are generally removed by conducting airways.
   ii. IgA present in bronchial secretions provide resistance to infection. IgA also maintains integrity of the mucosal lining the respiratory tract.
   iii. The epithelium of lungs contains protease activated receptors that on activation releases PGE_2, which in turn protects pulmonary epithelial cells. Particles more than 10 µm in diameter are removed by hairs in the nostrils.

8. Metabolic functions: Lungs perform a number of metabolic functions:
   i. Synthesis: Synthesize surfactant
   ii. Lysis: Lyse clot (local fibrinolytic system)
   iii. Synthesis and release: Synthesize and release following chemicals (local hormones) into systemic circulation:
      ▪ Histamine
      ▪ Kallikrein
      ▪ Prostaglandins
   iv. Removal from blood: Lungs do not metabolize epinephrine, dopamine, oxytocin, gastrin, ADH and angiotensin II. However, lungs partially remove prostaglandins, bradykinin, aceticholine and norepinephrine from blood.

9. Speech: Movement of air in the respiratory passage helps in the improvement of voice. Therefore, voice becomes thick with nasal intonation in nasopharyngitis and nasal obstruction.
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10. **Route for administration of anesthesia**: General anesthesia is usually administered via respiratory route.

**Symbols used in Respiratory Physiology**
Symbols widely accepted and commonly used in respiratory physiology are:

- **A**: Alveolar
- **a**: Arterial (systemic)
- **aw**: Airway
- **B**: Barometric
- **C**: Concentration or content in a liquid
- **c**: Pulmonary capillary
- **D**: Diffusion capacity
- **E**: Expired
- **F**: Fraction
- **f**: Respiratory frequency
- **I**: Inspired
- **P**: Pressure
- **Q**: Flow of blood
- **R**: Resistance
- **S**: Saturation of hemoglobin
- **V**: Volume of gas
- **V**: Rate of ventilation (volume of gas/unit time)
- **v**: Venous (systemic)
- **V**: Mixed systemic venous

**CHAPTER SUMMARY**

**Key Concepts**
1. The respiratory system physiologically is divided into upper and lower respiratory tract.
2. Upper respiratory tract is mainly the conducting zone and lower respiratory tract is the exchange zone.

**Important to Know (Must Read)**
1. In examination, **Long Questions** are usually not asked from this chapter.
2. Functions of lungs, Respiratory zone, may be asked as **Short Questions** in exam.
3. In Viva, examiner may ask… Which part of the lungs is known as conducting zone, what is its function. Which part of the lungs is known as respiratory zone, what is its function, Name the respiratory functions of lungs, Name the respiratory functions of lungs, Name the non-respiratory functions of lungs.
CHAPTER 104

Mechanics of Breathing

Learning Objectives

On completion of study of this chapter, the student MUST be able to:
1. List the muscles of inspiration and expiration, and give their mechanism of action.
2. Explain the alveolar and intrapleural pressure changes during quiet inspiration and expiration.
3. Define lung volumes and capacities and give their normal values.
4. Define lung compliance and explain the factors affecting it.
5. Understand the relaxation pressure-volume curve of lungs.
7. Understand the factors affecting airway resistance.
8. Define and give normal values of various dynamic lung volumes and capacities.

The student MAY also be able to:
1. Explain the relaxation pressure-volume curve of the lung, chest wall and respiratory system.
2. Describe the role surfactant in functions and dysfunction of the lung.
3. Describe the alteration in FEV1 in obstructive and restrictive lung diseases.
4. Explain the work of breathing of the lungs.

Scientist contributed

Henry Newell Martin (1848–1896), a great British Physiologist worked as Prof. of Physiology at Johns Hopkins University in the United States. He collaborated with George Nuttall. He developed the first isolated mammalian heart lung preparation (first described in 1881) which Starling later used to a great effect. He studied the details of mechanics of breathing. He demonstrated the functions of intercostal muscles in respiration.

Details of mechanics of breathing are complex phenomena. Therefore, in this chapter, we shall make every
effort to provide a simplified presentation of basic physi­cochemical principles that govern breathing and the factors that control mechanics of breathing.

**MUSCLES OF RESPIRATION**

Before understanding the basic principles of mechanics of breathing, we should first learn how the muscles involved in breathing help air to get into and come out of the lungs.

1. The lungs are located in the thoracic cavity, which is separated from the abdomen by the diaphragm, a large, thick and dome-shaped skeletal muscle.

2. The wall of the thoracic cavity is made up of the sternum anteriorly and vertebral column posteriorly with 12 pairs of ribs, and intercostal muscles (internal and external intercostals that are present between the ribs) hinged to the sternum and vertebral column.

3. As the rib cage is hinged between the vertebral column and sternum, it is raised and lowered during inspiration and expiration respectively.

4. The movement of rib cage along with diaphragm brings about the changes in various volumes and pressures in the thoracic cavity that result in inflow and outflow of air across the respiratory passages and lungs.

**Muscles of Inspiration**

Muscles of inspiration cause expansion of chest and increase the elastic recoil of chest wall. This makes the intrapleural pressure more negative that helps in expansion of the lungs due to which the air is sucked in. The inspiratory muscles are divided into two categories: primary and secondary (accessory).

**Primary Muscles of Inspiration**

The diaphragm and external intercostal muscles are primary muscles of inspiration.

**Diaphragm**

The diaphragm is the main muscle of inspiration, which is a skeletal muscle. Inflation of the lungs is caused primarily by contraction of the diaphragm. Contraction of diaphragm expands thoracic cavity in two ways.

1. The diaphragm is dome shaped and attached to the lower six ribs and the xiphoid process of the sternum. Thus, when it contracts, the dome is flattened and abdominal contents are pushed downward so that the thoracic cavity enlarges in its rostro-caudal extent. Thus, the vertical diameter of thoracic cage increases (Fig. 104.1).

2. Contraction of diaphragm also pushes the rib cage outward that enlarges the thoracic cavity in its antero-posterior and lateral planes.

   The ability of the diaphragm to expand the thoracic cage lies mainly in its dome-shaped configuration. During quiet respiration, the diaphragm moves towards abdomen by about 1 to 2 cm. However, during forced inspiration, descent of diaphragm occurs by about 10 to 12 cm that greatly increases the thoracic volume.

**Clinical Significance**

Inspiration is significantly compromised in following conditions.

1. Moderate to severe obesity, pregnancy (especially in third trimester), and tight clothing around thoracic and abdominal walls decrease expansion of lungs by reducing the effectiveness of diaphragm in enlarging the thoracic cage.

2. Diaphragm is innervated by phrenic nerves (C3–C5 cervical roots). Each lateral half of diaphragm is innervated by phrenic nerve of that side. Lesion of phrenic nerve results in paralysis of the diaphragm. It should be noted that when a phrenic nerve is damaged, the portion of diaphragm of that side moves up rather than coming down during inspiration. This grossly impairs ventilation.

**External Intercostals**

External intercostal muscles are present obliquely between ribs in forward and downward direction. Their attachment to lower ribs is more forward from the axis of rotation. Therefore, contraction of external intercostal muscles raises the lower ribs adequately. Contraction of external intercostal muscles has two effects:

1. The transverse and anteroposterior diameters of the thorax increase. This occurs by following two mechanisms:

   i. The 2nd to 10th ribs rotate upward and outward by a movement similar to the movement of the handle of a bucket (bucket-handle effect). This increases the transverse diameter of the thoracic cavity (Fig. 104.2A).
Section 10: Respiratory System

Muscles of Upper Respiratory Passage

Muscles of upper respiratory passage decrease airway resistance. Thus, they facilitate inspiration.

Muscles of Expiration

Expiration is a simpler process than inspiration. During inspiration, lungs and airways store enough energy in their elastic recoil, which is used for expelling air during expiration. When inspiration ends, the inspiratory muscles relax, the rib cage drops, and the thoracic volume decreases. The elastic recoil of the lungs is now unopposed, which helps lungs deflate. This results in expiration during normal breathing. Thus, quiet expiration is purely a passive process. Therefore, there are no primary muscles of expiration. However, expiratory muscles come into play during forced expiration. These muscles are called accessory muscles of expiration that include abdominal muscles, internal intercostals, and neck and back muscles.

1. **Abdominal muscles**: Muscles of abdominal wall that take part in expiration are internal oblique, external oblique, and transverse abdominis. Contraction of these muscles increases intra-abdominal pressure and pushes diaphragm upward into the chest cavity. This decreases rostro-caudal diameter of thorax and increases intrapleural pressure.

2. **The internal intercostals**: Internal intercostal muscles pull the rib cage downward and decrease thoracic volume both in anteroposterior and transverse diameter (opposite actions of external intercostal muscles). These muscles are especially helpful for forced expiration during coughing, vomiting, and strenuous-defecation.

3. **Neck and back muscles**: Muscles of neck and back that lower the pectoral girdle decrease cross-sectional area.

### Accessory Muscles of Inspiration

Accessory muscles assist in forced inspiration. These are as follows:

1. **Scalenes**: The scalene muscles of the neck are inserted into the upper two ribs. They are brought into play during deep breathing. They elevate the upper part of rib cage to further increase the thoracic volume.

2. **Sternocleidomastoids**: Sternocleidomastoids are inserted into the upper two ribs and top of the sternum. These muscles lift the sternum outward and elevate the rib cage. This contributes to the pump-handle effect and increases the thoracic volume.

3. **Neck and back muscles**: Contraction of neck and back muscles during forced respiration increase thoracic volume in two ways. Firstly, they elevate the pectoral girdle that increases the cross-sectional area of the thorax, and secondly, they extend the back that increases the vertical length of the thorax. Some important muscles of neck and back that assist forced respiration are posterior muscles of the neck, digastric muscles, mylohyoid, and posterior muscles of the upper back.

4. **Muscles of upper respiratory tract**: Contraction of alae nasi, levator palati, cheek muscles and other muscles of upper respiratory passage decrease airway resistance. Thus, they facilitate inspiration.

### Figs. 104.2A and B

Mechanism of bucket-handle (A) and pump-handle (B) movements of ribs of thoracic cage. Note, bucket-handle effect increases transverse diameter and pump-handle effect increases vertical diameter of thoracic cage.
of the thorax, and muscles that flex the trunk decrease rostrocaudal diameter of the chest cavity.

**Physiological Significance**

Following three important points should be noted for muscles of expiration.

1. **During forced inspiration**, accessory muscles are used to increase lung volume rather than to overcome the resistance to airflow, whereas during forced expiration accessory muscles are used mainly to overcome the resistance to airflow.
2. Quiet expiration is purely passive. However, exception to this is the individuals suffering from lung diseases like bronchial asthma in which airway resistance is high. They require active participation of expiratory muscles even in normal expiration.
3. The strength of expiratory muscles is important in endurance trainings like running, etc. Therefore, for long-distance runners, physical exercises are advocated to strengthen their abdominal and chest muscles as part of their training programmes.

**Pressure Changes during Breathing**

**Pressures in the Thoracic Cavity**

Various pressures exist in different structures in the thorax. The pressures are generated and modified in different phases of respiration. Changes in different pressures in the thoracic cavity that result in breathing are:

1. Intrapleural pressure
2. Transmural pressure
3. Alveolar pressure

**Intrapleural Pressure**

The pressure in the pleural space is the intrapleural pressure.

1. The **pleural space** is the space between the lungs and chest wall, i.e. the virtual space between the visceral and parietal pleura (refer Fig. 103.4; Chapter 103).
2. The intrapleural pressure also reflects the pressure in other regions of the thoracic cavity like the interstitial space surrounding the airways in the lungs, the space around the heart and great vessels, pressure inside and outside the esophagus when esophagus is not in peristalsis.
3. Thus, intrapleural pressure is the pressure everywhere in the thorax except the pressure in the airways, and in the lumen of the thoracic blood vessels and lymphatics.

**How is the Intrapleural Pressure created and Why is it Negative?**

The pleural space is a relative vacuum. The lungs have the tendency to collapse and the chest wall has the tendency to expand (Fig. 104.3). Therefore, the elastic recoil effects of lungs and chest wall are exerted equally but in opposite directions.

1. Lungs recoil inwardly and chest wall recoil outwardly. These equal and opposing forces cause the intrapleural pressure to be negative (less than atmospheric pressure).
2. Pleural pressure is negative during quiet breathing and more negative during deep inspiration.
3. During forced expiration only, intrapleural pressure may become positive.

**Normal Value**

In quiet breathing, intrapleural pressure (relative to atmospheric pressure) during expiration is about −2.5 to −4 mm Hg (approximately, −5 cm of H₂O) and during inspiration is about −6 mm Hg (approximately, −8 cm of H₂O) (Fig. 104.4).

However, during forced expiration, intrapleural pressure becomes positive and, during forced inspiration, it becomes further negative, may be up to −30 mm Hg.

**Measurement of Intrapleural Pressure**

The intrapleural pressure can be measured by inserting a needle into the intrapleural space. But, it is not safe to introduce a needle into the intrapleural space as it may result in pneumothorax. As intrapleural pressure reflects esophageal pressure, in practice it is measured by recording intraesophageal pressure by introducing a balloon catheter through mouth into the esophagus.

**Effects of Posture and Gravity**

**Upright Posture**

In upright posture, intrapleural pressure is about −2.5 cm H₂O at the base of the lung and −10 cm H₂O at the apex of the lung.

1. This apex to base increasing negative gradient of intrapleural pressure is due to the effect of gravity.
2. In standing position, the vacuum in intrapleural space is greater in the apex and less in the base of the lung, because gravity pulls the lungs downward (away from the apex of the thoracic cage).

3. This creates the intrapleural pressure gradient in vertical direction.

Supine Posture
In supine posture, this difference does not exist.
1. However, lying on one’s side creates the intrapleural pressure gradient in transverse direction.
2. As there is no gravitational force in the outer space, intrapleural pressure gradient is absent.

Significance of Intrapleural Pressure

Physiological Significance
It has two physiological significances.
1. The thoracic cavity is an airtight chamber. Therefore, expansion of the thoracic cage as occurs during inspiration causes the intrapleural pressure (the pressure between the lung and chest wall) to fall (to become more negative). A decrease in intrapleural pressure helps the lungs to expand, which in turn results in inflow of air into the lungs. Thus, fall in intrapleural pressure facilitates inspiration.
2. Intrapleural pressure maintains normal shape of chest wall and lungs as it resists the recoiling of these structures. Loss of normal intrapleural pressure results in lung collapse and barrel shaped chest.

Clinical Significance
The clinical significance of intrapleural pressure is observed when the chest wall is perforated and air enters the pleural space.
1. The normal pleural pressure is subatmospheric. Thus, when a connection is made between the atmosphere and the pleural space, air moves into the pleural space (air moves from regions of high to low pressure) and intrapleural pressure becomes atmospheric. This condition, in which air accumulates in the pleural space, is known as pneumothorax.
2. Due to loss of normal intrapleural pressure in this condition, the lungs collapse immediately due to inward recoiling, and the rib cage expands outward due to its outward recoiling (Fig. 104.5). In such a situation, the transpulmonary pressure is zero because the pressure difference across the lung is eliminated.

Causes of Pneumothorax
1. A gunshot wound that perforates the chest wall.
2. Injury with a knife in which the chest wall is punctured.
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3. In diseases in which the lung ruptures into the pleural cavity, e.g. rupture of a lung abscess or rupture of a parenchymal bullae by severe coughing.

4. During aspiration of pleural fluid for analysis, air enters into the pleural cavity due to faulty technique.

5. As part of therapy for some lung diseases, e.g. for treatment of tuberculosis, pneumothorax is purposefully created by inserting a sterile needle into the pleural space. For such purposes, nitrogen is injected into the pleural space to provide rest to the diseased lung.

**Transmural Pressure**

Transmural pressure is the pressure difference across an airway or across the lung wall. There are two major transmural pressures involved in breathing: transpulmonary pressure, and transairway pressure.

**Transpulmonary Pressure**

Transpulmonary pressure is the pressure difference across the lung wall (Fig. 104.6). This is measured by subtracting pleural pressure from alveolar pressure. Transpulmonary pressure is the pressure that keeps the lungs inflated and prevents the lungs from collapsing. An increase in transpulmonary pressure causes the inflation of lungs above the resting volume.

**Transairway Pressure**

Transairway pressure is the pressure difference across the airway, which is the difference between the pressure inside and outside the airway (Fig. 104.6). Transairway pressure is important in keeping the airways open during forced expiration.

**Alveolar Pressure**

The alveolar pressure is the pressure inside the alveoli.

1. Change in alveolar pressure moves air in and out of the lungs.
2. During inspiration, alveolar pressure decreases (becomes subatmospheric) that sucks air into the lungs, and, during expiration, the alveolar pressure increases that removes air from the lungs (Fig. 104.4).

**Normal Value**

During inspiration, it is approximately –1 mm Hg, and during expiration 1 mm Hg.

**Physiological Significance of Transmural and Alveolar Pressures**

At the end of expiration, when there is no flow of air into airways, the alveolar pressure is zero, i.e. equal to atmospheric pressure. Transpulmonary pressure, which is the difference in alveolar pressure and pleural pressure, is therefore +5 cm H₂O [0 – (–5) = +5] at the end of expiration.

1. Transpulmonary pressure is always positive in normal breathing.
2. Transpulmonary pressure is also referred to as distending pressure as it helps in inflation of lungs.
3. When transpulmonary pressure becomes more positive, the lungs are more inflated.

**Summary of Mechanics of Inspiration and Expiration**

**Sequence of Events Leading to Inspiration**

1. Diaphragm and other inspiratory muscles contract that cause expansion of thoracic cage which in turn makes intrapleural pressure more negative.
2. This increases transpulmonary pressure as a result of which inflation of lungs occurs.
3. Alveolar pressure decreases (becomes subatmospheric) and therefore, air flows into airways and lungs.
4. Inflow of air stops when alveolar pressure equalizes with atmospheric pressure (Flowchart 104.1).
5. The cessation of contraction of inspiratory muscles ends inspiration.

**Sequence of Events Leading to Expiration**

1. Due to relaxation of inspiratory muscles, rib cage drops.
2. Therefore, intrapleural pressure becomes less negative which decreases the transpulmonary pressure.
3. Lungs that were stretched in inspiration start deflating, due to which alveolar diameter decreases.
4. The alveolar pressure becomes greater than the atmospheric pressure that pushes air out of the lungs (Flowchart 104.2).
5. Airflow out of lungs continues till alveolar pressure equalizes atmospheric pressure.
**Types of Breathing**

Physiologically, breathing is of two types: Abdominothoracic and thoracoabdominal.

**Abdominothoracic Breathing**

Abdominothoracic breathing is usually seen in males.

1. During inspiration, diaphragm descends that pushes abdominal viscera down and, therefore, abdominal wall is raised.
2. Thus, abdominal movement becomes prominent. Contraction of abdominal muscles facilitates breathing.

**Thoracoabdominal Breathing**

This is predominant in females and children, as in them the movement of the chest wall is prominent during respiration.

1. In females, descent of diaphragm is resisted by the abdominal viscera; the exact cause of which is not known. Therefore, there is increased movement of the rib cage that increases thoracic cage volume. Hence, thoracic movement becomes prominent during breathing.
2. One contributing factor may be the free mobility of the ribs at sternocostal and costovertebral joints in females compared to males.
3. Also, diaphragm is more powerful in males that contributes to abdominothoracic breathing in males.

**LUNG VOLUMES AND CAPACITIES**

**Classification**

There are four lung volumes and four lung capacities (Fig. 104.7) as follows:

**Lung Volumes**

**Tidal Volume**

Tidal volume (TV) is the volume of gas inspired or expired during quiet breathing. This is about 500 ml in adults. About 150 ml of this volume fills the airways from the nose and mouth down to the respiratory bronchioles and this volume does not take part in gas exchange. This is called anatomical dead space. The remaining volume, i.e. 350 ml is available for alveolar ventilation.

**Inspiratory Reserve Volume**

The amount of air inspired with a maximal inspiratory effort in excess of the normal tidal inspiratory volume is called inspiratory reserve volume (IRV). The normal IRV is 3.3 liters in men and 1.9 liters in women.

**Expiratory Reserve Volume**

The volume of air that can be expired with a maximum expiratory effort after the normal tidal expiration is called expiratory reserve volume (ERV). The normal ERV is 1.2 liter in men and 0.7 liter in women.
Residual Volume
The volume of air left in the lungs at the end of maximal expiratory effort is the residual volume (RV). The normal value of RV in men is 1.2 liter and in women is 1.1 liter.

Lung Capacities

Vital Capacity
The maximum volume of air that can be expired after a maximal inspiratory effort is called the vital capacity (VC). The normal value of VC in men is about 4 liters and, in women, about 3 liters.

Forced Vital Capacity (FVC)
The total volume of air that can be expired with greatest force and speed after a maximal inspiration is the FVC. FVC differs very little from VC in the normal subject, but it is proportionately more reduced when there is airway obstruction with air trapping.

Physiological importance of FVC: The maximum volume of air that can be exhaled after a maximum inspiration is the VC. When expiration is performed as rapidly and as forcibly as possible, the volume is the FVC.
1. Normally, VC and FVC have same values.
2. To measure FVC, the individual inspires maximally and then exhales into the spirometer as forcefully, rapidly, and completely as possible.
3. From FVC, two other important lung functions are obtained: the forced expiratory volume of air exhaled in 1 second (FEV₁), and the forced expiratory flow (FEF₂₅–₇₅) that represents the expiratory flow rate over the middle half of the forced vital capacity, i.e. between 25 and 75%. Details of FEV₁ and FEF₂₅–₇₅ are discussed below.

Inspiratory Capacity
This is the maximum volume of air that can be inspired after tidal expiration. This is the sum of tidal volume and inspiratory reserve volume (TV and IRV). Normally, it is about 3.5 liter.

Functional Residual Capacity
This is the volume of air remaining in the lungs at the end of each tidal expiration. This is the sum of expiratory reserve volume and residual volume (ERV and RV). Normally, it is about 2.2–2.5 liters.

Physiological Importance of FRC
FRC is important for maintaining stable gas tension in the lung, so that at all times, enough air is available for gas exchange along the alveolocapillary membrane. It prevents sudden alteration in gas tension due to any brief interruption of respiration. Reduction in FRC may result in hypoxia. FRC increases when there is air trapping in lungs as seen in asthma or emphysema.

Total Lung Capacity
The volume of the air present in the lungs at the end of maximal inspiration is the total lung capacity (TLC). The normal value in men is about 6 liters and in women is about 4.2 liters.
**Measurement**

The lung volume and capacities are measured directly by simple spirometry. However, as lungs cannot be emptied completely following forced expiration, RV and FRC can not be measured directly by simple spirometry. RV and FRC are measured indirectly using a dilution technique involving helium (helium dilution technique). In this procedure, the subject is connected to a spirometer filled with 10% helium in oxygen (helium-oxygen mixture) and subject breathes from the spirometer. After the subject rebreathes, helium-oxygen mixture and equilibrates with the spirometer, the helium concentration in the lungs becomes same as in spirometer.

Applying the conservation of mass principle, we derive that:

\[ C_1 \times V_1 = C_2 \times (V_1 + V_2); \text{ where } C_1 \text{ is the initial concentration of helium in the spirometer, } V_1 \text{ is the initial volume of helium-oxygen mixture in the spirometer, } C_2 \text{ is helium concentration after equilibrium, and } V_2 \text{ is unknown volume in the lungs.} \]

Thus,

\[ V_2 = \frac{V_1 (C_1 - C_2)}{C_2} \]

1. **If the test begins at the end of a normal tidal volume**  
   (end of expiration), the volume of air remaining in the lungs represents functional residual capacity.
2. **If the test begins at the end of a forced vital capacity**,  
   then the test will measure residual volume.
3. Similarly, if the test starts after a maximal inspiration,  
   then \( V_2 \) would equal total lung capacity.

FRC can also be measured by body plethysmography.

Dynamic lung volumes and capacities are FEV\(_1\), PEFR, MMEFR, MVV, MEFVC, etc. (discussed below).

**Factors Affecting**

Lung volumes and capacities are influenced by various factors, such as age, gender, built, height, level of physical training, etc. (for details, refer chapter 114 “Pulmonary Function Tests”).

**MECHANICAL PROPERTIES OF LUNGS AND CHEST WALL**

The lung parenchyma, airways and pulmonary vasculature are embedded in elastic tissue. The inflation and deflation of lungs depend on their elastic properties. The physical principles that govern the process of breathing act mainly by affecting the mechanical properties of lungs and chest wall.

**Elastic Properties of the Lungs and Chest Wall**

The elastic properties of lungs and chest wall determine the lung volume and capacities. In an intact thoracic cage, the lungs have the tendency to collapse and the chest wall has the tendency to distend outward. These two forces acting in opposite direction precisely balance each other.

**Elastic Properties of Lungs**

Lungs are elastic tissues. An elastic tissue once stretched recoils back to its unstretched position like a spring. Similarly, lungs recoil when stretched.

1. The ability of the lungs to be inflated is called its stretchability or distensibility. Distensibility and elastic recoil are opposite to each other. Overstretching of the lungs causes loss of elastic recoil.
2. Lung elasticity depends on the presence of the amount of elastin and collagen fibers enmeshed in its parenchyma.
3. Elastin fibers are highly distensible and can be stretched easily, but collagen fibers resist stretch and limit lung expansion.
4. The arrangement of elastin fiber networks in the lung parenchyma is similar to the arrangement of fibers of a nylon stocking. When the stocking is stretched to fit into the leg, the length of individual fibers does not change much.
5. However, the arrangement of the nylon mesh in the stocking is such that it allows the stocking to be easily stretched and fit into the leg. If the nylon stocking is overstretched, it loses its elasticity and becomes loose (baggy). Similarly, an overstretched lung (baggy lung) as seen in emphysema, can not be deflated easily due to the loss of its ability to recoil.

**Lung Compliance**

Compliance means the ability to stretch (stretchability) or the ability to recoil. Lung compliance (distensibility and elastic recoil of the lung) can be assessed from the pressure-volume curve of the lung. This is similar to the inflation of a balloon in which for each unit of change in pressure, balloon is distended to a new volume. Lung compliance is a measure of its distensibility.

Compliance is represented by the volume change per unit pressure change, i.e.

\[ \text{Compliance} = \frac{\Delta V}{\Delta P}, \text{ where } \Delta V \text{ is the change in volume and } \Delta P \text{ is the change in pressure.} \]

**Measurement of Lung Compliance**

Two types of lung compliances are measured: static lung compliance and dynamic lung compliance.

**Static Lung Compliance Measurement**

A pressure-volume relationship is obtained for human lungs by simultaneously measuring changes in lung volume (with the help of a spirometer) and changes in pleural pressure (by measuring pressure changes in the esophagus with the help of a balloon catheter).

1. For recording pressure-volume curve, an individual inspires maximally to total lung capacity (TLC) and then expires slowly.
2. During the process of slow expiration, airflow is periodically interrupted (so that alveolar pressure is zero), and lung volume and pleural pressure are measured (Fig. 104.8).

3. Lung volume is recorded as percentage of TLC. The compliance (volume change per unit pressure change) recorded in these conditions is called static lung compliance, as airflow does not occur during the recording.

4. As lung is deflated from TLC, the deflation pressure-volume curve is obtained by this procedure.

5. To obtain the inflation pressure-volume curve, lung is inflated from functional residual capacity (FRC) to the TLC.

There are four stages in the inflation pressure-volume curve:

1. **Stage 1 (stage of stable lung volume):** The initial change in intrapleural pressure has no effect on lung volume. This is because a decrease in intrapleural pressure increases transpulmonary pressure and unless transpulmonary pressure is large enough to overcome the collapsing effect of surface tension created by the air-water interface in the airways, the decrease in intrapleural pressure has no effect on lung volume.

2. **Stage 2 (stage of opening of airways):** When intrapleural pressure decreases above ~8 cm H₂O, lung volume increases first due to opening of proximal airways and then by opening of distal airways.

3. **Stage 3 (stage of linear increase in lung volume):** When intrapleural pressure decreases further (becomes more negative) opening and expansion of all airways occur. This results in steep but linear increase in lung volume.

4. **Stage 4 (stage of cessation of inflation):** As increase in lung volume approaches TLC, further increase in the volume becomes less, and finally ceases. This occurs due to decrease in airway and chest wall compliance and limitation of muscle strength.

**Hysteresis Loop:** The difference between the inflation and deflation pressure-volume curves (Fig. 104.9) is known as hysteresis.

1. The hysteresis loop occurs because a greater pressure difference is needed to open an already closed airway than to keep airway open that has already been opened (to maintain airway opening, i.e. to prevent open-airway from closing).

2. For example, to keep open a previously opened airway at FRC requires a pressure of only about ~5 cm H₂O (as noted in the deflation limb of pressure-volume curve), whereas to inflate a collapsed lung at FRC requires a pressure of about ~15 cm H₂O (as noted in the inflation limb of pressure-volume curve). However, the hysteresis loop is much smaller during normal breathing and is located close to the deflation limb of the pressure-volume curve.

3. The slope of pressure-volume curve is the compliance and is the measure of distensibility of lungs.

**Dynamic Lung Compliance Measurement**
Dynamic lung mechanics are the mechanics in play when the lungs are in motion.

1. Dynamic lung compliance is measured by continuously recording the change in intrapleural pressure and lung volume.

2. In pressure-volume loop of dynamic record, the rise in volume in inspiration is in the form of a curve and returning to the starting point during inspiration is another curve.

3. The difference in inflation and deflation limbs of the curve produces a hysteresis, which is much similar...
to the pattern obtained in pressure-volume curve of static lungs, but the difference is that dynamic compliance of lung is less than its static compliance.

4. Dynamic lung compliance increases during exercise. However, dynamic compliance of chest wall does not differ significantly from its static compliance in normal conditions (Application Box 104.1).

**Application Box 104.1**

*Higher dynamic lung compliance is helpful in exercise:* Dynamic lung compliance during exercise is more than during the resting state. In resting state, alveolar surface area changes less due to tidal respiration. Therefore, smaller change in alveolar surface area, which is not adequate to bring more surfactant molecules to the surface, makes the lung less compliant. Whereas, during exercise, a larger change in tidal volume causes greater change in alveolar surface area, which incorporates more surfactant molecule to the air-liquid interface of the alveoli. Therefore, the lung is more compliant during exercise, which ensures better ventilation. Sighing and yawning also increase dynamic lung compliance.

**Regional Variation of Lung Compliance**

In upright posture, the lung compliance is less at the top portion of the lungs than at the base. This regional difference in compliance is due to the effect of gravity. As discussed above, due to the gravitational effect, the pleural pressure is less (more negative) at the apex than at the base of the lungs.

1. At the apex, transpulmonary pressure is therefore more, which causes the alveoli in the apical region to be more distended. This decreases compliance of lung at the apex. Thus, the apex of the lung is less distensible (Application Box 104.2).
2. Thus, at the base of the lung, there is a larger change in volume for the same pressure change, which ensures greater expansibility.

**Application Box 104.2**

Compliance facilitates ventilation: As discussed above, base of the lung is more distensible due to greater compliance. Consequently, during inspiration, a larger share of tidal volume goes to the base of the lungs. This is a major factor for greater ventilation at the base of the lungs in comparison to the apex.

**Factors Affecting Lung Compliance**

1. Distensibility and elastic recoil: More the lung is distensible and elastic more is the compliance. A stiff lung has less compliance.
2. Lung volume: The pressure-volume curve of the lung is nonlinear, which indicates that the compliance is not equal at all lung volumes. The compliance is high at low lung volumes and low at high lung volumes.
3. Lung size: In general, compliance is proportionate to the size of the lung. Thus, the lung of rat has less compliance than the lung of elephant. Children lung is less compliant than the adult lung. However, compliance expressed per unit tissue may be less in advancing age due to increased stiffness of the lung tissue.

4. **Surface tension inside the alveoli:** Surface tension mainly affects lung compliance (discussed below). Surface tension at the air-water interface of airways accounts for more than half of the lung compliance.

**Clinical Significance of Lung Compliance**

Diseases of lung alter the lung compliance.

**Low Compliance**

The low lung compliance indicates less distensibility. This means more work is required in a lung with low compliance to inflate the lungs to achieve the normal tidal volume.

1. In restrictive lung diseases, the compliance of the lungs is significantly low. For example, in pulmonary fibrosis, fibrous tissue is deposited in the lungs, which makes lungs stiff and difficult to inflate.
2. In a normal lung, tidal volume of 500 ml is achieved at about 2.5 cm H$_2$O of transpulmonary pressure.
3. In lung fibrosis, the same change in pressure produces a much less increase in lung volume (Fig. 104.10). This indicates that the static lung compliance in this condition is much less.

**High Compliance**

High compliance is also equally bad as low compliance. High compliance is seen in obstructive lung diseases like emphysema.

1. In emphysema, which occurs in chronic habitual smokers, the lung tissue (extracellular matrix including elastin) is destroyed by elastase released from macrophages. In this disease, it is much easy to inflate lung, since a higher lung volume is obtained at any particular pressure (Fig. 104.10). The static lung compliance is higher.
2. However, the elastic recoil of lungs is much less, due to which getting air out of lung is severely impaired.
Therefore, emphysematous lungs have abnormally high residual volume.

**Elastic Properties of Chest Wall**

Like lungs, chest wall has elastic properties, but the elastic recoil of the chest wall is in outward direction. Thus, chest wall has the intrinsic tendency to expand.

1. The outward recoil of chest wall balances the inward recoil of lungs. Therefore, if the elastic recoil of the chest wall is unopposed, as occurs in lung collapse, chest wall expands to about 70% of total lung capacity (TLC).
2. That means, when the volume is less than 70% of TLC, the chest wall starts recoiling outward.
3. It should be noted that, the outward elastic recoil of the chest wall is maximum at residual volume (and the inward elastic recoil of the lung is maximum at TLC).

At functional residual capacity (FRC), the elastic recoil of lung and chest wall balance each other. That means for the lung volumes at FRC, chest wall and lung have equal elastic recoil forces in opposite directions. Alterations in these elastic forces significantly affect FRC.

**Measurement of Thoracic Wall Compliance**

To measure thoracic wall compliance, first the compliance of thoracic cage and lungs are measured combine. Intrapulmonary pressure (which is the total pressure of lungs and thoracic wall) is measured for each lung volume inhaled. Then, lung compliance is measured separately (as described above). Thoracic wall compliance is then determined from the following formula:

\[
\frac{1}{\text{Thoracic wall compliance}} = \text{Total compliance} - \frac{1}{\text{Lung compliance}}
\]

So, thoracic wall compliance

\[
= \frac{\text{Lung compliance}}{\text{Total compliance} \times \text{Lung compliance} - 1}
\]

**Lung-Chest Wall Interaction**

During respiration, lung and chest wall move simultaneously in opposite direction. The change in volume of lungs and thoracic wall is equal as they move together. The pressure changes across the lung and chest wall during the respiratory movements are called transmural pressures (see above). These pressures are:

1. **Transpulmonary pressure**: This is the transmural pressure for lung. This is the pressure difference between the alveolar pressure and the intrapleural pressure. To increase its volume, lung requires positive transpulmonary pressure. When transpulmonary pressure is zero, the size of the lung is minimum. At zero transpulmonary pressure, though the lung is smallest in size, alveoli are not fully collapsed due to surface tension lowering property of surfactant.
2. **Transmural pressure across the chest wall**: This is the difference between intrapleural pressure and the pressure surrounding the chest wall (the body surface pressure or the barometric pressure). Transmural pressure across chest wall is usually negative as the intrapleural pressure is negative relative to the atmospheric pressure.
3. **Pressure across the respiratory system**: This is the sum of the pressure across the lung and across the chest wall. This is calculated as the difference between alveolar pressure and the barometric pressure.

**Relaxation Pressure-Volume Curves**

Interaction between these transmural pressures determines the lung-volumes and capacities, which can be studied by analyzing the relaxation pressure-volume curves of lung, chest wall and respiratory system (Fig. 104.9).

1. **At functional residual capacity** (FRC), transmural pressure across the respiratory system is zero, and at total lung capacity (TLC), both lung and chest wall pressures are positive.
2. This indicates that both lung and chest need positive transmural distending pressures.
3. The resting volume for chest wall is the 60% of TLC. This (the resting volume for chest wall) is the volume at which the transmural pressure for the chest wall is zero.
4. This means that at volumes less than 60% of TLC, chest wall tends to recoil outward and at volumes more than 60% of TLC, chest wall recoils inward.
5. It should be noted that at transpulmonary pressure of 20 cm of H\(_2\)O, lung reaches its elastic limit; therefore, the pressure curve flattens beyond this pressure.
6. The increase in transpulmonary pressure above 20 cm of H\(_2\)O does not increase lung volume much, as the distension of lung is limited by the connective tissues of the lung.
7. Further increase in pressure results in rupture of alveoli near the lung surface that can lead to pneumothorax (escape of air into the pleural space).

**ROLE OF SURFACE TENSION**

The force that pulls the surface molecules together of a liquid at an air-liquid interface is called surface tension. There is a thin layer of surfactant (a liquid film) lining the inner surface of the alveoli and the alveoli is filled with air; this forms the air-liquid interface.

1. The surface tension at the air-liquid interface in the lung alveoli is a major factor that influences lung compliance. In fact, surface tension at the air-liquid interface of lung airways accounts for more than half of the elastic recoil of lungs.
2. At the air-liquid interface of alveoli, surface tension is created because water molecules are strongly attracted to one another than to air molecules.
3. The surface tension in the alveoli produces a force, which is directed inward, and therefore, tends to decrease the alveolar size. Thus, surface tension favors collapse of alveoli.

The importance of surface tension in lung functions is best studied by analyzing the pressure-volume curves of lungs filled with air and liquid (Fig. 104.11). In this experiment, lungs are removed from chest and inflated or deflated at various pressures separately, first filled with air and then with saline. Surface tension is generated in the lung filled with air as air-liquid interface is created, whereas, surface tension is absent in the lung filled with saline as air-liquid interface is eliminated.

Two important points are noted in these pressure-volume curves:
1. **Hysteresis** (difference between inflation and deflation limbs of pressure-volume curve) is **very less** in saline-lung than air-lung. This indicates that in saline-lung, pressure needed to inflate the lung is almost same as to deflate the lung.
2. The **slope of inflation limb of saline-lung** is much steeper than the inflation limb of air-lung. This is because the static compliance is very high in saline-lung as lung was inflated with much less pressure (lung was more compliant due to elimination of surface tension). Thus, above evidences clearly signify that surface tension significantly affects compliance and work of breathing.

**Application of Laplace Law**

Surface tension also maintains alveolar stability. The Laplace law explains the relationship of surface tension with pressure inside a sphere like alveolus. The Laplace law states that the pressure in a spherical structure is equal to twice the wall tension divided by the radius, i.e. 

\[ P = \frac{2T}{r} \]

where, \( P \) is the pressure, \( T \) is surface tension and \( r \) is radius.

In lungs, all alveoli do not have same diameter, alveoli size also changes during inspiration and expiration, and alveoli are interconnected with each other.

1. If the surface tension was the same in all alveoli, according to the Law of Laplace, pressure would be greater in smaller alveoli than in bigger alveoli. The smaller alveoli would empty into the larger alveoli if they are interconnected and larger distending pressure would be needed to keep the smaller alveoli inflated (Fig. 104.12).
2. Thus, one can presume that larger alveoli tend to distend more and smaller alveoli tend to collapse, especially at low lung volumes. But, this does not happen. Surface tension is constant per unit surface area (i.e. 50 dyne/cm²). So, the surface tension of the alveoli decreases with the decrease in surface area.
3. Therefore, as the alveoli become smaller, the surface tension also reduces and as the alveoli distends (becomes larger), surface tension increases.
4. Thus, the smaller alveoli do not collapse as pressure \((2T/r)\) within the alveoli remains same and, therefore, volume of interconnected alveoli remains almost same.
Role of Surfactant in Pulmonary Mechanics

The inner lining of alveoli is coated by surfactant. The surfactant means a surface-active agent that decreases surface tension. The pulmonary surfactant is a thin liquid film of alveolar lining that decreases surface tension at the gas-liquid interface of alveoli and alters surface tension with change in alveolar diameter.

Pulmonary Surfactant

Structure and Composition

Pulmonary surfactant is a mixture of lipoprotein rich in phospholipid. The principal surface tension-lowering agent in surfactant is dipalmitoylphosphatidylcholine (DPPC), which is also called dipalmitoyllecithin (DPL).

1.Phospholipids of surfactant have a hydrophilic head and a hydrophobic tail. The hydrophobic tail consists of fatty acids. The tail of phospholipids faces toward the alveolar lumen (Fig. 104.13).

2. The alveolar surface tension is inversely proportional to the concentration of phospholipids in the surfactant.

Surfactant consists of:

1. **Lipids:** 90%
   - Dipalmitoylphosphatidylcholine (62%)  
   - Phosphatidylglycerol (5%)  
   - Other phospholipids (10%)  
   - Neutral lipids (13%)

2. **Proteins:** 8%
   - Albumin  
   - Immunoglobulin A (IgA)  
   - Apoproteins (SP-A, -B, -C, -D)

3. **Carbohydrates:** 2%

Source

Surfactant is synthesized in Type II pneumocytes. There are two types of cells in alveolar epithelium: type I and type II alveolar epithelial cells.

1. **Alveolar type II cells** are called type II pneumocytes. Though, the ratio of type I to type II epithelial cells is about 1:1, type I cells form about two-third of the surface area due to their flat surface.

2. However, type II cells are metabolically more active and contain more number of mitochondria. The special histological feature of type II cells is the presence of electron-dense lamellar inclusion bodies (Fig. 104.13).

3. In fact, the lamellar inclusion bodies store surfactant in type II cell.

Synthesis and Secretion

Palmitate, choline and glucose are substrate molecules for surfactant synthesis. The substrate molecules are taken up by alveolar type II cells from the circulating blood.

1. In mitochondria, they are synthesized into DPPC.

2. The surfactant is then stored in the lamellar inclusion bodies.

3. Lamellar inclusion bodies are continuously discharged onto the alveolar surface by constitutive exocytosis (exocytosis which is continuous and unregulated).

4. The released lamellar body-materials are converted into tubular myelin that forms the film of surfactant on the alveolar surface (steps of surfactant synthesis are summarized in Flowchart 104.3).

5. The active synthesis of lipid in the lung accounts for high rate of replacement of surfactant. Thus, an integrated layer of surfactant is continuously formed on the alveolar epithelium.

6. Some amount of surfactant is recycled (reuptake by type II cells) back.

Time of Surfactant Synthesis in Fetal Life

Lungs are among the last organs to develop during intrauterine life. Therefore, synthesis of surfactant occurs toward later part of pregnancy.

1. Surfactant synthesis starts at about 34 weeks of pregnancy, which is completed by about 90% at term (38–40 weeks). That means, maturation of surfactant production continues to the perinatal period.

2. Therefore, inadequate surfactant synthesis resulting in lung collapse is among the major causes of death in premature deliveries.

3. In premature newborns, adequate amounts of surfactant are not available to reduce surface tension and stabilize surface forces during breathing.

Regulation of Synthesis and Secretion

Surfactant synthesis is regulated by hormones, concentration of protein in the surfactant, stretching of lung and various pharmacological agents.
A. Hormonal factors
1. Glucocorticoid hormones: Cortisol stimulates surfactant synthesis. Secretion of glucocorticoids increases toward term. Number of glucocorticoid receptors in lungs also increases in third trimester of pregnancy.
2. Thyroxine: Thyroxine promotes surfactant synthesis. Maternal thyroid deficiency and cretinism decrease surfactant secretion.
3. Insulin: Insulin facilitates surfactant synthesis. Therefore, diabetes during pregnancy decreases surfactant secretion.

B. Quantity and quality of proteins in surfactant
Formation of phospholipid film of surfactant is greatly facilitated by proteins present in the surfactant. Surfactant proteins are albumin, immunoglobulin (IgA) and apoproteins (surfactant proteins).
1. There are 4 types of surfactant proteins (SP): SP-A, SP-B, SP-C, and SP-D. SP-B and SP-C control synthesis of monomolecular film of phospholipid.
2. SP-A controls reuptake of surfactant by type II cells.
3. The protein content of surfactant depends on concentration of protein in the plasma.

C. Stretching of lungs
Hyperinflation of lungs like yawning enhances surfactant synthesis. Yawning and sighing during infancy are effective stimuli for surfactant synthesis.

D. Pharmacological agents
- β adrenergic agonists enhance surfactant secretion
- Calcium stimulates surfactant secretion

E. Exercise
Practice of regular physical exercise stimulates surfactant synthesis in both children and adults.

Functions of Surfactant
1. Prevents lung collapse: Surfactant decreases surface tension at the air-liquid interface of alveoli. By decreasing surface tension, surfactant prevents lung collapse. During lung deflation, compression of surfactant molecules decreases surface tension. Especially at low lung volumes, the molecules are tightly compressed. During lung inflation, new surfactant is extruded onto the alveolar surface that forms a new film on the alveolar epithelial lining.
2. Promotes alveolar stability: In lungs, alveoli of different diameters are connected in parallel to each other. By law of Laplace, smaller alveoli could drain into larger alveoli and collapse. However, that does not happen and alveoli coexist. This alveolar stability is maintained by surfactant. Surfactant achieves this by lowering surface tension proportionately more in the smaller alveoli.
3. Helps to prevent edema in the lung: The inward force of surface tension that tends to collapse alveoli also tends to decrease interstitial pressure in the lungs. Decreased interstitial pressure pulls fluid out of capillaries and facilitates pulmonary edema formation. Surfactant reduces surface forces and thus prevents pulmonary edema formation. This is an important function of surfactant.
4. On work of breathing: Surfactant decreases the work of breathing, so that breathing becomes easier.
5. On immunity: IgA and apoproteins (SP-A and SP-D) in surfactant provide innate immunity by acting as opsonins. They promote phagocytosis of bacteria and viruses by alveolar macrophages.

Clinical Importance
1. Infant respiratory distress syndrome: After birth, the newborn makes strong inspiratory effort so that the lungs expand. Surfactant helps in lung inflation and prevents collapse of expanded lung. Thus, respiration continues throughout life after the first breath. Lung maturation is incomplete in premature infants. Also, hormonal disturbances like diabetes during pregnancy interfere with lung maturation. In such conditions, due to lack of surfactant synthesis, lung expansion becomes difficult after birth. Breathing becomes extremely labored due to high surface tension. It becomes difficult to inflate lungs. This is called infant respiratory distress syndrome (IRDS) or hyaline membrane disease. Pulmonary edema and atelectasis develop due to high surface tension.
Treatment of IRDS:

i. Administration of phospholipid by inhalation
ii. Inhalation of synthetic surfactant (or a surfactant preparation derived from bovine lungs)
iii. Glucocorticoid therapy (glucocorticoid promotes surfactant synthesis)

2. Atelectasis following surgery: During deep breathing, the lungs inflate to a larger volume and new surfactant molecules spread thoroughly on the alveolar surface, whereas, during shallow breathing, the spreading of surfactant is impaired. Therefore, patients recovering from anesthesia after surgery are often encouraged to breathe deeply to enhance the proper spreading of surfactant on the alveolar surface (Clinical Box 104.1).

Alveolar Stability

Alveolar stability is maintained by two factors:
1. Pulmonary surfactant (discussed above)
2. Principle of interdependence

   Interdependence is the mutual support among adjacent alveoli. Normally, alveoli are interconnected with surrounding alveoli. They support each other because of their structural arrangement (with many interconnecting links).
   - When one alveolus collapses, it prevents the collapse of adjacent alveoli. This is because, when alveoli tend to collapse, surrounding alveoli develop greater expanding forces. Thus, interdependence not only prevents atelectasis but also opens up alveoli that have already collapsed.
   - Alveolar interdependence is the major factor in adult lungs that maintain alveolar stability.
   - This is not important in newborns, as newborn lungs have fewer interconnecting links.
   - Surfactant is the major factor for maintaining alveolar stability in newborns and infants.

Properties of the Dynamic Lung

Properties of dynamic lung are studied when the lungs are in motion, associated with flow of air in and out of the respiratory tract. Under dynamic conditions (during air-flow), force is not only required to maintain the lung and chest wall at certain volume but also to overcome the inertia and resistance of tissues and air molecules.

Airflow in Airways

Flow occurs through a tube when the pressure difference exists at both ends of the tube. During inspiration, intrapleural pressure becomes negative that causes lung expansion due to which pressure decreases in the airways. This allows air to be sucked into the airway from outside. Two types of airflow occur in the lung: laminar flow and turbulent flow.

1. Laminar flow: When airflow is slow, laminar flow occurs. This is characterized by a streamlined flow. The flow is silent because layers of air molecules slide over each other. Normally, this type of flow occurs in small peripheral airways.
2. Turbulent flow: Turbulent flow occurs at high flow rates. This is normally observed in large airways, especially in the trachea and large bronchi. It consists of a disorganized pattern of airflow that produces sound. Therefore, in fast and deep breathing, the sound is heard both in inspiration and expiration.

Details of dynamics of inspiratory and expiratory flows and their importance are discussed above (in lung volumes and capacities).

Airway Resistance

The airflow causes resistance to air moving in the airways. It is defined as the ratio of driving pressure (ΔP) to airflow (V). Airway resistance (Raw) is expressed in cm H₂O/L per second. For total airway resistance (Raw), the driving pressure is the pressure difference between the mouth (Pmouth) and the alveoli (Pₐ).

\[
\text{Raw} = \frac{\Delta P}{V} = \frac{P_{\text{mouth}} - P_{\text{A}}}{V}
\]

Normal Value

In healthy individuals, the airway resistance is about 1–3 cm H₂O/L per second (with lungs at FRC). Airway resistance is more in children due to their smaller airways.

Site of Resistance

The primary site of airway resistance is the medium bronchi (lobar and segmental) and bronchi down to about the seventh generation (Fig. 104.14). As per Poiseuille’s law, the airway resistance increases with the fourth power of airway radius. Thus, one would expect that the small airways would offer more resistance to airflow. However, contribution of by small airways to the total airway resistance is only about 10 to 15%. This is because most of the small airways are aligned in parallel and the total surface area of small airways (like capillaries in vascular system) is more.

Clinical Importance

In the early stage, airway diseases mainly affect small airways. However, diagnosing diseases in the initial stage
becomes difficult because small airways contribute less to the total airway resistance. Airway diseases are diagnosed usually in their advanced stage.

**Factors affecting Airway Resistance**

1. **Diameter of airways**: Caliber of the airway is the most important factor affecting airway resistance. **Bronchodilation decreases** and **bronchoconstriction increases** resistance.

2. **Lung volume**: Lung volume is among the major factors affecting airway resistance because **caliber of airways is directly proportionate to the lung volumes**. Bronchi and small airways are embedded in lung tissue. Therefore, airway diameter increases with lung expansion. Thus, airway resistance **decreases during inspiration and increases during expiration**. It should be noted that airway resistance rises sharply at low lung volumes (Fig. 104.15).

3. **Bronchial smooth muscle tone**: Bronchial smooth muscle tone also affects airway resistance as it controls airway diameter. The smooth muscle tone of the airway is influenced by following factors:
   a. **Autonomic control**:
      i. **Parasympathetic**: Parasympathetic (vagal cholinergic fibers) stimulation causes **bronchial constriction** and increases mucus secretion. These effects are mediated by muscarinic receptors.
      ii. **Sympathetic**: Sympathetic Stimulation (adrenergic fibers) **causes bronchodilation** and inhibits glandular secretion. Activation of $\beta_2$ receptors causes bronchodilation increases bronchial secretion, whereas activation of $\alpha_2$ receptors inhibits secretion.
      iii. **Noncholinergic-nonadrenergic innervation**: Also, there is noncholinergic-nonadrenergic innervation of bronchiole that **causes bronchodilation**, most likely mediated by VIP.

   b. **Humoral factors**: Epinephrine, norepinephrine and other hormones that stimulate $\beta_2$-adrenergic receptors **cause dilation of airways**. Histamine causes constriction of alveolar duct and increases airway resistance.

   c. **Drugs**: Drugs, such as isoproterenol ($\beta_2$-adrenergic receptor agonists), produce bronchodilation. These drugs are usually used in the treatment of asthmatic attacks as they alleviate bronchial constriction.

   d. **Environmental factors**: Breathing chemical irritants, **dusts and smoke** particles cause reflex constriction of airways. Vagus nerve is the efferent pathway in the reflex arc that mediates bronchoconstriction in response to such irritants like cigarette smoke.

   e. **PCO$_2$ in breathing air**: Increased PCO$_2$ in the conducting airways causes **local bronchodilation**. However, bronchoconstriction caused by the decreased PCO$_2$ is of more importance.

4. **Viscosity and density of inspired gas**: Increase in gas density as occurs in **deep-sea diving increases airway viscosity**. The air density increases because of increased barometric pressure. Gas mixture containing oxygen-helium has low density. Therefore, a helium-oxygen mixture is often used during dive to make breathing easier. Oxygen-helium mixture is also used in the treatment of status asthmaticus.

5. **Phases of respiration**: Airway resistance is not significantly altered during quiet breathing. However, during forced expiration as seen in severe exercise, resistance is significantly increased. A high resistance is associated with decreased expiratory flow. The marked increase in resistance during forced expiration occurs due to compression of airways. This effect is well demonstrated by a **flow-volume curve** that depicts the relationship between airflow and lung volume during a forced inspiratory and expiratory effort (Fig. 104.16).
Flow-Volume Curve

A small loop of flow-volume curve is seen during tidal breathing, whereas, a large loop is observed during forced breathing.

1. **During forced inspiration**, inspiratory flow is limited only by effort; that means the force with which the individual inspires.

2. **During forced expiration** (forced vital capacity), flow rises sharply to a peak (peak expiratory flow) and then decreases linearly over most part of expiration as lung volume decreases.

3. The **first phase of expiratory flow-volume curve is dependent on effort**, but after the peak expiratory flow is reached, flow becomes independent of effort.

4. Thus, **during forced expiration, flow is mostly independent of effort**. This indicates the importance of dynamic airway compression during expiration.

5. Dynamic airway compression increases airways resistance, which effectively limits the forced expiratory flow (Clinical Box 104.2).

**Clinical Box 104.2**

**Bronchodilators are useful in asthma:** In bronchial asthma, the major pathologic consequence is the spasm of bronchial airway that increases airway resistance. Therefore, breathing becomes difficult (expiration is more difficult than inspiration). Hence, immediate treatment of asthma is the administration of bronchodilators. Airway resistance also increases in emphysema and bronchitis.

Timed Vital Capacity (FEV<sub>1</sub>)

This is also called **forced expiratory volume in 1 second** (FEV<sub>1</sub>). This is defined as the percentage of FVC expired in the specified time, e.g. FEV<sub>1</sub>, the percentage of air expired in 1st second; FEV<sub>2</sub>, in 2nd second and so on. It is one of the most useful tests to detect generalized airway obstruction.

1. This measures the FVC in relation to time and gives the percentage of FVC expired in the stipulated time. This is an index of air-flow rate.

2. In normal conditions, 72 to 85% of the forced vital capacity is expired in the first second, 95% in two seconds and 97% in three seconds (Fig. 104.17).

3. It is one of the most useful tests to detect generalized airway obstruction.

4. The ratio of FEV<sub>1</sub> to FVC is more important clinically than their individual measurements, which is normally about 0.8 as calculated from clinical spirogram (Fig. 104.18).

Clinical Importance of FEV<sub>1</sub>

FEV<sub>1</sub> is the single most useful test to detect generalized airway obstruction.

1. FEV<sub>1</sub> less than 72% indicates difficulty in exhaling air from the lungs due to obstruction. This is a hallmark of obstructive lung disease like bronchial asthma.

2. In restrictive lung disease like emphysema though FVC is decreased, FEV<sub>1</sub> as percentage of FVC remains normal (Fig. 104.19).

3. Thus, FEV<sub>1</sub> curve differentiates obstructive from restrictive lung diseases.
Section 10: Respiratory System

Maximum Mid-expiratory Flow Rate (MMEFR)

This is the maximum flow achieved during the middle third of the total expired volume.
1. This is expressed as forced expiratory flow at 25% to 75% of the lung volume (FEF_{25–75}). FEF_{25–75} indicates the patency of small airways.
2. The measurement of flow rate between 200 and 1200 mL (FEF_{200–1200 mL}) in liters per second indicates the patency of larger airways.

Maximum Voluntary Ventilation

This is also called as maximum breathing capacity (MBC).

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Maximum Voluntary Ventilation (MVV)

Indicates the maximum volume of gas that can be breathed per minute by maximal voluntary effort.

1. The normal value of MVV in adult male is 150 liters per minute and for adult female is 125 liters per minute.

Peak Expiratory Flow Rate

Peak expiratory flow rate (PEFR) is the maximum velocity of flow in liters per minute with which air is forced out of the lungs. PEFR is recorded by using peak-flow meter.
1. The normal value of PEFR is 400 to 600 L/min or 6 to 10 L/s.
2. PEFR is an index of patency of airways.
3. However, MMEFR is a more sensitive indicator than PEFR.

Maximum Expiratory Flow Volume Curve

Because of the limitation of FEV_{1}, certain other tests are performed to detect airways obstruction in its early phase.
1. These include V_{max 75}, 50% and 25% responses of maximum expiratory flow, which are studied by plotting maximum expiratory flow volume curve (MEFVC) by inhalation of helium. In MEFVC, flow is plotted against volume exhaled.
2. V_{max 75} is the flow rate at which 75% of the total FVC has been exhaled, V_{max 50} is the flow rate at which 50% of FVC remains to be exhaled and V_{max 25} is the flow rate at which 25% of the FVC has been exhaled. (TLC: Total lung capacity; RV: Residual volume; PEFR: Peak expiratory flow rate).

1. Maximum voluntary ventilation (MVV) indicates the maximum volume of gas that can be breathed per minute by maximal voluntary effort.
2. The normal value of MVV in adult male is 150 liters per minute and for adult female is 125 liters per minute.

Peak Expiratory Flow Rate

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3. When the individual performs at three different efforts (maximum, moderate and minimum), the MEFVC curves indicate that PEFR increases with effort.

4. However, at lower lung volumes (toward residual volume) the effort has no impact on flow-rate as all the three curves merge in the later part (Fig. 104.21).

5. This indicates that in expiratory maneuver, the early part is effort dependent and the later part is effort independent.

**Work of Breathing**

During breathing, work is done to expand the lungs and overcome the airway resistance. Work is involved during inspiration to expand the thoracic cavity, inflate the lungs, and to overcome airway resistance. The work done is expressed as a change in lung volume multiplied by the change in trans-pulmonary pressure. Thus, work \( W \) is equal to the product of pressure \( P \) and volume \( V \).

\[ W = P \times \Delta V, \]

where \( P \) is transpulmonary pressure and \( \Delta V \) is change in lung volume.

Energy is spent with muscular contraction to create a force (transpulmonary pressure) to increase lung volume (inflate the lungs). Therefore, more muscular work is done to create greater trans-pulmonary pressure, which is required to bring more air into the lungs. The pressure-volume curve depicts the work required for breathing in normal lung that spends about 5% of the body's total energy expenditure (Fig. 104.22A). During exercise, total energy expenditure for breathing is increased.
**Restrictive Lung Disease**

In restrictive lung disease, work of breathing is increased, as greater inspiratory effort is required due to marked decrease in lung compliance (Fig. 104.22B). Therefore, patients with a restrictive disorder economize their ventilation by taking rapid and shallow breaths.

**Obstructive Lung Disease**

In obstructive lung disease, work of breathing increases due to more expenditure of energy to overcome increased airway resistance (Fig. 104.22C). Patients with obstructive disorders economize their ventilation by taking slow and deep breaths.

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**CHAPTER SUMMARY**

**Key Concepts**

1. Contraction of diaphragm expands the thoracic cage and causes inflation of lungs. This decreases alveolar pressure and inspiration ensues.
2. Expiration is passive at rest that occurs due to elastic recoil of lungs.
3. Surfactant prevents collapse of lungs.
4. FEV₁ less than 72% indicates obstruction in the airway.

**Important to Know (Must Read)**

1. In examination, ‘Describe the mechanics of breathing with reference to the relaxation pressure-volume curve of the lung, chest wall and respiratory system’ may be asked as a Long Question.
2. Pulmonary surfactant, Timed vital capacity (FEV₁), Intrapleural pressure, Airway resistance, Work of breathing, Relaxation pressure-volume curve of the lung, Muscle of respiration, Lung volumes and capacities, Flow-volume curve, may be asked as Short Questions in exam.
3. In Viva, examiner may ask… Name the muscles of inspiration, name the muscles of expiration, What is intrapleural pressure, How is the intrapleural pressure created, Why is the intrapleural pressure negative, What is the normal value of the intrapleural pressure, What is the effect of posture and gravity on the intrapleural pressure, What is the significance of the intrapleural pressure, What is transmural pressure, what is its normal value, Define inspiratory reserve volume, what is its normal value, Define residual volume, what is its normal value, Define vital capacity, what is its normal value, Define inspiratory capacity, what is its normal value, Define functional residual capacity, What is its normal value, Define total lung capacity, what is its normal value, Define lung compliance, What is static lung compliance, What is dynamic lung compliance, Why is higher dynamic compliance helpful for exercise, Why does compliance facilitate ventilation, What are the factors affecting lung compliance, What is the clinical significance of lung compliance, What is the formula for measurement of thoracic wall compliance, What is the composition of surfactant, How is the surfactant synthesized and secreted, How is the surfactant synthesis and secretion regulated, What are the functions of surfactant, What is IRDS, how is it treated, What is atelectasis, how it is treated, Define airway resistance, What are the factors affecting airway resistance, Why bronchodilators are useful in asthma, What is FEV₁, what is its clinical importance, What is maximum midexpiratory flow rate, what is its significance, What is maximum voluntary ventilation, what is its normal value, What is peak expiratory flow rate, what is its normal value, What is work of breathing, how is it different in restrictive and obstructive lung disease.
**Important Terms**

**Ventilation:** Ventilation is a dynamic process that moves air in and out of the lungs.

**Minute ventilation:** Minute ventilation is the volume of air that enters lungs each minute. This is the **product of tidal volume and respiratory rate**.

If tidal volume is 500 ml, and respiratory rate is 12 per minute, the minute ventilation is 6 liters.

\[
\text{Minute ventilation} = \text{tidal volume} \times \text{respiratory rate} \\
= 500 \text{ (ml)} \times 12 \text{ (per/min)} \\
= 6000 \text{ ml/min}
\]

Normally, minute ventilation in adult is **5 L to 10 L per/min**, as the respiratory rate varies from 10 to 20 per min. However, tidal volume also varies with age, gender, body composition and metabolic activity. In children, tidal volume is 3–5 ml/kg of body weight. Respiratory rate is also high in children.

**Expired minute ventilation:** Expired minute ventilation is the amount of expired air per minute and at rest is same as minute ventilation as the inspired and expired tidal volume (the amount of air inspired in or expired out in quiet breathing) is same.

**DEAD SPACE**

The tidal volume occupies the respiratory tract that consists of conducting and respiratory airways. The fraction of tidal volume that occupies the conducting airways **does not take part in gas exchange**. For each 500 ml of air inhaled, about **150 ml remains in the conducting airways**, which is not used in gas exchange. This volume of wasted air is known as **dead space volume**.

1. Thus, dead space is defined as space within the airways that do not allow gas exchange with pulmonary capillary blood to occur. Since, the dead space is due to the anatomy of the airways, often it is referred to as **anatomical dead space**.
2. Due to the anatomical dead space, out of 500 ml of tidal inspiration, 350 ml of air enters the alveoli. Thus, the **normal dead space volume is 150 ml**.
3. The ratio of dead space volume to tidal volume in normal health is 25 to 30%. Thus, normally **30% of the minute ventilation does not participate** in gas exchange.

**Physiological Dead Space**

Some amount of inspired air in the alveoli does not participate in gas exchange. This happens as some alveoli due to
various reasons have little or no blood supply. Therefore, air in these alveoli also becomes part of the wasted air. The volume of air in these alveoli is referred to as alveolar dead space.

1. The sum of the anatomical dead space and alveolar dead space is called physiological dead space. Physiological dead space = anatomic dead space + alveolar dead space

2. Alveolar dead space is negligible in normal individuals. Therefore, in normal healthy individuals, anatomical dead space is almost equal with the physiological dead space.

3. However, in various lung diseases, alveolar dead space volume becomes high, in which the physiological dead space is more than anatomical dead space.

**Measurement of Dead Space**

The volume of dead space may be estimated by any of three methods: the Radford's formula, and the single-breath CO₂ and N₂ techniques.

**The Radford’s Formula**

Radford’s formula predicts the anatomical dead space of the individual. The anatomical dead space of an individual in milliliters is equal to his weight in pounds. Thus, for a person with body weight of 150 lbs (70 kg), the dead space is 150 ml. However, this prediction is fairly reliable for healthy individuals, but not in patients with respiratory problems.

**Single-Breath CO₂ Technique**

The volume of dead space (V_D) is usually measured by the single-breath technique that uses Bohr’s equation in the form of a gas such as CO₂. CO₂ used because it is almost absent in the inspired air, and its alveolar concentration is same as in systemic arterial blood. According to Bohr’s equation:

\[
V_D = (F_{AX} - F_{EX}) \frac{V_E}{F_{AX}}
\]

When, \(V_D\) is the volume of dead space, \(V_E\) is the volume of expired air, \(F_{AX}\) and \(F_{EX}\) represent the concentration of gas in the alveolar air and mixed expired air respectively. As alveolar concentration of gas is same as arterial blood, \(F_{AX}\) (concentration of substance in arterial blood) replaces \(F_{AX}\).

By using \(CO_2\) as the gas for this estimation,

\[
V_D = (P_{a}CO_2 - P_{E}CO_2) \frac{V_E}{P_{a}CO_2}
\]

When, \(P_{a}CO_2\) and \(P_{E}CO_2\) represent the PCO₂ in the arterial blood and mixed expired air respectively. However, alveolar concentration of \(CO_2\) can be obtained from PCO₂ of last 10 ml of expired air.

**Single Breath Nitrogen Technique**

The subject sitting comfortably breathes room air calmly for few breaths. Then, he is instructed to take a deep breath from a normal mid-inspiration during which he is switched over to a pure oxygen source (takes deep breath with pure oxygen). The subject is then asked to expire slowly and steadily to the maximum, so that the volume expired is his vital capacity. As he expires, a simultaneous record is made of the volume flow rate and the percentage content of \(N_2\) in the expired air, which is plotted as a graph (Fig. 105.1).

1. When the subject takes deep breath of pure oxygen, all the air in the dead space is replaced with oxygen, while the oxygen in the alveoli mixes with \(N_2\) (and \(CO_2\)) in the residual volume. The initial gas exhaled is the phase I, which fills the dead space and therefore does not contain \(N_2\).

2. The phase II is the mixture of dead space and alveolar gas, and phase III is the alveolar gas.

3. Phase III ends at closing volume, which is the lung volume above residual volume when airways in the lower parts of the lungs starts closing due to less transmural pressure in these dependent regions.

4. In phase IV, content of expired air increases, which ends at residual volume.

5. Dead space volume is the volume of gas expired in phase I and the volume expired till mid-portion of phase II.

Dead space can be calculated from the \(N_2\) curve obtained from the single-breath \(N_2\) test in two ways:

i. By constructing the equivalent dead space-alveolar air boundary

ii. By planimetry measurement
Construction of Equivalent Dead Space—Alveolar Air Boundary

Dead space is filled with pure oxygen prior to expiration. Therefore, appearance of \( N_2 \) in the curve indicates the air coming from alveoli. The boundary between dead space air and alveolar air is S-shaped because of some degree of mixing of alveolar gas with dead space air. However, an equivalent sharp boundary is constructed in such a way that the amount of \( N_2 \) in the dead space area is equal to the amount of \( N_2 \) in the alveolar air section. The dead space volume is then calculated as the volume expired up to this line, and is read from the volume recording.

Planimetry Measurement

The area representing dead space volume and the area representing total volume expired (\( V_E \)) are estimated with the help of a planimeter. The total area in the graph represents \( V_E \) (Fig. 105.2). Hence, the dead space volume from the corresponding area is determined by simple proportion.

ALVEOLAR VENTILATION

The volume of air that actually reaches the alveoli per minute is known as alveolar ventilation (\( V_A \)).

To know the volume of air that takes part in alveolar ventilation, first the dead space volume is subtracted from the tidal volume and then the volume is multiplied by breathing (respiratory) frequency.

To say, when tidal volume is 500 ml, dead space volume is 150 ml and respiratory rate is 12 /min:

\[
\text{Alveolar ventilation} = (500 \text{ ml} - 150 \text{ ml}) \times 12 = 350 \text{ ml} \times 12 = 4200 \text{ ml / min}
\]

Significance

Clinical Importance

Alveolar ventilation represents the amount of air reaching the alveoli. This is the air that takes part in gas exchange.

1. Even in the absence of lung diseases, if anatomic dead space volume is increased due to any cause, alveolar ventilation decreases.
2. For example, a patient on mechanical ventilator, alveolar ventilation decreases due to increased dead space volume (by tubing etc.). In such patients, if minute ventilation remains constant, alveolar gas exchange suffers.

Physiological Significance

In rapid and shallow breathing, though minute ventilation does not change much, alveolar ventilation is grossly impaired because most of the air inhaled is utilized to occupy the anatomic dead space (the air available to enter into alveoli is less).

1. Therefore, patients with rapid and shallow breathing develop hypoxia and hypercapnea.
2. In contrast, a subject with deep and slow breathing will have adequate alveolar ventilation though the minute ventilation does not change much. Such subjects have alveolar ventilation even greater than subjects with normal breathing.
3. Thus, to improve alveolar ventilation, it is important to increase the depth of breathing than to increase the frequency. In fact, during moderate to severe exercise, a trained athlete achieves the target alveolar ventilation by mainly increasing the depth rather than the frequency of breathing (Application Box 105.1).

Application Box 105.1

Pranayama increases alveolar ventilation: In most of yogic breathing exercises like pranayama (controlled breathing), emphasis is given to practice slow and deep breathing, which aims at increasing alveolar ventilation in addition to imbibing universal energy (prana), which is inspired consciously.

Measurement of Alveolar Ventilation

Alveolar ventilation is easy to calculate if dead space volume is known. However, dead space volume is not easily determined in a human subject.

1. Alveolar ventilation is calculated in the pulmonary function laboratory from the volume of expired carbon dioxide per minute and fractional concentration of carbon dioxide in the alveolar gas.
2. Because no gas exchange occurs in the conducting airways and the inspired air contains essentially no carbon dioxide, all of the expired carbon dioxide originates from alveoli.

Thus,

\[
V_{\text{CO}_2} = V_A \times F_{A\text{CO}_2}
\]
Where, \( V_A \) is the volume of carbon dioxide expired per minute and \( F_A^2CO_2 \) is the fractional concentration of carbon dioxide in alveolar gas.

Rearrangement of this provides the alveolar ventilation equation:

\[
V_A = V_A^2CO_2 / F_A^2CO_2
\]

The carbon dioxide concentration in the alveoli can be obtained by sampling the last portion of the tidal volume during expiration, the end-tidal volume, which contains alveolar gas.

**Alveolar Ventilation reflects PaCO_2**: It is important to note an inverse relationship exists between \( V_A \) and \( P_a^2CO_2 \).

1. If alveolar ventilation is halved, alveolar PCO_2 will double.
2. Clinically, the adequacy of alveolar ventilation is an index of arterial PCO_2.
3. An increase in \( P_a^2CO_2 \) reflects hypoventilation and a decrease in \( P_a^2CO_2 \) reflects hyperventilation.

**Clinical Abnormalities of Ventilation**

**Hyperventilation**

Hyperventilation occurs in many conditions. Usually, they are categorized into depression of respiratory centers by diseases or drugs (central hypventilation), and failure of the ventilatory apparatus (peripheral hypventilation).

Alveolar hypventilation occurs commonly in:

1. **Exacerbation of chronic lung disease** such as asthma, chronic bronchitis and emphysema.
2. **Depression of respiratory centers** as occurs in head injuries or by drugs such as barbiturates and opiates.
3. **Neuromuscular disorders** that weaken the respiratory muscles, e.g. myasthenia gravis, poliomyelitis, acute polyneuritis, and tetanus.

In hypoventilation, hypoxia occurs due to decreased oxygen supply and hypercapnia occurs due to decreased CO_2 removal.

**Hyperventilation**

Hyperventilation usually occurs due to stimulation of respiratory centers. However, voluntary hyperventilation and exercise induced hypventilation are common physiological causes of hyperventilation. Excess removal of CO_2 in hypventilation results in respiratory alkalosis.

**GAS EXCHANGE IN LUNGS**

Gas from atmosphere enters the airways starting from nostril down to the alveoli by bulk flow.

1. The driving force for this bulk flow is the pressure gradient, which is created due to the difference in barometric pressure (the pressure at the mouth) and the alveolar pressure.
2. Exchange of gas between the alveoli and the capillary blood occurs across the alveolar-capillary membrane by diffusion in response to partial pressure gradients of the gases.
3. For example, oxygen uptake from alveoli into the pulmonary capillary blood occurs due to partial pressure gradient of oxygen across the alveolocapillary membrane.

**Scientist contributed**

Antoine Laurent de Lavoisier (1743–1794) French chemist and biologist was the first scientist to show the significance of oxygen in combustion and in the gaseous exchange in the lungs. He had refuted the phlogiston theory of Stahl. With the help of Pierre Simon de Laplace (1749-1827) he had devised a calorimeter and measured the respiratory quotient. He was guillotined during French revolution.

Gas uptake from alveoli is determined by three factors:

1. Diffusion properties of the alveolar-capillary membrane
2. Partial pressure gradient for the gas
3. Pulmonary capillary blood flow

**Alveolar-Capillary Membrane**

Alveolar-capillary membrane (also called **respiratory membrane**) forms the blood-gas interface that separates blood in the pulmonary capillaries from the gas in the alveoli.

1. Diffusion of gases between alveolar air and pulmonary capillary blood takes place through alveolar-capillary membrane.
2. The alveolar-capillary membrane is exceedingly thin, and is mainly composed of alveolar epithelium, interstitial fluid layer, and capillary endothelium.
3. As the blood perfuses the alveolar capillaries and air ventilates the alveoli, oxygen and carbon dioxide move across the blood-gas interface by diffusion.

**Layers of Alveolar-Capillary Membrane**

From interior of the alveoli to the capillary blood, the alveolar-capillary interface consists of six layers. However, \( O_2 \) from hemoglobin molecule in the red cells of pulmonary capillaries to enter into alveolar lumen (or the transport in the reverse direction), passes through **10 layers** (Fig. 105.3):

1. Alveolar surfactant
2. Layer of alveolar epithelial cells
3. Basement membrane of alveolar epithelium
4. A thin layer of interstitial fluid
5. Basement membrane of capillary endothelium
6. Layer of capillary endothelial cells
7. Plasma
8. Red cell membrane
9. Intraerythrocyte fluid
10. Hemoglobin molecule
Chapter 105: Alveolar Ventilation and Gas Exchange in Lungs

Thickness of alveolar-capillary membrane: The thickness of alveolar-capillary membrane is normally 0.2 to 0.5µ. The thinness of the membrane allows easy diffusion of gases through it.

Factors Affecting Diffusion of Gases

Diffusion of gases through alveolar-capillary membrane depends mainly on **six factors**.

1. **Difference in partial pressure** of gas on both sides of the membrane, i.e. partial pressure gradient for the gas (discussed below)
2. **Diffusing capacity** of the membrane for the gas (discussed below)
3. **Surface area of the membrane**: Surface area for diffusion decreases in conditions like emphysema and increases in conditions in which there is more opening of number of capillaries as occurs in exercise.
4. **Solubility of the gas**: Solubility of the gas in the membrane is an important factor for diffusion. For example, CO₂ being more soluble diffuses easily.
5. **Thickness of the membrane**: Diffusion is inversely proportional to the thickness of the alveolar-capillary membrane. When thickness is doubled the diffusion is halved.
6. **Molecular weight of the gas**: Diffusion is inversely proportional to the molecular weight of the gas. Thus, gas with smaller molecule diffuses easily through the membrane.

Clinical Significance

Though the alveolar-capillary membrane is thin and does not interfere with gas exchange, diffusion is disturbed in diseased conditions due to some membrane pathology. This is called as **alveolar-capillary obstruction syndrome**. This occurs in two important types of defects:

1. **Thickness of the alveolar-capillary membrane**, as seen in diffuse interstitial fibrosis, asbestosis etc.
2. **Increased distance between alveolar and capillary membrane** due to interstitial edema as seen in heart failure etc.

In alveolar-capillary obstruction syndrome, diffusion of oxygen is decreased but not the carbon dioxide. Though both oxygen and carbon dioxide are highly soluble in lipids, **CO₂ is 20 times more soluble in water than oxygen**. Therefore, in conditions like interstitial edema in which more fluid accumulates in the space between alveolar and capillary membrane, diffusion of CO₂ is not hampered. Thus, in alveolar-capillary obstruction syndrome, hypoxemia develops without significant change in PCO₂.

Partial Pressure Gradients

**Diffusion Gradient**

The diffusion of gases depends on the difference of partial pressure of the individual gas across the alveolar-capillary membrane. This is also called as the **diffusion gradient** for the gas. For example, oxygen diffuses easily across the membrane because of a greater difference in PO₂ between the alveoli and pulmonary capillaries (**oxygen diffusion gradient**). Normally, the diffusion gradient for oxygen is **about 60 mm Hg**, which is the difference between the PO₂ of the alveolar air (160 mm Hg) and the arterial blood (100 mm Hg). The **diffusion gradient for carbon dioxide** across alveolar-capillary membrane (PvCO₂ – Paco₂) is **about 6 mm Hg**, which is much lower than that of oxygen.

Gases are dissolved in the liquid when they are exposed to such liquids like plasma. In this dissolved state in the liquid, gases exert a partial pressure.

1. **Henry’s law** states that the amount of gas dissolved in a liquid at a given temperature is directly proportional to the partial pressure and the solubility of the gas.
2. **Fick’s law** explains the diffusion of gases across the alveolar-capillary membrane. *Fick’s law states that* the volume of gas diffusing per minute across a membrane is directly proportional to the membrane surface area, the diffusion coefficient of the gas, and the partial pressure difference of the gas, and inversely proportional to membrane thickness.

**Partial pressure of gases** in different parts of respiratory systems is depicted in Table 105.1.

<table>
<thead>
<tr>
<th>Table 105.1: Partial pressure of gases (mm Hg) in different parts of respiratory system.</th>
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<tr>
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<tr>
<td>Inspired air</td>
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<td>Expired air</td>
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<td>Alveolar air</td>
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<td>Venous blood</td>
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<td>Arterial blood</td>
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<td>Capillary blood</td>
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</table>

**Diffusion Coefficient**

The diffusion coefficient of a gas is directly proportional to its solubility and inversely proportional to the square root of its molecular weight. Therefore, a highly soluble molecule or a smaller molecule diffuses rapidly. For example, the diffusion coefficient of carbon dioxide in aqueous solutions is about 20 times greater than that of oxygen because of its higher solubility, even though it is a larger molecule than O₂.

**Capillary Blood Flow**

Flow of blood in the pulmonary capillary significantly influences the oxygen uptake.

1. Normally, the **transit time** (time taken by the red cells to pass through the capillary), is approximately 0.75 sec, during which the gas tension in the blood equilibrates with the gas tension in the alveoli.

2. With increase in cardiac output, blood flow through the pulmonary capillaries increases that decreases the transit time. For example, **nitrous oxide** (N₂O), an anesthetic gas easily diffuses across the alveolar-capillary membrane and equilibrates in 0.1 s.

3. Thus, the only way to increase the transfer of N₂O from alveolar air into the blood is by increasing the blood flow. The amount of N₂O that can be taken up is entirely limited by blood flow, not by diffusion of the gas. Therefore, uptake of N₂O is flow-limited.

4. On the other hand, diffusion of **carbon monoxide** (CO) is different. CO also readily diffuses across the alveolar-capillary membrane, but due to its high affinity for hemoglobin it rapidly binds to hemoglobin. This decreases the partial pressure of CO in blood, for which the equilibrium for CO across the alveolar-capillary membrane is never reached in 0.75 s (transit time). Therefore, CO transfer is diffusion-limited (not limited by the blood flow).

5. The capillary oxygen tension equilibrates with alveolar oxygen tension well within the transit time, normally in one third of the available time, i.e. in 0.3 s. Thus, a wide safety margin is available for oxygen to ensure that the end-capillary PO₂ is fully equilibrated with alveolar PO₂.

6. During severe exercise, though the transit is reduced but still time is adequate to fully oxygenate the blood. Though, oxygen binds with hemoglobin less avidly than CO, oxygen equilibrates fast. Thus, oxygen uptake is flow limited.

**Diffusing Capacity (DC)**

The diffusing capacity provides a measure of the rate of gas transfer in the lungs per partial-pressure gradient.

**Measurement of DC**

CO is used to determine the lung diffusing capacity because of several advantages:

1. Its uptake is limited by diffusion and not by blood flow.
2. There is essentially no CO in the venous blood.
3. The affinity of CO for hemoglobin is 210 times greater than that of oxygen, which causes the partial pressure of carbon monoxide to remain essentially zero in the pulmonary capillaries.

To measure the diffusing capacity (DC) with CO, the equation is:

$$ D_C = \frac{V_{CO}}{P_{ACO}} $$

where $V_{CO}$ is CO uptake in ml/min and $P_{ACO}$ is alveolar partial pressure of CO.

The most common technique for making this measurement is called the single-breath test.

**Factors affecting DC**

Hemoglobin and capillary blood volume affect lung diffusing capacity. Diffusing capacity not only depends on the diffusion properties of the alveolo-capillary membrane, but also on hematocrit and capillary blood volume. A decrease in either the hematocrit or capillary blood volume decreases the diffusing capacity.
CHAPTER SUMMARY

**KEy Concepts**

1. Alveolar ventilation is the most crucial process of life as it determines the oxygenation of blood and CO₂ removal from blood.
2. Gas exchange in lungs depends on the integrity of alveolar-capillary membrane, partial pressure gradient of gasses, pulmonary capillary blood flow and diffusion capacity for the gas.
3. Dead space volume increases in artificial ventilation.

**Important to Know (Must Read)**

1. In examination, Long Questions are usually not asked from this chapter.
2. Dead space, Alveolar ventilation, Alveolar-capillary membrane, Diffusing capacity of gases, may be asked as Short Questions in exam.
3. In Viva, examiner may ask… Define minute ventilation, Define dead space, what is its volume, What is anatomical dead space, What is physiological dead space, What are the methods to measure dead space volume, What is alveolar ventilation, what is its volume, What is the clinical importance of alveolar ventilation, What is the physiological significance of alveolar ventilation, How does pranayama increase alveolar ventilation, How is the alveolar ventilation measured, In what conditions, hyperventilation occurs, In what conditions, hypoventilation occurs, What are the layers of alveolar-capillary membrane, What are the factors affecting diffusion of gases, What is the clinical significance of having a normal alveolar-capillary membrane, What is diffusion gradient for gases, What is diffusion coefficient for gases, When does the transfer of gases across alveolar-capillary membrane become limited by blood flow, When does the transfer of gases across alveolar-capillary membrane become diffusion-limited, What is diffusing capacity of a gas, How is the diffusing capacity measured, What are the factors that affect diffusing capacity.
The amount of blood ejected by heart into pulmonary circulation is same as the amount ejected into systemic circulation.

1. Though, the capacity of pulmonary circulation is significantly less than the capacity of systemic circulation, pulmonary vascular bed handles cardiac output in such a way that pressure is not high for the same volume.

2. Systemic circulation is a high-pressure circulation due to greater resistance offered by the blood vessels of the systemic vascular bed. The pulmonary circulation on the other hand is a low pressure circulation due to less resistance offered by the pulmonary vessels.

3. The total resistance in pulmonary circulation is about one-tenth of systemic circulation.

Functional Organization

The pulmonary arteries branch like that of airways so that each airway branch and arterial branch runs parallel to each other. Pulmonary capillaries are large in diameter and have multiple anastomoses. The arrangement of capillaries is such that each alveolus is surrounded by a capillary basket (Fig. 106.1). A branch of pulmonary artery divides further to form arterioles that in turn form extensive capillary lattice around a single alveolus, which drains into a branch of pulmonary veins. The pulmonary blood vessels with blood in them constitute about 40% of the total weight of the lungs.
Functions of Pulmonary Circulation

1. **Gas exchange**: The primary function of the pulmonary circulation is to bring venous blood from different parts of the body in contact with alveoli for exchange of gases, where CO₂ is removed from pulmonary capillary into alveoli and O₂ is added from alveoli into the capillary blood.

2. **Serves as a filter**: Pulmonary vessels filter thrombi and emboli that originate from venous compartment and right side of the heart. Endothelial cells of pulmonary vessels release fibrinolytic agents that lyse blood clots (thrombi) and thus prevent the entry of these thrombi and emboli into coronary, cerebral, and other important vessels.

3. **Metabolic functions**: Vasoactive hormones are metabolized in the pulmonary circulation. Angiotensin I is converted to angiotensin II in the lungs by angiotensin-converting enzyme (ACE) located mainly on the surface of the pulmonary capillary endothelial cells. Pulmonary endothelial cells inactivate bradykinin, serotonin, and prostaglandins E₁, E₂, and F₂α. Norepinephrine is also inactivated.

4. **Serves as a blood reservoir**: About 500 mL of circulating blood is present in the pulmonary circulation.

Special Features of Pulmonary Circulation

1. The pulmonary circulation is a low-pressure (mean pulmonary arterial pressure is 15 mm Hg), and low resistance system.

2. The pulmonary artery and its branches have thin walls. There is less smooth muscle in the walls of pulmonary arteries. Due to their thin walls and paucity of smooth muscles, pulmonary arteries are more compliant. The pulmonary arteries are much shorter and wider. Due to their high compliance pulmonary vessels can accommodate a relatively large amount of blood whenever required. For example, when a person assumes recumbent position from standing position, a large volume of blood shifts from lower limbs to lungs and the high compliance of pulmonary vessels accommodate the volume.

3. The pulmonary arterioles are also thin-walled and contain less smooth muscle. Therefore, the ability to constrict is less than thick-walled, muscular arterioles of systemic circulation.

4. The pulmonary veins are also thin-walled and highly compliant.

5. The arrangement of pulmonary capillaries is different. The capillaries form lattice in the alveolar wall so that blood flows as a thin sheet. Though, they comprise a dense capillary bed, they are not arranged as a network of tubular vessels as seen in systemic capillary bed. Thus, pulmonary capillaries do not form a capillary network. The walls of the pulmonary capillaries are very thin and therefore, they collapse if local alveolar pressure exceeds capillary pressure.

6. Change in pulmonary venous and left atrial pressures profoundly affects gas exchange. The pulmonary wedge pressure indirectly measures these pressures. The wedge pressure is recorded by introducing a Swan-Ganz catheter percutaneously through the right side of the heart into the pulmonary circulation, where the tip of the catheter literally wedges in small pulmonary artery. A continuous and close column of blood connects the probe’s end and the left atrium, the wedge pressure reflects left atrial pressure.

7. **Pulmonary vascular resistance**: Pulmonary vascular resistance is very low, which is about one-tenth that of systemic vascular resistance. The extremely low resistance is due to two factors:

   - (i) Pulmonary resistance vessels (arterioles) are thin, short and wide. Thus, they have high compliance.

   - (ii) The resting vasoconstrictor tone is very low or absent for which the arterioles are mostly dilated (in contrast to systemic arterioles and precapillary sphincters that are partially constricted because of their resting sympathetic tone).

8. A unique feature of the pulmonary circulation is that the pulmonary vascular resistance (PVR) falls with increased pulmonary arterial pressure as occurs in increased cardiac output. The ability to significantly decrease in pulmonary vascular resistance in response to increased pressure is due to two mechanisms:

   - (i) Normally, many capillaries are partially closed in the upper part of the lungs because of the low perfusion pressure. When blood flow increases, the collapsed vessels are opened. This decreases the overall pulmonary resistance. This is called the capillary recruitment and is the primary mechanism that accounts for the fall in pulmonary vascular resistance to increased cardiac output.

   - (ii) The second mechanism is capillary distension in response to increased pressure. This occurs because the pulmonary capillaries are exceedingly thin and highly compliant.

Physiological Significance of Low PVR

The decrease in pulmonary vascular resistance with increased cardiac output has three beneficial effects.

a. The decreased resistance decreases the velocity especially when the flow rate is high. This provides adequate time for pulmonary capillary blood to take up oxygen and dispose of carbon dioxide.

b. The capillary distension that decreases resistance increases the capillary surface area, which facilitates diffusion of gases along the alveolar-capillary membrane.

c. A high capillary pressure can cause pulmonary edema, and impair gas exchange. For example, during vigorous exercise cardiac output increases enormously that increases blood flow and pressure in pulmonary circulation. However, the decrease in pulmonary vascular
resistance decreases the load on the right ventricle and lowers the capillary pressure that prevents pulmonary edema.

Pulmonary Blood Flow

There is approximately 500 mL of blood present in pulmonary circulation at any given time, which is about 10% of the total circulating blood volume. The distribution of 500 mL of blood is as follows:
- In pulmonary arteries: 150 mL
- In pulmonary veins: 270 mL
- In pulmonary capillaries: 80 mL

Distribution of Pulmonary Blood Flow

In upright posture, blood flow increases from apex to the base of the lungs. This difference (the lowest blood flow at apex and highest blood flow at base) is due to the effect of gravity (details discussed below).

Factors Affecting Pulmonary Blood Flow

Factors that affect pulmonary blood flow are:
1. Pulmonary vascular resistance
2. Gravity
3. Alveolar pressure
4. Arterial to venous pressure gradient

Pulmonary Vascular Resistance

Pulmonary vascular resistance is affected by lung volumes, hormones and oxygen tension.

1. Lung Volumes

Pulmonary capillaries have less structural support. Thus, they can be easily distended or collapsed depending on the surrounding pressure. Functionally, pulmonary vessels are of two types: alveolar vessels (arterioles, capillaries, and venules), and extra-alveolar vessels (pulmonary arteries and veins). The extra-alveolar vessels are subjected to pleural pressure and alveolar vessels are subjected to alveolar pressure.

High Lung Volumes

At high lung volumes, the pleural pressure is more negative. This increases transmural pressure (pressure inside the vessel minus pressure outside the vessel) in the extra-alveolar vessels and vessels are distended. However, increased alveolar diameter at high lung volumes decreases transmural pressure in alveolar vessels. As a result, the alveolar vessels are compressed and pulmonary vascular resistance is increased.

Low Lung Volumes

At low lung volumes, pleural pressure is positive, which compresses the extra-alveolar vessels. This increases pulmonary vascular resistance. Thus, pulmonary vascular resistance also increases at low lung volumes. Pulmonary vascular resistance is lowest at functional residual capacity and increases at both higher and lower lung volumes.

2. Hormones

Serotonin, norepinephrine, histamine, thromboxane A\textsubscript{2}, and leukotrienes are potent vasoconstrictors. They increase pulmonary vascular resistance, particularly at low lung volumes when the vessels are already compressed. Adenosine, acetylcholine, prostacyclin (PGI\textsubscript{2}), and isoproterenol relax smooth muscle of blood vessel and decrease pulmonary vascular resistance.

3. Oxygen tension

Low oxygen tension increases pulmonary vascular resistance by causing vasoconstriction.

Effects of Gravity

Gravity significantly affects pulmonary blood flow. Due to effect of gravity, in upright posture pulmonary blood flow increases from apex to base of the lungs. This is because apex of the lungs remains above and base remains below the level of the heart. With 1 cm increase in height above the heart, the hydrostatic pressure falls by 0.74 mm Hg and with 1 cm decrease in height below the heart, the hydrostatic pressure increases by 0.74 mm Hg. Thus, pressure in the pulmonary artery at apex, which is about 10 cm above the heart, is 7.4 mm Hg less.

The arterial pressures in lungs at various levels are:
- At the level of the heart: 14 mm Hg
- At the apex of the lung: 6.6 mm Hg (10 cm above; 7.4 mm Hg less)
- At the base of the lung: 17.7 mm Hg (5 cm below: 3.4 mm Hg more)

Gravity influences both arterial and venous pressures. These variations in pressures not only influence blood flow but also the ventilation-perfusion ratio. As gravity causes change in lung perfusion from apex to base, physiologically, lung is divided into three zones: Zone 1 (upper zone), Zone 2 (middle zone) and Zone 3 (lower zone).

Upper Zone

The capillary pressure in the apex of the lungs is close to the atmospheric pressure in the alveoli.

1. The arterial pressure is just sufficient to maintain perfusion. If due to any reason the arterial pressure is decreased or the alveolar pressure is increased, capillaries collapse and no gas exchange occurs.
2. Gravity causes capillary beds to be under-perfused in the apex of the lungs. The upper zone (Zone 1) is well ventilated but not well perfused.
3. Therefore, this increases alveolar dead space. However, Zone 1 is usually very small or nonexistent in healthy individuals.
4. The area of Zone 1 increases in conditions in which alveolar pressure is increased or pulmonary arterial
pressure is decreased. For example, in hemorrhagic shock, Zone 1 is increased due to low blood pressure.

**Middle Zone**

The arterial and capillary pressures exceed alveolar pressure.

1. However, as pulmonary venous pressure is less than alveolar pressure, veins are mechanically constricted due to the pressure from outside.
2. This results in damming of blood in capillaries. But, pulmonary veins are compliant.
3. Therefore, veins collect the amount of blood that escapes into them through the constriction. This is called water-fall effect.
4. The middle zone (Zone 2) is present in the middle of the lungs, where blood flow is determined not by the arterial-venous pressure difference, but by the difference between arterial pressure and alveolar pressure.

**Lower Zone**

Alveolar pressure is lower than the pressure in all vessels (arteries, capillaries and venules), especially the venous pressure. Thus, blood flows by arteriovenous pressure difference. The increase in blood flow in this region is mainly due to capillary distension.

**Effects of Alveolar Pressure**

Alveolar pressure depends on lung volumes. Alteration in alveolar pressure due to change in lung volume changes pulmonary blood flow.

**Arterio-venous Pressure Gradient**

The difference between the pressure at arterial and venous compartment of pulmonary circulation determines the rate of blood flow. Arterial to venous pressure gradient is affected by alveolar pressure.

**Regulation of Pulmonary Blood Flow**

Pulmonary blood flow is regulated by active and passive factors.

**Active Factors**

Active factors are neural, hormonal and chemical.

**Neural Regulation**

Though the pulmonary circulation is richly innervated with sympathetic nerves, pulmonary vessel diameter is virtually unaffected by autonomic nerves in normal conditions.

1. This is because the resting sympathetic tone of pulmonary circulation is almost absent.
2. However, sympathetic stimulation causes mild vasoconstriction and parasympathetic stimulation causes mild vasodilation.

**Hormonal Regulation**

1. Major vasoconstrictors in pulmonary bed are serotonin, norepinephrine, endothelin, angiotensin, thromboxane A$_2$, and leukotrienes (Table 106.1). They decrease pulmonary blood flow.
2. Vasodilators that increase blood flow are adenosine, acetylcholine, prostacyclin (PG-I$_2$), bradykinin and nitric oxide.

**Chemical Regulation**

Hypoxia or alveolar hypoxia causes vasoconstriction of small pulmonary arteries. In contrast, hypoxia in systemic circulation and other parts of the body produces vasodilation.

1. The exact mechanism of this unique phenomenon of hypoxia-induced pulmonary vasoconstriction is not known. It is proposed that hypoxia directly causes contraction of pulmonary vascular smooth muscles.
2. The probable mechanism is that hypoxia inhibits K* channels that depolarize the muscle cells.
3. This opens the voltage-gated Ca$^{++}$ channels. Influx of Ca$^{++}$ causes vasoconstriction.
4. The hypoxia-induced vasoconstriction is accentuated by high carbon dioxide and low blood pH.

**Passive Factors**

Passive factors that regulate pulmonary blood flow are cardiac output, gravity and lung volumes. Increase in cardiac output increases pulmonary blood flow.

<table>
<thead>
<tr>
<th>Vasoconstrictors</th>
<th>Vasodilators</th>
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<tbody>
<tr>
<td>1. Catecholamines at α$_1$ receptors</td>
<td>1. Catecholamines at α$_2$ and β$_2$ receptors*</td>
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<tr>
<td>2. Purinergics at P$_{2X}$ receptors</td>
<td>2. Acetylcholine at M$_1$ receptors*</td>
</tr>
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<td>3. Tachykinin at NK$_2$ receptors</td>
<td>3. Purinergics at P$_{2Y}$ receptors*</td>
</tr>
<tr>
<td>4. Adenosine at A$_2$ receptors</td>
<td>4. Tachykinin at NK$_1$ receptors*</td>
</tr>
<tr>
<td>5. Angiotensin II at AT$_1$ receptors</td>
<td>5. VIP, CGRP, and ANP (at ANP$_A$ and ANP$_B$ receptors)</td>
</tr>
<tr>
<td>6. Endothelin at ET$_A$ receptors</td>
<td>6. Adenosine at A$_1$ receptors</td>
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<td>7. Serotonin at SHT$_1$ receptors</td>
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<td>8. Thromboxane at TP receptors</td>
<td>8. Bradykinin at B$_1$ and B$_2$ receptors*</td>
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<td>9. Endothelin at ET$_B$ receptors*</td>
<td>9. Histamine at H$_1$ receptors* and H$_2$ receptors</td>
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<td>10. Histamine at H$_1$ receptors* and H$_2$ receptors</td>
<td>11. Serotonin at SHT$_{2C}$ receptors*</td>
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<tr>
<td>12. Vasopressin at V$_1$ receptors*</td>
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*Vasodilators identified by star marks are dependent on vascular endothelium for their vasodilator properties.
Filtration Across Pulmonary Capillaries

Filtration of fluid across capillary walls in the pulmonary circulation is governed by Starling forces as occurs in systemic circulation (for details, refer Chapter 95). In addition to hydrostatic and osmotic pressure gradients, two more additional factors play role in fluid transfer across the pulmonary capillaries. These factors are surface tension and alveolar pressure. The alveolar surface tension pulls alveolar wall inward (facilitates alveolar collapse) that decreases interstitial pressure and therefore draws fluid into the interstitial space (favors filtration), whereas alveolar pressure compresses the interstitial space and increases the interstitial pressure (opposes filtration).

1. **Hydrostatic and osmotic pressure gradients**
   The hydrostatic pressure in pulmonary capillaries is low, which is about 8 to 10 mm Hg. As it is significantly less than the oncotic pressure, which is 25 mm Hg, this favors the net absorption of fluid from interstitial space into capillary blood.

2. **Role of alveolar surface tension**
   Alveolar surface tension favors filtration and counteracts the advantage of low hydrostatic pressure. The net result is that a small quantity of fluid circulates from the interstitium into the perivascular and peribronchial spaces and then from there passes into the lymphatic channels.

3. **Role of lymphatics**
   The lymphatic system is extensive and well-developed in lungs. The lymphatics are mainly located near the terminal bronchioles, which is favorable for them to drain excess fluid from peribronchial space.

Pulmonary Edema

Pulmonary edema develops when excess of free fluid accumulates in interstitial spaces and alveoli (Clinical Box 106.1). Pulmonary edema is caused by following mechanisms:

1. **Increased capillary hydrostatic pressure:** This is the commonest cause of pulmonary edema. Most frequently it results from an abnormally high pulmonary venous pressure as occurs in mitral stenosis or left heart failure.

2. **Increased alveolar surface tension:** High surface tension lowers interstitial hydrostatic pressure that favors filtration. Decreased surfactant synthesis increases the surface tension and causes pulmonary edema as occurs in ARDS.

3. **Decreased oncotic pressure:** Plasma colloid osmotic pressure decreases in hypoproteinemia as occurs in starvation.

4. **Increased capillary permeability:** This is a major cause of pulmonary edema. Increased capillary permeability occurs with pulmonary vascular injury as occurs in:
   - Oxidant damage, e.g. oxygen therapy, ozone toxicity
   - Inflammatory reactions, e.g. by the action of endotoxins
   - Neurogenic shock, e.g. head injury is a major cause of pulmonary edema.

Clinical Box 106.1

**Effects of pulmonary edema:** Pulmonary edema decreases gas exchange, which results in hypoxemia and hypercapnia. Pulmonary edema obstructs small airways, which in turn increases airway resistance. Lung compliance decreases in pulmonary edema because of interstitial swelling and increased alveolar surface tension. Work of breathing increases due to decreased compliance and airway obstruction.

Physiological Basis of Treatment of Pulmonary Edema

The aim of treatment of pulmonary edema is to reduce pulmonary capillary hydrostatic pressure. This is achieved by:

1. **Diuretics** that decrease blood volume
2. **Digitalis** that increases left ventricular function (usually the inability of left ventricle to pump blood effectively produces pulmonary edema)
3. **Vasodilators** that cause relaxation of systemic blood vessels.

Drowning

Fresh-Water Drowning

In fresh-water drowning, though aspiration of water occurs into the lungs, death does not occur due to pulmonary edema.

1. Death in drowning is usually due to ventricular fibrillation.
2. The aspirated water enters the alveoli and from there into the pulmonary capillary blood because of low capillary hydrostatic pressure and high oncotic pressure.
3. Entry of water into blood dilutes the plasma and produces hypotonic environment for red cells. This results in hemolysis.
4. Hemolysis in turn causes hyperkalemia and hypoahtremia.
5. Ventricular fibrillation occurs due to hyperkalemia and hypoxemia.

Salt-Water Drowning

In salt-water drowning, the aspirated water is hypertonic due to high Na⁺ and Cl⁻ content of sea water.

1. Hypertonic fluid in alveoli produces pulmonary edema.
2. The cause of death in sea water drowning is asphyxia.

VENTILATION-PERFUSION RATIO

As discussed above, there are regions in the lung where ventilation and blood flow are not properly matched. Due
to this physiological mismatching, a small fraction of cardiac output is not normally fully oxygenated. The matching of airflow to blood flow is studied by examining the ventilation-perfusion ratio.

Normal value: Ventilation-perfusion ratio is the ratio of alveolar ventilation to blood flow in lung. In a normal individual at rest, the alveolar ventilation ($V_A$) is 4 L/min and pulmonary blood flow (Q) or perfusion (which is same as cardiac output or strictly speaking the right ventricular output) is 5 L/min. Thus, the normal ventilation-perfusion ratio ($V_A/Q$) is 0.8. However, due to effect of gravity, regional differences in blood flow and alveolar ventilation exist in the lung.

1. In upright posture, the base of the lung is better ventilated and better perfused than the apex. Moreover, blood flow is proportionately greater than ventilation at the base, and ventilation is proportionately greater than blood flow at the apex (Fig. 106.2).
2. In fact, the blood flow exhibits about a 5-fold difference between the apex and base of the lung, whereas ventilation exhibits about a 2-fold difference.
3. This variation in the differences in ventilation and blood flow, results in regional difference the $V_A/Q$ ratio. The $V_A/Q$ ratio is 0.6 at the base and about 3 at the apex of the lungs.

Importance of VA/Q ratio

Physiological Importance

The ventilation-perfusion ratio is important for gas exchange in lungs.

- At the apex, the ratio is high because there is more ventilation relative to blood flow. Therefore, alveolar oxygen tension ($P_AO_2$) is high and carbon dioxide tension ($P_ACO_2$) is low at the apex of the lungs.

- At the base, the ratio is low as there is more perfusion relative to ventilation. Thus, some amount of the blood circulates through the base of the lungs without becoming fully oxygenated.

Clinical Importance

Due to differences in regional $V_A/Q$ ratios, some diseases are more localized to a part of the lungs. For example, tuberculosis occurs more commonly in the apex because favorable environment for Mycobacterium tuberculosis. The higher oxygen level facilitates growth of tubercular bacilli.

Effects of Change in $V_A/Q$ Ratio

Decreased $V_A/Q$ Ratio

When ventilation to an alveolus is reduced relative to its perfusion, the $V_A/Q$ ratio becomes less. In this situation, due to delivery of less oxygen, alveolar PO$_2$ falls and due to removal of less carbon dioxide PCO$_2$ rises (Figs. 106.3A and B).

Increased $V_A/Q$ Ratio

When perfusion is reduced relative to ventilation, $V_A/Q$ ratio increases, in which alveolar PCO$_2$ falls due to less carbon dioxide delivered into alveoli from blood and PO$_2$ increases as less oxygen is transferred from alveoli into the blood.

Venous Admixture and Physiologic Shunt

Like physiological dead space that causes wasted ventilation, there is wasted perfusion. Wasted perfusion refers to the quantity of the venous blood, which is not fully oxygenated. The mixing of unoxygenated blood with oxygenated blood is known as venous admixture.

Venous admixture occurs either due to a shunt or to a low ventilation-perfusion ratio.

Shunt

A shunt is a channel in which blood bypasses the lungs. There are two types of shunts.

Anatomical Shunt

In anatomical shunt, blood bypasses lungs through an anatomical defect. For example, in right to left shunt, admixture of blood occurs through the atrial or ventricular septal defect (Application Box 106.1). This also occurs from a branch of the pulmonary artery connecting directly to the pulmonary vein.

Physiological Shunt

In bronchial circulation, deoxygenated bronchial venous blood drains directly into the oxygenated blood of
pulmonary veins that accounts for physiologic shunt. Normally, there is physiological dead space as well as physiological shunt in the lungs.

**Application Box 106.1**

Anatomical shunt causes hypoxia: In normal healthy individuals, the maximum venous admixture (physiological shunt) is about 2% of cardiac output. However, in some bronchial diseases this amount can increase up to 20% of cardiac output and in congenital disorders with a right-to-left shunt it can increase up to 50% of cardiac output. In these conditions, gas exchange is grossly impaired that lowers oxygen tension in the arterial blood.

**Low Ventilation-Perfusion Ratio**

Low $\dot{V} / Q$ ratio occurs when a fraction of pulmonary capillary blood is not oxygenated due to low alveolar ventilation. Normally, this occurs at the base of the lung. This can also occur if an airway is partially obstructed that causes hypoventilation. The fraction of the blood that passes through a hypoventilated part of lung is not fully oxygenated. This results in venous admixture.

**BRONCHIAL CIRCULATION**

The bronchial circulation is the circulation that supplies blood to the walls of the conducting airways and surrounding tissues.

1. Bronchial circulation perfuses the upper respiratory tract. It does not supply the respiratory bronchiole or alveoli.
2. The venous return from the bronchial circulation is either by bronchial veins or by bronchopulmonary veins.
3. Bronchial circulation receives only 1% of the cardiac output. However, in some inflammatory disorders of the airways such as chronic bronchitis, it can be increased to 10% of cardiac output.
4. Bronchial arterial pressure is almost the same as the aortic pressure and bronchial vascular resistance is much higher than the resistance in the pulmonary circulation.

**Physiological Importance**

The bronchial circulation (not pulmonary circulation) is capable of undergoing angiogenesis (the formation of new vessels).

1. This is very crucial to provide collaterals to the lung parenchyma, when the pulmonary circulation is compromised.
2. When pulmonary blood flow is obstructed by a clot or embolus, the lung parenchyma supplied by these vessels survives due to perfusion by newly formed blood vessels.

**CHAPTER SUMMARY**

**Key Concepts**

1. The resting sympathetic tone in pulmonary circulation is less. Therefore, the vascular resistance is less. This helps in entire cardiac output to pass through this small circulation easily.
2. Ventilation-perfusion ratio is more in apex of lung and less at the base.
Important to Know (Must Read)

1. In examination, Long Questions are usually not asked from this chapter.
2. Ventilation–perfusion ratio, Special features of pulmonary circulation, Factors affecting pulmonary blood flow, Regulation of pulmonary blood flow, Pulmonary edema, Drowning, may be asked as Short Questions in exam.
3. In Viva, examiner may ask… What are the functions of pulmonary circulation, What are the special features of pulmonary circulation, What is the physiological significance of low pulmonary vascular resistance, What are the factors that affect pulmonary blood flow, What are the factors that affect pulmonary vascular resistance, What is the effect of gravity on pulmonary blood flow, What are the factors that regulate pulmonary blood flow, What does the neural mechanisms regulate pulmonary blood flow, How do the different hormones regulate pulmonary blood flow, What are the different chemicals that regulate pulmonary blood flow, Name the vasoconstrictors of pulmonary circulation, Name the vasodilators of pulmonary circulation, What are the passive factors that regulate pulmonary blood flow, What are the factors that regulate filtration across the pulmonary capillaries, What is the effect of hydrostatic and osmotic pressure gradients on filtration across the pulmonary capillaries, What is the effect of alveolar surface tension on filtration across the pulmonary capillaries, What is the role of lymphatics in filtration across the pulmonary capillaries, What are the causes of pulmonary edema, What is the effect of pulmonary edema on lung functions, What is the physiological basis of treatment of pulmonary edema, How do you differentiate between fresh water and salt water drowning, What is ventilation–perfusion ratio, What is the physiological importance of ventilation–perfusion ratio, What is the effect of decreased and increased ventilation–perfusion ratio, What is an anatomical shunt, What is a physiological shunt.
The transport of $O_2$ and $CO_2$ by the blood is often referred to as ‘gas transport’. This is an important step in the overall process of gas transfer between lungs and tissues. This is also the primary function of the circulatory system.

OXYGEN TRANSPORT

Oxygen is transported from lungs to the tissues in two forms: in combination with hemoglobin (Hb) in the red cell or physically dissolved in the plasma. Approximately 98% of the oxygen is transported in combination with hemoglobin and the remaining 2% in the physically dissolved form.

Scientist contributed

**Joseph Priestley** (1733–1804), a British scientist, had isolated the component of air, which was later called oxygen, and showed that it is essential for life. He observed that plants release it even under water.

In Physically Dissolved Form

In physically dissolved form in plasma, only about 2% of oxygen is transported. The amount of physically dissolved oxygen in the blood can be predicted from Henry’s law.

1. Henry’s law states that the amount of gas that dissolves in a liquid at a given temperature is proportional to the partial pressure of the gas.
2. Thus, the quantity of dissolved oxygen in arterial blood is calculated from the following equation:
   
   $$\text{Dissolved } O_2 (\text{mL/dL}) = \text{oxygen solubility } \times \text{ partial pressure of } O_2 \text{ in arterial blood (PaO}_2).$$
   
   $$= 0.003 \text{ (mL/dL of blood per mm Hg)} \times 95 \text{ mm Hg (normal PaO}_2 \text{ is 95 mm Hg)}$$
   
   $$= 0.3 \text{ mL/dL of blood}$$

   Thus, at PaO$_2$ of 95 mm Hg, the dissolved O$_2$ is 0.3 mL/dL. That means, in a normal healthy adult, 0.3 ml of O$_2$ is transported in dissolved form in 100 ml of blood (Application Box 107.1). As cardiac output is 5 L/min at rest, oxygen transported in dissolved form at rest is about 15 mL/min. Therefore, oxygen transported in dissolved form alone is grossly inadequate to meet the oxygen requirement of the body, which is about 250 mL/min at rest.

**Application Box 107.1**

Hyperbaric $O_2$ therapy improves tissue oxygenation: Though, 0.3 mL of O$_2$ is transported in dissolved form in 100 mL of blood, when 100% oxygen is inhaled in hyperbaric chamber, the dissolved oxygen is about 6 mL/dL of blood that accounts for supply of about 300 mL/min, which is adequate for tissue oxygenation.
In Combination with Hemoglobin

More than 98% of O₂ is transported in combination with Hb.
1. This becomes possible due to the binding affinity of hemoglobin for oxygen.
2. Hemoglobin that binds with oxygen is called oxyhemoglobin (HbO₂) and the hemoglobin that does not bind with O₂ is called deoxyhemoglobin (deoxy-Hb) or reduced-hemoglobin.

Oxyhemoglobin Formation

The hemoglobin molecule is a protein made up of four subunits that are bound together. Each subunit consists of a molecular group known as heme and a polypeptide attached to the heme. The four polypeptides of a Hb molecule are combinely known as globin.
1. Each heme group contains one atom of iron (Fe⁺⁺) to which oxygen binds (Fig. 107.1).
2. Since each iron atom can bind one molecule of oxygen, a single Hb molecule can bind four molecules of oxygen.
3. Hb binds with oxygen only when the iron is in ferrous (Fe⁺⁺) state. The Fe⁺⁺ iron in Hb is oxidized to ferric (Fe⁺⁺⁺) iron to form methemoglobin.
4. Thus, methemoglobin cannot bind oxygen. Methemoglobin formation occurs under the influence of various compounds like nitrites or sulfonamides. Methemoglobin is also formed spontaneously.
5. However, the enzyme methemoglobin reductase is present in red cell that reduces methemoglobin to Hb. Therefore, normally only about 1.5% of total Hb is methemoglobin.
6. Deficiency of methemoglobin reductase (a genetic defect) increases the methemoglobin concentration and decreases oxygen-carrying capacity. Oxygen binds rapidly and reversibly to hemoglobin to form oxyhemoglobin (HbO₂):

\[ O_2 + Hb \rightleftharpoons HbO_2 \]

During this process of HbO₂ formation, the heme remains in ferrous state. Thus, this process is oxygenation, not oxidation. As there are four subunits in Hb molecule, Hb reacts rapidly (in less than 0.01 s) with four molecules of oxygen to form HbO₄.

\[ \text{O}_2 \text{-Carrying Capacity of Hb} \]

Each gram of hemoglobin binds with 1.34 ml of oxygen. The maximum amount of oxygen that can be carried by hemoglobin is called the oxygen-carrying capacity.
1. In a healthy individual, the oxygen-carrying capacity of arterial blood is about 20 ml of O₂ per 100 ml of blood.

![Fig. 107.1: Structure of Hb molecule.](image-url)

![Fig. 107.2: Conversion of Hb molecule from T state to R state. As O₂ is added, salt bridges are successively broken and finally 2-3, BPG is expelled. T (taught) conformation of deoxy-Hb is changed to relaxed (R) conformation of oxy-Hb.](image-url)

blood, considering the hemoglobin concentration of 15 g% (1.34 mL × 15 g = 20.1 mL O₂/dl blood).

2. **Oxygen content** of Hb (HbO₂ content) is the amount of oxygen actually bound to hemoglobin, whereas **oxygen capacity** of Hb (HbO₂ capacity) is the amount of oxygen that can potentially bind with Hb.

3. The **percentage saturation** of hemoglobin with oxygen (SO₂) is the ratio of the quantity of oxygen actually bound (oxygen content) to the quantity that can be potentially bound (oxygen capacity) multiplied by 100.

\[
SO₂ = \frac{\text{HbO}_2 \text{ content}}{\text{HbO}_2 \text{ capacity}} \times 100
\]

For example, if oxygen content is 15 mL O₂ per dl blood and oxygen capacity is 20 mL O₂ per dl of blood, then the SO₂ of blood is 75%. Normally, in arterial blood, the percentage saturation of hemoglobin with oxygen is about 98%. Blood PO₂ and O₂ saturation are important indices of oxygen transport, which are best studied by analyzing oxyhemoglobin dissociation curve.

### Oxygen-Hemoglobin Dissociation Curve

Oxygen-hemoglobin (Oxy-Hb) equilibrium curve (dissociation or association curve) explains the relationship between **partial pressure of oxygen** (PO₂) in blood with **oxygen saturation of Hb**. Oxy-Hb equilibrium curve is an S-shaped curve over the range of PO₂ from 0 to 100 mm Hg (Fig. 107.3).

1. The **sigmoid shape** of the curve results from hemoglobin affinity for oxygen at various PO₂ levels. As PO₂ rises, the Hb saturation progressively increases.
2. However, the saturation is not linear with increase in PO₂ for which the curve becomes sigmoid-shaped.
3. The S-shaped oxyhemoglobin equilibrium curve enables oxygen to saturate hemoglobin under high partial pressures in the lungs and to give up large amounts of oxygen with small changes in PO₂ at the tissue level. The curve is divided into **two major phases**: the **steep phase** and the **plateau phase**.

### Steep Phase

The curve has a steep slope between PO₂ of 10 and 60 mm Hg. During this phase of the curve, combination of oxygen with Hb increases very rapidly as the PO₂ increases from 10 to 60 mm Hg. Oxygen saturation of Hb is about 90% at PO₂ 60 mm Hg.

### Significance of Steep Phase

In the steep phase, oxygen saturation of Hb is very high. Less increase in PO₂ leads to greater percentage saturation of Hb and, therefore, facilitates oxygen loading. Also, change in steep portion of the curve in reverse direction (that is, from 60 to 10 mm Hg) causes unloading of oxygen in the tissues. A small decrease in PO₂ in the tissue results in unloading of large amount of oxygen to the tissues. The steep phase allows large quantities of oxygen to be released from hemoglobin in the tissue capillaries where a lower capillary PO₂ prevails. This is especially achieved by **shifting the curve to right** (shift occurs mainly on the steep phase) by increased H⁺ or by increased CO₂.

### Plateau Phase

The curve begins to plateau at PO₂ around 60 mm Hg and flattens at PO₂ of 70 mm Hg. The increase in PO₂ above 60 mm Hg produces only a small increase in oxygen binding. Increase in PO₂ from 60 to 100 mm Hg in the plateau region of the curve illustrates that oxygen saturation and content remains apparently constant over a wide alteration in alveolar PO₂.

### Significance of Plateau Phase

There are two significances of plateau phase:

1. In different situations, like at high altitude or in pulmonary diseases in which a moderate hypoxia (decrease in PO₂ from 95 to 60 mm Hg) occurs, the total amount of oxygen carried by Hb decreases only by 5–10%, since Hb saturation is about 90% at PO₂ of 60 mm Hg. Thus, plateau in the curve provides a safety factor through which even a significant decrease in lung function can allow normal saturation of Hb.

2. Oxygen saturation and content remain fairly constant inspite of wide fluctuations in alveolar PO₂ (PAO₂). For example, if PAO₂ rises from 100 to 120 mm Hg, hemoglobin saturation increases only slightly (97-98%). This is the reason why oxygen content cannot be raised appreciably by hyperventilation or by breathing 100% oxygen because Hb is already completely saturated with oxygen at PO₂ of 100 mm Hg. This is true only for normal people at sea level. If a person has low arterial PO₂ due to lung disease or for his ascension to high altitude, hyperventilation or breathing 100% oxygen increases Hb saturation with oxygen as they have more deoxy-Hb initially.

![Fig. 107.3: Oxygen-hemoglobin dissociation curve. Note, at PO₂ of 27 mm Hg, Hb saturation of oxygen is 50% (P50). Hb is 89% saturated with oxygen at PO₂ of 60 mm Hg, above which increase in Hb saturation is marginal that results in plateau phase of the curve.](image-url)
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\[ P_{50} \]

\[ P_{50} \] is the level of \( \text{PO}_2 \) at which 50% of hemoglobin is saturated with oxygen.
1. \( P_{50} \) assesses the binding affinity of hemoglobin for oxygen.
2. In adults at sea level, the normal \( P_{50} \) occurs at a \( \text{PO}_2 \) of 27 mm Hg.
3. Alteration in the \( P_{50} \) value has a greater impact on the steep phase of the curve.
4. If \( P_{50} \) is high, it signifies the decrease in affinity of Hb for oxygen, which is seen in right-shift of the oxygen-hemoglobin equilibrium curve.
5. Conversely, if \( P_{50} \) is low, it signifies shift of the curve to the left, in which affinity of Hb for oxygen is more (Fig. 107.4).

Factors Affecting Hb-binding Affinity with Oxygen

Several factors influence the affinity of hemoglobin for oxygen.
1. The important factors are temperature, arterial PCO\(_2\), arterial pH and 2,3-DPG. A rise in PCO\(_2\), a fall in pH, and a rise in temperature, all shift the curve to the right.
2. The effect of carbon dioxide and hydrogen ions on the affinity of hemoglobin for oxygen is known as the Bohr effect. A shift of the oxyhemoglobin equilibrium curve to the right is physiologically advantageous at the tissue level, as affinity of O\(_2\) for Hb is lowered (increased \( P_{50} \)).
3. A rightward shift enhances the unloading of oxygen for a given \( \text{PO}_2 \) in the tissue, and a leftward shift increases the affinity of hemoglobin for oxygen, thereby lowering the ability to release oxygen to the tissues.
4. A simple way to remember the functional importance of these shifts is that an exercising muscle is warm and acidic and produces large amounts of carbon dioxide (high PCO\(_2\)), all of which favor the unloading of more oxygen to metabolically active muscles.

Temperature

Increase in temperature shifts the Oxy-Hb dissociation curve to the right and decrease in temperature shifts the curve to the left (Fig. 107.5). In other words, high temperature decreases the affinity of Hb for oxygen. This helps in release of oxygen in metabolically active tissues in which temperature is more.

pH

Alteration in blood pH shifts the Oxy-Hb dissociation curve. Christian Bohr and Neils Bohr in 1904 demonstrated that respiratory acidosis shifts oxy-Hb dissociation curve to the right. Since then, the decrease in oxygen affinity in acidosis is known as Bohr effect.
1. A decrease in pH shifts the curve to the right and increase in pH shifts the curve to the left (Fig. 107.6).
2. When blood passes through capillaries, CO\(_2\) enters red cell that decreases intracellular pH and shifts the Oxy-Hb dissociation curve to right.
3. It has been noted that under normal physiological conditions, binding of about 0.7 mole of H\(^+\) causes Hb to release 1 mole of oxygen.
4. Thus, when blood passes through tissue capillaries, the acidic environment facilitates release of oxygen from Hb into the tissues.

Carbon Dioxide

During cellular metabolism, CO\(_2\) is released into circulation that increases generation of H\(^+\) and decreases pH. This shifts the curve to the right. This helps in release of oxygen from Hb.
1. The shift to the right in acidosis (Bohr effect) is partly due to the effect of decrease in pH and partly to the direct effect of CO$_2$ on Hb.

2. CO$_2$ combines with unprotonated amino groups on Hb (Hb-NH$_2$) to form carbamino groups. The formation of carbamino group causes negative shift in the charge on one amino acid side chain that changes the conformation of Hb. This results in decrease in oxygen affinity of Hb.

3. In metabolically active tissues, metabolic vasodilation increases blood flow to the tissue increasing the oxygen transport to the area.

4. In the area, already persisting local hypercapnia and acidosis shift the curve to the right (decrease oxygen affinity for Hb) that facilitates oxygen delivery to the tissue.

2,3-DPG

Red blood cells lack mitochondria. Therefore, anerobic glycolysis occurs in red cells. During glycolysis, large quantities of 2,3-diphosphoglycerate (2,3-DPG), an organic phosphate compound is produced as the metabolic intermediary. This compound is called 2,3-biphosphoglycerate (2,3-BPG). Thus, 2,3-DPG level is much higher in red cells than in other cells. 2,3-DPG significantly affects affinity of hemoglobin for oxygen. In red cells, concentration of 2,3-DPG is almost same as that of Hb. In fact, 2,3-DPG binds to Hb in 1:1 stoichiometry, interacting with a central cavity formed by two β chains. 2,3-DPG has about 3.5 negative charges that interact with eight positively charged amino acids in the central cavity. This destabilizes the interaction of Hb with oxygen. Thus, binding of 2,3-DPG with Hb shifts the Oxy-Hb dissociation curve to the right (Fig. 107.7) and facilitates the release of oxygen. At the tissue level, an increase in 2,3-DPG facilitates unloading of oxygen from the red cell. Hypoxia stimulates glycolysis resulting in increased production of 2,3-DPG. Red cell 2,3-DPG increases in anemia, exercise and hypoxic conditions like high altitude, chronic lung disease, etc. Therefore, in these conditions, Oxy-Hb dissociation curve shifts to the right.

Factors that affect 2,3-DPG in Red Cells

1. pH: Acidosis inhibits red cell glycolysis and, therefore, decreases 2,3-DPG concentration.
2. Type of Hb: The γ chains of fetal Hb have less avidity for 2,3-DPG than β chains of adult Hb. Therefore, fetal Hb has higher oxygen affinity. This provides an advantage to fetus to extract oxygen from maternal blood in the placenta.
3. Fetal Hb shifts Oxy-Hb dissociation curve to left (Fig. 107.8).
4. Hormones: Growth hormone, thyroxine and testosterone stimulate the synthesis of 2,3-DPG.
5. Altitude: At high altitude, 2,3-DPG concentration increases substantially in red cells. This increases the availability of oxygen to the tissues.
6. PO$_2$: Hypoxia increases 2,3-DPG production.
7. Procedure of storage of blood: The 2,3-DPG concentration in red cells of blood stored in blood bank decreases. Therefore, transfusion of stored blood decreases oxygen transport, especially when transfused into hypoxic patients. However, if blood is stored in citrate-dextrose-phosphate solution, this effect is less than when stored in acid-citrate-dextrose solution.

Myoglobin

Myoglobin is the Hb pigment in muscle.

1. Though it is similar to Hb in structure, myoglobin binds, one molecule of O$_2$ per mole.
2. It shifts Oxy-Hb dissociation curve to the left and the curve becomes hyperbolic (loses sigmoid shape) (Fig. 107.8).

3. This helps in picking up of O$_2$ from blood.

4. Myoglobin is more in regularly exercising muscles, especially that are trained in isometric exercise. This helps the muscle to draw oxygen from myoglobin, especially when blood flow ceases for a longer duration during sustained muscle contraction.

5. Myoglobin is also believed to facilitate transfer of O$_2$ from blood to mitochondria.

**Effect of Carbon Monoxide (CO)**

The binding affinity of CO with Hb is 210 times more than oxygen. Moreover, CO interferes with oxygen transport by competing for the same binding sites on hemoglobin.

1. CO binds to hemoglobin to form carboxyhemoglobin (HbCO).

2. When the blood is 60% saturated with CO (carboxyhemoglobin), the oxygen content is reduced to less than 10 mL/dL.

3. As the partial pressure of CO approaches 1 mm Hg, Hb is fully saturated with CO. However, arterial PO$_2$ may be normal as oxygen diffusion gradient remains normal.

4. But, oxygen content is greatly reduced as it cannot bind to Hb. This grossly decreases the oxygen-carrying capacity.

5. Moreover, CO also shifts the Oxy-Hb dissociation curve to the left, which decreases oxygen release to the tissues.

6. Therefore, severe tissue hypoxia occurs in CO poisoning, which is fatal if not treated immediately (Clinical Box 107.2) (for details, refer to ‘Hypoxia’).

7. Normally, in healthy individuals, CO occupies 1–2% of Hb-binding sites.

8. However, in chronic cigarette smokers, traffic personnel and in residents of crowded traffic areas, CO concentration increases in plasma and CO occupies about 10% of Hb.

**Clinical Box 107.2**

**CO poisoning produces hypoxia:** CO produces anemic hypoxia, which is severely harmful for the following reasons:

1. CO has a strong affinity for hemoglobin, which forms carboxyhemoglobin. Therefore, Hb is practically not available for oxygen transport. Hence, it is often classified as anemic hypoxia.

2. CO is virtually undetectable, as it is an odorless, colorless, and nonirritating gas. Hence, subject suffers from CO poisoning without conscious knowledge of being poisoned.

3. PaO$_2$ remains normal in CO poisoning. Hence, feedback mechanism of hypoxia for corrective measures is absent.

4. Carboxyhemoglobin imparts bright-cherry red color to blood. This masks the clinical sign (paleness of skin) due to hypoxia. Hence, hypoxia is not detected clinically.

Thus, CO produces severe hypoxia without manifesting danger signals of it. The treatment for CO poisoning is breathing 100% oxygen. As O$_2$ and CO compete for binding with hemoglobin molecule, breathing a high oxygen concentration displaces CO from Hb and favors formation of oxyhemoglobin. Hyperbaric oxygen is also helpful. The 5% CO$_2$ may be added to inspired air as it stimulates ventilation, which lowers the CO.

**Factors that Shift Oxy-Hb Dissociation Curve**

**Factors that Shift the Curve to the Right**

1. Increased temperature
2. Decreased pH
3. Increased PCO$_2$
4. Increased 2,3-DPG
5. Hypoxia

Shift of the curve to right increases P$_{50}$ (decreased affinity of Hb for oxygen), which facilitates oxygen release.

**Factors that Shift the Curve to the Left**

1. Decreased temperature
2. Increased pH
3. Decreased PCO$_2$
4. Decreased 2,3-DPG
5. Fetal Hb
6. Carbon monoxide

Shift of the curve to left decreases P$_{50}$ (increases affinity of Hb for oxygen) that facilitates oxygen uptake and prevents oxygen release.

**Importance of Oxygen Saturation and Content**

**Oxygen Saturation**

Oxygen saturation is the ratio of the amount of oxygen bound to Hb to the maximum amount of oxygen that can bind Hb (100% oxygen capacity). At 100% oxygen capacity, heme groups of Hb are fully saturated with oxygen.

**Oxygen Content**

The oxygen content of blood is the volume of oxygen contained in unit volume of blood, which includes the oxygen bound to Hb and also dissolved in plasma. As the dissolved oxygen is negligible, the oxygen content depends...
on concentration of Hb and oxygen-binding capacity of Hb (Clinical Box 107.3).

Clinical Box 107.3
Oxygen content is important in anemic patients: Oxygen content is more important than PO₂ and oxygen saturation for oxygenation of tissues, when the patient is anemic. A patient with Hb concentration 7 g% or less may have a normal arterial PO₂, and Hb saturation but oxygen content will be grossly reduced. Oxygen saturation remains normal because oxygen content and capacity are proportionately reduced. Tissue suffers from hypoxia due to decreased oxygen content inspite of normal oxygen saturation.

Oxygen Extraction
Oxygen extraction is the amount of oxygen taken up by the tissues from the blood.
1. This is an index of oxygen consumption of the tissue. It is better quantified in terms of oxygen extraction ratio (OER).
2. OER, also called oxygen coefficient ratio, is the amount of oxygen extracted by the tissue divided by the amount of oxygen delivered.
3. In metabolically more active tissues like cardiac muscle, OER is as high as 85% at rest.

Applied Physiology

Measurement of Oxygen Saturation of Hb

Pulse Oximetry
Pulse oximetry is a noninvasive method of measurement of oxygen saturation of Hb. Oxygen saturation is continuously measured in hospitalized patients, especially patients in intensive care unit by this method. Instrument used is the pulse oximeter. The probe of the oximeter is attached usually to the finger-tip or ear lobule, where the pulsating blood vessels are accessible externally. Red and infrared lights are transmitted through the vascular bed, and pulsatile, nonpulsatile and total absorbances are calculated. The pulsatile component of absorbance represents the arterial oxygenated blood and the nonpulsatile component represents the deoxygenated capillary and venous blood.

Pulmonary Damage by Free Radicals
Though tissue oxygenation is essential for life, excess or inappropriate oxygen metabolism is harmful for the tissues. During synthesis of ATP, molecular oxygen is reduced to form water in mitochondria.
1. The reduction of oxygen is accomplished by addition of four electrons by the mitochondrial electron transport system.
2. However, leak in the electron transfer system allows oxygen to accept less than four electrons that form free radicals.
3. Free radicals cause damage to the tissues. Lung is frequently damaged by free radicals. Pulmonary capillaries are mainly damaged that results in pulmonary edema.

Reactive Oxygen Species and Antioxidants
A free radical is an atom or a molecule with an unpaired electron in its outermost orbit.
1. Superoxide radical (O₂⁻) and hydroxyl radical (OH⁻) are commonly produced free radicals in the body. Hydrogen peroxide also can generate hydroxyl radical.
2. Superoxide ion reacts with NO to form peroxynitrite, which is also a free radical. These free radicals are known as reactive oxygen species (ROS). ROS cause tissue damage and promote tissue degeneration. They are sometimes called pro-oxidants, as they are produced during the process of tissue oxidation.
3. However, there are antioxidants in the body that prevent the body from oxidative damage. Antioxidants are mainly enzymes, such as superoxide dismutase, catalase and peroxidases that neutralize ROS. Imbalance between ROS and antioxidants by either more production of ROS or decreased formation of protective enzymes results in oxidative stress (oxidative tissue damage).
4. ROS are also produced during inflammations, which occur mainly due to respiratory burst of neutrophils. Reperfusion injury deteriorates condition by generating local oxidative stress (Application Box 107.2).

Application Box 107.2
Reperfusion-induced injury produces further damage: Free radicals are produced during reperfusion-induced injury in which blood flow to tissue is reestablished following a period of tissue ischemia. This is one of the mechanisms of refractory shock, that occurs after irreversible stage of shock, and in traumatic shock, when blood flow is reestablished after a period of tissue ischemia (For details, refer to ‘Shock’ Chapter 101).

Carbon dioxide (CO₂) is produced mainly during aerobic cellular metabolism of glucose and during conversion of carbohydrate to fat. From tissue, CO₂ is transported to lung in the venous blood and then exhaled in the expired air. CO₂ is transported in blood in three forms:
1. Physically dissolved in the plasma (7%)
2. As bicarbonate ions in plasma and in red cells (70%)
3. As carbamino protein complexes (23%).
   - Carbamino compounds with plasma proteins
   - Carbamino-Hb in red cells

In Dissolved Form in Plasma
The amount of CO₂ dissolved in plasma is proportional to the partial pressure of CO₂ (PCO₂) as per the principle of Henry’s law. The solubility coefficient of CO₂ is 20 times that of oxygen, i.e. 0.06 mL/dL/mm Hg. Thus, in venous blood, 2.76 mL/dL (46 mmHg PCO₂ in venous blood x 0.06) is carried in simple solution. Due to absence of carbonic anhydrase in plasma, carbonic acid formation is less in
plasma. However, a small amount of CO₂ combines with plasma proteins to carbamino-protein complexes.

**As Bicarbonate Ions**

About 70% of CO₂ is transported in the form of *bicarbonate ions in the red cells*. This becomes possible due to presence of *large quantity of carbonic anhydrase in red cells*. From tissue, as soon as CO₂ is released into plasma, a bulk of it diffuses into the red cell. In red cells, it forms either *carbonic acid* (H₂CO₃) or *carbaminohemoglobin*. CO₂ combines with water to form H₂CO₃ (carbonic acid) by carbonic anhydrase. H₂CO₃ is then spontaneously converted into H⁺ and bicarbonate (HCO₃⁻).

\[
\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3 \rightleftharpoons \text{H}^+ + \text{HCO}_3^- 
\]

**Chloride Shift**

As carbonic acid readily dissociates to HCO₃⁻ and H⁺, bicarbonate concentration increases in red cells. HCO₃⁻ leaves the red blood cells and in exchange chloride (Cl⁻) enters the cell from the plasma to maintain electrical neutrality.
1. The exchange of HCO₃⁻ for Cl⁻ occurs by *chloride-bicarbonate exchanger* (anion exchanger) present in the red cell membrane (Fig. 107.9).
2. This exchanger is known as *anion-exchanger 1 (AE1)*, which was previously known as band-3 exchanger.
3. The chloride movement in exchange for HCO₃⁻ is known as the *chloride shift*.
4. As this phenomenon was described by Hamburger, chloride shift is also known as *Hamburger Shift*.
5. Cl⁻ accumulates in red cells and increases red cell size (Application Box 107.3).

**Application Box 107.3**

*Hematocrit of venous blood is more*: Due to accumulation of chloride in red cells leaving red cells, chloride content of red cells in venous blood is about 20% higher than the arterial blood. Chloride is osmotically active and, therefore, red cells imbibe water. This increases the size of red cells of venous blood. Thus, hematocrit of venous blood is about 3% more than that of the hematocrit of arterial blood.

The H⁺ formed during reaction cannot easily diffuse out of the cell because of the low permeability of the membrane to H⁺. Most of the H⁺ produced by the dissociation of bicarbonate is *buffered by hemoglobin*:

\[
\text{H}^+ + \text{HbO}_2^- \rightleftharpoons \text{HHb} + \text{O}_2 
\]

As H⁺ binds to hemoglobin, it decreases oxygen-binding of Hb and shifts the Oxy-Hb dissociation curve to the right. This *facilitates release of oxygen from hemoglobin into the tissue*. This also favors transport of carbon dioxide. Reverse process operates in pulmonary capillaries, where the oxygenation of hemoglobin favors the unloading of carbon dioxide.

The major reactions of CO₂ transport occur in the red cells. However, a large quantity of CO₂ is actually carried in the plasma in the form of bicarbonate.

**As Carbamino Compound**

Carbon dioxide in red cells also reacts with free amine groups (NH₂) of hemoglobin to form *carbaminohemoglobin* (HbNHCOOH):

\[
\text{CO}_2 + \text{HbNH}_2 \rightleftharpoons \text{HbNHCOOH} 
\]

Deoxygenated hemoglobin binds more CO₂ than the oxygenated hemoglobin. About 23% of CO₂ is transported in this form.

**CO₂ Dissociation Curve**

Carbon dioxide dissociation curve is plotted in a similar fashion to that of oxygen. Unlike oxygen dissociation, dissociation of CO₂ from blood is directly related to PCO₂. Therefore, CO₂ dissociation curve is almost a straight line in the normal arterial PCO₂ range (Fig. 107.10).

The major features of CO₂ dissociation curve are:
1. **Linear relationship between CO₂ content and PCO₂**: Within the physiological range of PCO₂ (40–46 mm Hg), the curve is *more linear and steeper* (whereas, Oxy-Hb dissociation curve is neither linear nor steeper within the physiological range of PO₂, i.e. from 40 to 100 mm Hg).
2. **Transfer of large quantity of CO₂**: Because of the shape of the CO₂ dissociation curve, *large amounts of CO₂ can be loaded or unloaded* from the blood with a small change in PCO₂. Also, blood can hold much more carbon dioxide than oxygen.
3. **Inverse relationship between PO₂ and CO₂ content**: The CO₂ dissociation curve shifts downward and to the right at a higher PO₂. The effect of change in PO₂ (or oxyhemoglobin saturation) on CO₂ content is known as the *Haldane effect*. The relationship between PO₂ and CO₂ content is inverse. At any level of PCO₂, the *total CO₂ content increases as PO₂ falls*. The advantage of this inverse relationship (the Haldane effect) is that it allows the blood to load more CO₂ in the tissues and unload more CO₂ in the lungs. Thus, when blood enters capillaries in the tissues and releases O₂, the CO₂-carrying capacity increases, and conversely, when blood enters pulmonary capillaries and picks up oxygen, the CO₂-carrying capacity decreases.
Haldane Effect

When blood passes through pulmonary capillaries, oxygen diffuses into the blood and forms Oxy-Hb.

1. Oxygenation of Hb shifts the $CO_2$ dissociation curve to the right and Hb begins to lose $CO_2$.
2. Thus, in the lungs, loading of $O_2$ facilitates unloading of $CO_2$.
3. This is called Haldane effect, as it is named after the great ancient respiratory physiologist John Scott Haldane (1860–1936).

Bohr Effect

Any factor that shifts Oxy-Hb dissociation curve to right, decreases affinity of oxygen for Hb.

1. At the tissue level, $CO_2$ enters the blood and shifts the curve to the right. This helps in unloading of oxygen from Hb and facilitates tissue oxygenation. This phenomenon is called Bohr Effect as was initially described by Christian Bohr (1855–1911), who has showed experimentally the influence of $CO_2$ tension on blood-binding of oxygen.
2. This occurs mainly due to decreased pH in the tissue.
3. Increased temperature of blood in the tissue also contributes.
4. In tissue, acidification of blood decreases affinity of $O_2$ to Hb, as deoxygenated Hb binds $H^+$ more actively than that of oxyhemoglobin.
5. The $PCO_2$ level in the tissue rises, the curve shifts to the right and $P_{50}$ rises. Thus, Bohr’s effect helps in unloading of $O_2$ and loading of $CO_2$.

Key Concepts

1. Oxygen is transported mainly in combination with Hb, which is primarily affected by the level of pH, Hb, $pCO_2$, temperature, $H^+$, 2,3-DPG.
2. About 70% is transported as bicarbonate ion.
3. Haldane effect helps in loading of $O_2$ and Bohr effect helps in loading $CO_2$ at lungs and tissue level respectively.

Important to Know (Must Read)

1. In examination, ‘Describe the mechanism of transport of oxygen in blood from lungs to the tissues;’ ‘Describe the mechanism of transport of carbon dioxide in blood from tissues to the lungs’ are usually asked as Long Questions.
2. Oxygen-hemoglobin dissociation curve, $CO_2$ dissociation curve, Chloride shift, $P_{50}$, Haldane effect, Bohr effect are asked as Short Questions in exam.
3. In Viva, examiner may ask... State Henry’s law, What are the different forms in which oxygen is transported in blood, How is the oxyhemoglobin formed, What is the oxygen-carrying capacity of hemoglobin, What are the different phases of O$_2$-Hb dissociation curve, What is the significance of plateau phase, What are the factors affecting Hb-binding affinity with oxygen, What is the effect of temperature on the O$_2$-Hb dissociation curve, What is the effect of pH on the O$_2$-Hb dissociation curve, What is the effect of $CO_2$ on the O$_2$-Hb dissociation curve, What is the effect of 2,3-DPG on the O$_2$-Hb dissociation curve, What are the factors that affect 2,3-DPG in red cells, What is the effect of myoglobin on the O$_2$-Hb dissociation curve, What is the effect of carbon monoxide on the O$_2$-Hb dissociation curve, How does CO poisoning produce hypoxia, What are the factors that shift the O$_2$-Hb dissociation curve to the right, What are the factors that shift the O$_2$-Hb dissociation curve to the left, What is oxygen saturation, What is oxygen extraction, Why is oxygen content important in anemic patients, How is the oxygen saturation of Hb measured, What are the different forms in which CO$_2$ is transported in blood, Why is the Hematocrit of venous blood more, What is chloride shift, What is Hamburger shift, What is Haldane effect, What is Bohr effect.
The control of respiration is critical for survival of living creatures. Regulation of respiration differs from regulation of other systemic functions for having both automatic and voluntary control mechanisms.

1. Automatic breathing is produced by rhythmic discharge of motor neurons that innervate and drive muscles of respiration. Cyclical excitation of respiratory muscles influences the volume of the thorax that in turn regulates breathing.

2. Control of excitation of respiratory motor neurons and muscles is the result of activities in the brain, especially in the brainstem. Activities of muscles of respiratory apparatus are mainly controlled by discharge from brain centers.

3. Therefore, transection of spinal cord at its origin from the brain (at medullary-spinal junction) completely abolishes breathing.

4. Activities of respiratory centers are influenced by the chemical composition of blood, especially \( \text{PO}_2 \), \( \text{PCO}_2 \), and pH, and other nonchemical factors like impulses from proprioceptors, etc.

**Scientist contributed**

John Scott Haldane (1860–1936), the great English respiratory physiologist, had studied the oxygen transport in the blood and the respiratory gas exchange in animals. With John Gilles Priestely (1880-1941), he discovered that the respiratory reflex is triggered by an excess of \( \text{CO}_2 \) in the blood, rather than a lack of \( \text{O}_2 \). He described in details of regulation of respiratory drive by \( \text{CO}_2 \) and its effects on blood \( \text{H}^+ \) concentration. Effect of ‘loading of oxygen in the lung facilitating unloading of carbon dioxide’ is named after him and popularly known as Haldane effect. His monumental work on high altitude physiology, diving physiology, oxygen therapy, and carbon monoxide poisoning led to a sea change in clinical medicine and improved safety and reduced mortality and morbidity in many high risk situations. He is referred to as ‘Father of Oxygen Therapy’.

**Sources:**

Overview of Control of Breathing

The rhythmic discharge of neurons from brain to the motor neurons in the spinal cord that in turn activates respiratory muscles occurs spontaneously.

1. **Neurons in the medulla generate appropriate motor signals** that drive the cranial and spinal motor neurons innervating respiratory muscles.

2. The area in the medulla that generates this respiratory rhythm is called as the central pattern generator (CPG) of respiration (Fig. 108.1).

3. During normal breathing, inspiration is brought about by activation of inspiratory muscles, especially the diaphragm. The termination of inspiration occurs due to cessation of excitation of inspiratory muscles, following which expiration occurs passively by elastic recoil of the lungs and chest wall. However, expiration becomes an active process when more ventilation is required, as occurs during exercise.

4. The neural control of these breathing patterns depends on coordinated cyclical changes in the activity of cells primarily located in the medulla, in and around DRG-VRG complex.

5. The medullary neurons drive the respiratory motor neurons that in turn alter the activity of chest wall, diaphragm and conducting airways.

6. The change in ventilation in response to respiratory muscle activities alters chemical composition of blood, especially $\text{PO}_2$, $\text{PCO}_2$, and $\text{pH}$ that provide feedback signals via peripheral and central chemoreceptors to the CPG and other pontomedullary respiratory centers.

7. Alteration in ventilation also provides feedback signals via vagus nerve, spinal nerves and proprioceptive pathways.

8. The feedback signals in turn appropriately modify the output signals from respiratory centers to adjust ventilation according to the need of the body. Thus, control of breathing is broadly divided into three categories:
   - Neural control
   - Chemical control
   - Nonchemical (reflexes from lungs and chest wall) control

**NEURAL CONTROL OF RESPIRATION**

There are two separate neural control mechanisms: The voluntary control and the automatic control.

1. The centers for voluntary control are located in the cerebral cortex. The cortex controls respiratory muscles via corticospinal tract.

2. The centers for automatic control are located in brainstem.

3. The neurons of brainstem respiratory areas converge on spinal cord motor neurons. The neurons that control inspiration converge on $C_3$–$C_5$ motor neurons (phrenic nerve) and thoracic motor neurons that supply external intercostal muscles and the neurons that control expiration converge mainly on thoracic motor neurons that supply internal intercostal muscles.

**Medullary Respiratory Centers**

Medulla controls the basic rhythm of respiration. Two different groups of neurons have been well defined in medulla. According to their anatomic locations they are called as dorsal respiratory group and ventral respiratory group of neurons (Fig. 108.2). In addition, CPG is located in the medulla in and around dorsal and ventral group of neurons.

**Dorsal Respiratory Group**

Dorsal respiratory group (DRG) is named for its dorsal location in the medulla.

1. It is located bilaterally in and around the nucleus tractus solitarius (NTS). DRG primarily contains inspiratory neurons. NTS receives sensory inputs from all thoracic and abdominal viscera.
2. DRG neurons receive sensory inputs from different parts of respiratory system including peripheral chemoreceptors and related respiratory structures via 10th and 9th cranial nerves.
3. Thus, DRG is primarily responsible for inspiration and for integration of sensory information that originates from respiratory system.
4. Neurons from DRG directly project to the cell bodies of phrenic nerve motor neurons in the spinal cord.

**Ventral Respiratory Group**

Ventral respiratory group (VRG) of cells are located in the ventrolateral medulla in the region of the nucleus ambiguous (retroambigous and paraambigous) and retrofacialis.
1. VRG has a larger collection of neurons that extends almost the entire length of medulla. The VRG contains both inspiratory and expiratory neurons.
2. The cells in the nucleus retrofacialis and retroambigous are active during expiration and cells in the nucleus paraambigous are active during inspiration.
3. VRG has three regions: Rostral expiratory, middle inspiratory and caudal expiratory regions.

- The **rostral expiratory region** of VRG (also called as Bötzinger complex) drives the expiratory activity of caudal region.
- The **caudal expiratory region** in turn projects to the spinal motor neurons that innervate muscles of expiration.
- The **middle inspiratory region** projects to the spinal motor neurons that innervate inspiratory muscles, and also to cell bodies of neurons that innervate muscles of pharynx and larynx. Thus, the middle region not only controls inspiration, but also increases the caliber of the upper respiratory tract during inspiration.

The DRG and VRG are located on both sides in the medulla that control the activities of respiratory apparatus of their respective sides. However, cross-communication between the centers of both sides helps them to work synchronously and harmoniously. Thus, respiratory movements of both sides of chest occur simultaneously and symmetrically.

**Central Pattern Generator**

1. It appears that CPG is present in the pre-Bötzinger complex, a group of pacemaker cells located bilaterally between nucleus ambiguous and lateral reticular nucleus (Fig. 108.3). The details of activities of CPG are not fully understood. Presently, it is widely accepted that, CPG is responsible for generating normal respiratory rhythm. The neurons (pacemaker cells) in the pre-Bötzinger complex discharge rhythmically to activate phrenic motor neuron in the spinal cord. Rhythmic activation of phrenic motor neurons during inspiration is abolished by destroying pre-Bötzinger complex or by making section below it and above phrenic motor neurons.
It also appears that CPG is influenced by an integrator, which is located within the medullary reticular formation. Integrator receives and processes inputs from chemoreceptors and higher brain centers like cortex, hypothalamus and limbic system. The integrator in turn controls mainly the frequency and amplitude of respiratory pattern.

**Pontine Respiratory Centers**

In pons, there are two respiratory centers: pneumotaxic and apneustic. The pneumotaxic and apneustic centers in pons modulate the output from medullary respiratory centers, but they are not essential for normal respiratory output.

**Pneumotaxic Center**

Pneumotaxic center is located in nucleus parabrachialis medialis and Kölliker-Fuse nucleus that are present in the rostral pons (Fig. 108.4).
1. Neurons in pneumotaxic center are active during both inspiration and expiration.
2. The exact function of pneumotaxic center is not clearly known. Nevertheless, pneumotaxic center coordinates respiration by switching between inspiration and expiration.
3. Normally, pneumotaxic center inhibits apneustic center.
4. Suprapontine lesion in experimental animals does not change respiratory rhythm indicating that brain centers above pons do not appreciably influence basic rhythm of respiration.

**Apneustic Center**

Apneustic center is present in the caudal pons.
1. Stimulation of apneustic center results in prolonged inspiratory effort interrupted by brief expiration (apneusis).
2. This indicates that apneustic center strongly activates medullary inspiratory center.
3. However, apneustic center is tonically inhibited by pneumotaxic center, and therefore, inspiratory drive originating in apneustic center is checked (Fig. 108.5).

**Role of Vagus Nerve**

Vagus nerve carries afferent fibers from lung and airways. During inspiration, stretching of lung parenchyma increases discharge of afferent fibers in vagus nerve originating from lung.
1. The afferent vagal impulse inhibits the medullary inspiratory neurons. Therefore, magnitude of inspiration increases following vagotomy and vagal stimulation results in inhibition of respiration.
2. However, disruption of vagal afferents (abolition of vagal feedback) prolongs the activity of motor neurons supplying inspiratory muscles i.e., efferent activity in phrenic nerve, without affecting their magnitude.
3. Vagus nerve and pneumotaxic center keep the activity of apneustic center in check.
4. As apneustic center normally activates inspiratory drive, which is inhibited by vagus nerve, vagotomy stimulates inspiration.
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5. Further, vagotomy following lesion of pneumotaxic center results in apneusis (Fig. 108.6)

Scientist contributed

**Henry Head** (1861–1940) demonstrated the function of the vagus nerve in the regulation of respiration. He had also analyzed referred pain from visceral disturbance, and investigated symbolic formulation and expression in aphasia.

Source:

**Control by Higher Centers**

Control of respiration by higher centers in the brain is more complex than the control of rhythmic pattern of respiration. Though many centers influence the activity of medullary respiratory centers, the influence from reticular activating system (RAS) and limbic system is more prominent. RAS exerts a tonic drive on CPG, which is increased during arousal from sleep. The drive from limbic system increases during emotional reactions.

**Integration of Neural Mechanisms**

Transection of brainstem below medulla completely abolishes respiration and respiration continues though irregularly following lesion above the medulla. This indicates that the basic rhythm generator for respiration is located in medulla. Lesion above pons does not affect respiration, which indicates that the controlling centers are located mainly in pons (Fig. 108.6).

Normal breathing is a stereotyped phenomenon. The respiratory cycle consists of two phases: inspiration and expiration. CPG, which is located in the DRG-VRG complex, is responsible for generating normal respiratory rhythm. The pacemaker cells in the pre-Bötzinger complex discharge rhythmically to activate phrenic motor neurons in the spinal cord that initiate and generate inspiratory breathing patterns.

**Inspiration**

During inspiratory phase, phrenic nerve activity increases gradually in two seconds that stops suddenly just at the onset of expiration.
1. The inspiratory neurons exhibit constant discharge throughout inspiration (constant inspiratory neurons) or ramp like discharge during inspiration (inspiratory ramp neurons).
2. Inspiratory ramp activity correlates with phasic activity in the phrenic nerve. Increase in ramp activity ensures smooth increase in lung volume in inspiration.
3. Phrenic nerve activity becomes silent during expiration except in the early part of it when some burst activity occurs (Fig. 108.7).

Expiration

Expiration has two phases. As soon as inspiration is terminated, some inspiratory muscle activity persists (or renewed) in the very early phase of expiration and serves to control initial part of expiratory airflow. This effect slows as lung volume progressively decreases.
1. In the second phase of expiration, inspiratory muscle activity totally ceases during which expiratory airflow occurs due to passive recoil of lungs in quiet breathing or due to contraction of expiratory muscles in forced breathing.
2. The duration of expiration depends on the degree of inhibition of inspiratory neurons in DRG-VRG complex, which is maximal at the beginning of expiration that slowly declines as expiration continues.
3. Finally, inhibitory effect becomes inadequate to prevent the onset of inspiration.
4. The decline of inhibition that triggers the onset of inspiration is influenced by several factors.

5. The duration of expiration depends on neural information originating during expiration and the pattern of the preceding inspiration.

Control Mechanisms of Breathing

Different control mechanisms regulate breathing under various conditions and alter breathing patterns to meet metabolic need of the body.
1. The essential pattern of breathing is generated in CPG, which is located in the medulla. The CPG is extensively influenced by numerous control mechanisms.
2. Apart from CPG, it is proposed that an integrator (or controller) is also located in the medulla that projects to and influences the activity of CPG. Inputs arriving from different brain areas (especially from cortex and limbic system), proprioceptors, intrapulmonary receptors and changes in blood gases influence the controller of respiration.
3. For example, in exercise, the medullary pattern generator driven by cortical inputs controls the excitation of the muscles used during exercise in proportion to the degree of exercise needed. By sensing the magnitude of the carbon dioxide load in the systemic blood, medullary pattern generator accordingly drives ventilation.
4. Intrapulmonary receptors provide feedback signal to respiratory control centers to regulate the degree of breathing.
5. Proprioceptive inputs from joints (due to increased joint movement during exercise) also provide feedback control of breathing.

The controller also adjusts rate and depth of breathing to reduce the work expenditure for ventilation of a given magnitude.
- To achieve this, the controller utilizes afferent neural signals about lung volume, rate of change of lung volume, and change in trans-pulmonary pressures, which are provided by lung and chest wall mechanoreceptors.
- The pattern generator that controls the rhythm of chest wall muscle activity during respiration also controls the activity of muscles of upper airways (of nose, pharynx, and larynx).
- Excitation of these muscles during inspiration causes airway widening and decreases resistance from the nostrils to the larynx.

During normal quiet breathing, in the first phase of expiration, when there is renewed (or sustained) inspiratory muscle activity, active adduction of the vocal cord occurs that in turn causes expiratory braking. However, during forced breathing as seen in exercise-induced hyperpnea, the vocal cords are separated throughout expiration that decreases expiratory resistance and makes expiration easier.
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CHEMICAL CONTROL OF BREATHING (Control by $H^+$, PCO$_2$, and PO$_2$)

The hydrogen ion concentration and respiratory gas composition of the arterial blood profoundly influence respiration. In general, breathing activities are directly related to PCO$_2$ and $H^+$ and inversely related to arterial blood PO$_2$.

1. The two sets of receptors that detect these chemical changes in blood are peripheral and central chemoreceptors.
2. Hypoxia, hypercapnia and acidosis stimulate respiration that in turn raise PO$_2$, lower PCO$_2$ and raise pH.
3. Responses to carbon dioxide and blood pH depend mainly on the central chemoreceptors that are located in the brainstem and responses to hypoxia depend mainly on peripheral chemoreceptors that are located in carotid and aortic bodies.

Peripheral Chemoreceptors

Structure of Chemoreceptors

Peripheral chemoreceptors are located in the carotid and aortic bodies. They respond to changes in PO$_2$, PCO$_2$ and pH in arterial blood. However, they are more sensitive to decreased arterial PO$_2$ (hypoxia) though they also sense increase in PCO$_2$ and decreased pH. Carotid and aortic bodies send signals to the dorsal respiratory group (DRG) of neurons in medulla to increase ventilation.

Carotid Bodies

Functions of carotid bodies as chemoreceptors were first reported by Corneille Heymans for which he got Nobel Prize in Physiology in 1938. Carotid bodies are very small chemosensitive organs having width of about 2 mm and weight of about 2 mg. They are located at the bifurcations of the common carotid arteries near the base of the skull on both sides (Fig. 108.8). Afferent nerves from the carotid bodies travel to the brainstem in the glossopharyngeal nerves.

Two special features of carotid bodies are:
1. They receive unusually high blood flow, which is highest in the body per unit-weight of the tissue. The blood flow is about 2 L per 100 g of tissue/min, which is approximately 40 times the blood flow to the brain.
2. They have a high metabolic rate, which is about three times greater than that of brain.

Due to the greater blood flow, the carotid bodies easily detect minor changes in PO$_2$, PCO$_2$ and pH of blood. However, in spite of greater metabolism, the composition of PO$_2$, PCO$_2$ and pH in the carotid body is virtually same as that of arterial blood due to their much higher blood flow.

Carotid body consists of two types of cells: Type I and Type II. These cells are surrounded by fenestrated sinusoidal capillaries (Fig. 108.9).

Type I Cells

The type I cells are the chemosensitive cells in carotid body. They are also called as glomus cells.
1. Glomus cells are spherical in shape and have the diameter of about 10 μm. They are neuroectodermal in origin and structurally resemble chromaffin cells of adrenal medulla and neurons of peripheral nervous system.
2. They contain voltage-gated ion channels on their membrane and many granules in their cytoplasm.

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Fig. 108.8: Location of carotid body. It is located at bifurcation of common carotid into internal and external carotids. Carotid sinus is the initial dilated portion of internal carotid artery.

Fig. 108.9: Histology of carotid body. Note, glomus (type I) cells are supported by type II cells and closely surrounded by fenestrated capillaries. Release of neurotransmitter from glomus cell triggers action potential in 9th cranial nerve.
containing catecholamines (norepinephrine, dopamine), substance P, met-enkephalin and acetylcholine. These chemical substances, especially catecholamines (mainly dopamine) are released from glomus cells in response to hypoxia.

3. Dopamine released by glomus cells acts on \( D_2 \) receptors present on membrane of 9th nerve ending and triggers the action potential in carotid sinus nerve.

4. The close contact of fenestrated capillaries with glomus cells helps the cells to easily monitor the changes of gasses in the arterial blood.

**Type II Cells**
The type II cells are also called sustentacular cells. They surround the clusters of glomus cells. They are the supporting cells for glomus cells as glial cells support neurons in the brain.

**Aortic Bodies**
Aortic bodies are located along the ascending aorta and arch of the aorta. They are innervated by vagal afferents. They also contain glomus cells similar to that of carotid body. However, the responses of aortic chemoreceptors to stimuli are somewhat different from the carotid chemoreceptors. Also, aortic chemoreceptors are relatively less effective than carotid chemoreceptors in the regulation of respiration.

**Sensitivity of Chemoreceptors**
The chemoreceptors respond to hypoxia, hypercapnia and acidosis. It is interesting to note that the mechanism of chemoreceptor stimulation is different from the mechanism of sensory transduction as described in sensory system in neurophysiology.

1. The sensor for chemical stimuli is the glomus cell, which responds to all three chemical changes in blood.

2. The final pathway of the mechanisms for three stimuli is also the same in which inhibition of \( K^+ \) channel results in depolarization-induced opening of voltage-gated \( Ca^{++} \) channels that in turn releases neurotransmitters and causes stimulation of afferent fibers.

3. However, the mechanism that causes inhibition of \( K^+ \) channel by each stimulus is different.

**Hypoxia**
Hypoxia is the major stimulus for activation of peripheral chemoreceptors. With normal blood pH and \( PCO_2 \), increase in \( PO_2 \) above normal range does not have significant effect on breathing, but decrease in \( PaO_2 \) less than 100 mm of Hg results in progressive increase in the firing rate of afferent nerves (Fig. 108.10) that increases ventilation. However, the change in ventilation to alteration in alveolar \( PO_2 \) is not linear. The response becomes prominent when alveolar \( PO_2 \) falls below 80 mm Hg. The response is most effective at \( PO_2 \) less than 60 mm Hg (Fig. 108.11). Below this level, ventilatory response curve is steep and almost linear.

Mechanisms of less rise in ventilation when \( PO_2 \) falls from 100 to 60 mm Hg:

1. In hypoxia, hemoglobin (Hb) is less saturated with \( O_2 \). Oxy-Hb is a stronger acid than Hb. Thus, with fall in arterial \( PO_2 \), the \( H^+ \) of arterial blood falls due to less saturation of Hb with \( O_2 \). The fall in \( H^+ \) inhibits respiration.

2. Increased ventilation due to hypoxia decreases \( PCO_2 \) that in turn inhibits ventilation.

Therefore, unless hypoxia is strong enough to overcome the inhibitory effect of decreased \( H^+ \) and \( PCO_2 \) in arterial blood on ventilation, its effect on ventilation is not prominent. When \( PO_2 \) is less than 60 mm Hg, hypoxic drive becomes strong enough to override these inhibitory effects.
**Significance**

The response of peripheral chemoreceptors to oxygen depends on PaO\(_2\), and not on the oxygen content. Therefore, in anemia or carbon monoxide poisoning, the two conditions that exhibit reduced oxygen content but have normal PaO\(_2\), have little effect on the ventilation response curve.

**Mechanism**

Hypoxia inhibits K\(^+\) channel. The accumulation of K\(^+\) in the glomus cell results in depolarization that activates voltage-gated Ca\(^{++}\) channels. Increased Ca\(^{++}\) influx causes neurotransmitter secretion that stimulates the afferent nerve.

Inhibition of K\(^+\) channel by hypoxia occurs by three possible mechanisms (Fig. 108.12):

1. The oxygen sensor in the glomus cell is a heme-containing protein, which is closely associated with K\(^+\) channel. This protein is normally bound to O\(_2\). In hypoxia, the heme-containing protein loses its O\(_2\), which leads to inhibition of K\(^+\) channels.
2. Hypoxia increases cAMP in the glomus cells. Increased intracellular cAMP inhibits cAMP-sensitive K\(^+\) channels.
3. Hypoxia inhibits mitochondrial NADPH oxidase in the glomus cells. NADPH oxidase is essential for reduction of glutathione. This increases the ratio of reduced glutathione (GSH) to the oxidized glutathione (GSSG), which directly inhibits K\(^+\) channels.

**Effect of Change in PACO\(_2\)**

At lower level of alveolar PCO\(_2\) (37 mm Hg), no stimulation of ventilation occurs by hypoxia until the alveolar PO\(_2\) is less than 60 mm Hg, and below this the response curve is steep and linear with progressive decrease in PO\(_2\) (Fig. 108.13). At higher level of alveolar PCO\(_2\) (49 mm Hg), an inverse relationship is observed between ventilation and alveolar PO\(_2\), in which decrease in PO\(_2\) proportionately increases ventilatory response and increase in PO\(_2\) linearly decreases ventilatory response.

**Hypercapnia**

The rate of discharge of carotid and aortic chemoreceptors increases linearly with increase in PaCO\(_2\). This directly increases the ventilatory response to carbon dioxide. CO\(_2\) is produced by metabolisms. Thus, increased metabolism stimulates respiration, which in turn removes CO\(_2\) from the body. In fact, ventilation remains elevated till arterial PCO\(_2\) returns to normal.

1. Increase in PaCO\(_2\) stimulates peripheral chemoreceptors by increasing CO\(_2\) in glomus cells. In fact, H\(^+\) formed form H\(_2\)CO\(_3\) in the glomus cells is the actual stimulus rather than the molecular CO\(_2\). Effect of PaCO\(_2\) on ventilation is mediated up to 40% by peripheral chemoreceptors.
2. It should be noted here that hypoxia affects ventilation mainly via peripheral chemoreceptors, whereas hypercapnia affects ventilation mainly via central chemoreceptors.
3. The effect of CO\(_2\) on ventilation is studied by inhaling gas mixture containing CO\(_2\). Increase in alveolar PCO\(_2\) increases arterial PCO\(_2\) that stimulates ventilation. Hyperventilation removes CO\(_2\) from blood and decreases alveolar PCO\(_2\).
4. However, alveolar PCO\(_2\) does not become normal and remains elevated. Therefore, stimulation of ventilation
5. When CO₂ content of atmospheric air is high (CO₂ content of inspired air is more than 7%), PCO₂ of alveolar air and arterial blood increase abruptly. Elimination of CO₂ from body becomes ineffective in this condition and CO₂ accumulates in blood.

6. High hypercapnia causes CNS depression. Initially, there will be headache and confusion, which fast leads to coma. The condition is called CO₂ narcosis.

**Mechanism**

Increase in PaCO₂ increases entry of CO₂ into the glomus cells. CO₂ forms H⁺ in the cell. This results in decrease in intracellular pH (increased H⁺). Increased cytosolic protons (H⁺) block K⁺ channels and inhibit K⁺ efflux. This causes depolarization of the cell, which causes opening of voltage-gated calcium channels. Calcium influx leads to increased cytosolic Ca²⁺ and Ca²⁺-mediated neurotransmitter release that lead to increased nerve traffic in 9th nerve (Flowchart 108.1).

**Effect of Hypoxia on CO₂ Response**

With decrease in alveolar PO₂, the slope of the curve increases and shifts to left. That means, hypoxia increases the sensitivity of ventilatory response to hypercapnia (Fig. 108.15).

**Acidosis**

Peripheral chemoreceptors are sensitive to change in arterial pH. Acidosis alone stimulates ventilation in the absence of hypoxia or hypercapnia, as occurs in metabolic acidosis. Acidosis also increases the sensitivity of chemoreceptors to PaCO₂.

**Mechanism**

Acidosis (decrease in pH) inhibits acid-extruding transporters, like Na-H⁺ exchanger. This results in increased intracellular acid load (accumulation of H⁺ in the cell). Increased proton inhibits K⁺ channels.

**Other Factors**

Poisons that inhibit metabolic respiratory chain like cyanide strongly stimulate peripheral chemoreceptors. Fall in blood pressure below 60 mm Hg, with or without change in PaO₂, stimulates chemoreceptor activity. This effect is mediated
more through aortic bodies. Afferent impulses from chemoreceptors interact with neural inputs of other reflexes like lung stretch reflex, baroreceptor reflex, etc. that especially help in regulation of cardio-respiratory functions.

Scientist contributed

August Krogh (1874–1949) the great physiologist from Denmark demonstrated that blood capacity for oxygen is influenced by carbon dioxide tension. He clearly showed the importance of the capillary vasomotion. Krogh was awarded the Nobel Prize in Physiology and Medicine in 1920.

Central Chemoreceptors

Central chemoreceptors are located as bilaterally paired cell groups just below the surface of the ventrolateral medulla immediately caudal to the pontomedullary junction. Therefore, they are also called medullary chemoreceptors or chemosensitive areas (Fig. 108.16).

1. On each side, the cell groups are divided into rostral, intermediate and caudal chemosensitive zones. Three areas were described by three physiologists (Rostral area by Mitchell, intermediate area by Schláfke and caudal area by Loeschcke).

2. The medullary chemosensitive neurons respond to change in the H⁺ of the surrounding interstitial fluid. It should be noted that the cerebral interstitial fluid H⁺ concentration is the function of PCO₂ in the cerebral arterial blood and the bicarbonate concentration of cerebrospinal fluid.

3. Recently it has been discovered that the chemosensitive neurons are also located in or around the other brain-stem nuclei such as nucleus tractus solitarius, nucleus ambiguous, nucleus ceruleus and hypothalamus.

Mechanism of Stimulation

Cerebrospinal fluid (CSF) is essentially protein-free. Phosphate concentration of CSF is less than its plasma level, whereas CSF bicarbonate concentration is almost same as that of plasma. Therefore, bicarbonate serves as the main buffer in CSF.

1. Change in H⁺ concentration of blood is poorly reflected in CSF, as H⁺ penetrates very slowly into the brain. However, molecular carbon dioxide diffuses readily into CSF.

2. CO₂ that enters the CSF is immediately hydrated to form H₂CO₃. H₂CO₃ dissociates to form H⁺ and HCO₃⁻. Thus, blood PCO₂ influences the pH of CSF.

3. In fact, concentration of H⁺ in interstitial fluid in the brain parallels the PCO₂ level in arterial blood. The concentration of H⁺ in CSF stimulates central chemoreceptors that increase respiration and the magnitude of stimulation is proportional to its concentration.

The pH of CSF is 7.3, which is slightly less than that of blood (7.4). It is primarily determined by the bicarbonate content and the level of PCO₂ in CSF. Therefore, the change in CSF pH occurs less than the change of pH of plasma in metabolic acid-base disturbances, whereas in respiratory acid-base disturbances, the change in CSF pH is same as that of blood. This is because in metabolic disturbances, the CSF bicarbonate change is about 40% of the change that occurs in blood and in respiratory disturbances, CSF bicarbonate change is same as that in blood.

Integrated Responses to Hypoxia, Hypercapnia and Acidosis

In the preceding discussions, though the effects of hypoxia, hypercapnia and acidosis on peripheral and central chemoreceptors have been described separately, in real life, the individual changes of these chemical compositions of blood occur rarely. Hypoxia is usually associated with hypercapnia and acidosis. Moreover, the effects are mediated by simultaneous activation of peripheral and central chemoreceptors (Flowchart 108.2).

1. The ventilatory response to hypercapnia is more in the presence of hypoxia, and the degree of increase in ventilation varies with the degree of hypoxia (Fig. 108.15, as depicted above).

2. Similarly, the ventilatory response to hypoxia increases in the presence of hypercapnia. Due to this interdependence, the subsequent increased ventilation blunt the response to hypoxia, unless PaCO₂ is held constant as PaCO₂ decreases due to increased ventilation.

3. The low PaCO₂ blunts the response to hypoxia mainly by affecting central chemoreceptors. The best example
is the hypoxic response at high altitude. Hyperventilation occurs due to hypoxic stimulation of peripheral chemoreceptors.

4. Subsequently, increase in pH (respiratory alkalosis) created by the hyperventilation allows hypoxic stimulation to be less effective. Also, H⁺ effects on CO₂ response is additive in nature. The CO₂ response curve shifts to left with decrease in pH as the chemoreceptor sensitivity increases. About 40% of response to CO₂ is abolished if the decrease in arterial pH by CO₂ is prevented to occur.

   The interaction between the chemoresponses to hypoxia, hypercapnia and acidosis is best studied in metabolic and respiratory acidosis.

Metabolic Acidosis

Metabolic acidosis is caused due to accumulation of non-volatile acids like lactic acid, ketoacid, etc.

1. In the initial phase, hyperventilation occurs by stimulation of the peripheral chemoreceptors by H⁺. As penetration of H⁺ into CSF is poor, the fall in blood pH does not stimulate the central chemoreceptors directly.

2. Hyperventilation effect brought about by peripheral chemoreceptors decreases PaCO₂ that in turn results in compensatory alkalosis. Due to decreased PaCO₂, pH of CSF increases.

3. This paradoxical rise of CSF pH as a result of reduced PaCO₂ actually restrains hyperventilation. In the later phase (if acidosis persists for days), CSF bicarbonate concentration is adjusted downward, and ultimately, ventilation increases further due to stimulation of central chemoreceptors as the paradoxical alkalosis of CSF is eliminated.

4. Thus, profound hyperventilation occurs in metabolic acidosis, for example Kussmaul breathing as seen in diabetic ketoacidosis.

Respiratory Acidosis

Respiratory acidosis occurs due to accumulation of carbon dioxide.

1. Respiratory acidosis occurs commonly in chronic obstructive lung diseases like emphysema, asthma, etc. or due to failure of respiratory apparatus to ensure adequate gas exchange like muscle weakness (myopathies, neuropathies) or due to failure of respiratory centers to respond to carbon dioxide as occurs during anesthesia and following brain injury.

2. In such conditions, in the acute phase, the response is an acute increase in minute ventilation proportional to the rise in PaCO₂.

3. If hypercapnia continues for few days, acute-severe hyperventilation subsides due to compensatory increase in CSF bicarbonate. This increases pH of CSF, which shifts the response curve to right. This results in decrease in ventilation (central adaptation).

4. Therefore, in chronic lung diseases, in spite of persistent hypercapnia, the central chemoreceptor drive for ventilation is less. However, the drive for ventilation in such conditions is maintained via hypoxia stimulating the peripheral chemoreceptors.

5. In such patients, if the hypoxic component alone is corrected by administering oxygen, the hypoxic stimulus for ventilation will be lost that may result in greater hypoventilation.

6. This results in further hypercapnia and severe acidosis.

7. Therefore, more appropriate treatment in such cases is to provide mechanical assistance for restoring adequate ventilation.

Hormonal Control of Respiration

Many hormones influence respiration. Female gonadal hormones, especially progesterone stimulate ventilation. Therefore during pregnancy and secretory phase of menstrual cycle, ventilation is considerably more. Progesterone is believed to stimulate medullary respiratory centers. Progesterone also increases body temperature that stimulates ventilation. Thyroxine, cortisol and catecholamines also influence respiration.
from environmental insults. The receptors are lung, airway and chest wall mechanoreceptors and chemoreceptors that respond to changes in blood pH and gas tensions.

**Receptors for Respiratory Reflexes**

Receptors for respiratory reflexes are of three types: slowly adapting receptors, rapidly adapting receptors, and C fiber endings. These receptors are mainly innervated by myelinated and unmyelinated fibers in vagus nerve.

**Slowly Adapting Receptors**

The slowly adapting receptors are located within the smooth muscle of conducting airways. They are sensory terminals of myelinated afferent fibers. As they respond to airway stretch, they are also known as pulmonary stretch receptors.

1. They discharge in response to increased airway transmural pressure, and sense the changes in lung volume.
2. They adapt slowly to the stretch imposed on them. When stimulated, they inhibit inspiration and prolong expiration. This is called Hering-Breuer reflex.

**Hering-Breuer Reflex**

This reflex was described by Hering and Breuer in 1868, who found that lung inflation decreases tidal volume and increases respiratory frequency. There are two Hering-Breuer reflexes: Hering-Breuer inflation reflex and Hering-Breuer deflation reflex.

1. In Hering-Breuer inflation reflex, steady increase in lung volume (lung inflation) results in increase in duration of expiration. Marked inflation of lungs with sustained pressure may even abruptly terminate inspiration in progress in addition to the prolongation of expiration.
2. In Hering-Breuer deflation reflex, marked deflation of lung results in decrease in duration of expiration. As described above, the receptors are slowly adapting stretch receptors in lung parenchyma and airways and afferent and efferent pathways are vagus nerve.

**Significance of Slowly Adapting Receptors**

Increased lung volume stimulates slowly adapting receptors that excite the inspiratory off-switch. This effect shortens inspiration when tidal volume is larger than normal. The slowly adapting receptors are involved in regulating expiratory time, expiratory muscle activation, and functional residual capacity. Stimulation of these receptors also relaxes airway smooth muscle, reduces vasomotor tone and increases heart rate.

**Rapidly Adapting Receptors**

The rapidly adapting receptors are sensory terminals of myelinated afferent fibers that are found in the larger conducting airways.

1. They respond to a sudden maintained inflation with rapid increase in firing rate. However, their firing rate rapidly declines when the volume change is sustained. The firing rate decreases fast, with about 20% decrease occurring in the first second.
2. They are very sensitive to different chemical stimuli like histamine, serotonin, bradykinin and prostaglandins released locally in response to allergy and inflammation.
3. Frequently they are called irritant receptors as they respond to irritation of the airways by various noxious substances, such as smoke, dust, ammonia, etc. Receptors are also stimulated by acute congestion and inflammation.

**Significance of Rapidly Adapting Receptors**

Rapidly adapting receptors play important role in detecting pathological processes that involve irritation, congestion and inflammation of airways. Activity of these receptors is inversely proportional to lung compliance and they are considered as the sensors of compliance change, especially in pathological states as they are almost inactive during quiet breathing. In general, stimulation of rapidly adapting receptors results in excitatory responses like coughing, gasping, and prolonged-inspiration.

**C Fiber Endings**

C fiber endings are terminals of unmyelinated nerves. They innervate the receptors in alveoli and conducting airways. They are of two types in the lungs.

1. The first category is pulmonary C fibers that are located adjacent to alveoli and are accessible from the pulmonary capillary circulation. They are also called juxtapulmonary capillary receptors or J receptors (described by Indian scientist Dr. A. S. Paintal).
2. The second category is the bronchial C fibers that are located in airways and accessible from the bronchial circulation.

Both the categories of receptors are stimulated by lung injury, marked inflation, acute pulmonary vascular congestion, and certain chemical agents.

**Significance of C-Fiber Endings**

Pulmonary C fibers are more sensitive to mechanical processes like edema, congestion, and embolism and less sensitive to chemical stimulation like products of inflammation, whereas the bronchial C fibers are more sensitive to chemical stimulation.
1. When they are stimulated, they cause rapid shallow breathing, bronchoconstriction, increased airway secretion, and cardiovascular depression.

2. Acute stimulation results in apnea and a marked hypotension. Skeletal muscle tone is also abruptly reduced.

**Chest Wall Proprioceptors**

Chest wall proprioceptors provide information about chest movement and muscle tension, especially when the breathing is effortful. Intercostal muscles contain plenty of muscle spindles that are rare in the diaphragm. These muscle spindles adjust breathing by controlling feedback motor neuron activities. Chest wall proprioceptors also play an important role in the perception of breathing-effort.

**Other Proprioceptors**

Joint movements, both active and passive stimulate respiration. Impulses in the ascending fibers in sensory pathways from proprioceptors in the muscle, tendons, joint ligaments, etc. to the thalamus, give collaterals to brainstem respiratory centers. Therefore, increased activity in these ascending pathways stimulates respiration. This is one of the mechanisms for hyperventilation that occurs during exercise.

**Receptors in Upper Airways**

Receptors from upper part of the airways, especially from nasopharynx, trachea and bronchi influence respiration during coughing and sneezing.

**Coughing**

Coughing could be voluntary or a reflex (involuntary) phenomenon to clear the irritants from respiratory tract. It occurs with a deep inspiration, which is immediately followed by forced expiration against a closed glottis. The intrapleural pressure increases to 100 mm Hg or more and then glottis opens instantaneously causing an explosive outflow of air at a velocity of about 600 miles per hour. Coughing is intact in individuals with transplanted lungs (Application Box 108.1).

**Sneezing**

Sneezing is usually an involuntary phenomenon to remove the dusts and other irritants from upper part of airways. The mechanism is almost same as coughing, but the glottis remains continuously opened through out the reflex act.

**Afferents from Viscera**

Respiration is temporarily inhibited and glottis is closed during swallowing and vomiting that prevents food particle or vomitus to enter the respiratory tract. Afferents from viscera and diaphragm mediate these responses.

**Hiccup**

For hiccup reflex, inspiratory muscles including diaphragm contract spasmodically, causing an instantaneous inspiration. During this process, glottis closes abruptly, which produces the typical sound associated with hiccup. The physiologic significance of hiccup is not known, though it occurs during fetal life and postnatal life. Usually, holding breath firmly stops hiccup; but there are intractable hicups that sometimes respond to centrally acting analgesics and dopamine antagonists.

**Yawning**

This is a peculiar reflexive respiratory act associated with deep inspiration and typical prolonged musical sound, which is often infectious. The exact mechanism and physiological basis of yawning is not known, though it is also observed in fetus, fish, tortoise and mammals. The probable functions of yawning are:

1. **Removes excess CO₂ from blood and activates the body:** It is believed that yawning occurs when there is excess accumulation of CO₂ in the blood. Yawning removes CO₂ from the body and improves oxygenation of tissues. Yawning usually occurs when people are fatigued or tired and yawning temporarily activates them. But, the exact mechanism of this psychophysical activation is not known.

2. **Heralds sleep:** Just before going to sleep, people often yawn. Yawning occurs frequently in sleeplessness condition.

3. **Improves lung expansion:** It is proposed that deep inspiration and stretching of the thorax during yawning causes expansion of the lungs and improves air flow into under ventilated alveoli. Thus it is suggested to prevent collapse of these alveoli and prevent atelectasis; though experimental evidences do not prove it.

4. **Improves venous return:** Yawning improves venous return probably by increasing abdominal and thoracic pump activities. Thus, yeaning is good for heart.

5. **Nonverbal communication:** A specific nonverbal message can be delivered to others through yawning, which is true for both animals and human beings.
Chapter 108: Regulation of Respiration

Afferents from Baroreceptors
Afferent fibers from baroreceptors located in carotid sinus, aortic arch, ventricles and atria relay in medullary respiratory cardiovascular centers, in addition to their relay in medullary cardiovascular centers. Usually, impulses originating from these baroreceptors inhibit respiration, which has temporary and mild effect. Hyperventilation that occurs due to chemoreceptor stimulation is not baroreceptor-mediated.

Afferents from Higher Centers
Afferents from limbic system and hypothalamus terminate in brainstem respiratory centers. They mediate breathing responses during pain and emotion. Afferents from neocortex to respiratory motor neurons in the spinal cord mediate the voluntary control respiration. These cortical neurons do not terminate on brainstem respiratory centers.

Breathing During Sleep
Sleep occurs due to removal of excitatory influences that arise from the brainstem reticular formation. Reticular activating system has tonic excitatory drive on medullary respiratory neurons. Therefore, one may expect that sleep would result in general respiratory inhibition. Breathing patterns are different in REM sleep and slow-wave sleep.
1. In general, during sleep, as the stimulus of wakefulness is removed, breathing is depressed with the deepening of sleep and when wakefulness returns as sleep lightens, breathing is activated by the carbon dioxide accumulated during the interval of sleep.
2. This periodic pattern of breathing if exaggerated is known as Cheyne-Stokes breathing, which usually occurs in slow wave sleep.
3. In slow-wave sleep, in stages 1 and 2, there is periodic variation in depth of breathing. However, in stages 3 and 4, breathing is slow, deep and regular. In REM sleep, breathing is rapid and irregular.

Responses to CO_2, Hypoxia and Airway Irritation during Sleep
In general, ventilatory responses to CO_2 are decreased during sleep. In Non-REM sleep, the decreased sensitivity to CO_2 is mainly due to reduction in wakefulness-stimulus. Breathing remains responsive to carbon dioxide during slow-wave sleep though the sensitivity is decreased.
1. In fact, during slow wave sleep, in the absence of the wakefulness-stimulus, carbon dioxide-stimulus provides the major background medullary excitation for stable breathing to continue. Therefore, alterations in the carbon dioxide-stimulus in diseased conditions result in depression in breathing during slow-wave sleep.
2. During REM sleep, breathing is regulated by the behavioral control systems.
3. Ventilatory responses to hypoxia are decreased during both slow-wave and REM sleep. However, the irregular and rapid breathing of REM sleep remains unaffected by hypoxia. During sleep, hypercapnia is a potent stimulus than hypoxia for arousal.
4. Responses to airway irritation alter during sleep. Stimuli that cause cough, tachypnea, and airway constriction during wakefulness usually cause apnea and airway dilation during sleep.

Upper Airway Obstruction during Sleep
During REM sleep, skeletal muscle tone is reduced. Tone of laryngeal and pharyngeal muscles is also decreased. Muscles of tongue relax. This increases the possibility of obstruction of the upper airways during REM sleep. Airway obstruction during sleep results in snoring. Repeated and prolonged obstruction results in significant hypercapnia and hypoxemia that causes repeated arousals from sleep.

Sleep Apnea Syndrome
In adult sleep apnea syndrome, marked loss tone of pharyngeal muscles occurs during REM sleep causing obstruction of airway during inspiration. This produces apnea. This is called obstructive sleep apnea.
1. Obesity contributes to the obstructive sleep apnea.
2. The person wakes up and breathes normally for sometime and sleeps again to have another bout of apnea. Thus repeated apnea occurs during sleep.
3. These people develop morning headache and fatigue due to frequent apnea in the night.
4. Sleeplessness occurs during the day (day somnolence).
5. In central sleep apnea, the primary cause is the decreased neural output from medullary respiratory center to the phrenic motor neurons that supply diaphragm.

CHAPTER SUMMARY

Key Concepts
1. Respiration has both voluntary and involuntary control mechanisms. Neural mechanisms are located mainly in the brainstem. These center receive inputs from higher centers mainly, hypothalamus and limbic systems.
2. Chemical control of respiration depends on blood levels of CO_2 and H^+ and O_2. However, brain level of H^+ is the main stimulus.
3. Peripheral chemoreceptors are more sensitive to pCO_2 than and pO_2.
Important to Know (Must Read)

1. In examination, Describe the neural regulation of respiration, Describe the chemical regulation of respiration, are usually asked as Long Questions.

2. Medullary respiratory centers, Pontine respiratory centers, Effect of transaction of brainstem at various levels on respiration, Effect of hypoxia on ventilation, Effect of hypercapnea on ventilation, Hering-Breuer reflex, are asked as Short Questions in exam.

3. In Viva, examiner may ask… Name the medullary respiratory centers, What is the function of dorsal respiratory group of neurons in medulla, What is the function of ventral respiratory group of neurons in medulla, What is the location and function of the central pattern generator, Name the pontine respiratory centers, What is the location and function of the pneumotaxic center, What is the location and function of the apneustic center, What is the role of vagus nerve in the regulation of respiration, How do the pneumotaxic, apneustic and medullary neurons interact, What is the effect of hypoxia on ventilation, What is the mechanism of increased ventilation by hypoxia, Why is there not much rise in ventilation when the pO$_2$ falls from 100 mm Hg to 60 mm Hg, What is the effect of hypercapnea on ventilation, What is the mechanism of increased ventilation by hypercapnea, Name the central chemoreceptors, What is the effect of metabolic acidosis on respiration, Name the hormones and their mechanisms that influence respiration, What are the slowly adapting receptors, What is Hering-Breuer inflation reflex, What is Hering-Breuer deflation reflex, What is the significance of Hering-Breuer reflex, What are the rapidly adapting receptors, what is their significance, What are the J receptors, what is their significance, What is sleep-apnea syndrome, Why coughing remains intact in patients with lung transplant.
Paul Bert was the first scientist to describe that the harmful effects of high altitude are due to low oxygen tension. It is important to note that the percentage of oxygen does not alter at high altitude rather the barometric pressure decreases. Therefore, the responses at high altitude are due to decreased tension of oxygen ($P_{O_2}$) in inspired gas. Hypoxic symptoms appear at 10,000 feet and become severe at 15,000 to 18,000 feet. Above 20,000 feet, barometric pressure is around 50% of the normal value. However, normally the effects are not severe when the ascent is slow while camping at different heights.

Scientist contributed
Paul Bert (1833–1886), the French physiologist developed fundamental studies on relation of oxygen tension to physiological processes, which made the foundation for study of aviation and high attitude physiology.

COMPENSATORY MECHANISMS
At high altitude, decrease in $P_{O_2}$ decreases oxygen supply to the tissues. To deliver normal quantity of oxygen to the tissues, many compensatory mechanisms are activated.
1. Immediate and important amongst them is hyperventilation. Hyperventilation induced by hypoxia is not significantly prominent unless alveolar $P_{O_2}$ decreases below 60 mm Hg.
2. Normally drop in alveolar $P_{O_2}$ to 60 mm Hg occurs at an altitude of approximately 4500 m (14,000 feet).

Stages of Compensation
The hyperventilation induced by hypoxia at high altitude appears in two stages:

In First Stage
In the first stage that appears immediately on exposure to hypoxia ventilation increases instantly though the increase in ventilation is small compared to the increase in ventilation in the second stage. The hyperventilation in first stage is mainly due to hypoxic stimulation of the carotid bodies. The magnitude of increase in ventilation is less because the hypoxic stimulation of peripheral chemoreceptor is opposed by the decrease in arterial $PCO_2$ that occurs due to excess removal of carbon dioxide induced by hyperventilation. This also increases arterial pH. Decreased arterial $PCO_2$ and alkalosis together blunt the hypoxic response.

In Second Stage
In second stage, ventilation increases slowly in 8 to 10 hours and then remains sustained. The sustained increase in ventilation is due to the ventilatory acclimatization at high altitude. Ventilatory acclimatization is a physiological response that occurs during prolonged exposure to
hypoxia. After about 2 weeks, hyperventilation induced by hypoxia reaches a stable plateau. There are two mechanisms for ventilatory acclimatization.

1. **Chemoreceptor mechanism**: The pH of CSF is more alkaline in the acute phase. However, ventilation stimulated by hypoxia brings pH close to normal by the movement of bicarbonate out of the CSF. In prolonged exposure to hypoxia, sensitivity of carotid body to arterial PO\(_2\) also alters.

2. **Renal mechanism**: The alkaline blood pH that occurs due to hypoxia-induced hyperventilation opposes the hypoxic response that decreases ventilation. However, kidney compensates pH by excreting more bicarbonate. This decreases blood pH toward normal in about 3 days. Thus, the effects due to alkaline pH are minimized. This allows hypoxic drive to further increase minute ventilation and then attain a steady state.

**Acclimatization at High Altitude**

When a person ascends to high altitude and stays there for longer periods, he slowly gets adapted to the new environment. This process of adaptation is called acclimatization. The acclimatization at high altitude starts within twelve hours and may take several days or weeks to complete. The maximum height up to which adaptation occurs is 18000 feet. Beyond this level, the subject needs \( \text{O}_2 \) inhalation for survival. Adaptive changes mainly affect respiratory and cardiovascular systems, and blood and tissues. The primary objective of these changes is to increase oxygen supply to the tissues.

**Respiratory Changes**

Hypoxia stimulates peripheral chemoreceptors.

1. This increases pulmonary ventilation by increasing rate and depth of respiration.

2. Consequently, \( \text{CO}_2 \) is washed off and **alkalosis develops** (respiratory alkalosis). Alkalosis counteracts the stimulating effects of hypoxia.

3. The kidney corrects blood pH by excreting HCO\(_3^-\) in urine. Thus, urine becomes alkaline. This helps the response of the respiratory center to hypoxia to continue so that pulmonary ventilation is maintained at higher levels.

4. Ventilation also steadily increases due to active transport of H\(^+\) into the CSF. Though ventilatory response decreases slowly after four days, it remains permanently above the pre-exposure level.

After about 4 days of acclimatization, ventilatory response slowly increases, which also depends on the level of altitude. As the altitude increases, magnitude of response increases. **Ventilatory equivalent (VE)** is a better index of ventilatory response. VE is the ratio of expired minute volume (\(V_E\)) to the \(\text{O}_2\) consumption (\(\text{VO}_2\)). VE increases with increase in height of altitude (Fig. 109.1).

**Cardiovascular Changes**

In the early phase, heart rate, cardiac output and blood pressure increase.

1. These changes occur due to activation of sympathetic axis by hypoxia.

2. Gradually, heart rate returns to normal. However, some people may continue to have high blood pressure. Increased cardiac output helps to increase blood flow.

3. Hypoxia causes vasodilation in the systemic circulation that adds to increase in blood flow and oxygen supply to the tissues.

4. Though increased red cell mass improves oxygenation of tissue, significant polycythemia (increased hematocrit) increases viscosity of blood, which in turn increases the workload on the heart. This leads to cardiac hypertrophy.
Tissue Changes

Following tissue changes occur, especially in the skeletal muscles:
1. Increase in number of capillaries
2. Increase in the number of mitochondria in the cells
3. Increase in activity of oxidative enzymes like cytochrome oxidase
4. Increase in myoglobin content
5. Angiogenesis (release of chemicals from the hypoxic tissue stimulate formation of new blood vessels).

Other Changes

- Described in “Environmental Physiology”

HIGH ALTITUDE ILLNESS

Types of Illness

High altitude illness includes acute mountain sickness, chronic mountain sickness, high altitude cerebral edema, and high altitude pulmonary hypertension and edema.

Acute Mountain Sickness

Sudden exposure to high altitude leads to development of certain signs and symptoms that are together referred to as “Acute Mountain Sickness”. Usually, people develop fatigue, dyspnea, nausea, vomiting, diarrhea, headache, insomnia and palpitations. Loss of co-ordination, memory and judgment are not uncommon. Euphoria and emotional changes are also observed. These symptoms appear within 4 to 8 hours of arrival at high altitude and may last for several days. In severe hypoxia or in susceptible individuals, pulmonary and pulmonary cerebral edema develop.

Chronic Mountain Sickness (Monge’s Disease)

This is seen in long-term exposure to high altitude. These people develop malaise, fatigue, hypervolemia, exercise intolerance, polycythemia, pulmonary hypertension, right ventricular hypertrophy and heart failure that are collectively referred to as chronic mountain sickness.

Pulmonary Hypertension and Edema

Pulmonary hypertension occurs due to prolonged exposure to hypoxia. Alveolar hypoxia causes pulmonary vasoconstriction. In chronic hypoxia, smooth muscles in pulmonary arteries undergo hypertrophy and hyperplasia that cause narrowing of the arterial lumen. In severe hypoxia, the pulmonary veins are also constricted. Increased alveolar capillary pressure leads to pulmonary edema. Pulmonary hypertension also increases the workload on the right side of the heart, which may cause right ventricular hypertrophy, and in severe cases right-sided heart failure.

Cerebral Edema

The increased capillary permeability leads to cerebral edema, which may be associated with disorientation and ataxia. In severe cases, coma may occur due to herniation of brain through tentorium.

Treatment of High Altitude Illness

The individual who does not tolerate high altitude habitation should be brought to the low altitude at the earliest. The condition immediately improves by descent to lower altitude. If the features of sickness still persist, especially cerebral and pulmonary edema continues, following treatment should start.

1. Diuretics: Diuretics are very useful for the treatment of cerebral and pulmonary edema. Acetazolamide is the preferred diuretic. It inhibits carbonic anhydrase and increases bicarbonate excretion in urine, thus decreases alkali load. It also decreases the formation of CSF.
2. Steroids: Glucocorticoid decreases cerebral edema.
3. Oxygen therapy: Hyperbaric oxygen is very useful if pulmonary edema is present.
4. Nifedipine: Calcium channel blockers like nifedipine help to reduce pulmonary arterial pressure.
5. Other drugs: To improve the general conditions.

CHAPTER SUMMARY

Key Concepts

1. Ascent to high altitude is the commonest physiological cause of hypoxia.
2. Though compensatory mechanisms are activated to deliver O₂ to tissues, acute ascent to a very high altitude may be fatal.

Important To Know (Must Read)

1. In examination, Long Questions are usually not asked from this chapter. But, ‘Describe the physiological changes following acute ascent to high altitude’ may be a question.
2. Physiological changes at high altitude, Acclimatization at high altitude, Monge’s disease, are asked as Short Questions in exam.
3. In Viva, examiner may ask... What are the mechanisms for ventilator acclimatization at high altitude, What are the respiratory changes as acclimatization to high altitude, What are the hematological changes as acclimatization to high altitude, What are the cardiovascular changes as acclimatization to high altitude, What are the tissue changes as acclimatization to high altitude, What are the features of acute mountain sickness, What are the features of chronic mountain sickness, What is the mechanism of pulmonary hypertension and edema, What is the mechanism of cerebral edema, What is the physiological basis of treatment of high altitude illness.
HYPOXIA

Hypoxia is defined as **deficiency of O<sub>2</sub> at the tissue level**. Tissues suffer from hypoxia when supply of adequate O<sub>2</sub> to them is decreased or when they fail to utilize the available O<sub>2</sub>.

**Types of Hypoxia**

Hypoxia is classically divided into 4 categories.

1. Hypoxic hypoxia.
2. Anemic hypoxia.
3. Stagnant hypoxia.
4. Histotoxic hypoxia.

**Hypoxic Hypoxia**

**Definition**

When PO<sub>2</sub> of arterial blood is reduced, the hypoxia is called hypoxic hypoxia.

**Mechanism of hypoxia**

In hypoxic hypoxia, PO<sub>2</sub> of arterial blood is decreased, due to which the delivery of O<sub>2</sub> to the tissue is reduced. This occurs either due to decreased O<sub>2</sub> in the inspired air or due to diseases of the respiratory apparatus that decrease O<sub>2</sub> supply to the tissue.

It is seen in following conditions:

1. **Low PO<sub>2</sub> in the inspired air**, e.g., at high altitude or in closed chambers. As one ascends rapidly to 3000 m (10,000 ft), hypoxia develops due to decline in alveolar PO<sub>2</sub> to about 60 mm Hg. Ascent to more than 5000 m (15,000 ft) produces severe hypoxic symptoms.
2. **Hypoventilation**, e.g. airway obstruction, paralysis of respiratory muscles, and depression of respiratory center, scoliosis etc.
3. **Diffusion defect**, e.g. pulmonary edema, pulmonary fibrosis, lung collapse etc.

The last three mechanisms (2–4), are also called as respiratory hypoxia.

**Anemic Hypoxia**

**Definition**

When PO<sub>2</sub> of arterial blood is normal, but the hemoglobin to carry O<sub>2</sub> is not adequate in amount, anemic hypoxia develops.

**Mechanism of anemia**

Conditions that lead to anemic hypoxia are:

a. Anemia (decreased Hb concentration in blood)

b. Carbon monoxide poisoning

c. Altered Hb, e.g. Methemoglobin

**Mechanism of Hypoxia**

In anemic hypoxia, as the PO<sub>2</sub> of inspired air is normal and the diffusion capacity of lung is normal, the PO<sub>2</sub> of
arterial blood is normal. But, the O₂ supply to the tissue is reduced, as adequate Hb is not available to transport O₂. However, in mild to moderate anemia, usually O₂ supply to the tissue is not severely affected, as there is concomitant increase in 2,3-DPG in the red cells. Such patients develop severe hypoxia during exercise due to their limited ability to deliver O₂ to the tissue.

The affinity of CO for Hb is about 210 times greater than the affinity of O₂. CO combines with hemoglobin to form carboxyhemoglobin or carboxyhemoglobin (COHb). In CO poisoning, release of CO from COHb is very slow due to the high affinity of CO to Hb. Therefore, O₂ cannot bind with hemoglobin. Thus, in CO poisoning, though the hemoglobin content is normal, Hb is not available to deliver O₂ to the tissue. Therefore, CO poisoning is classified as anemic hypoxia. Hb-dissociation curve (for whatever oxy-Hb is available) shifts to left signifying the decreased release of O₂ from Hb.

**Stagnant Hypoxia (Hypoperfusion Hypoxia)**

**Definition**

When hypoxia occurs due to decreased blood flow to the tissues, this is called stagnant hypoxia (stagnation of flow). This is also called hypoperfusion hypoxia, as it causes decreased perfusion of tissues due to stagnation of blood flow. This is sometimes also called ischemic hypoxia.

Conditions associated with stagnant hypoxia:

**Mechanism**

In stagnant hypoxia, O₂ content of arterial blood is normal. Hypoxia occurs due to stagnation of blood in circulation. Therefore, this is also called circulatory hypoxia. It occurs either due to decreased cardiac output or decreased blood flow secondary to some other causes. In chronic or complete stagnant hypoxia, local acidosis develops due to accumulation of the lactate that inhibits cellular metabolism.

Stagnant hypoxia is seen in:
1. Heart failure
2. Shock
3. Vascular obstruction (that causes specific organ hypoxia)

**Histotoxic Hypoxia**

**Definition**

When tissue cannot utilize oxygen inspite of normal O₂ supply, the resulting hypoxia is called histotoxic hypoxia. As tissue is unable to utilize O₂, the venous tension of O₂ is high. Usually, hypoxia occurs in tissue poisoning as produced by cyanides or similar poisons.

**Mechanism**

Mechanism of hypoxia depends on the cause of hypoxia.
1. **Cyanide poisoning:** Cyanide inhibits cytochrome oxidase. Therefore, tissue oxidation is paralyzed.
2. **Diphtheria:** In severe diphtheria, diphtheria toxin inhibits the synthesis of one of the cytochromes and therefore, prevents O₂ utilization.

Cyanide poisoning is treated by **methylene blue or nitrites.** They form methemoglobin, which in turn reacts with cyanide to form **Cyanmethemoglobin,** which is a non-toxic compound. **Hyperbaric O₂ therapy** is also useful in cyanide poisoning.

**Effects of Hypoxia**

Hypoxia mainly affects CNS, especially the higher centers. Effects of hypoxia depend on the severity, duration and type of hypoxia.

**Acute and Subacute Hypoxias**

*Features of acute hypoxia resemble acute alcoholism.* Impaired judgment and motor incoordination are major manifestations of acute hypoxia.

1. If hypoxia persists for a longer period, the features are fatigue, drowsiness, nausea, headache, hyperventilation and dyspnea, apathy, inattentiveness, delayed reaction time, reduced work capacity, confusion and disorientation. In severe hypoxia, hypertension occurs.
2. In severe and prolonged hypoxia, brainstem is depressed and death results from respiratory failure.
3. **Anaerobic glycolysis** results in formation of more lactic acid that leads to **metabolic acidosis.** Stimulation of chemoreceptors by hypoxia produces hyperventilation and causes **respiratory alkalosis.** However, combined with metabolic acidosis, serum bicarbonate level decreases.

**Effects on Cells**

Transcription factors known as **hypoxia inducible factors (HIFs)** are produced by hypoxia.

1. HIFs have α and β subunits. Normally, in the presence of adequate oxygen in the tissues, α subunits are rapidly removed from the cells.
2. In hypoxic conditions, dimerization of α and β subunits occurs in the cells. The α-β dimers cause induction of genes that produce erythropoietin and angiogenic factors.
3. This is one of the causes of angiogenesis or neovascularization in hypoxic tissues.

**Effects on Brain**

Brain tissue is highly sensitive to hypoxia.

1. Sudden fall in inspired P O₂ to less than 20 mm Hg, causes loss of consciousness in about 15 seconds. If hypoxia continues, death can occur in 4 to 5 minutes.
2. This can typically occur in sudden drop in cabin pressure in a plane flying above 16,000 meter.
3. However, hypoxia of lower intensities, cause the symptoms similar to that of acute alcohol intoxication such as disorientation, impaired judgment, headache, drowsiness etc.
Oxygen therapy is indicated in hypoxia. It is very useful in acute and severe hypoxia, especially when hypoxia is associated with dyspnea.

**Methods**

Oxygen is administered by following methods.

1. **Using oxygen tent**: It is very useful in children, as they usually do not tolerate mask or cannula. It is also useful in administering hyperbaric oxygen.
2. **Using oxygen mask**: Oxygen enters the mask at a higher velocity so that oxygen is drawn through the holes in the mask.
3. **Mechanical ventilator**: When patient is semiconscious or comatose, oxygen is administered from a mechanical ventilator through an endotracheal or tracheostomy tube.
4. **Through an intranasal tube**: A cannula is inserted into the nostril, which is connected to the oxygen cylinder. Administration of oxygen through cannula is useful in conditions in which hypoxic drive is essential to drive ventilation, as oxygen content of inspired air never reaches 100% by this method.

**O₂ Therapy in Different Forms of Hypoxia**

**Hypoxic Hypoxia**

As hypoxia is due to decreased PO₂ of arterial blood, oxygen therapy is very useful in hypoxic hypoxia. Especially, it is very essential for hypoxia caused by low PO₂ in the inspired air, hypoventilation and impaired diffusion in the lung.

1. In such conditions, administration of O₂ increases the pressure gradient between alveoli and the blood that facilitates O₂ entry into the blood.
2. However, O₂ therapy is not beneficial in hypoxic hypoxia due to A-V shunts, as admixture occurs after oxygenation in the lungs.
results in premature retinopathy. Visual defects also occur due to formation of opaque vascular tissue in the eye. If given for a longer period, 100% $O_2$ causes **bronchopulmonary dysplasia** and lung cysts, especially in infants treated for respiratory distress syndrome.

### Mechanism of Toxicity

With pure $O_2$ therapy, oxidizing free radicals like super oxide anion ($O_2^-$), and hydrogen peroxide ($H_2O_2$) accumulate in the body in excess amounts. They oxidize the polyunsaturated fatty acids and destroy the cellular enzymes. As a result, toxic effects due to $O_2$ therapy develop.

<table>
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<th>CHAPTER SUMMARY</th>
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<td><strong>Key Concepts</strong></td>
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<td>1. Hypoxia is a stimulus for erythropoietin production. Therefore, physiological hypoxia such as exposure to high altitude may sometimes be good for health.</td>
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<td>2. CO poisoning is an example of anemic hypoxia, as free Hb is not available in this condition.</td>
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**Important to Know (Must Read)**

1. In examination, ‘Describe the different types of hypoxia and add a note on 100% $O_2$ therapy’ may be asked as a **Long Question**.
2. Hypoxic hypoxia, anemic hypoxia, stagnant hypoxia, histotoxic hypoxia, oxygen therapy, effect of 100% $O_2$ therapy, are asked as **Short Questions** in exam.
3. In **Viva**, examiner may ask… Classify hypoxia, Define hypoxic hypoxia and state its mechanism, what are the conditions in which hypoxic hypoxia is seen, Define anemic hypoxia and state its mechanism, what are the conditions in which anemic hypoxia is seen, define stagnant hypoxia and state its mechanism, what are the conditions in which stagnant hypoxia is seen, define histotoxic hypoxia and state its mechanism, what are the conditions in which histotoxic hypoxia is seen, what are the effects of hypoxia on cells and brain, what are the methods of $O_2$ therapy, what is hyperbaric oxygen therapy and what is its use, what is the effect of 100% $O_2$ therapy on CNS, respiratory system and special sense, what is the mechanism of toxicity following 100% $O_2$ therapy.
CAUSES AND TYPES OF HAZARDS

At sea level, the atmospheric pressure is 760 mmHg (1 atmosphere) and the pressure in the lungs is equal to this pressure. When the individual dives into the sea, he is exposed to a high pressure, which is proportional to the depth to which he dives.

1. For every descent of 33 feet depth (10 meters), there is increase in pressure by 1 atmosphere. Thus, at 100 feet depth, the pressure is about 4 (3+1) atmospheres. This high pressure in the deep sea causes some hazards.

2. People using caisson (the chamber in which they work under-water) are also exposed to similar high pressure-hazards as pressure in caissons is increased to keep the water out of the chamber.

3. Also, at deeper region of the sea, air or mixtures are inhaled at high pressure to equalize the external pressure on the thorax and abdomen so that adequate chest expansion is maintained during respiration.

4. The inhaled gas at high pressure dissolves in the body fluids in large quantities and causes certain hazards.

   The hazards of deep sea diving are:
   1. Nitrogen narcosis
   2. High pressure nervous syndrome
   3. Acute oxygen toxicity
   4. Dysbarism
   5. Air embolism

Nitrogen Narcosis

During deep sea diving, gases are inhaled at high pressures.

1. Inhalation of 100% O₂ at high pressure causes CNS depression.
2. Therefore, normally a mixture of 20% O₂ and 80% N₂ is inhaled at high partial pressures. At high pressure, N₂ produces narcotic effects. Up to 100 feet (4 atmosphere) the dissolved N₂ produces euphoria.
3. However, narcotic effects of N₂ start at about 120 feet and become severe at or below 250 feet (8.5 atmospheres).
4. The features are similar to alcohol intoxication. Though the manual agility remains unaffected, intellectual functions are altered. The narcotic effects of N₂ are due to its anesthetic effect.

High-Pressure Nervous Syndrome

As dissolved N₂ at deeper regions of sea produces narcotic effects, a mixture of helium and O₂ is preferred for following reasons.

1. The narcotic effect of helium is one-fifth of that of N₂.
2. Resistance to air flow is less as helium is lighter than N₂ (density of helium is 2, whereas density of nitrogen is 14). Thus, the work of breathing is less with helium mixture.
3. Helium is less soluble in body fluids.
Inhalation of helium mixture during deep dives causes high-pressure nervous syndrome (HPNS).
1. HPNS occurs due to the anesthetic effects of helium at high pressure.
2. Helium like xenon, krypton, neon etc, is inert at atmospheric pressures and anesthetic at high pressure. The anesthetic effect is due to their lipid solubility and direct effect on membrane of neurons.
3. HPNS is characterized by tremors, drowsiness and incoordination. The activity in EEG is depressed and manual dexterity is impaired.

**Acute O**₂ **Toxicity**

Gases are inhaled under high pressure to maintain chest and abdominal expansion during deep sea diving. CO₂ is usually removed from the gas mixture, as its accumulation in the body is fatally toxic. However, breathing 100% O₂ at a higher-pressure is also toxic.
1. Acute oxygen toxicity occurs mainly due to the effects of high-pressure 100% oxygen on CNS.
2. The features of oxygen toxicity are nausea, dizziness, irritability, disorientation, disturbed vision, and convulsions. Pulmonary damage also occurs.
3. Therefore, in gas mixtures to be inhaled at high pressure the concentration of O₂ is reduced to about 20% to prevent its toxicity.

**Dysbarism (Caissons Disease)**

Dysbarism or decompression sickness is a condition, which occurs when the subjects exposed to high atmospheric pressures are suddenly brought to low atmospheric pressures. For example, deep-sea divers or workers in caisson (caisson is a watertight chamber used for performing construction works under water) returning to sea surface rapidly from deep sea level. Rapid ascent from sea level to about 9000m in an unpressurized cabin of an airplane can also cause decompression sickness.

Pressure at sea level is 1 atmosphere. Pressure increases by 1 atmosphere for every 10 meter depth of water. Thus, the pressure at 10 meter depth: 1 + 1 atmosphere, at 20 meter depth: 2 + 1 atmosphere, at 50 meter depth: 5 + 1 atmosphere, and so on. Deep-sea divers usually breathe 80% N₂ from the gas mixtures.
1. When the diver descends into the deep sea, N₂ dissolves in the body fluids, which is proportional to the depth of descent. N₂ is lipid soluble. Therefore, it also dissolves in the lipid structures of the tissues.
2. If the return to the surface is slow, the gases that come out of the solution diffuse into the blood and are eliminated by the lungs.
3. If the return (decompression) is rapid, the escape of gases from the solution is quick and bubbles are formed in tissues and blood. This causes damage to the tissues.
4. Bubbles in the tissues cause severe pain in the tissues. Pain occurs mainly in muscles and joints. This is called decompression sickness or caisson disease.
5. In severe cases, bubbles in the blood vessels decrease blood flow to brain and spinal cord resulting in sensory and motor deficits in the form of paresthesia, motor weakness paralysis and respiratory failure.
6. Bubbles in the pulmonary vessels produce dyspnea and a feeling of respiratory compression, and bubbles in the coronary vessels produce myocardial ischemia.
7. Features of decompression sickness usually appear within half an hour of coming to the surface.

**Note:** The important factor contributing to the severity of decompression sickness is not the absolute decrease, but the percentage decrease in pressure. A rapid decrease upto 50% of the original pressure is safe but decrease below 45% is harmful.

**Prevention**

Decompression sickness can be prevented by slow recompression (gradual ascent to the sea level). Reduction in the pressure should not exceed 50%.

**Treatment**

As soon as the symptoms develop, the subject should be recompressed immediately in a pressurized cabin. Quick recompression should be followed by slow decompression. Hyperbaric oxygen is also helpful. Though recovery is usually complete, some residual neural deficits may persist.

**Air Embolism**

When a diver breathing from a tank at increased pressure suddenly ascends to the surface holding his breath, gas in the lungs expand rapidly and rupture pulmonary veins. Air escapes into the circulation and causes air embolism.
1. Also, sudden reduction in external pressure from atmospheric to subatmospheric level can cause rapid expansion of gas in the lungs and produce air embolism, which typically occurs when a pressurized cabin of an aeroplane or a rocket ruptures at high altitude.
2. Air embolism can also occur during a rapid ascent from shallow depth of 5 m. Therefore, to prevent this, a diver can take breath at the surface and then dive into the water holding the breath and come out of it holding the same breath.

**SCUBA**

Self-contained under-water breathing apparatus (SCUBA) is a tank and valve system, used by deep-sea divers.
1. It consists of cylinders containing mixture of compressed helium and oxygen.
2. The valve arrangement helps the subject to inhale and exhale gases. O₂ and helium mixtures are frequently used as they are less toxic during deep sea diving.
CHAPTER SUMMARY

KEY CONCEPTS
1. From the deep sea divers should ascent slowly to the surface.
2. Quick recompression followed by decompression is the treatment of caisson’s disease. Hyperbaric oxygen helps.

IMPORTANT TO KNOW (MUST READ)
1. In examination, Long Questions are usually not asked from this chapter.
2. Nitrogen narcosis, High-pressure nervous syndrome, Acute \( O_2 \) toxicity, Caisson’s disease, SCUBA, Decompression sickness, are asked as Short Questions in exam.
3. In Viva, examiner may ask..... What are the hazards of deep sea diving, what is nitrogen narcosis, what are its features, what is high-pressure nervous syndrome, what are its features, what is acute \( O_2 \) toxicity, what are its features, what is Caisson’s disease, what are its features, what are the modes of prevention and treatment of Caisson’s disease, what is air embolism, what are its features, what is SCUBA.
Asphyxia
Asphyxia is a condition in which hypoxia is combined with hypercapnia. Usually, asphyxia develops due to mechanical obstruction of airway as occurs in suffocation, strangulation, drowning, foreign body impaction in the trachea or larynx, and traumatic compression of the chest.

Stages of Asphyxia
Asphyxia is divided into three stages:
1. Stage of exaggerated breathing
2. Stage of convulsion
3. Stage of exhaustion and collapse

Stage of Exaggerated Breathing
Hypercapnia and hypoxia stimulate respiratory centers strongly. Therefore, breathing becomes deep and rapid. Participation of accessory muscles of respiration becomes prominent. Patient becomes severely dyspneic and looks intensely anxious. Cyanosis develops and eyes become prominent. This stage lasts for about 1 or 2 minutes.

Stage of Convulsion
The respiratory efforts become violent. Generalized muscular convulsions occur. Increased sympathetic discharge and catecholamine secretion, cause tachycardia and rise in blood pressure. This phase lasts for about a minute. Consciousness of the patient may be lost.

Stage of Exhaustion and Collapse
Continuation of hypoxia to the vital centers in the brain results in suppression of cardiorespiratory activities. Convulsions cease abruptly and respirations become gasping. Respiratory efforts cease and blood pressure falls. The pupils are widely dilated and pulse becomes feeble. Reflexes are abolished. The pause between respiratory gasps becomes longer, until finally the patient takes his last breath. This stage lasts for about 5 minutes.

Hypercapnia
Definition and Causes
Hypercapnia is the retention of CO₂ in the body. CO₂ is a major product of metabolism. Therefore, conditions of hypermetabolic states such as fever and excess carbohydrate intake generally produce hypercapnia.

Causes
1. Hypercapnia is common in conditions of hypoventilation in which either the ventilation is depressed as seen in respiratory failure, brain injury, narcotic poisoning etc. or the ventilation is inadequate as occurs in myocardial failure, polyneuropathies, diseases of the chest wall etc.
2. Hypercapnia usually does not occur in pulmonary fibrosis, as CO₂ is more soluble than O₂. However, severe destruction or thickening of alveolar-capillary membrane as seen emphysema or fibrosis can directly impair gas exchange and produce hypercapnia and hypoxia.
3. Hypoxia with normocapnia is termed as type I respiratory failure and hypoxia with hypercapnia is called as type II respiratory failure.
4. In fever, with rise in body temperature by 1°C, CO₂ production increases by 13%. However, respiration is also stimulated in fever and excess CO₂ is removed from the body. Therefore, if ventilation is compromised in such situations, CO₂ accumulates in the body and hypercapnia results.
5. In high carbohydrate intake, production of CO₂ is increased due to increase in respiratory quotient.

Effects of Hypercapnia
Hypercapnia stimulates respiration.
1. However, if severe and prolonged, hypercapnia leads to respiratory depression. The patient develops decreased sensory perceptions and confusion.
2. Finally, this leads to coma and respiratory depression that further increases the level of PCO₂.
3. Profound hypercapnia produces respiratory acidosis, during which plasma bicarbonate may exceed 40 mEq/L.
4. This occurs as HCO₃⁻ reabsorption from kidney tubule becomes more than its excretion, that increases plasma HCO₃⁻ and tries to compensate acidosis to some extent.

Hypocapnia

Causes
Hypocapnia is CO₂ washout from the body that results from hyperventilation. As hyperventilation increases oxygen intake, there is simultaneous increase in PO₂.
1. Temporary hyperventilation occurs on sudden exposure to hot environment. Hyperventilation is common in neurotic patients.
2. Another example is voluntary hyperventilation (performed for experiments) in which considerable hypocapnia (PCO₂ may fall as low as 15 mm Hg) is associated with significant increase in PO₂ in arterial blood, which may rise up to 140 mm Hg.

Effects
1. Hypocapnia produces vasoconstriction and reduces cerebral blood flow by more than 30%.
2. Cerebral ischemia may cause dizziness, light headedness and sensory abnormalities mainly in the form of paresthesia.
3. Hypocapnia causes depression of vasomotor and respiratory centers.
4. Cardiac output is decreased due to decreased coronary blood flow.
5. Features may also occur due to associated respiratory alkalosis that results from hyperventilation. Plasma bicarbonate level is decreased.
6. However, due to inhibition of secretion of acid in the renal tubule, HCO₃⁻ reabsorption is decreased. Features of hypocalcemic tetany such as Chvostek sign and carpopedal spasm may develop due to decreased ionic calcium level.

Drowning
Drowning is suffocation caused by immersion in water.
1. In fresh-water drowning, water enters the lungs and from lungs water is rapidly transferred to blood causing faster hemolysis and hemolysis.
2. In seawater drowning, hypertonic water in the lungs draws fluid from vascular space into the lung that causes hemoconcentration. However, in few cases of drowning, first grasp of water into the respiratory tract causes immediate reflex laryngospasm and water does not enter the lungs. The patient dies of asphyxia.

Respiratory Acidosis
Any condition that decreases the removal of carbon dioxide from the body through lungs causes increase in PCO₂. This leads to respiratory acidosis.
1. Depression of the respiratory centers as produced by narcotics and barbiturates causes respiratory acidosis.
2. Diseases that affect respiratory apparatus like chronic obstructive lung diseases, pneumothorax, pleural effusion, chest wall deformities etc., and diseases that impair diffusion of gases through alveolar-capillary membrane like pulmonary fibrosis, pulmonary edema etc. can also produce respiratory acidosis.
3. The increase in PCO₂ stimulates respiratory centers and increase rate and depth of respiration provided the primary defect is not in the respiratory center. Elimination of CO₂ through lungs restores blood pH.

Respiratory Alkalosis
The basic cause of respiratory alkalosis is excess elimination of acid via respiratory tract. The decrease in H⁺ concentration is due to hyperventilation that decreases PCO₂. A physiological cause of respiratory alkalosis is ascension to high altitude.

ABNORMAL RESPIRATIONS

Periodic Breathing
Cyclical repetition of apnea and shallow breathing (like hyperpnea) in normal individuals is called periodic breathing. This is typically seen following voluntary hyperventilation performed for 2 to 3 min. The cycles of apnea-hyperpnea continue for sometime.
Chapter 112: Respiration in Abnormal Conditions and Abnormal Respirations

1. Apnea occurs due to removal of CO\(_2\) during hyperventilation that removes the CO\(_2\) drive on ventilation.
2. During the period of apnea, PCO\(_2\) accumulates and PO\(_2\) falls in the blood that stimulates ventilation via chemoreceptor stimulation.
3. This initiates respiration and PO\(_2\) returns to normal.
4. The elimination of hypoxic drive stops breathing (apnea), which again leads to accumulation of PCO\(_2\) and produces hyperventilation.
5. Thus cycles of apnea-hyperpnea continue till PCO\(_2\) gradually returns to normal (Fig. 112.1B). Periodic breathing in abnormal condition is called Cheyne-Stokes respiration.

**Cheyne-Stokes Respiration**

Periodic breathing that occurs in diseases and abnormal condition is called Cheyne-Stokes respiration. Though it occurs in deep sleep in some normal persons, it is more common in congestive cardiac failure, uremia and brain diseases.

1. The patients have increased sensitivity to CO\(_2\) due to disruption of neural pathways.
2. Accumulation of CO\(_2\) causes hyperventilation that lowers PCO\(_2\).
3. Decreased PCO\(_2\) removes the CO\(_2\)-drive on ventilation and produces apnea, which consequently increases the PCO\(_2\) again.
4. The increased sensitivity of respiratory mechanism to PCO\(_2\) produces hyperventilation and the cycle continues.

**Causes**

Cheyne-Stokes respiration is seen in:

1. Premature infants.
2. Unacclimatized persons at high altitude.
3. During deep sleep in some people.
4. Heart failure.
5. Renal failure.

In a normal person periodic breathing is prevented because respiratory center alters the depth of respiration in such a way that arterial PCO\(_2\) does not drop below critical value. If arterial PCO\(_2\) goes below critical level, apnea appears.

**Other Abnormal Breathings**

**Kussmaul Breathing**

The pattern of respiration seen in diabetic ketoacidosis is called Kussmaul breathing (described by Kussmaul).

1. Accumulation of metabolic acids such as acetoacetic acid and β-hydroxy butyric acid produces metabolic acidosis that stimulates respiratory centers.
2. This leads to rapid and deep respiration or ‘air hunger’ as described by Kussmaul.

**Biot’s Respiration**

In this type of abnormal breathing, 3 to 4 cycles of normal respiration is followed by abrupt onset of apnea (Fig. 112.1C). Before apnea, usually deep gasps occur. It is seen in meningitis, diseases affecting medulla of the brain, increased intra cranial pressure, morphine poisoning and damage to the brainstem.

**Sleep Apnea Syndrome**

In adult sleep apnea syndrome, marked loss of tone of pharyngeal muscles occurs during REM sleep causing obstruction of airway during inspiration. This produces apnea.

1. The person wakes up and breathes normally for sometimes and sleeps again to have another bout of apnea.
2. Thus repeated apnea occurs during sleep.
3. These people develop morning headache and fatigue due to frequent apnea in the night. Sleeplessness occurs during the day.

**Sudden Infant Death Syndrome**

Sudden infant death syndrome (SIDS) is believed to be a type of sleep apnea in premature infants. Loss of rhythmic activity of the respiratory center leads to apnea in sleep and causes death of the infant. The exact cause is not known though it is commonly seen in premature babies with cardiac arrhythmias complicating long QT syndrome. It is also common in mothers who are chronic smokers.

**Hysteric Hyperpnea**

Spontaneous hyperpnea of sudden onset occurs in patients with hysteria. This results in washing out of CO\(_2\) leading to
alkalosis and convulsions. This is treated by allowing the patient to breath into a facial mask till he recovers.

**Ondine’s Curse**

Some patients with brain injury lose their automatic control on breathing but retain voluntary breathing. Such patients require respiratory assistance intermittently. As depicted in *German mythology*, the unfaithful lover of Ondine, a water nymph was cursed by the king of water nymphs that he will lack automatic breathing and will live till he breathes voluntarily. Ultimately the disloyal lover slept after a long period of sleeplessness and died as he lost the voluntary breathing.

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**CHAPTER SUMMARY**

**Key Concepts**

1. Though voluntary hyperventilation can produce periodic breathing, its manifestation in diseases indicates severe metabolic problems.
2. Kussmaul’s breathing is seen in diabetic ketoacidosis, & Biot’s respiration indicated injury to respiratory cents.

**Important to Know (Must Read)**

1. Long Questions are not asked.
2. Asphyxia, Hypercapnia, Hypocapnia, Periodic breathing, Cheyne-Stokes respiration, may be asked as Short Questions in exam.
3. In Viva, examiner may ask… What is asphyxia, what are its stages, what are the features of asphyxia in different stages, Define hypercapnia and state its causes, Define hypercapnia and state its causes, what is respiratory acidosis, what is respiratory alkalosis, what is periodic breathing, what is Cheyne-Stokes respiration, in what conditions is it seen, what is Kussmaul breathing, in what conditions is it seen, what is Biots respiration, in what conditions is it seen, what is sleep apnea syndrome, what is sudden infant death syndrome, what is hysteric hypernea, what is Ondines curse, in what conditions is it seen.
ARTIFICIAL RESPIRATION

In respiratory arrest, artificial ventilation is given to the subject till normal rhythmic respiration is restored. Brain cannot survive long in the absence of O$_2$ supply. Hence, there is a need of giving artificial ventilation immediately. Artificial respiration is useful when functions of heart and dynamics of circulation are normal.

Methods of Artificial Ventilation

Two types of artificial ventilatory methods are practiced: the instrumental methods, and the manual methods.

Instrumental (Mechanical) Methods

When artificial ventilation is required for longer period, instrumental methods are used. There are two types of mechanical methods: positive pressure and negative pressure methods.

Positive Pressure Method

This is usually used in operation theaters during surgery. In this method, the lung is inflated by pumping air or air-O$_2$ mixture at positive pressure. The disadvantage is that it impairs venous return.

Negative Pressure Method

This is achieved by alternate compression and relaxing of chest wall. Some of the negative pressure methods are:


Drinker’s Method

(Iron lung chamber method): The apparatus consists of an airtight chamber and the pressure inside the chamber is alternatively reduced and increased. The patient is placed inside the chamber with his neck and head outside. When the pressure inside is increased, expiration takes place due to compression and when the pressure is reduced below atmospheric pressure, inspiration takes place.

Braggpaul Method

This consists of a hollow elastic rubber bag, which is wrapped round the chest. When a pump inflates the bag, the chest is compressed and air is expelled out. When the pressure is released the chest passively enlarges and air is drawn in.

Boyle’s Apparatus

In the hospitals, this is used for giving artificial ventilation. This is an automatic instrument in which, rate and depth of ventilation and composition of air inhaled can be varied.

Manual Methods

Though there are many manual methods, Holger-Neilson method (back pressure–arm lift method), mouth-to-mouth breathing and mouth-to-nose breathing are usually practiced (described below).
CARDIOPULMONARY RESUSCITATION

The supportive and specific treatment given immediately to patients, in whom for some reasons the cardiac and ventilatory activities have stopped, is called cardiopulmonary resuscitation (CPR). CPR requires adequate life saving procedures that are administered by a well-trained and experienced team.

Indications

CPR is indicated in conditions of cardiorespiratory arrest. The common situations that can lead to cardiorespiratory arrest include:
1. Primary cardiac arrhythmias
2. Arrhythmias associated with myocardial infarction
3. Acute and healed myocardial infarction
4. Myocarditis
5. Cardiomyopathy (dilated and hypertrophied)
6. Electric shock
7. Poisoning
8. Acute psychological trauma
9. Drowning
10. Pulmonary embolism
11. Muscular relaxation during surgery
12. Anaphylactic shock
13. Head injury
14. Accident
15. Cardiac surgery
16. Cardiac tamponade
17. Acute pulmonary edema
18. Airway obstruction
19. Chronic lung disease (rare)

The common mechanisms of cardiac arrest or cessation of heart functions are cardiac asystole and ventricular fibrillation. Features of cardiac arrest are the absence of heartbeats and pulsation of large arteries. Pallor, cyanosis and gasping movements followed by total arrest of respiration and dilated pupils are the signs.

Objectives of CPR

1. To provide adequate pulmonary ventilation so that partial pressure of oxygen in the arterial blood is maintained.
2. To initiate and facilitate pumping of the heart so that effective circulation is maintained.

Procedure

Steps of CPR

CPR is carried out in three steps.
1. Initial evaluation and basic life support
2. Advanced life support
3. Post-resuscitation measures

Initial Evaluation and Basic Life Support

Initial Evaluation

This includes assessment of state of consciousness, respiratory movements, skin color and the state of arterial pulses. If the cardiorespiratory arrest is due to foreign body impaction in the respiratory tract, Heimlich maneuver is performed to dislodge the obstructing body.

Basic Life Support

This should start immediately. A clear airway and initiation of breathing are essential parts of successful resuscitative measures. Unless adequate ventilation is achieved, attempts to restore circulation become futile. The steps are:
1. Assess the responsiveness of the patient by gently shaking the subject.
2. Position the patient on a firm and flat surface.
3. Open the mouth of the subject and remove vomitus, blood, mucus or debris if present in the oral cavity.
4. Extend the neck of the subject to increase the patency of the airway. To extend the neck, place the palm of your one hand on the patient’s forehead and apply firm pressure to tilt the head backward. At the same time, place the palm of the other hand under the chin to support it (head tilt-chin lift maneuver). This raises the tongue away from the spine and opens the airway.

To ventilate the lungs of the patient, perform any of the following three maneuvers. Mouth to mouth breathing is performed in adults and mouth to nose breathing is preferred in infants and children. Holger Nielson method is usually not performed unless there is a respiratory problem as seen in drowning.

5. Mouth to mouth respiration:
   - Clear the airway
   - Extend the neck
   - Close the nostrils (of the patient) by pinching with the thumb and the index finger of the right hand
   - Take a deep breath
   - Apply your mouth close to the subject’s mouth and exhale forcefully into the his mouth
   - Look for chest expansion and abdominal distension
   - Repeat and maintain the breathing at a rate of 10-15 per minute.

Mouth to nose respiration: Usually, it is performed for children. The steps are same as those of mouth-to-mouth respiration except that the mouth of the patient is closed and the rescuer breathes into the nostrils of the subject.

Holger Nielson method: (Manual manipulation of the thorax by back pressure-arm lift method):

- Lay down the patient in prone position.
- Abduct the arms at the shoulders and flex the elbows.
- Turn the head to one side testing on the hands.
- Kneel down with one knee near the patient’s arm and straighten yourself to raise the subject’s arm until resistance is felt (Figs. 113.1A and B). During this maneuver, the thorax of the patient expands and the intrathoracic pressure drops. Thus, inspiration takes place.
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- Then, gently drop the patient’s arm
- Place your hands with fingers spread apart on the back of the subject in the mid-axillary space, and slightly compress to produce expiration (Figs. 113.1C and D).
- Repeat the whole cycle 10-12 times per minute.
  1. Palpate the carotid pulse. Palpation for at least 10 seconds is recommended to ensure that slow, irregular, or very weak pulses are not missed.
  2. **External cardiac massage:** If the carotid pulse is not felt, perform external cardiac massage. If the patient is in bed, place a hard board under the patient. The hands are then positioned about 3 cm above the xiphoid process and to the left with the shoulders of the rescuer vertically above the chest of the subject. With the heel of the hand, and with fingers off the chest, the sternum is compressed 4-5 cm thrusting straight down towards the spine. The recommended compression rate is 80-100 per minute. The rescuer responsible for airway management should assess the adequacy of compression by periodically palpating for the carotid pulse. The **compression-ventilation ratio is 5:1**.
  3. If pulses return, ventilation should be continued as required.

**Advanced Life Support**

Advanced life support comprises of primary and adjunctive therapies.

**Primary Therapies**

Primary therapy of advanced life support includes:
  1. Defibrillation
  2. Airway management and oxygen therapy
    - **Defibrillation:** This is one of the most important modalities of treatment of CPR. It should be started as early as possible. The time between the onsets of the arrest to the successful defibrillation is the major determinant of survival in cardiac arrest due to ventricular fibrillation. A fibrillating heart cannot pump blood, as effective contractions do not occur. Defibrillation converts fibrillation into flutter or normal rhythm so that effective ventricular contractions occur and heart pumps blood. Adrenaline and sodium bicarbonate are administered intravenously.

    **Airway management and oxygen therapy:** 100% oxygen should be administered and endotracheal intubation should be carried out by a qualified individual as soon as possible. But, the basic life support should not be delayed or interrupted for more than 30 seconds for intubation.

**Adjunctive Therapies**

Adjunctive therapies include following.
  1. **Self-induced cough:** When the arrest is detected before loss of consciousness, self-induced vigorous coughing can produce minimum blood flow to the brain temporarily until definitive treatment is initiated.
  2. **Precordial thump:** A quickly applied solitary precordial thump may convert ventricular fibrillation or asystole to a rhythm.
  3. **Atropine sulphates:** Atropine sulphate (0.5 mg) is injected intravenously every 5 minutes for the treatment of bradycardia. It may also help in the treatment of cardiac asystole.
  4. **Sodium bicarbonate:** It is given as 1 mEq/kg intravenously every 10 minutes for the treatment of cardiac arrest due to hyperkalemia or acidosis.
  5. **Pacemakers:** It helps in patients with problems of abnormal impulse formation. Pacing is carried out early during resuscitation in conditions of refractory bradyarrhythmias or persistent asystole.

There are different methods to assist ventilation in CPR like Sylvester-Brosche Method, Drinker’s Method, Paul-Bunne method and Rocking method. But these methods are not routinely followed. Despite resuscitative efforts, a patient in cardiac arrest may not recover and regain spontaneous ventilation and circulation. Persistent deep unconsciousness and absence of respiration, reflex response or papillary reaction to light suggest cerebral death. In these conditions, resuscitative efforts are unproductive.

**Post-Resuscitation Care**

In advanced hospital set up, respiratory support is usually not required for a longer time and hemodynamics stabilize promptly. Post-resuscitation measures aim at treating the underlying disease that produced cardiopulmonary arrest. Usually in cardiac arrest for longer period, hypoxic encephalopathy and cerebral edema occur. Recently, induction of hypothermia is practiced to reduce metabolic demands and cerebral edema.
CHAPTER SUMMARY

**Key Concepts**

1. External cardiac massage of CPR should be done by experts who have the training of it. Otherwise it will be ineffective and may fracture ribs.
2. Adjunctive therapy for CPR and assisted ventilation should start simultaneously.

**Important to Know (Must Read)**

1. In examination, **Long Questions** are usually not asked from this chapter.
2. Cardiopulmonary resuscitation (CPR), Holger-Nielson method of artificial respiration, Negative pressure methods of CPR, may be asked as **Short Questions** in exam.
3. In **Viva**, examiner may ask are the mechanical methods of artificial ventilation, What are the manual methods of artificial ventilation, What is CPR, What are the indications of CPR, What are the objectives and steps of CPR, What are the components of advanced life support, What is defibrillation, What is airway management and oxygen therapy, What are the adjunct therapies, What is post-resuscitation care.
The primary function of the lung is to maintain tension of oxygen and carbon dioxide of the arterial blood within the normal range.

1. This is achieved by uptake of oxygen from the inspired air and giving up of carbon dioxide in the expired air. The fundamental mechanisms involved in attaining this goal are ventilation, diffusion and perfusion.

2. The assessment of the type and degree of functional impairment caused by various diseases may be as important in the diagnosis, management, and prognosis of some of the pulmonary disorders as a detailed history with roentgenographic, bacteriological, and pathological examination.

3. Though some of the pulmonary function tests (PFTs) require sophisticated equipment and procedures, most of the tests are simple, non-invasive and inexpensive.

Classification of PFTs

Classification of PFTs is based on the lung functions assessed by the tests. Accordingly, various tests are grouped under three broad categories: tests that assess ventilation, tests that assess gas exchange in the lungs and tests that assess pulmonary circulation.

1. Tests that assess ventilation
   a. Lung volumes
      i. Tidal volume
      ii. Inspiratory reserve volume
      iii. Expiratory reserve volume
      iv. Residual volume
   b. Lung capacities
      i. Vital capacity
      ii. Inspiratory capacity
      iii. Functional residual capacity
      iv. Total lung capacity
   c. Mechanics of breathing
      i. Timed vital capacity
      ii. Maximum mid-expiratory flow rate
      iii. Maximum voluntary ventilation
      iv. Peak expiratory flow rate
      v. Flow-volume curve
      vi. Closing volume
      vii. Maximal expiratory pressure
      viii. Maximal inspiratory pressure

2. Tests that assess gas exchange in lungs
   a. Assessment of ventilation-perfusion matching
   b. Assessment of diffusing capacity
   c. Determination of arterial blood gasses

3. Assessment of pulmonary blood flow and pressure
Uses of PFTs

PFTs help in:
1. Diagnosis of respiratory dysfunctions.
2. Monitoring the progress of the disease.
4. Assessing the health status of the person prior to recruitment to military or police services.
5. Determining the efficacy of physical training.
6. Studying the prevalence of respiratory diseases in the community or respiratory industrial hazards.
7. Assessing the respiratory fitness of the patients for general anesthesia prior to surgery.
8. Finalizing medico-legal cases to decide fitness or degree of compensation.

Normal Values of PFTs

Normal values of various parameters of PFTs in adult male subjects:

- TV : 500 mL
- IRV : 3000 mL
- ERV : 1100 mL
- FVC : 4600 mL
- FEV₁ : > 80% of FVC
- PEFR : 400 to 600 L/min
- RMV : 6000 mL
- MVV : 125 to 170 L/min
- Breathing reserve (MVV – RMV) : 115 to 160 L/min

Dyspneic index : > 90% (< 60% indicates dyspnea at rest).

Values vary greatly for race, age, gender, height, build and training of the individual. All lung volumes and capacities are about 15 to 25% less in females. The values are usually more in athletes and tall persons and less in non-athletes and poorly-built persons.

Assessment of Ventilation

The process of ventilation is concerned with movement of air in and out of alveoli, the gas exchanging units of the lung. Its adequacy depends on the lung volumes and capacities and the mechanics of breathing. The various lung volumes and capacities are indices of static dimensions of the lung at various stages of inflation. The mechanics of breathing deal with static as well as dynamic mechanical properties of the respiratory apparatus.

Lung Capacities

1. **Vital capacity**: The maximum volume of air that can be expired after a maximal inspiratory effort is called the vital capacity (VC). The normal value of VC in men is about 4 liters and in women is about 3 liters (Refer Fig. 104.7, Chapter 104).
2. **Forced vital capacity (FVC)**: The total volume expired with greatest force and speed after a maximal inspiration is the FVC. FVC differs very little from VC in the normal subject, but it is proportionately more reduced when there is airway obstruction with air trapping.
3. **Inspiratory capacity**: This is the volume of gas that can be inspired from the resting expiratory level. This is the IRV+TV.
4. **Functional residual capacity**: This is the volume of air remaining in lungs at the end of resting expiratory level.
5. **Total lung capacity**: The volume of air present in the lungs at the end of maximal inspiration is the total lung capacity (TLC). The normal value in men is 6 liters and in women is 4.2 liters.

Mechanics of Breathing

In carrying out the process of ventilation certain forces are required to overcome the elastic recoil of the lung and thorax, the non-elastic resistance caused by movement of tissues during breathing, and also the airway resistance.

Compliance

The elastic recoil of the lung, which is measured under static condition is called compliance. The compliance is defined as the volume change per unit transpulmonary pressure difference between the esophageal or intrapleural pressure and the mouth pressure. The compliance measures the relative stiffness and distensibility of the lungs and thorax. If the volume change per unit pressure
change is higher than normal, the tissues are more distensible and if it is less then the tissue are stiffer than normal. The patients with decreased compliance put more respiratory effort to achieve adequate alveolar ventilation and therefore they are dyspneic.

Airway Resistance

This represents the frictional resistance to airflow through the conducting air passages. The patients with increased airway resistance often present serious mechanical problems and develop dyspnea. The degree of dyspnea depends on the severity of the increased airway resistance.

The measurement of the following respiratory parameters gives a fair idea of the mechanics of breathing in clinical practice.

1. Timed vital capacity (FEV$_1$): This is also called forced expiratory volume in 1 second (FEV$_1$). This is defined as the percentage of air expired in the specified time, e.g. FEV$_1$ (FEV in first second) This test measures the vital capacity in relation to time and gives the portion of the vital capacity expired in a specified time, say one, two or three seconds.
   - This is an index of air-flow rate. In normal conditions, 80 to 85% of the forced vital capacity is expired in the first second, 95% in two seconds and 97% in three seconds (Refer Fig. 104.17, Chapter 104).
   - It is one of the most useful tests to detect generalized airway obstruction.

2. Maximum mid-expiratory flow rate (MMFR): This is the maximum flow achieved during the middle third of the total expired volume. This is expressed as forced expiratory flow at 25% to 75% of the lung volume (FEF$_{25-75}$%). FEF$_{25-75}$% indicates the patency of small airways. The measurement of flow rate between 200 and 1200 mL (FEF$_{200-1200}$ mL) in liters per second indicates the patency of larger airways.

3. Maximum voluntary ventilation (MVV): This is also called as maximum breathing capacity (MBC). MVV indicates the maximum volume of gas that can be breathed per minute by maximal voluntary effort. The normal value of MVV in adult male is 150 liters per minute and for adult female is 125 liters per minute.

4. Peak expiratory flow rate (PEFR): It is the maximum velocity in liters per minute with which air is forced out of the lungs. PEFR can be read directly from the dial of the peak-flow meter. The normal value of PEFR is 400 to 600 L/min or 6 to 10 L/s.

5. Maximum expiratory flow volume curve (MEFVC): Because of the limitation of FEV$_1$, certain other tests are performed to detect airways obstruction in its early phase. These include $V_{max}$ 75%, response of maximum expiratory flow volume (MEFV) curve to inhalation of helium, and closing volume. In MEFVC flow is plotted against volume exhaled. $V_{max}$ 75% is the volume achieved after exhaling 75% of the total FVC (Refer Fig. 104.20, Chapter 104).

6. Closing volume (CV): This test detects small airway obstruction by measuring the volume of gas remaining in the lung after closure of small airways in the gravity dependent lung areas.

7. Maximal inspiratory pressure (MIP): Patient exhales completely to RV and than tries to inspire maximally against an occluded airway. The pressure generated in this maneuver is the MIP. It assesses the strength of inspiratory muscles.

8. Maximal expiratory pressure (MEP): Patient inhales completely to TLC and than tries to expire maximally against an occluded airway. The pressure generated in this maneuver is the MEP. It assesses the strength of expiratory muscles.

Measurement of Ventilatory Functions

Most of the pulmonary functions are measured by spirometry. A recording spirometer is a spirometer with recording kymograph. The spirometer consists of a hollow double-walled vessel. The space between the two walls contains water to maintain an airtight seal. In the space between the two walls is placed an inverted hollow cylindrical bell of 9 liters capacity. The bell is attached to a counterbalance with a chain, which passes over a pulley. The counterbalance carries a pen for writing on the kymograph paper. The kymograph operates at the speeds of 60 and 1200 mm per minute. The spirometer records the volume and capacities of the lung. However, now-a-days pulmonary functions are measured by computerized spirometer.

RV, FRC and TLC can not be measured by spirometry as they include volume of gases present in the lungs even after maximal expiration. They are measured by helium dilution technique and total body plethysmography.

Abnormalities of Ventilation

There are two major patterns of ventilatory abnormalities: restrictive and obstructive patterns. In the obstructive disease, the hallmark of dysfunction is the decrease in expiratory flow rates, particularly the MMFR and FEV$_1$/FVC and the hallmark of the restrictive disease is the reduction in FVC.

Features of Obstructive Diseases

1. TLC is normal or increased.
2. RV is elevated due to trapping of air during expiration.
3. Ratio of RV/TLC is increased.
4. VC is frequently decreased (not due to decreased lung volumes but due to increased RV).
5. FEV$_1$ is less than 80% of TLC (Refer Fig. 104.19, Chapter 104).
6. FEV$_1$/FVC decreases.
7. MMFR decreases.
In the early phase of obstructive disease which originates in the small airways, FEV$_1$/FVC may be normal. However, decreased in MMFR and an abnormal configuration in the terminal portion of forced expiratory flow volume curve may indicate the presence of the disease.

Features of Restrictive Diseases

1. Decreased TLC
2. Decreased VC
3. Decreased RV
4. Preservation of forced expiratory flow rates, especially FEV$_1$ expressed, as percentage of FVC is normal or supernormal (Refer Fig. 104.19, Chapter 104).

The restrictive diseases can be broadly subdivided into parenchymal and extraparenchymal disease. Extraparenchymal dysfunction is again of two categories, the extraparenchymal dysfunction in inspiration and the extraparenchymal dysfunction in inspiration plus expiration.

Restrictive Parenchymal Dysfunction

1. Decreased TLC
2. Decreased RV
3. Decreased VC
4. Normal or increased FEV$_1$/FVC

Restrictive Extraparenchymal Dysfunction

Inspiratory dysfunction: In extraparenchymal inspiratory dysfunction, which occurs usually due to either inspiratory muscle weakness or a stiff chest wall, the adequate distending forces are prevented from being exerted on an otherwise normal lung. Therefore, TLC is reduced but RV is not affected, and expiratory flow rates are preserved.

Inspiratory plus expiratory dysfunction: This usually occurs in expiratory muscle weakness or due to deformed chest wall that is abnormally rigid at lung volumes below FRC. Therefore, RV is often significantly elevated. The ratio of FEV$_1$/FVC may be affected depending on the strength of the expiratory muscles.

List of common obstructive and restrictive diseases:

1. Obstructive diseases
   a. Asthma
   b. COPD (chronic bronchitis, emphysema)
   c. Bronchiectasis
   d. Bronchiolitis
   e. Cystic fibrosis
2. Restrictive diseases
   a. Parenchymal restrictive diseases
      i. Pulmonary fibrosis
      ii. Pneumoconiosis
      iii. Radiation-induced interstitial lung disease
   b. Extraparenchymal restrictive diseases
      i. Neuromuscular diseases
         • Myasthenia gravis
         • Paralysis of diaphragm
         • Muscular dystrophy
   ii. Chest wall diseases
      • Kyphoscoliosis
      • Obesity
      • Ankylosing spondylitis

Alterations in Lung Volumes and Capacities

Lung volumes and capacities show a wide range in the normal population depending on the age, sex, and height of the subject. Indian population shows significant lower values as compared to their western counterparts. Predicted nomograms are available based on these variable factors. Deviation up to 20% from the predicted value for a given age, sex, and height is commonly seen in normal subjects. However, in a particular individual, even 5% change from his preexisting value may be of significance. Therefore, serial measurement of these values is of great importance in diagnosis.

Conditions that Decrease Vital Capacity

1. Loss of functioning lung tissue
   - Interstitial pulmonary fibrosis
   - Chest deformity
   - Neuromuscular disease
   - Thickened pleura
2. Loss of distensibility of lung tissues or pleura
   - Atelectasis
   - Consolidation
   - Pulmonary edema
   - Pulmonary resection

Conditions that Decrease Static Lung Compliance

1. Pulmonary edema
2. Chronic pulmonary congestion
3. Kyphoscoliosis
4. Fibrothorax
5. Interstitial fibrosis
6. Atelectasis

Patients with decreased compliance have to put in more respiratory muscular effort to achieve adequate alveolar ventilation. Therefore, very often they are dyspneic.

Conditions that Increase Airway Resistance

1. Bronchial asthma
2. Chronic bronchitis
3. Emphysema
4. Other diseases that are characterized by airway obstruction.

Patients with increased airway resistance are usually dyspneic, which depends on severity of airway obstruction.

Conditions that Decrease FVC

FVC decreases in conditions in which there is obstruction to the airways resulting in air trapping, e.g. bronchial asthma.
**Conditions that Decrease FEV<sub>1</sub>**

FEV<sub>1</sub> is an important test to detect **generalized airway obstruction**. As it is effort dependent it should be performed properly to get the appropriate result. However, it is not specific for detecting small airway obstruction.

FEV<sub>1</sub> decreases in **obstructive diseases of the lung**, as in bronchial asthma.

**Change in FEV<sub>1</sub>/FVC**

The ratio of FEV<sub>1</sub>/FVC is approximately 0.75 to 0.80. This is a more **sensitive indicator of airway obstruction** than FVC or FEV<sub>1</sub> alone.

**Alteration of MMFR (FEF<sub>25−75%</sub>)**

This is a sensitive indicator of **patency of the small airways**. This is slowed in small airway obstruction.

**Variation in FEF<sub>200−1200</sub>**

This is the flow rate between 200 and 1200 mL of FVC. It is one of the sensitive indicators of **patency of larger airways**. It is slowed in large airway obstruction.

**Reduction in PEFR**

As it measures peak expiratory flow rate during peak of expiration, it decreases in **airway obstruction**. MMFR and FEF<sub>200−1200</sub> are better indicators than PEFR.

**Decline in MVV**

The normal value of MVV is 150 liters in males and 125 liters in females. However, the value can be fallacious if the patient does not cooperate or fails to use maximum effort to carry out the test. MVV decreases in patients with subjective dyspnea.

**Assessment of Gas Exchange**

**Ventilation-Perfusion Relationship**

Even under normal conditions, the inspired air is not distributed evenly throughout lungs. In erect posture, resting ventilation per unit volume of lung is greater at the bases than at the apices. The difference becomes less in lying posture and during exercise. In disease states, the distribution becomes more uneven resulting in hypo- and hyperventilated areas. Such non-uniform distribution of inspired gas leads to decreased oxygen tension in the arterial blood. The uniformity of distribution of inspired air is measured by **nitrogen washout method**. An alveolar gas sample after 7 minutes of breathing oxygen normally contains less than 2.5% of nitrogen. The higher the percentage of nitrogen in the alveolar sample, the greater is the degree of non-uniformity of distribution of inspired gas.

The perfusion is also not uniform in erect posture due to the effect of gravity. The normal ratio of ventilation to perfusion is 0.8. But greater degree of non-uniform perfusion occurs in diseases like pulmonary embolism and diseases that destroy lung tissues. The arterial blood gas tension is primarily affected by the relationship of ventilation with perfusion. Alteration in this ratio mainly affects PaO<sub>2</sub> than PaCO<sub>2</sub>.

**Diffusion**

Diffusion is the physical process by which gas move across a membrane from the region of higher partial pressure to the region of lower partial pressure. In lungs, oxygen moves from alveoli to the pulmonary capillaries to combine with the hemoglobin. Similarly, carbon dioxide moves in the opposite direction. The diffusing capacity of carbon dioxide is 20 times than that of oxygen. Therefore, diffusion problems usually do not produce carbon dioxide retention.

Measurement of the arterial blood gas tension is essential in the evaluation of pulmonary functions. The normal value of PaO<sub>2</sub> is 90 to 95 mm Hg, and of PaCO<sub>2</sub> is 36 to 44 mm Hg.

**Hypoxemia**

Decrease in PaO<sub>2</sub> is called hypoxemia. It occurs in four conditions (detailed discussed below);

1. Decreased inspired PO<sub>2</sub>
2. Hypoventilation
3. Shunting (when desaturated blood effectively bypasses oxygenation at alveolar-capillary level): it is common in cyanotic congenital heart disease.
4. Ventilation-perfusion mismatching as occurs in COPD, asthma etc.

**Conditions that Decrease Pulmonary Diffusing Capacity**

1. When the total surface area of the alveolar capillary membrane is reduced
   - Emphysema
   - Pulmonary embolism
   - Thrombosis of pulmonary capillaries
   - Following surgical removal of lung tissues
2. When there is defect in the alveolar-capillary membrane (thickening of the membrane)
   - Asbestosis
   - Sarcoidosis
   - Progressive systemic sclerosis
   - Collagen diseases
   - Interstitial edema
   - Interstitial fibrosis
   - Diffuse metastatic lesions of the lung

**Increase in Pulmonary Diffusing Capacity**

1. Exercise
Gas Sampling and Analysis

Sampling of Alveolar Air
Sampling of alveolar air is performed by two methods:
1. Haldane-priestley method: The subject makes a forced expiration through a long narrow tube of about 3 feet length and 2.5 cm diameter. He closes the mouthpiece with his tongue. A sample of air contained near the mouthpiece of the tube is withdrawn via a side tube into a sampling tube. Thus, the last to be expelled from the lung is considered as alveolar air.
2. Rahn-Otus method: This is a procedure of continuous sampling. Breathing through a mouthpiece fitted with inspiratory and expiratory valves collects a sample of the end-tidal volume. The samples are passed into gas analyzers.

Sampling of Expired Air
Douglas bag is used for this purpose. A two-way valve is fixed to the Douglas bag and then connected to the mouth of the subject. Atmospheric air is inhaled and then exhaled into the bag.

Respiratory Gas Analysis
Samples of inspired air (atmospheric), mixed expired air (from the Douglas bag) and alveolar air (collected by Haldane-Priestly method) are taken for analysis of partial pressure of oxygen, carbon dioxide and nitrogen. Gas analyzers are available to give the partial pressure of different gases.

Blood Gas Analysis
The oxygen content of the blood is determined by Haldane’s gas analyzer. The venous blood is collected and sent for analysis. The oxygen carrying capacity of the blood is estimated by Van Slyke gasometric method. The partial pressure of carbon dioxide is also determined with the help of gasometers.

Scientist contributed
Heinrich Gustav Magnus (1802–1870) made the first quantitative analysis of blood gases, showing relative amounts of oxygen and carbon dioxide in arterial and venous blood.


Assessment of Pulmonary Blood Flow
Assessment of pulmonary circulation depends upon measuring pulmonary vascular pressures and cardiac output. These measurements are usually done in intensive care units with the facilities of invasive monitoring. With a flow-directed pulmonary arterial (Swan-Ganz) catheter, the pulmonary arterial and pulmonary capillary wedge pressures can be measured directly. The cardiac output is obtained by thermodilution method. The pulmonary vascular resistance (PVR) is calculated as:

$$PVR = 80 (PAP - PCW)/CO$$

Where,
- PAP = Mean pulmonary arterial pressure in mm Hg
- PCW = Pulmonary capillary wedge pressure in mm Hg, and
- CO = Cardiac output in L/min

The normal PVR is 50-150 dynes.s/cm$^5$.

Disturbances in Pulmonary Circulation
Pulmonary vascular resistance ($PVR$) increases by four different mechanisms:
1. Pulmonary vasoconstriction: Pulmonary arterial or ateriolar vasoconstriction in response to alveolar hypoxia increases PVR.
2. Pulmonary thromboembolism: Intraluminal thrombi in pulmonary vessels decrease the luminal cross sectional area and increase PVR.
3. Vascular hypertrophy: Proliferation of smooth muscle within the vessel wall decreases luminal cross sectional area and increases PVR.
4. Pulmonary injury: Destruction of small pulmonary vessels either by scarring or by loss of alveolar wall decreases total cross sectional areas of the pulmonary vascular bed and increases PVR. Increase in PVR increases pulmonary arterial pressure that in turn decreases right ventricular output.

Conditions that Increase PVR
1. Heart diseases: Cardiac conditions that elevate left arterial pressure such as mitral stenosis increase PVR.
2. **Lung diseases**: Respiratory conditions that cause chronic pulmonary hypoxemia increase PVR, as seen in,
   - COPD (chronic obstructive pulmonary diseases)
   - Interstitial lung disease
   - Chest wall diseases, e.g. Kyphoscoliosis
   - Obesity hypoventilation
   - Sleep apnea syndrome

3. **Diseases affecting pulmonary vessels**:
   - Recurrent pulmonary embolism
   - Scleroderma (occludes small pulmonary arteries and arterioles)

**Respiratory Causes of Hypoxemia**

The causes of hypoxemia are classified into respiratory and non-respiratory. **Respiratory dysfunction** is the most common cause of hypoxemia in adults. **Non-respiratory causes** include anemia, carbon monoxide poisoning, and a decreased inspired oxygen tension (as occurs at high altitude).

**Normal A-aO\textsubscript{2} Gradient**

Normally, Hb is 100% saturated with oxygen when the blood passes the pulmonary capillaries, so that end-capillary PO\textsubscript{2} is same as alveolar PO\textsubscript{2}. But, the blood in the pulmonary veins that returns to the left atrium has a lower PO\textsubscript{2} than pulmonary end-capillary blood. Therefore, the average oxygen tension (PaO\textsubscript{2}) in systemic arterial blood is about 95 mm Hg and Hb is 98% saturated.

The difference between alveolar oxygen tension (P\textsubscript{A}O\textsubscript{2}) and arterial oxygen tension (P\textsubscript{a}O\textsubscript{2}) is the **alveolar-arterial oxygen gradient** (A-aO\textsubscript{2} gradient). Because alveolar PO\textsubscript{2} is normally 100 to 102 mm Hg and arterial PO\textsubscript{2} is 85 to 95 mm Hg, a normal A-aO\textsubscript{2} gradient is 5 to 15 mm Hg.

The respiratory causes of hypoxemia may be due to:

1. Regional hypoventilation
2. Occlusion of a major artery in the lungs by a large blood clot
3. Shunts
4. Generalized hypoventilation
5. Diffusion block.

**Regional Hypoventilation**

Regional hypoventilation is the most common physiological factor for respiratory causes of hypoxemia and reflects a local ventilation-perfusion imbalance resulting from a partially obstructed airway. Therefore, a fraction of the blood that passes through the lungs does not get fully oxygenated, resulting in an increase in venous admixture. Normally, only a small amount of venous admixture occurs. Patients with abnormally low ventilation-perfusion ratio have a high A-aO\textsubscript{2} gradient, low PO\textsubscript{2}, and low O\textsubscript{2} content.

**Large Blood Clot**

Another cause for a regional low ventilation-perfusion ratio is the occlusion of a major artery in the lungs by a large blood clot. This restricts a greater portion of the cardiac output to another part of the lungs, resulting in over-perfusion with respect to alveolar ventilation and increases venous admixture.

**Shunts**

The next common cause of hypoxemia is a shunt, either a right-to-left cardiac shunt or an intrapulmonary shunt due to obstruction by a foreign object or a tumor. The shunt causes a high A-aO\textsubscript{2} gradient, low PO\textsubscript{2}, and low O\textsubscript{2}. Breathing 100% O\textsubscript{2} in a patient with a shunt, does not correct low arterial PO\textsubscript{2} because the high oxygen mixture does not come in contact with the shunted blood.

**Generalized Hypoventilation**

Generalized hypoventilation is a common cause of hypoxemia that occurs when alveolar ventilation is low. This occurs in chronic obstructive pulmonary diseases such as emphysema or in depressed respiration as a result of a head injury or a drug overdose, for example morphine. As ventilation is depressed, there is associated increase in arterial PCO\textsubscript{2} with a simultaneous decrease in arterial pH. However, the differentiating feature is that in generalized hypoventilation A-aO\textsubscript{2} gradient is normal. The cause of hypoxemia is entirely due to generalized hypoventilation. It is best corrected by placing the patient on a mechanical ventilator, breathing room air.

**Diffusion Block**

The infrequent cause of hypoxemia is a diffusion block. This occurs when the diffusion distance across the alveolar-capillary membrane is increased or the permeability of the alveolar-capillary membrane is decreased. It is characterized by a low PaO\textsubscript{2}, high A-aO\textsubscript{2} gradient and high PaCO\textsubscript{2}. Pulmonary edema is among the common causes of diffusion block.

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**CHAPTER SUMMARY**

**Key Concepts**

1. FEV\textsubscript{1} is the most sensitive and non-invasive test of lung function to differentiate obstructive from restrictive disease.
2. Blood gas analysis is a very sensitive measure of lung function, especially of diffusion capacity.
### Important to Know (Must Read)

1. In examination, **Long Questions** are usually not asked from this chapter. But, ‘Describe the principle, brief methods and merits of various lung function test’; may come as along question.

2. Timed vital capacity, Lung volumes and capacities, Blood gas sampling, Assessment of V-P ration, Hypoxemia may be asked as **Short Questions** in exam.

3. In **Viva**, examiner may ask… Classify PFTs, name the tests that assess ventilation, name the tests that assess gas exchange in lungs, what are the uses of PFTs, State the normal values of PFTs, what are the features of restrictive lung diseases, what are the features of obstructive lung diseases, name some common restrictive lung diseases, name some common obstructive lung diseases, name the conditions that decrease vital capacity, name the conditions that decrease static lung compliance, name the conditions that increase airways resistance, what is hypoxemia, name the conditions where hypoxemia occurs, name the conditions that decrease pulmonary diffusing capacity, name the condition that increase pulmonary diffusing capacity, name the methods for sampling alveolar air, name the methods for sampling expired air, name the condition that increase pulmonary vascular resistance.
Neurophysiology

**Part A: Introduction to Neurophysiology**
- 115. Functional Organization of Nervous System
- 116. Synaptic Transmission in Central Nervous System

**Part B: The Sensory System**
- 117. Introduction to Sensory System and Physiology of Receptors
- 118. Sensory Communication to Spinal Cord
- 119. Ascending Pathways
- 120. Physiology of Pain, Itch and Temperature
- 121. Trigeminal System
- 122. Thalamus
- 123. Sensory Cortex
- 124. Sensory Abnormalities

**Part C: The Motor System**
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- 126. Segmental Organization of Motor System
- 127. Muscle Spindle and Golgi Tendon Organ
- 128. Spinal Reflexes
- 129. Descending Pathways
- 130. Regulation of Posture and Movement
  - Spinal Integration
  - Medullary Integration
  - Midbrain Integration
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**Part D: Hypothalamus, EEG and Sleep; Limbic and Higher Functions; CSF**
- 134. Functions of Hypothalamus
- 135. Physiology of Reticular Activating System
- 136. Electroencephalogram and Sleep
- 137. Limbic System
- 138. Physiology of Learning and Memory
- 139. Physiology of Language and Speech
- 140. Association Cortex, Cerebral Asymmetry, Lobes of the Brain, and Cortical Plasticity
- 141. Cerebrospinal Fluid
“Heaven in its rapture dreams of perfect earth,  
Earth in its sorrow dreams of perfect heaven. 

..........Since God has made earth, earth must make in her God; 
What hides within her breast she must reveal.  
i claim thee for the world that thou hast made”

Sri Aurobindo (in ‘SAVITRI’)
Learning Objectives

On completion of study of this chapter, the student **MUST** be able to:
1. Name different parts of CNS & give their functions.
2. Understand the functions of nervous system.
3. Name the glial cells & give their functions.
5. Classify neurons.

The student **MAY** also be able to:
1. Describe the structure and functions of neurons and glial cells.

Nervous system is a well-organized **system of communication** that allows the individual to interact with his external environment. It is also a **system of integration** that coordinates functions of various internal organ systems of the body. Neural connections between body parts provide the basis for anatomical and physiological communications that help in smooth execution of most of the systemic functions such as gastrointestinal functions, secretion of hormones, functions of heart, lungs and kidney, and musculoskeletal system and so on.

Scientists contributed

Camillo Golgi (1843–1926) and Santiago Ramón y Cajal (1852–1934)

The **Nobel Prize in Physiology or Medicine 1906** was awarded jointly to Italian neuroanatomist and neurophysiologist Camillo Golgi and neuroanatomist and neurophysiologist of Spain Santiago Ramón y Cajal “in recognition of their work on the structure of the nervous system”

Divisions of Nervous System

The nervous system consists of three **divisions**: Central nervous system, peripheral nervous system, and autonomic nervous system.

Central Nervous System

The central nervous system (CNS) includes brain and spinal cord. Brain is situated in the skull, which continues into the vertebral canal as the spinal cord.

Brain

Brain is divided into three parts (Fig. 115.1):

**Prosencephalon**

This is the **forebrain**, which consists of the **telencephalon** (the two cerebral hemispheres) and the **diencephalon** (thalamus, hypothalamus, metathalamus, and subthalamus).
1. The **telencephalon** is meant for perception of sensations, cognition, learning and memory, and planning and programming of responses.
2. The **diencephalon** is primarily meant for relay of information to cortex, and control of autonomic and endocrine functions.

**Mesencephalon**

This consists of the **midbrain** structures, which includes corpora quadrigemina, cerebral peduncles, substantia nigra, tegmentum and many midbrain nuclei. Midbrain contains central pattern generator for locomotion and nuclei for righting reflexes. It also contains a part of reticular formation.
Section 11: Neurophysiology

Rhombencephalon
This is the hindbrain, which consists of metencephalon (the pons and the cerebellum) and myelencephalon (the medulla oblongata). The midbrain, pons, and medulla are combinely known as brainstem. The main functions of brainstem are control of cardiovascular and respiratory functions, motor activities, sleep-wakefulness and visceral functions.

Spinal Cord
Spinal cord starts from the base of the skull as the extension of medulla and continues till the body of first lumbar vertebra. Thus, spinal cord does not run the full length of the vertebral column in adults.
1. The space at the end of spinal cord, especially between L1 and L2 vertebral segments is used in tapping cerebrospinal fluid during lumbar puncture.
2. Spinal cord has 31 segments, each having a motor and a sensory nerve root. The nerve roots combine to form the 31 bilaterally symmetrical pairs of spinal nerves.
3. Spinal cord contains all the ascending and descending tracts.
4. The main function of spinal cord is to receive sensory inputs from peripheral structures via somatic nerves and transmit them to the brain, and convey the signals originating from brain motor and autonomic areas to the target structures.

Peripheral Nervous System
The peripheral nervous system (PNS) includes somatic nerves that come out of the spinal cord to innervate different body structures like skin, muscles, bones, etc. The main function of PNS is to collect and convey information from peripheral structures to CNS via sensory axons and convey motor signals from CNS to muscles and related structures via motor neurons.

Autonomic Nervous System
The autonomic nervous system (ANS) consists of the nerve fibers originating from spinal cord and brain stem that innervate visceral structures. These fibers accompany the somatic and cranial nerves to the visceral organs. The ANS has sympathetic and parasympathetic divisions that work reciprocally to regulate visceral functions (for details, refer chapters 31 and 32 respectively).

Functions of The Nervous System
The major functions of the nervous system are:
1. Communication between body parts and integration of systemic functions.
2. Appropriate regulation of body functions.
3. Coordinated interaction of the body with the environment.

The neural regulation is the major controlling mechanism of many functions and processes of the body. Nervous system achieves its objectives through neurons that are designed for rapid transmission of information from one body part to the other.

CNS accomplishes its functions through four major processes (Fig. 115.2):
1. Recognition of stimulus and transmission of information in the form of nerve impulse to CNS.
2. Processing of information in CNS and perception of sensations.
3. Generation of appropriate command signal in CNS.
4. Specific responses in accordance to the command signal conveyed by CNS.

**Stimulus Recognition and Transmission of Information to CNS**

This is the process by which we become aware of our environment through various sensations.

1. **Sensation** is the basic recognition of the stimulus. It is a perceptible change in some form of physical energy in the environment. Sensory detection starts with application of a stimulus.
2. The **receptors** transduce environmental energy into the action potentials in the sensory neurons. This becomes possible due to the presence of different modalities of receptors in the body that detect changes in their environment. The forms of stimuli that are detected by receptors include mechanical, chemical, photic (light), auditory (sound), thermal (temperature), and electrical.
3. **Sensory neurons** transmit information in the form of nerve action potentials from receptors to the CNS via sensory pathways. Details of these mechanisms are discussed in physiology of sensory system.

**Information Processing and Generation of Command Signal**

Information received by CNS is processed by different mechanisms in different steps. The general outline of these events is as follows:

1. The action potential (sensory signal) transmitted is first relayed in the thalamus and then processed in sensory cortex. The processed signal is then transformed into other signals in sensory networks in the brain.
2. Integration of the processed signals into appropriate command signals through detailed planning and programming mechanisms.
3. Transmission of the command signal to the effector organs for implementation of the plan.
4. Learning based on sensory inputs and then storage of learned information in memory for future utilization of the knowledge (described in detail in the higher functions).

**Responses**

Responses consist of movement of body parts (motor activities), change in visceral functions (autonomic responses) or even the change in behavior of the individual. Thus, the responses may be an internal change such as alteration in cognition, behavior, etc., or an external change such as motor activities. Details of these mechanisms are discussed in physiology of motor system.

**CELLULAR COMPONENTS OF CNS**

Despite its complexity, CNS consists of only two principal cell types: Glial cells and neurons. Glial cells of nervous system are called neuroglia. Neuroglia support and protect the neurons and maintain homeostasis of fluids that bath the neurons. Neurons transmit impulses in the form of action potentials. Neuroglia are 10 – 30 times plentiful than neurons.

**Glial Cells**

Glial cells neither conduct action potential nor form functional synapse with other cells. However, they can be passively polarized in response to nearby neural activity. Though glial cells generally provide support for neurons, their functions are complex and not completely understood. Glial cells can multiple (Application Box 115.1)

**Functions of Glial Cells**

1. They form the mechanical matrix in which neurons are embedded.
2. They play metabolic and nutritive roles for neurons.
3. They may help to regulate blood flow through the brain.
4. They may act as a sink or source of ions.
5. They insulate axons and synapses and electrically isolate them from one another.
6. They phagocytose neural debris.

**Types of Glial Cells**

Four types of glial cells have been described in CNS: astrocytes (astroglia), oligodendrocytes (oligodendroglia), ependymal cells and microglia. Schwann cells are glial cells in PNS.

**Astrocytes**

Astrocytes are so named because of their star shape (Fig. 115.3A). They are found abundantly throughout the brain and spinal cord. Processes of astrocytes surround the neurons and their axons, and often terminate on the wall of blood vessels.

**Functions of astrocytes** are as follows:

1. They provide the mechanical matrix.
2. They serve metabolic and nutritive functions for neurons.
3. Synapses in CNS are usually surrounded by the processes of astrocytes. Thus, astrocytes electrically insulate synapses and separate them from one another.

**Oligodendrocytes**

Oligodendrocytes are found close to the myelinated axons in the brain and spinal cord.

1. The processes of oligodendrocytes wrap many times around an axon to form the myelin sheath (Fig. 115.3B). This sheath not only insulates axons from one another, but also limits current flow across the axon membrane (axolemma).
2. Because of this myelination, action potential is conducted in a saltatory fashion in myelinated fibers, which is much faster than the transmission of impulse in unmyelinated fibers.

3. Oligodendrocytes provide myelin sheath for neurons in CNS in a much similar way the Schwann cells do for peripheral nerves.

**Microglia**

Microglia are the smallest cells in the central nervous system (Fig. 115.3C). They are the scavenger cells in brain. If the nervous tissue is damaged or infected, these cells enlarge and become mononuclear phagocytes to eliminate debris and organisms.

**Ependymal Cells**

Ependymal cells line the surfaces of the brain’s ventricles and central canal of the spinal cord (Fig. 115.3D). Their function is unclear.

**Application Box 115.1**

Gliomas: Neurons cannot multiply, whereas neuroglia can. Following brain injury, neuralgia multiply to fill the space formerly occupied by neurons. Excessive multiplication of glial cells results in gliomas. Gliomas are common malignant tumors of brain.

**Neuron**

The neuron is a nerve cell. It is the functional unit of the nervous system.
**Soma**

The soma or the cell body consists of nucleus and cytoplasm.

1. **Cytoplasm** contains many organelles like endoplasmic reticulum, a prominent Golgi apparatus, many mitochondria and cytoskeletal elements that include microfilaments (neurofilaments) and microtubules.
2. The special granules present in the cytoplasm are Nissl bodies that are modified rough endoplasmic reticulum and act as biosynthetic apparatus for synthesis of proteins in the neurons. The Nissl bodies are present throughout the soma except in the axon hillock.
3. **Nucleus** is centrally or eccentrically placed and contains a nucleolus.

**Dendrites**

These are tapering processes of variable complexity that arise from the soma.

1. They account for more than 90% of the total surface area of the neuron. The parts of dendrites that are close to the cell body (proximal dendrites) contain Nissl granules and Golgi apparatus, whereas the parts that are present away from the soma (distal dendrites) contains no Nissl granules.
2. The dendrites contain mainly the microfilaments and microtubules.
3. Dendrites and cell bodies are the main parts of the neuron for receiving information and therefore form the receiving or receptor zone of the neuron. Dendrites conduct impulses toward the cell body.

**Axon**

The axon is the longest process of the neuron. It arises from the soma at a specialized region called axon hillock.

1. The axon hillock is the tapered part of the cell body that gives rise to axon. The differences between the axon hillock (proximal part of the axon) and the proximal dendrites (proximal part of the dendrites) are that the axon hillock does not contain Nissl granules, endoplasmic reticulum and Golgi apparatus, whereas the proximal dendrites contain all these cell organelles.
2. The axon hillock continues as unmyelinated proximal part of the axon called as the initial segment.
3. The initial segment is the spike initiation zone of the neuron as action potential normally arises here due to summation of electrical activities that have occurred in the cell body and dendrites.
4. The interior of the axon is called as axon cylinder that contains axoplasm. The axoplasm contains mitochondria, neurofibrils and the vesicles. Nissl bodies are not found in the axon. The neurotransmitter substances are synthesized in the soma and transported in the axoplasm by means of axonal flow to the synapse.
5. If axons are covered by myelin sheath, the nerve fibers are known as myelinated fibers and without the cover, they are unmyelinated fibers.

**Types of Neuron**

Neurons are classified according to the number of processes, length of axon, functions of neurons and patterns of dendrites.

**A. According to the Arrangement of Axon or Processes**

According to the arrangement and number of processes, the neurons are classified into unipolar, pseudounipolar, bipolar, and multipolar neurons (Fig. 115.5).

- **Unipolar neurons**: They have a single process (Fig. 115.5A). They are usually found in invertebrate. In vertebrates, they are found in ANS.
- **Pseudounipolar neurons**: In pseudounipolar neurons, axon after originating from soma splits into central and peripheral processes (Fig. 115.5B). The example is dorsal root ganglion cell (primary sensory neurons with cell bodies in dorsal root ganglion).
- **Bipolar neurons**: In these neurons, two processes arise from the cell body (Fig. 115.5C). The example is the bipolar cell of retina.
- **Multipolar neurons**: In multipolar neurons, many processes arise from the soma (Fig. 115.5D). The example is a spinal motor neuron.
B. According to the Length of Axon

According to the length of the axon, neurons are classified into two categories: Golgi type 1 and type 2.

Golgi type 1: These are the neurons with short axons. Dendrites of these neurons terminate near the soma. The example is cortical inhibitory neurons.

Golgi type 2: Axons of Golgi type 2 neurons are long. Cortical motor neurons (neurons that give rise to corticospinal tract) are the examples.

C. According to Function

According to functions, neurons can be divided into sensory and motor neurons.

Sensory neurons: These are the neurons that carry impulses from the receptors to the central nervous systems. These are called afferent neurons (afferent fibers).

Motor neurons: These are the neurons that carry impulses from the central nervous system to the target organs. These are called efferent neurons (efferent fibers).

D. According to Dendritic Pattern

According to dendritic pattern, two types of neurons are present: pyramidal cells and stellate cells.

Pyramidal cells: Dendrites of these cells spread like pyramids. The example is hippocampal pyramidal neurons.

Stellate cells: Radial shaped spread of dendrites occurs in these cells. The examples are cortical stellate cells.

CHAPTER SUMMARY

Important to Know (Must Read)

1. In examination, Long Questions are usually not asked from this chapter.
2. Types of glial cells and their functions, Types of neurons and their functions may come as Short Questions in exam.
3. In Viva, examiner may ask... What are the divisions of CNS, Parts of each component of CNS and their functions, What are types of glial cells, Each type of glial cells and their functions, How do you classify neurons, Each type of neurons and their functions.
CHAPTER 116
Synaptic Transmission in Central Nervous System

Learning Objectives
On completion of study of this chapter, the student must be able to:
1. Give the classification of synapses.
2. Draw a labeled diagram of a synapse.
3. Describe the mechanism of synaptic transmission.
4. List the properties of synapses.
5. Give the examples and mechanisms for different types of synaptic inhibitions.
6. Classify neurotransmitters (NTs).
7. Understand the functions of common NTs.
The student may also be able to:
1. Explain the properties of synapses.
2. Describe the receptors, functions and dysfunctions of neurotransmitters.

Synapses in CNS
Communication, the major function of the nervous system, is achieved through synaptic transmission, the transmission of impulses through synapses.
1. Synapses are the neuro-neuronal junctions through which information from one neuron passes to the other.
2. Through the synapses, neuronal messages are conveyed to the appropriate target structures in the CNS. Command signals from CNS to the peripheral organs are also conveyed through the synapses.
3. The higher functions of CNS (e.g. processing and integration of information, learning, memory, etc.) are also possible because of activation and alteration of activities at synapses.
4. Formation of new synapses and synaptic modifications continue throughout the life of an individual.

Types of Synapses
There are innumerable synapses in the nervous system. On average, each neuron forms about 2000 synapses. As there are about $10^{11}$ neurons in CNS, the total number of synapses may be about $2 \times 10^{14}$. These synapses are of different types and have various names. Synapses are usually classified, as follows:

According to Part of the Neurons Involved in the Formation of Synapse
Based on the parts of the neurons involved in the formation of synapse, four types of synapses are found in CNS: axodendritic, axosomatic, axoaxonic and dendrodendritic (Figs. 116.1A to C).
**Axodendritic Synapse**

In this type of synapse, axon of presynaptic neuron connects with dendrite of postsynaptic neuron. This is the **commonest synapse** in CNS. The axon terminal may synapse with the spinous process of the dendrite (axosynaptic synapse) or with the shaft of the dendrite (shaft synapse) (Fig. 116.1A).

**Axosomatic Synapse**

In this variety, axon of presynaptic neuron synapses with cell body of postsynaptic neuron (Fig. 116.1B). This is also a common form of synapse in CNS.

**Axoaxonic Synapse**

In axoaxonic type, axon of presynaptic neuron synapses with axon of postsynaptic neuron (Fig. 116.1C).

**Dendrodendritic Synapse**

In this type, dendrite of presynaptic neuron connects to the dendrite of postsynaptic neuron.

In CNS, axodendritic synapse is the common type of synapse followed by axosomatic synapse. For example, in cerebral cortex, about 80% of neurons synapse on dendrites, whereas only 15% end on cell bodies. Axoaxonic synapse is less common and dendrodendritic synapse is the rare one. The ratio of synapses to neuron in human forebrain is about 40000 : 1.

**According to the Nature of Transmission**

Synapses are of three types according to the mode of transmission of the impulse.

- **Chemical synapse**: Transmission of the impulse occurs through release of neurotransmitters.
- **Electrical synapse**: Transmission of the impulse occurs through gap junctions.
- **Conjoint synapse**: Partly electrical and partly chemical.

**According to the Number of Neurons Involved**

1. One neuron ends on another (one to one).
2. Multiple neurons terminate on a single neuron (many to one).
3. One neuron contacts multiple neurons (one to many).

Example of ‘one to one’ synapse is the neuromuscular junction, ‘many to one’ is the usual type found in CNS and ‘one to many’ is less frequent. In ANS, usually ‘one to one’ synapse is found in parasympathetic and ‘one to many’ type is found in sympathetic system.

**MECHANISM OF SYNAPTIC TRANSMISSION**

According to the nature of transmission, synapses are categorized into chemical and electrical synapse. As chemical synapses are very common, and electrical synapses are sparse in CNS, the mechanism of synaptic transmission discussed in this section is through the chemical synapse.

**Functional Anatomy of Synapse**

The neuron from which the information passes through the synapse is the presynaptic neuron and the neuron, which receives the information, is the postsynaptic neuron. The part of the presynaptic axon terminal forming the synapse is called presynaptic membrane and that of the postsynaptic neuron is called the postsynaptic membrane. The space between presynaptic and postsynaptic membrane is called the synaptic cleft. The presynaptic axon terminal, postsynaptic membrane and the synaptic cleft form the synapse (Fig. 116.2).

**Presynaptic Axon Terminal**

The presynaptic membrane is the part of an axon terminal of the presynaptic neuron. The terminal of presynaptic neuron typically ends in small bulbous enlargement called terminal bouton or synaptic knob, which is about 1 mm in diameter. Synaptic bouton contains two specialized structures:

- **Numerous synaptic vesicles** that contain neurotransmitters.
- **Dense tufts** or projections that are made up of filamentous proteins, which contact larger filaments in the axoplasm.

**Synaptic Vesicles**

There are three types of synaptic vesicles: i) small vesicles with clear-core, ii) small vesicle with dense-core, and iii) large vesicle with dense-core. Small-clear vesicles contain...
acetylcholine, GABA, glycine or glutamate. **Small-dense vesicles** contain catecholamines and **large-dense vesicles** contain neuropeptide.

1. **Large vesicles** are present throughout the presynaptic terminals and are released by exocytosis from all parts of the presynaptic membrane.

2. **The small vesicles** are located close to the presynaptic membrane and are released to the synaptic cleft through the active zone by exocytosis.
   - The small vesicles after discharging their content are recycled back into the terminal. The vesicles are endocytosed and then fused with the endosomes.
   - New vesicles that are formed from endosomes by budding are refilled with the transmitter chemicals.
   - Vesicles then move close to the active zone and undergo **docking and priming** (Fig. 116.3) before discharging their content into the synaptic cleft.

**Dense Tufts**

These are filamentous projections present in close contact with vesicles and play an important role in exocytosis of vesicles. Dense tufts are present mainly in active zone (see below).

**Active Zone**

Presynaptic terminal also contains few large mitochondria. A region of presynaptic membrane is modified to form active zone, which contains many proteins and calcium channels. Release of vesicles containing neurotransmitters occurs mainly through the active zone (Application Box 116.1).

**Application Box 116.1**

**Presynaptic receptors are autoreceptors:** There are receptors in the presynaptic membrane as receptors are present in the postsynaptic membrane. These receptors sense the release of neurotransmitters from presynaptic nerve terminal and control their rate of secretion. Therefore, they are called autoreceptors. Usually, they inhibit the secretion of neurotransmitters when the rate of secretion is more. For example, noradrenaline secretion is inhibited from noradrenergic nerve terminal by α₂ receptors. Sometimes, they also stimulate the secretion of neurotransmitters.

**Synaptic Cleft**

The synaptic cleft is the gap between pre- and post-synaptic membranes, which is about 20 to 50 nm wide. This space is filled with extracellular fluid. Transmitter molecules released from the presynaptic terminal diffuse across the cleft to reach the postsynaptic receptors.

**Postsynaptic Membrane**

The postsynaptic membrane usually is a part of a dendritic spine, but may be a part of a cell body (soma) or part of an axon, which contains receptors for the neurotransmitters.

1. The area of the postsynaptic membrane modified for synaptic transmission is called **postsynaptic density.**

2. Postsynaptic density is the **cluster of receptors** for transmitter embedded within the postsynaptic membrane. 

**Neurotransmitter** is the chemical substance used for transfer of information through the synapse. The neurotransmitter **amplifies the effect of the action potential** arriving at the synapse. Due to this amplification, presynaptic action potential after passing through the synaptic cleft **stimulates the postsynaptic neuron.** The arrangement in the synapse is such that the neurotransmitter released from presynaptic nerve ending acts on a wide area of the postsynaptic membrane and activates a large number of receptors (ion channels) to activate postsynaptic potential (Application Box 116.2).

**Role of Neurexins**

Neurexins are proteins attached to the presynaptic membrane.

1. There are **neurexin receptors** in the postsynaptic membrane to which neurexins bind.

2. Thus, neurexins that link presynaptic and postsynaptic membranes provide **structural stability to the synaptic architecture.**

3. In fact, the systematic organization of the synapse depends partly on neurexins. In many vertebrates,
neurexins are produced by a single gene that codes for the α isoform.
4. Neurexins are coded by three genes and there are both α and β isoforms. As each of the genes has two regulatory regions and extensive alternative splicing of their mRNAs occurs, there are more than 1000 different neurexins.
5. It is proposed that neurexins not only bind the synapses together, but also provide basis for synaptic specificity.

Application Box 116.2

**Specific binding proteins for postsynaptic receptors:** Postsynaptic receptors are present in clusters close to the presynaptic nerve terminal that secretes the neurotransmitter. This occurs due to presence of specific binding proteins for the receptors on the postsynaptic membrane. For example, for glutamergic receptors, the binding protein is PB2-binding protein, for GABA, receptors binding protein is gephyrin, for nicotinic acetylcholine receptors binding protein is rab3.

Steps of Synaptic Transmission

Synaptic transmission is the process by which information from presynaptic neuron passes to the postsynaptic neuron through the synapse. In a chemical synapse, it occurs due to release of neurotransmitter from presynaptic nerve terminal that initiates action potential in the postsynaptic neuron. The mechanism of synaptic transmission can be divided into presynaptic and postsynaptic mechanisms.

**Presynaptic Mechanisms**

**Five major steps** are involved in the presynaptic mechanism of synaptic transmission.

1. Vesicles containing neurotransmitter molecules that are concentrated at active zone of the presynaptic axon terminal undergo docking and priming. **Docking** is the process by which vesicles attach with the membrane and **priming** is the process by which the vesicles become ready to discharge their content in response to a stimulus.
2. The action potential that arrives at presynaptic axon terminal **depolarizes the presynaptic membrane**.
3. Depolarization of membrane causes **opening of voltage-gated calcium channels** that allows calcium to enter the axon terminal through the active zone.
4. Increase in calcium concentration in the presynaptic terminal increases **calcium-mediated exocytosis** of the vesicles. Calcium causes fusion of vesicles to the presynaptic membrane by causing contraction of microfilaments in the dense tuft that facilitates their movement, and then help to discharge their content into the cleft.
5. **Kiss and Run Discharge:** Discharge of synaptic vesicular contents takes place through a small hole in the cell membrane, which immediately closes rapidly.
   - In this process, the main vesicle remains inside the cell. This is called kiss-and-run discharge.
   - Following discharge, some vesicles are quickly recovered by endocytosis and refilled locally, in which the endocytic process is short-circuited.
6. Transmitter is released into the synaptic cleft in a quantized amount that diffuses passively across the cleft to the postsynaptic membrane. Quantal release of neurotransmitter is sometimes called Dale’s phenomenon. From the **calcium influx to the transmitter release takes about 200 µs**.

**Role of Membrane Proteins**

Normally, small synaptic vesicles recycle in the presynaptic nerve terminal. The mature vesicles move to the active zone, dock and get primed. When action potential arrives, calcium influx facilitates fusion of vesicles with presynaptic membrane that causes discharge of granular content into the synaptic cleft.
1. Vesicle membrane then gets **coated with clathrin** and taken up by endocytosis into the presynaptic terminal where it is reutilized for neurotransmitter packaging (Fig. 116.3).
2. Fusion of synaptic vesicle with cell membrane is facilitated by synaptobrevin, a **v-snare protein** present in vesicular membrane, and syntaxin, a **t-snare protein** present in the cell membrane.
3. In fact, synaptobrevin attaches and interacts with syntaxin for docking and priming of vesicles.
4. Various other synaptic proteins (SNAP 25 connected with syntaxin, and α/γ SNAPs connected with synaptobrevin) facilitate the interaction between synaptobrevin and syntaxin (Fig. 116.4).
5. A **multi-protein complex** regulated by small GTPase like rab3 also participates in the process.

**Clinical Significance**

Many neurotoxins inhibit release of neurotransmitters by preventing attachment of synaptobrevin and syntaxin.

1. For example, tetanus and botulinum toxins act on synaptobrevin and syntaxin that in turn prevents fusion of vesicles with membrane that blocks release of neurotransmitters from presynaptic terminals. **Botulinum toxins** are of seven types (A to G).
2. A and B botulinum toxins act on SNAP 25 and prevent the interaction between synaptobrevin and syntaxin that in turn prevents docking and priming of vesicles. **Botulinum toxin C** acts on syntaxin and prevents its attachment with synaptobrevin.
3. **Botulinum toxin B, D, F and G** act on synaptobrevin and prevents its attachment with syntaxin. Thus, botulinum toxins produce flaccid paralysis by inhibiting release of acetylcholine at neuromuscular junction.
4. **Tetanus toxin** acts on synaptobrevin and prevents its attachment with syntaxin. Tetanus toxin prevents release of other neurotransmitters from vesicles at different synapses in CNS, and therefore produces spastic paralysis (Clinical Box 116.1).
Clinical Box 116.1

Clinical uses of botulinum toxin: For its muscle relaxation effect, botulinum toxin in small doses is used for the treatment of conditions that produce muscle hyperactivity, for example, injection of the toxin into lower esophageal sphincter in achalasia cardia produces relaxation of the sphincter. In low doses, it is also used to decrease the facial wrinkles by causing relaxation of facial muscles.

Postsynaptic Mechanisms

The following events take place in postsynaptic cell.

1. Neurotransmitter binds with the receptors in the postsynaptic membrane and brings about conformational change in the receptor that either opens an ion channel or triggers a cascade of biochemical reactions that generate a second messenger, which in turn generates change in ionic permeability of the cell.

2. Some of the transmitter molecules diffuse away from the postsynaptic receptor that are cleared either by enzymatic degradation or taken back into the presynaptic cell by endocytosis (Application Box 116.3).

After the binding of the neurotransmitter with receptors, the ion channels in the postsynaptic membrane open up and movement of ions occurs. Depending on the ion (cation or anion) and the direction of their movement, the membrane potential of the postsynaptic membrane changes either towards depolarization or hyperpolarization. This change in membrane potential, also called synaptic potential, creates signal in the postsynaptic neuron.

Fig. 116.4: Priming and docking. Note the role of syntaxin and synaptobrevin

Synaptic Potential is of longer duration than an action potential. When it is excitatory, it can cause repeated firing of initial segment of the postsynaptic neuron. Synaptic potentials are of two types: excitatory postsynaptic potential (EPSP) and inhibitory postsynaptic potential (IPSP).
Excitatory Postsynaptic Potential

If the potential changes in the postsynaptic membrane during synaptic transmission is towards depolarization, the potential is called EPSP. For example, if initially the RMP was −70 mV, which becomes −60 mV during synaptic transmission, then it is EPSP (Fig. 116.5).

1. The latency of EPSP is about 0.5 ms. EPSP reaches its peak at 11.5 ms after the entry of afferent impulses into the spinal cord, which then declines exponentially. A single EPSP leads to a change of 0.5–2 mV only. Though the EPSP produced at one synaptic knob is small, EPSP at many synaptic knobs summate.
2. EPSP of optimum magnitude leads to excitation of the postsynaptic neuron.
3. During this potential, excitability of postsynaptic neuron increases to other stimuli, and therefore, the potential is called excitatory postsynaptic potential.
4. EPSPs are produced by transmitters like acetylcholine, nor-adrenaline etc.

Ionic Basis

EPSP is caused by opening of Na⁺ or Ca²⁺ ion channels in the postsynaptic membrane that results in influx of Na⁺ or Ca²⁺ and causes depolarization. EPSP is also caused by closure of K⁺ ion channels.

Slow EPSP

Slow EPSP has been observed in autonomic ganglia and cortical neurons. They have the latency of 100–500 ms, and they persist for several seconds. They are usually due to decreased K⁺ conductance.

Inhibitory Postsynaptic Potential

During synaptic transmission, if the potential of the postsynaptic membrane is carried towards hyperpolarization, then the potential is called IPSP (Fig. 116.5).

1. This is because the hyperpolarization leads to inhibition of the postsynaptic neuron. For example, if the RMP was −70 mV, and after transmission it becomes −80 mV, then the potential is IPSP, which is of −10 mV.
2. Actually, a very small change occurs due to an IPSP, i.e. about 0.5 mV. During this potential, excitability of postsynaptic neuron decreases to other stimuli, and therefore, the potential is called inhibitory postsynaptic potential.
3. IPSP, like EPSP is a local response and can be summed. IPSPs are caused by transmitters like GABA (gamma amino butyric acid), glycine, etc. IPSP reaches its peak at 11.5 ms after the application of stimulus, which then decreases exponentially.

Ionic Basis

IPSP is produced by opening of Cl⁻ channels (Cl⁻ enters into the cells along the concentration gradient). Opening of K⁺ channels that result in K⁺ efflux can produce IPSP. Closure of Na⁺ or Ca²⁺ channels also produce IPSP.

Slow IPSP

Slow IPSPs are also observed in autonomic ganglia and cortical neurons. They usually occur due to increased K⁺ conductance.

Reversal Potential

As it is known, the IPSP is mediated by Cl⁻ influx. However, in an experimental set up, Cl⁻ influx occurs only when the membrane potential is less than −70 mV, for example the RMP at −50 mV. At the membrane potential of −70 mV, influx or efflux of Cl⁻ across the membrane disappears, as the E_Cl is −70 mV. If the membrane potential becomes more negative, such as at RMP of −90 mV, Cl⁻ efflux takes place that makes the interior of the cell positive. This is called the reversal potential.

Genesis of Action Potential

The message arriving at postsynaptic neuron usually comes from a large number of presynaptic neurons. These neurons can end on any part of the soma and dendrites of the postsynaptic neuron.

1. In CNS, many excitatory and inhibitory inputs continuously and simultaneously arrive at the postsynaptic membrane. All the EPSPs or IPSPs produced at various postsynaptic membranes act together to summate electronically.
2. The soma of the neuron integrates these potentials and the algebraic sum of these depolarizing and hyperpolarizing potentials finally produce the change in resultant potential.
3. If the resultant potential is depolarizing and the change is about +15 mV, the firing level is reached that leads to genesis of a propagated spike potential.
4. However, a full phased action potential is generated only at initial segment, as this part of the neuron has the lowest threshold.
The action potential propagates in two directions, toward axon terminal down the axon (orthograde propagation) and towards the cell body (retrograde propagation). The propagation of action potential into the soma cleans the soma for subsequent renewal of postsynaptic activities.

**Role of Dendrites**
Dendrites are the extensions of soma.
1. They increase the surface area for presynaptic knobs to end on them. Thus, more the number of dendrites, better the integration of excitatory and inhibitory activities.
2. Number and pattern of dendritic spine change during development. A special type of neural protein called neurolignin controls synapse formation.
3. Alteration in dendritic spine occurs in learning and long-term potentiation (Application Box 116.4). It has been recently observed that the propagated action potentials are also initiated in few dendrites.
4. It has also been noted that protein synthesis, which usually occurs in soma, also occurs in ribosome in dendritic spine that can alter inputs from glutaminergic neurons.

**Application Box 116.4**
Change in dendritic spine occurs in motivation, learning and long-term memory. Pattern and arbor of dendritic spines can change in different conditions. New dendrites can appear and grow or they can also disappear even in minutes and hours. From the cell body, strands of mRNA move into the dendrites and cause dendritic structural change by influencing protein synthesis that alters the effects of individual synapses on the spine. Changes in dendritic spines have been observed to provide the physiological basis in motivation, learning and long-term memory.

**Transmission through an Electrical Synapse**
In electrical synapses, the pre- and post-synaptic membranes come close together to form gap junctions. Gap junctions are low resistance channels through which ions pass easily. Electrical activities of one neuron can pass to the other directly through gap junctions. There are sparse electrical synapses in CNS.

Transmission through electrical synapse differs from that of a chemical synapse by following ways:
1. EPSP generated in the postsynaptic neuron by electrical transmission has short latency than the EPSP generated in the chemical transmission.
2. In chemical synapse, the transmission is usually unidirectional, whereas in electrical synapse the transmission is bidirectional.
3. The signal is not magnified in electrical transmission. As magnification or modification of signal occurs in the chemical synapse, chemical synapses are superior to the electrical synapses in transmission of impulse.
4. The time taken for impulse to travel an electrical synapse is much less than the chemical synapse.

**Properties of Synapses**
The synapses or rather the synaptic transmissions exhibit the following properties:

**Law of Forward Conduction**
Through a synapse, impulse travels in one direction only i.e. from presynaptic to postsynaptic neuron. This is called law of forward conduction. However, transmission of impulse is bidirectional in electrical synapses.

**Synaptic Delay**
The time required for the impulse to be transmitted through the synapse is called synaptic delay.
1. It is about 0.5 ms. Synaptic delay occurs due to the time spent in entry of Ca$^{++}$ into the presynaptic knob, release of neurotransmitter into the synaptic cleft and for the action of neurotransmitter on the postsynaptic membrane to produce the postsynaptic potential.
2. The knowledge about synaptic delay helps to find out the number of synapses present in a neural pathway (monosynaptic, disynaptic or polysynaptic reflex arc).

**Law of Divergence and Convergence**
Divergence means one to many projections (Fig. 116.6B). This means information from one presynaptic neuron passes to many postsynaptic neurons.
Convergence means many to one projection, in which many presynaptic neurons project to a single postsynaptic neuron (Fig. 116.6A). Divergence and convergence are very common in CNS and form the physiological basis for summation and facilitation at synapses.
**Postsynaptic Potentials**

During synaptic transmission, if the potential of the postsynaptic membrane is carried towards depolarization, is called **excitatory postsynaptic potential** (EPSP) or, if the potential of the postsynaptic membrane is carried towards hyperpolarization, then it is called **inhibitory postsynaptic potential** (IPSP).

**Synaptic Inhibitions**

In CNS, neurons are highly interconnected, and therefore, any trivial stimulus would lead to an widespread activation of many neurons. **Synaptic inhibition prevents this explosive situation** by stabilizing the neurons and by preventing unnecessary spread of impulse.

There are two different types of synaptic inhibitions: direct inhibition vs. indirect inhibition, and feedback inhibition vs. feedforward inhibition.

**Direct and Indirect Inhibitions (Postsynaptic and Presynaptic Inhibitions)**

**Direct Inhibition**

When synaptic inhibition occurs through formation of IPSP on the postsynaptic neuron, is called **direct inhibition** as the postsynaptic neuron is directly inhibited (postsynaptic inhibition).

1. Example of direct inhibition is inhibition of anterior horn cells (AHC) by the descending inhibitory fibers in the spinal cord or by the afferent inputs originating from the Golgi tendon organs that influences AHC via an inhibitory interneuron.
2. In both these situations, there is release of inhibitory transmitter from presynaptic neurons (Fig. 116.7A).

**Indirect Inhibition**

Indirect inhibition occurs when the passage of impulse through a synapse is inhibited by another earlier impulse originating from a separate neuron terminating on presynaptic ending (Fig. 116.7B).

1. This inhibition occurs due to decreased release of neurotransmitter from the presynaptic nerve terminal (presynaptic inhibition).
2. Indirect inhibition could also be due to the effects of previous postsynaptic neuron discharge. For example, the neuron can be refractory to excitation because it is in its refractory period as it has just fired. During after-hyperpolarization cells are less excitable. In spinal neurons, after-hyperpolarization may be prolonged, especially after repeated firing.

**Mechanisms of Presynaptic Inhibition**: For presynaptic inhibition, three mechanisms of have been proposed.

1. **Activation of the presynaptic receptors facilitate Cl⁻ conductance**, which decreases the magnitude of the action potential reaching the excitatory ending. Thus Ca²⁺ entry is decreased and consequently the quantity of neurotransmitters released is reduced.
2. Activation of the presynaptic receptors also causes voltage-gated K⁺ channels to open. The resulting K⁺ efflux decreases the Ca²⁺ influx.
3. Direct inhibition of transmitter release can occur, independent of Ca²⁺ influx into the excitatory ending.

GABA was the first neurotransmitter shown to produce presynaptic inhibition. There are two GABA receptors: GABA_A and GABA_B. GABA acting through its GABA_A receptors produces presynaptic inhibition by increasing Cl⁻ conductance. Through GABA_B receptors, it produces presynaptic inhibition via a G protein that produces an increase in K⁺ conductance (Application Box 116.5). Other neurotransmitters also produce presynaptic inhibition by G protein-mediated effects on Ca²⁺ channels and K⁺ channels.

**Application Box 116.5**

**Baclofen causes presynaptic inhibition**: Baclofen, a GABA_B receptor agonist, is useful in the treatment of the spasticity of spinal cord injury and multiple sclerosis. It is administered intrathecally via an implanted pump. It inhibits neuronal transmission by causing presynaptic inhibition.

**Feedback and Feedforward Inhibitions**

**Feedback Inhibition**

Renshaw cell inhibition in the spinal cord is an example of feedback inhibition.

1. This is a **negative feedback inhibition** in which collateral from spinal motor neurons ends on an inhibitory interneuron, known as the **Renshaw cell**, which in turn inhibits the discharge of the same motor neuron (Fig. 116.8).
2. A collateral from Renshaw cell also inhibits neighboring motor neuron, which is called **lateral inhibition**.
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Feedforward Inhibition

The example of feedforward inhibition is the inhibition of Purkinje cell output by parallel fibers originating from granule cells in the cerebellum.

1. Mossy fiber inputs stimulate granule cell, which through its parallel fibers activates Purkinje cells. Output of Purkinje cell is inhibitory as neurotransmitter secreted by Purkinje cells is GABA.

2. Thus, stimulation of Mossy fiber-parallel fiber pathway finally activates the inhibitory output of Purkinje cells. This is called feedforward inhibition.

Synaptic Facilitation

This is the process by which transmission through a synapse is increased. It is usually caused by increase in the transmitter release from the presynaptic terminal. Therefore, this is also called presynaptic facilitation. Prolongation of action potential at presynaptic ending helps voltage gated Ca\(^{+}+)\) channel to remain open for a longer duration. This ensures more Ca\(^{+}+)\) influx that in turn increases transmitter release from the presynaptic ending. Thus, transmission through the synapse is facilitated.

Action of Serotonin: Serotonin released at an axon terminal causes synaptic facilitation by increasing intraneuronal cAMP levels. This induces phosphorylation of one group of K\(^{+}\) channels that closes the K\(^{+}\) channels. This slows the process of repolarization and prolongs the duration of action potential.

Summation

Summation literally means to add up. Since synaptic potentials are graded, individual potential changes can summate. The phenomenon of summation is seen in neuronal networks involving multiple synapses in which synapses are shared by many neurons. There are two types of summations: temporal summation and spatial summation.

Temporal Summation

In temporal summation, the same input stimulates the postsynaptic neuron repetitively in such a way that the second synaptic potential arrives before the postsynaptic cell recovers from the first synaptic potential. This happens because action potentials arrive in rapid succession at the presynaptic terminal, which in turn stimulates the postsynaptic membrane in quick succession so that resultant postsynaptic potential overlap in time. Thus, postsynaptic potential is elevated to the firing level.

Spatial Summation

In spatial summation, instead of repeated stimulation by the same input, two or more separate inputs arrive simultaneously at the postsynaptic membrane. In this situation, if the inputs have the same sign (either all are excitatory or all are inhibitory), the postsynaptic response evoked by all inputs becomes larger than response that would have occurred in response to their individual application. This type of summation is called spatial summation.

Thus, temporal summation occurs when a single presynaptic knob is stimulated repeatedly and spatial summation occurs when many synaptic knobs converging on one postsynaptic membrane are stimulated simultaneously.

Occlusion

When a presynaptic neuron (say A) fires, five postsynaptic neurons discharge and when another presynaptic neuron (say B) fires separately, another five postsynaptic neurons discharge. However, as two presynaptic neurons share their discharge zone (common discharge zone of A and B), simultaneous firing of A and B results in activation of eight postsynaptic neurons instead of ten (Fig. 116.9). This is called occlusion.

Habituation, Sensitization and Potentiation

The phenomena of habituation, sensitization and potentiation are also observed in synaptic transmission. In habituation, the synaptic transmission is decreased but in sensitization and potentiation, the transmission is increased.

Habituation

Habituation is the gradual decrease of transmitter release in response to repeated transmission of impulses. This is probably caused by prolonged inactivation of the Ca\(^{+}+)\) channels in the synaptic knob or due to decreased number of vesicles in the presynaptic terminal.

Sensitization

Sensitization occurs when a transmission is accompanied by a painful or unpleasant sensation. The response
increases more and more by the mechanism of presynaptic facilitation. Sensitization is short lived and is explained on the basis of increased $\text{Ca}^{++}$ entry into the presynaptic terminal.

**Potentiation**

Potentiation is enhanced synaptic transmission for a prolonged duration.

1. In post-tetanic potentiation, the duration is shorter in comparison to other potentiation called long-term potentiation. Post-tetanic potentiation is due to increased $\text{Ca}^{++}$ in the presynaptic knob in response to the arrival of a series of stimuli that causes increased transmitter release.

2. Long-term potentiation occurs due to increased $\text{Ca}^{++}$ in the postsynaptic neuron due to opening of NMDA (N-methyl-D-aspartate) channels (e.g. by glutamate) along with increase of intracellular proteins.

**Synaptic Plasticity**

There are several ways by which synaptic transmission is modified to suit the need of the body, and to learn to react against a particular stimulus in an appropriate manner. These changes are memorized for a variable period by the concerned cells that are involved in the process of learning and memory. This involves a long-term change in the synapses, which is also called synaptic plasticity.

**NEUROTRANSMITTERS**

Neurotransmitters are the chemical substances liberated at the nerve endings that help to transfer the message in the form of nerve impulse from the presynaptic neuron to postsynaptic neuron. Neurotransmitter substances are also released from neurons supplying a muscle or a gland. Chemicals secreted from neurons if enter the blood to circulate as hormones, the chemicals are called neurohormones, e.g. norepinephrine, ADH, GnRH, etc.

There is large number of chemicals in the nervous system that act as transmitters. However, a chemical to be labeled as a neurotransmitter should have the following criteria:

1. The chemical must be synthesized in the neuron concerned,
2. Should be stored in the presynaptic endings,
3. Should be released at the synapse,
4. Should have specific receptors on the postsynaptic membrane, and
5. Should be disposed by a suitable mechanism as soon as its action is over.

The chemicals that are liberated from the nerve endings directly on the target organs or secreted into the ECF are called neurosecretions. Chemicals liberated at the neuromuscular junctions are also called neurotransmitters. Another term, neuromodulator is used to mark the chemicals, which are used to modify the activities of the postsynaptic neurons. In the following discussion, the chemicals considered are neurotransmitters.

**Classification**

Neurotransmitters may be classified in different ways. The following classification is based on their chemical structure:

1. **Acetylcholine**
2. **Amines**
   - Norepinephrine, Epinephrine, Dopamine
   - Serotonin, Histamine
3. **Amino acids**
   - **Excitatory amino acids**
     - Glutamate, Aspartate
   - **Inhibitory amino acids**
     - Glycine, GABA
4. **Polypeptides**
   - ADH, Oxytocin
   - Tachykinins like Substance P
   - CRH, TRH, GnRH
   - Somatostatin
   - Endorphins, Enkephalins
   - CGRP, Neuropeptide-Y
   - Angiotensins
   - Activins, Inhibins

[Fig. 116.9: Mechanism of occlusion. Activation of presynaptic neuron A stimulates a set of five postsynaptic neurons and activation of presynaptic neuron B stimulates another set of five postsynaptic neurons, when A and B are activated separately. But, as two presynaptic neurons share their discharge zone (common area of A and B), simultaneous activation of A and B results in activation of eight postsynaptic neurons instead of ten. This is called occlusion.]
Acetylcholine

Distribution

Acetylcholine (Ach) is found as neurotransmitter in many parts of the nervous system, as given below.
1. All autonomic preganglionic neurons,  
2. Postganglionic parasympathetic neurons,  
3. Postganglionic sympathetic neurons supplying sweat glands and blood vessels of skeletal muscles,  
4. Axons of motor neurons arising from spinal cord (neuromuscular junctions),  
5. Many parts of the brain including Betz cells of the motor cortex and basal ganglia and  
6. Amacrine cells in retina.

Synthesis

Ach is synthesized by acetylation of choline in the presynaptic ending:

\[
\text{Choline + Acetyl-CoA} \rightarrow \text{Acetylcholine+CoA}
\]

Ach is then stored in the clear vesicles in the presynaptic endings. It is released by exocytosis initiated by Ca\(^{++}\) influx in response to action potential arriving at presynaptic nerve terminal. The Ach released into the synaptic cleft binds to the cholinoceptive receptors located on the postsynaptic membrane (or on motor end plates or on cells of target organs).

Receptors

The cholinergic receptors are of two types: nicotinic and muscarinic.

Nicotinic Receptors

These receptors behave like ligand gated ion channels but with some differences. The receptors at motor end plate but not at ganglia can be blocked by α bungarotoxin, whereas mecamylamine blocks receptors at the ganglia but not at the neuromuscular junction.
1. The nicotinic receptors are members of a superfamily of ligand gated ion channels that includes GABA\(_A\), glycine and few glutamate receptors.  
2. Each nicotinic receptor is made up of five subunits (α, β, γ, δ and ε) that form a central channel, which permits Na\(^+\) and other ions to pass through when the receptor is activated. There are 16 known subunits for these 5 subunits of nicotinic receptors.
3. These are: α\(_1\) - α\(_9\), β\(_1\) - β\(_3\), γ, δ and ε. In fetus, nicotinic receptor is made up of two α\(_1\), one β\(_2\), one γ and one δ subunits. In adults, the γ subunit is replaced by δ subunit, which decreases the time duration of channel opening, but increases the rate of conductance of ions through the channel.
4. The receptors in the autonomic ganglia usually contains α\(_4\) subunits in addition to other subunits. Nicotinic receptors in the brain are located in the postsynaptic membrane, especially in glutaminergic axon terminals.
5. A special characteristic of neuronal nicotinic receptors is that they are highly permeable to Ca\(^{++}\).

Muscarinic Receptors

These receptors are found in brain, involuntary muscles, glands, etc.
1. Five subtypes of muscarinic receptors such as M\(_1\), M\(_2\), M\(_3\), M\(_4\) and M\(_5\) have been described.  
2. Though specific blockers are available for each receptor subtypes, atropine blocks all.  
3. These receptors are serpentine membrane proteins coupled to G proteins, K\(^+\) channel, etc., for their intracellular activities.

In the synaptic cleft, Ach is rapidly hydrolyzed to choline and acetate by specific enzyme, the cholinesterase. The choline is taken up by the presynaptic nerve ending (reuptake) and reutilized for synthesis of new Ach molecule. For details of distribution and functions of acetylcholine in brain, refer Chapter 120.

Catecholamines

Catecholamines are epinephrine (adrenaline), norepinephrine (nor-adrenaline) and dopamine. All of them have a catechol nucleus (1, 2-dihydroxybenzene). They are found in the synapses in brain, spinal cord and peripheral nerves. Norepinephrine is mainly secreted by the postganglionic sympathetic endings and epinephrine by adrenal medulla.

The catecholamines are stored in dense core granules in the presynaptic terminals and are released by exocytosis. They bind to their respective receptors in the postsynaptic membrane.
1. The epinephrine and norepinephrine bind to the adrenergic receptors, all of which are serpentine membrane proteins.
2. The adrenergic receptors are divided into two main types: α and β. The α adrenergic receptors are of two subtypes: α\(_1\) and α\(_2\), and β adrenergic receptors are of three subtypes: β\(_1\), β\(_2\), and β\(_3\). These receptors are found not only on the postsynaptic membrane but also on presynaptic membrane (especially the α\(_2\) receptors).
3. The catecholaminergic receptors are present in almost all tissues of the body. The receptors bind with circulating transmitters or the transmitters secreted from the nerve endings.
4. The enzymes monoamine oxidase (MAO) and catechol-o-methyl transferase (COMT) metabolize epinephrine and norepinephrine respectively. Norepinephrine is also taken up by the presynaptic nerve endings (reuptake).

**Dopamine**

Dopamine is secreted by dopaminergic neurons and bind to dopaminergic receptors on the postsynaptic membrane, mostly in the synapses in brain. Nigrostriatal dopaminergic neurons in the basal ganglia are important in normal motor functions.

1. **Five types of dopamine receptors** have been described such as D₁, D₂, D₃, D₄ and D₅. All dopaminergic receptors are membrane proteins and many of them have subtypes.

2. It is observed that the number of D₂ and D₃ receptors increases in schizophrenia as the use of D₂ receptor blocker is very helpful in the treatment of schizophrenia, and D₂ receptor blockers also bind to D₄ receptors.

**Gamma Aminobutyric Acid**

Gamma aminobutyric acid (GABA) is a major inhibitory neurotransmitter in brain and retina.

**Synthesis and Metabolism**

It is formed from glutamate by decarboxylation by the action of enzyme glutamate decarboxylase. GABA is present in body fluids as β-aminobutyrate. It is metabolized mainly by transamination reaction by the enzyme GABA tansaminase to succinate in the Kreb cycle.

**Receptors**

GABA has **three receptors** called GABA₉, GABA₈ and GABA₇. GABA₉ and GABA₈ receptors are present in the brain and GABA₇ is present in retina.

1. GABA₉ and GABA₈ receptors are ion channels and have five subunits surrounding a pore similar to that of nicotinic receptor. These are membrane proteins that on activation lead to increased Cl⁻ conductance. Different tranquilizer drugs like benzodiazepines act on these receptors.

2. GABA₇ receptors are metabotropic receptors coupled to G proteins that on activation produce increased K⁺ conductance, decreased Ca²⁺ influx and decreased adenylyl cyclase activity.

**Functions**

GABA is a major inhibitory neurotransmitter in brain.

1. The actions of GABA such as increased Cl⁻ conductance, increased K⁺ conductance and decreased Ca²⁺ influx all lead to hyperpolarization of neuron, causing generation of IPSP.

2. Recently it has been observed that chronic low level stimulation of GABA receptor in brain is aided by GABA present in intestinal fluid, which controls neuronal discharge of billions of neurons in the brain by decreasing the signal to noise ratio. Also it is observed that GABA activity declines with age, which decreases the specificity retinal neuronal responses.

3. **Benzodiazepine drugs** that are used as sedatives, muscle relaxants, anticonvulsants and anti-anxiety medicine act by increasing Cl⁻ conductance via GABA₉ receptors. They bind to α subunit of GABA receptors. A second set of benzodiazepine receptors has been found in peripheral tissues and steroid secreting endocrine cells. These are called **peripheral benzodiazepine receptors** (PBR). PBR may be involved in steroid synthesis.

4. **Alcohol and barbiturates** also act partly by increasing Cl⁻ conductance through Cl⁻ channels (Clinical Box 116.2).

5. **Progesterone and deoxycorticosterone** in high doses induce sleep and act as anesthetics by acting through GABA₉ receptors.

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**Clinical Box 116.2**

Anesthetics act via GABA receptor: Alcohol, barbiturates and many volatile inhaled anesthetics increase Cl⁻ conductance by acting on GABA₉ and glycine receptors. Some anesthetics act by inhibiting NMDA and AMPA receptors instead of acting on GABA receptors. Local anesthetics produce anesthesia by blocking Na⁺ channels in peripheral nerves.

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**Glycine**

Glycine is both inhibitory and excitatory neurotransmitter. Glycine receptor is a pentamer having two subunits: the α subunit that binds with the ligand and the β subunit.

1. Glycine receptor is a Cl⁻ channel that increases chloride conductance and produces IPSP. Hence, glycine is mainly an inhibitory transmitter. It causes direct postsynaptic inhibition in brainstem and spinal cord.

2. However, it also acts as an excitatory transmitter in some parts of the brain where it influences the activity of NMDA (N-methyl-D-aspartate) receptors, which is bound to glutamate.

3. For example, in dorsal horn of spinal cord, glycine binds with NMDA receptor and facilitates pain transmission.

**Neurons causing direct inhibition**

Recently neurons causing direct inhibition have been categorized into 3 categories: neurons that secrete glycine, neurons that secrete GABA and neurons that secrete both glycine and GABA. GLYT2, the glycine transporter is present in neurons secreting glycine, and GAD, the GABA transporter is present in neurons secreting GABA.

**Glutamate**

Glutamate is the common excitatory neurotransmitter in CNS that causes about 75% of excitatory transmission in brain. It is formed from α-ketoglutarate in the Kreb’s cycle. It accumulates in synaptic vesicle by the transporter BNP₁. Glutamate released from nerve terminal is taken up by astrocytes that convert it first to glutamine and
then transfer glutamate to nerve ending where it is converted to glutamate. Glutamate is used for making lesion (Clinical Box 116.3).

**Clinical Box 116.3**

| Glutamate in excess produces lesion in brain: | Glutamate is removed by astrocytes from synapses. However, if glutamate accumulates in higher concentration, they act on cell bodies of the neuron and intensely increase Ca” influx into the cell that causes cell death. Thus, glutamate in higher concentration acts as an excitotoxin. Therefore, glutamate is used to produce excitotoxic lesion in the brain in experimental animals. This type of response is also seen in ischemia, hypoxia, hypoglycemia and trauma. This is one of the mechanisms of damage to brain tissue that occurs in stroke.

**Serotonin**

Serotonin or 5-hydroxytryptamine (5HT) is a transmitter in CNS, enteric nervous system and retina. It is an amine produced from the amino acid tryptophan by hydroxylation and decarboxylation. Serotonin acts through its seven receptors named as: 5HT₁, 5HT₂, 5HT₃, 5HT₄, 5HT₅ and 5HT₆. There are six types of 5HT₁ receptor subtypes like 5HT₁A, 5HT₁B, 5HT₁C, 5HT₁D, 5HT₁E and 5HT₁F. There are three receptor subtypes: 5HT₂A, 5HT₂B, 5HT₂C and 5HT₂D. There are two 5HT₃ receptors: 5HT₃A and 5HT₃B.

1. Most of the receptors act by activating adenylyl cyclase and phospholipase C.
2. Serotonergic transmission in the raphespinal pathway (nucleus raphe magnus to spinal cord neurons) is an important component of endogenous analgesia system.
3. Serotonin is a mood elevator and lysergic acid diethylamide (LSD), the hallucinogenic agent is a serotonin agonist, which acts through 5HT₂ receptors.
4. Serotonin is also a circulating chemical secreted by many cells (enterochromaffin cells, platelets, basophils, etc.) and takes part in physiological processes like contraction of smooth muscles, formation of platelet plug etc.

**Histamine**

Histamine acts as neurotransmitter as well as a circulating hormone. Histaminergic neurons are present mainly in hypothalamus and limbic system.

1. There are three receptor types: H₁, H₂ and H₃.
2. Histamine controls behavioral functions and also has important peripheral actions like smooth muscle contraction (leading to bronchospasms etc.), secretion of HCl in stomach, increased capillary permeability, arteriolar dilatation etc.

**Substance P and Other Tachykinins**

Substance P is a major tachykinin. It is a polypeptide containing 11 amino acids. It is found in many parts of CNS, intestine and peripheral nerves.

1. There are other tachykinins such as neurokinin A, neurokinin B, neurokinin K and neurotensin α.
2. There are 3 types of neurokinin receptors: NK-1, NK-2 and NK-3. NK-1 and NK-2 receptors are G protein coupled receptors.
3. NK-1 is the receptor for substance P, NK-2 is the receptor for neurokinin K and NK-3 is the receptor for neurokinin B.
4. Substance P activates phospholipase C and increases intracellular concentration of IP₃ and DAG.

**Functions of Substance P**

1. Substance P causes transmission of pain in the dorsal horn of spinal cord.
2. In nigrostriatal pathway in basal ganglia, it is a cotransmitter with dopamine and its concentration is proportionate to the concentration of dopamine.
3. In hypothalamus, it causes neuroendocrine modulation.
4. It mediates axon reflex in skin.
5. It mediates peristalsis in intestine.
6. It suppresses mode. Recently, NK-1 receptor antagonist has been used as antidepressant.

**Calcitonin-Gene Related Peptide**

Calcitonin-gene related polypeptide (CGRP) is a polypeptide, which has two forms: CGRP α and CGRP β. CGRP β is mainly present in GI tract. CGRP α is present along with substance P in primary sensory afferent neurons in spinal cord and in the neurons of blood vessel. It is also present in thalamus and median forebrain bundle. In spinal cord it modulates transmission of pain. It is potent vasodilator.

Though the same gene produces CGRP and calcitonin, CGRP has little calcium lowering effect and Calcitonin has little vasodilation effect.

**Neuropeptide Y**

Neuropeptide Y (NP-Y) is a polypeptide containing 36 amino acids distributed widely in brain and ANS. Cell bodies of NP-Y secreting neurons are highly concentrated in arcuate nucleus of hypothalamus that project to paraventricular nuclei. NP-Y is potent orexigenic neurotransmitter that profoundly increases food intake. NP-Y antagonists are used to decrease food intake. NP-Y acts through Y receptors that are G protein coupled. They are known 4 Y receptors for NP-Y: Y₁, Y₂, Y₄ and Y₅.

**Purines and Pyrimidines**

Known transmitters in this group are adenosine, ATP, uridine, and adenosine metabolites.

**Adenosine**

Adenosine is a general depressant in CNS and potent vasodilator in many regional circulations.

1. It has four receptor subtypes: A₁, A₂A, A₂B and A₃.
2. All the receptors are G protein coupled. Adenosine acting through A₂A and A₂B receptors increase cAMP formation and acting through A₁, and A₃ receptors decrease cAMP formation.
3. Theophylline and caffeine in tea and coffee exert stimulatory effect by blocking adenosine receptors.
4. A1 receptor antagonists are used in the treatment of stroke to decrease glutamate release and prevent excitotoxin effects of glutamate.

**ATP**

ATP has recently been emerged as a neurotransmitter in CNS. In ANS it causes rapid synaptic responses and in habenula it causes fast neurophysiological responses.

1. ATP acts through P2X and P2Y receptors that are widely distributed in the body.
2. P2X receptors are ligand-gated ion channel receptors that have seven subtypes: P2X1 to P2X7.
3. There are eight P2Y receptor subtypes: P2Y1, P2Y2, P2Y4, P2Y6, P2Y11, P2Y12, P2Y13 and P2Y14.
4. Presence of P2X receptors in dorsal horn of spinal cord indicates the role of ATP in transmission of sensory information in the spinal cord.

**Nitric Oxide**

Nitric oxide (NO), also called endothelium-derived relaxing factor (EDRF) is released from endothelial cell of blood vessels. It is also found as a transmitter in brain.

1. NO is formed from arginine by NO synthase. NO synthase requires NADPH, which is also called as NADPH diaphorase (NAD).
2. In brain it acts as a signal for postsynaptic neurons to communicate with presynaptic neurons in mediating long-term potentiation and long-term depressions.

**Co-transmitters**

The chemicals that are secreted along with the neurotransmitters are called co-transmitters. Examples are release of VIP with Ach or neuropeptide-Y with norepinephrine, etc. Sometimes, cotransmitters facilitate the actions of regular transmitters in synapses. Aspartate, enkephalins, prostaglandins, etc. are also described as transmitters in the CNS.

### CHAPTER SUMMARY

**Important to Know (Must Read)**

1. 'Describe the mechanism of synaptic transmission in CNS' or 'Describe the properties of synapses' may come as a **Long Question**.
2. Types of synapses, Mechanism of synaptic transmission, Presynaptic mechanisms, Synaptic receptor proteins, Synaptic potentials, EPSP & IPSP, Properties of synapses, Acetylcholine as a neurotransmitter may come as **Short Questions** in exam.
3. In **Viva**, examiner may ask... What are the types of synapses, What is structure of a synapse, What are the major steps of synaptic transmission, Details of presynaptic mechanism and post synaptic mechanisms, Synaptic receptor proteins and their function, What is docking and priming, Types of synaptic potentials, EPSP & IPSP; Properties of synapses, Types of synaptic inhibition with examples, Types of synaptic facilitation, Classify neurotransmitters, Receptors and functions of each neurotransmitter, what are the transmitters and what are their functions.
CHAPTER 117

Introduction to Sensory System and Physiology of Receptors

LEARNING OBJECTIVES

On completion of study of this chapter, the student **MUST** be able to:
1. Define sensation and appreciate the dimensions of sensation.
2. Define and classify receptor.
3. Appreciate the structure and functions of important receptors.
4. Understand the mechanism of receptor potential.
5. List and understand the properties of receptors.

The student **MAY** also be able to:
1. Describe the structure and functions of each receptor.
2. Describe the properties of receptors.

GENERAL PRINCIPLES

Sensory system makes us aware of our external and internal environments. The process of this awareness is initiated by the application of a stimulus.

1. The afferent endings (**the receptor**) sense the stimulus and then convert it into the action potential (**impulse**) by means of transduction.
2. The impulse is carried to the spinal cord through sensory nerves and from there to the brain by means of **ascending neurons**. In the central nervous system, the sensory information is processed in various centers and finally brings a change in behavior via efferent pathways.
3. **The sensory system consists** of the receptors that receive and transduce the stimulus into action potential in sensory neurons, the **afferent pathways** (sensory neurons) that transmit the stimulus in the form of action potential to the CNS, the **neurons in the CNS** that modulate sensory information and the **areas in the brain** that recognize the stimulus.

Sensation

The **basic recognition of a stimulus** is defined as sensation. Clinically, sensation is called **esthesis**, a Greek word, which means ‘feeling’. Accordingly absence of sensation is called **anesthesia**, abnormal sensation is called **parasthesia**, sensation of movement is called **kinesthesia**, etc.

**Types of Sensations**

Sensation is broadly classified into **three categories**: special sensation, visceral sensation, and somatic sensation.

**Somatic Sensations**

The somatic sensations (**somesthesia**) arise from receptors present on the body surface, in the body wall, muscles, tendons, bones, joints, and connective tissues. These include sensation of touch, pain, temperature, vibration, joint movement (proprioception), etc.

**Visceral Sensations**

Visceral sensations originate from stimulation of receptors in the **viscera**. Usually, receptors are located in the wall of the viscera (if the viscera have a wall) or in the **connective tissue** of the viscera. The visceral organs are present in the skull, thorax, abdomen and pelvis.

**Special Sensations**

The special sensations originate in the **special sense organs** and include vision, audition, olfaction, gustation (taste), and the vestibular senses. These are sensed by special sensory receptors present in eye, ear, nose and tongue.
Perception
Appreciation and interpretation of sensation is called perception. It involves first recognition and then comparison, discrimination and integration of the sensations.

Sensory Modulation
Sensation elicited by a stimulus differs in quality and intensity depending on the afferent pathways carrying the sensation, CNS areas activated by it and the nature of processing of information in the CNS areas. This may result in either accentuation or inhibition of the sensations. This type of control of sensation is known as sensory modulation.

Dimensions of Sensation
Sensation has several aspects like modality, intensity, affect and acuity.

Modality
The quality or type of sensation is known as its modality. For example, modality of tactile sensation is touch.

Intensity
This is the degree of perception of a stimulus. The sensation that spontaneously decreases in intensity quickly when the stimulus is applied for a little longer duration is said to be rapidly adapting, the sensation that decreases in intensity slowly is slowly adapting, and the sensation that does not change its intensity is said to be non-adapting. For example, pain is non-adapting whereas touch is rapidly adapting.

Affect
The emotional component of a sensation is called affect. The sensation that do not have emotional component is said to be neutral. The affect may be positive (when it evokes pleasant emotional response), or negative (when it evokes unpleasant emotional response). For example pain, which is associated with unpleasant sensation has negative affect.

Acuity
The precision of stimulus localization is called acuity. It varies with the concentration of receptors (innervation density) and the receptive field size of the area where the stimulus is applied and the size of the cortical area representing that somatic body part.

RECEPTORS
Definition
Receptors are transducers that convert various forms of energy in the environment into action potentials in sensory neurons.

Classification
Receptors are classified in various ways such as by function, by adequate stimulus or by location.

A. Based on Function
Receptors are classified into four categories: exteroceptors, interoceptors, proprioceptors and teleceptors.

Exteroceptors
These receptors are present in the skin and subcutaneous tissue and are concerned with a change in the external environment close to the body. These are present in the cutaneous sense organs.

Interoceptors
These are the receptors that detect change in the internal environment of the body. For example, the baroreceptors detecting change in blood pressure, the osmoreceptors detecting change in osmolality of the body fluids, etc.

Proprioceptors
These receptors provide information about the position of the body in space at any given time. Proprioceptors are mechanoreceptors present in the muscles, tendon and joints. Sense of position also depends on kinesthesia, which is the sensation of movements of body parts, i.e. dynamic movements and static position of the body.

Teleceptors
These are the receptors that receive stimuli or the sensation that are present far away from the body. For example, the auditory receptors that detects sound coming from a distance.

B. Based on Adequate Stimulus
There are different types of adequate stimuli (forms of energy). These are touch-pressure, cold-warmth, pain, chemical and photons. They are detected by the presence of various sense organs in the skin and the subcutaneous tissues, and in epithelia. The major receptors are mechanoreceptors, thermoreceptors, nociceptors, chemoreceptors and photoreceptors.
Mechanoreceptors
Mechanoreceptors respond to application of a mechanical stimulus; for example, touching or stroking the skin. The mechanoreceptors are broadly divided into three categories: expanded endings, encapsulated endings and naked nerve endings.

Expanded endings: These are Merkel’s disks and Ruffini endings.
Encapsulated endings: These are Pacinian corpuscles, Meissner’s corpuscles, and Krause’s end-bulbs.
Naked Nerve endings: The expanded and encapsulated endings are not very much essential for elicitation of the cutaneous sensations. All the four cutaneous sensory modalities can be elicited from the areas that contain only naked nerve endings.

Thermoreceptors
These receptors are sensitive to changes in the temperature of the skin. Accordingly, there are two types of thermoreceptors: the warmth receptor and the cold receptor. These receptors are active over a broad range of change in temperature.

Nociceptors
They respond to painful stimuli, the stimuli that are harmful (damages the tissue or threaten to produce damage) to the organism.
1. There are two types of nociceptors: Aδ-mechanical nociceptors and C-polymodal nociceptors.
   - Aδ-mechanical nociceptors respond to fast pain, for example, sharp pain due to pricking. They do not respond to pain activated by thermal or chemical stimuli.
   - On the other hand, C-polymodal nociceptors respond to several types noxious stimuli including thermal and chemical ones.

Chemoreceptors
Receptors that respond to chemical stimuli are chemoreceptors. Examples are taste receptors, olfactory receptors, etc.

Photoreceptors
Stimulus for photoreceptors is the light (photons). Example is rods and cones of retina.

C. Based on Location
Superficial: Receptors are located in the skin. Examples are touch and pressure receptors.
Deep: Receptors are present in muscles, bone, tendons etc.
Visceral: Receptors are located in the viscera. Example is visceral pain receptors.

Important Mechanoreceptors
Pacinian Corpuscles
These are mechanoreceptors located in the skin and deep tissues. They mediate the sensation of vibration and pressure.

Structure
Pacinian corpuscle consists of a body composed of concentric layers (lamellae) much like an onion. There are 20–70 lamellae in each corpuscle arranged concentrically (Fig. 117.1).

Dimensions
0.5–2 mm long (it is the largest mechanoreceptor) and 0.7 mm in diameter
The distal end of a primary afferent fiber penetrates into the concentric lamellae of Pacinian corpuscle. The cell body of this Aδ fiber lies in the dorsal root ganglion and the axon enters the spinal cord through dorsal root. The fiber is myelinated but the ending which lies in the concentric lamellae of the Pacinian corpuscle is unmyelinated.

Function
This is a rapidly adapting mechanoreceptor capable of receiving high frequency vibration. Therefore, it senses vibration and fine touch. It also senses pressure sensation. The distal end of the axon, not the lamellae of the Pacinian corpuscle is the actual transducer. Thus, when the concentric lamellae are stripped away, the force applied to the nerve ending continues to result in the genesis of receptor potential.

Meissner’s Corpuscle
This is a relatively large receptor located in the dermal ridges of the glabrous (hairless) skin.
Section 11: Neurophysiology

Structure
The dimensions are:
Length: 150 µm
 Diameter: 50 µm
This is an encapsulated ending having a single layered capsule (Fig. 117.2) into which the ending of the afferent nerve penetrates. The afferent fiber is myelinated but ending is unmyelinated. The characteristic of the ending is that it is branched to form a complex structure inside the corpuscle.

Function
This is a rapidly adapting receptor that senses vibration sensation. It also senses the sensation of contact (touch).

Merkel’s Disks
These are the most superficial mechanoreceptor present in the epidermis of glabrous and hairy skin.

Structure
They are formed by flattened terminations of primary afferent axons (Fig. 117.3). The fibers are myelinated but lose their myelin sheath before entering the epidermis.
1. The afferent ending branches to form cup like flattened structures called Merkel disks which synapses with the special modified cells, the Merkel cells.
2. The Merkel cells along with the Merkel disk are combinely called as Merkel apparatus or Iggo-dome receptor (because they elevate the epithelium of the skin).

Function
Merkel disks are slowly adapting mechanoreceptor that senses touch-pressure. The functions of the associated Merkel cells are not clearly known. Because the Merkel cells form a synaptic junction like structure with the Merkel disks, they are assigned the role of primary electro-mechanical transduction.

Ruffini Endings
These are smallest mechanoreceptors present in both glabrous and hairy skin.

Structure
These are expanded endings of the afferent Aβ nerve fibers. The myelinated nerve fiber and its unmyelinated endings branch out to form a complex expanded structure (Fig. 117.4).

Function
These are slowly adapting receptors that usually receive the sensation of crude touch.

Krause’s End-Bulb
These are rapidly adapting mechanoreceptors present in the dermis and are widely distributed in the skin.

Structure
These are encapsulated receptors into which the primary afferent nerve penetrates. The capsule is made up of modified cells that encircle the branched unmyelinated endings of Aβ myelinated nerve fiber (Fig. 117.5).

Function
These are rapidly adapting mechanoreceptors that mediate the sensation of touch and pressure.

Golgi-Mazzoni Corpuscle
These are first identified in the tendons and muscles, but afterward also found in skin.

Structure
These are encapsulated receptors consisting of 10–15 lamellae. They are 150–250 µm in diameter.
Chapter 117: Introduction to Sensory System and Physiology of Receptors

Function
These are rapidly adapting mechanoreceptors. The function is not exactly known but probably they are involved in the elicitation of the sensation of touch-pressure.

Hair Follicle Endings
The endings of the afferent fibers are often present adjacent to the hair follicles. The pressure on the hair distorts and stimulates the associated afferent endings in the hair follicle. Just by bending a hair (without touching skin) therefore touch sensation can be well elicited.

There are three types of hair follicles innervated by three morphologically distinct afferent endings: simple hair-follicle, nonsinus facial hair follicle and sinus hair follicle.

Simple Hair Follicle
These are hair follicles without erectile tissue. They are innervated by unmyelinated nerve terminals of many myelinated axons. The receptors are functionally rapidly adapting mechanoreceptors.

Sinus Hair Follicle
These are associated with hairs having large diameter and erectile tissue at the base surrounding the follicle. These follicles are rich in nerve supply. Slowly and rapidly adapting endings are present in these follicles. Thus, they have the properties of both slow and rapid adaptation. The hairs with these follicles are called vibrissae or tactile hairs.

Nonsinus Hair Follicle
These are associated with spray like terminals resembling Ruffini endings. They are present mainly in the skin of the face. They behave like slowly adapting mechanoreceptors.

Receptor Potential
Receptors convert environmental energy into action potential in the sensory nerves. On application of stimulus (change in energy), a potential change is observed in the receptor. This is usually a nonpropagated depolarizing potential that resembles an EPSP. This is called receptor potential or generator potential.

1. When the stimulus intensity is increased, the magnitude of the potential is proportionately increased. When it reaches about 10-15 mV, the action potential is generated in the sensory nerve (Fig. 117.6).

2. Pacinian corpuscles, due to their large size are the best-studied receptors for studying the genesis of receptor potential.

3. It has been experimentally observed that for the generation of receptor potential, the lamellae of the pacinian corpuscles are not required. It is the unmyelinated nerve terminal that generates the receptor potential (Figs. 117.7A to C).

4. It is further observed that the receptor potential forms action potential in the sensory nerve at the first node of Ranvier, which is located in the lamellae of the pacinian corpuscle.
Section 11: Neurophysiology

Mechanism of Genesis of Receptor Potential
The pressure on the Pacinian corpuscle causes mechanical distortion of the lamellas which in turn opens stretch sensitive sodium channels in the nerve terminal, the actual receptors. This increases influx of sodium ions that results in the production of receptor potential. At the first node of Ranvier, the action potential is generated. The magnitude and frequency of action potential is proportionate to the intensity of the stimulus.

Properties of Receptors

Specificity
Receptors are specific to a particular type of stimulus. For example, pain receptors are stimulated by application of a painful stimulus and touch receptors by tactile stimulation.

Adequate stimulus
The particular form of energy to which the receptor is most sensitive is called adequate stimulus. For example, adequate stimulus for rods and cones is the light.

Adaptation
When a stimulus of constant strength is applied continuously to a receptor, frequency of action potential in the sensory nerve decreases. This is called adaptation (desensitization). Based on this property, receptors are classified to two types: phasic and tonic receptors.

Phasic Receptors
The receptors that adapt rapidly are called phasic receptors. For example, touch and pressure receptors.

Tonic Receptors
The receptors that adapt slowly (or do not adapt at all) are called tonic receptors; for example, the baroreceptors present in the carotid sinus and aortic arch or the pain receptors.

Acuity
The precision of stimulus localization is called acuity. It depends on the number of receptors present in the area where the stimulus is applied.

Intensity
When a stimulus is applied, receptors discharge depending on the strength of stimulus. When the stimulus strength is less, receptors that are present close to the site of stimulus and receptors with low threshold, discharge. When the stimulus strength is more, the activated neurons fire at more rate, and receptors that are present some distance away from the stimulus, also discharge. This is called receptor recruitment.

Weber-Fechner Law
The magnitude of sensation felt is proportionate to the log of the intensity of the stimulus. This is called Weber-Fechner law.

Law of Projection
No matter where a specific sensory pathway is stimulated along its course to the cortex, the sensation formed is referred to the location of the receptors. This is called the law of projection.

1. This means, irrespective of the site of application of the stimulus in the sensory pathway, the sensation evoked is felt at the nerve endings (the receptors).
2. This forms the basis of phantom limb. In phantom limb phenomenon, the limb actually does not exist (as the limb has been amputated), but the patient complains that he feels pain or itch in the limb.

Doctrine of Specific Nerve Energy
The sensation evoked by a stimulus that generates impulse in the specific sensory pathway depends on the precise area of the brain that is eventually activated by the stimulus. This was first described by Muller, therefore, it is called Muller's doctrine of specific nerve energy.

1. For example, when the sensory fiber for touch is stimulated anywhere along its course to the cortex, the sensation evoked is touch.
2. It becomes possible because the pathways are specific and discreet from the sense organ to the cortex.
Chapter 117: Introduction to Sensory System and Physiology of Receptors

Important to Know (Must Read)

1. ‘Classify receptors and give the structure and functions of each, and describe the mechanism of genesis of receptor potential’ or ‘Describe the properties of receptors’ may come as a Long Question.
2. Receptor potential, Pacinian corpuscle, Properties of receptors may come as Short Questions in exam.
3. In Viva, examiner may ask… Define a receptor, What are the types of sensations, What are the dimensions of sensation, Classify receptors, What are the structure and functions of each receptor, What is a receptor potential, How is receptor potential generated in Pacinian corpuscle, What are the properties of receptors, About each property.

Scientist contributed

Johannes Müller (1801–1858) was a great teacher in Physiology at Bonn. He distinguished himself by expounding the principle of receptor properties and specific nerve effect, which is popularly known as Muller's doctrine of specific nerve energy. He also studied the mechanism of secretion, showing the relation of blood capillaries to glandular structures. He discovered chondrin and glutin, studied digestion and recognized cellular characteristics.

Sensory Unit and Receptive Field

1. Sensory unit is defined as a single sensory axon and all its peripheral branches.
2. Receptive field of a sensory unit is the area from which a stimulus produces a response in that unit. Usually, there is some degree of overlap of sensory units of the nearby regions.

Recruitment of Sensory Unit

Receptors spread over a large area are activated when the strength of stimulus is increased. This, not only activates the sense organs that are in direct contact with receptor but also the sensory units present in the surrounding areas. This is called recruitment of sensory units or receptor recruitment.
CHAPTER 118

Sensory Communication to Spinal Cord

Learning Objectives

On completion of study of this chapter, the student MUST be able to:
1. Name the afferent fibers in peripheral nerves and give their functions.
2. Understand the involvement of nerve in axotomy, demyelination, ischemia and compression.
3. Appreciate the arrangement of afferent and efferent neurons in the spinal cord.
4. Understand the importance of spinal cord laminae.

Somatic sensations are carried from the receptors via sensory fibers in the peripheral nerves to the spinal cord. They enter spinal cord via dorsal root (Fig. 118.1). From spinal cord, they are carried to the different centers in the brain in various ascending pathways. In this chapter, we discuss how sensory informations are conveyed from the peripheral structures to the spinal cord and how they are organized in spinal cord.

Peripheral Nerve

The somatosensory signals travel to the CNS via the peripheral nerves.
1. The peripheral nerves contain axons of the pseudounipolar sensory neurons. The cell bodies of these neurons are located in the dorsal root ganglia (DRG).
2. The sensory fibers have different diameter and conduction velocity depending on the receptors they innervate and the sensation carried by them.
3. Sensory nerve originating from a particular segment of a spinal cord innervates a specific dermatome (Fig. 118.2). That means, an area of the skin is supplied by a particular sensory nerve. This is called as the somatosensory map of the body.
4. Therefore, lesion of a particular segment of the spinal cord, or a particular dorsal root or a particular nerve results in loss of sensation from the corresponding skin area supplied by the nerve. This helps in localizing the nerve or the spinal cord segment involved in the disease process.

Afferent Fibers in Peripheral Nerves

The afferent fibers that transmit cutaneous sensations are broadly divided into three categories:
- Large myelinated Aα and Aβ fibers: These fibers transmit impulses generated in mechanoreceptors.
- Small myelinated Aδ fibers: These fibers transmit impulses from nociceptors (fast pain) and cold receptors. Some fibers also transmit impulses generated in mechanoreceptors.
- Small unmyelinated C fibers: These fibers primarily transmit slow pain, and temperature. Few fibers also transmit impulses generated in mechanoreceptors.
Clinical Importance

Peripheral nerves are affected by four major types of disease processes: axotomy, demyelination, ischemia and compression.

Axotomy

Axotomy is the transection of nerve fiber. This usually happens in cut injuries.

1. The part of the axon distal to axotomy degenerates, which is called Wallerian degeneration (for details, refer Chapter 24).
2. Degeneration also occurs in the proximal part of the neuron, which includes swelling of cell body, chromatolysis in soma, and retraction of axon from the point of injury.
3. During the process of regeneration, sprouting of new fibers starts from the proximal axon.
4. The peripheral axons regenerate faster than the central axons. Sensory fibers regenerate faster than the motor fibers.

Demyelination

Demyelination occurs in many neuropathies. Distal axonopathy (degeneration of distal part of peripheral neurons) occurs commonly in diabetes and chronic alcoholism. Usually, these diseases manifest in the form of sensory loss in the distal parts of the limbs (distal sensory neuropathy).

Ischemia

Ischemia for more than 15 minutes results in loss of touch, temperature and fast pain. Slow pain is usually not affected, as unmyelinated C fibers are less sensitive to hypoxia than myelinated fibers. Acute ischemia inhibits axoplasmic transport and impulse propagation, known as conduction block, whereas chronic ischemia results in degeneration of nerve fibers.

Compression

Compression of nerve fiber is called compressive neuropathy. In compressive neuropathy, large diameter fibers (fibers>5 µm in diameter) are affected early and more severely than the small unmyelinated fibers. Therefore, usually slow pain is not affected initially. Example of compressive neuropathy is entrapment of median nerve in carpal tunnel (carpal tunnel syndrome).

Note: In ischemia and compression, elimination of touch sensation occurs first and then the pain sensation,
whereas local anesthetics first eliminate the pain sensation and then the touch. Small unmyelinated fibers are more sensitive to local anesthetics but less sensitive to hypoxia and mechanical compression than the large diameter fibers.

**DORSAL ROOT**

The afferent fibers that travel in the peripheral nerve enter the spinal cord via the dorsal root.
1. The cell bodies of the afferent neurons are present in the DRG. The distal axons are the peripheral nerve fibers. The proximal axons are the dorsal root fibers that enter the spinal cord.
2. The region of the skin innervated by a single dorsal root ganglion is called a dermatome (Fig. 118.2).
3. Usually, there is no significant overlapping of the dermatomes of the adjacent ganglia. This helps in mapping (detecting the defect according to dermatomal innervation) the sensory loss of a particular segment of the spinal cord and thereby locating the disease process.
4. However, the distribution of innervations of muscles (myotomes) or bones (sclerotomes) by individual dorsal root ganglia is loose, as there is greater overlapping.

**Clinical Significance**

The dorsal roots may be affected by compression, transection or various other disease processes. The features of dorsal root involvement depend on severity and distribution of the fibers. For example, mild compression causes irritation whereas more compression causes damage to the dorsal root fibers.

**Compression of Dorsal Root**

Compression of dorsal root is not uncommon. This occurs usually from the herniation of an intervertebral disk. The injury produced by compression results in pain in all the affected dermatomes. Irritation of the dorsal root can produce paresthesia or hyperesthesia in the corresponding dermatome, whereas damage to the dorsal root can cause frank segmental anesthesia.

**Other Diseases Affecting Dorsal Root**

Dorsal root damage can also occur in following pathological processes.
- Traction
- Inflammation
- Ischemia
- Infection (Tabes dorsalis)
- Degeneration

**SPINAL CORD**

Afferent fibers enter spinal cord through the dorsal root and efferent fibers leave the spinal cord through the ventral root. Thus, dorsal root is sensory and ventral root is motor. This is called Bell-Magendie Law.
1. However, there are evidences that few afferent fibers are also present in ventral root.
2. The ventral root afferents are usually small unmyelinated nociceptors arising from visceral structures.

**Scientists contributed**

Charles Bell (1774–1842), a brilliant anatomist and neurosurgeon, Charles Bell demonstrated the motor functions of ventral spinal nerve roots. He was the first scientist to study and differentiate the sensory and motor functions of nerves roots. A similar observation was also noted by his contemporary Physiologist, Francois Magendie (described below). Hence, the theory proposing “Dorsal root of spinal cord is sensory and ventral root of spinal cord is motor in function” is popularly known as Bell-Magendie Law.

Francois Magendie (1783–1855) a contemporary of Bell, clarified the functions of spinal nerve roots. He had analyzed the phenomena of vomiting, mechanism of absorption, pharmacology of localization of drug action, noted the process of anaphylaxis and studied pathology as part of physiology.

**Spinal Connections**

Afferent fibers (the first order of neurons of sensory pathway), after entering the spinal cord may have the following three destinations (Fig 118.3):
1. They ascend directly in the dorsal column of the spinal cord (without relay on second order neuron) as a major ascending tract (dorsal column pathway) to reach the second order of neuron in the medulla.
2. They terminate in the dorsal horn and relay on the second order of neuron in the same segment and the same side of the spinal cord. The second order neuron then crosses to the opposite side and ascend in the anterolateral system.
3. They may ascend or descend few segments in the spinal cord before contacting the second order of neurons.
4. Many afferent neurons originating from muscle spindle, Golgi tendon organs or joint receptors mostly directly or sometimes indirectly contact the corresponding motor neurons in the anterior horn cells.
5. Some afferent fibers also make connections with the interneurons present in the spinal cord segment or with the terminals of the fibers in the descending pathways originating from different areas of the brain.

**Somatotopic Organization**

Fibers are somatotopically (somatotopic means representing a body part) arranged in the spinal cord. The afferent fibers arising from distal part of the extremities and the
ventral surface of the trunk terminate in or occupy the medial part of the spinal cord. The fibers coming from the proximal part of the extremities and the dorsal body surface terminate laterally in the spinal cord.

**Spinal Cord Laminae**

There are different laminae in the dorsal and ventral horns of the spinal cord. The laminae in the dorsal horn are called **sensory laminae** as they accommodate the afferent fibers.

1. **Sensory laminae** are lamina I to VI. The laminae in the ventral horn are called **motor laminae** as they accommodate efferent fibers.
2. The **motor lamina** is mainly lamina IX. However laminae VII and VIII also contribute to motor laminae, but contain mainly interneurons.
3. Lamina X is the **intercommissural lamina**.

The spinal laminae were first described by Rexed in 1954. Therefore, this cytoarchitectural arrangement in the spinal cord is known as **Rexed laminae**. The nerve fibers with different diameters terminate preferentially in the different sensory laminae in the dorsal horn of the spinal cord (Fig 118.4).

**Lamina I**

Lamina I receives small nociceptive Aδ and C fiber inputs. Therefore, this lamina responds primarily to the **noxious stimuli**. The cells of lamina I project their axons to a variety of spinal and supraspinal nuclei. They send axons to lateral cervical nucleus, dorsal column nuclei, thalamus, etc.

**Lamina II**

Laminae II is known as **substantia gelatinosa**. It receives primarily C fiber inputs. Thus, most of the lamina II cells respond to nociceptive or strong mechanical stimuli. The axons of laminae II terminates locally, a few neurons project to the other segments of the spinal cord.

**Laminae III–VI**

These laminas mainly receive myelinated A fiber inputs. These neurons respond to **fine touch, vibration and proprioception**. The axons of these cells ascend up in the somatosensory pathway in the dorsal column. In addition, lamina V receives inputs from nociceptors.

**Descending Influences on the Spinal Cord Laminae**

The descending influences come primarily from the **cortex** via corticospinal tract and from the **brainstem** via extrapyramidal tracts.

1. These descending tracts primarily terminate (directly or indirectly) on the motor neurons located in the ventral horn (anterior horn cells). However, a significant number of descending fibers also terminate on the cells of the dorsal horn laminae (the sensory laminae).
2. The main purpose of this termination of the descending tract is to bring about **sensory-motor coordination** or to modify the sensory input entering into the central nervous system according to the motor need of the body. This provides a feedback for sensory motor regulations.

3. The following are the sites of termination of the descending tracts in the sensory laminae, in addition to their terminations on motor neurons and other spinal neurons:

<table>
<thead>
<tr>
<th>Tracts</th>
<th>Terminations</th>
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<tbody>
<tr>
<td>1. Corticospinal</td>
<td>III, IV, V, VI</td>
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<tr>
<td>2. Raphespinal</td>
<td>I, II, V, VI, VII</td>
</tr>
<tr>
<td>3. Medullary reticulospinal</td>
<td>I, II, V, VI, VII</td>
</tr>
<tr>
<td>4. Pontine reticulospinal</td>
<td>V, VII, VIII</td>
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**CHAPTER SUMMARY**

**Key Concepts**

1. The fibers in the dorsal column are sensory fibers, having cell bodies in DRG. Invariably they terminate on ascending sensory neurons or on the segmental motor neurons.

2. Sensory fibers originating from one spinal cord segment have specific dermatomal distribution.

**Important to Know (Must Read)**

1. **Long Questions** are not asked from this chapter.
2. **Short Questions** are usually not asked from this chapter.
3. In **Viva**, examiner may ask... Types of sensory neurons in peripheral nerves, What is axotomy and what changes occur in a neuron following axotomy, What are the spinal cord laminae and how are they arranged, How a primary sensory afferent terminate in spinal cord.
Learning Objectives

On completion of study of this chapter, the student **MUST** be able to:
1. Appreciate the arrangement of neurons in ascending pathways.
2. List the sensations carried in dorsal column pathway and anterolateral system.
3. Draw a labeled schematic diagram to trace the pathway for dorsal column and anterolateral system.
4. Understand the physiology of important ascending pathways.
5. Define various abnormalities of sensations and give their common causes.

The student **MAY** also be able to:
1. Describe the connections and functions of ascending pathways.
2. Describe the connections and functions of dorsal column nuclei.
3. Describe the physiology of each sensation.
4. Explain the physiological basis of abnormalities of dorsal column sensations.

General Aspects

Arrangement of Neurons

The ascending pathways carry sensations from the peripheral structures to the different areas in the brain, mainly via thalamus to the sensory cortex. These pathways are simply a *set of neurons arranged in series*. Usually, they have **three sets of neurons**: the first order, the second order, and the third order of neurons.

1. These neurons are arranged sequentially in the central nervous system.
2. Some of the pathways also have the higher order of neurons (fourth order neurons) for further processing of information in other parts of the cerebral cortex.

First Order of Neurons

These are **primary afferent neurons**.

1. They **start from the receptor** and travel in the peripheral nerves to reach the spinal cord via the dorsal root.
2. **Cell bodies are located in the DRG**.
3. After entering the spinal cord, they terminate on the second order of neurons in the spinal cord laminae itself or ascend up in dorsal column (refer Fig. 118.3; Chapter 118) to terminate on the second order of neurons in the medulla.

Second Order of Neurons

They are either **located in the spinal cord** or **in the brainstem**.

1. The primary function of these neurons is to ***transmit impulse from the first order of neurons to the thalamus***.
2. As a general rule, the **second order of neurons typically cross the midline** (either in the spinal cord or in the medulla).
3. Thus, these neurons transmit the impulses originating in one side of the body to the opposite side of the thalamus.

Third Order of Neurons

These neurons **originate from the specific nuclei in the thalamus** and terminate in the specific areas **in the sensory cortex**.

1. Sensations transmitted from different parts of body are relayed in the thalamus before being projected into the cortex.
2. Therefore, thalamus is considered as the **main sensory relay center** in the brain.
Higher (Fourth) Order of Neurons
These neurons are usually not included in the sensory pathways. However, sometimes a higher order of neuron originates from the terminals of third order of neurons in the sensory cortex and project to other areas of the brain, especially to the cortical sensory association area, for further processing of the sensory information.

Classification
The ascending pathways are typically divided into three categories: dorsal column pathways, anterolateral systems and other ascending pathways.

Dorsal Column Pathways
Fibers ascending in the dorsal column of the spinal cord are included in this pathway. This is also called lemniscal system as fibers occupy medial lemniscus in the brainstem.

Dorsal column pathways transmit following sensations:
1. Fine touch
2. Vibration
3. Proprioception
4. Tactile localization
5. Tactile discrimination
6. Stereognosis

Anterolateral Systems
It is divided into two parts: anterior spinothalamic tract and lateral spinothalamic tract.
1. The anterior spinothalamic tract carries the sensation of crude touch.
2. The lateral spinothalamic tract carries the sensation of pain and temperature.

Other Ascending Pathways
These include many other ascending pathways such as:
1. Spinocerebellar tract (dorsal and ventral)
2. Spinoreticulothalamic tract
3. Spinocervicothalamic tract

First Order of Neuron
Neurons arriving from lower extremity and lower part of the trunk ascend up in the gracile fasciculus, whereas the neurons arriving from upper extremity and upper part of the trunk ascend in the cuneate fasciculus.
1. The gracile fasciculus is located medially in the spinal cord and carries sensations from hindlimb and trunk.
2. The cuneate fasciculus is situated laterally that transmits impulses from upper limb and upper part of the trunk.
3. The first order of neurons terminate in the nucleus gracilis and nucleus cuneatus in the medulla.
Second Order of Neuron

The cell bodies of these neurons are present in the nucleus gracilis and cuneatus in the medulla.
1. These two nuclei are known as the dorsal column nuclei.
2. The fibers originating from these nuclei cross the midline and pass on to the opposite side in the medulla and ascend up in the medial lemniscus to reach thalamus.
3. The second order of neurons, thus transmit impulses to the contralateral thalamus.

Dorsal Column Nuclei

There is a somatotopic organization in the dorsal column nuclei with face placed laterally and trunk and hind limb medially. Recently, two cell types have been described in dorsal column nuclei. Accordingly, the dorsal column nuclei are divided into two different zones: cluster and non-cluster zones.

Cluster Region

This is the main part of the dorsal column nuclei in which cells are arranged in clusters.
1. This region receives direct input from the fibers in the dorsal column pathways.
2. The second order of neurons from this region cross over to the opposite side to reach thalamus.
3. This region informs the thalamus and cortex about the sensory stimulation.

Non-Cluster Region

The non-cluster region is present more rostrally in the dorsal column nuclei.
1. This region receives inputs from descending fibers from the cortex and from fibers in the dorsal column pathways.
2. The projection from this region is mainly to the non-thalamic areas like cerebellum, tectum, pretectum, inferior olive, red nucleus and areas that are involved in motor control.
3. Thus, main function of this region is to provide direct sensory input to the different motor areas of the brain to bring about immediate regulation of movement by appropriate sensory feedback.

Third Order of Neuron

These neurons originate from the specific nucleus (the VPL nucleus) in the thalamus. The neurons project to the somatosensory areas of the cerebral cortex.

ANTEROLATERAL SYSTEM

Phylogenetically, this is older than the dorsal column system. It is divided into anterior spinothalamic tract that carries the sensation of crude touch, and lateral spinothalamic tract that carries the sensation of pain and temperature (Fig. 119.2).

First Order of Neuron

These are primarily the afferent fibers originating from nociceptors, thermoreceptors, and mechanoreceptors.
1. The fibers enter the spinal cord through dorsal root and the cell bodies are present in the DRG.
2. In the spinal cord, fibers terminate on the second order of neurons that are present on the same side of the dorsal horn of the spinal cord.
3. The cell bodies of second order of neurons are located mainly in the laminae I, II and V in the dorsal horn.

Second Order of Neuron

The cell bodies of these neurons are present in the dorsal horn of the spinal cord.
1. The axons cross the midline in the same spinal segment and ascend up in the opposite side of the anterolateral funiculus to reach the thalamus.

2. The fibers carrying the sensation of crude touch are placed anteriorly and therefore called anterior or ventral spinothalamic tract.

3. The fibers carrying the sensations of pain and temperature are placed laterally and therefore called lateral spinothalamic tract.

**Third Order of Neuron**
The neurons originate from the VPL, midline and intralaminar nuclei of the thalamus and project to the specific areas in the sensory cortex.

**PATHWAYS FOR SPECIFIC SENSATIONS**

**Touch**

**Receptors**
Touch can be elicited from the skin areas containing no specialized receptors. Thus, it is observed that the free nerve endings mediate touch sensation.

1. However, it has been known that Meissner’s corpuscle and pacinian corpuscles are rapidly adapting touch receptors, whereas Merkel disk and Ruffini endings are slowly adapting touch receptors.

2. The Na⁺ channel BNC1 is closely linked with touch receptors. This ion channel belongs to a group of proteins called degenerins that if hyperexpressed cause degeneration of the neurons.

3. The receptors are activated by mechanical pressure.

**Distribution of Receptors**
Touch receptors are abundantly present in hands especially in fingertips, and lips, and are less in number in the proximal part of the limbs and trunk of the body.

1. Receptors are very less on back of the trunk. Usually, the receptors are present in more numbers around the hair follicles. Therefore, movement of hair is a potent stimulus to elicit touch sensation.

2. They are also present in skin and subcutaneous tissues of the hairless areas.

**Pathway**
The fibers carry the touch sensations are Aβ fibers.

1. The fibers ascend in two pathways: the dorsal column pathway for the fine touch and the anterior spinothalamic tract for the crude touch (for details, see above).

2. The touch sensation carried in the dorsal column is well localized and have better discriminative aspects, whereas, the touch sensation carried in the anterolateral system is poorly localized and has less discriminative aspects.

**Proprioception and Kinesthesia**

**Definitions**

1. **Proprioception** is defined as the afferent input arising from stimulation of muscle, tendon, and joint mechanoreceptors that inform about the movement of joints and body parts in space.

2. **Kinesthesia** is defined as the sensation arising from movement of the body parts in relation to one another.

3. These two terms are used to describe the sense of position and movement of the parts of the body. This excludes the sense of position and orientation of the body obtained by the stimulation of visual and vestibular receptors.

**Receptors**
The receptors are slowly adapting spray endings in the joints and muscles that resemble the Golgi tendon organs. The pacinian corpuscles in the synovia and ligaments are also included in the proprioceptors. The touch receptors in the skin overlaying the joint may also be the part of the receptors.

**Pathway**
Proprioception (sensation of movement of body parts due to joint movements) is transmitted in the dorsal column of the spinal cord. From medulla (nucleus gracilis and cuneatus) a significant proprioceptive projection goes to the cerebellum. Therefore, diseases affecting dorsal column produce ataxia, as they interrupt the fibers to cerebellum.

**Vibration**
Application of mechanical stimuli oscillating rapidly from 2 to 400 Hz elicits this sensation, which is described as trembling or vibrating.

1. Ability to feel mechanical vibration is also called pallesthesia.

2. The sensation elicited in the lower range of this frequency is flutter and at higher frequency range is sense of vibration.

3. The sense of vibration is appreciated when a vibrating tuning fork is applied to the skin especially on the bony prominences.

4. Vibratory sense is a composite sensation comprising of touch and rapid alterations of deep-pressure sense.

**Receptors**
The receptors are touch receptors that are rapidly adapting, especially Pacinian corpuscles.

**Pathway**
Sense of vibration is carried in the dorsal columns.
Clinical Importance

1. Vibratory sensibility and proprioception are closely related. The diseases that decrease proprioception also decrease vibratory sensibility and vice versa.

2. In cortical lesions, proprioception and sense of vibration are affected early and impaired severely.

3. Depression of the threshold for vibratory sensibility is an early symptom of degeneration of the spinal cord involving the dorsal column. Such degenerations are seen in chronic diabetes mellitus, vitamin deficiencies, especially deficiency of vitamin B<sub>12</sub> (pernicious anemia) and early part of tebes dorsalis.

Two-Point Discrimination

The minimal distance that permits two-touch stimulus to be perceived as two separate points, when applied simultaneously on the skin, is called as two-point discrimination. Two-point discrimination threshold is a measure of tactile acuity.

Receptors

The receptors are fine touch receptors. However, identifying two points depends on the intactness of the cortex.

1. The magnitude of two-point threshold is different in different body parts.

2. The body parts with abundant touch receptors have the smallest magnitude. For example, on the back the two points must be separated 65 mm or more to be distinguished as separate points, whereas on the finger tips this distance is reduced to as low as 3 mm.

3. This also depends on the degree of representation of body parts in the sensory cortex.

Pathway

The sense of two-point discrimination is carried by dorsal column pathway.

Clinical Significance

This sensation is abolished when cortex is damaged. Two-point discrimination, tactile localization and stereognosis are severely impaired in cortical lesions as they are integrated in cortex. Therefore, they are called cortical sensations.

Stereognosis

The ability to identify known objects by handling them with eyes closed is called stereognosis.

Receptors

Receptors are touch and pressure receptors.

Pathway

This sensation is transmitted by dorsal column pathway to the brain.

Clinical Significance

Inability to identify the object by touch is called astereognosis or tactile agnosia. Inability to identify an object by sight is called visual agnosia, inability to identify sounds or words is called auditory agnosia and inability to identify position of limb is called positional agnosia.

1. Stereognosis is one of the important cortical sensations. Therefore, impaired stereognosis is an early sign of cortical pathology, which may occur with intact touch and pressure sensations.

2. This is also compromised in diseases that affect dorsal column of the spinal cord.

Abnormalities of Tactile Sensations

Anesthesia

Definition

Anesthesia means complete loss of all forms of sensations. Hypesthesia refers to partial loss of sensations. Loss of a particular sensation is depicted specifically with a prefix; for example, thermoanesthesia indicates loss of temperature sensation or pallanesthesia means loss of vibratory sense.

Causes

A. Hypesthesia

This is usually seen in central lesions like lesions at thalamus, internal capsule and cortex. It affects the distal parts of the limbs more than the peripheral parts.

B. Complete anesthesia

Usually occurs in peripheral nerve lesions. The commonest cause is leprosy. Lesion of peripheral nerve results in anesthesia corresponding to the distribution of sensory fibers. Complete anesthesia also occurs in complete transection of the spinal cord where anesthesia is seen in the limbs and trunk below the level of the lesion.

Dissociated Anesthesia

Definition

When the sensation of pain and temperature is lost with the preservation of touch, the condition is known as dissociated anesthesia.

Causes

1. Syringomyelia

2. Intramedullary tumors

3. Brainstem lesions (syringobulbia)

4. Thrombosis of the posterior inferior cerebellar artery.

Physiological Basis

Dissociated anesthesia occurs in diseases when spinal cord grey matter surrounding the central canal is damaged.
1. The fibers of dorsal column pathway are spared in the disease process as they are placed in the periphery of the spinal cord. Therefore, tactile sensibility is preserved.

2. The fibers of spinothalamic tract (that carry the sensation of pain and temperature) while crossing over to the opposite side in the spinal cord are placed close to the central canal.

3. These fibers are damaged in the disease process that results in loss of pain and temperature.

Hemianesthesia
Definition: Loss of sensations that affect face, arm, trunk and leg of one side of the body is called hemianesthesia.

Causes: Usually seen in lesions of the thalamus, internal capsule or cortex of contralateral side.

Hyperesthesia
When the response to the sensory stimulation is exaggerated, the condition is called hyperesthesia. The increased sensitivity to various stimuli indicates heightened activity of sensory apparatus like increased receptor sensitivity. It is seen in thalamic lesions.

Hyperpathia
Exaggerated perception of sensations is called hyperpathia. It is interchangeably used for hyperesthesia.

Alloesthesia
Definition
When a tactile or a painful stimulus delivered on the side of hemisensory loss is experienced on the corresponding area of the opposite side, is called alloesthesia or allesthesias.

Causes
1. Lesion of right side putamen (usually that occurs due to putamen hemorrhage)
2. Anterolateral lesion of the cervical spinal cord (this may be due to existence of few uncrossed spinothalamic fibers).

Paresthesia
Definition
When the sensations are abnormal and distressing (touch may produce an unpleasant sensation almost amounting to pain) the condition is called paresthesia or dysesthesia. This may occur in the form of pricking, numbness, or band like sensations around the trunk.

Causes
1. Nerve compression: This is the commonest cause of paresthesia. It occurs when peripheral nerves are stretched or subjected to pressure. The commonest example is the experience of paresthesia (numbness and pricking) when sitting posture is maintained for a longer time with legs crossed.
2. Spinal tumors
3. Subacute combined degeneration of spinal cord
4. Disseminated sclerosis
5. Thalamic lesions

Agraphestesia
Definition
Loss of graphesthesia, i.e. the loss of sense of cutaneous localization and figure writing (sense of writing figures on the body). Graphestesia is the function of posterior column.

Cause
This is commonly seen in lesion of the sensory cortex.

Astereognosis
Definition
Inability to identify a known object by palpation with eyes closed even though the primary tactile sensibility is intact.

Cause
It is seen in cortical disease. However, it should be differentiated from stereoaesthesia, in which lesions of the spinal cord or brainstem interrupt proprioceptive and tactile sensations.

Abnormalities of Proprioceptive Sensations
Proprioceptive sensation means the sensation of joint movement, or the sense of position of different parts of the body in space. The sense of vibration is closely linked to proprioceptive sensation.

The loss of proprioceptive sensation can occur without loss of other sensations. It occurs in lesions of the posterior column.

It is typically seen in:
1. Tabes dorsalis
2. Subacute combined degeneration of spinal cord
3. Proprioception and vibration sense are severely affected in lesion of the sensory cortex

Romberg Sign
Inability to maintain a balanced standing position with feet together and eyes closed is called Romberg sign. This displays the lack of sense of position in both the legs. The patient should be tested in bare-foot. This is called sensory ataxia. However, ataxia also occurs in cerebellar disease and vestibulopathy, and should be differentiated from posterior column lesion.
CHAPTER SUMMARY

KEY CONCEPTS

1. Ascending pathways carry specific sensations in specific fiber systems.
2. The fibers for dorsal column pathways ascend up in the same side of spinal cord. Therefore, lesions in spinal cord affect the sensation of the same side.
3. The fibers for anterolateral system ascend up in the opposite side of spinal cord. Therefore, lesions in spinal cord affect the sensation of the opposite side.

Important to Know (Must Read)

1. With the help of a labeled schematic diagram, trace the pathway for dorsal column sensations, and describe the physiology of touch sensations; and ‘With the help of a labeled schematic diagram, trace the pathway for crude touch and pressure, and give a note on dissociated anesthesia’ may come as Long Questions.
2. Dorsal column nuclei, Dorsal column pathways, Anterolateral system, Dissociated anesthesia, may come as Short Questions.
3. In Viva, examiner may ask… Name the sensations carried in dorsal column pathways, Name the sensations carried in anterolateral system, Trace the pathway for each dorsal column sensation, Trace the pathway for crude touch, What are the receptors for various sensations and their pathways, Define anesthesia and what are the causes, Define dissociated anesthesia and what are the causes, Define hyperesthesia and what are the causes, Define alloesthesia and what are the causes, Define paraesthesia and what are the causes, Define agraphesthesia and what are the causes, Define astereognosis and what are the causes, What is Rhomberg sign.
CHAPTER 120
Physiology of Pain, Itch and Temperature

Learning Objectives
On completion of study of this chapter, the student MUST be able to:
1. Define and classify pain.
2. Name nociceptors and give their distribution and properties.
3. Draw labeled diagrams of pain pathways.
4. List the differences between somatic and visceral pain.
5. Define referred pain and understand its theories.
6. Understand the mechanism of pain recognition and perception.
7. Describe the endogenous analgesia systems.
8. Understand the physiology of itch and temperature.
The student MAY also be able to:
1. Explain the differences between somatic and visceral pain.
2. Explain the theories of referred pain.

PAIN

General Concepts
Generally, pain is the earliest indication of morbidity. Physiologically, pain is a protective phenomenon of the nature. Experience of pain, with or without a disease, is almost universal. Pain, either due to punishment or due to natural injury, leaves a scar in the memory. Pain is expressed in different terms and languages. Usually, it is described like throbbing, burning, piercing, excruciating, lacerating, aching, etc. However, often it is difficult to describe the exact feeling of pain. It is said that pain is better felt than expressed.

Definition
Pain is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage. This is the definition of pain given by International Association for the Study of Pain (IASP).

Nociceptive Stimuli
Stimulus that elicits pain is called noxious or nociceptive stimulus. Nociceptive means the one, which is potentially damaging. Thus, a stimulus that is capable of damaging or causing harm to the tissue is a nociceptive stimulus and the sensation elicited is called nociception (pain sensation).

Nociceptive stimuli are different for different tissues, as listed below:
- For skin: Pricking, cutting, crushing, burning, freezing, etc.
- For GIT: Inflammation of mucosa, distension or spasm of smooth muscle and traction on the mesenteric attachment.
- In skeletal muscle: Ischemia, necrosis, hemorrhage, injection of irritating solution and injury of the connective tissue sheath.
- In cardiac muscle: Ischemia to or inflammation of the muscle.
- In joints: Inflammation of synovial membrane, exposure of synovial membrane to hypertonic saline and stretching or tearing of ligaments around the joint.
- For blood vessels: Artery or vein pierced by needle, inflammation of artery or vein, obstruction of artery or vein as occurs in thrombotic or embolic occlusion, excessive arterial pulsation as seen in migraine, etc.
- In brain: Traction on cerebral arteries or meningeal structures.
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Types of Pain

**Fast Pain vs Slow Pain**
A painful stimulus first elicits a sharp-localized pain, which is followed by diffuse-dull pain. The first component is called fast pain or first pain and the second component is called the slow pain or second pain. The fast pain is transmitted by Aδ fibers and the slow pain is transmitted by C fibers.

**Superficial Pain vs Deep Pain**
The pain elicited in the superficial structures like skin and subcutaneous tissues is the superficial pain, which is usually the fast pain. The pain felt in the deeper structures like bones, muscles, connective tissues, etc. is the deep pain. Deep pain is usually the slow pain that occurs due to stimulation of C fibers in the deeper structures of the body.

**Somatic Pain vs Visceral Pain**
Pain originating in the somatic structures is the somatic pain and pain originating in the visceral structures is the visceral pain (details of visceral pain are described below).

**Peripheral Pain vs Central Pain**
Pain occurring due to direct stimulation of receptors or nerves is the peripheral pain. Example is the neuropathic or neurogenic pain (as seen in neuralgias). Stimulation of central pain fibers resulting in pain is called central pain. Example of central pain is the pain below the level of lesion in spinal transection.

**Physiologic Pain vs Pathologic Pain**
1. **Acute pain** is sometimes referred to as physiologic pain. Acute pain has sudden onset and subsides faster with the normal healing process or due to treatment. It is called physiologic pain or ‘good pain’ as it serves an important protective mechanism.
2. **Chronic pain** is often referred to as pathologic pain or ‘bad pain’ as it persist even after the healing from the injury or following treatment as usually they do not respond to usual anti-inflammatory analgesics. There are two categories of pathologic pain: neuropathic and inflammatory.
3. **Neuropathic pain** is due to nerve damage as occurs in chronic diabetes, ischemia or toxins.
4. **Inflammatory pain** is due to inflammatory diseases such as rheumatoid arthritis. Usually, inflammatory pain is accompanied by hyperalgesia and allodynia (details given at the end of this chapter).

Receptors and Peripheral Fibers

**Types of Nociceptors**
Receptors for pain are called nociceptors. Nociceptors are the free nerve endings. They are distributed widely throughout most parts of the body. However, there are few tissues like brain (only the neural tissue of the brain) that are devoid of nociceptors. Though the brain tissue does not contain nociceptors, the coverings of the brain are rich in these receptors.

The nociceptors are broadly divided into three categories: Aδ mechanical nociceptors, multimodal C fiber nociceptors and other nociceptors.

**Aδ Mechanical Nociceptors**
These are the terminals of Aδ fibers. The Aδ fibers are small myelinated axons that discharge only in response to intense mechanical stimuli (but, not to thermal or chemical stimuli). The fibers are usually 1–5 mm in diameter with conduction velocity of 10–30 m/s. Aδ fibers conduct the fast pain.

**Polymodal C Fiber Nociceptors**
The polymodal nociceptors are activated by high-intensity mechanical, chemical and thermal (both hot and cold) stimuli. As they respond to varieties of stimuli, they are polymodal in nature. These are terminals of C fibers, which are fine-unmyelinated fibers having diameters of about 0.3–1.2 mm and conduction velocity of 0.5–2 m/s. The C fiber nociceptors usually respond to thermal and chemical stimuli. They respond to high skin temperature (at least 45°C) and locally applied chemicals. The C fibers carry the slow pain.

**Other Nociceptors**
These include thermal nociceptors (Aδ and C fiber terminals that respond to very low and high temperature, i.e. < 5°C and > 45°C respectively), Aδ fibers responding to heat and non-multimodal C fibers responding to strong mechanical stimuli:
1. Three types of receptors for thermal pain have been cloned: 1. Cold and methanol-sensitive receptor 1 (CMR 1), 2. VR-1, and 3. VRL-1.
2. No. 2 and 3 are vanilloid receptors for nociceptive heat that are sensitive to vanillins, a group of chemical substances including capsaicin that induce pain.

**Vanilloid Receptors**
Vanilloid receptor–1 (VR–1) at C fiber terminals have been described recently:
1. These receptors are so named as they respond to vanillin, a group of pain producing compounds that include capsaicin.
2. VR–1 responds not only to nociceptive stimuli such as capsaicin, but also to increase in temperature > 43°C, and change in pH.
3. Another type, called VRL–1 receptors isolated from C fibers respond to temperature above 50°C, but not to capsaicin.
4. Thus, it appears that there are multiple receptors in C fiber endings responding to various stimuli or possibly, there may be different C fiber systems.

**TRP Family of Ion Channels**
CMR 1, VR-1 and VRL-1 receptors belong to the transient receptor potential family (TRP family) of ion channels. These are excitatory ion channels:
1. Especially, VR-1 has a PIP$_2$ binding site. When the quantity of PIP$_2$ bound to VR-1 is decreased, the sensitivity of this thermal nociceptor is increased.
2. However, the mechanism of action of these receptors is fully understood.
3. In general, depolarization in the cutaneous receptors could be due to inhibition of K$^+$ channels that decreases K$^+$ efflux or activation of Na$^+$ channels that causes Na$^+$ influx or inhibition of Na$^+$-K$^+$ pump.
4. Depolarization of cool receptors is observed to be due to Ca$^{++}$ influx.

**Spinal Cord Termination of Pain Fibers**
The cell bodies of pain fibers are located in DRG. Fibers enter spinal cord via dorsal horn. In case of cranial pain fibers, the afferents project to the nucleus of trigeminal nerve (the medullary analogue of dorsal horn). In the dorsal root, pain fibers occupy the most lateral part of the root. In the dorsal horn, A$b$ fibers terminate in laminae I and V and C fibers terminate in lamina II (Fig. 120.1). In the spinal cord, the ascending pain fibers occupy the lateral fasciculus. However, the thinnest pain fibers (C fibers) form a discrete bundle in the spinal cord, the tract of Lissauer. The Lissauer’s tract also contains few deep sensory and propriospinal fibers.

**Specificity of Nociceptors**
Many theories have been forwarded to explain the specificity of nociceptors and nociceptive pathways. These are:

- **Specificity theory**: This theory states that the pain sensation has a specific sensory modality, specific receptors, and a specific pathway in the CNS. Stimulation of these receptors and pathway produces the sensation of pain.
- **Overstimulation theory**: This theory explains that the pain results from over-stimulation of various types of afferent ending. Therefore, according to over-stimulation theory, pain can be transmitted via different afferent pathways.
- **Pattern theory**: This theory states that the pain sensation can be elicited by stimulation of receptors by a particular pattern. However, finally it has been agreed that specificity theory explains all the aspects of pain. Thus, the receptors for pain are specific and also the pathways.

**Dermatomal Distribution of Pain Fibers**
Pain fibers are distributed in the somatic structures as per the sensory map of the body (refer Fig. 118.2; Chapter 118), which is useful to the physician to locate the site of lesion:
1. The distribution of pain fibers from deep structures however does not fully correspond to the cutaneous pattern, though they also follow a segmental pattern.
2. For example, $T_1$ to $T_4$ nerve roots mainly innervate thoracic organs like heart and lungs, $T_6$ to $T_8$ nerve roots are for upper abdominal organs and $T_{10}$ to $L_2$ nerve roots are for lower abdominal viscera.

**Localization**
Pain is localized like other sensory modalities:
1. Sharp pain is better localized than the dull pain, because it preserves its specific dermatomal representation and also its somatotopic organization in the CNS.
2. However, in general, pain is relatively poorly localized, because pain endings have low innervation density and pain pathways exhibit extensive branching than any other sensory pathways.
3. Visceral pain is very poorly localized than the somatic pain due to less number of receptors present in visceral structures and also due to their poor cortical representation.

**Pain Pathways**
Pain is transmitted to the higher centers in the brain in the lateral spinothalamic tract of the anterolateral system. The pain pathways are divided into two types: the paleospinothalamic pathway and neospinothalamic pathway.

**Paleospinothalamic Pathway**
Phylogenetically, paleospinothalamic is the oldest pathway. This pathway mainly carries the sensation of slow pain.
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This is the slow conducting multineuron system that mediates the poorly localized pain from deep somatic and visceral structures. The fibers are mostly C fibers (Fig. 120.2).

First Order Neurons
First order neurons enter the spinal cord and terminate mainly in the laminae II of the dorsal horn.

Second Order Neurons
Second order neurons decussate and ascend in the contralateral spinothalamic pathway. The fibers are more medially placed in the spinothalamic tract. In the brainstem on their way to thalamus, fibers project to three major nuclear groups forming three subsystems:
1. At the level of medulla, the collaterals from the second order of neurons heavily project to the reticular formation; therefore, this pathway is also called as spinoreticulothalamic pathway.
2. Fibers also project heavily to the midbrain nuclei (spinomesencephalic fibers) like midbrain reticular formation, parabrachial nuclei and periaqueductal gray.
3. Fibers also project to hypothalamus forming spinohypothalamic fiber system.

Third Order Neurons
In the thalamus, the fibers terminate mainly in the medial nuclear group (the midline and intralaminar nuclei; also called as non-specific nuclei) from where the third order of neurons arise and project to different areas of the cortex including limbic cortical areas.

Special Features
1. Because of non-specific projection of paleospinothalamic fibers that mainly transmits slow pain; the slow pain is poorly localized.
2. Through its substantial projection to the reticular formation, the slow pain keeps a person awake.
3. Also, through its connection with limbic system, paleospinothalamic pathway evokes the emotional experience of pain and through hypothalamic connections it mediates autonomic responses.
4. This pathway mediates motivational-affect component of pain.
   Thus, the paleospinothalamic pathway mediates arousal, affective aspects and autonomic responses of pain.

Neospinothalamic Pathway
The neospinothalamic tract is most developed in primates. This pathway carries mainly the fast pain. The fibers are mostly Aδ fibers.

First Order Neurons
First order of neurons terminate mainly in the lamina I and V in the dorsal horn of the spinal cord (see Fig. 120.1). The neurotransmitters released at the terminals of primary nociceptive afferents (1st order of neurons) are glutamate and neuropeptides (of which most important is substance P).

Second Order Neurons
Second order neurons cross over to the opposite side in the same segment of the spinal cord and ascend in the lateral spinothalamic tract. In the spinal cord, there is a topographic organization of fibers:
1. The fibers from lower body parts are placed laterally and fibers from upper body parts are located more medially in the lateral fasciculus.
2. The fibers from sacral spinal segments occupy the most lateral part and the fibers from cervical region are present in most inner part in the spinothalamic tract (Fig. 120.3).
3. Second order neurons terminate in lateral nuclear group (the ventrobasal and posterior nuclear complexes; or also called VPL nuclei or specific nuclei) of the thalamus.
4. On their way to thalamus, the neospinothalamic tract project sparsely to the midbrain reticular formation, periaqueductal gray, and hypothalamus.
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Third Order Neurons

The third order of neurons originate from specific thalamic nuclei and project to the postcentral gyrus (the sensory cortex). The topographic organization of these fibers in the thalamus and cortex is very concrete, and in the sensory cortex, the neurons are organized in modality specific columns. Therefore, the fast pain is better localized.

Special Features

1. The topographic organization of neospinothalamic fibers in the thalamus and cortex is very concrete, and in the sensory cortex, the neurons are organized in modality specific columns. Therefore, the fast pain is better localized.
2. The neospinothalamic pathway for its termination in the specific and discrete areas in thalamus and cortex, subserves the sensory-discriminative aspects of pain that is the localization of pain and detection of quality and intensity of the noxious stimuli.

Deep Pain

Pain originating from nociceptors in the deeper somatic structures such as muscles, tendons, bones, periosteam, internal ligaments, etc. is the deep pain:

1. It differs from superficial pain by the nature of pain evoked by the stimuli, which is usually dull aching with poor localization. This is mainly due to deficiency of Aδ fibers in the deeper structures and the relatively less number of receptors in the deep structures.
2. Like visceral pain (see below), often deep pain is associated with autonomic symptoms as autonomic fibers accompany the neurons originating these deeper body structures.
3. Pain from a deeper body part is usually accompanied by contraction of surrounding skeletal muscles. Prolonged contraction of these muscles induces muscle ischemia and aggravates the pain (Clinical Box 120.1).

Clinical Box 120.1

Muscle pain is common pathological pain: Muscle contraction for a longer duration produces pain due to ischemia, which usually subsides once blood flow is re-established. This is usually due to accumulation of Lewis factor or P factor in the ischemic muscles. The exact chemical nature of P factor is not known, though accumulation of K+ is the most likely factor. Common example is angina pectoris induced by exertion and relieved by rest. Intermittent claudication of leg muscles in persons suffering from occlusive vascular disease, which appears with leg exercise and disappears with rest.

Visceral Pain

Pain originating from the visceral structures is usually aching in quality or sometimes may be burning (peptic ulcer pain) or anginal (coronary ischemia) in nature. However if intense, pain may be sharp or penetrating type:

1. Usually, visceral pain is diffuse in nature and poorly localized and associated with autonomic symptoms.
2. Visceral pain often radiates or referred to other structures.

Scientist contributed

Henry Head (1861–1940), had pioneered in the study of visceral pain; especially he analyzed the referred pain originating from viscera. His studies on skin sensation and on loss and regeneration of sensations after cutting a nerve stimulated many pupils in physiology. He had demonstrated the function of vagus nerve in regulation of respiration.


Receptors

The receptors for pain are similar to the nociceptors in the somatic structures, but the receptors are much less in number. Due to the paucity of nerve endings in viscera, the margins of the painful zone are not well delineated and the pain is poorly localized.

Stimulus

Stimulus for visceral pain is usually the distension of the organ, if it is a hollow viscus. Chemical irritation or ischemia also causes visceral pain.

Afferent Fibers

Afferent fibers carrying pain sensations from viscera reach CNS via autonomic fibers. The cell bodies are located in the DRG and homologous cranial nerve ganglia. The cranial nerves that carry visceral pain fibers are vagus, glosso-pharyngeal, and facial nerves:

1. Visceral pain from esophagus, stomach, small intestine and proximal colon is largely carried in vagus nerve and terminate in the nucleus of solitary tract (NTS) before projecting to thalamus.
2. Pain fibers from abdominal viscera also accompany the sympathetic fibers. Therefore, visceral pain is associated with autonomic features like sweating, alteration in blood pressure, etc.
3. Pain fibers from pelvic structures accompany lumbo-sacral roots.
4. The trigeminal nerve carries the sensation from face and eye.

**Ascending Pathway**

From spinal cord, the fibers travel along the lateral spino-thalamic tract, the same pathway as that for the somatic sensation:
1. From thalamus, fibers project to cortex via thalamo-cortical radiation.
2. In the cortex, the representation of visceral structures is poorly organized and the cortical areas for visceral sensation are intermixed with the somatic areas.

**Special Characteristics of Visceral Pain**

1. As the pain receptors are less in number in the viscera, the visceral pain is poorly localized.
2. The important stimulus for elicitation of visceral pain is the distention of viscera. Inflammation of viscera also increases the sensitivity to pain.
3. Visceral pain is usually unpleasant, because, it has an affective component, and the afferent fibers stimulate the vomiting center in the brain.
4. Visceral pain is usually associated with autonomic symptoms like change in blood pressure, sweating, etc. because the afferent fibers travel in sympathetic and parasympathetic pathways. Thus, visceral pain causes autonomic changes.
5. Visceral pain (especially abdominal pain) causes muscle spasm and rigidity. It is especially observed, if the inflammatory process that produces pain also involves the peritoneum. Muscle guarding (abdominal wall rigidity) as seen in acute abdomen is a protective mechanism that prevents further injury to the viscera.
6. Visceral pain often radiates to other structures (referred pain).

**Referred Pain**

Visceral pain may be referred to the somatic structures that are located away from the viscera:
1. The pain perceived in the somatic structure due to visceral irritation or injury is called the referred pain.
2. Usually, the visceral pain is not referred to the skin overlying the viscera, but to the other areas of the skin innervated by the same embryonic spinal segment. For example, the referred pain is typically seen in acute myocardial infarction or in acute cholecystitis.
3. In acute myocardial infarction, the pain is referred to the inner aspect of the left arm, and in acute cholecystitis to the tip of the right shoulder.
4. In cholecystitis, the inflamed gall bladder irritates the diaphragm, which stimulates the phrenic nerve. Therefore, cholecystitis pain radiates to the tip of the shoulder, as the shoulder and diaphragm develop from the same dermatomal segments.

The referred pain is explained by the following theories:
1. Dermatomal theory
2. Convergence theory
3. Facilitation theory
4. Experience theory

**Dermatomal Theory**

Visceral pain is referred usually to a structure that develops from the same embryonic segment (dermatome). This is called dermatomal theory. For example, the heart and the inner aspect of the arm develop from the same embryonic segment. Therefore, the pain of acute myocardial infarction radiates to the ulnar border of the left arm.

**Convergence Theory**

The visceral and the somatic afferent fibers converge on the second order of neuron in the spinothalamic tract, as the number of second order neurons is less than the afferent fibers (the first order neurons). Therefore, the fibers carrying the pain sensation from the somatic structures also carry the pain sensation arising from the visceral structures (Fig. 120.4). The cortex sometimes cannot differentiate the site of origin of the pain sensation because the ascending fibers projecting to cortex from both structures are the same. Therefore, signal conveyed by brain for perception is also referred to the somatic area in addition to the projection to the viscera.

**Facilitation Theory**

The collaterals arising from the visceral afferent fibers project to the spinothalamic neurons that receive afferents from the somatic structures (Fig. 120.5). Therefore, the pain sensation arising from somatic structure is facilitated (strengthened) by activity in the visceral afferents. Thus, minor activity in the somatic afferent can cause pain.
Experience Theory
Experience plays a role in the genesis of referred pain. According to this theory, pain instead of being felt at its usual site, may be referred to some other structure or area in which the patient had experience of pain earlier. For example, the pain due to the inflammation of abdominal viscera is usually referred to the midline. But, in patients with previous history of abdominal surgery, the pain is referred to the surgical scar, which may not be in the midline.

Mechanism of Pain Recognition and Perception

Pain Recognition
Nociceptive stimuli damage the tissues, which are recognized by the body as sensation of pain. Proteolytic enzymes are released from damaged tissues:
1. These enzymes act on tissue proteins and cells locally to release many substances that activate nociceptors.
2. The chemical substances that mediate pain are histamine, prostaglandins, serotonin, kinins and other polypeptides like leukotrienes (Fig. 120.6). Locally released potassium ions from the damaged cells also activate nociceptors.
3. Activation of nociceptors directly releases polypeptide mediator by the primary afferent fibers that sensitizes nociceptors.
4. The mediator is the substance P released from C-fiber endings in skin. Substance P enhances pain perception. Substance P also mediates other features of inflammation as well (for details, refer ‘Triple Response’ Chapter 98)

Modulation of Pain Perception
The threshold for pain perception may be same in all, but is lowered in inflammation.
1. Local anesthetics and many centrally acting analgesics (and also placebos) act by raising the pain threshold.
2. Distraction that turns the attention away from the painful part of the body decreases awareness to pain.
3. Strong emotion also decreases pain perception probably by acting on descending adrenergic system (for details see below). However, the degree of emotional reaction varies in individual.
   The conscious awareness of pain occurs when the impulse reaches thalamocortical level. Recognition of noxious stimulus is the function of thalamus, and appreciation of intensity, localization and discrimination are the functions of sensory cortex.

Endogenous Pain Control Mechanisms
Recently, endogenous analgesia systems have been described. Prominent among them are neuronal analgesia (descending pain-inhibiting) systems and opioid systems.

Descending Pain Modulating Systems
Neuronal analgesia systems were described following discovery by Raynold that stimulation of ventrolateral periaqueductal gray (PAG) in rats produces profound analgesia. Subsequently other areas were found to have analgesic properties:
1. Important among them are nucleus raphe magnus and nucleus paragigantocellularis in medulla.
2. Two descending analgesia systems have been described: raphespinal serotonergic pathway and ceruleospinal norpinephrinergic pathway.

Descending Raphespinal Serotonergic Pathway
This pathway originates from the frontal cortex and hypothalamus and projects to the cells in the periaqueductal region of the midbrain.
1. Fibers from PAG project to the nucleus raphe magnus and reticularis magnocellularis in the medulla.
2. From raphe nucleus, fibers descend down to the spinal cord via raphespinal pathway (Fig. 120.7).
3. Fibers of raphespinal pathway are serotonergic and they terminate in laminae I, II, and V of dorsal horn. In the spinal cord, raphespinal fibers mainly terminate...
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on the interneurons that by presynaptic mechanisms inhibit the transmission of impulses from primary afferent fibers to second order of neurons.

4. These interneurons release enkephalins as their neurotransmitters that inhibit the release of substance P from the presynaptic ending. Thus, transmission of impulses in the pain pathway in the dorsal horn is inhibited.

This endogenous pain-inhibiting pathway is activated by prolonged pain that chronically activates the ascending pain pathways. Fibers from ascending pain pathway terminate on PAG and raphe nucleus that in turn activates descending endogenous analgesia system. Therefore, in chronic pain, intensity of pain automatically decreases (autoinhibition of pain).

**Descending Ceruleospinal Norepinephrinergic Pathway**

Cell bodies of descending noradrenergic fibers are located in the locus ceruleus of dorsolateral pons. These neurons do not receive inputs from PAG. The fibers project to dorsal horn of spinal cord to inhibit the transmission of nociceptive impulses from primary afferents.

**Endogenous Opioid Systems**

Morphine is a potent endogenous analgesic peptide. It acts at synapses of the nociceptive pathways by binding to specific sites, the opiate receptors. The binding of morphine molecules to an opiate receptor decreases nociceptive synaptic excitability. There are **three major classes of opiate receptors**: μ, δ and κ. Genes encoding these receptors are found to be members of G protein-coupled class of receptors. Morphine is a potent agonist at the μ receptor:

1. Opiates such as morphine act **pre- and postsynaptically** to inhibit the transmission of impulses from Aδ and C fibers.
2. **Increased potassium conductance** of postsynaptic membrane produces postsynaptic inhibition and inhibition of release of substance P from terminals of sensory neurons results in presynaptic inhibition. These effects are reversed by naloxone, the narcotic antagonist that binds to μ receptor.
3. Acupuncture and acupressure produce analgesia by facilitating the release of endogenous opioids. There are many opioid peptides synthesized endogenously in our body. These are collectively known as endorphins (endogenous morphine like substances).
4. The **important endorphins** are enkephalins (leu-enkephalin and met-enkephalin), β-endorphin, γ-endorphin, dynorphin, α-Neoendorphins, etc.
5. The **endorphin receptors** are particularly present in the spinal cord, PAG of midbrain and raphe nucleus in the medulla. The endorphins produce **profound analgesia** when they are secreted from different parts of the brain in response to different stimuli.
6. However, the major disadvantage of opioid is that on chronic use they produce addiction and tolerance (Clinical Box 120.2)

**Clinical Box 120.2**

**Chronic opioid use produces addiction and tolerance**: Repeated use of opioid analgesics results in addiction and tolerance, mechanisms of which are not clearly known. It has been proposed that tolerance results from an uncoupling of opioid receptor from its associated G proteins. A protein called **β-arrestin-2** that phosphorylates G protein is responsible for development of addiction, but not tolerance.

**Gate Control Theory of Pain**

According to this theory, pain can be modulated by the peripheral mechanisms, especially by gating the impulses in the spinal cord. This theory postulates that the collaterals from large myelinated afferent fibers associated with tactile sensibility produce presynaptic inhibition of Aδ and C fibers in the dorsal horn of spinal cord. Thus, activity in the large afferent fibers regulates (as a gate in the spinal cord) the transmission of impulses originating in the pain receptors. Acupuncture analgesia acts by gate control mechanism and release of endorphins (Clinical Box 120.3).
Stress-induced Analgesia

During stress, reaction of the individual to pain is suppressed. This results in analgesia. Stress induced analgesia occurs by both opioid and nonopioid mechanisms. Stress activates descending analgesia systems.

Specific Pain Syndromes

Terminologies

Hyperalgesia: Hyperalgesia refers to increased sensitivity and lowered threshold to painful stimuli. Inflammation of skin is among the common causes of hyperalgesia.

Hypalgesia: Hypalgesia or hypoalgesia refers to decreased sensitivity and raised threshold to painful stimuli.

Analgesia: Complete loss of pain sensation. Analgesia may not be associated with loss of other sensations.

Hyperpathia: This is a defect in pain perception, which is associated with an increased reaction to the pain stimulus once it is perceived. In general term, it is the exaggerated response to pain. However, in this condition there is also an increased reaction to other stimuli.

Allodynia: This is a state in which there is excessive response to even mild stimuli. For example, a stimulus like light touch which is never painful elicits pain in allodynia.

While assessing pain of any cause, the following points are carefully noted and appropriately addressed by the physician:

1. The mode of onset of pain.
2. The quality of pain.
3. The location of pain, and referred pain, if present.
4. The intensity and duration of pain.
5. The provoking and relieving factors.

Causalgia

Causalgia is a burning pain that usually develops following a traumatic peripheral nerve injury. The pain is continuous and often accompanied by hyperalgesia and allodynia. It is interesting to note that causalgia persists even after the complete recovery from the nerve damage. The sympathetic fibers are proposed to be responsible for maintaining causalgia, as it is relieved by sympathectomy or by using adrenergics blockers. The noradrenergic sympathetic fibers overgrow into the dorsal root ganglia of sensory nerves from the injured area. Therefore, sympathetic discharge results in pain in the injured area.

Inflammatory Pain

Commonest cause of pain is inflammation. Inflammation occurs following injury. Mediators of inflammation like cytokines and substance P increase sensitivity and lower the threshold for pain in the injured area. Thus, a minor painful stimulus elicits severe pain. These substances increase the pain perception.

Neuropathic Pain

Injury to the nerve results in neuropathic pain. Neuralgia is a general term for neuropathic pain. Trigeminal neuralgia due to herpes zoster (including causalgia) is an example of neuropathic pain. Neuropathic pain occurs in many nerve diseases. Diabetic neuropathy, nerve injury, different types of polyneuropathies, root irritation as occurs in disk prolapse and spinal cord injury are other examples of neuropathic pain. Neuropathic pain is frequently associated with hyperesthesia (increased cutaneous sensitivity), hyperalgesia, hyperpathia and allodynia. Usually, neuropathic pain does not respond well to analgesic treatment. Epidural injection of mixture of analgesic and steroid is usually helpful. In severe cases, nerve section (section of the nerve that carries sensation from the site of injury), dorsal rhizotomy (cutting the pain fibers in the dorsal root) or anterolateral cordotomy or spinotalamic tractotomy (cutting the ascending fibers of spinothalamic tract in the spinal cord) are considered.

Tic Douloureux

This is also known as trigeminal neuralgia. This is severe and stabbing type of acute facial pain that persists briefly. Most patients develop this syndrome following compression of the trigeminal nerve roots (for details, see Chapter 121; Trigeminal System).

Thalamic Syndrome

The patients with thalamic syndrome experience chronic, severe and extremely unpleasant pain that occurs either spontaneously or in response to a trivial stimulus.

1. The pain is felt in the brain, in the absence of any peripheral pathology.
2. It occurs due to ischemic or hemorrhagic lesions of posterior thalamic nuclei following obstruction of the thalamogeniculate branch of posterior cerebral artery.
3. Pain is cured by surgical destruction of the posterior thalamic nuclei by stereotaxic surgery.

Toothache

Teeth have plenty nociceptors. Teeth are innervated by axons of maxillary and mandibular divisions of trigeminal nerve. The fibers include unmyelinated C fibers, and myelinated Aδ and Aβ fibers. The fibers enter the tooth through the root apex, branched within the pulp and then terminate in the plexus of the pulp. Enamel and cementum contain no nerve fibers. The pulp is protected and covered by enamel. Thus, exposure of pulp or pulp inflammation is
painful. Toothache is caused by heat, cold, inflammation, and mechanical probing of the dental pulp.

**ITCH**

**Receptors and Pathway**

**Definition**
It is defined as an unpleasant sensation that evokes the desire to scratch. This is a distinct sensory modality. It can be evoked by mechanical or chemical stimuli. In contrast, tickle is a pleasurable sensation.

**Receptors**
Itch is produced by stimulation of itch receptors that are the endings of unmyelinated C fibers (these are not the same C fibers that are responsible for pain):
1. Itching can be produced by mechanical stimulation of the skin and by application of various chemical agents. Itching invariably occurs when plasma concentration bile salt is high.
2. Histamine is the most important chemical agent that produces itching (antihistaminics are prescribed to stop itching), though itching can occur without histamine.
3. Kinins also produce severe itching.

**Pathway**
The pathway for transmission of sensation of itch and tickle is lateral spinothalamic tract.

**TEMPERATURE**

**Receptors and Pathway**

**Receptors**
There are two types of temperature receptors:
1. The warmth receptors, the receptors that respond maximally to the temperature above the body temperature, and,
2. The cold receptors, the receptors that respond maximally to temperature below body temperature. Cold receptors respond to the temperature ranging from 10 to 40°C and warm receptors respond from 30–50°C (Fig. 120.8).
3. The sense organs are naked nerve endings. Three special sets of temperature receptors have been recently described.
   1. Cold and methanol sensitive receptors 1 (CMR 1): These are the receptors for moderate cold.
   2. VR 1: respond to very high temperature (nociceptive thermoceptors).
   3. VRL 1: respond to moderate to high temperature. All these three set of receptors (CMR 1,VR 1 and VRL 1) are ion channels and they belong to transient receptor potential (TRP) subfamily.

**Pathway**
The afferents for cold are Aδ and C fibers, and afferents for heat are C fibers. The temperature sensation is transmitted by lateral spinothalamic tract.

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**CHAPTER SUMMARY**

**KEY CONCEPTS**
1. Pain is a protective phenomenon, and an early sign of morbidity.
2. Pin fibers while ascending up to the thalamus, give collaterals to many brainstem, hypothalamic and limbic nuclei. These fibers mediate the autonomic and emotional responses associated with pain.
3. Endogenous analgesia systems are activated with activation of pain pathways.

**Important to Know (Must Read)**
1. With the help of a labeled schematic diagram, trace the pathway for pain, and describe the physiology of pain; and With the help of a labeled schematic diagram, trace the pathway for endogenous analgesia system, and give a note on opiate receptors’ may come as Long Questions.
2. Anterolateral system, Ascending pain pathways, Nociceptors, Visceral pain, Referred pain, Endogenous neural analgesia system, Endogenous opioid system, may come as Short Questions.
3. In Viva, examiner may ask... Define pain, Types of pain, Types of nociceptors, Tract of ascending pain pathways, Topographic organization pain fibers in spinal cord, Differences between somatic pain and visceral pain, Definition and theories of referred pain, Mechanism of pain perception, Name the neural analgesia systems, Trace the pathway for endogenous neural analgesia system, Endogenous opioid system, Endorphins, Opioid receptors, What is hyperalgesia, hypoalgesia, causalgia, neuropathic pain, tic dolorouex, thalamic syndrome, Receptor and pathway for itch, Receptor and pathway for itch.
CHAPTER 121

Trigeminal System

**Learning Objectives**

On completion of study of this chapter, the student **MUST** be able to:
1. Understand the importance of trigeminal system in learning physiology of pain and analgesia.
2. Draw a labeled schematic diagram of trigeminal pathways.
3. Appreciate the distribution of sensory segments as innervated by three divisions of trigeminal nerve.
4. Understand the thalamic representation of trigeminal nerve.
5. Understand the physiological basis of trigeminal neuralgia.

**Trigeminal Pathway**

A complete somatosensory representation of the body includes inputs from the trigeminal nerve.

1. Trigeminal nerves supply face, oral cavity, and head. Trigeminal pathway is different from the pain sensory pathway from other body parts. Trigeminal nerve has three branches that supply three different parts of the face (Fig. 121.1).
2. The neurons of trigeminal nerve are **pseudobipolar neurons**. They have their cell bodies in the semilunar or gasserian ganglion (trigeminal ganglion). The proximal axons divide into ascending and descending branches to reach two brainstem nuclei.
3. These brainstem nuclei are: **spinal nucleus** (spinal V), and the **main sensory nucleus** (main sensory V) of the trigeminal nerve (Fig. 121.2).

**Spinal V**

The caudal nucleus is the spinal nucleus of the trigeminal nerve, which has **three sub-divisions**:

1. The lowermost is the **caudal sub-division** of spinal V that receives **many nociceptive afferent fibers** from the face. Therefore, to relieve untreatable chronic facial pain as occurs in trigeminal neuralgia, surgical lesion of the caudal sub-division spinal nucleus of trigeminal nerve is performed.
2. The middle sub-division is the **interpolar sub-division**, which receives mechanosensitive and nociceptive inputs from teeth and gums, and the mucous membrane of the mouth.
3. The uppermost is the **oral sub-division** that receives nociceptive inputs mainly from the oral cavity. The spinal nucleus receives mainly the nociceptive inputs via small diameter fibers. Therefore, this is considered as the **spinothalamic pathway** of the trigeminal system.
**Main Sensory V**

This nucleus is situated rostrally. It receives ipsilateral projections from the low threshold mechanoreceptors from face and teeth. The fibers are large diameter fibers. Therefore, the main sensory V is considered to be the trigeminal homologue of dorsal column nuclei.

1. The input from trigeminal nuclei reaches thalamus via trigeminal lemniscus (Fig. 121.2), which runs along the medial lemniscus.

2. The projections are primarily contralateral thalamus. In thalamus fibers terminate in the specific nuclei i.e. ventral posterior and lateral (VPL) and posterior (PO) nuclei of thalamus. The VPL receives inputs from the rostral sub-divisions of spinal V and from main sensory V, whereas the PO receives inputs from the caudal sub-divisions of spinal V (Fig. 121.2).

The VPL nucleus of the thalamus projects to the ventrolateral portion of the somatosensory cortex (SI). The cortical representation of the face is relatively enormous, due to its high innervation density. The representation of mouth and tongue is also larger. Both SI and SII receive trigeminal inputs from the thalamus.

**APPLIED PHYSIOLOGY**

**Trigeminal Neuralgia**

This is a condition of severe and stabbing type of acute facial pain that persists briefly.

1. The pain is initiated by application of an innocuous stimulus to the trigger zone, which is a very specific and restricted area on the face. The trigger zone may be located on the cheek, nose, lip, oral mucosa, teeth, or scalp. The trigger zone may not have a particular relation to the area from which the pain is perceived.

2. Most patients develop this syndrome following compression of the trigeminal nerve roots. The root compression causes nerve irritation or degeneration that results in episodic bursts of severe pain.

3. If adequate control is not achieved with analgesic, surgical release of trigeminal compression or lesions of trigeminal ganglion may be considered.

**CHAPTER SUMMARY**

**Key Concepts**

1. Trigeminal pathway exclusively innervates face (both sensory and motor).

**Important to Know (Must Read)**

1. Usually Long Questions are not asked from this chapter.

2. Trigeminal pathway may come as a Short Question.

3. In Viva, examiner may ask… Innervation of the face, What are the area of the face supplied by different trigeminal nerve divisions, How trigeminal system differs from other body pain pathways, What is the cause and treatment of trigeminal neuralgia.
Chapter 122
Thalamus

Learning Objectives
On completion of study of this chapter, the student MUST be able to:
1. Name the major nuclear groups in the thalamus and give their functions.
2. Appreciate the functional organization of thalamus.
3. List the functions of thalamus.
4. Correlate the functions of thalamus with thalamic dysfunctions.
5. Give the structure and functions of epithalamus and subthalamus.

Functional Organization
Thalamus is an egg-shaped nuclear structure sitting obliquely atop the brain stem. It is known as the sensory relay station. It receives ascending sensory inputs and projects them to the sensory cortical areas. Afferent fibers of the ascending reticular formation also project to thalamus and thalamus receives input from the cortex, mainly from the layer VI. There are two thalami on both sides. Anteriorly they are separated by the third ventricle and the corpora quadrigemina is present between their posterior parts.

Thalamic Nuclei
The thalamus is composed of a number of discrete nuclei.

5. The **dorsal nuclear group** contains lateral posterior nucleus, lateral dorsal nucleus and pulvinar.
6. The **ventral nuclear group** is comprised of ventral anterior, ventral lateral and ventral posterior nuclei, and medial and lateral geniculate bodies (Figs. 122.3A and B).
7. The ventral posterior group, also known as **ventrobasal complex**, consists of ventroposterolateral and ventroposteromedian groups of nuclei (Flowchart 122.1). However, physiologically there are **four groups of nuclei**: 1. **Specific relay nuclei**: Ascending afferent inputs synapse on these nuclei. The axons arising from them transmit the afferent impulse to cortex. This nuclear
Chapter 122: Thalamus

Fig. 122.2: Nuclei of thalamus (A: Anterior; VA: Ventral anterior; VL: Ventral Lateral; VPL: Ventral posterolateral; DL: Lateral dorsal; PL: Lateral posterior). Purple arrows indicate afferent fibers.

Figs. 122.3A and B: View of thalamic nuclei from superior aspect (A) and coronal section (B).

Flowchart 122.1: Schematic division of thalamic nuclei.
The anterior nucleus receives inputs from the hypothalamus through the mammillothalamic tract and relays the information to the cingulate gyrus.

2. Association nuclei: This nuclear group consists of pulvinar, dorsal nuclei and lateral posterior nucleus (Fig. 122.8).
   - These nuclei receive inputs from sensory cortex and limbic system and project diffusely to the association cortex or to sub-cortical structures.
   - The pulvinar projects to the inferior parietal lobe.
   - The dorsolateral nucleus is reciprocally connected to the cingulate gyrus.

3. Non-specific nuclei: These include intralaminar (IM) (Fig. 122.9), midline (Fig. 122.10), centromedian (CM) nuclei, and reticular nuclei (RET).
Chapter 122: Thalamus

Fig. 122.7: Major connections of medial geniculate body of thalamus.

Fig. 122.8: Major connections of dorsal nucleus of thalamus.

Fig. 122.9: Major connections of intralaminar nuclei of thalamus.

Fig. 122.10: Major connections of midline nuclei of thalamus.

Fig. 122.11: Major connections of lateral nuclei and pulvinar of thalamus.

They receive inputs mainly from reticular formation and paleospinothalamic tract, and also inputs from striatum, hypothalamus and other thalamic nuclei.

They project diffusely to wide areas of cerebral cortex, thalamus and limbic system.

4. Motor nuclei: These include lateral, ventral anterior and ventral lateral nuclei (Fig. 122.11).
The ventral lateral nucleus receives input from the cerebellum through the dentato-rubro-thalamic tract and projects to the motor cortex area 4 and 6 (Fig. 122.12). The ventral anterior nucleus receives inputs from basal ganglia and projects to the premotor cortex. The thalamic nuclei are also divided into extrinsic and intrinsic nuclei. The specific relay nuclei are known as extrinsic nuclei and rest others as intrinsic nuclei. It should be noted that all somatosensory information reaches all of these different types of thalamic nuclei.

**THALAMIC ORGANIZATION AND FUNCTIONS**

The ascending somatosensory pathways terminate in certain thalamic nuclei. The sensory signals mostly arise from contralateral receptors of the body.

1. Axons from cells in the dorsal column nuclei cross the brain stem immediately at the level of the medulla and travel in the contralateral medial lemniscus to terminate primarily in the ventral-posterolateral nucleus (VPL) of the thalamus.
2. The fibers of the anterolateral system (lateral and anterior spinthalamic tracts) travel in the contralateral anterolateral funiculus of the spinal cord to terminate in the VPL, posterior (PO) and intralaminar (IM) nuclei.
3. Reticulo-thalamic fibers of the spinoreticulothalamic pathway remain largely uncrossed and terminate in IM, midline or centromedian (CM) nuclei, and in the thalamic reticular nuclei (RET).

The VPL and PO project to cortical regions. IM diffusely projects to cortex and RET is reciprocally connected with other thalamic nuclei.

**Ventral Posterolateral Nucleus (VPL)**

The VPL receives input from many of ascending sensory pathways. Each pathway has a typical pattern of termination in VPL nucleus. The central core of VPL nucleus receives largely cutaneous inputs. The dorsal aspect of VPL receives mostly deep inputs, whereas anterior surface receives muscle inputs. Thus, the VPL consists of a core of cutaneous inputs and a shell of deep inputs. The afferent fibers from the face, arm and leg are represented in a medial to lateral manner in the VPL nucleus.

**Posterior Nuclei (PO)**

PO receives nociceptive inputs. Cells in PO respond to a variety of somatic and non-somatic modalities. PO is mainly involved in the transmission of nociceptive impulses. Large lesion in PO produces analgesia and stimulation of PO produces pain sensations. However, there is no somatotopic organization in PO.

**Intralaminar Nuclei and Reticular Nuclei**

Cells in intralaminar nuclei (IM) have large, somatic receptive fields. They are activated by nociceptive and other sensory stimuli. Responses in IM are strongly affected by the level of arousal, attention and affect. Cells in reticular nuclei (RET) respond to a variety of somatosensory modalities. IM has diffuse but strong projection to the cortex, striatum and limbic system. RET does not project to cortex but is reciprocally connected to other thalamic nuclei. Thus, RET is involved in the modulation of thalamic activity.

**Functions of Thalamus**

All afferent impulses on their way to the sensory cortex terminate in the thalamus.

1. **Relay station for all somatic sensations:** Thalamus conveys the sensory information to the cortex through thalamocortical projections (Table 122.1). Thus, it is the major relay station for sensory inputs in humans and higher order of animals, whereas in lower order of animals, it acts as the center for sensory integration.
2. **Relay of special sensations:** Thalamus is the relay center for all special sensations except olfaction. The lateral and medial geniculate bodies receive the visual and auditory afferents respectively. The VPL nucleus receives the taste afferents.
3. **Arousal mechanisms:** Thalamocortical projects from nonspecific nuclei to cortex contributes to reticular activating system that activates arousal mechanisms and keeps the individual awake.
4. **Subcortical Perception of Sensations:** Subcortical perception of pain, temperature, pressure (crude touch) occurs to some extent in thalamus. Thalamus is not only the relay station, but also the integration center for these sensations. Therefore, these sensations remain intact considerably in cortical lesion.
5. **Motor Functions:** Thalamus is an integral part of motor loop of the brain in which globus pallidus of basal ganglia projects mainly to VPL nucleus of thalamus (via pallidothalamic tract) and thalamus projects...
to the motor cortex (via thalamocortical fibers), which projects back to the basal ganglia. Through motor loop thalamus influences postural movements. Thalamus also links cerebellum and motor cortex via dentato-rubro-thalamo-cortical tract. Through this connection, thalamus influences planning and programming of movements.

6. Memory and Emotion: Anterior thalamus is a constituent of Papez circuit. It receives input from mammillary body of limbic system via mamillothalamic tract. Through this connection, thalamus is concerned with recent memory and emotion.

7. Synchronization of EEG: Stimulation of intralaminar thalamic nuclei at low frequency causes synchronization of EEG waves recorded from ipsilateral cortex. This is called recruiting response. However, high frequency stimulation causes arousal and desynchronization.

8. Role in Sleep: A circuit linking the thalamus and cortex (thalamocortical loop) is important in generating the pattern of brain activity in sleep-wake cycle. Inhibitory thalamic reticular neurons are proposed to be the part of this neuronal network that causes induction of sleep.

9. Sensory Motor Coordination: Thalamus receives all sensory inputs from the body and closely interacts with basal ganglia, cerebellum and motor cortex. Therefore, thalamus is one of the major structures in the brain for coordination between sensory and motor functions, especially in the sensory feedback for correction and improvement in motor output.

10. Language and Speech: Dorsal lateral nucleus of thalamus is reciprocally connected with parietal lobe of the brain, and therefore is concerned with language and speech and complex integrated functions.

### Table 122.1: Connections and functions of major thalamic nuclei.

<table>
<thead>
<tr>
<th>Nuclear Group</th>
<th>Major Afferents</th>
<th>Major Efferents</th>
<th>Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Specific Nuclei</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lateral-ventral cortex, movement.</td>
<td>Dentatothalamic tract, and pallidothalamic tract</td>
<td>Thalamocortical tract (to motor area 4 and 6)</td>
<td>Proprioceptive input to control of voluntary movements</td>
</tr>
<tr>
<td>Postero-ventral</td>
<td>Spinothalamic fibers, trigeminothalamic fiber, &amp; medial lemniscus.</td>
<td>To sensory cortex (area 3, 1 and 2)</td>
<td>Relay of all somatosensory inputs including face.</td>
</tr>
<tr>
<td>Dorsal-lateral Pulvinar</td>
<td>From parietal lobe</td>
<td>To parietal lobe</td>
<td>Language and speech. Integrates somatic, auditory and visual information.</td>
</tr>
<tr>
<td></td>
<td>From parietal, temporal and occipital lobes</td>
<td>To parietal, temporal and occipital lobes</td>
<td></td>
</tr>
<tr>
<td>LGB</td>
<td>From optic tract</td>
<td>To visual cortex</td>
<td>Vision</td>
</tr>
<tr>
<td>MGB</td>
<td>From cochlea and inferior colliculi</td>
<td>To auditory cortex</td>
<td>Audition</td>
</tr>
<tr>
<td><strong>B. Nonspecific Nuclei</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior group</td>
<td>Mamillothalamic tract</td>
<td>To cingulated gyrus</td>
<td>Memory and emotion</td>
</tr>
<tr>
<td>Midline group</td>
<td>All ascending fibers, &amp; from hypothalamus and reticular formation</td>
<td>To neocortex, BG and hypothalamus</td>
<td>Integration of somatic and visceral sensations, and arousal.</td>
</tr>
<tr>
<td>Intralaminar</td>
<td>From RAS &amp; BG</td>
<td>To neocortex and prefrontal cortex</td>
<td>Arousal and motor functions.</td>
</tr>
<tr>
<td>Dorso-medial synthesis</td>
<td>From hypothalamus &amp; prefrontal cortex</td>
<td>To prefrontal cortex</td>
<td>Association center for of crude somatic sensations.</td>
</tr>
</tbody>
</table>

### APPLIED ASPECTS

#### Thalamic Syndrome

Lesion of the VPL occurs due to thrombosis of posterolateral branch of the posterior cerebral artery.

1. This results in severe impairment of the discriminative touch and pressure sensations of contralateral side, whereas diffuse touch, temperature, and pain sensations are often less impaired.
2. Also, there occurs decreased muscle tone, profound weakness of the muscles and ataxia as VPL nucleus receives afferent impulses from cerebellum and relays them to the motor cortex, area 4 and 6.
3. The emotion may be affected. This is called thalamic syndrome.
4. Vascular lesions usually spare the medial thalamus including VPM nuclear group. Therefore, sensations in the face and head often remain intact.

#### Other Deficits

When the thalamus is damaged, not only thalamic functions are lost, but also many cortical functions are affected, as cortex is intimately connected with thalamus.

Electrical lesion of intralaminar nuclei relieves chronic suffering type of pain, though the acute perception of pain remains intact.

### THE EPITHALAMUS

The epithalamus consists of the structures that form the roof of the third ventricle. These include the pineal gland, the habenular nuclei and the striae medullaris. Externally, it is seen above the superior colliculi.
THE SUBTHALAMUS

The subthalamus is located between the upper end of substantia nigra and the thalamus. Subthalamus contains sensory fasciculi, rostral extensions of midbrain nuclei, fiber bundle from cerebellum and globus pallidus, and subthalamic nuclei. Fibers in the supramamillary commissure interconnect subthalamic structures. The subthalamic nucleus (body of Luys) has reciprocal connection with the globus pallidus. Lesion of subthalamic nucleus results in hemiballismus (for detail, see Basal Ganglia).

CHAPTER SUMMARY

KEY CONCEPTS

1. Though thalamus is major relay center of all sensations, it interacts and integrates with many brain areas and functions.
2. The specific nuclei (ventral, lateral, anterior, posterior group) convey sensations to sensory cortex.
3. The nonspecific nuclei (intraluminal and midline group) interact with reticular formation and cortex for arousal mechanisms.

Important to Know (Must Read)

1. With the help of a labeled schematic diagrams, describe the connections and functions of thalamus’ may come as a Long Question.
2. Thalamic nuclei, Connections and functions of thalamus, Functions of thalamus, thalamic syndrome, may come as Short Questions.
3. In Viva, examiner may ask... Name the thalamic nuclei, Connections and functions of each nuclear group of thalamus, What are the specific and nonspecific nuclei of thalamus and what their functions, What is thalamic syndrome.
Chapter 123

Sensory Cortex

Learning Objectives
On completion of study of this chapter, the student MUST be able to:
1. Name cortical sensory areas and give their functions.
2. Understand the connections and effects of lesion of each sensory area.
3. Appreciate the organization of sensory homunculus.

Sensory Cortical Areas

The cortical areas associated with processing of somatic sensory informations collectively form somatosensory cortex. Third order neurons of ascending sensory pathways project to the somatosensory cortex. The primary somatosensory cortical areas are SI and SII. There are other somatosensory cortical areas that are called secondary somatosensory cortical areas. There are minimum four distinct areas in the cortex that receive somatosensory inputs. These are:

Primary somatosensory area I (SI): SI is present in the post-central gyrus, especially on the posterior bank and floor of the central sulcus. SI includes the Brodmann’s area 3, 1, and 2 (Fig. 123.1). Area 3 has two sub-areas: 3a and 3b.

Primary somatosensory area II (SII): SII is located in the wall of the Sylvian fissure (superior bank of the lateral sulcus).

Somatosensory association cortex (Brodmann’s area 5): This is present in the posterior parietal lobe. This is also called higher somatosensory cortex, as it largely receives inputs from SI and SII. Lesion of this area produces more subtle deficits of sensations.

Supplementary sensory area: This is part of the posterior parietal association area located on the medial wall of the parietal lobe.

Other somatosensory areas: Other areas include,
1. Precentral gyrus (area 4): Though this is the primary motor cortex, it also receives somatosensory signals.
2. Cortical areas surrounding SII (area 7b): The cells in this area also respond to somatosensory stimuli.

Connections of Somatosensory Areas

Somatosensory areas are interconnected to each other (Fig. 123.2) as follows:
1. All sub-areas of SI make reciprocal and specific point-to-point connection with the VPL nuclear group in the thalamus. The main projection to the thalamus is from the area 3a.
2. SII receives inputs from ventrobasal nucleus of the thalamus.
3. There is a serial flow of information from area 3b to area 5. Area 3a is reciprocally connected with area 1 and 2.
4. SI and SII have extensive reciprocal connections.
5. Area 5 receives input only from the lateral posterior nucleus of the thalamus, which does not receive direct somatosensory inputs.
6. SI and SII are connected with their homologous areas in the opposite hemisphere via corpus callosum.
7. SI, SII, area 5 and supplementary sensory areas project to the precentral gyrus (area 4 and 6) that is involved in regulation of movement. Thus, this provides an opportunity in the CNS for sensory motor coordination.
8. Both SI and SII also receive monoaminergic input from brainstem nuclei (especially from locus ceruleus and raphe nuclei).
9. Sensory cortical areas are extensively connected with each other.

**Somatosensory Area I (SI: Area 3, 1 and 2)**

This is the primary somatosensory area that resides in the parietal lobe on the posterior bank and floor of the central sulcus.

**Input**

SI receives inputs mainly from following thalamic and cortical areas:
1. VPL nucleus of the thalamus
2. SII area, and
3. Brainstem nuclei (locus ceruleus and raphe nucleus)

**Output**

SI projects mainly to the following thalamic and cortical areas:
1. VPL nucleus of the thalamus
2. Supplementary sensory area

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**Sub-Regions (Based on Modality Specificity)**

SI is divided into area 3, 1, and 2. Area 3 is further subdivided into area 3a and 3b. This division of SI is based on their modality specificity. That means whether they respond to cutaneous (from the skin) or deep (from muscles and joints) stimuli.

- **Area 3a and 2**: Respond mainly to deep stimulation (i.e. from the muscles and joints).
- They respond poorly to cutaneous stimulation.
- **Area 1**: Responds to both cutaneous and deep stimulation.
- **Area 3b**: Responds mainly to cutaneous stimulation.

**Topographic Organization**

Entire body is represented twice in SI (once in area 3b, and again in area 1). The topographic representation is called the sensory homunculus (Fig. 123.3).
1. In this sensory map in brain, face is represented in the lateral part, hand and upper extremity are represented in the dorsolateral part of the postcentral gyrus, and lower extremity on the medial surface of the hemisphere.
2. The greatest area of the map is devoted for face, especially lips (area devoted for speech), and hand, especially the digits (cutaneous sensations from areas involved in skilled activities).
3. The cortical receiving areas for trunk and back are small.

**Columnar Organization**

Especially in SI, the neurons are arranged along a line perpendicular to the cortical surface, which is known the columnar organization. Cortical columns that are located adjacent process different sensory modalities. For example, the column in the area 3b responds to cutaneous stimulation (that come from rapidly adapting mechanoreceptors), whereas the column in the area 3a responds to deep stimulation (mainly from slowly adapting mechanoreceptors).

**Functions**

SI is involved in the initial processing of the somatosensory input. It also processes higher sensory orders like perception of the direction of an applied stimulus. The functions of SI are best studied by producing lesions.

**Effects of Lesions**

Cortical lesions do not completely abolish somatic sensation. Proprioception and fine touch are most affected by cortical lesions. Temperature and pain sensibility are least affected. Cortical sensations (tactile localization, tactile
discrimination, and stereognosis) are almost abolished. However, damage to different parts of SI produces different sensory deficits.

1. Lesion of SI of one side results in clear deficit in all aspects of somesthesis in the contralateral body parts.
2. With selective lesions of area 3, there is failure to learn the discriminative task even after repeated trials.
3. Lesion of area 1 causes significant impairment of hard-soft, or smooth-rough discrimination, but no deficit in other aspects of sensory learning.
4. Damage to postcentral gyrus also causes impairment of kinesthesia (inability to appreciate passive position and movements of different body parts).
5. Damage to area 5 specifically affects stereognosis, with other tactile sensation remaining intact.
6. There is least impairment of pain and temperature sensation in cortical lesion.

Somatosensory area II (SII)

SII is located in the superior wall of the Sylvian fissure (the fissure that separates temporal lobe from frontal and parietal lobe). The topographic organization is such that head is represented at the inferior end of the postcentral gyrus and the feet at the bottom of the sylvian fissure.

Most of the neurons of SII respond to light mechanical stimulation of the hair or skin (rapidly adapting mechanoreceptors). They poorly respond to deep stimuli.

Lesion of SII results deficit in learning based on tactile discrimination. It seems that SII has no significant effect on the processing of information in SI.

Association Cortex

Association sensory cortex is present in the parietal lobe (parietal association cortex). It receives inputs mainly from SI, but also from SII and visual cortex. The major function of the parietal association cortex is to coordinate the relationship of the body to extrapersonal space, e.g., hand-eye coordination. In the non-dominant hemisphere, the association cortex is involved in spatial relations, whereas in the dominant hemisphere it is concerned with language.

A lesion of parietal association cortex of the nondominant hemisphere produces impairments in the ability to relate to extrapersonal space. For example, if the subject is asked to copy a geometry, the figures are distorted. The person develops constructional apraxia (defect in constructing a picture) and hemineglect syndrome (he denies existence of the opposite side of the body).

**Fig. 123.3:** Sensory homunculus. Note, larger cortical representations are from face and hands.
CHAPTER SUMMARY

**Key Concepts**

1. The hand and face are more represented in sensory cortex, as these parts are most used by human beings.

**Important to Know (Must Read)**

1. Usually **Long Questions** are not asked from this chapter.
2. Connections and functions of sensory cortex, may come as a **Short Question**.
3. In **Viva**, examiner may ask… Name the sensory areas in the cortex, What are the primary and secondary sensory areas in the cortex, How is body represented in the sensory homunculus, What are the effects of lesion of SI, SII.
CHAPTER
124
Sensory Abnormalities

LEARNING OBJECTIVES
On completion of study of this chapter, the student MUST be able to:
1. Correlate the knowledge of sensory physiology in understanding the abnormalities of sensory system.
2. Name the defects produced at various levels of sensory pathways.
3. Understand the sensory deficits produced by lesion at different levels of sensory system.
4. Understand the physiological basis of sensory function tests.

FUNCTIONAL ASPECTS
Study of sensory physiology helps us to understand how are the stimuli perceived, processed and integrated by the nervous system.
1. It also helps us learn the physiological basis of diagnosis and management of the sensory abnormalities. It is not only important to detect the nature of the deficit, but also to localize the site (the level of the sensory neuraxis) of sensory deficit.
2. The detection and localization of lesions of the sensory system depend on the distribution and type of sensory loss.
3. The disease may affect the nerve, the nerve roots, the spinal cord, the brainstem, the thalamus, and the cortex.

Scientist contributed
CHARLES EDOUARD BROWN-SÉQUARD (1817–1894) pioneered in the study of sensory pathway and effects of their lesions. He also studied glands of internal secretion, showing indispensability of the adrenals. He also studied the functions of sympathetic nerves. He is remembered for his classic description of pathways of conduction in tracts in spinal cord. The dysfunction of hemisection of spinal cord is popularly known as Brown-Séquard syndrome.

Lesions at Different Levels

Single Peripheral Nerve Lesion
Features of lesion of a peripheral nerve depend on whether the nerve is predominantly cutaneous, muscular or mixed.

1. Lesion of a cutaneous nerve results in sensory loss in the corresponding areas of distribution of that particular nerve.
2. However, deficit is always less than the anatomic distribution because of overlap from the adjacent nerve.
3. Perception of deep pressure and passive movements is usually not affected, as these modalities are mediated by nerve fibers from subcutaneous structures and joints.
4. If the nerve is affected by compression, large fibers carrying touch and pressure are affected whereas small fibers carrying pain and temperature remain intact.

Multiple Nerve Lesions (Polyneuropathy)
Diabetic, inflammatory and vasculitic neuropathies are common form of polyneuropathies. Usually, the sensory impairment is bilateral.
1. As the long and large fibers are most affected, sensory deficits are more marked in legs and feet in lower limb and hands in upper limb.
2. Sensory loss involves all modalities of sensation. If the loss is distal and symmetrical, glove and stocking anesthesia (anesthesia of hands and feet of both sides) occurs.
3. However, if the degeneration is more demyelinating than the axonal type, paresthesia is an early feature. As large fibers (kinesthetic fibers that carry sense of position and vibration) are more affected, sensory ataxia may occur. In chronic cases, due to prolonged analgesia, trophic ulcers develop.
Nerve Root Lesion (Radiculopathy)

Segmental anesthesia occurs in root lesions corresponding to the involvement of the segment of the spinal cord from where the nerve root arises. This usually occurs in compression of a single sensory nerve root as occurs in herniation of intervertebral discs. However, considerable overlap from adjacent root usually occurs. Therefore, lesion of a single sensory root does not produce complete loss of sensation in area of the skin.

Lesion of Sensory Ganglia

Dorsal root ganglia are affected in many inflammatory, toxic and neoplastic diseases. This produces same sensory loss as that of root lesion, but loss of sensation in proximal parts of the body is more pronounced.

Spinal Cord Lesions

Features of spinal cord lesion depend on the part of the spinal cord involved in the disease process. Accordingly following sensory spinal cord syndromes are observed:
1. Tabetic syndrome (one side of dorsal column affected)
2. Complete spinal sensory syndrome (complete transection of spinal cord)
3. Brown-Séquard syndrome (hemisection of spinal cord)
4. Syringomyelic syndrome (area surrounding central canal is affected)
5. Posterior column syndrome (both sides of dorsal column affected)
6. Anterior spinal artery syndrome (ventral half of the cord is affected)

Tabetic Syndrome

In tabes dorsalis, destruction of large proprioceptive and other posterior column fibers of one side (Fig. 124.1A) results in loss of sense of position and vibration. Numbness and paraesthesia may occur. Romberg sign may be positive. This is typically seen in neurosyphilis. Tabetic syndrome also occurs in diabetes.

Posterior Column Syndrome

 Destruction fibers in the posterior column (Fig. 124.1B) results in loss of vibratory and position sense, without affecting pain and temperature. Stere anesthesia, impairment of graphesthesia and tactile sensibility also occur. This resembles the cortical lesion, but loss of vibration differentiates it.

Brown-Séquard Syndrome

Sensory deficit: Brown-Séquard Syndrome occurs in hemisection of the spinal cord (Fig. 124.1C). It is usually seen in injury to the spinal cord or in tumors of the spinal cord that affects only half of the body. On the side of lesion, the fine-touch sensation, proprioceptive sensations (sensations from tendons, muscles, joints and vibration sense) and tactile discrimination are lost. On the opposite side, pain and thermal sensations are lost. This occurs because sensation for fine-touch, proprioception, and two-point discrimination ascend up in the dorsal column of the same side, whereas the sensation for pain and temperature ascend up in the anterolateral system in the opposite side of the spinal cord.

Motor deficit: There is also damage to corticospinal tract on the side of hemisection of spinal cord. This causes paresis (muscle weakness) and spasticity of muscles of the same side of the body.

Complete Spinal Sensory Syndrome

In complete section of spinal cord (Fig. 124.1D), all forms of sensation are abolished below the level of lesion. Usually, a narrow zone of hyperesthesia occurs at the upper margin of anesthetic zone.

Syringomyelic Syndrome

Syringomyelia is a disease in which there is lesion around the central canal of the spinal cord (Fig. 124.1E). The lesion interrupts the lateral spinothalamic fibers (for pain and temperature) without affecting fibers of dorsal column. Thus, pain and temperature are lost without affecting touch and postural sensibility (dissociated sensory loss).

Anterior Spinal Artery Syndrome

Anterior spinal artery supplies ventral part of the spinal cord. Thus, obstruction or hemorrhage of this artery causes infarction of anterior half of the cord (Fig. 124.1F).
This results in anterior myelopathy. Usually, pain and temperature sensation are lost without affecting proprioceptive sensations below the level of lesion. Spastic paralysis occurs due to involvement of corticospinal tract.

**Brainstem Lesion**

Lesion at the medulla affects descending trigeminal tract and the crossed lateral spinothalamic tract on one side of the brain stem. This results in crossed sensory loss, i.e., loss of pain and temperature on one side of the face and on the opposite side of the body.

In the upper medulla, pons and lower midbrain, the crossed spinothalamic and trigeminothalamic tracts run together. Thus, lesion at these levels causes loss of pain and temperature on the opposite half of the face and body without affecting other sensations (as the medial lemniscus is spared).

In the upper brainstem, spinothalamic tract and medial lemniscus become confluent. Thus, lesion at this level results in contralateral loss of all superficial and deep sensations (posterior column sensations and pain and temperature).

**Thalamic Lesion**

Severe and extensive lesion of the thalamus results in gross impairment of sensory modalities on the opposite side of the body.

1. The threshold for pain may be raised, but a less painful stimulus may cause an exaggerated response (hyperalgesia). The touch sensation may induce unpleasant sensation (paresthesia).
2. This is called thalamic syndrome, which occurs in the lesion of lateral and ventral nuclei.

**Cortical Lesion**

The cortex is mainly involved in integration of finer aspect of sensations, especially the spatial and discriminatory sensibility.

1. Tactile localization, two-point discrimination and stereognosis are therefore called as cortical sensations.
2. The cortical lesion results in topagnosia (inability to localize touch and pain stimuli), astereognosis and widening of two-point discrimination. Proprioception and sense of vibration are also impaired. Sensory inattention, extinction or neglect is characteristic manifestation of parietal lobe lesion.

**Sensory Function Tests**

Functions of sensory system can be assessed by performing clinical examination of sensory system, sensory nerve conduction studies and by studying somatosensory evoked potentials.

**Clinical Examination of Sensory System**

A thorough clinical examination of sensory system should help the clinician to establish the nature and level of sensory loss. All sensory modalities should be examined over all dermatomes (sensory segments) of the body separately (for details, refer ‘Clinical Examination of Sensory System’ in the Textbook of Practical Physiology, by G K Pal and Pravati Pal, Universities Press).

**Sensory Nerve Conduction**

Sensory nerve conduction studies by electrophysiological testing are very helpful in confirming the diagnosis. Thorough analysis of sensory nerve action potential and determination of conduction velocities are very useful for diagnosing sensory neuropathy. Recording of H and F responses are useful for detecting radiculopathy (for details, refer ‘Nerve Conduction Studies’ as described in Textbook of Practical Physiology by G K Pal and Pravati Pal, published by Universities Press).

**Somatosensory Evoked Potentials**

Somatosensory evoked potentials (SEP) demonstrated defects in conduction of large diameter fibers in the peripheral nerves, brain stem thalamus and cortex. SEP is especially useful to know whether the defect is in the peripheral or central conductive pathway (for details, refer ‘Somatosensory Evoked Potentials’ as described in the Textbook of Practical Physiology by G K Pal and Pravati Pal, published by Universities Press).

**CHAPTER SUMMARY**

**Key Concepts**

1. Sensory deficits depend on the site of lesion, type of disease process and the severity of the disease.

**Important to Know (Must Read)**

1. Usually Long Questions are not asked from this chapter.
2. Brown-Séquard syndrome, may come as a Short Question.
3. In Viva, examiner may ask... What is Brown-Séquard syndrome, how is it produced and what are the features, featured of sensory deficits in lesion of different parts of the spinal cord, features of thalamic syndrome, effects of cortical lesions, name sensory function tests.
CHAPTER 125
Introduction to and Organization of Motor System

LEARNING OBJECTIVES
On completion of study of this chapter, the student MUST be able to:
1. Understand the general organization of motor system.
2. Name the types of movement and give their characteristics.
3. Appreciate the role of feedback control systems in improvement of motor functions.
4. Understand the role of each component of motor system in execution of motor functions.

Ability to move is an essential feature of animal life. Locomotion or movement is crucial for survival of organisms. Fulfilling fundamental needs of life and appropriate interaction to environment needs execution of suitable and coordinated movements. Through precise and harmonious movements, one expresses his best abilities like an artist drawing a beautiful picture or a surgeon performing a successful operation etc.

1. Motor system deals with the study of physiology of movement. Motor physiology involves initiation, execution, and control of movements.
2. For any movement to be appropriately carried out, a stable and maintained posture is the basic necessity.
3. With the help of stable postural background and with appropriate postural adjustments, coordinated movement becomes possible.
4. Therefore, motor physiology deals with the study of control of movement and posture together.

Scientist contributed
CHRISTIAN WILHELM BRAUNE (1831–1904), a German anatomist and physiologist studied the details of various aspects of human movement (Locomotion). He gave mathematical analysis of mechanics of motion. With Otto Fischer (1861–1917), he made classical account of human locomotion.

VARIOUS ASPECTS OF MOVEMENT
Types of Movements
Movements can be broadly divided into two categories: the automatic movements, and the volitional movements.

Automatic Movements
The automatic movements are reflexive in nature. An example of an automatic movement is a rapid response to a nociceptive stimulus.

- They have short latency
- They are stereotyped in nature
- They are executed rapidly
- Usually, they cannot be modified
- They are triggered by a specific sensory stimulus

Volitional Movements
The movements that are under voluntary control are the volitional or intentional movements. They can easily be modified. An example of a volitional movement is painting, or threading a needle.

- Have long latency
- Are slow in execution
- Can be modified easily
4. Are under voluntary control
5. Are rarely triggered by a specific sensory stimulus
6. May be affected by factors like attention, emotion and motivation.

What is Movement?
Movement is defined as displacement of the body parts that results in change in position of the body as a whole or a part of the body. Movement is produced by the contraction and relaxation of the muscles.

1. The muscles that facilitate a particular movement (decrease the angle at the joint) are called as agonists.
2. The muscles that oppose the movement (increase the angle at the joint) are called as antagonists. In fact, movement is facilitated by relaxation of the antagonist muscle.

How Does the Movement Occur?
Movement occurs due to the motor signals generated in the motor neurons in the spinal cord. This is accomplished by the activities initiated in the central nervous system.

1. For a basic or reflexive movement to be executed, the motor signal generated in the spinal cord is conveyed to the appropriate muscles via motor neurons.
2. However, for complex or volitional movements to be appropriately executed, first, the signals are generated in the forebrain (mainly in motor cortex) and brainstem, and then, the signals are relayed to the spinal cord.
3. Spinal cord interacts with supraspinal influences for the integration and refining of the final output signal (Fig. 125.1). Thus, the precision and stability of movements are achieved by various feedback control mechanisms.

Feedback Control Systems
The feedback systems are meant to improve quality of movement. The accuracy and stability in execution of motor activities are the objectives of feedback control systems. The feedback mechanisms can be divided into three types: 1. Local feedback, 2. Central feedback, and 3. Special Sensory feedback.

The Local Feedback
The local feedback is the lowest level of feedback systems. It is exclusively integrated in spinal cord (Fig. 125.2).
1. The afferent signal does not interact with supraspinal signals originating from various parts of CNS. Thus, it acts fast and controls gross reflexive movement.
2. The local feedback operates for movements in which the speed is more important than the accuracy of the movement.
3. For example, during withdrawal of body parts in response to a noxious stimulus, the body part must be withdrawn faster no matter how precisely it is done.
4. However, the pattern of movement depends on the strength and nature of afferent inputs.

The Central Feedback
This is the second level of feedback control system. It is integrated in spinal cord, brainstem and cortex (Fig. 125.2).
1. In this feedback mechanism, the afferent signal satisfactorily interacts with the other signals arising from different parts of the supraspinal segments.
2. For processing and integration of signals at various levels, the speed of execution is considerably delayed. However, with slowness, the precision of movement is achieved.
3. Thus, central feedback mechanism operates when accuracy of movement is of paramount importance than the speed of execution.
4. For example, for threading a needle, the movement needs proper balance and coordination to achieve its precision.

Fig. 125.1: General schematic representation of control of movement. Note, efferent signals originating in cortical and brainstem areas, determine the motor output signal from spinal cord that finally produces movement.

Fig. 125.2: Local and central feedback mechanisms in motor control.
Special Sensory Feedback

This feedback mechanism involves special sensory information like visual and auditory inputs.
1. The movement **becomes most accurate** with control by this feedback system. For example, to hit a nail with a hammer becomes easier and accurate with eyes open.
2. In this system, the inputs from special sensory structures interact with different parts of the central nervous system to improve the accuracy of the movement.

ORGANIZATION OF MOTOR SYSTEM

The components of motor systems are: muscle and its efferent connections, the segmental circuit (in the spinal cord), the brainstem controlling centers, the basal ganglia, cerebellum, and cerebral cortex (Fig. 125.3).

Muscle and its Efferent Connections

The important components of the motor system are the muscles and their efferent connections (the motor neurons). **Tone of muscle depends on its intact innervation.**
1. Without muscle tone, the adequate force cannot be generated for execution of movements.
2. Therefore, lesion of the motor neurons that abolishes muscle tone and function results in **complete paralysis.**
3. Thus, muscle and the motor neurons are fundamental parts of the motor system.

Spinal Segmental Circuit

Sensory signals arising from muscle enter the spinal cord through muscle afferents.
1. These sensory inputs directly or indirectly influence the motor neurons that in turn innervate the same muscle.
2. This circuit of neural connection constitutes the **local or segmental spinal circuit.**
3. The segmental circuit is **very essential for all rapid reflexive movements.**
4. Also, the local circuit in the spinal cord generates and controls basic neural patterns required for genesis and coordination of limb movements.

Brainstem Controlling Centers

The activities of motor neurons and interneurons in the spinal cord are largely influenced by the **descending inputs** arising from the brainstem motor nuclei.
1. The descending pathways from the brainstem to the spinal cord are mainly the **extrapyramidal systems.**
2. The descending pathway from brainstem reticular nuclei is the reticulospinal tract and from vestibular nuclei is the vestibulospinal tract.
3. These two extrapyramidal pathways profoundly influence the activities of the motor neurons in the spinal cord that mainly control postural movements.

Cortex
The motor cortex directly controls the spinal cord motor neurons through the corticospinal tracts.
1. It also strongly influences the brainstem nuclei (via corticobulbar projections) from where the extrapyramidal tracts originate.
2. Thus, motor cortex both directly and indirectly regulates the peripheral motor activities. Sensory cortex projects to the motor cortex and also contributes to the corticospinal fibers.
3. The inputs from somatosensory cortex to the motor cortex provide feedback information to descending motor signals for alteration and improvement of motor performance.
4. Thus, cortex is one of the levels of sensory motor coordination.

Basal Ganglia
Basal ganglia are important subcortical structures that strongly influence motor activities.
1. They do not receive any direct somatosensory input from the spinal cord. However, basal ganglia project to motor cortex via the thalamus and strongly influence the motor output to spinal cord.
2. Basal ganglia are involved in initiation, smoothening and coordination of the movement.
3. In humans, diseases of the basal ganglia as seen in Parkinsonism produce significant impairment of control of posture and movement.

Cerebellum
Cerebellum is situated posterior to the motor neuraxis.
1. It receives inputs from almost all sensory modalities and projects heavily to the brainstem motor nuclei, and motor cortex. Therefore, cerebellum plays a crucial role in the regulation of posture and movement.
2. It controls almost all the aspects of movement, starting from planning, programming, and initiation to the smoothening and coordination, and termination of movement.
3. Therefore, diseases of the cerebellum significantly display abnormalities of all aspects of movements.

Thalamus
Thalamus is the major sensory relay station in the brain.
1. The sensory inputs arising from different body parts first relay in the thalamus before projecting to the cortex.
2. Thalamus also receives inputs from cerebellum and basal ganglia.
3. Thus, thalamus plays an important role in sensory-motor coordination.

CHAPTER SUMMARY

**Key Concepts**
1. Movement occurs due to muscle contraction that occurs by action potential generated in motor neurons.
2. Motor neurons are controlled by signals generated at spinal and supraspinal centers.

**Important to Know (Must Read)**
1. Usually Long Questions are not asked from this chapter.
2. ‘Feedback control system for movement’ may come as a Short Question.
3. In Viva, examiner may ask… Define movement, Name the types of movements, What are the characteristics of automatic and volitional movements, What are the Feedback control system for movement, and how are they organized, What are the different levels of organization of movement.
Learning Objectives

On completion of study of this chapter, the student MUST be able to:
1. Understand the organization of motor system at spinal segments.
2. Classify muscles physiologically.
3. Define upper and lower motor neurons and give their functions.
4. Understand the importance of motor unit recruitment.
5. Appreciate the topographical organization of motoneuron pool and spinal interneurons.

Scheme of Organization

Motor system is broadly organized at three levels: spinal cord, brainstem and forebrain. Integration of motor system at the level of spinal cord is the major component of the regulation of motor functions.

1. In the spinal cord, each segment has its own neural circuitry for motor control. This is called segmental organization.
2. Organization of motor control above the spinal cord segment (mainly by brainstem and forebrain areas) is called suprasegmental organization.

Components of Organization

Each segment of spinal cord has its input and output connections with various muscles.

1. From muscles, input reaches spinal cord via muscle afferents (Ia, Ib and other afferents).
2. From spinal cord, output reaches muscles via motoneurons.
3. In the spinal cord, between afferent neurons and motoneurons are many interneurons (Fig. 126.1).
4. In addition to the inputs from afferents, interneurons and motoneurons are the targets of descending fibers from various suprasegmental centers.
5. Organization of neuronal connections at every spinal cord segment is a unit of segmental organization. Segmental organization includes muscles, afferent neurons, motoneurons, motoneuron pool, and spinal interneurons (Fig. 126.2).

Muscles

According to their functions and anatomical positions, muscles are classified into various groups.

1. Usually, they are categorized as flexors-extensors, adductors-abductors, pronators-supinators depending on the movement of body parts they perform.
2. Physiologically, most important muscles are classified as flexors and extensors.
   - Extensor group of muscles is especially important for maintenance of posture and flexor group of muscles for withdrawal reflexes.
By definition, **extensors** are muscles that increase the angle at the joint, and **flexors** are muscles that decrease the angle at the joint.

- Extensors of lower limbs resist gravity and therefore are the main **antigravity muscles**.

3. The **tone of antigravity muscles is important for maintaining posture**. According to electrochemical properties and ability to sustain exercise, muscles are classified into fast twitch-fatigue resistant, slow twitch-fatigue resistant, fast twitch-fatigable and slow twitch-fatigable types. However, from neurophysiological point of view, muscles are best classified as medial-lateral or proximal-distal groups.

### Medial or Proximal Group of Muscles

The medial or proximal groups of muscles include the axial and girdle muscles, and proximal limb muscles.
1. Axial muscles are muscles attached to axial skeletons and girdles of the body.
2. These muscles are involved in postural adjustments as they produce body and whole limb movements.
3. The **medial extensors** perform antigravity function, and therefore are important for **control of posture**.

### Lateral or Distal Group of Muscles

The lateral or distal muscles include the intrinsic muscles of the digits and the distal muscles of the extremities. These muscles subserve the manipulatory activities, i.e. the **skilled voluntary movements**.

Thus, to summarize, the **proximal group of muscles control posture and distal group of muscles control skilled voluntary activities**. In fact, for understanding motor physiology, the whole nervous system is divided into medial/proximal and lateral/distal distinctions.

### Afferents

The main afferent fibers for motor activities are Ia afferents that arise from muscle spindles. Type II and Ib afferents also play a role in motor control. Flexor reflex afferents mediate withdrawal reflexes.

### Motor Neurons

- **Motor Neurons**: The motor neurons (also called, motoneurons) are neurons that are involved in control of motor functions. They are broadly divided into **two categories**: Upper motor neurons and lower motor neurons.

#### Upper Motor Neurons (UMNs)

UMNs are motor neurons that are derived from various motor areas of brain like motor cortex, brainstem motor nuclei etc. and **terminate directly or indirectly (via interneurons) on lower motor neurons** in the spinal cord (Fig. 126.3).
1. Axons of these neurons **form the descending pathways**.
2. Some important examples of these pathways are corticospinal, rubrospinal, vestibulospinal, and reticulospinal tracts.

#### Lower Motor Neurons (LMNs)

LMNs originate in the anterior horn of the spinal cord or in cranial nerve motor nuclei, and **innervate various skeletal muscles** (Fig. 126.3).
1. The cell bodies of motor neurons are located in the ventral horns of the spinal gray matter (anterior horn cells) and brainstem nuclei.
2. Though some of the fibers in the descending pathways terminate directly on motor neurons, most of the fibers terminate on interneurons, which in turn project to motor neuron.
3. There are **two types of lower motoneurons**: α motoneurons and γ motoneurons.

#### α Motoneurons

The α motoneurons constitute the **final common path of motor output signals** from the CNS, the route by which central neural activity influences the skeletal muscles.
1. The α motor neurons innervate extrafusal muscle fibers, which are responsible for force generation. One alpha motor neuron innervates 10 to 1,000 muscle fibers, depending on the muscle.

2. A Motor unit constitutes a motor neuron, its axon, the branches of the axon, the neuromuscular junctions at the distal end of each axon branch, and all of the extrafusal muscle fibers innervated by that motor neuron (Fig. 126.4).

3. When a motor neuron generates an action potential, all of its muscle fibers are activated (Application Box 126.1).

**Physiologic Significance:** According to their cell body size and axon diameter, α motor neurons are divided into two types: the larger and smaller motoneurons.

- The larger motoneurons have fast conduction velocities and are active in high-effort force generation. They innervate fast-twitch, high-force but fatigable muscle fibers.

- The smaller motor neurons have lower thresholds to synaptic stimulation, have slower conduction velocity, and innervate slow-twitch, low-force and fatigue resistant muscle fibers.

Thus, all muscle fibers of a motor unit belong to one category, i.e. either fast-twitch or slow-twitch type.

- This property of homogeneity of motor unit is decided by the type of motor neuron. Following denervation, muscle fiber type (twitch type) may change if the muscle is reinnervated by an axon sprouted from another type motor neuron.

4. The γ motor neurons do not receive inputs from sensory afferents; rather descending pathways influence them. Thus, supraspinal motor centers regulate muscle tone mainly by altering the discharge of γ motor neurons.

**Motoneuron Pools**

Spinal motoneurons are final common path for central motor outputs. The cell bodies of motoneurons are located in the ventral horn of the spinal cord, which are known as anterior horn cells. The anterior horn cells constitute the motoneuron pools.

**Topographical Organization**

There is a topographical organization of motoneurons in the spinal cord with regard to the target muscles they innervate. Accordingly, motoneuron pool is divided into two parts: the medial group and the lateral group.

1. **The medial group** of anterior horn cells innervates the axial and proximal muscles, and the **lateral group** of anterior horn cells innervates the distal muscles (Fig. 126.5).

2. Thus, **medial group of anterior horn cells control posture** whereas **lateral group of anterior horn cells control skilled voluntary activities**.

3. Consequently, lateral motoneuron pool is enlarged to occupy about 70% of the ventral horn in the lower cervical and upper thoracic spinal cord segments (the **brachial enlargement**), as these segments supply intrinsic muscles of hand that are involved in manipulatory activities.

4. Lower down in the spinal cord, in the ventral horn, the motoneuron pool is devoted for medial group cells as successively more proximal limb muscles are innervated by them.

It is important to remember the inputs that preferentially project to the more **medial motoneuronal cell group primarily control posture** and inputs that distribute to the **lateral motoneuronal cell group principally control skilled activities**.

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**Application Box 126.1**

**Motor unit recruitment:** The smaller motor neuron with small motor unit size has the low threshold. Therefore, when synaptic activity is low, they are activated first. This produces low-force tonic contractions in slow-twitch, fatigue resistant muscle fibers. When drive increases in the descending pathways, first the rate of discharge increases in the initially activated motor neurons and then additional motor units of the same type are activated. When, still higher force is needed to be generated in the muscle, the larger motor neurons (with large motor unit size) are recruited. This phenomenon of orderly recruitment of motor unit follows the size principle; the smaller motor units are activated first and then the larger motor units. Thus, antigravity muscles mainly contain slow-twitch muscle fibers as they function for the continuous postural support. Muscles with fast-twitch fibers like many flexors are capable of producing high-force contractions by rapidly recruiting motor units.

**γ Motor Neurons**

The γ motor neurons innervate the intrafusal fibers that are fibers of the muscle spindle.

1. They constitute 30% of fibers arising from anterior horn cells.

2. They do not contribute to the total force generated in the muscle. Rather, they modulate the sensitivity of muscle spindle and thereby regulate muscle length and muscle tone.

3. The γ motor neurons with intrafusal fibers constitute the fusimotor system.
Spinal Interneurons

Interneurons in the spinal cord are placed in between the dorsal and ventral horns.

1. Interneurons that are concerned mainly in motor functions are placed more toward the ventral horn and interneurons concerned with sensory functions are placed more toward the dorsal horn.
2. Most of the descending influences on the motoneurons are exerted indirectly via interneurons, rather than their direct projections to the spinal motoneurons.
3. Thus, interneurons are major targets of descending tracts.

Topographical Organization

Like motoneuronal cell groups, interneuronal cell groups are arranged topographically in the spinal cord. Interneurons are divided into two cell groups: ventromedial interneuronal cell group and dorsolateral interneuronal cell group (Fig. 126.5).

1. The ventromedially situated interneurons project preferentially to the medial group of motoneurons and dorsolaterally placed interneurons project to lateral group of motoneurons.
2. Consequently, ventromedial interneuronal cell group is primarily involved in controlling posture and dorso-lateral interneuronal cell group in regulating skilled voluntary activities.

CHAPTER SUMMARY

Key Concepts

1. The segmental organization of motor system, at the spinal cord level, is the key component of motor control, as all the inputs and outputs between spinal cord and muscle are part of this organization.
2. All descending fibers target the segmental components of the motor control.

Important to Know (Must Read)

1. Usually Long Questions are not asked from this chapter.
2. Upper motor neuron, Lower motor neuron, y motor neuron, Motor units, Motoneuron pool, may come as a Short Question.
3. In Viva, examiner may ask… What are the components of segmental organization of motor control. How do you classify muscle physiologically and clinically, What are the functions of proximal and distal group of muscle, Define upper motor neuron and lower motor neuron and give examples, y motor neuron, Define motor units, What is motor unit recruitment and what is its significance, How is motoneuron pool organized.
CHAPTER 127
Muscle Spindle and Golgi Tendon Organ

Learning Objectives
On completion of study of this chapter, the student **MUST** be able to:
1. Understand the importance of muscle spindle and Golgi tendon organ in motor physiology.
2. Draw a labeled schematic diagram of muscle spindle.
3. Describe the function of muscle spindle.
4. Understand the role of γ motor neuron in the control of muscle tone.
5. Appreciate the structure and function of Golgi tendon organ.

The student **MAY** also be able to:
1. Explain the role of γ motor neuron in motor control.
2. Describe the role of Golgi tendon organ in motor control.

Muscle Spindle
Muscle spindles are specialized sense organs present in all skeletal muscles in mammals. Muscle spindles are present in large number in muscles that serve fine movements. They are also found in good number in muscles involved in control of posture, especially the muscles rich in slow-twitch fiber types.

1. The muscle spindles are named because of their **long spindle (fusiform) shape**.
2. The fibers of the muscle spindle are known as **intrafusal fibers** as they are present inside the fusiform capsules of the spindle. Thisdifferentiates spindle fibers from the **extrafusal fibers**, the regular contractile units of the muscle that are present outside the fusiform capsule of the spindle. Extrafusal fibers are the regular muscle fibers.
3. Muscle spindles are the **receptors that respond to change in muscle length and the velocity of lengthening**.

Structure of Muscle Spindle
About 2 to 12 fibers are present in a muscle spindle. Each muscle spindle is enclosed in a connective tissue capsule. The muscle spindle is about 100 µm in diameter and 5 to 10 mm in length. The muscle spindle lies in parallel to the regular muscle fibers and their distal ends are attached to the tendon of the muscle or to the sides of extrafusal fibers (Fig. 127.1). This parallel arrangement and special attachment help the spindle to respond to muscle stretch.

Intrafusal fibers are of **two types**: the nuclear bag fibers, and the nuclear chain fibers.

Nuclear Bag Fibers
Nuclear bag fibers are longer than the nuclear chain fibers (Fig. 127.2). These fibers are called **nuclear bag fibers** as
they are **dilated at their center to form a bag like structure** that contains multiple nuclei.

1. Usually, **two nuclear bag fibers** are present in a spindle: the nuclear bag fiber 1, and the nuclear bag fiber 2.

2. The **bag fiber 1** has **low myosin ATPase activity** and responds best in the dynamic phase of muscle stretch.

3. The **bag fiber 2** has **high myosin ATPase activity** and responds best in the static phase of muscle stretch.

**Nuclear Chain Fibers**

These are thinner and shorter fibers.

1. They are present by the side of the nuclear bag fiber and do not have a definitive bag.

2. The **nuclei in these fibers are arranged in a row in the form of a chain**, for which they are called **nuclear chain fibers**.

3. Usually, four or more nuclear chain fibers are present in a spindle.

**Location of Receptors**

The **central noncontractile part** of the muscle spindle contains the receptors, whereas the **peripheral parts** contain **contractile elements**. Contractile units are absent in the central portion. Thus, stretch of the central part of the spindle activates the muscle spindle.

**Innervation of Muscle Spindle**

The muscle spindles have both afferent (sensory) and efferent (motor) innervations.

**Afferent Innervation (Sensory Fibers)**

Afferent fibers for muscle spindle are Ia and II fibers. Ia fibers have larger diameter (12 to 20 µm) than type II fibers (6 to 12 µm) and have faster conduction velocity. There are **two types of sensory endings** in each muscle spindle: the primary endings, and the secondary endings.

**Primary Endings**

The primary endings are the terminals of **type Ia afferent fiber**.

1. These are also called **annulospiral endings** as they are coiled spirally around the center of the intrafusal fibers.

2. Type Ia afferent fiber has two branches.

3. One branch of Ia fiber terminates on nuclear bag fiber 1, and the other branch terminates on the nuclear bag fiber 2 and the nuclear chain fibers.

**Secondary Endings**

The secondary endings are the terminals of **type II afferent fiber**.

1. They are also called **flower-spray endings**, as they appear like flowers.

2. They are usually present on the nuclear chain fibers.

3. The secondary endings are located in paracentral part of the spindle.

**Efferent Innervation (Motor Fibers)**

The spindles are innervated by a separate set of motor neurons, the **γ motor neurons**. They are also called **fusimotor fibers**.

1. These are small nerves arising from the anterior horn cells and are having the diameter of 3–6 µm. They constitute only about 30% of the fibers in the ventral root (70% of fibers are α motor neurons).

2. Because of their size and number, they comprise the **small motor nerve system**.
3. The γ motor neurons supply the peripheral parts of intrafusal fibers.

4. The terminals of the γ efferent fibers on the nuclear bag fiber 1 form the plate ending (as they end on the motor endplates), and on the nuclear bag fiber 2 and chain fiber form the trail ending.

Types of γ motor neurons: The γ motor neurons are of two types: the dynamic γ motor neurons that terminate on the nuclear bag fiber 1, and the static γ motor neurons that innervate the nuclear bag fiber 2 and nuclear chain fiber.

1. Stimulation of dynamic γ motor neurons increases response in type Ia afferent fibers (only during the dynamic phase of muscle stretch; i.e. during change in muscle length).

2. Stimulation of static γ motor neurons increases response in type II afferent fibers (only during the static phase of muscle stretch; i.e. the maintained stretch).

3. Thus, γ motor neurons monitor the muscle length and the speed of change in length.

The muscle spindle is also innervated by β efferent fibers and there are both dynamic and static β efferents.

**Afferent Discharge Patterns**

Two patterns of afferent discharge are obtained following stimulation of motor neurons: the dynamic, and the static patterns. Both γ and β motor neurons produce these two types of responses.

1. Stimulation of dynamic fusimotor fibers results in dynamic response and stimulation of the static fusimotor fibers produces static response.

2. Fibers from nuclear bag fibers show dynamic response and fibers from the nuclear chain fibers show static response (described below).

**Functions of Muscle Spindle**

Muscle spindles are receptors that are sensitive to stretch. When the muscle spindle is stretched, the sensory endings are distorted and action potential is generated in the afferent fibers. The frequency of afferent signal is proportional to the degree of stretching. Spindle is stretched by the stretch of the muscle, as the spindle is present in parallel with the extrafusal fibers. Therefore, stretch of the muscle initiates the activities in the spindle afferents.

1. The spindle afferents directly terminate on the cell bodies of α motor neurons in the spinal cord. Therefore, spindle afferent activity stimulates α motor neurons that in turn results in muscle contraction (Flowchart 127.1).

2. On the contrary, when muscle contracts, the spindle activity (firing in the spindle afferents) is less, as the muscle spindle is no more stretched.

3. Thus, stretch of the muscle causes muscle contraction by stretching the muscle spindle whereas contraction of the muscle causes muscle relaxation by inhibiting the spindle.

**Effects of Stimulation of γ Motor Neurons**

Muscle also contracts in response to stimulation of γ motor neurons.

1. But, in this situation, muscle contraction is not a direct response to the γ motor neuron discharge.

2. The stimulation of γ motor neuron causes contraction of the peripheral portions (that contains the contractile elements) of the muscle spindle.

3. This in turn stretches the central portions of the muscle spindle, which contains primary endings. This distorts the primary endings in the muscle spindle, which in turn increases the discharge of the afferent fibers.

4. Afferent fibers stimulate α motor neurons and cause muscle contraction (Flowchart 127.2).

5. Thus, γ motor neurons increase the sensitivity of the spindle to stretch.

6. It should thus be noted that the muscle contracts by stimulation of α motor neurons directly or by stimulation of γ motor neurons indirectly.

7. In fact, the sensitivity of the muscle spindle to stretch varies with the state of γ efferent discharge. Therefore, stimulation of γ motor neuron in a stretched muscle leads to maximum spindle response (Fig. 127.3).
Spindle Responses

The primary endings on the nuclear bag fibers and nuclear chain fibers are stimulated in response to stretch, but the patterns of responses are different.

**Dynamic Response**

The endings on the nuclear bag regions show a dynamic response, that is, they discharge more rapidly when the muscle is being stretched and less in response to sustained stretch.

**Static Response**

The primary endings on the nuclear chain fibers show a static response, that is, they discharge at an increased rate throughout the period of stretch of the muscle (Fig. 127.4).

Thus, the primary endings are capable of exhibiting both dynamic and static responses, which means they respond to both change in length and the velocity of lengthening of the muscle. The static response is the response to muscle stretch, i.e. change in muscle length at a maintained stretch, whereas the dynamic response is the response to the rate at which the muscle is being stretched, i.e. the velocity of lengthening of the muscle.

Control of \( \gamma \) Efferent Discharge

\( \gamma \) motor neurons do not receive input from primary sensory afferents. Rather, they are primarily influenced by the descending pathways.

1. Activity in the descending pathways increases the sensitivity of the muscle spindle by adjusting the rate of \( \gamma \) motor neuron discharge.
2. However, activities originating in the sensory pathways also indirectly influence \( \gamma \) motor neuron discharge by activating the descending influences. For example, in Jendrassik’s maneuver, the process in which the tendon reflexes are better elicited by trying to pull the hands apart when the flexed fingers are hooked, the rate of \( \gamma \) motor neuron discharge increases.
3. In Jendrassik’s maneuver, proprioceptive input from the hand activates the supraspinal centers, which in turn increases γ efferent discharge by stimulating the descending pathways.

4. Tendon reflexes are also exaggerated in anxiety, as anxiety increases γ efferent discharge.

α–γ Colinkage
The descending pathways that stimulate the γ motor neurons also stimulate the α motor neurons.

1. Therefore, γ efferent discharge increases along with the increased discharge of α motor neurons. This is called α–γ colinkage (α–γ coactivation; Fig. 127.5).

2. Because of this α–γ co-interaction, muscle spindle continues to discharge even when the muscle is in contracted state.

3. Thus, spindle adjusts motor neurons discharge throughout the period of muscle contraction.

Golgi Tendon Organ
Location
Golgi tendon organs (GTO) are found in the tendon of the muscles.

Structure
The GTOs are formed by the terminals of the group lb afferent fibers (Fig. 127.6).
1. The diameter of GTO is about 100 mm and the length is about 1 mm. These sensory endings are arranged in series with that of the muscle fibers.

2. The lb fibers originating from GTO terminate indirectly on α motor neurons via interneurons.

3. As the interneurons are inhibitory, stimulation of lb fibers inhibit the motor neuron activity.

Function
Because of their arrangement (in series) with the muscle, GTO can be activated either by muscle stretch or by contraction of the muscle.

1. However, a strong muscle contraction is a stronger stimulus than the passive stretch of the muscle (Fig. 127.7). The passive stretch does not effectively stimulate GTO because the elastic muscle fibers take up much impact of the stretch. But, the afferents from GTO discharge actively in response to muscle contraction as muscle contraction stretches the tendon to a greater extent.

2. The actual stimulus for activation of GTO is the force that develops in the tendon (by muscle contraction or muscle stretch).

Thus, GTO signals the force of muscle contraction, whereas the muscle spindle signals the muscle length. Therefore, GTO provides the force feedback whereas muscle spindle provides the length feedback (Fig. 127.8).
**CHAPTER SUMMARY**

**Key Concepts**

1. Muscle spindle is the receptor for muscle contraction, in response to stretch.
2. γ motor neuron increases the spindle sensitivity to stretch.
3. Muscle spindle provides the length feedback, and GTO provides the force feedback.

**Important to Know (Must Read)**

1. With the help of labeled schematic diagrams, describe the structure and functions of muscle spindle may come as a Long Question.
2. Structure and functions of muscle spindle, Fusimotor neuron, Spindle responses, Control of γ motor neuron discharge, Golgi tendon organ may come as Short Questions.
3. In Viva, examiner may ask… What is the structure of muscle spindle, What are the fibers in muscle spindle, What is afferent and efferent innervation of muscle spindle, What are the functions of muscle spindle, Fusimotor neuron, What are the spindle responses, What is static and dynamic γ motor neuron, What are the influences of descending pathways on γ motor neuron discharge, What are the static and dynamic spindle responses, What is Jendrassik’s maneuver, How it works, Structure and functions of Golgi tendon organ, What is the meaning of length feedback, and force feedback.
Spinal Reflexes

**Learning Objectives**

On completion of study of this chapter, the student **MUST** be able to:

1. Name the spinal reflexes.
2. Draw the schematic diagrams of stretch reflex and inverse reflex and explain their importance in motor functions.
3. Understand the importance of withdrawal reflex.
4. List the differences between stretch and withdrawal reflexes.
5. Explain the significance and mechanism of after discharge of withdrawal reflex.
6. Mention the properties of spinal reflexes.
7. Understand the mechanism of inhibitions in the spinal cord.

The student **MAY** also be able to:

1. Explain the properties of spinal reflexes.
2. Describe the role of spinal reflexes in motor control.

Spinal reflexes play a crucial role in the control of posture and movements. Many posture-regulating mechanisms control motor functions mainly by **altering the threshold and sensitivity of spinal reflexes**.

A spinal reflex is defined as a **stereotyped motor response to a specific stimulus like stretch of the muscle**. Typically, the spinal reflexes include receptors, afferents, interneurons, motor neurons, and the muscle (Fig. 128.1).

**The reflexes integrated in the spinal cord are:**

1. Stretch reflex (Myotatic reflex)
2. Inverse stretch reflex (Inverse myotatic reflex)
3. Withdrawal reflex (Flexion reflex)
4. Positive supporting reaction
5. Negative supporting reaction

The last two reflexes are not observed in normal subjects, rather elicited in spinal human/animal.

**Scientist contributed**

Ivan Mikhailovich Sechenov (1829–1905), a Russian physiologist, was Professor of Physiology at St. Petersburg and Moscow. He studied the role of **spinal reflexes** on locomotion. He demonstrated **cerebral inhibition of spinal reflexes**. Sechenov laid the foundation of Russian Physiology.

**STRETCH REFLEX (MYOTATIC REFLEX)**

**Definition:** The reflex contraction of the muscle to stretch when a skeletal muscle with its intact nerve supply is stretched is called the **stretch reflex**.
1. The stretch reflex is the key reflex in the regulation of posture and movement. The **stimulus** is the stretch of the muscle and the **response** is the contraction of the stretched muscle.

2. The stretch of muscle initiates activities in the afferent nerves that directly stimulate the motor neurons.

3. Therefore, stretch reflex is a **monosynaptic reflex** (a single synapse between the afferent and efferent limbs).

4. There are two types of stretch reflexes: the **phasic stretch reflex** and the **tonic stretch reflex**.

**Phasic Stretch Reflex**

The phasic stretch reflex is elicited by stimulating the primary endings of the muscle spindle.

**Stimulus**

The stimulus for phasic stretch reflex is the **sudden stretch** of the muscle.

**Reflex Arc**

The receptor is the muscle spindle and afferent is the group Ia afferent fiber (Fig. 128.2).

1. In the spinal cord, afferent fiber divides into two main branches.
2. The one of the branches of Ia fiber directly terminates monosynaptically on the homonymous motor neurons (i.e. the motor neuron that supplies the protagonist muscle), and the other branch terminates disynaptically via an inhibitory interneuron (Golgi bottle neuron) on the heteronymous motor neuron (i.e. the motor neuron supplying the antagonist muscle).

3. Thus, activity in the Ia afferent fiber **stimulates the homonymous motor neuron** that causes contraction of the protagonist muscle, and **inhibits the heteronymous motor neuron** that causes relaxation of the antagonist muscle.

4. Therefore, when the agonist muscle contracts, simultaneously the antagonist muscle relaxes.

5. **Relaxation of antagonist muscle facilitates movement** caused by contraction of agonist muscle.

6. Such an innervation that ensures activation of a set of motor neuron and inhibition of another set of motor neuron is called **reciprocal innervation**, and the inhibition of antagonist muscle is called reciprocal inhibition.

**Function**

Reflex contraction of muscle in response to a **sudden stretch** is the phasic stretch reflex.

1. The phasic reflex contraction results in **rapid limb movement**. The examples are **tendon reflexes**. The tendon reflex is elicited by tapping the tendon, which provides a quick stretch of the muscle.

2. By making rapid and transient movements, phasic stretch reflex ensures **immediate corrections of spinal motor output** in the moment-to-moment motor control.

**Clinical Significance**

Tendon reflex reflects the **general excitability of motor neurons**.

1. When excitability of the motor neurons is altered in different pathological conditions the tendon reflexes are depressed or exaggerated.

2. This helps the physician to diagnose the nature of the **neurological deficit based on the response of the tendon reflexes**. Especially, the excitability of γ motor neurons is more important as it controls spindle sensitivity.

3. **Exaggeration of tendon reflex is an index of increased γ motor neuron discharge** and depression of reflex indicates decreased γ motor neuron activity.

**Tonic Stretch Reflex**

**Stimulus**

The tonic stretch reflex is elicited by a **sustained stretch** of the muscle.

**Reflex Arc**

It is same as that of the phasic stretch reflex except that the receptors are both primary and secondary endings.

1. Therefore, the afferents are both Ia and II afferent fibers from the muscle spindle.

2. Group II afferents make direct connections with the α motor neurons. Thus, this is also a monosynaptic reflex.
Function

Tonic stretch reflex contributes to the muscle tone.
1. **Muscle tone** is the resistance of muscle to stretch.
2. Tonic stretch reflex is also important for regulation of posture.
3. To maintain standing position after assuming the position, the extensors of knees should contract so that knees remain extended and legs do not bend. This is achieved by the action of gravity on medial extensors of the thigh. Due to the effect of gravity, extensor muscles (antigravity muscles) are stretched.
4. The sustained stretch of extensors results in sustained contraction of these antigravity muscles that maintains extension at knee joint. Thus, the standing position is maintained.

After assuming standing posture for a longer duration, fatigue sets in. Due to fatigue, gradually the knees bend that further stretches the quadriceps muscles. The flexion at knee joints elicits additional tonic stretch reflex that, in turn, causes added sustained contraction of quadriceps. This maintains further extension of knees and prevents the person from falling. Thus, stretch reflex helps to restore and maintain the posture for a very long period.

- For example, a traffic police who is standing for a longer duration flexes his knees due to physical fatigue. This stretches his quadriceps and elicits tonic stretch reflex. Contraction of quadriceps opposes the flexion at the knee and restores his posture. Thus, tonic stretch reflex maintains posture.

In summary, the phasic stretch reflex mediates tendon jerk and controls rapid corrections in motor output, whereas the tonic stretch reflex maintains body position despite alteration in load on the body.

**INVERSE STRETCH REFLEX (INVERSE MYOTATIC REFLEX)**

**Definition:** Relaxation of the muscle in response to a strong stretch is called **inverse stretch reflex.** This is also known as **autogenic inhibition.**

**Reflex Arc**

**Receptors**
Receptors are **Golgi tendon organs** (GTO). These are netlike collection of knobby nerve endings located in the fascicles of a tendon. There are 3–25 muscle fibers per tendon organs.

**Stimulus**
The stimulus for inverse stretch reflex is a **stronger stretch.** An active muscle contraction also elicits this reflex.

**Reflex Circuit**
Afferent fibers from GTO are **Ib fibers** (Fig. 128.3).

**Fig. 128.3:** Schematic representation of an inverse stretch reflex.

1. Ib fibers terminate on the **inhibitory interneurons** that, in turn, project to the homonymous α motor neurons.
2. This results in inhibition of the agonist muscle. This is a disynaptic reflex.

**Functions**

Golgi tendon organ monitors force developed in the muscle.
1. The force is detected either by strong stretch or by an active contraction. Stimulation of the GTO inhibits the agonist muscle through its reflex connections.
2. The agonist muscle relaxes in response to activation of GTO.
3. Thus, a stronger stretch imparted on the muscle automatically inhibits the muscle. Therefore, the reflex is also called autogenic inhibition.

**Physiological Significance**

Muscle spindle (stretch reflex) monitors muscle length and GTO (inverse stretch reflex) monitors muscle tension, i.e. the force of contraction.
1. Thus muscle spindle provides length feedback and GTO provides force feedback (refer Fig. 127.8, Chapter 127).
2. Stretch reflex and inverse stretch reflex by acting together maintain optimal motor responses (of muscle tension and muscle length) for postural adjustments.
3. Inverse stretch reflex, by allowing the muscle to relax, prevents rupture of muscle when the muscle is stretched to greater extents.

**Effect of γ Motor Neuron on Stretch Reflexes and Muscle Tone**

The γ motor neurons control the activity of stretch reflexes by altering the sensitivity of muscle spindle to stretch.
1. Spindle afferents do not influence the activities of γ motor neurons, as they have no direct contact with them. However, descending influence from supraspinal segments increases the discharge of γ motor neurons, which, in turn, increases the sensitivity of the spindle to stretch.
2. In upper motor neurons paralysis, for example following spinal transection (in the recovery phase), the increased y motor neuron discharge (due to loss of inhibitory suprasegmental inputs on the y motor neurons) increases the reflex activity. The muscle becomes hyper-reactive due to increased phasic stretch reflex activity and becomes hypertonic due to increased tonic stretch reflex activity.

**Muscle Tone**

**Resistance of the muscle to stretch** is called tone.

1. Muscle becomes hypertonic or spastic when the resistance to stretch is more due to hyperactive stretch reflexes (increased y motor neuron discharge).
2. Muscle becomes hypotonic (flaccid) when the motor neurons supplying the muscle are damaged or when the discharge of y motor neurons is decreased.

**Clasp-Knife Phenomenon**

When a hypertonic muscle as seen in upper motor neuron paralysis is stretched, muscle contracts, but if the stretch is continued then muscle relaxes.

1. For example, flexing the elbow of such a patient first meets with greater resistance, but if the flexion continues then the muscle relaxes and suddenly the resistance force disappears (flexion occurs easily).
2. This type of high resistance followed by sudden collapse is known as clasp-knife phenomenon as it resembles the closing a clasp-knife.
3. Physiologically, this is called lengthening reaction as the response occurs due to lengthening of spastic muscle.

**Physiological Basis**

As the muscle tone is more, resistance against flexion of the limb is more. However, when flexion is continued, further stretch of the triceps muscle activates inverse stretch reflex that relaxes the muscle due to autogenic inhibition. Thus, the resistance force against flexion suddenly disappears.

**Clonus**

This is defined as a regular rhythmic contraction of a muscle subjected to a sudden and maintained stretch. The best example is the ankle clonus. This is elicited by dorsiflexing the foot and trying to maintain the dorsiflexion, which results in rhythmic planter flexion at the ankle.

1. It is typically seen in upper motor neuron paralysis in which the spindles are hyperactive.
2. When the muscle is suddenly stretched the spindle discharges and muscle contracts. Muscle contraction stops the spindle discharge and muscle relaxes. But, maintained stretch stimulates the spindle again and the process is repeated.

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**WITHDRAWAL REFLEX**

Withdrawal reflex is an example of polysynaptic spinal reflex. This is also called flexor withdrawal reflex because activation of it causes withdrawal of the body parts by flexing the limbs.

**Receptors and Stimuli**

The receptors are nociceptors that are present in the skin, muscle, tendon, or joints. Noxious or painful stimulation of skin, subcutaneous tissue or muscle elicits this reflex.

**Reflex Circuit**

The afferents are called flexor reflex afferents (FRA).

1. This includes type III and IV somatic afferents. The afferents, after entering the spinal cord divides into two branches to terminate on two separate sets of interneurons (Fig. 128.4).
2. One branch activates a set of interneurons that, in turn, excites α motor neurons supplying flexor muscles of the same side.
3. Another branch activates a separate set of interneurons that, in turn, inhibits α motor neurons supplying the same side extensor muscles.
4. A branch of the interneuron (commissural interneurons) excited by FRA crosses the midline to enter the opposite side of the ventral horn and terminate on opposite set of interneurons.
5. That means, the commissural interneurons activate the interneurons that, in turn, stimulate motor neurons...
Reflex activity continues even after the withdrawal of the stimulus. 

Muscle spindle

Single reciprocal innervation

Less

Double reciprocal innervation

Withdrawal reflex is a protective

Ia afferents

Flexor muscles of the same side and extensor muscles of the opposite sides.

Flexor reflex afferents (FRA)

Flexor muscles of the opposite side and extensor muscles of the same side and flexion of the other limb of the opposite side.

The same muscle

Reflex stops as the stimulus is withdrawn

Reflex activity continues even after the withdrawal of the stimulus. This occurs due to after discharge.

Widespread response

Nonlinear and may be widespread

Local sign:

Widespread: If the stimulus is still stronger, the opposite pattern of action in the opposite limb is called crossed extension reflex or Phillipson’s reflex.

The flexion response of the ipsilateral limb results in withdrawal of the body parts from the source of noxious stimulus and the extension of the opposite limb maintains balance during the act.

Withdrawal reflex is prepotent

Motors of the opposite side will continue to flex when the body part is withdrawn from the source of noxious stimulus.

Motors of the contralateral side will continue to extend when the body part is withdrawn from the source of noxious stimulus.

Withdrawal reflex is linear or stereotyped

The response to the withdrawal reflex is specific to a set of muscles.

Withdrawal reflex is not prepotent

The withdrawal reflex can be inhibited by the flexion response of the opposite limb.

Withdrawal reflex is absent

Withdrawal reflex is a protective reflex that protects body parts from damage caused by noxious (potentially harmful) stimuli. Flexion of the stimulated limb withdraws the limb and extension of the opposite limb supports or balances the body.

Withdrawal reflex is prepotent

This means when this reflex is activated, temporarily other reflex activities in the spinal cord almost stop at that moment, so that the spinal cord carries out the reflex responses precisely and without delay (Table 128.1).

Withdrawal reflex is absent

The pattern of response of flexor withdrawal reflex depends on the nature and the location of the stimulus. For example, abduction occurs when the medial surface of the limb is stimulated, and adduction occurs when lateral surface is stimulated. This is called local sign.

Withdrawal reflex is prepotent

Afterdischarge: A stronger stimulus not only causes widespread response but also prolongs the response. The prolongation of response is due to prolonged and repeated firing of the target motor neurons. This process is called afterdischarge. Afterdischarge occurs due to two mechanisms (Fig. 128.5):

i. Involvement of many interneuronal pathways of different length between afferent and efferent neurons. The pathways containing less number of interneurons stimulate the motor neuron early (as the impulses arrives at motor neuron early), and the pathways containing more interneurons stimulate the motor neurons later (as the impulses arrive at motor neuron later through these pathways) (Fig. 128.5). Thus, motor neurons are stimulated repeatedly.

ii. Presence of reverberating circuits in the interneuronal pathways in the spinal cord. The branches of the interneurons projects onto the other interneurons that project back to the same interneurons. Thus, stimulation of interneurons reverberates the impulses circuitously. Therefore, motor neurons are stimulated repeatedly.

Table 128.1: Differences between Stretch reflex and Flexion (withdrawal) reflex.

<table>
<thead>
<tr>
<th>Receptors</th>
<th>Latency</th>
<th>Target muscle</th>
<th>Reciprocal innervation</th>
<th>Response</th>
<th>Duration</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle spindle</td>
<td>Short (as it is monosynaptic)</td>
<td>The same muscle</td>
<td>Single reciprocal innervation</td>
<td>Linear or stereotyped</td>
<td>Reflex stops as the stimulus is withdrawn</td>
<td>Specific to a set of muscles</td>
</tr>
<tr>
<td>Ia afferents</td>
<td>Less</td>
<td>Widespread</td>
<td>Double reciprocal innervation</td>
<td>Nonlinear and may be widespread</td>
<td>Reflex activity continues even after the withdrawal of the stimulus. This occurs due to after discharge.</td>
<td>Less specific (involves many muscle groups).</td>
</tr>
<tr>
<td>Flexor reflex afferents (FRA)</td>
<td>Long (as polysynaptic)</td>
<td>Flexor muscles of the same side and extensor muscles of the opposite sides.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. Receptors
2. Latency
3. Target muscle
4. Reciprocal innervation
5. Response
6. Duration
7. Specificity
8. Local sign
9. Importance

Flexing contralateral extensor muscles and activate interneurons that, in turn, inhibit motor neurons supplying contralateral flexor muscles.

Response

As per the reflex connections described above, stimulation of flexor reflex afferents causes flexion of the ipsilateral limb due to activation of flexor group of muscles and inhibition of extensor group of muscles.

1. If the stimulus is stronger, extension of the contralateral limb occurs due to activation of extensor group of muscles and inhibition of flexor group of muscles.
2. This opposite pattern of action in the opposite limb is called crossed extension reflex or Phillipson’s reflex.
3. The flexion response of the ipsilateral limb results in withdrawal of the body parts from the source of noxious stimulus and the extension of the opposite limb maintains balance during the act.

Widespread response: If the stimulus is still stronger, impulses irradiate upward and downward in the spinal cord and activate motor neurons that supply muscle of other limbs to produce widespread response.

1. This results in extension of the other limb of the same side and flexion of other limb of the opposite side. This is one of the examples of irradiation of impulse in the spinal cord generated by the stimulus and also of the recruitment of motor neurons or motor units.
2. This is also an example of divergence of impulses in the spinal cord. That is, impulses in FRA project to many interneurons and motor neurons in the spinal cord and finally results in flexion of the same limb and with extension of the opposite limb, and extension of the other limb of the same side and flexion of the other limb of the opposite side.
3. Various patterns of these limb movements provide postural support and balance for immediate and appropriate withdrawal of body parts.

Special Features of Withdrawal Reflex

1. Protective reflex: Withdrawal reflex is a protective reflex that protects body parts from damage caused by noxious (potentially harmful) stimuli. Flexion of the stimulated limb withdraws the limb and extension of the opposite limb supports or balances the body.
2. Prepotent: Withdrawal reflex is prepotent. This means when this reflex is activated, temporarily other reflex activities in the spinal cord almost stop at that moment, so that the spinal cord carries out the reflex responses precisely and without delay (Table 128.1).
3. Local sign: The pattern of response of flexor withdrawal reflex depends on the nature and the location of the stimulus. For example, abduction occurs when the medial surface of the limb is stimulated, and adduction occurs when lateral surface is stimulated. This is called local sign.
4. Afterdischarge: A stronger stimulus not only causes widespread response but also prolongs the response. The prolongation of response is due to prolonged and repeated firing of the target motor neurons. This process is called afterdischarge. Afterdischarge occurs due to two mechanisms (Fig. 128.5):
   i. Involvement of many interneuronal pathways of different length between afferent and efferent neurons. The pathways containing less number of interneurons stimulate the motor neuron early (as the impulses arrives at motor neuron early), and the pathways containing more interneurons stimulate the motor neurons later (as the impulses arrive at motor neuron later through these pathways) (Fig. 128.5). Thus, motor neurons are stimulated repeatedly.
   ii. Presence of reverberating circuits in the interneuronal pathways in the spinal cord. The branches of the interneurons projects onto the other interneurons that project back to the same interneurons. Thus, stimulation of interneurons reverberates the impulses circuitously. Therefore, motor neurons are stimulated repeatedly.
Properties of Spinal Reflexes

The following are the properties of spinal reflexes:

Adequate Stimulus

The quality of a stimulus that evokes a reflex response is a very precise one. This is called the adequate stimulus for that particular reflex. For example, stretch of the muscle is the adequate stimulus for stretch reflex.

Convergence

Convergence literally means many to one projection (refer to Fig. 116.6A; Chapter 116). This is defined as the termination of several neurons on one target neuron. For example, many group Ia afferent fibers from muscle spindle of a particular muscle converging monosynaptically on a single α motor neuron.

Divergence

Divergence literally means one to many projections (refer to Fig. 116.6B; Chapter 116). This is defined as termination of a single neuron on many target neurons. The example of divergence is the termination of flexor reflex afferent on many motor neurons via different sets of interneurons in the spinal cord.

Facilitation

Convergence provides the physiological basis of facilitation. Activities in a single afferent fiber may not be able to excite the target motor neurons. But, when many afferent neurons projecting on a single motor neuron are stimulated simultaneously the motor neuron is activated. This is called facilitation.

Inhibition

Inhibition means, decrease in activities of the target neurons. An example of inhibition is the reciprocal inhibition in the spinal cord. Another example is the Renshaw cells inhibition (for details, refer to Chapter 116).

1. The motor neurons in the ventral horn give collateral to the interneurons that, in turn, project back to the same motor neuron. The interneurons (Renshaw cell) inhibit the output of the same motor neurons that stimulate them.
2. Inhibitions are classified as direct (post synaptic) and indirect (presynaptic) inhibitions or feedback (e.g. Renshaw cell inhibition) and feedforward inhibitions (for details, refer to Chapter 116).

Summation

There are two types of summations: spatial and temporal.

Spatial Summation

If the strength of the stimulus arriving at the synapse from an afferent neuron is not adequate, target motor neuron is not activated. However, it brings the target neurons to the subliminal fringe. If, other afferent neurons arriving at the same target neuron are stimulated simultaneously, the activities are said to summate on the target motor neuron and the motor neuron discharges. This is called spatial summation.

Temporal Summation

The impulse arriving in one afferent neuron may not be able to stimulate the target neuron; but, when the same afferent neuron is stimulated repeatedly in rapid succession, the target neuron is activated. This is called temporal summation.

Occlusion

When one afferent neuron is stimulated, it activates the motor neurons that are present in its discharge (central) zone and brings the motor neuron to the partially excited state that are present in the subliminal fringe (peripheral) zone. When the discharge zone of the neighboring afferent neurons overlaps, occlusion (decrease in the number of target neurons activated) occurs (for details, refer to Chapter 116), and when the discharge zone of the neighboring afferent neurons overlap facilitation (increase in the number of target neurons activated) occurs. This occurs in simultaneous activation of many closely situated neurons that have overlaps of their zones.

Habituation

Usually, the reflex responses are stereotyped. However, with experience, reflexes can also be modified. This is
called **habituation**. Habituation and sensitization forms the physiological basis of learning and memory in the CNS.

**Sensitization**

Decreased response of the target neuron to an afferent stimulation, when the stimulus is applied repeatedly is called **sensitization**.

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**Final Common Pathway**

The motor neurons that supply the skeletal muscle especially the α motor neurons are considered to be the **final common path** for the motor output. This is because all the neural influences arriving from different parts of the CNS, regulate muscle activity through these motor neurons.

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**CHAPTER SUMMARY**

**Key Concepts**

1. Spinal stretch reflexes are for immediate reflexive movements, and muscle tone. Descending pathways alter motor activity mainly by altering the activity of spinal reflexes.
2. Withdrawal reflex is a protective reflex.

**Important to Know (Must Read)**

1. **Long Questions** are usually not asked from this chapter.
2. Stretch reflex, Inverse stretch reflex, Withdrawal reflex, Afterdischarge, Lengthening reaction, Clasp knife phenomenon, Properties of spinal reflexes, Summation, Inhibition may come as **Short Questions**.
3. In **Viva**, examiner may ask… Name the spinal reflexes, Receptor, Stimulus, Reflex Arc, Function and importance of each spinal reflex, Details of stretch reflex, Reciprocal inhibition, Differences between stretch reflex and withdrawal reflex. Mechanism of afterdischarge, Lengthening reaction, Clasp-knife phenomenon, Mechanism of clonus, Properties of spinal reflexes, Special features of withdrawal reflex, Summation, Types of inhibition, Occlusion, Convergence and divergence.
Supraspinal influences on motor control are mediated through descending pathways:
1. Descending pathways are fibers passing downward from different parts of the brain that influence spinal cord neurons.
2. However, all descending tracts are not motor pathways like ceruleospinal and raphespinal tracts that predominantly influence sensory functions.
3. Supraspinal centers controlling motor activities are broadly divided into two categories: the motor cortex and the brain stem centers.

Therefore, pyramidal tract disease clinically refers to the disease that interrupts fibers of the corticospinal tracts.
3. Extrapyramidal tracts include reticulospinal, vestibulospinal, rubrospinal, and tectospinal tracts. **Confusion of pyramidal-extrapyramidal classification:** Pyramidal-extrapyramidal classification of descending pathways is not appropriate physiologically:
1. This is because, all the fibers of corticospinal tracts do not pass through pyramid, and especially most of the fibers in the anterior corticospinal tract bypass the pyramid.
2. Moreover, the pyramidal tract signs seen in patients suffering from pyramidal tract disease are not similar with the features of the isolated lesion of corticospinal tract produced in experimental animals. Hence, this clinical classification of pyramidal tract is not synonymous with the corticospinal tract.
3. More confusion is created for the extrapyramidal system. Anatomically, extrapyramidal tracts are fibers that do not pass through the pyramid in the medulla. Extrapyramidal tracts originate from the brainstem. Clinically, extrapyramidal diseases refer to the diseases of basal ganglia and cerebellum.
4. However, the major descending pathway involved in basal ganglia function is the corticospinal tract rather than the extrapyramidal tracts.
5. Thus, classification of pyramidal versus extrapyramidal pathways is **physiologically incorrect**.

Physiologically, descending pathways are divided into two: **lateral system** and **medial system** pathways. This classification is based on the termination of the descending pathways on the motor neurons in the spinal cord, and the function of the pathways.

### Lateral System Pathways

Lateral system pathways are pathways that **descend down in the lateral column** of the spinal cord:

1. These include **lateral corticospinal tract** and **rubrospinal tract**.
2. The fibers of these tracts are placed in the lateral funiculus in the spinal cord and fibers **terminate on the motor neurons that are placed laterally** in the ventral horn of the spinal cord, i.e. on the lateral group of motor neurons (Fig. 129.1).
3. Thus, these tracts are involved in the **regulation of skilled voluntary movements** as lateral group of motor neurons innervate the distal limb muscles.

### Medial System Pathways

Medial system pathways are pathways that **descend down in the medial and anterior columns** of the spinal cord. These pathways include:

1. Reticulospinal tract
2. Vestibulospinal tract
3. Tectospinal tract
4. Anterior corticospinal tract

The fibers of these tracts are placed in the medial and anterior funiculi in the spinal cord and they **terminate on the motor neurons that are placed medially** in the ventral horn, i.e., on the medial group of motor neurons (Fig. 129.2). Therefore, these tracts are involved in **regulation of posture** as the motor neurons of medial group innervate the proximal limb muscles and the muscles of the axial skeleton of the body.

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### MEDIAL SYSTEM PATHWAYS

#### Corticospinal Tracts

**Introduction**

Traditionally, corticospinal tract, pyramidal tract and upper motor neurons are used interchangeably. But, strictly speaking, these tracts are not altogether synonymous:

There are **two groups** of corticospinal tracts: the lateral and anterior corticospinal tracts.

1. The **lateral corticospinal tract** (LCST) constitutes about 80% of the fibers in the corticospinal pathway. This is the most important descending pathway for the control of skilled voluntary activities.
2. The **anterior or ventral corticospinal tract** constitutes only 20% of the fibers in the corticospinal pathway and is involved in control of posture.

**Origin**

The fibers of corticospinal tract **originate from the primary motor cortex (area 4)**, especially from the large motor cells of Betz in the fifth layer of the precentral gyrus:

1. There are about 30,000 Betz cells in the cortex, whereas about one million axons are present in corticospinal tract, which clearly indicate that the corticospinal fibers also originate from other areas in the cortex.
2. The **other cortical motor areas** include **premotor cortex** (lateral part of area 6), **supplementary motor area** (medial part of area 6), **primary somatosensory cortex** (area 3, 1, and 2) and **parietal cortex** (area 5 and 7).
3. Motor cortex contributes to 60% of fibers (30% from area 4 and 30% from area 6) and sensory cortex contributes to 40% of fibers in the corticospinal tract.

**Course**

Fibers arising from different parts of the cortical areas converge through **the posterior limb of the internal capsule** (Fig. 129.3):
Chapter 129: Descending Pathways

1. As the fibers in the subcortical areas appear in a radiating pattern, they are collectively known as corona radiata.
2. After passing through internal capsule, fibers descend down in the ventral brainstem as the cerebral peduncles. In the medulla, fibers pass through the pyramid (medullary pyramid).
3. About 80% of the fibers after passing through the pyramid, immediately decussate (cross over to the opposite side) and descend down in the lateral funiculus of the spinal cord. This forms the lateral corticospinal tract. The lateral corticospinal fibers have monosynaptic connections with anterior horn cells.
4. The remaining 20% of the fibers descend down ipsilaterally (do not decussate) in the anterior funiculus of the spinal cord. This constitutes the ventral or anterior corticospinal tract. The fibers cross over to the opposite side only at the spinal cord segments through their termination on the interneurons.

Termination

Fibers of lateral corticospinal tract terminate on lateral group of motor neurons in the ventral horn of spinal cord:
1. About 30% of Lateral CST fibers terminate directly on motor neurons and 70% fibers terminate through interneurons. These motor neurons innervate the distal limb muscles.

2. The fibers of ventral corticospinal tract do not directly terminate on the motor neurons, rather they end on the interneurons in the same side of the spinal cord, which in turn cross over to the opposite side and terminate on the medial group of motor neurons. Few fibers from the interneurons terminate on the same side of medial group of motor neurons. These motor neurons supply the proximal limb muscles and axial muscles of the body.

Functions

The motor cortex is mainly involved in initiation, planning and control of movement. Corticospinal tracts transmit central command signal from motor cortex to the spinal cord interneurons and motor neurons:
1. Lateral corticospinal tract controls the skilled voluntary movements of the body.
2. The anterior corticospinal tract controls posture.

Effects of Lesions

Lesion of lateral corticospinal tract results in impairment of skilled voluntary activities like writing, painting, etc. But, as the rubrospinal tract is intact, the subject recovers after few days or weeks:
1. However, isolated lesion of lateral corticospinal tract is very uncommon in humans. In addition, diseases that affect corticospinal tract also affect the corticobulbar tracts that influence activities of extrapyramidal systems. Thus, a pure corticospinal tract disease is not seen in humans.

2. **Lesion of anterior corticospinal tract** in animals results in inability to maintain posture while walking, climbing, etc. But in human beings, postural deficit following lesion of anterior corticospinal tract is not prominent because of two reasons; firstly, this tract is not well developed in humans and secondly, other major posture regulating pathways especially the reticulospinal tract and vestibulospinal tracts are still intact.

**Clinical Importance**
Corticospinal pathway may be interrupted anywhere along its course from cortex to spinal cord:

1. However, the lesion of corticospinal tract at the internal capsule (capsular lesion) is the commonest pyramidal tract lesion:
   - As the fibers coming from different parts of the cortex pass through a narrow tunnel in the posterior limb of the internal capsule, disease of the internal capsule results in complete interruption of corticospinal fibers.
   - This leads to contralateral hemiplegia.
2. It should be noted that, the ascending fiber systems from basal ganglia and cerebellum pass close to the internal capsule. Therefore, extrapyramidal systems are also affected in addition to involvement of corticospinal fibers.
3. Consequently, pyramidal tract disease due to capsular lesion is often termed as complete upper motor neuron paralysis.
4. The usual cause of capsular lesion is the rupture of Charcot’s artery (lenticulostriate branch of middle cerebral artery; this is also called the artery of cerebral hemorrhage as it accounts for more than 60% of the causes of intracerebral hemorrhage).

**UMN Paralysis**
Paralysis that results from lesion of descending fibers between their origin from cortical motor areas and their termination on anterior horn cells in the spinal cord is called as upper motor neuron paralysis. Clinically, pyramidal (corticospinal) tract lesion is referred to as upper motor neuron paralysis.

**Features of UMN Paralysis**
1. Increased muscle tone (spasticity).
2. No muscle atrophy (mild atrophy may occur in the long run due to disuse of the muscle, called disuse atrophy).
3. Muscles are usually affected in groups (individual muscles are never affected).
4. Tendon reflexes are exaggerated.

5. Superficial reflexes are lost
6. Extensor planter response (Babinski’s sign positive)
7. No fascicular twitches
8. No denervation potential in EMG
9. Normal nerve conduction studies

However, if only the corticospinal fibers are interrupted by the disease process that affects medullary pyramid (or, experimentally produced lesion of medullary pyramid), most of the above-mentioned features are not observed. The most prominent features of such a lesion are paralysis or weakness of the distal muscles of the limbs and positive Babinski’s sign. Spasticity is not seen, muscle tone even may be decreased. This indicates that descending fibers passing through pyramid are mostly the fibers of lateral corticospinal tract. The features described for pyramidal tract disease indicate the involvement of corticobulbar fibers that influence brainstem motor nuclei in addition to the interruption of corticospinal fibers.

**Physiological Basis of Features of UMN Paralysis**

**Spasticity**
Spasticity occurs due to increased discharge of motor neurons and increased excitability of the motor neuron pool:

1. In upper motor neuron lesions, not only the corticospinal fibers are interrupted, but also the corticoreticular fibers are damaged.
2. Normally, corticoreticular fibers (fibers from motor cortex to the brainstem reticular formation, especially to the pontine reticular nuclei that forms pontine reticulospinal tract) inhibit reticulospinal pathway.
3. Therefore, interruption of corticoreticular pathway facilitates reticulospinal activity. Normally, pontine reticulospinal tract excites the proximal extensor muscles.
4. Loss of inhibitory corticoreticular influence makes the reticulospinal tract more facilitatory, and therefore, muscle tone increases. Hence, spasticity and hypertonia are important features of UMN paralysis.

**Absence of Muscle Atrophy**
Muscle atrophy occurs when either the blood supply or the nerve supply to a muscle is disrupted.

1. In UMN paralysis, the nerve supply is not interrupted (the lower motor neurons remain intact).
2. Therefore, atrophy is not a feature of UMN paralysis.
3. However, in long standing cases, mild atrophy occurs due to disuse of the muscle. This is called disuse atrophy.

**Exaggeration of Deep Tendon Reflexes**
Usually, the upper motor neurons are inhibitory to the lower motor neurons.

1. In UMN paralysis, loss of these inhibitory influences increases the motor neuron discharge.
2. Especially, the increased $\gamma$ motor neuron discharge increases the sensitivity of the muscle spindle to stretch. This results in increased deep tendon reflex.
Loss of Superficial Reflexes

Superficial reflexes are long polysynaptic reflexes that involve different parts of CNS (unlike stretch reflexes that are monosynaptic and integrated at the level of spinal cord):
1. The afferent pathways of superficial reflexes ascend up in the ascending sensory systems.
2. The efferent pathways are the descending motor pathways that finally terminate in skeletal muscles.
3. As efferent pathway is disrupted in UMN paralysis, the superficial reflexes are abolished.

Extensor Planter Response

Corticospinal tract excites the flexor motor neurons and inhibits the extensor motor neurons supplying the muscles of the digits of the limbs. Therefore, normally stroking the sole of the foot elicits plantar flexion. In UMN paralysis, disruption of corticospinal influence on the lumbosacral motor neurons causes dorsi-flexion of big toe and fanning of other toes (extensor planter response; Babinski’s sign positive).

Rubrospinal Tract

Origin
Rubrospinal tract originates from red nucleus, located in the midbrain. Red nucleus receives strong excitatory input from motor cortex and cerebellum.

Course
Immediately after originating from red nucleus, fibers cross over to the opposite side at the same level (Fig. 129.4). [Note: Both lateral descending systems, viz, lateral corticospinal and rubrospinal tracts, cross over to opposite side]. After descending down through contralateral brain stem, fibers occupy the lateral column of the spinal cord. The fibers terminate on lateral group of motor neurons that innervate distal limb muscles. Rubrospinal tract excites flexor group of muscles and inhibits extensor muscles.

Functions
Rubrospinal tract controls skilled voluntary movements.

Applied Physiology
In experimental animal, lesion of rubrospinal tract produces deficit in the distal limb muscles, especially in the flexor group of muscles. However, if the lateral corticospinal tract is intact, the deficit persists temporarily.

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Vestibulospinal Tracts
Vestibulospinal tracts (VST) originate from vestibular nuclei. There are four vestibular nuclei: lateral, medial, superior and inferior. Vestibular nuclei receive inputs from otolith organs and semicircular canals. Therefore, VST maintain body posture in response to change in head position and acceleration of the body. Vestibular nuclei are also connected reciprocally with superior colliculi, cerebellum and reticular formation. Therefore, they also control eye position during head movement and balance of the body during movement.

There are two important vestibulospinal tracts: lateral vestibulospinal and medial vestibulospinal tracts.

Lateral Vestibulospinal Tract

Origin
Lateral vestibulospinal tract (LVST) originates from lateral vestibular nucleus (Deiter’s nucleus) in the brainstem.

Course
This tract descends down ipsilaterally through the brainstem and spinal cord. The fibers occupy the ventral funiculus of the spinal cord and terminate on the medial group of interneurons and motor neurons in the ventral horn of spinal cord (Fig.129.5). This tract extends throughout the rostrocaudal extent of the spinal cord.
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Functions
It excites motor neurons that supply the proximal group of muscles (especially the extensor muscles of the limb). Thus, LVST controls posture. The input to lateral vestibular nucleus comes mainly from semicircular canals and otolith organs of the inner ear. Therefore, LVST controls body posture especially the head position in response to angular acceleration.

Clinical Importance
In decerebrate animals (following midcollicular section), LVST becomes hyperactive due to loss of inhibitory controls from the cortical areas on it. Therefore, extensor rigidity is the hallmark of decerebrate animals. In humans, brainstem lesion due to stroke or injury facilitates vestibulospinal tract activity that manifests in the form of neck, arms and leg rigidity.

Medial Vestibulospinal Tract
Origin
Medial vestibulospinal tract (MVST) originates from medial vestibular nucleus.

Course
MVST descends down ipsilaterally in the brainstem and ventral funiculus of the spinal cord. It extends up to the midthoracic spinal cord segments. The fibers of MVST terminate on the medial group of interneurons and motor neurons (Fig. 129.5).

Functions
MVST controls body posture. The input to medial vestibular nucleus comes mainly from the semicircular canals. Therefore, MVST adjusts body posture especially the head position in response to angular acceleration.

Reticulospinal Tracts
Core of brainstem contains reticular formation. Reticular formation receives inputs from spinal cord, vestibular nuclei, cerebellum, hypothalamus, tectum and cortex, and projects mainly to cortex, thalamus, and spinal cord:

1. Projection of reticular nuclei to spinal cord is important in control of motor activity as they profoundly affect motoneuronal excitability, especially of the γ motor neurons. In this regard, two reticular nuclei are important: nucleus reticularis pontis in pons and nucleus gigantocellularis in medulla.
2. Accordingly, there are two main reticulospinal tracts: the pontine reticulospinal tract, and the medullary reticulospinal tract. Reticulospinal tracts are most important medial system pathways for control of posture.

Pontine Reticulospinal Tract
Origin
Pontine reticulospinal tract originates from nucleus reticularis pontis oralis and nucleus reticularis pontis caudalis located in the pontine reticular formation.

Course
This tract descends down ipsilaterally in the spinal cord. The fibers terminate on the medial group of interneurons and motoneurons that innervate the proximal and axial groups of muscles of the body (Fig. 129.6).

Functions
The function of pontine reticulospinal tract is similar to that of lateral vestibulospinal tract. It excites the motor neurons of the proximal extensor muscles that are involved in regulation of posture.

Medullary Reticulospinal Tract
Origin
Medullary reticulospinal tract originates from nucleus gigantocellularis located in the reticular formation of the medulla.

Course
The tract descends down ipsilaterally in the ventral funiculus of the spinal cord to terminate on the medial group of interneurons and motoneurons that innervate the proximal group of muscles.
Functions
This tract is mainly inhibitory. It inhibits the activities of the motor neurons that innervate extensor neurons.

Tectospinal Tract

Origin
Tectospinal tract originates from the tectum or superior colliculus (from deep layers).

Course
Immediately after originating from superior colliculus, fibers cross-over to the opposite side below the peri-aqueductal gray (Fig. 129.7). The fibers then descend down in the ventral funiculus of the spinal cord to terminate on the medial group of interneurons and motor neurons. This is the smallest of all descending tracts as it extends up to the midcervical region of the spinal cord.

[Note: All medial descending pathways descend down ipsilaterally in brain and spinal cord except, tectospinal tract that crosses over to opposite side].

Functions
Superior colliculus mainly receives visual inputs. Therefore, tectospinal tract regulates contralateral movement of the head in response to visual stimuli.

OTHER DESCENDING PATHWAYS

Monoaminergic Pathways

This includes monoaminergic (raphespinal and ceruleospinal tracts) pathways.

Raphespinal Tract

Origin
This tract originates from nucleus raphe magnus in the medulla.

Course
The fibers descend down ipsilaterally and terminate on the interneurons in the dorsal horn (refer Fig. 120.7; Chapter 120) that inhibit the transmission of impulses in the nociceptive pathway. Few fibers also terminate on the interneurons in the ventral horn that are excitatory to the motor neurons.

Functions
The fibers of the raphe spinal pathway are serotonergic. They inhibit the nociceptive transmission in the spinal cord (endogenous pain inhibiting system). Through their termination on the motor neurons, they are involved in sensory-motor coordination at the level of spinal cord.
Ceruleospinal Tract

**Origin**
Fibers originate from the nucleus locus ceruleus and nucleus subceruleus.

**Course**
Fibers mainly descend down ipsilaterally in the spinal cord to terminate on the interneurons and motor neurons.

**Functions**
The fiber of this pathway contains norepinephrine. The pathway is inhibitory to the nociceptive afferents, and also to the motor neurons. It alters the excitability of the motor neurons to different stimuli and brings sensory-motor coordination in the spinal cord.

**LOWER MOTOR NEURON PARALYSIS**
Lower motor neurons (LMN) paralysis occurs in diseases that cause destruction of anterior horn cells or their axons in dorsal root, nerve plexuses, or the peripheral nerves. The examples of LMN disease are nerve lesion as occurs in nerve injury or diseases of the nerve, poliomyelitis, motor neuron disease, and lesion of nerve roots.

**Features of LMN Paralysis**
1. Flaccid paralysis (muscles are hypotonic).
2. Pronounced muscle atrophy.
3. Individual muscles are affected depending on the muscles supplied by that particular nerve.
4. Tendon reflexes are diminished or absent.
5. Superficial reflexes are lost.
6. Flexor planter response (Babinski’s sign is not elicited)
7. Involuntary movements like fascicular twitches are observed.
8. Denervation potentials (fibrillation, fasciculation, and sharp waves) are seen in EMG.
9. Nerve conduction is decreased or absent.

**Physiological Basis**
1. As the lower motor neurons are interrupted, the innervation to the muscle is lost. Therefore, pronounced muscle atrophy occurs as secretion of nerve growth factors is abolished and muscle function is lost.
2. The muscles that are innervated by the nerve are only affected, not the muscles of one limb or side of the body as seen in UMN paralysis (Table 129.1).
3. Loss of motor neurons disrupts the reflex arc of the stretch reflexes, as well as superficial reflexes.
4. Therefore, both the tendon and superficial reflexes are diminished or lost in LMN paralysis. The denervation abolishes influence of γ motoneurons that results in hypotonia (flaccidity of muscles), an important feature of LMN paralysis.
5. Usually, muscular paralysis is associated with sensory changes, because the nerve that carries the motor impulses from the spinal cord also transmits sensory information to the spinal cord.
6. Nerve conduction is decreased because of damage to the nerve fibers.
7. Babinski’s sign is not elicited due to loss of motor neuron activity, but if at all present, is of normal flexor type.
8. As the muscle is denervated the denervation potentials (fibrillation or fascicular twitches) are observed in EMG recordings.

**Patterns of Paralysis**
Paralysis or plegia means complete loss of voluntary movement, whereas paresis refers to the weakness of muscles (incomplete paralysis). Depending on the distribution of parts of the body involved, paralysis is divided into following categories.

**Monoplegia**
Monoplegia refers to weakness or paralysis of all the muscles of one limb (leg or arm). Paralysis of an individual muscle or a group of muscle is not monoplegia. Examples of monoplegia are crural (leg) monoplegia that occurs due to trauma, myelitis, disc-prolapse or tumor of thoracolumbar segments of the spinal cord or brachial (arm)
monoplegia that occurs due to diseases affecting cervical segments. Monoplegia may also occur due to a central cortical defect (thrombotic or embolic infarction, or a circumscribed tumor or abscess).

Hemiplegia
Hemiplegia means paralysis of one half of the body. This is the commonest form of paralysis that involves arm, leg and sometimes the face on one side of the body. Usually it occurs due to lesion of the corticospinal pathway at the internal capsule that results in contralateral hemiplegia (as discussed above).

Paraplegia
Paraplegia refers to paralysis of both lower limbs. It usually occurs due to spinal cord injury or diseases that transect the cord. Rarely the diseases of motor cortex, cauda equina, or peripheral nerves cause paraplegia.

Quadriplegia
Quadriplegia or tetraplegia indicates paralysis of all four extremities. It usually occurs due to transection of spinal cord in the upper cervical segments. Disease of the upper motor neurons bilaterally in the cervical cord, brainstem, or cerebrum can also cause quadriplegia. Diplegia is a special form of quadriplegia in which the legs are affected more than the arms. Triplegia occurs most often as a transitional condition in the development of or partial recovery from tetraplegia.

Isolated Paralysis
Isolated paralysis of one or more muscle groups occurs due to disease of a particular nerve or the branch of the nerve.
Chapter 130

Regulation of Posture and Movement

Learning Objectives

On completion of study of this chapter, the student MUST be able to:
1. Outline the organization of motor control.
2. Classify postural reflexes.
3. List the postural reflexes integrated at different levels of neuraxis.
4. Describe the features in spinal, decerebrate, midbrain and decorticate preparations.
5. Explain the mechanism of decerebrate rigidity.
6. Name the cortical motor areas and give their functions.
7. Appreciate organization of body parts in motor homunculus.
8. Understand the role of various components of neuraxis in regulation of posture and movement.

The student MAY also be able to:
1. Describe the mechanisms of regulation of posture and movement.
2. Explain the dysfunctions of regulation of posture and movement.

Movement occurs due to muscle contraction. Activities in the muscle depend on the discharge of the spinal motor neurons. Spinal motor neurons are continuously influenced by the impulses arriving from various supraspinal centers via descending fibers. These influences originate in brainstem, cortex, cerebellum, and basal ganglia. The movements are planned in the cortex, basal ganglia, and cerebellum (Fig. 130.1). The command is conveyed to the spinal cord motor neurons from the cortex directly via corticospinal tract, and from basal ganglia and cortex via corticobulbar pathways and from cerebellum via its projection to the brainstem nuclei that influence the activities of extrapyramidal system. These upper motor neurons regulate movement by constantly altering the activities of the spinal motor neurons according to the need of the situation.

The supraspinal inputs controlling the spinal motor neurons carry out three important functions:
1. Initiation and execution of voluntary activities.
2. Coordination and smoothing of movements.
3. Postural adjustments to provide a stable background for movement.

The motor plan is conveyed to spinal motor neurons as motor command signal that initiates motor activities.

Fig. 130.1: Overall organization of motor control.

Once movement is initiated, activities in the muscle, tendon, ligaments, joints, and skin provide sensory feedback to the CNS.
Scientist contributed

Charles Scott Sherrington (1857–1952) was the first neurophysiologist to systematically analyze the functions of nervous system. He proposed the concept of integration of body functions of organism as a whole is the coordinated action of nervous system. He analyzed the stretch reflex, described arrangement of motor fibers in lumbosacral plexus and studied functions of many parts of the brain. Many experimental studies of nervous system are named after him, for example, decerebrate preparation for the study of medullary integration of motor function is known as Sherringtonian decerebration. He shared Nobel Prize in Physiology or Medicine in 1932 with Edgar Douglas Adrian for discoveries regarding the functions of neurons.

1. Via **dorsal and ventral spino cerebellar tracts**, feedback inputs directly reach spino cerebellum. Cerebellum immediately interacts with motor cortex and instantaneously corrects motor activities by comparing initial plan with success (**motor adjustments**) via extrapyramidal pathways (Fig. 130.2).

2. **Proprioceptive inputs** reach sensory cortex via dorsal column pathways that project to the motor cortex. Motor cortex via its corticospinal and corticobulbar projections makes necessary changes in motor output for smooth execution of movement.

3. **Smoothening and coordination of movement and postural adjustments** during movement are regulated mainly by the **extrapyramidal systems** that originate from the brainstem.

4. The major extrapyramidal pathways are vestibulospinal, reticulospinal, and tectospinal tracts. As the activities in these tracts are profoundly influenced by **cerebellum and basal ganglia**, these structures are largely considered as **extrapyramidal structures**.

LEVELS OF INTEGRATION

The mechanisms involved in regulation of posture and movement are integrated at the following levels of the CNS:

1. Spinal cord
2. Medulla
3. Midbrain
4. Cortex
5. Basal ganglia
6. Cerebellum

At the spinal cord level, afferent inputs produce **simple reflex responses**. Higher up in the neuraxis, afferent inputs produce **alteration in motor behavior** and occurrence of **more complex responses**. The influence of a particular center in the neuraxis on posture and movement is best studied by separating (by making section in experimental animals) the center from its higher centers.

When the neuraxis is transected at a particular level, motor activities carried out by the centers below the section are **usually accentuated**. This is called **release phenomenon**, which facilitates the final motor output.

The facilitation of motor activity is due to **two main reasons**:

i. Release of the center from the inhibitory control of higher centers.
ii. **Denervation hypersensitivity** of the center below the transection.

**Postural Reflexes**

Postural reflexes are integrated at different levels in the CNS that on activation produce appropriate motor responses. These reflexes maintain a balanced posture of the body, provide a stable background for movement and adjust body posture constantly during the movement.

The postural reflexes are broadly classified into **two broad categories**: **phasic** and **static reflexes**.

**Phasic or Dynamic Reflexes**

These are the reflexes that are short-term in nature and produce transient movements for immediate change in posture and rapid postural adjustments. The examples are:

- Phasic stretch reflex,
- Righting reflexes,
- Vestibular placing reactions, etc.

**Tonic or Static Reflexes**

These reflexes produce sustained contraction of the muscle and mainly provide a stable background for maintaining
a posture. They also adjust posture during movement. The examples are:
• Tonic stretch reflexes,
• Tonic neck reflexes,
• Tonic labyrinthine reflexes, etc.

**SPINAL INTEGRATION**

The spinal cord plays a crucial role in the regulation of posture and movement. It helps in execution of four important motor functions:

1. Spinal cord contains neural circuitry that generates basic postural reflexes, called **spinal reflexes**. Spinal reflexes produce immediate change in motor output that leads to rapid responses. For example, withdrawal of body parts in response to a noxious stimulus.

2. Spinal cord contains neural circuitry that can alter motor neuron output to maintain the tone of the muscle. Especially, the change in γ motor neuron activity alters the muscle spindle sensitivity to stretch that in turn greatly influences muscle tone. However, the γ motor neuron activity is primarily influenced by activities in the descending fibers.

3. Spinal cord contains neural circuitry (pattern generator for locomotion) that on appropriate stimulation produces basic movements like walking.

4. Tonic stretch reflex executed in spinal cord is essential for maintaining posture.

**Spinal Preparation**

The role of spinal cord in regulation of movement and posture is studied in a spinal animal. A **spinal animal** is the animal (usually performed in a cat) in which influence of brain on spinal cord is removed by making a section below the medulla. Ideally, in the spinal preparation, the section is made below the C₅ so that the respiration (diaphragm activity) remains intact. However, usually, section is made below the mid-thoracic level so that cardiovascular activity also remains intact and it becomes easy to maintain the preparation.

**Three phases** are distinctly marked in spinal preparation:

1. The stage of shock (stage of flaccidity)
2. The stage of recovery (stage of reflex activity)
3. The stage of failure

**Stage of Shock**

As soon as the section is made, animal enters into the stage of shock (**spinal shock**). This is also called as the stage of flaccidity. Spinal transection causes immediate and permanent loss of sensations and voluntary movements below the level of lesion. In all vertebrates, transection of the spinal cord is followed by a period of spinal shock during which all spinal reflex responses are profoundly depressed.

**Duration of Shock**

The duration of spinal shock is proportionate to the degree of encephalization of motor function. Accordingly, the duration of shock is different in different species.

- In frogs: few minutes (2–4 minutes)
- In dogs and cats: few hours (2–4 hrs)
- In monkeys: few days (2–4 days)
- In humans: few weeks (1–4 weeks)

**Mechanism of Shock**

The exact mechanism of spinal shock is not known. However, evidences suggest that spinal shock results from immediate abolition of tonic bombardment of motor neurons by descending fibers. The loss of supraspinal, more precisely the supra-segmental influences on the spinal cord in the acute phase causes shock, which is evident from the following observations:

1. The **extent and duration of areflexia** is much greater in species that have more developed suprasegmental control of spinal cord.
2. After recovery from spinal shock following the initial transection, if a second transection is made caudal to the first one, the **areflexia of the previously affected muscle does not occur again**. This is because the second transection cannot further compromise suprasegmental influences. Thus, it indicates that loss of influences of descending pathway is the major cause in the genesis of spinal shock.
3. Neurophysiological evidences indicate that motor neuron excitability is depressed after spinal transection.

**Features of Shock**

Below the level of the lesion:

1. The muscles are completely paralyzed (**flaccidity**).
2. All the reflexes are abolished (**areflexia**).
3. Complete loss of all sensation (**anesthesia**)

At the level of lesion: Cramp like pain is present.

**Other Features**

- The **bladder and rectum are generally paralyzed**. The sphincter vesicae, however, frequently retains its function or recovers rapidly.
- The **penis remains flaccid** and erection becomes impossible.
- Section below T₁ leads to gross fall in BP which resembles to the degree of hypotension observed following destruction of vasomotor center. As the vasoconstrictor fibers leave the spinal cord between T₁ and L₂, section below L₂ produces little fall in BP.
- Absence of muscle pump activity due to muscle paralysis significantly decreases blood flow and venous return. **Cardiac output decreases grossly**. Therefore, legs become blue and cold.
- If the lesion is at T₅, impulses coming from abdominal viscera are interrupted. Therefore, griping sensation or distension of the viscera cannot be appreciated.
Chapter 130: Regulation of Posture and Movement

Stage of Recovery
Smooth Muscle Function
As the stage of shock passes off, activity returns first in smooth muscle. The sphincter vesicae recovers very soon, but detrusor muscle regains slowly. The paralyzed blood vessels regain tone and BP slowly returns to normal.

Reflex Responses
The first reflex response to appear is slight contraction of leg flexors in response to a noxious stimulus. Adductors also respond to painful stimulus. In some patients, knee jerk recovers first.

Mechanism of Recovery
Mechanism of reflex recovery from spinal shock is not clearly known. It may possibly be due to the segmental influences that become more effective during the recovery. This occurs due to the axonal sprouting. The sensory fibers that enter the cord give more collaterals and spinal interneurons sprout additional terminals. These nerve terminals occupy the synaptic space vacated by degeneration of sectioned descending fibers.

Muscle Tone and Size
Tone in skeletal muscle returns after 2 to 3 weeks in humans. Tone reappears first in the flexor muscles of lower limbs, then in the flexors of upper limbs and trunk. Extensor muscles remain flabby for a longer period. Due to increased flexor tone, the body adopts a position of flexion. Thus, the paralysis in this stage is referred to as paraplegia in flexion. Though paralyzed, muscles usually do not undergo wasting due to constant reflex activity.

Reflex Movements
Spontaneous involuntary flexor movements of the limb occur. The small toes are separated and raised. Contraction of flexor group of muscles occurs, which is accompanied by the reciprocal inhibition of the extensor muscles.

Flexor Reflex
The reflex response that returns first is the flexor reflex to a noxious stimulus. Stimulation of the lateral aspect of the sole of the foot leads to Babinski sign with dorsiflexion of big toe, abduction of other toes, flexion of knee and hip and abduction of the thigh. The antagonistic muscles are inhibited. Flexor reflex is a protective withdrawal reflex, which removes the limb from the site of injury.

Mass Reflex
By scratching any point on the inner aspect of the thigh or lower anterior abdominal wall, a very widespread response is readily elicited. This is called mass response, which consists of:
• Flexor spasm of lower extremities and contraction of the anterior abdominal wall.
• Evacuation of bladder and bowel, those occur partly due to increased abdominal pressure.
• Profuse sweating below the level of lesion. Sweat fibers to head and neck arise from T1-5 and to arm from T5-9. Thus, with a lesion at T1 or above, the whole body sweats when mass reflex is activated. Mass reflex appears late, maybe after several months of transection.

Coitus Reflex
This is elicited by stimulation of glans penis, or the skin around the genitals. The response consists of swelling and stiffening of penis, withdrawal of testis (because of contraction of cremasteric muscle) and curling up of the scrotal skin by the action of the dartus. Flexors of hip and abductors of thigh also contract. Seminal emission may occur. However, full intercourse never becomes possible.

Deep Reflexes
Knee jerk returns in 1 to 5 weeks after the recovery of flexor response. Quadriceps muscle, though contract inadequately, relax immediately and, therefore, limb drops soon. Ankle jerk returns later. Ankle clonus may be present in the mild form.

Extensor Responses
Increase in extensor activity occurs later. Generally, in six months, tone appears and slowly becomes marked in extensors. This leads to appearance of extensor spasm. The body assumes an attitude in extension. In this stage, the condition is called paraplegia in extension. The following findings are observed in this stage:
• Ankle and knee jerks become exaggerated
• If limb muscles are stretched passively and abruptly (e.g. if the flexed thigh is suddenly extended), a reflex extension of it or both limbs occurs. Contraction involves both extensor and flexor muscles that convert the limbs into “solid pillars”.
• Mass reflex as described above may not be obtained any more.
• Stimulation of glans penis produces the genital response as seen in the recovery of flexor group of muscles, but occurs without seminal emission.

Autonomic Reflexes
1. Micturition reflex becomes hyperactive. Though reflex evacuation of bladder occurs, bladder is not emptied completely. Hyperactive bladder reflex for a long duration keeps the bladder in a shrunken state that results in hypertrophy and fibrosis of the bladder wall and decreases bladder capacity. This type of bladder is called spastic neurogenic bladder.
2. Defecation reflex also regains, but reflex contraction of rectum does not empty it completely.
3. Blood pressure remains normal at rest. However, due to lack of feedback regulation by the baroreceptor reflex, wide swing in pressure occurs in various conditions.
4. Sweating occurs and skin becomes healthy.

Evacuation of bladder and bowel occurs in various conditions.
Supporting Reactions

Once the spinal reflexes reappear, their threshold steadily drops. A minor noxious stimulus may cause prolonged withdrawal response. Repeated flexion movement may occur for a prolonged period. Stretch reflexes also become hyperactive. An example of this is supporting reactions. There are two supporting reactions:

1. **Positive supporting reaction (PSR):** When a finger is placed on the sole of the foot of a spinal animal and then withdrawn, the limb of the animal extends following the examining finger. This is called PSR or 
   magnet reaction (as the limb is withdrawn like a magnet). The receptors are tactile receptors and afferent pathways involve both tactile and proprioceptive afferents. The motor responses transform the limb into a rigid pillar that resist gravity. Other limbs also become rigid and support the animal. Therefore, this is called supporting reaction.

2. **Negative supporting reaction:** The disappearance of positive supporting reaction is an active phenomenon, and called negative supporting reaction. This is initiated by stretch of the extensor muscles. On the basis of positive supporting reaction, spinal cats and dogs can be made to stand and walk though ineptly, for about two minutes.

Locomotion Generator

As spinal animals can stand and walk with appropriate stimulation, it indicates that a circuit intrinsic to the spinal cord produces locomotion. This is called central pattern generator (CPG) for locomotion. There are two spinal cord CPGs, one is located in the cervical regions for upper limbs and the other in the lumbar region for lower limbs. The CPGs are group of interneurons that activate the motor neurons. CPGs on stimulation produce alternate contractions of flexors and extensors of limbs, which is needed for locomotion. Experimentally, it is also observed that injection of glutamate or L-dopa into the spinal cord produces rhythmic action potentials for locomotion in motor neurons. CPGs in spinal cord are activated by discharge of a locomotor center present in the midbrain, called mesencephalic locomotion generator (MLG). In spinal animal, as the influence of MLG on CPG is interrupted, CPG requires appropriate stimulation to be activated.

Stage of Failure

In chronic spinal patients, due to lack of proper nutrition and hygiene, and repeated general infections or toxemia, failure of reflex activity develops.

1. **It becomes difficult to elicit reflexes as threshold for all reflexes is raised.** The mass reflex disappears.
2. Muscle wasting occurs and muscles become flaccid.
3. Bedsores develop at pressure points, which results in decubitus ulcers. Immunity is suppressed due to protein depletion. Poor healing of ulcers leads to septicemia.
4. Prolonged immobilization and lysis of protein matrix of bones cause hypercalcemia and hypercalciuria. This results in formation of stone in the urinary tract that causes repeated urinary infection. Frequent septicemia and uremia cause death of most of the patients. However, early and appropriate use of modern antibiotics and glucocorticoids, and proper nutrition and nursing care prolong the life of such patients.

MEDULLARY INTEGRATION

Medulla mainly integrates the tonic reflexes that maintain posture. The role of medulla in regulation of posture and movement is best studied by producing a midcollicular lesion (section between superior and inferior colliculi) in an experimental animal. The preparation is known as decerebrate preparation and the procedure is called decerebration.

Features of Decerebrate Preparation

1. No phenomenon similar to spinal shock occurs in decerebrate preparation.
2. Following the mid-collicular section, severe spasticity is immediately observed in the extensor group of muscles of the body. This is called decerebrate rigidity. Rigidity is so prominent that the limbs are fully extended and the spine is hyperextended.
3. Righting reflexes are absent. Therefore, animal lies on the ground.
4. **Tonic reflexes are prominently marked.**

Mechanism of Decerebrate Rigidity

Rigidity occurs due to increased activity of motoneurons that facilitate stretch reflexes. Facilitation of stretch reflexes occurs due to two mechanisms:

1. Increased excitability of the motor neuron pool in general.
2. Increased rate of y motor neuron discharge.

Increased general excitability of motor neurons occurs due to facilitation of descending neural inputs converging on the anterior horn cells. In the brainstem, the reticular nuclei for motor control are largely divided into two areas: the large facilitatory area, which is mainly located in pons (that gives rise to pontine reticulospinal tract) and the small inhibitory area (Fig. 130.3), which is located in medulla (that gives rise to medullary reticulospinal tract).

1. The facilitatory area discharges spontaneously whereas the inhibitory area does not. Discharge of inhibitory area requires inputs from the cortex, basal ganglia and cerebellum. Basal ganglia do not directly influence the brainstem reticular activity. Basal ganglia
projects to the motor cortex and via cortical projection to brainstem, it controls the medullary reticular activity.

2. Normally, **medullary reticulospinal fibers inhibit motor neurons** in the spinal cord. As the cortex drives the inhibitory (the medullary) reticular area, the cortex and basal ganglia are classified under inhibitory brain areas.

3. Likewise, cerebellum is also classified under inhibitory brain areas as it also drives the inhibitory reticular area. Thus, three areas (cortex, basal ganglia and cerebellum) drive the inhibitory center in medulla. In midcollicular lesion, the influence of two (cortex and basal ganglia) out of the three inhibitory centers on medullary reticular formation is removed (Fig. 130.4).

- Thus, inhibitory output of the medullary reticulospinal tract becomes less inhibitory, whereas the discharge of facilitatory pontine reticulospinal tract continues (as it discharges spontaneously).
- Consequently, the net discharge of reticulospinal tract shifts more towards facilitation. The output of γ motor neurons is mainly influenced by reticulospinal tract.
- Therefore, γ motor neuron discharge increases following decerebration. This causes decerebrate rigidity.

**Fig. 130.3**: Control of output of reticulospinal tract activity. The facilitatory reticular area discharges spontaneously; whereas the inhibitory reticular area to discharge effectively requires input from cortex, basal ganglia, and cerebellum. Basal ganglia influences reticulospinal activity via its projection to the motor cortex. As cortex, basal ganglia and cerebellum drive the inhibitory medullary reticular area, they are classified under inhibitory brain areas (as indicated by ‘minus’ sign). Output of vestibulospinal tract is facilitatory.

**Fig. 130.4**: Mechanism of decerebrate rigidity. In midcollicular lesion, the influence of cortex and basal ganglia on inhibitory reticular area is abolished (only cerebellar drive remains). Therefore, inhibitory output of the medullary reticulospinal tract becomes less inhibitory, whereas facilitatory area continues to discharge spontaneously. Consequently, the net output of reticulospinal tract becomes more facilitatory. As motor neurons are primarily driven by reticulospinal tract influence, decerebration causes severe rigidity.

1. Extensor muscles are the most important components of posture regulating system as they maintain erect posture of the body by keeping the limbs extended.
2. The **tone of these muscles**, which is a static postural reflex, is highly essential to support the animal against gravity. Therefore, these muscles are called **antigravity muscles**.
3. In humans, the major antigravity muscles are the **extensors of the lower limbs**.
4. The increased extensor rigidity in decerebrate preparation indicates that medulla controls the tone of the antigravity muscles that are involved in maintaining posture.

**Medullary Reflexes**

Reflexes integrated in medulla for control of motor activities are mainly **static postural reflexes**. Activities of these reflexes are accentuated following midcollicular lesion. These reflexes are:

1. Extensor rigidity
2. Tonic labyrinthine reflexes
3. Tonic neck reflexes

**Extensor Rigidity**

The tone in the extensor muscles (antigravity muscles) is a **prominent static postural reflex**, which is essential for maintaining posture against gravity.

**Importance of Decerebrate Rigidity**

Rigidity observed in decerebrate animal is **more marked in the extensor muscles**.
1. This extensor muscle tone is mainly due to the discharge of motor nuclei located in pons and medulla. Reticulospinal and vestibulospinal tracts primarily contribute to muscle tone.

2. **Reticulospinal tract** controls tone of antigravity muscles via its influence on \( \gamma \) motor neurons and vestibulospinal tract via \( \alpha \) motor neurons in the spinal cord (Application Box 130.1).

3. The \( \gamma \) motor neurons control muscle tone by regulating muscle spindle sensitivity.

4. Hence, \( \gamma \) motor neuron discharge, and therefore the reticulospinal tract activity is the principal regulator of muscle tone.

**Application Box 130.1**

**Experimental Deafferentation:** The vestibulospinal tract mainly influences \( \alpha \) motor neurons and reticulospinal tract influences \( \gamma \) motor neurons in the spinal cord. This can be experimentally proved by making **dorsal rhizotomy that causes deafferentation.** When afferent fibers (Ia) are interrupted by deafferentation, the input from muscle spindle to spinal cord is abolished. As \( \gamma \) motor neurons control muscle tone via spindle sensitivity, deafferentation removes the influences through \( \gamma \) motor neurons. Therefore, following dorsal rhizotomy, influence of reticulospinal tract on muscle tone is abolished, which indicates that this tract acts through \( \gamma \) motor neurons. However, influence of vestibulospinal tract on muscle tone is not affected (as it terminates mainly on \( \alpha \) motor neurons that contact extrafusal fibers) indicating that this tract acts through \( \alpha \) motor neurons (Fig. 130.5).

**Tonic Labyrinthine Reflexes**

The decerebrate animal cannot right itself, and therefore remains static in the position in which it is placed. If the **position of the animal is changed passively, the pattern of rigidity in the limbs alters.** This is called **tonic labyrinthine reflexes.** The rigidity is maximum in supine position and minimum in prone position.

**Receptors**

*Otolith organs* of the vestibular apparatus are receptors for tonic labyrinthine reflexes.

**Stimulus**

*Action of gravity on the otolith organs*, as altered by change in body position is the potent stimulus for this reflex.

**Reflex Pathway**

The effects are mediated by *vestibulospinal tracts*.

**Response**

When the animal is placed on its back, the rigidity is maximum in the extensor muscles (all four limbs are maximally extended). If the animal is turned to either side, the rigidity decreases and rigidity becomes minimum in the prone position.

**Importance**

These reflexes help animal to maintain the tone of muscle, especially in erect posture.

**Tonic Neck Reflexes**

This is the change in pattern of rigidity when position of the head is changed in relation to the body.

**Receptors**

*Proprioceptors* in the upper part of the neck are receptors for tonic neck reflexes.

**Stimulus**

*Change in head position* that stimulates proprioceptors in the neck.

**Reflex Pathway**

The reflex is mediated via *reticulospinal and tectospinal pathways*.

**Response**

If the head is ventroflexed, the upper limbs flex and the hind limbs extend. If the head is turned to one side, the limb of that side (the jaw limb) is extended while the contralateral limb (occipital limb) is flexed. **Extension of the head** causes extension of the forelimbs and flexion of the hind limbs.

**Importance**

The change in rigidity due to change in head position in relation to the body helps the animal to maintain posture in that particular position. When the animal looks above, for example, looking for an object in the tree, during which extension of the head extends the forelimb and flexes the hindlimb. This helps the animal to maintain posture in that...
Chapter 130: Regulation of Posture and Movement

1. **Labyrinthine RR**
   - **Stimulus**: Tilting of head (effect of gravity)
   - **Receptors**: Otolith organ
   - **Center**: Midbrain
   - **Responses**: Head kept level

2. **Neck RR**
   - **Stimulus**: Stretch of neck muscle
   - **Receptors**: Muscle spindle
   - **Center**: Midbrain
   - **Responses**: Righting of shoulders, thorax and then pelvis

3. **Body RR**
   - **i. Body on head RR**: Pressure on the side of the body
     - **Receptors**: Exteroceptors
     - **Center**: Midbrain
     - **Responses**: Righting of head
   - **ii. Body on body RR**: Pressure on the side of the body
     - **Receptors**: Exteroceptors
     - **Center**: Midbrain
     - **Responses**: Righting of body even head held sideways

4. **Limb RR**
   - **Stimulus**: Stretch of the limb muscles
   - **Receptors**: Muscle spindle
   - **Center**: Midbrain (red nucleus)
   - **Responses**: Righting of the body

5. **Optical RR**
   - **Stimulus**: Visual cues
   - **Receptors**: Visual receptors
   - **Center**: Visual cortex
   - **Responses**: Righting of head

Similarly, when the animal looks downward for an object on the ground or below the ground level, flexion of head causes extension of hind limb and flexion of the forelimbs. This helps the animal to maintain posture in that position.

**MIDBRAIN INTEGRATION**

Midbrain mainly integrates the **phasic postural reflexes**. These are righting reflexes, vestibular placing reflex and grasp reflex. **Central pattern generator for locomotion (CPG)** is situated in midbrain (mesencephalic generator for locomotion) that drives the spinal CPG.

**Midbrain Preparation**

The influence of midbrain on posture and movement is best studied by making a section at the superior border of the midbrain. The animal is called **midbrain animal**.

**Features of Midbrain Animal**

1. No features of shock
2. Extensor rigidity (as seen in decerebrate animal) is present only when the animal is at rest. Rigidity disappears on activation of phasic postural reflexes, e.g. when animal walks.
3. The animal can right itself (righting reflexes are preserved) and change its posture.
4. Grasp reflex and vestibular placing reaction are intact.

**Midbrain Reflexes**

**Righting Reflexes**

These are a series of reflexes that operate to correct the body position and maintain it by keeping the animals head upright. Maintaining stable head position is an integral part of the posture regulating mechanisms. Righting reflexes keep the head in a stable position and the eyes fixed at visual targets despite movement of the body. They tend to restore the position of the body when it is altered, by stimulating proprioceptors, tactile receptors and vestibular receptors. The responses are initiated by vestibular stimulation, stretching of neck muscle, pressure on the side of the body or on the limbs, and stimulation of visual receptors. Righting reflexes are series of responses integrated in midbrain (Table 130.1). Following are the righting reflexes:

- **Labyrinthine Righting Reflex**
  - **Stimulus**: Tilting of the head that stimulates otolith organs.
  - **Response**: Contraction of neck muscles that keep the head level.

- **Body on Head Righting Reflex**
  - When the animal is laid on its side, the pressure on that side of the body initiates reflex righting of the head. This reflex is operated even after labyrinthectomy. This is called body on head righting reflex.

- **Body on Body Righting Reflex**
  - Pressure on the side of the body rights the body even if the head is prevented to right. This is called body on body righting reflex.

- **Limb Righting Reflex**
  - Pressure on the limbs rights the body.

- **Optical Righting Reflex**
  - The righting reflexes are best operated with eyes open (even in the absence of labyrinthine or body stimulation). This is the optical righting reflex. The center for this reflex is not the midbrain, but the cortex. Therefore, this reflex depends on the intact cortical functions.
**Grasp Reflex**
When a stick or an object is brought close to the limbs of a midbrain animal, the animal grasps the object and the limbs are extended. This is the grasp reflex. This reflex is a **primitive reflex** that helps the animal to stand up and **support the posture**. In humans, it is better observed **during infancy and early childhood**.

**Vestibular Placing Reaction**
When a blindfolded animal is brought down from a height rapidly, the forelimbs of the animal extend and the toes spread, which assist the animal to steadily land on the ground.

- **Receptors**: Vestibular receptors.
- **Stimulus**: Linear acceleration of the body.
- **Pathway**: Vestibulospinal tract.
- **Response**: Extension of forelimbs and spreading of the toes. This helps the animal to **land steadily on the ground**.

**Mesencephalic Locomotion Generator**
The generator for locomotion is located in the midbrain. This is called **mesencephalic locomotion generator** (**midbrain locomotion center**). This midbrain center drives the spinal locomotion generator. It organizes the command signals that arrive from the motor cortices via corticobulbar fibers and accordingly influences spinal motor neurons mainly via reticulospinal tract. Locomotion is not only activated and initiated but also altered rapidly during swift motor activities like running, swimming etc., by adjustment of activities of phasic postural reflexes. All these postural mechanisms are integrated in midbrain (Fig. 130.6).

**CORTICAL INTEGRATION**
For the command signal for movement to be conveyed to spinal motor neurons, first, the motor plan is generated in the cortex. Motor cortex interacts with basal ganglia and cerebellum for finalizing the plan and program of the movement, and later, interacts with the sensory cortex and association cortex to fine-tune and modify the command signal for implementation of the initial plan. Information is also transmitted partly to the premotor cortex and supplementary motor area, for generating further motor signal for initiating appropriate movement. Motor signal is then conveyed to spinal motor neurons via descending motor pathways.

**Decortication**
The role of motor cortex in regulation of posture and movement is best studied by removing the cerebral cortex in experimental animals. The surgical procedure is called **decortication** and the animal is called **decorticate animal**.

**Features of Decorticate Animal**
1. No features of shock.
2. All the reflex activities of midbrain animal are intact.
3. Rigidity (**decorticate rigidity**) is very minimal and present only at rest.
4. **Hopping and placing reactions** are severely impaired.
5. Striking defect is the **inability to react in terms of past experience**.
6. Conditioned reflexes are absent, but can be reestablished with special training.
7. Temperature regulation is intact.
8. Visceral homeostatic mechanisms are intact.
   As the hypothalamus is intact in decorticate animal, it is easier to maintain such an animal due to intact visceral homeostatic mechanisms including the process of temperature regulation.

**Decorticate Rigidity**
Cortex inhibits medullary reticulospinal tract. Therefore, removal of cortex results in facilitation of y motor neuron discharge. Rigidity is seen only at rest and disappears when phasic postural reflexes are activated.

**Hopping and Placing Reactions**
When a normal animal (or a human being) is pushed laterally, the animal takes short steps to balance the body (**hopping reaction**) and then places its feet firmly on the ground (**placing reaction**) to attain and maintain the new stable position. These reflexes are seriously impaired following decortication. This indicates that center for integration of hopping and placing reactions is the motor cortex.
   Thus, in summary, spinal reflexes including supporting reactions are integrated in spinal cord, antigravity
Table 130.2: Integration of motor functions at various levels of CNS as studied in different preparations.

<table>
<thead>
<tr>
<th>Reflexes</th>
<th>Spinal preparation</th>
<th>Decerebrate preparation</th>
<th>Midbrain preparation</th>
<th>Decorticate preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Spinal reflexes</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2. Antigravity reflexes</td>
<td>Absent</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3. Righting reflexes</td>
<td>Absent</td>
<td>Absent</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4. Conditioned reflexes</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>±</td>
</tr>
<tr>
<td>5. Hopping and placing reactions</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Grossly impaired</td>
</tr>
</tbody>
</table>

(static postural) reflexes are integrated in medulla, righting reflexes are integrated in midbrain, and hopping and placing reactions in cortex as studied in above-mentioned preparations (Table 130.2).

Cortical Motor Areas

Cortical motor areas include a large number of areas in the brain that are involved in or associated with movement (Fig. 130.7). These include:

1. Primary motor cortex (precentral gyrus)
2. Premotor cortex
3. Supplementary motor area
4. Somatosensory cortex
5. Frontal eye field

Primary Motor Cortex

Primary motor cortex (area 4) is present in the precentral gyrus. Corticospinal and corticobulbar tracts mainly originate from this area of the cortex. This area is involved in planning and initiation of voluntary movements.

Connections

Motor neurons originating from area 4 project to premotor cortex, brain-stem and spinal cord. It is reciprocally connected with thalamus, sensory cortex and supplementary motor area (Fig. 130.8). The connections are such that the cortical motor areas control the muscle activity of the opposite side of the body.

Motor Homunculus

The motor areas of various parts of the body are represented in the precentral gyrus. The areas for face are represented bilaterally, whereas representation of rest of the body is unilateral. The representation is such that the feet remain at the top of the gyrus and the face at the bottom (Fig. 130.9). The cortical representation is proportionate to the skill with which the body part is involved in voluntary activities. Therefore, hand and digits occupy a large area in the cortex since they are involved in execution of skilled activities. As human beings are specialized in speech, the lips, jaw, and tongue also occupy a significantly large area in the cortex. This is called the vocalization of the cortex.
**Premotor Cortex**

Pre-motor cortex (**lateral area 6**) receives information from supplementary motor area and primary motor cortex and projects to brainstem areas that are concerned with postural control (Fig. 130.10). It **sets posture at the beginning of a planned movement**. Thus, it helps the individual to **prepare for execution of movement**.

**Supplementary Motor Area**

This is the **medial portion of area 6**. It receives input from somatosensory cortex. It is involved in higher motor functions like **planning and programming the motor sequences** and it **controls bimanual tasks** (Fig. 130.11). Lesion of this area produces difficulty in performing complex motor activities, and the activities that involve bimanual coordination.

**Primary Somatosensory Cortex**

The somatosensory cortex (**area 3, 1, and 2**) projects to the supplementary motor area and pre-motor cortex. Lesion of this area produces **defect in motor performance** that involves **execution with learned sequences of events**. For example, eating with knife and fork is severely affected. Fibers in the corticospinal tract that originate from somatosensory cortex terminate more in the dorsal horn. These fibers modulate afferent inputs into the CNS.

**Posterior Parietal Cortex**

Posterior parietal lobe (**area 5 and 7**) contributes to the corticospinal tract and is also connected with motor cortex. This area utilizes complex sensory information for producing movements.
**Frontal Eye Field**

This is the area 8. It receives input from primary and premotor cortices and projects to pontine nuclei that control eye movements and superior colliculus that integrates saccadic movements (Fig. 130.12).

**CHAPTER SUMMARY**

**Key Concepts**

1. In motor neuraxis, the higher centers inhibit the lower centers. Therefore, lesion of center causes excitation of motor activity below the centers, due to release phenomenon.
2. Spinal cord is for integration of postural reflexes and spinal CPG, medulla is for control of tone extensor muscles and midbrain is for righting reflexes and mesencephalon CPG.

**Important to Know (Must Read)**

1. ‘Describe the mechanisms of regulation of posture and movement’ or ‘Describe the spinal and medullary integration of regulation of posture and movement’ may come as a Long Question.
2. Central pattern generator (CPG) of locomotion, Spinal reflexes, Supporting reactions, Medullary reflexes, Decerebrate rigidity, Righting reflexes, Cortical reflexes, Moot homunculus, Motor cortex, Cortical motor areas, may come as Short Questions.
3. In Viva, examiner may ask... Classify postural reflexes, What is release phenomenon, Name the postural reflexes integrated in spinal cord, medulla, midbrain and cortex, What are the stages of spinal preparation and what are the features in each stage, Central pattern generator (CPG) of locomotion, Name spinal reflexes, Supporting reactions, Name medullary reflexes, Mechanisms of decerebrate rigidity, Name righting reflexes, Name cortical reflexes, Moot homunculus, Connections and functions of motor cortex, Cortical motor areas.
Learning Objectives

On completion of study of this chapter, the student **MUST** be able to:
1. Name the parts of basal ganglia.
2. List the inputs, outputs and internal connections of basal ganglia.
3. Understand the influence of direct and indirect pathways on motor functions.
4. List the functions of basal ganglia.
5. Understand the physiological basis of etiology, features and management of Parkinsonism.
6. Mention the abnormality of Huntington's disease.
7. Define chorea, athetosis and ballism.

The student **MAY** also be able to:
1. Describe the role of basal ganglia in motor function.
2. Explain the physiological basis of dysfunctions of basal ganglia.

Basal ganglia are a group of deep subcortical nuclei located at the base of forebrain. They are primarily involved in control of posture and movement.
1. Unlike cerebellum, basal ganglia do not receive inputs directly from the spinal cord.
2. They also do not directly project to the brainstem areas that control motor activities.
3. They receive inputs mainly from cortex and project to the cortex via thalamus.
4. Basal ganglia project mainly to brainstem areas (that give rise to so-called extrapyramidal tracts) via their cortical connections.
5. Therefore, basal ganglia **mainly influence extrapyramidal functions**, and, consequently, they are classified as important **extrapyramidal structures**.
6. Lesion of basal ganglia produces abnormal movements and severe deficits in control of posture.

ANATOMICAL ORGANIZATION

Basal ganglia mainly include **caudate nucleus, the putamen and the globus pallidus** (Fig. 131.1).
1. Subthalamic nucleus, and substantia nigra are also included in basal ganglia. The caudate nucleus and putamen are together known as **striatum** (neostriatum),
and putamen and globus pallidus are combinely known as lenticular nucleus.

2. Globus pallidus is divided into an external part (globus pallidus externus) and an internal part (globus pallidus internus).

3. Substantia nigra is divided into two parts: pars compacta and pars reticulata.

**Inputs**

In contrast to cerebellum, basal ganglia do not receive direct sensory input from the peripheral structures, spinal cord or sensory nuclei in the brainstem.

- The main inputs to basal ganglia come from cerebral cortex (Fig. 131.2).
- Basal ganglia also receive inputs from thalamus, dorsal raphe nucleus and pedunculopontine region of the brainstem.
- Most afferent information enters basal ganglia via striatum (caudate nucleus and putamen).

**The afferent fibers are:**

1. **Corticostriate projection:** Basal ganglia receive afferents from all parts of cerebral cortex via corticostriate projection. Putamen receives input mainly from the sensorymotor cortex and caudate nucleus receives inputs from remainder of the cortex.

2. **Thalamostriate projection:** The centromedian nucleus of thalamus projects to the striatum via thalamostriate projections.

3. **Raphestriate projection:** Striatum also receives input from dorsal raphe nucleus.

4. **Pedunculostriate projection:** Pedunculopontine nucleus of brainstem projects to basal ganglia.

**Outputs**

The principal output nucleus of basal ganglia is the internal segment of globus pallidus (Fig. 131.3).

1. The fibers project mainly to the ventral-lateral, ventral-anterior, and centromedian nuclei of thalamus from where fibers project to prefrontal and premotor cortices (extrapyramidal pathways mainly originate from these cortical areas).

2. The output from the internal segment of the globus pallidus to the thalamus is inhibitory, whereas the output from the thalamus to the cerebral cortex is excitatory.

3. Pars reticulata portion of substantia nigra projects to thalamus.

4. Basal ganglia also project to pedunculopontine nucleus, habenula and superior colliculus.

The main feature of input and output of basal ganglia is that cerebral cortex projects to striatum, striatum projects to internal segment of globus pallidus, globus pallidus projects to thalamus, which projects back to the cortex, completing the motor loop.

**Connections within Basal Ganglia**

Major connections within the basal ganglia are as follows (Fig. 131.4):

1. **Nigrostriatal projection:** The pars compacta portion of substantia nigra projects to striatum. The nigrostriatal projection is dopaminergic. Degeneration of this system produces Parkinsonism.

2. **Striatonigral projection:** Striatum also projects to substantia nigra. The striatonigral projection is inhibitory and neurotransmitter secreted in this pathway is GABA. Degeneration of this pathway produces Huntington’s disease.

3. **Projection from subthalamic nucleus** to globus pallidus internus: Subthalamic nucleus projects to internal segment of globus pallidus. This projection is excitatory.

**Neural Pathways through Basal Ganglia**

The inputs, interconnections, and outputs constitute two important neural pathways through the basal ganglia that
are involved in control of motor activities: the direct pathway, and the indirect pathway.

**Direct Pathway**

Cortex projects to striatum. Striatum projects to globus pallidus internus, which projects to thalamus and thalamus in turn projects to motor cortex. This is the direct pathway (Fig. 131.5).

1. The projection of cortex to striatum is excitatory (glutaminergic).
2. Projection of striatum to globus pallidus internus is inhibitory (GABAergic), and projection from globus pallidus internus to the thalamus is inhibitory (GABAergic).
3. Thus, stimulation of striatum results in stimulation of thalamus by disinhibition. The direct pathway is stimulated during movement. Usually, neurons in the striatum have less resting background activity.
   - During movement, they are activated by inputs from cortex and thalamus.
   - Activation of striatum inhibits globus pallidus internus.
   - However, pallidal neurons themselves are inhibitory to thalamus.
   - Therefore, activation of striatum finally excites the thalamic neurons.
   - Consequently, the **target neurons in the motor cortex are stimulated** via thalamocortical projection.

**Indirect Pathway**

This involves connection from striatum to the external segment of globus pallidus, which projects to subthalamic nucleus. Subthalamic nucleus projects to internal segment of the globus pallidus, which in turn projects to thalamus (Fig. 131.5).

1. In this pathway, **striatum inhibits globus pallidus externus**, which inhibits subthalamic nucleus.
2. Subthalamic nucleus activates globus pallidus internus.
3. Therefore, **stimulation of striatum activates globus pallidus internus** through this indirect pathway.
4. The final output of striatum through this **indirect pathway is inhibitory** (as globus pallidus internus activation inhibits thalamocortical projections).

**Modulation by Nigrostriatal Projections**

The direct and indirect pathways have opposite effects. Normally, there is a balance between these two pathways. Alteration of activity in either of the pathways leads to imbalance in motor output from basal ganglia. Therefore, in basal ganglia disorders, both **hypo- and hyperkinetic features** are observed.

1. Another important connection of these pathways is the **nigrostriatal projections**.
2. The dopaminergic projections from pars compacta of substantia nigra to the striatum appear to have an excitatory influence on the direct pathway and inhibitory influence on the indirect pathway.
3. It also modulates the input from cortex to the striatum.
4. Therefore, projection from substantia nigra to striatum is important physiologically.
5. The neurons in the striatum are cholinergic.
6. In striatum, the ratio of acetylcholine and dopamine keeps the striatal neurons active. Alteration in this ratio results in abnormalities in motor activities.

Subdivisions of Striatum

Striatum is subdivided into two zones: striosomes, and matrix.

Striosomes

Limbic system mainly projects to the striosomes of striatum. Therefore, striosomal part of basal ganglia is concerned with limbic functions.

Matrix

This zone receives projection from motor cortex. Thus, matrix zone of basal ganglia is connected with motor functions.

FUNCTIONS OF BASAL GANGLIA

1. The neurons of basal ganglia are observed to discharge before the movement begins. This indicates that basal ganglia is involved in planning and programming of motor activities.
2. Basal ganglia controls posture. Basal ganglia-thalamocortical projection to brainstem influences descending pathways that control posture. Diseases of basal ganglia result in profound postural abnormalities.
3. Basal ganglia inhibits stretch reflexes by stimulation of caudate nucleus. This is achieved by stimulation of inhibitory motor cortex through thalamocortical feedback pathway and by stimulation of inhibitory reticular formation.
4. Neostriatum regulates subconscious gross movements,
5. Basal ganglia also play a role in cognitive functions. This is especially performed by the caudate nucleus through its connections with the frontal portion of the neocortex. Lesion of caudate nucleus results in deficit in performance based on learning.
6. Lesion of head of the left caudate nucleus is associated with dysarthritic aphasia (difficulty in articulating words).
7. Globus pallidus provides inputs for appropriate muscle tone for skilled movements.
8. Substantia nigra is the center for coordination of impulses essential for skilled movements.
9. Basal ganglia controls associated movements that occur automatically and normally with various body movements.
10. Lesion of basal ganglia in animals does not lead to significant motor deficit. However, diseases of the basal ganglia in humans result in severe motor dysfunctions.

DYSFUNCTIONS OF BASAL GANGLIA

Parkinson’s Disease

Parkinson’s disease (as described by James Parkinson) results from degeneration of nigrostriatal dopaminergic neurons. With age, there is progressive loss of dopamine and dopamine receptors in the basal ganglia. When this processes is accentuated, Parkinsonism results.

Scientist contributed

James Parkinson (1755–1824) was an English surgeon, most famous for his 1817 work, *An Essay on the Shaking Palsy* in which he was the first to describe ‘paralysis agitans’, a condition that was later renamed Parkinson’s disease.

Causes

1. Idiopathic: The cause of degeneration of dopaminergic neurons is not exactly known.
2. Drugs: It occurs secondary to chronic use of many drugs like phenothiazine, D₂ receptor blockers, etc.
3. MPP: It has been recently described that methylphenylpyridinium (MPP) concentration in the brain of Parkinsonism is high. MPP is formed from methylphenyl-terahydropyridinium (MTP) by the action of monoamine oxidase B (MAO-B) enzyme. MPP rapidly accumulates in the neurons of basal ganglia and destroys them.

Features

Both hypokinetic and hyperkinetic movements are observed in Parkinsonism.

Hypokinetic Movements

1. Akinesia: Akinesia is defined as difficulty in initiating movements and decreased spontaneous movements.
2. Bradykinesia: Bradykinesia is defined as slowness of movement.
3. Decreased associated movements: Examples of associated movements are swinging of the arms during walking or the facial expressions during speaking. These associated movements are grossly diminished in Parkinsonism. The patient suffering from Parkinsonism speaks without any emotional expression (expressionless face or mask face).

Hyperkinetic Movements

1. Rigidity: Rigidity of Parkinsonism is different from spasticity that occurs in UMN paralysis. In rigidity, the motor neuron discharge is increased in both agonists and
Section 11: Neurophysiology

1. **Antagonists**: Therefore, the limbs offer resistance to passive bending throughout the movement (lead pipe rigidity). Sometimes, cogwheel rigidity (series of catches during passive movement) is also seen. However, the clasp-knife spasticity (sudden loss of resistance while moving a rigid limb) of UMN paralysis is never seen.

2. **Tremor**: Tremor occurs due to regular alternating contractions of antagonist muscles, at a frequency of about 8 per second. Typically, tremor is observed only at rest. Once patient initiates the movement, tremor disappears. This is described as resting tremor.

3. **Festinant gait**: Patient walks in an attitude that as if he is trying to catch the center of gravity. Usually, he bends forward but does not fall, instead takes short and shuffling steps (Fig. 131.6).

**Treatment**

1. **Replacement of dopamine**: Dopamine cannot cross the blood brain barrier. Therefore, **L-dopa**, a precursor of dopamine that easily crosses blood brain barrier, is the drug of choice for Parkinsonism. It also helps repair dopamine deficiency. Other dopamine agonists like **bromocriptine** are also used.

2. **Anticholinergics**: Though Parkinson’s disease occurs due to dopamine deficiency in the striatum, the alteration in the ratio of dopamine to acetylcholine plays an important role. Therefore, injection of anticholinergics that decreases acetylcholine concentration in the basal ganglia and reestablishes acetylcholine dopamine ratio, improves the symptoms.

3. **Deprenyl**: Deprenyl inhibits monoamine oxidase B, therefore, prevents formation of MPP from MPTP.

4. **Transplantation of adrenal**: Transplantation of adrenal medulla from one of the adrenal gland of the patient into his basal ganglia helps in regenerating the dopaminergic neurons.

5. **Implantation of fetal basal ganglia**: Implantation of tissue from the basal ganglia of fetuses into the basal ganglia of the patient improves the condition.

6. **Transplantation of glomus cells**: Recently, for treatment of Parkinsonism, glomus cells from carotid body are isolated and transplanted into basal ganglia. Glomus cell releases dopamine locally. This has been found to be encouraging.

**Huntington’s Disease**

This is a genetic defect of autosomal dominant type that occurs due to a defective gene on the chromosome 4. The gene codes for huntingtin, an abnormal protein that causes the disease.

**Cause**

The disease occurs due to degeneration of GABAergic striatonigral pathway. GABAergic and cholinergic neurons are lost in the striatum.

**Features**

**Age**: Disease usually starts between the age of 30–40 and progresses uniformly till death occurs within 10–15 years. Disease has three important features: chorea, dementia and slurred speech.

**Chorea**: Chorea is defined as rapid involuntary and dancing movements. The chorea is called Huntington’s chorea. It is believed that loss of GABA-ergic neurons in the striatum removes its inhibitory influence on the globus pallidus, which reduces the activities in the thalamic nucleus that results in chorea.

**Dementia**: Dementia occurs due to simultaneous and progressive loss of cholinergic neurons in the cerebral cortex.

**Slurring speech**: Gradually, speech is slurred in Huntington’s disease.

**Treatment**

There is no definite treatment of Huntington’s chorea. The disease is progressively fatal.

**Other Dysfunctions**

**Ballism**

Ballism is defined as involuntary movements that are flailing, intense, and violent in nature. Usually, it occurs suddenly. Ballism occurs when the subthalamic nucleus is damaged.

**Hemiballism** is common than ballism and occurs due to hemorrhage in the subthalamic nucleus of the opposite side of the brain.
Athetosis
Athetosis is defined as continuous but slow writhing movements. It occurs due to damage to striatum.

Chorea
Chorea is defined as rapid involuntary dancing movements. It occurs due to damage to caudate nucleus.

CHAPTER SUMMARY

Key Concepts
1. Basal ganglia is meant for planning and programming of movements. However, it also profoundly influences posture through its indirect projects to brainstem.
2. Glomus cell implantation is promising in the treatment of Parkinsonism.

Important to Know (Must Read)
1. 'Describe the connections, functions and dysfunctions of basal ganglia’ may come as a Long Question.
2. Basal ganglia, Internal connects of basal ganglia, Parkinsonism may come as Short Questions.
3. In Viva, examiner may ask… Name the parts of basal ganglia, What is neostriatum and what is its function, Inputs to basal ganglia, Outputs from basal ganglia, What is the motor loop, What are the direct and indirect pathways of basal ganglia, Causes, features, and treatment of Parkinsonism, Causes and features of Huntington’s disease, What is ballism, hemiballism, athetosis and chorea.
Cerebellum literally means the 'little brain'. Cerebellum is situated posterior to the brainstem. Cerebellum is vital for regulation of posture and movement.

1. It receives inputs of almost all sensory modalities.
2. From spinal cord, it receives proprioceptive inputs. It also receives special sensory inputs from visual, auditory and vestibular structures.
3. It projects to almost all areas of brain that are involved in control of motor activities.
4. Thus, cerebellum plays a critical role in motor control by integrating sensory and motor information in the brain.
5. Therefore, cerebellum strongly influences all aspects of movement, starting from the rate, range, force, and direction to the termination of movement.
6. Hence, damage to cerebellum results in severe incoordination of movement.
7. Cerebellum directly projects to the brainstem nuclei that give rise to major descending pathways.
8. Therefore, damage to cerebellum results in severe postural abnormalities.
9. Cerebellum also regulates vestibulo-ocular reflex and motor learning.

Scientist contributed

Johan Evangelista Purkinje (1787–1869), a Czech physiologist, created the world's first Department of Physiology at the University of Breslau in Prussia (Poland) in 1839 and the world's first official physiology laboratory in 1842. He is best known for his 1837 discovery of Purkinje cells, large neurons with many branching dendrites found in a cerebellum. Purkinje also pioneered in subjective visual phenomena. He described germinal vesicle in egg embryo and classified fingerprints. He described ciliary epithelial motion and its function. He studied the structure of cerebellum. He is also popular for describing the Purkinje fibers in the ventricle of heart and Purkinje images in the eyes.

CEREBELLAR ORGANIZATION

Cerebellum is located in the posterior cranial fossa, behind the brainstem.

1. It is connected to midbrain through superior cerebellar peduncle, to pons through middle cerebellar peduncle and to the medulla through inferior cerebellar peduncle (Fig. 132.1).
Chapter 132: Cerebellum

2. The surface area of cerebellum is about 75% of the cerebral cortex, but in weight it is only 10% of the cortex. Thus, cerebellar cortical tissue is much folded.

3. There are two main fissures in the cerebellum that divide it into two major parts: the posterolateral fissure that separates flocculonodular lobe from rest of the cerebellum and the primary fissure that separates the anterior lobe from the posterior lobe.

Functional Divisions and Functions of Cerebellum

Functionally, cerebellum is divided into three major subdivisions: vestibulocerebellum, spinocerebellum and cerebrocerebellum (Fig.132.2).

Vestibulocerebellum

This is also called archicerebellum, as phylogenetically it is the oldest part.
1. It consists of flocculonodular lobe.
2. This part of cerebellum is called vestibulocerebellum for its extensive and reciprocal connection with the vestibular nuclei.
3. It is concerned with equilibrium and learning induced changes in vestibulo-ocular reflex.

Spinocerebellum

This is also called paleocerebellum, as it is intermediate in development.
1. It consists of the vermis and the paravermal regions of cerebellum.
2. It is called spinocerebellum, as it receives proprioceptive and other sensory inputs from all the body parts through the spinal cord.
3. It also receives inputs from the motor cortex, where motor planning is carried out. By comparing plan with performance, it smooths and coordinates movement.
4. The vermal portion of spinocerebellum projects to the brainstem areas that control axial and proximal limb muscles. Therefore, vermal spinocerebellum controls posture.
5. The paravermal region of spinocerebellum projects to the brainstem nuclei that influence distal limb muscles. Therefore, paravermal spinocerebellum controls skilled voluntary movements.
Cerebrocerebellum
This is also called neocerebellum, as it is newest phylogenetically.
1. It consists of the two main cerebellar hemispheres.
2. This is called cerebrocerebellum for its connections with the cortex.
3. Cortex projects to neocerebellum via the pontine nuclei; hence, this is also called corticopontocerebellum.
4. As it interacts with the cortex, it is involved in planning and programming of the movements.

Functional Histology
Cerebellum is divided into the outer cortex and the inner part containing deep cerebellar nuclei.

Cerebellar Cortex
The cerebellar cortex has three layers: outer molecular layer, middle Purkinje cell layer, and inner granular layer (Fig. 132.3).

Molecular Layer
This layer contains interneurons that are basket cells and stellate cells.

Purkinje Cell Layer
This layer contains Purkinje cell.
1. Purkinje cells are the largest neurons with extensive dendritic branches.
2. Dendrites of Purkinje cells enter into the molecular layer. The axons of the interneurons of the molecular layer project to the dendrites of the Purkinje cells.
3. Purkinje cells also receive inputs directly from the climbing fibers.
4. Purkinje cells are the only cells that project from the cortex of cerebellum to the deep cerebellar nuclei. Thus, Purkinje cells are connecting links between cerebellar cortex and deep cerebellar nuclei.

Granular Cell Layer
This layer contains granule cells and Golgi cells (interneurons).
1. The Golgi cells project to the granule cells and modify granular cell output.
2. The granule cells receive inputs from the mossy fibers and project to the Purkinje cells, basket cells, stellate cells and Golgi cells via parallel fibers.

Deep Cerebellar Nuclei
There are four deep cerebellar nuclei (Fig. 132.4).

Nucleus Fastigius
The nucleus fastigius is present in the deep vermal portion of the cerebellum. The vermal cortical portion of spinocerebellum projects to the fastigial nucleus.

Nucleus Globosus and Nucleus Emboliformis
The globos and emboliform nuclei are combinely known as nucleus interpositus. The paravermal portion of spinocerebellum projects to nucleus interpositus.
**Nucleus Dentatus**

This is present in the hemispheric portion of the cerebellum. It receives inputs from neocerebellum. The name of the nucleus is ‘dentate’ for its appearance, which has teeth-like serrated morphology.

The deep cerebellar nuclei project to the different parts of the brainstem and thalamus (discussed in Cerebellar Outputs).

**Cerebellar Connections**

**Cerebellar Inputs**

Cerebellum receives somatosensory inputs from almost all parts of the body and inputs of all sensory modalities including special sensory inputs. The cerebellar afferents are:

1. **Vestibulocerebellar tract:** Through this tract, cerebellum receives impulses directly from the vestibular apparatus and also from the vestibular nuclei.
2. **Dorsal spinocerebellar tract:** This tract conveys proprioceptive and exteroceptive impulses from different parts of the body to cerebellum.
3. **Ventral spinocerebellar tract:** This pathway also conveys proprioceptive and exteroceptive impulses from different parts of the body.
4. **Cuneocerebellar tract:** This tract originates from lateral cuneate nucleus in the caudal medulla and conveys proprioceptive inputs from head and neck.
5. **Tectocerebellar tract:** This tract conveys visual information from superior colliculus and auditory information from inferior colliculus to the cerebellum.
6. **Pontocerebellar tract:** Impulses from motor cortex reach cerebellum via pontine nuclei.
7. **Olivocerebellar tract:** Proprioceptive inputs from the whole body reaches cerebellum via inferior olive. Inferior olivary nucleus is located in the rostral medulla that receives input from the vestibular system, spinal cord and cerebral cortex. It projects to cerebellum via climbing fibers.

**Mode of Inputs**

Inputs to cerebellum reach via three routes: mossy fibers, climbing fibers and other inputs (Table 132.1).

**Mossy Fiber Inputs:** Mossy fibers are major source of inputs to cerebellum. These fibers carry direct proprioceptive inputs from all parts of the body and also convey input from cerebral cortex. Mossy fibers project mainly to the granule cells.

**Climbing Fiber Inputs:** Climbing fibers convey inputs from inferior olivary nucleus to cerebellum. Inferior olive receives proprioceptive input from all parts of the body. Climbing fibers project to Purkinje cells of cerebellum.

**Other Inputs:** Cerebellum receives monoaminergic inputs, and inputs from thalamus and other parts of the brain. These fibers project to the deep cerebellar nuclei.

**Table 132.1: Cerebellar inputs via different fiber systems.**

<table>
<thead>
<tr>
<th>Fiber systems</th>
<th>Tracts</th>
<th>Nature of input</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Through climbing fibers</td>
<td>Olivocerebellar tract</td>
<td>Proprioceptive inputs from whole body via relay in inferior olivary nucleus</td>
</tr>
<tr>
<td>II. Through mossy fibers</td>
<td>1. Dorsal spinocerebellar tract</td>
<td>Proprioceptive and exteroceptive inputs</td>
</tr>
<tr>
<td></td>
<td>2. Ventral spinocerebellar tract</td>
<td>Proprioceptive and exteroceptive inputs</td>
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<td></td>
<td>3. Vestibulocerebellar tract</td>
<td>Inputs from vestibular nuclei</td>
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<tr>
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<td>4. Tectocerebellar tract</td>
<td>Visual information from superior colliculus and auditory inputs from inferior colliculus</td>
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<td></td>
<td>5. Cuneocerebellar tract</td>
<td>Proprioceptive inputs from head and neck</td>
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<td></td>
<td>6. Corticopontocerebellar tract</td>
<td>Inputs from cortex via pontine nuclei</td>
</tr>
<tr>
<td>III. Other fiber systems</td>
<td>1. Monoaminergic inputs</td>
<td></td>
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<tr>
<td></td>
<td>a. Serotonergic inputs</td>
<td>From nucleus raphe magnus</td>
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<tr>
<td></td>
<td>b. Noradrenergic inputs</td>
<td>From nucleus locus ceruleus</td>
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<tr>
<td></td>
<td>2. Thalamic and other inputs</td>
<td>From thalamus and other brain areas</td>
</tr>
</tbody>
</table>
Cerebellar Outputs
Different parts of cerebellum project to various descending pathways via deep cerebellar nuclei (Fig. 132.5). Deep cerebellar nuclei are the output pathway of cerebellum.

Output from Vestibulocerebellum
Vestibulocerebellum directly projects to the vestibular nuclei without any relay in the deep cerebellar nuclei. Thus, vestibulocerebellum directly controls vestibulospinal tract activity.

Output from Spinocerebellum
1. The vermal portion of the spinocerebellum projects to fastigial nucleus, which, in turn, projects to pontine reticular formation and vestibular nuclei in the brainstem. Thus, vermal part of spinocerebellum controls the activity of pontine reticulospinal tract and vestibulospinal tract.
2. The paravermal portion of the spinocerebellum projects to the nucleus interpositus, which, in turn, projects to the red nucleus. Thus, paravermal part of the spinocerebellum controls the activity of rubrospinal tract.

Internal Connections of Cerebellum
Cerebellum receives inputs from two sources: the climbing fibers (from olivary nucleus), and the mossy fibers.
1. Purkinje cells are stimulated directly by climbing fiber input, whereas mossy fibers stimulate Purkinje cells indirectly via granule cell-parallel fiber pathways (Fig. 132.6).
2. Mossy fibers project to granule cell. Granule cells via its parallel fibers provide excitatory input to the basket and stellate cells, and Purkinje cell.
3. Basket and stellate cells that are activated by mossy fiber-parallel fiber pathway finally inhibit Purkinje cell. This is an example of feed-forward inhibition.

Output from Cerebrocerebellum
The cerebellar hemisphere projects to the dentate nucleus, which, in turn, project to the motor cortex via thalamus. Thus, cerebrocerebellum controls the activity of the corticospinal tract.

As different parts of the cerebellum project to all the motor nuclei in the brainstem and to the motor cortex, cerebellum controls activities of all the descending pathways (corticospinal and extrapyramidal systems). Therefore, diseases of the cerebellum affect both regulation of posture and skilled voluntary movements.
4. Granule cell also stimulates the Golgi cells (the interneurons in granular cell layer), which, in turn, inhibit the activity of granule cells. This is an example of local feedback inhibition and is meant to regulate the granule cell output.

**Excitatory Output from Cerebellum**

The Purkinje cell output to the deep cerebellar nuclei is inhibitory because the neurotransmitter secreted by Purkinje cells is GABA.

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1. However, deep cerebellar nuclei receive excitatory inputs from mossy fibers and climbing fibers, and from other sources.
2. Therefore, inspite of inhibition by the Purkinje cells, the output of deep cerebellar nuclei to the brainstem is always excitatory.
3. The internal circuitry of cerebellar neurons is designed mainly to modulate the excitatory output of the deep cerebellar nuclei.
4. Therefore, lesion of the cerebellum in human beings results in hypotonia.

**Purkinje Cell Activity**

Purkinje cells exhibit two types of action potentials: the simple spikes, and the complex spikes (Figs. 132.7A and B).

- **Simple Spikes:** Simple spike action potential is generated in response to stimulation of mossy fiber-parallel fiber input.
- **Complex Spikes:** Complex (multi-peaked) spike action potential is generated in response to stimulation of climbing fiber input that comes from olivary nucleus. These complex spike action potentials are involved in motor learning as climbing fiber activity is observed to be increased when a new motor task is learned. They also produce long-term adjustment in motor responses.

**Functions of Cerebellum**

1. **Control of postural balance and equilibrium:** This is the function of vestibulocerebellum, which has extensive and reciprocal connection with the vestibular nuclei. Afferents from vestibular apparatus in the inner ear project to vestibulocerebellum via vestibular nuclei.
2. **Vestibulo-ocular reflex:** Vestibulocerebellum is concerned with learning induced changes in vestibulo-ocular reflex.
3. **Smoothening and coordination of movement:** This is the function of spinocerebellum that receives proprioceptive and other sensory inputs from all the

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**Fig. 132.6:** Inputs and internal connections of cerebellum. Note, inspite of inhibitory inputs from Purkinje cells, the output of deep cerebellar nuclei is always excitatory. BC: Basket cell; SC: Stellate cell; GC: Golgi cell.

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**Figs. 132.7A and B:** Purkinje cell responses. (A) Simple spike; (B) Complex spike.
body parts through the spinal cord. It also receives inputs from the motor cortex, where motor planning is carried out. By comparing plan with performance, it smoothens and coordinates movement.

4. **Control of posture**: The vermal portion of spinocerebellum projects to the brainstem areas that control axial and proximal limb muscles. Therefore, vermal spinocerebellum has profound influence on posture.

5. **Control of skilled voluntary movements**: The paravermal region of spinocerebellum projects to the brainstem nuclei that influence distal limb muscles. Therefore, paravermal spinocerebellum controls skilled voluntary movements.
   - Cerebellum controls all aspects of movement starting from rate, range, force, and direction to termination of movement. Although functionally cerebellum has three lobes (vestibulo-, spino- and neocerebellum), they work in a coordinated manner, that means it acts as “comparator of a servo mechanism”.
   - Cerebellum receives information from corticospinal output transmitted to the muscles, receives proprioceptive inputs from muscles (via spinocerebellar tracts) that informs about ongoing movements and position of the limbs, and also receives all special sensory inputs (visual, auditory and vestibular inputs).
   - Cerebellum projects to cortex via red nucleus and pontine nuclei (Fig. 132.8). Cerebellum coordinates all the cortical and spinal information and appropriately modifies the ongoing movements via its influence on all descending pathways.
   - It sends error signal to the cortex for alteration in programming of the movement for any desirable change in motor outputs to be achieved.

6. **Planning and programming of the movements**: This is the function of neocerebellum that interacts with the cortex. Hence, neocerebellum controls planning and programming of the movements.

7. **Control of muscle tone and stretch reflexes**: Cerebellum influences the activity of the major descending medial system pathways through its output from fastigial nucleus, especially the vestibulospinal and reticulospinal tracts.
   - As vestibulospinal tract mainly controls $\alpha$ neuron activity and reticulospinal tract controls $\gamma$ neuron activity in the spinal cord, cerebellum is one of the major sites of $\alpha-\gamma$ co-linkage.
   - In human beings, the output of deep cerebellar nuclei to the to the brainstem motor nuclei is excitatory that facilitates muscle tone. Therefore, cerebellar disorder produces hypotonia.
   - Though cerebellum has profound influence on all descending brainstem pathways, its influence on stretch reflexes is minimal, except in some patients it is pendular in whom knee jerk is observed (pendular knee jerk). Therefore, stretch reflexes remain usually normal in cerebellar disorder.
Chapter 132: Cerebellum

Climbing fiber activity
However, cerebral cortex via corticospinal tract depends on the part due to dysmetria, the correction of which is needed.
Therefore, a cerebellar hemisphere influences the output of opposite cerebral cortex.
- Thus, each cerebellar hemisphere influences the output of opposite cerebral cortex.
- However, cerebral cortex via corticospinal tract that decussates to opposite site just after passing through pyramid controls the motor functions of contralateral half of the body.
- Therefore, due to double decussation, each cerebellar hemisphere controls movements of its own side of the body.

9. Learning and improvement of motor skill: Cerebellum plays a critical role of comparing information of the ongoing movements and the changes required to improve performance.
- Hence, for every activity, cerebellum improves the learning and performance.
- Cerebellum also controls long-term adjustment of motor skills.
- Especially, climbing fiber inputs that produce complex spikes in Purkinje cells (Figs. 132.7A and B) is involved in motor learning.
- Climbing fiber activity is increased every time a new activity is learned.

10. Eyeball movement: The paraflocculus and pyramis of cerebellum are concerned with movement of eye ball especially in upward direction. Stimulation of these parts of cerebellum causes upward eye movement of the ipsilateral side. Especially, visual judgment of distance is the function of cerebellum, which is more developed in monkeys.

11. Vestibular functions: For its dense and reciprocal connection with vestibular reflex, vestibulocerebellum is involved in control of all vestibular functions, such as balance during movement, execution of vestibulococular reflex, vestibular postural reflexes, and change in body posture and movement in response to head movement and acceleration.

Features of cerebellar disorder depend on the part of cerebellum affected and whether the cortex or the deep cerebellar nuclei are involved in the disease process. Effects of lesion of one side cerebellar hemisphere manifest on the ipsilateral side of the body.
In general, cerebellar disorders have the following features:
1. No paralysis (voluntary movements are intact, though defective)
2. Usually, reflexes are normal, except that sometimes, pendular knee jerk is elicited.
3. No sensory deficit.
4. Hypotonia is a usual feature.
5. Ataxia: Motor deficit in cerebellar disorder manifests mainly in the form of ataxia, which is defined as a defect in coordination due to errors in the rate, range, force, and direction of movement. If only cerebellar cortex is involved in the disease process, ataxia is temporary. But, if the lesion involves deep cerebellar nuclei, the ataxia almost becomes permanent.

Ataxia manifests in the following forms:
   ii. Scanning speech: Ataxia involving muscles of speech manifests in the form of scanning speech. Patient scans the syllables while speaking.
   iii. Dysmetria: When patient attempts to touch an object, usually the hand overshoots instead of reaching the target. This is called dysmetria (inability to measure the length or distance). This is also called past pointing.
   iv. Intention tremor: Due to dysmetria, the corrective measures are immediately initiated, but this time hand overshoots in the opposite direction. Repeated overshoot and correction results in intention tremor (hand oscillating back and forth). Tremor is not seen at rest.
   v. Rebound phenomenon: This results due to inability to put on brake (suddenly stop) of the ongoing movement. For example, if the patient is asked to flex his limb against resistance and then asked to stop immediately by withdrawing the resistance, he cannot stop, rather his arm moves with a wide arc. This is called rebound phenomenon.
   vi. Adiadochokinesia: Inability to perform alternate movements rapidly is called adiadochokinesia. For example, patient cannot perform supination and pronation rapidly.
   vii. Decomposition of movement: Inability to perform movement that involves more than one joint simultaneously. Therefore, cerebellar patient dissects such complex movement and performs movement at each joint slowly and separately.

6. Inability to carry out long-term adjustment in motor response.
7. Defect in vestibulo-ocular reflex leads to pathological nystagmus.

CEREBELLAR DISORDERS
Diseases affecting flocculonodular lobe result in abnormalities in maintaining equilibrium. For example, stimulation of vestibulocerebellum or vestibular nuclei leads to the motion sickness. Intractable motion sickness, in fact, is cured by selective removal of flocculonodular lobe.
8. **Charcot’s triad**: Presence of nystagmus, intention tremor and scanning speech (or lalling speech like a baby) is seen in cerebellar disorder and disseminated sclerosis that affects cerebellar functions.

9. **Friedreich’s ataxia**: This is a form of hereditary ataxia in which spinocerebellar tract degenerates producing the ataxia as described above.

**Cerebellar Function Tests**

Many clinical tests detect cerebellar functions. These are:

1. Test for coordination
   - In upper limbs: Finger-nose test, making circle in the air, etc.
   - In lower limbs: Knee-heel test, walking on a straight line, etc.

2. Tests for postural stability
   - To stand erect with feet closed but eyes open.

3. Assessment of various aspects of ataxia by eliciting different movements as described above.

4. Assessment of gait and speech.

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**CHAPTER SUMMARY**

**Key Concepts**

1. Cerebellum influences all components of movement. Therefore, cerebellar disorder manifests in all forms of motor deficits, except paralysis and gross sensory loss.

2. Cerebellum receives inputs from all sensory modalities and projects to all descending pathways.

3. Ataxia is the major feature of cerebellar disorder.

**Important to Know (Must Read)**

1. ‘Describe the connections and functions of cerebellum’ usually comes as a Long Question.

2. Purkinje cells, Spinocerebellum, Vestibulocerebellum, Cerebellar nuclei, Internal connects of cerebellum, Functions of cerebellum
   - Cerebellar function tests may come as Short Questions.

3. In Viva, examiner may ask… Name the parts of cerebellum and say their functions, Connections and functions of vestibulocerebellum, Connections and functions of spinocerebellum, Connections and functions of neocerebellum, Layers of cortex of cerebellum, Cerebellar nuclei, Inputs to cerebellum, outputs from cerebellum, Internal circuitry of cerebellum, Structure and functions of Purkinje cells, Feedfoward and lateral inhibition in cerebellum, Functions of cerebellum, Cerebellar function tests, What is ataxia, Features of cerebellar disorder.
Vestibular Apparatus

**Learning Objectives**

On completion of study of this chapter, the student **WILL** be able to:

1. Name the components of vestibular apparatus and give their functions.
2. Understand the mechanism of action potential generation in hair cells.
3. Understand the physiological significance of hair cells arrangement and their responses in otolith organs and semicircular canals.
4. Trace the vestibular pathways.
5. Name vestibular reflexes.
6. Understand the vestibular dysfunctions.
7. List the vestibular function tests.

Vestibular apparatus is located in the bony labyrinth of the inner ear. Though ear is popularly known for its special sensory function of audition, it is equally important for its nonauditory sensory and motor functions, i.e. the **maintenance of equilibrium** at rest and **balance** during movement.

1. The receptors for equilibrium and balance are **hair cells** that are located in vestibular apparatus. Vestibular apparatus consists of **semicircular canals** and **otolith organs**.
2. They detect the sense of position and motion of the head. Movement of head occurs with the movement of the body.
3. Thus, vestibular apparatus indirectly detects the position and movement of the body. Vestibular receptors are stimulated by **linear and angular acceleration**.
4. Linear acceleration stimulates otolith organs and angular acceleration stimulates semicircular canals.
5. They maintain equilibrium by activating postural and ocular reflexes.

![Fig. 133.1: Components of vestibular apparatus.](image)

**Scientist contributed**

Robert Bárány (1876–1936) the otolaryngologist and physiologist from Austria had worked extensively in physiology and pathology of vestibular apparatus. **The Nobel Prize in Physiology or Medicine 1914 was awarded to Robert Bárány “for his work on the physiology and pathology of the vestibular apparatus”**.

**Functional Anatomy**

Vestibular apparatus is enclosed in the bony labyrinth of temporal bone. Vestibular apparatus is also known as **membranous labyrinth**. The membranous labyrinth contains **endolymph**. The endolymph contains high concentration of potassium, which is similar with that of the intracellular fluid. The space between the membranous labyrinth and the bony labyrinth contains **perilymph**, which resembles extracellular fluid in its composition.

1. The vestibular apparatus consists of the **otolith organs** and three **semicircular canals** (Fig. 133.1).
2. Otolith organs are referred to the two sacs like structures called **saccule and utricle**.

3. Saccule and utricle contain calcium carbonate crystals (crystals of the ear or **otoliths**), for which they are called the otolith organs. The saccule communicates with the cochlea via the ductus reuniens.

4. Three semicircular canals on each side are arranged in three mutually perpendicular planes. Accordingly, they are named as the **horizontal, anterior and posterior canals** respectively. Their arrangement ensures that at least any one of them of either ear can detect change in rotational acceleration in any plane.

### Hair Cells

The hair cells are **receptors in vestibular apparatus**.

1. Hair cells have a large number of cilia arranged according to their length (Fig. 133.2). The longest cilium is the **kinocilium**, which is located to one extreme end. Other cilia are called **stereocilia**, which are arranged in a graded length with longest stereocilium remaining next to kinocilium.

2. Hair cells have **directional sensitivity**. When cilia **bend towards the kinocilium** hair cells are depolarized or stimulated and when cilia **bend away from the kinocilium** **hair cells are hyperpolarized** or inhibited (Fig. 133.3).

3. Hair cell activity is conveyed to the higher centers through eighth cranial nerve.

4. Bending of cilia towards kinocilium opens K⁺ channels, through which Ca²⁺ enters hair cells and produce depolarization. Bending of cilia away from kinocilium closes K⁺ channels, and prevents entry of Ca²⁺ that produce hyperpolarization.

**Otolith Organs**

The receptors in otolith organs are hair cells. Hair cells in saccule and utricle are located in the **macula**, the sensory epithelium.
1. The macular hair cells are covered with an otolith membrane, which is a gelatinous mass containing crystals of calcium carbonate, known as otoliths or otoconia (Fig. 133.4).

2. The cilia of the hair cells project into the gelatinous mass and are embedded in the otolith membrane. The macula of the saccule is oriented vertically and macula of the utricle is oriented horizontally (Fig. 133.5).

3. Hair cells of otolith organs are stimulated by linear acceleration.

4. Thus, saccule detects linear acceleration in vertical direction and utricle detects linear acceleration in horizontal direction.

5. They also detect change in head position, like tilting or bending of head.

**Mechanism of Action**

The cilia of hair cells are embedded in otolith membrane that contains otoliths.

1. Otoliths are heavier than endolymph. Thus, specific gravity of the otolith membrane is more than that of the endolymph.

2. Therefore, a change in the direction of the gravitational pull exerted on the otolith membrane bends the cilia of hair cells.

3. Otolith organs detect change in head position and linear acceleration.

**Change in Head Position**

With change in head position like tilting of head, change occurs in the direction of the gravitational pull exerted on its otolith membrane. This results in gravitational movement of otolith membrane (Fig. 133.6A). As cilia are entrenched in otolith membrane, head-tilt bends the cilia of some hair cells towards the kinocilium. This increases action potential frequency in eighth nerve.

**Linear Acceleration**

During linear acceleration of the head, due to higher specific gravity of the otolith membrane, the membrane lags behind because of greater inertia imparted to it than the endolymph (Fig. 133.6B). This causes bending of cilia that are embedded in the otolith membrane. Thus, action potential frequency in eighth nerve increases. Saccule detects linear acceleration in vertical direction; e.g. experiencing acceleration while using the lift, and utricle detects linear acceleration in horizontal direction; e.g. experiencing acceleration in a car when the car starts moving.

**Semicircular Canals**

In semicircular canals, hair cells are located in ampulla, the dilated end of each canal.

1. The ampullae open into the utricle. Each ampulla contains crista terminalis located on a pendular hillock (as it appears like a small hill).

2. Hair cells are present in the crista ampullaris along with the supporting cells (Fig. 133.7).

3. The cilia of the ampullary hair cells are embedded in a gelatinous mass known as cupula, which is an encapsulated-inverted cup like structure that forms a watertight space between the canal and the utricle.

4. However, the gelatinous mass of cupula does not contain otoliths. Thus, the specific gravity of cupular fluid is same as that of endolymph.

**Mechanism of Action**

The cupula extends from crista to the top of ampulla and moves back and forth with the movement of the fluid in the canal.

1. When head rotates to one side, canals being part of the head automatically rotate to that side. However, the endolymph in the canals due to its natural inertia of a gelatinous fluid does not move immediately for about 20 seconds. Therefore, initially endolymph lags behind, as if the endolymph moves in an opposite direction to that of the direction of canal movement (Fig. 133.6C).

2. Thus, if head rotates to the right, for initial 20 seconds endolymph practically rotates to the left. Cupular fluid having the same specific gravity as that of endolymph
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moves along the direction of endolymph. The cupular deflection bends the cilia of the hair cells.

3. In the ampulla, the **kinocilia are located towards the utricle**. Therefore, displacement of cupula towards the utricle bends cilia towards the kinocilium and stimulates hair cells (Fig. 133.8).

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**Figs. 133.6A to C:** Mechanism of stimulation of hair cells of otolith organs during change in head position (A) and linear acceleration (B); and of hair cell of SCC during head rotation.

**Fig. 133.7:** Structure of ampulla of semicircular canal.

**Fig. 133.8:** Mechanism of activation of hair cells of semicircular canal during rotation of head. (SCC: Semicircular canal).
4. Thus, head rotation is detected in the first 20 seconds. After the initial lag period, the movement of endolymph equalizes with the movement of the canal. Therefore, rotation is not detected after the initial phase.

5. When head rotation stops, endolymph in the canal continues to move in the same direction for about 20 seconds, which causes deflection of cupula away from the utricle and the hair cells are inhibited. However, arrangement of hair cells in the canals of both the ears is such that the beginning of rotation is detected by the hair cells in the ear towards which rotation takes place, and termination of rotation is detected by the hair cells in the opposite ear.

Vestibular Pathways

Vestibular information is conveyed to CNS via vestibular division of the 8th cranial nerve. The cell bodies of afferent fibers are located in the Scarpa’s ganglion (vestibular ganglion). These neurons are bipolar neurons. The central axons travel in 8th cranial nerve, which enter the brain at the level of the pons and terminate in vestibular nuclei in the brainstem (Fig. 133.9). There are four vestibular nuclei: lateral, medial, superior and inferior.

Fig. 133.9: Vestibular pathways. (SCC: Semicircular canals).

VESTIBULAR REFLEXES

Vestibular reflexes are broadly divided into two categories: Postural and visual reflexes. The activation of postural reflexes maintains balance and equilibrium during movement to provide postural stability for movement, and activation of visual reflex provides stability of visual images in spite of bodily movements.

Postural Reflexes

Postural reflexes activated by vestibular apparatus are:
1. Tonic labyrinthine reflex (discussed in chapter 130)
2. Labyrinthine righting reflex (discussed in chapter 130)
It happens by assuming a Vestibular Assessment of postural balance tests. This occurs due to lesion of 8th cranial nerve when actually no rotation is occurring. Vestibular system Vertigo is defined as illusion of motion, usually rotation that senses position and rotation of head, if becomes abnormal vertigo occurs. It occurs in following conditions:
1. **Physiological vertigo**: Usual example is motion sickness. However, motion sickness in severe form is pathological.
2. **Central positional vertigo**: Lesion of 8th cranial nerve and vertebrovascular insufficiency are the usual cause.
3. **Peripheral or labyrinthine vertigo**: This occurs due to disease of vestibular apparatus, e.g. Ménière’s disease.
4. **Benign positional vertigo**: It happens by assuming a particular position of the head. Usually it occurs in elderly.

### Vestibular Function Tests

1. **Clinical tests**: Assessment of postural balance tests vestibular functions. For example, ask the subject to walk on a straight line, or stand erect with feet together but with eyes open. (with eyes closed, the test is Romberg sign that assesses postural imbalance other than cerebellar and vestibular origin).
2. **Rotational stimulation (Postrotatory Nystagmus)**: Vestibular stimulation can be produced by rotating the patient in a Bárány chair. The subject seats on the chair with his head bent 30° forward as the horizontal semicircular canals remains horizontal in this position and can be stimulated most effectively by rotation around a vertical axis. Subject’s eyes are closed or he uses Frenzel lenses to avoid the effect of optokinet nystagmus. The chair is revolved at the rate of one revolution per two seconds for 10 revolutions, and then suddenly stopped. If vestibular function is normal, nystagmus will be observed for 20–30 s.
3. **Caloric stimulation (Caloric nystagmus)**: Vestibular stimulation can be produced by irrigating ear canal alternatively with warm and cold saline. The test is based on Bárány’s theory that warming the endolymph decreases its specific gravity, which sets up convection currents that produce movement of endolymph as occurs during angular acceleration. Patient is kept in supine position with his head bent forward by 30°. In this position the horizontal SCC remains in vertical position. External auditory canal is syringed gently first with cold (30° C) water for 30 seconds and then with warm water (44° C) with a gap of 5 seconds between them. The procedure leads to nystagmus if semicircular canal function is normal. With warm water, nystagmus occurs to the irrigated side for about 90 seconds.
4. **Electronystagmography**: This provides more accurate assessment of labyrinth functions.

### Pathway for VOR

The first order neurons start from vestibular apparatus (afferent in vestibular nerve) and terminate in the vestibular nuclei in brainstem. The second order neurons project from vestibular nuclei to oculomotor nerve nucleus. The third order neurons originate from oculomotor nucleus to innervate extraocular muscles. The latency of the reflex is about 10 ms.

### Motion Sickness

Motion sickness occurs in susceptible individuals due to overstimulation of the vestibular apparatus.
1. Very often it is encountered while traveling in ship or a fast moving vehicle like bus.
2. It heralds with nausea and palpitation, and culminates with sweating, dizziness and vomiting. However, repeated vomiting occurs if person continues the journey.
3. Antiemetics may give mild relief. Closing the eye helps in reducing the symptoms.
4. In severe cases, labyrinthectomy or ablation of vestibulocerebellum is performed to cure the disease.

### Vertigo

Vertigo is defined as illusion of motion, usually rotation when actually no rotation is occurring. Vestibular system
CHAPTER SUMMARY

**KEY CONCEPTS**

1. Vestibular apparatus is the structure for maintenance of equilibrium and posture, especially during rotation and acceleration. Vestibular disequilibrium even at rest.
2. Vertigo is the main feature of dysfunction of vestibular apparatus.

**Important to Know (Must Read)**

1. ‘Describe the connections and functions of vestibular apparatus’ may come as a Long Question.
2. Vestibulocerebellum, Hair cells, Otolith organs, SCC, Vestibular reflexes, Motion sickness, Vertigo, Vestibular function tests, may come as Short Questions.
3. In Viva, examiner may ask… Name the parts of vestibular apparatus and say their functions, Connections and functions of vestibulocerebellum, Structure and mechanism of action of Hair cells, Functions of otolith organs, Functions of SCC, Vestibular reflexes, Cause and treatment of motion sickness, Types of vertigo, Vestibular function tests.
CHAPTER 134

Functions of Hypothalamus

Learning Objectives

On completion of study of this chapter, the student MUST be able to:
1. Name the major nuclear groups of hypothalamus.
2. List the functions of hypothalamus.
3. Understand the role of hypothalamus in endocrine, autonomic and reproductive functions, and in regulation of temperature, circadian rhythm, sleep and food and water intake.
4. Describe the regulation of food intake.
5. Understand the abnormalities of hypothalamic functions.

The hypothalamus is a small structure, but it subserves many important functions of the body. The hypothalamus is an essential component of regulation of many homeostatic functions, visceral functions, behavior, sleep-wakefulness, body rhythms (circadian and seasonal) and reproductive functions.

1. For its widespread connections and functions, the hypothalamus is considered as a major integrating center in the brain.
2. Through its connection with pituitary gland it controls major endocrine and reproductive functions, through its connections with brainstem it controls cardiovascular, respiratory, autonomic and thermoregulatory functions, through its connections with limbic system it controls behavioral functions, and through its connections with cortical areas it controls higher functions including learning and memory.

Functional Anatomy

Hypothalamus is a small structure in the brain lies on each side of the third ventricle. It weighs about 10 g.
1. It is located below the thalamus (Fig. 134.1) and hence called hypothalamus.
2. Along with thalamus and subthalamus it forms the diencephalon. The hypothalamus consists of a large number of nuclei and nuclear groups (Fig. 134.2).

It comprises of four main nuclear groups (Fig. 134.3).

- Anterior group: Includes the preoptic, supraoptic and paraventricular nuclei.
- Middle group: Consists of the tuberal, arcuate, ventromedial and dorsomedial nuclei.
- Posterior group: Contains supramammillary, mammillary and posterior hypothalamic nuclei.
- Lateral group: Comprises of lateral preoptic area and lateral hypothalamic nuclei.
Connections of Hypothalamus

Hypothalamus through its afferents and efferents is connected with almost all parts of the brain. Following are the major connections of hypothalamus (Fig. 134.4):

1. **Fornix**: Through fornix hypothalamus is reciprocally connected with limbic system.
2. **Medial forebrain bundle**: Hypothalamus is extensively and reciprocally connected with brainstem including reticular formation and limbic system, especially the septum via median forebrain bundle. Median forebrain bundle also connects brainstem with the cerebral cortex.
3. **Periventricular system**: Reciprocally connects midbrain and sensory pathways to hypothalamus.
4. **Mammillothalamic tract**: This tract conveys information from mammillary body of hypothalamus to the anterior nucleus of thalamus.
5. **Retinohypothalamic fibers (optic nerve)**: It carries visual information from retina to suprachiasmatic nucleus of hypothalamus.
6. **Mammillotegmental tract**: Connects mammillary body to tegmental reticular nuclei of midbrain.
7. **Hypothalamohypophyseal tract**: Connect supraoptic and paraventricular nuclei of hypothalamus to posterior pituitary.
8. **Tubero-infundibular tract**: Connects accurate and ventromedial nuclei of hypothalamus to infundibulum.
9. **Dorsal noradrenergic bundle**: Connects locus ceruleus to dorsal hypothalamus.
10. **Serotonergic neurons**: Connect raphe nuclei to hypothalamus.
11. **Mesolimbic dopaminergic system**: Connects third ventricle to medial hypothalamic nuclei and within hypothalamic nuclei.
12. **Corticohypothalamic fibers**: Connect cerebral cortex to hypothalamus directly.

Hypothalamic Region Outside Blood-Brain Barrier

The hypothalamic areas present outside the blood-brain barrier (BBB) are:

1. Organum vasculosum of lamina terminalis (OVLT)
2. Subfornical organ (SFO)
3. Area postrema

These hypothalamic areas are present in the ventral part of median eminence. Since portal vessels arise in the...
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median eminence, the regions in median eminence of the hypothalamus are present outside the blood-brain barrier. These regions of the hypothalamus also detect change in osmolality of blood and are involved in regulation of blood volume and water intake.

FUNCTIONS OF HYPOTHALAMUS


Endocrine Functions

Hypothalamus controls endocrine functions via its connections with pituitary gland. It controls both anterior and posterior pituitary functions. Hypothalamus is connected with anterior pituitary through portal hypophysial vessels and with posterior pituitary via hypothalamohypophysial tract.

Control of Anterior Pituitary Functions

Hypothalamus controls secretion of all anterior pituitary hormones by secreting various releasing and inhibiting hormones. 1. These hormones are synthesized in the hypothalamus and transported to the median eminence, from where they enter the portal plexus of the superior hypophyseal artery. 2. From there, these hormones travel in the long portal vessels to reach the anterior pituitary (for details refer Chapter “Pituitary Gland”). 3. The main releasing and inhibiting hormones are corticotropin releasing hormone (CRH) that regulates ACTH secretion, growth hormone releasing hormone (GRH) that controls GH secretion, somatostatin that inhibits GH, TSH and prolactin secretion, thyrotropin releasing hormone (TRH) that stimulates TSH secretion, prolactin releasing hormone (PRH), prolactin inhibiting hormone (PIH) and gonadotropin releasing hormone (GnRH) that stimulates LH and FSH secretion (Details of their functions are described in “Hypothalamus”). 4. Many of these hormones act as neurotransmitters in cerebral cortex and limbic system, and few of them also have peripheral actions.

Control of Posterior Pituitary Functions

Hypothalamus secretes two hormones: antidiuretic hormone (ADH) and oxytocin. 1. These hormones are synthesized by the neurons of supraoptic and paraventricular nuclei of the hypothalamus. 2. They are transported along their axons (hypothalamohypophysial tract) to the posterior pituitary, where they are stored and released into circulation at the time of need.

Autonomic Functions

Sympathetic Control

Hypothalamus has profound influence on sympathetic functions (Application Box 134.1). In general, stimulation of lateral and posterior hypothalamic areas results in sympathetic responses. 1. Stimulation of lateral area results in general sympathetic activation in the form of piloerection, rise in BP, increase in heart rate, sweating, papillary dilation, and increase in secretion of catecholamines. Recently, it has been suggested that a separate hypothalamic system exists exclusively for secretion of adrenaline and noradrenaline. 2. Stimulation of posterior hypothalamus results in activation of emotional behavior pattern, such as aggression, fear or rage, which may be due to its connection through limbic system. However, these behavioral responses are also seen as part of autonomic responses in true life.

Parasympathetic Control

Stimulation of anterior hypothalamus results in parasympathetic responses.
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Circadian Rhythm

Circadian rhythm means 24 hours fluctuation in body functions, i.e. day-night variation or variation during light-dark cycle. Many body functions show a circadian pattern. Some of these important functions include sleep habit, corticosteroid secretion, variation in body temperature and melatonin secretion. Most of these circadian functions are regulated by the hypothalamus.

1. Hypothalamic nucleus regulating and maintaining the circadian rhythmicity is the *suprachiasmatic nucleus* (SCN). Therefore, SCN is known as biological clock.
2. The accuracy of execution of this biological clock is achieved by light and darkness through retinohypothalamic fibers.
3. These fibers convey the retinal information about light and darkness via optic chiasm to the SCN. From hypothalamus, information is conveyed to various neuroendocrine structures for maintaining circadian rhythm of various functions.
4. Nocturnal secretion of melatonin is believed to provide important hormonal signal for regulation of other functions.

The physiological basis of SCN seems to be the rhythmic discharge exhibited by this nucleus. It has been observed that neurons of SCN continue to discharge rhythmically in vitro even after its removal from the brain. It has been recently proved that, there are specific genes in neurons of SCN that regulate the rhythmic discharge pattern. Four such genes have been identified in mammals. In the neurons of SCN, genes are activated diurnally that synthesize new proteins. The proteins that are formed enter cytoplasm and modify cell functions. Change in cytoplasmic activity alters their discharge patterns. Later, these proteins return back to nucleus and inhibit the genes that produce them. Thus, genes that control rhythmic discharge of SCN are also regulated by negative feedback mechanism so that the biological clock acts within the physiological range.

Temperature Regulation

Regulation of body temperature depends on the balance between the mechanisms that control heat loss and heat gain. Many peripheral and central mechanisms play role in temperature regulation. In the central mechanisms, hypothalamus plays the key role (Application Box 134.2).

Role of Anterior Hypothalamus

Anterior hypothalamus detects body temperature by sensing the temperature of blood and by receiving inputs from thermoreceptors, especially cold receptors located in skin, deep tissues, spinal cord and other brain areas. If the temperature is above the set point, the anterior hypothalamus activates mechanisms that promote heat loss, especially by causing cutaneous vasodilation and sweating. Heat loss in turn decreases body temperature back toward normal.

Role of Posterior Hypothalamus

The posterior hypothalamus activates mechanisms that increase heat production and promote heat gain. Posterior hypothalamus depends mainly on the information provided by the cutaneous receptors. If the environmental temperature is low, the posterior hypothalamus activates heat conservation by cutaneous vasoconstriction and piloerection. It also increases release of catecholamines from the adrenal medulla.

Many endogenous pyrogens like cytokines act on preoptic area of hypothalamus to produce fever. These cytokines, such as interleukins, increase the local release of prostaglandins that produce fever by raising the temperature range of set point. In fact antipyretics act by decreasing the production of prostaglandins.

Regulation of Food Intake

Regulation of food intake is an intricate phenomenon because of the complex nature of control of eating behavior. Though food intake mainly depends on the individual’s appetite to eat, eating behavior depends on many other physiological and nonphysiological factors. Some of these important factors include psychological, genetic, environmental and social factors, quality of food, nutritional state of the individual, energy expenditure, and interest for food and appetite of the individual at the time of taking food. The long-term regulation of food intake is aimed at maintaining a balance between the intake and energy expenditure so that body weight is maintained within its normal range. However, to make it a simpler one, we classify the factors regulating food intake into three major categories: neural factors, hormonal factors and metabolic factors. Hypothalamus plays the key role in integrating all these factors.

Neural Factors

Role of Hypothalamus

Hypothalamus is the major regulator of food intake. Two areas in hypothalamus control food intake: the feeding center and the satiety center.
Feeding Center
Feeding center is the lateral hypothalamus (LH), located in the bed nucleus of median forebrain bundle at its junction with the pallidohypothalamic fibers. LH is called feeding center as its stimulation greatly increases food intake. On the contrary, experimental lesion of LH results in fatal anorexia in otherwise healthy animals.

Satiety Center
The ventromedial hypothalamus (VMH) is called satiety center, because stimulation of it causes cessation of eating, whereas lesion of it causes severe hyperphagia that results in morbid obesity. The obesity due to VMH lesion is called hypothalamic obesity.

Food intake results from the interaction between the feeding and satiety center. Normally, satiety center decreases food intake by inhibiting feeding center. Feeding center is believed to be chronically active. Following feeding, activity of satiety center increases that in turn inhibits feeding center and finally stops feeding.

However, it should be remembered that appetite for food is different from the desire for food. Appetite is controlled by the neural mechanism described above, whereas the desire for food per se is a complex phenomenon. People often eat out of their desire to eat even when they are not hungry. Uncontrolled desire for eating results in excess calorie intake that leads to obesity.

1. The desire to eat food is controlled by extrahypothalamic structures, such as limbic system. Preliminary reports from our laboratory (Dr GK Pal et al) suggest that hormones or neurotransmitters secreted from limbic areas of brain influence hypothalamic feeding areas. It is not only the excess food intake per se but also the desire for food plays role in the genesis of obesity. This is supported by the fact that there are people who do not develop obesity inspite of high intake.

2. We propose that a hormone is secreted from limbic areas of brain that in addition to its effect on stimulation of feeding center in hypothalamus, influences body weight gain by directly controlling deposition of fat in the fat cells (adiposity).

3. Presently, we designate the hormone as "Greed Hormone" till its detail structure and functions are established scientifically. Identification of this hormone and its effects on hypothalamic feeding areas will have promising impact on obesity research.

Role of Other Centers
Extrahypothalamic areas in the brain have been recently described to control food intake. Mainly, the mesolimbic areas like amygdala (basolateral and central nuclei), caudate nucleus, nucleus accumbens, septal areas (lateral and medial septal nuclei), etc. Dr Pal, et al at JIPMER, Pondicherry, India, have worked extensively on nucleus accumbens and caudate and the neurotransmitters injected into these nuclei, in the regulation food and water intake. It has been hypothesized that many of these areas influence food intake partly though not fully through hypothalamic centers.

Role of Vagus Nerve
Vagus nerve provides sensory signals regarding the filling of the stomach to the hypothalamic regulatory centers. Therefore, stretching of stomach wall by accumulation of food is the immediate factor to inhibit feeding.

Hormonal Factors
Though there is a long list of hormones that influence food intake, following important and well-established hormones are discussed here. Most of these hormones are either secreted from hypothalamus or act on hypothalamus to influence food intake.

A. Hormones that increase food intake
1. Neuropeptide Y
2. Orexins
3. Ghrelin
4. Melanin concentrating hormone (MCH)
5. Agouti-related peptide (AGRP)
6. Galanin
7. Growth hormone-releasing hormone (GHRH)

B. Hormones that decrease food intake
1. Estrogen
2. Dopamine
3. α-MSH
4. CART (cocaine and amphetamine-regulated transcript)
5. CRH
6. Gut hormones
7. Oxytocin
8. CCK
9. Peptide YY
10. Leptin

Leptin
Leptin is the key hormone for regulation of food intake and body weight. Leptin is a polypeptide containing 167 amino acids, released from white fat cells (adipocytes). Increase in adipose tissue mass increases plasma leptin level and decrease in fat store decreases leptin concentration. The receptors for leptin are located in hypothalamus. Leptin, by acting on hypothalamus, decreases food intake and increases energy consumption. The arcuate nucleus of hypothalamus is the sensor to detect plasma leptin concentration.

Physiological responses to high leptin level: High leptin level as occurs in obesity decreases food intake, increases energy expenditure and increases sympathetic activity.

Physiological responses to low leptin level: Low leptin level as occurs in starvation increases food intake, decreases energy expenditure and increases parasympathetic activity.
Leptin mediates its effects on food intake by mainly inhibiting the release of neuropeptide Y (NPY) from hypothalamus. Leptin response to starvation is mainly mediated by NPY and response to obesity is mediated by MSH. Hypothalamus controls food intake according to the adipose tissue mass (degree of obesity) in the body. Hypothalamus senses adiposity via leptin and accordingly controls food intake and energy expenditure of the body. This is called lipostatic hypothesis of food intake, according to which food intake is inversely proportional to adiposity.

Other actions of leptin are as follows:
1. Regulates the onset of puberty.
2. Stimulates thyroid function.
3. Controls glucocorticoids secretion.
4. Increases the activity of uncoupling of protein in the brown adipose tissue cells.

The last three actions increase peripheral energy expenditure.

**Neuropeptide Y**

Neuropeptide Y (NPY), a polypeptide containing 36 amino acids, is a strong orexigen (orexigen is a substance that increases food intake). It is secreted mainly from hypothalamus. NPY containing neurons are present abundantly in arcuate nucleus. NPY stimulates food intake by stimulating the feeding center. NPY also inhibits action of various anorexigenic agents on hypothalamus. NPY has five receptor subtypes: \( Y_1, Y_2, Y_3, Y_4, \) and \( Y_5 \). NPY exerts its effects physiologically via \( Y_1 \) and \( Y_4 \) receptors. However, \( Y_1 \) receptor mainly mediates the food intake. It has been observed that NPY mRNA increases in hypothalamic neurons during feeding and decreases during satiation.

**Orexin**

There are two forms of orexin: Orexin A and Orexin B. Orexins strongly stimulate food intake. Orexins are formed in the lateral hypothalamus.

**Ghrelin**

Ghrelin is a polypeptide secreted from stomach and hypothalamus. Recently it has been discovered to be a potent orexigen. It stimulates food intake and body weight gain. During fasting, ghrelin concentration increases in plasma and, during feeding, concentration decreases.

**Estrogen**

Estrogen is secreted from ovary. It is a potent anorexigenic agent. It crosses BBB to act on hypothalamic centers. It inhibits feeding center and also decreases release of NPY. Therefore, hyperphagia and obesity are usual features of ovariectomized rats. In human beings also, obesity develop following menopause, due to estrogen withdrawal.

**Dopamine**

Dopamine is also a strong anorexigenic agent. It inhibits food intake by acting on hypothalamus and limbic system. However, injection of dopamine into nucleus caudatus and accumbens increases food intake (Dr GK, Pal et al. 1991–94). Thus, dopamine may be the neurotransmitter for drive (motivation) for food in mesolimbic areas and inhibitory neurotransmitter in hypothalamic feeding areas.

**Gut Hormones**

Hormones secreted from GI tract inhibit food intake. Following ingestion, food passes through the GI tract and results in release of gut hormones. These gut hormones inhibit food intake by inhibiting hypothalamic feeding center. This is called gut peptide hypothesis regulation of food intake. These hormones are mainly GRP, glucagons, somatostatin and CCK. CCK is most important among them.

**Cholecystokinin**

Cholecystokinin (CCK) acts peripherally and centrally to inhibit food intake. Peripherally, it acts on visceral receptors and centrally acts on hypothalamus. There are two types of CCK receptors: CCK-A and CCK-B. Peripheral receptors are mainly CCK-A, whereas central receptors are both CCK-B and CCK-A. In hypothalamus, CCK-B receptors are present in more numbers. Therefore, CCK-B antagonist profoundly inhibits satiety and increases food intake.

**Metabolic Factors**

**Plasma Glucose**

Plasma glucose concentration is the important metabolic factor for regulation of feeding. Discharge of ventromedian hypothalamus (satiety center) partly depends on its glucose utilization. Following feeding, plasma glucose concentration rises that in turn increases the activity of neurons of satiety center. Increased discharge of satiety center inhibits the feeding center that finally inhibits food intake. Thus, the person feels sated. Food intake remains inhibited till food glucose concentration remains high. This is called glucostatic hypothesis of regulation of food intake. Hypothalamus by sensing plasma glucose concentration not only regulates food intake, but also indirectly regulates plasma glucose concentration. Therefore, hypothalamus is called glucostat. Hypoglycemia is a potent stimulus for food intake.

**Malonyl CoA**

Malonyl CoA accumulation in the tissue inhibits food intake. Malonyl CoA is produced from acetyl CoA. Malonyl CoA is converted to fatty acid by fatty acid synthase. Therefore, currently the focus of research is to develop an agent, which will inhibit fatty acid synthase, so that malonyl CoA can accumulate and inhibit feeding. Malonyl CoA is also seen to inhibit hypothalamic NPY synthesis by inhibiting the formation of NPYm-RNA. It also induces weight loss and decreases fat store.
Amino Acids and Fatty Acids
Among the chemical signals that give rise to satiety are certain amino acids and fatty acids absorbed from GI tract.

Body Temperature
Decrease in body temperature increases food intake and increase in body temperature inhibits food intake. This is called thermostatic hypothesis of food intake. Therefore, anorexia is a common feature of fever due to any cause. In fact, change in food intake changes body temperature (by changing body metabolism) and vice versa.

Regulation of Water Intake
Hypothalamus plays an important role in water intake. Two major factors that affect water intake are plasma osmolality and extracellular fluid volume (mainly blood volume). The hypothalamus responds to change in both these factors. Thus, hypothalamus is the major thirst center in the brain. OVLT (organum vasculosum of lamina terminalis) and SFO (subfornical organ) are the hypothalamic areas that respond to thirst.

Change in Osmolality
Osmoreceptors are located in the anterior hypothalamus. Osmoreceptors detect the change in the osmolality of plasma. Hyperosmolality of plasma stimulates hypothalamic thirst center that increases water intake. Hyperosmolality also increases ADH secretion that decreases loss of water in urine by increasing water reabsorption from kidney tubule.

Change in Fluid Volume
Volume change is detected by volume receptors in the right and left atria and pulmonary vessels. Hypovolemia (decrease in blood volume) increases secretion of rennin from JG cells of kidney that in turn forms angiotensin II (A II). A II is a strong dipsogen (dipsogen is a substance that stimulates thirst). All stimulates OVLT and SFO to increase water intake.

Control of Reproductive Functions
Hypothalamus secretes gonadotropin-releasing hormone (GnRH) that plays a crucial role in reproductive functions and sexual behaviors.
1. GnRH secreting neurons are located in preoptic area and these neurons contain receptors for gonadal hormones that regulate GnRH secretion. GnRH secretion begins at puberty and then continues throughout the reproductive life. GnRH is essential for attainment of reproductive functions in both males and females.
2. Hypothalamus also controls sexual behavior. Stimulation of medial forebrain bundle in experimental animals induces penile erection and sexual drive, whereas lesion of anterior hypothalamus abolishes this sexual behavior.
3. Hypothalamic testosterone implants restore normal sexual functions in castrated male rats. In females, medial preoptic area of the hypothalamus regulates sexual behavior.
4. Hypothalamic estrogen implants induce estrous heat in ovariectomized rats. However, sexual functions in males and females are further influenced by limbic system.

Other Functions

Influence on Emotion
Limbic system is the principal seat of emotion. Hypothalamus forms one of the output pathways of limbic system for emotional responses. Suitable stimulation of lateral hypothalamus activates rage reactions in animals. Self-stimulation experiments have established that the reward system in hypothalamus is located in lateral preoptic area.

Role in Sleep
There are three subcortical regions that on appropriate stimulation induce slow wave sleep. Diencephalic sleep zone is one among them. The major part of this zone is the posterior hypothalamus. Stimulation of preoptic area of the hypothalamus also induces sleep. By regulating circadian rhythm, hypothalamus controls sleep.

Role of Hypothalamus in Immunity
Hypothalamus influences immunity by controlling the secretion of cortisol via hypothalamo-pituitary-adrenal (cortex) axis. Many stressful stimuli suppress immunity by stimulating hypothalamic CRH release that increases ACTH secretion from anterior pituitary that in turn promotes cortisol synthesis and secretion from adrenal cortex.

Applied Physiology
Hypothalamic dysfunctions are usually known as hypothalamic syndromes. Hypothalamic syndromes are broadly divided into two categories: Global hypothalamic syndrome and partial hypothalamic syndromes.

Global Hypothalamic Syndrome
In global hypothalamic syndromes, the disease process involves either all parts or a large part of hypothalamus. Primary tumors or metastatic carcinoma, lymphoma, or granulomatous diseases like sarcoidosis affecting hypothalamus result in such syndromes. Usually, patient dies due to failure of visceral homeostatic mechanisms and failure to regulate body temperature.

Partial Hypothalamic Syndrome
Partial hypothalamic syndromes occur due to selective lesion of a specific part of hypothalamus, usually resulting in deficiency or overproduction of a single hormone. Some of these important syndromes are described below:
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- **Diabetes insipidus (DI):** Neurogenic DI occurs in hypothalamic or pituitary tumors affecting secretion of ADH.
- **Neurogenic salt wasting:** It occurs as part of SIADH (syndrome of inappropriate ADH secretion).
- **Precocious puberty:** Hypothalamic tumor resulting in premature and excess secretion of GnRH is one of the causes of precocious puberty.
- **Body weight alteration:** Lesion of lateral hypothalamus result in failure to eat and lesion of medial hypothalamus causes overeating. Such lesions occur in various brain tumors like craniopharyngioma.
- **Anorexia nervosa and bulimia:** Severe functional alteration in hypothalamic nuclei results in either anorexia nervosa or bulimia. These are more of behavioral disorder than organic hypothalamic disease.
- **Disturbance in temperature regulation:** Bilateral lesions of anterior parts of hypothalamus result in severe hyperthermia.
- **Hypothalamic cardiovascular disorder:** Hypothalamus with its limbic connection can mount a massive sympathoadrenal discharge to provoke arrhythmias and hypertension, as occurs in extreme emotional stress.
- **Neurogenic pulmonary edema:** Lesion of caudal hypothalamus causes pulmonary edema. There occurs rapid rise in pulmonary vascular resistance, which may be fatal unless treated immediately.

**CHAPTER SUMMARY**

**Key Concepts**

1. Hypothalamus is the main controller of visceral functions. As the master of endocrine orchestra, it controls all endocrinal and reproductive functions.
2. It mediates all limbic and autonomic influences.

**Important to Know (Must Read)**

1. ‘Describe the nuclear groups, connections and functions of hypothalamus’ may come as a Long Question.
2. Role of hypothalamus on food intake, Role of hypothalamus on temp. regulation, Role of hypothalamus on circadian control, Role of hypothalamus on endocrine functions, Role of hypothalamus on reproductive functions, may come as Short Questions.
3. In Viva, examiner may ask… Name the nuclear groups of hypothalamus, What are the functions of hypothalamus, Details of mechanisms of all hypothalamic functions, such as food intake, temp. regulation, circadian control, endocrine functions, reproductive functions etc.
CHAPTER 135

Physiology of Reticular Activating System

Learning Objectives

On completion of study of this chapter, the student MUST be able to:
1. Understand the functional organization of brainstem reticular formation.
2. Remember the components and functions of brainstem reticular activating system (RAS).
3. List the functions of brainstem reticular formation.
4. Appreciate the importance of RAS in body physiology.

Organization of Reticular Formation

In the brainstem, there are specific groups of cell bodies of neurons that form various nuclei for cranial nerves (cranial nerve nuclei), integration for motor and sensory activities (brainstem motor nuclei, olivary nuclei, etc.) and relay nuclei for ascending or descending pathways.

1. Excluding these specific cell groups, there are cell groups in the core of the brainstem that constitute brainstem reticular formation. This reticular formation is formed by a diffuse system of neurons having widely branching axons.
2. Characteristically, these neurons have very long dendrites and axons. They have long ascending branches projecting to the thalamus, hypothalamus and cortex and long descending branches projecting to the spinal cord (Fig. 135.1).

Nuclear Column and Connections

The neurons are organized into two columns: medial and lateral columns.

Medial Column

Medial group of cells constitute gigantocellular part (large cell group) that contains the raphe nuclei and central group of nuclei.
1. They receive afferents from all sensory pathways, especially from spinoreticular tracts (Fig. 135.2).
2. Fibers from this part project upwards as the reticular activating system (RAS).
3. They project downward as the medial and lateral reticulospinal tracts (Fig. 135.3).

Lateral Column

Laterally placed is the parvocellular part containing small-celled nuclei. This part contributes to sleep-wakefulness.

Functions of Reticular Formation

1. Control of Motor Activities

Reticular formation gives rise to reticulospinal tracts (RSTs). RSTs are important components of medial system pathways that are involved in regulation of posture. Fibers of RST originate from the large-celled neurons of reticular formation. Motor cortex, basal ganglia and the cerebellum control posture by influencing the activity of RST. There are two RSTs: pontine and medullary.

Pontine RST

Pontine RST facilitates spinal stretch reflexes by exciting the extensor group of muscles (antigravity muscles), and, therefore, important for maintaining posture.

Medullary RST

Medullary RST inhibits spinal motor neurons that innervate extensor group of muscles.
Chapter 135: Physiology of Reticular Activating System

1. Especially for pain sensation, along with alertness for the sensation, there is simultaneous activation of endogenous analgesia system.

2. Control of Sensory Activities

Many of the ascending sensory pathways provide their collaterals to reticular formation. Therefore, when a sensory stimulus is applied, it not only evokes conscious perception of the sensation, but also by activating RAS, it makes the individual aware of the nature and other aspects of the sensation.

1. Especially for pain sensation, along with alertness for the sensation, there is simultaneous activation of endogenous analgesia system.
2. The neurons of raphe nucleus in reticular formation are activated by paleospinothalamic and spinoreticulothalamic fibers. Consequently, activation of raphe-spinal pathway inhibits transmission of pain in the dorsal horn of spinal cord.

3. Control of Cardiovascular and Respiratory Functions
Cardiovascular and respiratory centers are located in the brainstem reticular formations.

**Cardiovascular Centers**
The vasomotor center (VMC) located in the medullary reticular formation controls heart rate, cardiac output, and vascular tone. Therefore, VMC plays a critical role in the regulation of blood pressure. Cardioinhibitory centers also regulate heart functions and blood pressure.

**Respiratory Centers**
The inspiratory and expiratory groups of neurons are located in the central gigantocellular cells in medulla. The pneumotaxic and apneustic centers are located in pontine reticular formation. All these centers control various aspects of respiration.

4. Control of Visceral Functions
Visceral functions are controlled mainly by the hypothalamus. Reticular formation, through its extensive hypothalamic connections, controls visceral functions. Vomiting and swallowing reflexes are integrated in medullary reticular formation.

5. Sleep and Wakefulness
Fibers projecting from reticular formation to the cortex via nonspecific thalamic nuclei maintain wakefulness. Therefore, this neuronal system is called reticular activating system (RAS). Decreased activity of RAS induces sleep, and damage to RAS causes coma.

**Components of RAS**
There are four major components of the reticular activating system (RAS):

1. **Ascending Reticular Activating Neurons**
   From central group of reticular nuclei (magnocellular part), ascending neurons project to the intralaminar and midline nuclei of the thalamus (nonspecific nuclei).
   - From these nonspecific thalamic nuclei, fibers project to all parts of the cerebral cortex and limbic system. Few fibers from reticular formation bypass thalamus and project directly to the cortex (Fig. 135.1).
   - These neuronal projections form the major part of brainstem ascending reticular activating system, which maintains arousal of the being.
   - Increased activity in these projecting neurons causes excitation of cortical neurons and creates alertness and wakeful state.
   - Diminished activity in these systems induces sleep.

2. **Monoaminergic and Cholinergic Fibers**
   Locus ceruleus is located in the reticular formation at the junction between pons and medulla.
   - The noradrenaline-secreting neurons originate from locus ceruleus.
   - Major monoaminergic and cholinergic systems of the brain are located in the reticular activating system.
   - These fibers are involved in integration of various sensory and behavioral activities.

3. **Fibers from Parvocellular Part**
   Fibers originating from small-celled neurons located in lateral part of the brainstem reticular formation (parvocellular part) are active only during the awakened state and silent during sleep. This indicates that they control sleep-wakefulness.

4. **Serotonergic Neurons**
The serotonergic neurons originating from raphe nuclei project to all parts of CNS.
   - These neurons are active during deep sleep.
   - This indicates that these neurons are involved in genesis of sleep.
   - Histaminergic neurons projecting from hypothalamus to all parts of CNS play a major role in arousal.

**CHAPTER SUMMARY**

**Key Concepts**
1. Reticular activating system (RAS) is the center of alertness. Damage to this area leads to unconsciousness.
2. RAS projects to cortex, hypothalamus, and limbic system for modulation of sleep, visceral, and autonomic functions.

**Important to Know (Must Read)**
1. ‘Describe the nuclear groups, connections and functions of RAS’ may come as a Long Question.
2. ‘Functions of RAS’ may come as Short Questions.
3. In Viva, examiner may ask… Name the nuclear groups of RAS, Afferents and efferents of RAS, Functions of RAS, etc.
Electroencephalogram and Sleep

**Learning Objectives**

On completion of study of this chapter, the student **WILL** be able to:

1. Understand the functional organization cortical and subcortical neurons in the genesis of EEG.
2. Name the EEG waves and their mechanism of genesis.
3. Name the stages of sleep and draw the EEG waves in different stages.
4. Understand the mechanisms of NREM and REM sleep.
5. List the differences between NREM and REM sleep.
6. Understand the theories of sleep.
7. Understand the physiological basis of sleep disorders.

**Electroencephalogram (EEG)** is the record of spontaneous electrical activities generated in the cerebral cortex that are picked up from brain’s surface through electrodes placed on designated sites in the scalp. These electrical activities reflect the electrical currents that flow in the extracellular spaces in the brain. These electrical currents reflect the summed effects of innumerable excitory and inhibitory synaptic potentials upon the cortical neurons. These spontaneous activities of cortical neurons are greatly influenced by the afferent inputs arising from thalamus and brainstem reticular formation. These afferent impulses entrain the cortical neurons to produce most of the characteristic rhythmic EEG waves.

**Electroencephalography** is the procedure of recording EEG. **Electroencephalograph** is the sensitive device that records EEG. EEG is the best diagnostic tool available for assessing the abnormalities of electrical activities of the brain. Therefore, EEG is very helpful in diagnosing epilepsies and for studying sleep and sleep disorders.

As the EEG waves are of very low voltage, they require more amplification before recording.

**Procedure of Recording**

**Hans Berger**, a German psychiatrist in 1929 for the first time demonstrated that electrical activities of the human brain could be recorded using external electrodes on the scalp, which he termed as electroencephalogram (EEG).

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**Scientist contributed**

**Hans Berger** (1873–1941): The discovery of electroencephalography (EEG) in 1929 by the German psychiatrist Hans Berger was a historical breakthrough providing a new neurologic and psychiatric diagnostic tool, without which the making of neurologic diagnosis and planning neurosurgical operative procedures would now be difficult.


1. EEG leads may be bipolar (comparing the potentials between two active leads) or unipolar (measuring the potential changes at a single lead against a reference lead placed on the ear or nose or chin).
2. EEG electrodes are solder or *silver-silver chloride disc* of 0.5 cm diameter.
3. Recording is done with subject preferably in recumbent position with his head and neck supported to ensure that the posterior electrodes are secure.
4. Usually, four leads are attached to the scalp by means of adhesive material on standard skull locations on each side.
5. A **multi-channel pen recorder** is used to record the activities from the **eight leads simultaneously**.
6. The EEG waves are analyzed manually or by using a computer.
EEG waves are described in terms of their frequency, which usually ranges from 1 to 30 Hz, and amplitude, which ranges from 20 to 100 µV. Characteristics of EEG wave vary according the state of consciousness.

1. When the individual is fully alert (sensory inputs are maximum), the waves are mostly of high frequency and low amplitude with as many units asynchronized.
2. When the person is minimally alert as in deep sleep (least sensory input), the waves are of low frequency and high amplitude, and synchronized.
3. Absence of EEG waves indicates brain death.

EEG wave patterns are classified into four types: α, β, θ and δ according to their frequency. The characteristic features of the various EEG rhythms are as follows:

**Alpha Rhythm**

Frequency ranges from 8 to 13 Hz and amplitude from 50 to 100 µV (Fig. 136.1). This is the most prominent EEG rhythm seen in a normal adult at rest (awake but relaxed) with eyes closed. It is found in the posterior half of the brain, especially in the parieto-occipital regions.

**Alpha Block**

The alpha rhythm disappears upon opening of the eyes or the subject engaging in mental effort such as mental arithmetic. The regular alpha rhythm is replaced by irregular low voltage activity. This phenomenon is known as alpha block or desynchronization. This is also called arousal or alerting response.

**Factors affecting a Wave Frequency**

Frequency of alpha rhythm is decreased by hypoglycemia, hypothermia, high arterial pressure and low levels of glucocorticoids. High blood glucose, increased body temperature, low arterial pressure and high levels of glucocorticoids increase frequency of alpha rhythm.

**Beta Rhythm**

Frequency of beta waves ranges from 13 to 30 Hz with low amplitude ranging from 5-10 µV. This is seen in adults, when the eyes are open. These waves appear in posterior regions. Beta rhythms are sometimes seen in the frontal regions regardless of whether the eyes are closed or open.

**Theta Rhythm**

Frequency of theta waves ranges from 4 to 8 Hz with large amplitude. Usually, theta rhythm is seen in normal children. It also occurs during moderate sleep. It may sometimes appear in adults when they are severely disappointed or depressed.

**Delta Rhythm**

Frequency of delta waves ranges from 0.5 to 4 Hz and amplitude from 20 to 200 µV. Delta rhythm occurs normally during deep sleep. Its appearance in an alert state in adult, suggests a serious organic brain damage.

**EEG Rhythms in Infants and Children**

The EEG recordings in children show wide range of patterns. Usually, in awakened infants, there is fast beta rhythm. The rhythm speeds up during childhood and theta rhythm appears. As the child matures, the theta rhythm is replaced by faster alpha rhythms. The alpha rhythm of adults gradually appears during adolescence. The theta rhythm is prominent in the temporal or parietal region, while alpha rhythms are in the occipital region.

**Neural Basis of EEG**

EEG is the summated synaptic potentials that are generated in the activated pyramidal cells. This is because of the typical arrangement of the pyramidal cells.

1. Pyramidal cells are oriented parallel to one another, and their dendrites are oriented perpendicular to the surface of the cortex.
2. Synaptic activity at any point along a dendrite may be depolarizing or hyperpolarizing.
3. This local potential change creates a difference between the active site (called the sink) and the remainder of the dendritic shaft, which serves as a passive current source.
4. The potential difference between the ‘sink’ and ‘source’ is similar to an electrical dipole, and it is proposed that the flow of current between them is responsible for the EEG wave.

**SLEEP AND EEG**

Sleep is defined as temporary state of unconsciousness (more accurately, withdrawal of conscious from the physical world) from which the subject can be aroused with appropriate sensory stimuli. Though, about one-third of
our life is spent in sleep, the time spent in sleep is not wasted as sleep is essential for normal growth and development of mind and body (Application Box 136.1).

1. An individual is kept awake by activation of RAS. Sleep occurs due to periodic shutdown of RAS by influences from brainstem and other regions of brain.

2. Aserinsky, Dement and Kleitman in 1953 through EEG and polygraphic analysis, described that normal sleep consists of recurring cycles of different stages, each of which is characterized by specific EEG changes associated with autonomic and endocrine changes. They discovered that some particular stages of sleep are accompanied by rapid eye movements (REMs), which do not occur in the other stages.

3. Accordingly, the sleep was divided into two phases: REM sleep and non-REM sleep. The non-REM sleep is known as slow wave sleep, whereas REM sleep is known as paradoxical sleep.

Application Box 136.1
Sleep potentiates growth: Growth hormone secretion is more in sleep though the secretion is less in REM period. As infants and children spend more time in sleep, this helps them to attain adequate growth. Sleep deprivation invariably leads to growth deficiency.

Sleep Cycles

The EEG pattern recorded during sleep varies in a cyclic fashion, which repeats in about every 90 minutes. Thus, there are about four cycles in normal 6 to 7 hours of sleep.

1. In normal individuals, sleep cycle begins with slow-wave sleep or Non-REM sleep. There are four stages of slow-wave sleep: stages 1 to 4. A person when falls asleep, passes sequentially through these four stages of increasingly deep sleep.

2. After that, the sleep lightens and he enters into REM period.

3. With completion of REM phase, sleep cycle completes.

4. The REM phase is followed by the next new cycle, i.e. with stage 1 of non-REM sleep.

5. Thus, the cycle repeats in every 70 to 90 minutes.

6. There are differences in the proportion of time spent in the various sleep stages in different age groups. Moreover, each individual has his or her own characteristic pattern.

Usually, there is a predominance of deep slow wave sleep during the early part of the night, and the first REM sleep may occur after an hour. But, REM stage becomes prevalent during the later part of the night. In general, REM sleep occupies about 25% of total sleep period.

As the individual goes to sleep, he advances from stage 1 to stage 4 of slow wave sleep. These are four stages of progressively deepening sleep (it is difficult to wake up the subject in slow wave sleep) during which EEG pattern becomes progressively slower in frequency and higher in amplitude (Fig. 136.2).

Stage 1
First, subject becomes drowsy; the EEG shows a change from beta to alpha rhythm. Immediately, light sleep begins during which the alpha rhythm is replaced by high frequency and low amplitude EEG waves.

Stage 2
The subject then enters into stage 2 sleep. The amplitude of EEG waves slightly increases in this stage. The hallmarks of EEG pattern in stage 2 are sleep spindles and K-complexes.

Sleep Spindles
Sleep spindles are bursts of alpha-like waves having frequency of 12–14 Hz and amplitude of about 50 µV, each lasting for about two seconds. They are called sleep spindles because of their characteristic waxing and waning amplitude.
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K-Complexes
K-complexes are high amplitude-sharp waves appearing irregularly in EEG.

Stage 3
This is the stage of moderately deep sleep. The rhythm of the EEG waves shows lower frequency and higher amplitude.

Stage 4
This is the stage of deep sleep, with delta rhythm predominant over the whole scalp. Maximum slowing (lowest frequency) with large amplitude waves is seen in this phase. It is very difficult to wake up the subject in this stage. In young adults, maximum time of sleep is spent in stage 4 (Fig. 136.3).

Autonomic and other Changes during Slow Wave Sleep
1. Decrease in heart rate and blood pressure
2. Fall in body temperature
3. Slow and regular respiration
4. Increase in gastrointestinal activity, which indicates increased parasympathetic activity.
5. Decreased muscle tone
6. No rapid-eye movements (NREM stage)
7. Sleep is deep in stage III and IV.

Control Mechanisms of Slow Wave Sleep
Mechanisms controlling slow wave sleep may be broadly divided into neural and chemical mechanisms.

Neural Control Mechanisms
Neural control mechanisms of slow wave sleep may be subdivided into three categories: afferent control, central control and circadian control.

Afferent Control
It is known that repeated and monotonous stimulation of mechanoreceptors or afferents from these receptors at a frequency less than 10 Hz produces sleep. This is mediated by low frequency stimulation of brainstem and other areas (see below) that activates the sleep producing areas in the brain.

Central Control
There are three brain areas that on appropriate stimulation produce slow wave sleep. These sleep zones are diencephalic zone, medullary zone and basal forebrain zone.

Diencephalic zone: This zone is formed by posterior hypothalamus and intralaminar and anterior thalamic nuclei close to hypothalamus. Low frequency stimulation of this zone produces an EEG pattern that resembles slow wave sleep.

Medullary zone: The reticular formation at the level of NTS in medulla constitutes the medullary sleep zone. Low frequency stimulation of this zone produces slow wave sleep.

Basal forebrain zone: Preoptic area of hypothalamus and diagonal band of Broca form this zone, where stimulation (either high or low frequency) produces slow wave sleep.

Circadian Control
It was observed in experimental animals that destruction of the suprachiasmatic nucleus disrupts many behavioral and circadian rhythms including the sleep-wake cycle. The suprachiasmatic nucleus is the biological clock, which is influenced by light-dark cycle through retinohypothalamic pathway. Thus, day-night variation controls sleep by influencing inherent rhythmic discharge of the biological clock (for details, see hypothalamic functions).

Chemical Control Mechanisms
Centers for mechanisms governing NREM and REM sleep lie in pons, which is influenced by serotonin, norepinephrine, acetylcholine, adenosine and prostaglandins.

Serotonin: It is clearly known that serotonin agonists suppress sleep and serotonin antagonists induce slow wave sleep. Serotonergic projection from raphe nucleus to thalamus and cortex plays role in the control of sleep.

Norepinephrine: Cells in the locus ceruleus release norepinephrine. Fibers from locus ceruleus project to spinal cord and also to the cortex. The ascending fibers on stimulation prevent sleep and descending fibers inhibit motor neurons that produce hypotonia during slow wave sleep.

Administration of L-DOPA (catecholamine precursor) causes an increase in wakefulness. Stimulation of the adrenergic fibers of the reticular formation leads to EEG arousal and spinal facilitation. Inhibition of catecholamine synthesis leads to a decrease in EEG arousal, i.e. a move towards EEG synchronization. Considerable evidences suggest that decrease in brain monoamine causes increase in REM sleep (not slow wave sleep) and vice versa.

Acetylcholine: Cholinergic neurons projecting rostrally from dorsal pontine tegmentum are part of the RAS. Acetylcholine secreted from these neurons play a role in the control of sleep. It is proposed that cholinergic mechanism selectively promote REM sleep.
Adenosine: Adenosine induces sleep. Caffeine, which is an antagonist of adenosine, is known to produce alertness.

Prostaglandin: Increased concentration of PGD₂ in the medial preoptic area induces sleep and increased concentration of PGE₂ decreases slow wave sleep and produce wakefulness.

Paradoxical Sleep (REM Sleep)

After completion of slow wave sleep, a new pattern EEG waves starts suddenly. During this stage the slow waves are replaced by rapid low-voltage EEG activity. This is called paradoxical sleep, because the EEG activity is very rapid like β rhythm as seen in awakened state; still it is difficult to awaken the individual. In fact, threshold for arousal by sensory stimuli is raised during this stage. This phase lasts for about 10 to 15 minutes.

Features of Paradoxical Sleep

1. Paradoxical sleep is characterized by rapid and roving eye movements, even visible under the closed eyelids. Therefore, paradoxical sleep is also called rapid eye movement (REM) sleep.
2. The EEG pattern shows a desynchronized high frequency and low-voltage like fast β rhythm. Yet it is difficult to arouse the individual from sleep as the threshold for sensory stimuli is raised. However, the subject is likely to wake up spontaneously from REM sleep than at any other stage.
3. Another characteristic features of REM sleep is the appearance of PGO spikes in EEG (Fig. 136.2). PGO spikes are phasic potentials that occur in groups of three to five. They originate in pons and through lateral geniculate body travel to occipital cortex. Therefore, they are called ponto-geniculo-occipital spikes or PGO spikes.
4. If the subject is awakened from REM sleep, he commonly reports that he was dreaming. The dream is easily remembered in this stage, but usually it is not of frightful type, which occurs in slow wave sleep. The REM sleep is customarily considered as dream sleep.
5. Sympathetic system is stimulated; Heart rate and blood pressure are increased, and respiration is rapid and sometimes may be irregular (Table 136.1).
6. In males, penile erection occurs. This is used in diagnosing impotence, in which erection does not occur in REM sleep.
7. Muscle tone is profoundly depressed. Hypotonia is widespread. However there are two exceptions. The two groups of skeletal muscles not involved in hypotonia are the extraocular muscles (that cause rapid eye movements), and muscles of the middle ear ossicles (that protect the inner ear). Hypotonia occurs due to active inhibition of motor neurons by a group of neurons located close to the locus ceruleus in brainstem. The stretch reflexes are inhibited.

Mechanisms for Paradoxical Sleep

Neural Mechanisms

The EEG pattern of REM sleep resembles EEG recording during alerting response. Form PET scanning in humans; it is observed that neuronal activity increases in pons, amygdala and cingulate gyrus during REM sleep. The mechanisms that generate REM sleep are located in the pontine reticular formation. Rapid eye movements, the hallmark of REM sleep are triggered by potentials originating from pons in the form of PGO spikes.

Chemical Mechanisms

Acetylcholine seems to mediate REM sleep. In cat, administration of atropine suppresses paradoxical sleep, whereas injection of physostigmine (which promotes acetylcholine activity) increases the duration of REM sleep. It is also seen that decrease in brain monoamine causes increase in REM sleep. Reserpine, a drug that depletes monoamine stores, blocks slow wave sleep, and hypotonia and EEG desynchronization seen in REM sleep. However, reserpine enhances the rapid eye movements by facilitating PGO activity. Thus, the role of monoamines in paradoxical sleep appears to be complex one. Barbiturates decrease the duration of REM sleep.

### Table 136.1: Differences between NREM and REM sleep.

<table>
<thead>
<tr>
<th></th>
<th>NREM sleep</th>
<th>REM sleep</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Timing in sleep-cycle</td>
<td>Occurs first</td>
<td>Occurs after NREM sleep</td>
</tr>
<tr>
<td>2. Duration in normal adults</td>
<td>75% of total sleep</td>
<td>25% of sleep</td>
</tr>
<tr>
<td>3. Autonomic symptoms</td>
<td>Sympathetic inhibition (low HR, BP, respiration)</td>
<td>Sympathetic excitation (high HR, BP, respiration)</td>
</tr>
<tr>
<td>4. Eyeball movement</td>
<td>No eye movement</td>
<td>Rapid eye movement occurs</td>
</tr>
<tr>
<td>5. Dreams</td>
<td>Dreams are not memorized</td>
<td>Dreams well memorized</td>
</tr>
<tr>
<td>6. Muscle tone</td>
<td>Is inhibited</td>
<td>Is profoundly depressed</td>
</tr>
<tr>
<td>7. Type of sleep</td>
<td>Enters into deep sleep</td>
<td>Sleep lightens</td>
</tr>
<tr>
<td>8. EEG waves</td>
<td>Slow wave-high amplitude (in stage 3 and 4)</td>
<td>High frequency-low voltage</td>
</tr>
<tr>
<td>9. Mechanism</td>
<td>Inhibition of RAS</td>
<td>Activation of pontine reticular formation</td>
</tr>
</tbody>
</table>
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Effect of Age on Sleep

The duration of REM sleep and stage 4 sleep decreases gradually with advancing age. Newborns and infants sleep 18–20 hours a day of which 50% is spent in REM sleep (Fig. 136.4).

Functions of Sleep

Popper and Eccles concluded that “sleep is a natural repeated unconsciousness that we do not even know the reason for”. However, sleep is essential for normal functioning of the body, for following reasons. Sleep deprivation results in many dysfunctions.
1. Sleep restores strength, both physical and mental.
2. Ability to think and concentrate is enhanced with adequate sleep.
3. Sleep consolidates learning and memory.
4. Adequate sleep promotes growth, as during sleep growth hormone secretion is more.
5. Dreams are produced during sleep. However, utility of dreams depends on the ability to remember and interpret them.

Theories of Sleep

Sleep-Wake Cycle

Sleeping or remaining awake is due to the alteration between the state of sleep and state of wakefulness of sleep-wake cycle. This rhythmical cyclic alteration is very unique phenomenon of living worlds, in both animals and humans. It is also believed that even plants sleep. The exact mechanism of induction of sleep or to arouse from sleep is not known. Though many theories have been forwarded to explain the shift from one state to the other between wakefulness and sleep, nothing is fully conclusive. However, one fact is established that sleep-wake cycle is entrained with the diurnal rhythm, which means sleep-wakefulness is synchronized to the day-night variation in the environment.

Circadian Rhythm plays an important role in shift between sleep-wake cycles, at least in human beings. Following theories have been proposed to explain the sleep-wake cycle:
1. Role of circadian rhythm
2. Role of pineal gland
3. Thalamocortical loop
4. Alteration in neurochemicals

Circadian Rhythm

Many mammalian functions and behaviors are linked to the circadian rhythm. The center of circadian rhythm is the suprachiasmatic nucleus (SCN) of hypothalamus. SCN is so named for its location bilaterally above the optic chiasm. For its major influence on physiological alterations in diurnal rhythm, SCN is designated as the biological clock.
1. Experimental findings suggest that the peaks of circadian activity of SCN correlate with the light-dark cycle. It has been observed that sleep-wake cycle depends on the time of exposure to the bright light.
2. If the individual is exposed to bright light during the day, sleep-wake cycle is usually not affected; if exposed to bright light after the evening, onset of sleep is delayed; if exposed in the early morning (before dawn), onset of next sleep period is accelerated.
3. Therefore, it was suggested that the exposure to light (photic stimulation retina) is an important determinant of sleep-wake cycle. The retinal stimulation day stimulates retinohypothalamic pathway that activates SCN, which keeps the individual awake by activation of reticular activating system.
4. Cessation of retinohypothalamic activation with the onset of night (loss of photic stimulation of retina) induces the onset of sleep.
5. It has been proposed that SCN initiates the neurohumoral signals that entrain the circadian rhythm of sleep-wake cycle.
6. This photic stimulation theory of retinohypothalamic pathway may be true for human beings as many animals like rats sleep mostly during day and remain awake mostly in the night.

Role of Pineal Gland

Earlier it was thought that pineal gland is a small vestigial organ in the brain like that of vermiform appendix in GI tract. Recent evidences suggest that pineal plays important roles in hypothalamic control of hormonal secretion and regulation of circadian rhythm.
1. It has been observed that activity of sympathetic fibers to pineal gland is entrained with the light-dark cycle of the environment via retinohypothalamic connections of SCN and melatonin secretion from pineal gland.
2. The alteration in melatonin secretion varies with the light-dark cycle. The secretion is more in the evening and first half of night and less in the second half of the night and first half of the day.
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3. It is substantiated that the melatonin secretion from pineal is controlled by post-ganglionic sympathetic fibers innervating pineal gland that norepinephrine, which increases cAMP by acting on β receptors on the pinealocytes.

4. Melatonin is released into circulation from the fenestrated capillaries of pineal gland is suggested to act on brain reticular activating neurons to mediate sleep-wake cycle (Flowchart 136.1).

5. Melatonin acting on ML₁ receptors inhibits cAMP formation and acting on ML₂ receptors stimulates formation IP₃ and DAG. In infants, pineal gland is larger in size (Application Box 136.2).

Application Box 136.2

Displacement of pineal sands indicates space occupying lesion in brain: Pineal gland regresses in adults and calcium carbonate and phosphate crystals are deposited in pinealocytes. These small concretions of pinealocytes are called pineal sands that can be detected by X-ray film of skull. Therefore, any space occupying lesion such as brain tumor if large enough to shift pineal gland from its position can be detected by noting displacement of pineal gland sands in skull X-ray.

Role of Thalamocortical Loop

The neural circuit between thalamus and cortex has been proposed to be an important component of pattern generator of sleep-wake cycle.

Role of Neurochemicals in Brainstem

The neurons in brainstem RAS projecting to various areas in the cortex secrete norepinephrine (NE), serotonin and acetylcholine.

1. It has been observed that concentration of NE and serotonin secreted from these neurons modulate the release of acetylcholine and this controls the rhythm of sleep-wake cycle.

2. Decreased acetylcholine keeps the individual awake and increased acetylcholine induces sleep (Flowchart 136.2).

3. The serotonergic neurons originating from raphe nucleus and NE secreting neurons from locus ceruleus in the brainstem inhibit the cholinergic neurons in pontine RAS and decrease the content of acetylcholine that keeps the individual awake.

Role of Hypothalamic Neurochemicals: Secretion of histamine from posterior hypothalamic neurons and GABA from preoptic neurons of hypothalamus play a crucial role in activation of thalamocortical neurons mediating sleep-wake cycle. In this mechanism, decreased GABA concentration increases histamine release that activates thalamocortical neurons and keeps the individual awake and increased GABA level inhibits histamine secretion that suppresses thalamocortical neurons and induces sleep (Flowchart 136.3).

Flowchart 136.1: Circadian control of sleep-wake cycle. Note, photic stimulation of retina through retinohypothalamic pathway causes sympathetic activation of pineal gland and releases melatonin that controls sleep-wake cycle rhythm. SCN: suprachiasmatic nucleus; SCG: Superior cervical ganglion.

Flowchart 136.2: Modulation of acetylcholine content of pontine reticular activating system (RAS) by serotonin secreted from neurons projecting from raphe nucleus and norepinephrine (NE) secreted from neurons projecting from locus ceruleus in the brainstem control the sleepwake cycle.
Clinical Usage of EEG

**Diagnosis of Various Types of Epilepsies**

Epilepsy (seizure or fit) is defined as the intermittent disorder of cerebral function associated with a sudden uncontrolled discharge of cerebral neurons, which may or may not be accompanied by loss of consciousness. The epileptogenic focus in the cerebral cortex discharges irregular slow waves or sometimes, high voltage waves that can be recorded in EEG.

There are two main groups of epilepsy:

1. **Generalized seizures** with loss of consciousness associated with generalized synchronous EEG discharge from both hemispheres, e.g. grand mal and petit mal epilepsies.

   **Grand mal epilepsy** is characterized by immediate loss of consciousness followed by sustained contraction of limb muscles (tonic phase) and then jerky movements due to rhythmic contraction-relaxation of limb muscles (clonic phase). In EEG, fast activities are recorded in tonic and slow activities in clonic phase.

   **Petit mal epilepsy (absence seizure)** manifests in the form of short-lived loss of consciousness with mild or no motor activity. EEG recording shows doublets consisting of a spike and a dome. Three such doublets occur typically per second.

2. **Focal epilepsy**, the manifestations of which depend on the site of the cortex from which the discharge occurs, e.g. temporal lobe epilepsy and Jacksonian (motor cortex) epilepsy.

   The value of EEG is to diagnose epilepsy, confirm the type of epilepsy, and to identify the cortical site that may be the focus of the abnormal discharge of epilepsy. However, EEG recording between the attacks may be normal.

**Intracranial Space Occupying Lesion**

Cerebral tumors do not directly produce abnormal electrical activity. They compress adjoining neurons. Therefore, they suppress normal rhythms of adjacent compressed neurons that manifests as irregular or slow waves. This helps in localizing cerebral tumors. Fluid collection such as subdural hematoma can suppresses neurons and produce local abnormal EEG waves recorded from the underlying cortex.

**Diagnosis of Sleep Disturbances**

Analysis of sleep, and diagnosis of sleep disorders (see below) are accomplished with the help of EEG.

**Sleep Disorders**

There are many sleep disorders that occur in different phases of sleep. Somnambulism, nocturnal enuresis and night terrors occur during NREM (slow wave sleep) phase, and bruxism and REM sleep behavioural disorder occur in REM phase of sleep.

**Insomnia**

The chronic inability to sleep in spite of adequate opportunity to do so is called insomnia. There are two types of insomnia: **primary insomnia**, in which there is abnormality in normal sleep mechanism (normal nocturnal sleep is disturbed chronically), and **secondary insomnia** in which the sleep disturbance is secondary to medical or psychological disorders.

**Narcolepsy**

Narcolepsy is a condition in which there is episodic sudden loss of muscle tone accompanied with irresistible urge to sleep. Sleep may start with REM phase of sleep, which never occurs in normal individuals in whom slow wave sleep always precedes REM sleep. Cataplexy is a condition in which there is a sudden loss of power of movement and posture while the subject is awake, followed by irresistible desire to sleep. Even, there may be brief period of paralysis at the beginning or end of the sleep. Catalepsy is usually triggered by strong emotional excitement, which persists for few seconds or minutes. Three-fourth of narcoleptic patients present first with catalepsy. Narcolepsy has a strong genetic susceptibility. Hypothalamus of narcoleptic subjects contain less hypocretin (orexin) producing neurons.

**Bruxism**

This is nocturnal grinding of teeth that is usually associated with dreams in REM sleep. Sometimes it occurs during daytime sleep. It can occur in all ages. EMG studies have revealed that masseter and temporalis muscles contract excessively during bruxism.
Nocturnal Enuresis
Bed-wetting in the night with daytime continence is a frequent disorder in childhood. It may persist into adult life. Boys are affected more than girls in a ratio 4:3. It occurs usually after 3 to 4 hours of sleep in the stage 3 or stage 4 of slow wave sleep. Though, initially it was thought to be functional, recent studies have revealed that intravesicular pressure periodically rises to much higher level in aneuretics than in normal individuals, which indicates that there is failure of neural regulation of micturition in sleep. It is preceded by a burst of rhythmic delta waves in EEG, associated with general body movements.

Parasomnic Disturbances
These include several disorders that occur only during sleep. These are somnolescent starts, sensory paroxysms, nocturnal paroxysmal dystonia, sleep paralysis, night terrors and nightmares, somnambulism, and REM sleep behavior disorder.

Somnolescent Starts
At the beginning or in the early part of sleep (in the early stages), certain jerky motor activities occur in some individuals due to excitation of certain motor center. This is called somnolescent motor starts. It may involve one or both legs or trunk, but less often the arms. It is associated with frightening dreams or sensory experience. These are different from epilepsy in sleep or nocturnal myoclonus.

Sensory Paroxysms
Sensory experiences occurring in paroxysm in sleep are due to excitation of sensory centers. Patient usually gets up in sleep with the feeling that the sensation is transmitted through the body. Sudden flash of light or crashing sound, or sensation of turning or lifting of the body are experienced. Abnormality usually involves labyrinthine vestibular mechanism.

Nocturnal Paroxysmal Dystonia
Characterized by choreoathetotic or ballistic dystonic movements that occur in NREM sleep in paroxysm. Patient appears awake with fearful or astonished expressions like night terrors. It affect any age in both sexes, and usually nonfamilial.

Sleep Paralysis
Sleep paralysis occurs usually during transition from sleeping to the waking state. During this phase, patients are unable to activate their muscles. It occurs towards end of REM sleep. Respiratory and diaphragmatic function and eye movements are usually not affected.

Night Terrors and Nightmares
Night terror (pavor nocturnus) and nightmares are usually the problems of childhood. Usually, this occurs after falling asleep, in the stage 3 or 4 of NREM sleep. Child awakens abruptly in a state of intense fright with marked tachycardia (150-170/min) and tachypnea and then he sleeps automatically. The episode occurs only for 1 to 2 minutes, and child does not remember anything in the morning.

Somnambulism
Somnambulism is the condition in which the sleeping individual is able to stand and walk about. This is called sleep walk, which is just opposite of cataplexy. Usually patient sits up at the edge of the bed, but sometimes he gets down from the bed and really walks with eyes open. He may even perform some familial act without outward show of emotion. Usually, it is seen in children and is benign; but in adults it may be an aggressive motor act. During the act they do not respond to external stimuli. It occurs in stage 4 of slow wave sleep in the first third part of sleep when usually dream does not occur. Next morning, patient does not recall anything.

REM Sleep Behavior Disorder
This parasomnic disorder occurs more commonly in adults or older men, characterized by vigorous and often dangerous motor activity that accompanies vivid dreams. They do not have history of childhood sleepwalking. Characteristically, they become aggressive, angry and shout loudly or even become violent and cause injury to themselves or to the bedmate. The violent episodes occur during the REM phase, during which hypotonia fails to occur.

Obstructive Sleep Apnea
Obstructive sleep apnea (OSA) is a common sleep disorder of middle aged people in which time spent in stage 1 is more. Normally, the duration of stage 1 of NREM sleep is about 10% of total duration of night sleep. In OSA, this duration increases to 30–50% and there is marked reduction in stage 3 and 4 of NREM sleep (slow wave sleep). There is reduction in muscular tone and respiratory drive at the onset of sleep that causes obstruction of upper airway, especially of the pharynx (decreased tone of pharyngeal muscles). Apnea (cessation of breathing) occurs for more than 10 seconds during frequent episodes of obstruction. Apnea causes brief arousal from sleep that reestablishes the airway tone. But, airway obstruction is repeated often throughout the night that decreases the duration of sleep and sleep is usually disturbed. Therefore, the subject feels often sleepy during day time.

Hypersomnia (Excessive Sleep)
Patient sleeps for days to weeks continuously. It usually occurs due to damage to brainstem RAS, or to the neurons in the subthalamus or hypothalamus. Recently, it has been suggested that destruction of dopaminergic neurons in the substantia nigra resulting in overactivity of serotonergic neurons of raphe nucleus causes hypersomnia. This is different from sleeping sickness that occurs in trypanosomiasis.
## CHAPTER SUMMARY

### Key Concepts

1. EEG is useful for detecting the sleep stages and diagnosing sleep disorder.
2. It is nice to spend enough time in slow wave sleep that promotes growth.

### Important to Know (Must Read)

1. **Long Questions** are usually not asked from this chapter.
2. EEG waves, NREM sleep, REM sleep, Theories of sleep, may come as **Short Questions**.
3. In **Viva**, examiner may ask… Who first time described sleep, Name the EEG waves and give their significance, What is alpha rhythm, What are the sleep stages, Stages of NREM sleep, EEG waves in different stages of sleep, REM sleep, PGO spikes, What is paradoxical sleep, Differences between NREM and REM sleep, Effects of age on sleep, Functions of sleep, All types of disorders of sleep.
ChAPTeR 137
Limbic System

Learning Objectives
On completion of study of this chapter, the student **MUST** be able to:
1. Name the components of limbic system.
2. Understand the distribution and functions of different mesocortical neurotransmitter systems.
3. Name the components and functions of Papez circuit.
4. Describe functions of limbic system.
5. Understand abnormalities of limbic functions.
The student **MAY** also be able to:
1. Describe the connections and functions of limbic system.
2. Describe various neurotransmitter systems of limbic cortex.
3. Explain the physiological basis of limbic dysfunctions.

Phylogenetically, limbic system is the oldest part of cortical and subcortical areas of the brain that mainly controls emotional responses. For a medical student, study of physiology of limbic system is really challenging because of its complex internal connections (connections within its components) and multiple neuronal networks and neurotransmitter systems, and its intricate connections with various parts of CNS.

Scientists contributed
James Papez (1883–1958) was an American neurologist and neuroanatomist, is most famous for his 1937 description of the Papez circuit which is a neural pathway in the brain thought to be involved in the cortical control of emotion. He proposed that the circuit connecting the hypothalamus to the limbic lobe was the basis for emotional experiences.

PD MacLean redefined the circuit as the “visceral brain” which consisted of the limbic lobe and its major connections in the forebrain–hypothalamus, amygdala, and septum.

General Aspects
Functional Anatomy
The limbic system comprises limbic lobe of the cortex and subcortical structures associated with it (Flowchart 137.1).

Limbic Lobe
The structures of limbic lobe include cingulate gyrus, subcallosal and parahippocampal gyrus including subiculum, and hippocampal formation that includes dentate gyrus and hippocampus (Fig. 137.1).

Subcortical Structures
The subcortical structures associated with limbic functions are categorized into two parts: diencephalic components and other components (Fig. 137.2).

Diencephalic Components: Diencephalic components of the limbic system are the hypothalamus (including mammillary body), epithalamus and anterior thalamus.

Other Components: Other components include the amygdala, nucleus accumbens, septal nuclei, the prefrontal cortex (anterior and inferior components of the frontal lobe) and the habenula.

The limbic structures are interconnected by circuitous tracts, which were initially described by Papez, hence called Papez circuit (Fig. 137.3). The major circuitous tract of limbic system connects hippocampus to the mammillary body of the hypothalamus, the hypothalamus to the anterior thalamic nuclei via the mammillothalamic tract, and the anterior thalamus to the cingulate gyrus by thalamic projections. The circuit is completed by cingulate gyrus...
Flowchart 137.1: Classification of structures in the limbic system.

Fig. 137.1: Components of limbic lobe.

Fig. 137.2: The limbic system and its associated subcortical structures.
projections to hippocampus. The minor circuitous tracts of limbic system connect other structures within the major circuit.

**Important Physiological Aspects**

1. Septum is an important component of limbic system.
2. Hippocampus is connected to septal nuclei through fornix.
3. Brainstem has reciprocal connections with the hypothalamus. Ascending fibers from brainstem send collaterals to the limbic system.
4. Brainstem projections provide visceral and somatic sensory signals including pain and temperature and sensory information from sexual organs to the limbic system.
5. Information about learning and memory from cortex, especially from prefrontal cortex is referred to the limbic system mainly through cortico-hippocampal connections.

**MAJOR AMINERGIC AND CHOLINERGIC SYSTEMS**

Major fiber systems in the brain connecting various parts with each other are the monoaminergic and cholinergic fibers. The monoaminergic fibers are usually catecholaminergic (noradrenergic, adrenergic and dopaminergic), serotonergic and histaminergic fibers. Neurons projecting from reticular formation innervate almost all parts of the CNS and these projecting fibers are mostly axons of monoaminergic neurons. The limbic system and basal ganglia are richly innervated by these neurons. In the limbic system, monoaminergic neurons play a major role in determining emotional and other behavioural responses.

**Dopaminergic Systems**

The major dopaminergic systems are: mesocortical, tuberoinfundibular, nigrostriatal, and incertohypothalamic systems.

**Mesolimbic or Mesocortical System**

The mesocortical system of dopaminergic neurons originate in the ventral tegmental area of the midbrain region of the brainstem and project to the limbic system (olfactory tubercles, septal nuclei, amygdala, nucleus accumbens) and limbic cortex (frontal and cingulate cortices) (Fig. 137.4). This dopaminergic system plays an important role in motivation and drive (Application Box 137.1). Especially, dopaminergic neurons in the more ventral structures such as the septal and accumbens are associated with the brain’s reward system.

**Application Box 137.1**

**Drug abuse and use:** Due to its importance, dopamine is significant for drug use and drug abuse.

1. **Drug abuse:** Drugs that increase dopaminergic transmission stimulate the brain’s reward system. For example, cocaine inhibits dopamine reuptake, and amphetamine promotes dopamine release and inhibits its reuptake. Repeated administration of these drugs chronically stimulates reward systems and motivation. Therefore, these drugs are potential candidate for drug addiction (drug abuse).

2. **Drug use:** The mesolimbic dopaminergic system is major site of action of neuroleptic drugs that are used to treat schizophrenia and other psychotic conditions. Amphetamine that promotes dopamine release causes schizophrenia and antischizophrenic drugs act by blocking D_{2} and D_{4} receptors.

**Tuberoinfundibular System**

The tuberoinfundibular system of dopaminergic neurons is located within the hypothalamus. The cell bodies are in
the arcuate nucleus and periventricular nuclei fibers terminate in the median eminence. The tuberoinfundibular system controls secretion of hypothalamic releasing factors into a portal system. Especially, it inhibits prolactin secretion.

**Nigrostriatal System**

Cell bodies are located in the pars compacta of substantia nigra and fibers project to neostriatum (caudate and putamen) (Fig. 137.4). This dopaminergic pathway is essential for maintaining normal muscle tone, posture and voluntary movements (For details, see Basal Ganglia’).

**Incertohypothalamic System:** This system connects zona incerta of lateral septum with hypothalamus. It is involved in motivation and ingestive behavior.

**Noradrenergic System**

Noradrenergic neurons secrete norepinephrine. These neurons are mainly located in locus ceruleus, subceruleus, and other brainstem nuclei. Two major noradrenergic systems have been described: locus ceruleus system, and tegmental system.

**Locus Ceruleus System**

Cell bodies of noradrenergic neurons are located mainly in the locus ceruleus from where fibers project to all parts of the CNS (Fig. 137.5).

1. Fibers project to spinal cord, cerebellum, hypothalamus (paraventricular, supraoptic and periventricular nuclei), thalamus, basal forebrain and the whole of neocortex.
2. The fibers ascending from locus ceruleus form dorsal noradrenergic bundle.
3. This system is involved in modulation of sensory and motor functions, and influences cardiovascular regulation and other autonomic functions.
4. The ceruleospinal noradrenergic pathway is a component of endogenous analgesia system.

**Tegmental System**

This system includes neurons that are located in dorsal and ventral tegmentum, dorsal motor nucleus of vagus, and nucleus tractus solitarius (NTS).

1. Fibers originating from ventral tegmentum form ventral tegmental system and fibers originating from lateral tegmentum forms lateral tegmental system.
2. The fibers of tegmental systems project to spinal cord, brainstem, all parts of hypothalamus and basal forebrain.
3. The fibers ascending from tegmental systems form ventral noradrenergic bundle. Ventral tegmental system is involved in regulation of secretion of ADH and oxytocin.
4. It also controls hypothalamic secretion that in turn regulates hypophysiotropic secretion. Fibers of dorsal motor nucleus of vagus, and NTS control cardiovascular and respiratory functions.
5. Noradrenergic transmission controls mode and behavioural functions (Application Box 137.2).

**Application Box 137.2**

**Drug abuse:** Drugs that interfere with noradrenergic transmission significantly influence the mood and affect (the emotional state like euphoria, depression, anxiety, etc.). Drugs like reserpine that decreases brain norepinephrine produce depression and drugs that increase norepinephrine availability like MAO (monoamine oxidase) inhibitors produce elevation of mood. Therefore, such drugs are candidates for drug abuse.

**Adrenergic System**

Cell bodies of neurons secreting epinephrine are located in the medulla and project to spinal cord, hypothalamus, thalamus and periaqueductal gray. Exact role of these neurons are not known.

**Serotonergic Systems**

Cell bodies of serotonergic neurons are located in the midline raphe nucleus of the brainstem (Fig. 137.6).

1. The fibers project to spinal cord, hypothalamus, limbic system, neocortex and cerebellum. Serotonin is an important mood elevator (Clinical Box 137.1).
2. Serotonin suppresses sleep. Serotonin neurons are most active during awake state and least active during slow wave sleep (not during REM sleep).
3. Serotonin also stimulates prolactin secretion, inhibits pain transmission in the dorsal horn of the spinal cord and controls circadian rhythm by influencing function of suprachiasmatic nucleus.
Chapter 137: Limbic System

Clinical Box 137.1

Antidepressant and ecstasy drugs: Drugs that increase serotonin transmission are effective antidepressant agents. For example, the serotonin agonist LSD (lysergic acid diethylamide), a popularly known hallucinogen acts by activating 5-HT\(_2\) receptors. The ecstasy drug, MDMA (3,4-methylene dioxymethamphetamine) produces euphoria by releasing serotonin. Drugs that inhibit norepinephrine reuptake also inhibit serotonin reuptake.

Histaminergic System

Cell bodies of histaminergic neurons are located in tuberomammillary nucleus of posterior hypothalamus. The fibers project to olfactory bulb and all part of cortex. Histamine is proposed to be involved in regulation of limbic functions, especially in arousal and sexual behavior, regulation of blood pressure, water intake, pain and anterior pituitary secretion. Recently, it has been observed that histamine release from posterior hypothalamus inhibits thalamocortical projection neurons that induce sleep.

Cholinergic Neurons

Cholinergic neurons are plentiful available in basal telencephalon, thalamus and cortex. Distribution of these neuron closely resemble to that of monoaminergic neurons.

1. A major cholinergic system of neurons projects from nucleus basalis of Meynert to amygdala and neocortex (Fig. 137.7).
2. Both nicotinic and muscarinic receptors are present in brain. Cholinergic neurons are involved in many physiological functions such as learning, memory (Clinical Box 137.2), motivation, perception, cognition and REM-sleep.
3. Muscarinic blockers produce hallucination.

FUNCTIONS OF LIMBIC SYSTEM

PD MacLean in 1949 called the limbic system as ‘the visceral brain’ for its close association with hypothalamus and visceral autonomic functions. Limbic system is primarily involved in motivation, addiction, emotions of rage and fear, sexual behavior and autonomic responses.

Motivation

There are different areas in the brain that on stimulation produce either pleasurable feeling (rewarding) or unpleasant (aversive) feelings.

1. The system that produces feeling of pleasure is called the reward system and the system that produces displeasure is called avoidance system.
2. About, 35% of brain areas are rewarding, 5% aversive and 60% neutral in nature.
3. These areas are detected by self-stimulation studies in animals, usually in monkeys, by electrodes implanted in their different brain areas (animals are allowed to deliver current to brain areas through the electrodes by pressing a bar).

Reward System

The reward system is also called approach system.

1. It consists mainly of dopaminergic pathways starting from ventral tegmental area to the nucleus accumbens.
2. Frontal cortex and hypothalamic nuclei also belong to this system. In humans, when limbic structures are stimulated during brain surgery, they reported positive emotional feelings.

3. Nucleus accumbens is found to be the major reward center in which dopamine is the major neurotransmitter for it. In rats using Skinner box procedure, electrical stimulation of ventral tegmentum, median forebrain bundle, nucleus accumbens and prefrontal cortex are found to leads to repeated bar pressing that are part of the reward system (Fig. 137.8).

4. Dopaminergic agonists facilitate the rate of self-stimulation and D3 dopaminergic receptor antagonist decrease self-stimulation.

5. Also, reports from our laboratory (Experimental NeuropHysiology Res Lab, JIPMER, Pondicherry, India) indicate that dopaminergic stimulation of nucleus accumbens stimulates feeding and drinking behaviors in Wistar albino rats, though dopamine is an anorectic neurotransmitter in other parts of the brain.

Avoidance System
Structures of avoidance system includes lateral portion of posterior hypothalamus, dorsal midbrain and entorhinal cortex. In humans, the feeling on stimulation of these areas ranges from fear to terror. Therefore, this system is also called punishment system.

Addiction
Addiction is defined as the compulsion to repeatedly use a substance in spite of knowing the negative impact of the substance on health.

1. Addiction commonly occurs to opiates such as morphine and heroin. Other usually addictive drugs are cocaine, amphetamine, ethyl alcohol and nicotine.

2. Irrespective of the chemical nature of the substance, the major mechanism for addiction is the increase in dopamine concentration in the reward system of the brain.

3. The primary center for the neural mechanism of addiction is the nucleus accumbens.

4. Ventral tegmental area projecting to nucleus accumbens through mesocortical dopaminergic fibers is the major afferent pathway for addiction (Fig. 137.9).

5. Projection from frontal cortex, amygdala and hippocampus also form part of this system. Dopamine released at the nerve terminals act on the D3 dopaminergic receptors in the nucleus accumbens.

Emotions of Fear, Anxiety, Rage and Aggression

Fear
Centers for fear reaction are located in hypothalamus and amygdala.

1. Following experimental destruction of amygdala, animals become fearless and autonomic reactions associated with fear are not elicited.

2. Amygdaloid nuclei also encode the memories that evoke fear.

3. In humans, left amygdala is activated by looking at the ugly faces that evoke fear, which does not occur by looking at joyful faces.

Anxiety
Anxiety is the normal emotional reaction to a stimulus in which there is uncertainty or doubt about future and apprehension for the unknown. Anxiety is part of routine normal life.

1. However, inappropriate or excessive anxiety in any condition is abnormal. The seat of anxiety is the frontal part of temporal lobes.

2. The α2-GABA receptors have been identified to mediate anxiety.

3. Benzodiazepines, the known anxiolytics, act by increasing chloride conductance in these receptors.

Rage and Placidity
Rage is the anger reaction in which a person loses his temper in response to a minor stimulus.
1. **Placidity** is the emotional state in which the individual is not disturbed even in response to a major irritating stimulus. *Destruction of neocortex, ventromedian hypothalamus and septal nuclei* results in rage in response to a minute stimulus, whereas *bilateral lesions of amygdaloid nuclei* lead to greater placidity.

2. In 1928, Bard produced ‘Sham Rage’ (rage or aggression without emotional expression) in cat by removing cerebral hemisphere and keeping the hypothalamus and brainstem intact, in which the animal reacted to all stimuli by expressing anger and autonomic overactivity.

3. Later, Bard and Mountcastle found that placidity produced by amygdaloid lesion in monkey could be converted into rage by destroying *ventromedian hypothalamus*.

### Aggression

Aggression is the violent form of rage. Gonadal hormones, especially *testosterone*, are known to produce aggressive behaviors.

1. Aggression decreases following castration.
2. Fight-or-flight response, rage and aggression are fighting behaviors that can be elicited by *stimulation of hypothalamic and amygdalar nuclei*.
3. If the connections of frontal cortex to the limbic system are removed, aggressiveness becomes more intense and permanent.
4. In contrast, *bilateral amygdalectomy results in permanent placidity*.

### Sexual Activity

The sexual activities include sexual reflexes and behavior. *Sexual reflexes* are coordinated mainly in spinal cord and brainstem.

1. **Sexual behavior** is controlled primarily by the limbic system. However, sexual behavior is poorly understood for its complex integrating mechanisms.
2. Though, this is an instinctive behavior **controlled mainly by limbic system**, in human beings the higher cortical centers override limbic influences and refine the behavior. Also, this behavior is conditioned by social and psychological factors.
3. Copulatory responses can be activated in animals by stimulating parts of the hypothalamus, olfactory system, and other limbic areas, that results in mounting behaviors in males and lordosis (arching the back and raising the tail) in females.

### In Females

In females, sexual behavior is still more complex. Female animals usually do not seek for mating always throughout their estrous cycle. Suddenly in the heat phase, sexual activity increases in females and they deeply seek for mating. In human also, sexual appetite increases in females in periovulatory phase, though the basal desire for mating remains throughout the menstrual cycle. *Lesion of anterior hypothalamus abolishes sexual activity in female animals*.

Olfactory stimuli are important in initiating sexual activity. Anterior and pre-optic areas of hypothalamus initiate endocrine regulation of the gonads. Hormonal secretion of odorants, the *pheromones* in females signal the onset of estrus cycle. Pheromones increase sexual receptivity to the male. The odorants are powerful stimulants that in low concentration initiates mating behavior in males. The olfactory system, through its direct connections with the limbic system integrates the behavioral, endocrine, and autonomic responses of sexual behavior.

### Autonomic Responses

Hypothalamus, for its major role in autonomic functions, is called the *head ganglion of ANS*.

1. Insular and prefrontal areas of cortex are involved in the regulation of autonomic functions.
2. Autonomic component of emotional responses are *controlled by amygdala*.
3. Parts of limbic system that project to *brainstem and spinal cord centers* have major influence on autonomic functions.
4. Therefore, alteration in cardiovascular and respiratory functions is an integral part of autonomic responses.

### DISORDERS AFFECTING LIMBIC SYSTEM

Alterations in brain’s monoaminergic systems resulting in neurochemical imbalances in catecholamines, acetylcholine, and serotonin have been observed in major psychiatric disorders, including affective disorders and schizophrenia. Therefore, the most effective drugs used in treating psychiatric disorders are agents that alter monoaminergic transmission.

### Types of Limbic Dysfunctions

#### Affective Disorders

The affective disorders include *depression, mania* or manic-depressive psychoses (MDP). In MDP, periods of depression follow the periods of mania in a cyclic pattern. It has been detected that, in MDP, during depression periods brain *NE concentration* is decreased and in manic periods, NE transmission is increased. Both in depression and mania, there is *decreased brain serotonin transmission*. The antidepressant drugs like MAO
inhibitors or serotonin reuptake inhibitors stimulate both noradrenergic and serotonergic neurons of limbic system. Acute treatment of mania is the use of drugs that block dopamine receptors and long-term treatment for mania is lithium. Lithium inhibits regeneration of the second messenger phosphatidylinositol in neuronal membranes by blocking the hydrolysis of inositol-1-phosphate.

Schizophrenia
Schizophrenia refers to a group of closely related psychotic disorders characterized by a particular type of disordered thinking, affect and behavior. The usual features are thought disorder, inappropriate emotional response, and auditory hallucinations. Though the biochemical discrepancy resulting in schizophrenia is not properly known, neuroleptic drugs that block dopamine receptors in the limbic system are known to ameliorate the features of schizophrenia.

Physiology of Emotions
Emotion is a state of feeling that manifests mentally and physically through cardiovascular and other autonomic changes. Emotional activation and responses occur in three phases: stimulus recognition and evaluation, emotional responses and emotional experience.

Stimulus Recognition and Evaluation
First, the stimulus should be perceived, which is called recognition or the awareness of sensation. Then, the next step is the stimulus evaluation, the process by which the output is compared with stored information. The stored information is available either by experience or by inheritance. The knowledge and experience that are acquired are used for emotional expression. The inherited information also helps in emotional integration, for example inherent fear in monkeys for snakes.

Emotional Responses
Emotional response has three components: affect, conation and physical changes. Affect is the feeling of emotion itself, conation is the urge to take immediate action and the physical changes are the sympathetic effects like rise in heart rate, BP and respiration, and sweating. However, emotional manifestations mainly depend on the type of emotional response.

Emotional response may be a natural response, a defense response and a conditional response.

Natural Response
Natural emotional response is the normal response of fear, anxiety or pleasure encountered in daily life. Though, the natural stimulus for emotion involves the neocortical perceive-cognitive mechanisms like the non-emotional sensory processes, the natural emotion is associated with visceral and behavioral effects. In humans, cingulate gyrus is believed to play an important role in emotion as it interacts with neocortex. Stimulation of cingulate cortex during neurosurgery has been reported to evoke normal pleasure or fear responses and cingulectomy produced for psychotic or neurotic patients is reported to produce diminished emotional responses.

Defense Response
Defense response is the response for a natural attack or responses to prevent from an attack. In experimental animals, the defense response is elicited by stimulation of hypothalamic areas that in cat elicits hissing, arching of the back, piloerection and dilation of the pupil. This is associated with autonomic cardiovascular responses consisting of an increased heart rate and blood pressure, and a large increase in skeletal muscle blood flow. Respiratory rate and depth also increase.

Conditioned Response
This is the emotional response attached to a conditioned stimulus. Most of our emotional responses are conditioned emotional responses as they are usually evoked by a conditioned stimulus.

Emotional Experience
Emotional experiences are stored effectively in the memory. The subsequent emotional reactions to the similar stimuli mostly depend on the knowledge of the previous experiences.

Control of Emotional Responses
Though the emotional responses are profoundly influenced by external factors like social and cultural influences, they are mainly mediated by neural and hormonal mechanisms. The neural control mechanisms for emotion are divided into peripheral and central mechanisms.

Peripheral Control of Emotion
The main output pathway for emotional responses is the autonomic nervous system. The pattern of autonomic activation depends on the type of emotion. The emotion of fear and rage closely resembles the effects of injection of norepinephrine. Thus, it is clear that emotional responses of fear and anger are mediated by sympathetic activation.

Central Control of Emotion
Emotional responses have been experimentally seen to occur in the absence of cortex. Hypothalamus and other limbic structures are important for expression of emotion. As suggested by Papez, for emotional expression and experience, the sensory information from environment passes through the thalamus to the hypothalamus. From hypothalamus, projections are divided into ascending and descending outputs.
**Chapter 137: Limbic System**

**Ascending Output:** The ascending output reaches anterior thalamus via mammillothalamic tract and from there it projects to cingulate cortex. The cingulate cortex interacts with the cortical signals. The cingulate gyrus projects to hippocampus. The circuit is completed by the hippocampus projecting back to the hypothalamus.

**Descending Output:** The descending output of the hypothalamus is directed to the brainstem and spinal cord centers from where autonomic fibers originate. The autonomic output is primary basis for manifestations of emotional expression.

**Role of Limbic System**

Limbic system receives both exteroceptive and interoceptive sensory inputs.

1. **Exteroceptive inputs** reach limbic system via two ways; first, through collaterals arising from ascending sensory projections before the fibers reach cortical areas, and second, form sensory cortex.

2. **Interoceptive inputs** reach hypothalamic and amygdalar nuclei of limbic system from brainstem especially from the nucleus tractus solitarius.

   The major output of limbic system to autonomic control areas is via its connections with brainstem and spinal cord. In addition, amygdala has direct connections with hypothalamus and brainstem autonomic areas. The Papez circuit forms the limbic-hypothalamic connections for emotional expression through autonomic pathways.

**Applied Physiology**

**Psychosurgery**

In 1935, John Fulton observed that frontal lobotomy in monkeys cures experimentally induced neurosis. Since then, selected lesions of the brain, especially of the limbic areas have been performed for the treatment of psychiatric disorders, which are popularly known as psychosurgery. Psychosurgery is usually used for the treatment of psychiatric illness in which extremes of aggression is the main symptom.

**Types of Emotional Disturbances**

1. Disturbances of emotionality due to perceptual or cognitive abnormalities
   a. Perceptual dysfunction
      - Illusion
   b. Cognitive disorder
      - Delusion

2. Disinhibition of emotional expressions
   a. Emotional lability
   b. Pathological laughing and crying

3. Rage reaction and aggressiveness

4. Apathy and placidity
   a. Klüver-Bucy syndrome
   b. Other syndromes

5. Altered sexuality

6. Endogenous fear, anxiety, depression and euphoria

**Klüver-Bucy Syndrome**

Klüver-Bucy syndrome (first described by H Klüver and PC Bucy) is experimentally induced in rhesus monkey by bilateral temporal lobectomy, particularly involving the amygdala.

1. Animal exhibits placidity and inability to recognize object visually inspite of good vision (visual agnosia), but will pick up almost all objects and explore them orally.

2. They also show hypersexuality and hyperphagia (omniphagic).

3. The striking abnormality is to examine everything orally.

4. Animal fails to ignore peripheral stimuli (hypermetamorphosis), and therefore, respond to every stimulus and explore everything.

5. Similar picture is observed in human beings following bilateral surgical removal of temporal lobes, cerebral atrophies and meningoencephalitis following toxoplasmosis, herpes simplex or AIDS.

### CHAPTER SUMMARY

**Key Concepts**

1. Emotional responses, autonomic reaction and visceral of the body are influenced by limbic system.

2. Nucleus accumbens is the major reward center and dopamine is the key neurotransmitter for motivation.

**Important to Know (Must Read)**

1. ‘Describe the connections and functions of limbic system’ or ‘Describe the aminergic and cholinergic systems of the brain’ may be a Long Question.

2. Limbic functions, Papez circuit, Motivation, Emotion, Addiction, Reward system, Avoidance system, Autonomic responses may come as Short Questions.

3. In Viva, examiner may ask... Name the structures of limbic system, What are the limbic functions, What are the components of Papez circuit, Physiology of Motivation, Physiology of Emotion, Physiology of Addiction, Physiology of Reward system, Physiology of Avoidance system, Types of autonomic responses, Neurotransmitter systems and their functions, Causes and features of Klüver-Bucy syndrome.
Learning Objectives

On completion of study of this chapter, the student **WILL** be able to:

1. Define and classify learning and memory.
2. Give the types and significance of conditioned reflexes.
3. Describe the mechanism of learning and memory.
4. Name the brain areas involved in different types of learning and memory.
5. Understand the physiological basis of abnormalities of learning and memory.

**GENERAL CONCEPTS**

Learning and memory are closely associated higher functions in human beings. This is because the process of learning involves the storage of new information in memory and its retrieval at appropriate time, and the process of memory involves repeated acquirement of new knowledge (learning). It is known that frequent learning of newer facts increases the horizon of memory. Thus, learning utilizes memory and memory utilizes learning. Though learning involves memory, it is certainly more than the memory per se. Memory is the simple repetition of what has already been performed or stored, whereas learning involves more than the simple use of memory. It uses all less-understood processes like reasoning, cognitive processes and common sense:

1. The **common sites of learning and memory** in the human brain are the association areas of the cerebral cortex and sub-cortical structures in the temporal lobe, including the hippocampus and amygdala.
2. The association cortical areas imbibe sensory information from the somatosensory cortex, and visual, auditory, and olfactory cortices. They also receive information about emotional feelings from limbic system.
3. These information are integrated with previous experiences of learned skills and are then stored in the memory.

**Definitions**

**Learning**

Learning is defined as **acquirement of information or knowledge by experience** that results in the alteration of behavior. Learning is the sincere attitude and readiness to perform an assigned task. It depends mostly on motivation that creates adequate interest and attention to promote learning.

**Memory**

Memory is defined as **retention of learned information and experiences**. The stored information should be retrieved and utilized at any time in life whenever needed. Thus, memory has **four stages**:

1. **Registration of memory that includes proper perception and attention**: Failure of learning and memory occurs due to impaired perception and attention because the material to be learned is never registered and assimilated (Application Box 138. 1).
2. **Integration and retention**: Learned experiences are processed and integrated by various structures in CNS and then retained at appropriate place in the brain in the form of short-term or long-term memory.
3. **Recognition and recall**: At the appropriate time and place, memory is recalled for proper use.
4. **Reutilization**: Memory (the learned experience) is utilized for improvement of further learning.

**Application Box 138.1**

**Anterograde and retrograde amnesias**: When information cannot be correctly registered and cannot be retained for more than few minutes, *anterograde amnesia* (failure of learning) occurs, and when there is defect in recall and reproduction of memories that have been formed several days, weeks or years before, *retrograde amnesia* occurs (for details, see below).

### TYPES OF MEMORY

Memory is broadly divided into explicit or **declarative memory** and implicit or **nondeclarative memory**:

I. **Explicit or declarative memory** (memory of facts, i.e. semantic memory, and memory of events (episodic memory)
   1. Short-term memory
   2. Long-term memory

II. **Implicit or nondeclarative memory**
   1. Priming
   2. Procedural memory
      a. Skills and habits
   3. Associative learning
      a. Classical conditioning
      b. Operant conditioning
   4. Nonassociative learning
      a. Habituation
      b. Sensitization

Explicit memory and many forms of implicit memory involve short-term and long-term memory.

### Explicit Memory

1. The explicit memory, also known as **declarative memory** is connected with awareness.
2. It has two forms: the memories of events (episodic memory), and the memories of facts (semantic memory).
3. The declarative memory is dependent on the hippocampus and other parts of the medial temporal lobes of the brain for its retention.

### Short-term Memory

1. Short-term memory is the memory that lasts for seconds to hours, during which processing in the hippocampus and elsewhere lays down long-term changes in synaptic strength.
2. **Working memory** is a form of short-term memory that makes information available for a brief period.
3. As a result of repeated training, short-term memory can be transferred into long-term memory, which depends on a process called **consolidation**.
4. During short-term memory, the memory traces are subject to disruption by trauma and various drugs.

### Long-term Memory

1. Long-term memory is the one that stores information for years together, and sometimes for life.
2. Long-term memory traces are remarkably resistant to disruption.
3. This is broadly divided into explicit and implicit memory. Some forms of implicit memories also involve short-term and long-term memories.

### Implicit Memory

1. The implicit memory is not associated with awareness and is therefore also called as **reflexive or nondeclarative memory**.
2. It includes skills, habits, priming and conditioned reflexes etc.
3. Explicit memory is initially required to develop implicit memory. For example, a learner of motorcycle riding initially remembers the steps of changing the gear (he changes gear with conscious knowledge) till it becomes a reflexive habit to do so (he changes gear without awareness). Once skill is acquired, the acts become unconscious and automatic.

### Priming

Priming is the facilitation of recognition of words or objects by prior exposure to them. An example is improved recall of a word when presented with first few letters of it.

### Procedural Memory

Includes skills and habits, which once acquired become unconscious and automatic.

### Nonassociative Learning

The organism learns about a single stimulus. Examples are habituation and sensitization.

**Habituation**:

1. Habituation is a simple form of learning in which repeated application of a neutral stimulus elicits less and less response.
2. The response that was first studied was gill withdrawal in *Aplysia* when the gill is stroked. After a few strokes, the response is not seen. The withdrawal is an aversive response, and if the stroke is harmless, the animal gets habituated to it.
3. Habituation implies learning and therefore can be studied for its cellular mechanisms. It can be short-term, or it can be prolonged if exposure to the benign stimulus is repeated many times.
4. Habituation is a classic example of nonassociative learning.

**Sensitization**:

1. Sensitization is the opposite reaction in which repeated application of stimulus evokes greater and greater response.
2. Sensitization occurs especially when the stimulus to which habituation has developed is coupled with a pleasant or unpleasant stimulus. For example, application of noxious stimulus to gill results in greater withdrawal of gill (an increased responsiveness).

3. Sensitization may occur as a transient response, or if it is reinforced by additional pairings of the noxious stimulus and the initial stimulus, it can exhibit features of short-term or long-term memory.

**Associative Learning**

The organism learns about the relation of one stimulus with other. The classical example is conditioned response.

**Conditioned Reflexes**

**Definition 1**

Reflex response to a stimulus that hardly elicited any response in the past, but presently the response to the stimulus is acquired by pairing the stimulus with another stimulus that normally produces the response, is called a conditioned response.

**Types of Conditioned Reflexes**

There are two types of conditioned responses: classical conditioning and operant conditioning. Conditioned reflexes have two components that are associated with emotional responses and motor responses. The emotional responses are regulated by amygdala and the motor responses are controlled by cerebellum.

**Classical Conditioning**

1. In classic conditioning, in the beginning, there is a stimulus that normally elicits a specific innate response (the response which is already present without training). The stimulus is called unconditioned stimulus (UCS).
2. Later, an arbitrary stimulus that normally does not produce any response, on application does produce a significant response when paired with the UCS. The stimulus is called conditioned stimulus (CS).
3. The typical example of classic conditioning is the Ian Pavlov's experiment on salivation in dog. First, Pavlov produced salivation in dog by placing a piece of meat in its mouth. Then, he rung a bell just before placing meat in dog’s mouth, and repeated the procedure a number of times till the animal was made to salivate with bell-ringing, and finally without even placing meat in the mouth.
4. In this experiment, salivation in dog by placing meat in the mouth is the UCS and bell ringing is the CS. Salivation in response to CS (sound of the bell) occurred by pairing it sufficient number of times with UCS (placing meat in the mouth).

5. Finally, CS produced the response even in the absence of UCS, which was initially evoked only by UCS.

**Scientist contributed**

Ivan Petrovich Pavlov (1849–1936), a skillful experimental physiologist, Pavlov clarified understanding of the mechanics of digestion, and of the functional operation of the alimentary tract, which led to detailed studies on nervous relations of the alimentary tract, and to the important concepts of conditioned reflex action. He is popularly known for the conditioned experiments of salivation he carried on dogs. Pavlov had received the Nobel Prize in Physiology or Medicine in 1904.

**Inhibition of Conditioned Reflex:** Conditioned reflexes can be inhibited in two ways: internal and external inhibitions:

1. If the CS is presented indefinitely without UCS, the response decreases and eventually stops. This is called internal inhibition or extinction.

2. Conditioned reflex response can also be abolished if animal is disturbed externally just after the application of CS. This is called external inhibition.

**Reinforcement of Conditioned Reflex:** It is difficult to maintain conditioned reflex indefinitely:

1. However, if CS is paired repeatedly with UCS from time to time, conditioned reflex becomes permanent (reinforcement of conditioned reflex).

2. Conditioned reflex can also be strongly formed by associating UCS with a pleasant or unpleasant affect. Accordingly, there are two types of reinforcements: Positive and negative reinforcement. If the UCS is associated with a pleasant affect, positive reinforcement occurs and if associated with an unpleasant affect, negative reinforcement occurs (Application Box 138.2).

**Operant Conditioning**

1. In this type of conditioning, animal is trained to carry out a task for either to receive a reward or to avoid a punishment.
2. The UCS may be a pleasant or unpleasant event. The CS is applied as a signal in the form of light or sound that alerts the animal to perform.
3. BF Skinner had extensively studied this type of conditioning. The animal, usually a rat is kept in the Skinner box, in which provision is made in such a way that pressing a bar results in delivery of food pallet, or prevention of an electric shock.
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4. Initially, the response occurs by chance. However, later response occurs with greater probability as reward follows the response (animal learns that food is obtained by pressing the bar or the shock is prevented). Thus, the reinforcement may be a positive reinforcement (by pressing bar animal gets food), or a negative reinforcement (by pressing bar animal prevents electric shock).

5. Conditioned motor response results in avoidance of electric shock. Therefore, this is also called conditioned avoidance reflex or aversion conditioning (Application Box 138.3).

6. Another example of negative reinforcement (conditioned avoidance reflex) if food aversion conditioning, in which animal severely develops aversion to a particular food, if the taste of food (UCS) is coupled with injection of a chemical that develops physical illness (CS).

Application Box 138.3
Physiological utility of operant conditioning: Aversion conditioning occurs in human beings. Food aversion is very strong in humans if the food is associated with illness developed by application of a CS. It develops strongly even in single pairing of UCS with CS, and even persists after separation of CS from UCS. This helps in avoiding ingestion of poisonous foods.

MECHANISMS OF LEARNING AND MEMORY

Mechanisms underlying learning and memory may be divided into cellular (molecular), neural and biochemical mechanisms.

Molecular Mechanisms

Learning and memory are initiated and established by several neurochemical changes like increased synaptic connection, neurotransmitter secretion, formation of intracellular second messenger, protein synthesis and gene activation.

Sensitization and Habituation

Habituation and sensitization occur due to change in neurotransmitter release at the sensory neuron terminals due to alteration in intracellular calcium.

Habituation:
1. Habituation occurs due to decreased neurotransmitter release from the presynaptic sensory ending in response to repeated application of a particular stimulus. Serotonin secretion decreases from the modulator neuron.
2. The stimuli gradually inactivate calcium channel resulting in decreased calcium content at the presynaptic terminal that in turn inhibit neurotransmitter release.

Sensitization:
1. Sensitization occurs due to prolongation of action potential in the sensory endings that result in increase in intracellular calcium, which in turn increases neurotransmitter release.
2. Serotonin released from modulator neurons has been identified to be the mediator.
3. In sensitization, serotonin secretion is increased at the target sensory neurons. In the sea snail Aplysia, the noxious stimulus causes discharge of serotonergic neurons that end on presynaptic endings of sensory neurons.
4. Thus, sensitization is due to presynaptic facilitation. Serotonin activates cyclic AMP in the sensory neuron terminal. Cyclic AMP phosphorylates one set of K⁺ channels that closes the K⁺ channels. This results in slowing of repolarization and prolongation of action potential. This facilitates voltage dependent calcium influx into the sensory terminal that increases release of transmitter by exocytosis.
5. The short-term prolongation of sensitization is due to a calcium-mediated change in adenylyl cyclase that leads to a greater production of cAMP.
6. The long-term potentiation also involves protein synthesis and growth of the presynaptic and postsynaptic neurons and their connections.

Conditioned Reflexes

1. In classical conditioning, pairing of UCS with CS causes biochemical changes in target neurons. The basic mechanism involved is the prolongation of action potential that causes presynaptic facilitation.
2. For the classic conditioned reflex to develop, it is important that the UCS should come soon after the CS to cause a temporal association.
3. The UCS acts on neurons that are activated by CS. UCS increases calcium in the presynaptic terminal. The long-term increase in presynaptic calcium alters adenylyl cyclase activity.
4. Thus, when CS activates the presynaptic neuron, adenylyl cyclase is activated to a greater extent that forms more and more cAMP. Increased cAMP causes phosphorylation of a set of K⁺ channels and closes the channel. This slows the repolarization and prolongs the action potential.

Post-tetanic Potentiation

1. This is the production of enhanced postsynaptic potentials in response to stimulations. This enhancement lasts upto 60 seconds and occurs after a brief (tetanizing) train of stimuli in the presynaptic neuron.
2. The tetanizing stimulation causes Ca²⁺ to accumulate in the presynaptic neuron to such a degree that the intracellular binding sites that keep cytoplasmic Ca²⁺ low are overwhelmed.
Learning and Long-term Potentiation

The physiological process of learning is inadequately explained due to the complex mechanisms involved in it:
1. Repeated stimulation of presynaptic neurons results in change in excitability of postsynaptic neurons by altering the rate of discharge, new protein synthesis and neurotransmitter release. These changes are found to be associated with learning.
2. Long-term potentiation (LTP) is an important process for establishment of learning and memory. Unlike posttetanic potentiation, it is initiated by an increase in intracellular Ca$^{++}$ in the postsynaptic rather than the presynaptic neuron. Increased excitability and change in intracellular protein formation by repeated synaptic stimulation are known mechanisms of LTP.
3. In LTP, the initial step is the phosphorylation of a number of proteins that are stimulated by formation of receptor-mediated second messengers. Phosphorylation of proteins activates various intracellular enzymes and alters neuronal excitability. In the later stage of LTP, the synaptic connections between neurons increase.
4. LTP occurs in the hippocampus in mammals. This is a process of potentiation of impulse transmission in neuronal pathways in hippocampus that lasts for days to weeks when they are stimulated at a high frequency. The potentiation is mediated by calcium influx.

Hippocampal LTP

Hippocampal LTP is of two types: the mossy fiber LTP and Schaffer collateral LTP:
1. The mossy fiber LTP is NMDA independent. It is mediated by presynaptic mechanisms that involve cAMP and I$_{h}$, a hyperpolarization-activated cation channel.
2. The Schaffer collateral LTP is initiated by increased intracellular calcium in the postsynaptic neuron and depends on NMDA receptors. Increased calcium level makes glutamate receptors accessible to glutamate molecules.
   In the amygdala NMDA-independent LTP is produced in GABAergic neurons.

Long-term Depression

Though long-Term Depression (LTD) was first described in the hippocampus, it was subsequently demonstrated in all the fibers as for LTP:
1. LTD is just the opposite of LTP. It is mainly characterized by a decrease in synaptic strength.
2. It is demonstrated by slower stimulation of presynaptic neurons and there is smaller rise in intracellular Ca$^{++}$ compare to that as occurs in LTP.
3. In cerebellum, LTD requires phosphorylation of the GluR2 subunit of AMPA (α-Amino-3-hydroxy-5-Methylisoxazole-4-Propionic acid) receptors, which may be involved in motor learning.

Neural Mechanisms

Brain Regions Involved

Three brain regions that have been thoroughly studied in experimental animals (especially in monkey and rat) to understand learning and memory are: prefrontal cortex, inferotemporal cortex and hippocampus.

Prefrontal Cortex
1. Removal of the frontal lobes in monkeys resulted in delayed response to different memory tasks. It was suggested that spatial short-term memory resides in the frontal lobes.
2. It was further investigated by ablation studies that spatial short-term memory (see below) is the function of dorsolateral frontal cortex.
3. Thalamic fibers concerned with memory project to prefrontal cortex and from there to the basal forebrain. From basal forebrain, fibers project to amygdala, hippocampus and neocortex. These fibers are mainly cholinergic fibers.

Inferotemporal Cortex
1. Lesion of this part of the cortex interferes with visual discrimination, whereas tactile, auditory or olfactory cues remain unaffected.
2. The integrity of pre-frontal and inferotemporal cortices is required for performance of tasks that are relatively difficult. Thus it appears that neural substrates for learning are task specific.

Hippocampus
1. Hippocampus is an important component of the Papez circuit, which is extensively connected with, hypothalamus, thalamus, amygdala and septum.
2. The combined lesions of the hippocampus and the amygdala produce significant amnesia than the individual lesions.
3. The neural basis of learning involves the substrates of reward. The hippocampus and medial forebrain bundle are important structures of the reward system.
4. Prefrontal cortex, the seat of working memory is connected with hippocampus and parahippocampal portion of medial temporal lobe.
5. Bilateral destruction of ventral hippocampus in humans causes striking deficit in short-term memory. They have intact working memory and remote memory. They are capable of learning new tasks and retaining pre-lesion remote memories. However, they can not form new long-term memory.
6. Hippocampal connections with mammillary body (hypothalamus), amygdala and thalamus are also involved in memory.
7. Lesion of mammillary body or thalamus causes impairment of recent memory. Hippocampal connection with amygdala is concerned with emotions related to memory.
By MRI and evoked potential studies, recently it has been observed in humans that activity in left parahippocampal cortex and left frontal lobe increases when they recall words and activity in right parahippocampal cortex and right frontal lobe increases when they recall pictures. New neurons are formed in hippocampus in response to learning and memory (Application Box 138.4)

Application Box 138.4
New brain cells are formed in hippocampus: Now it has been documented that the new neurons are formed from stem cells throughout life in two brain structures: the olfactory bulb and the hippocampus. The process of formation of new neurons is called neurogenesis. In hippocampus, neurogenesis has been observed with learning and memory and decrease in the number of new neurons formed in hippocampus is proposed to decrease the hippocampal memory production.

Neural Mechanisms of Declarative and Procedural Memory

Declarative Memory
1. Declarative memory refers to the memory of events and facts and the ability to knowingly access them.
2. Declarative memory is integrated in medial portion of the temporal lobe.
3. Patients, who have undergone bilateral medial temporal lobectomy, for example for the treatment of intractable temporal lobe epilepsy lose their declarative memories or become incapable in forming new declarative memory, but retain procedural memory.

Procedural Memory
1. Procedural memory refers to the ability to learn and remember new skills and procedures.
2. Procedural memory is integrated in different parts of the brain, depending on type of tasks learned and remembered.
3. Learning and remembering new motor skills and habits require the striatum, motor cortex, and cerebellum.
4. Remembering emotional components associated with tasks and skills require the amygdala. Learning the conditioned reflexes requires the cerebellum and cortex. The medial temporal lobe is not involved in procedural memory.

Neural Mechanisms of Short-Term and Long-term Memory

Short-term Memory
Declarative memory is divided into short-term and long-term memory. Short-term memory is that which can be recalled for seconds to minutes and long-term memory is that which can be recalled for days to years.
1. Learned experiences that are newly and recently acquired can be easily recalled using short-term memory. For example, before an individual dials a telephone number, first he sees the number and repeats that mentally till the number is dialed, and then he forgets the number quickly once he starts talking on phone. This is a form of working memory. However, if the number is repeatedly used or is an important number, the number is stored in the memory for a longer duration.
2. Thus, the permanent storage of information is based on its importance or its repeated use or on whether it is associated with an important or emotional event. For memory to become more permanent, processing occurs in subcortical areas that mainly involve hippocampus.

Working Memory
1. Working memory makes the information available for a brief period.
2. The center for working memory is the prefrontal cortex.
3. Working memory has two components: verbal component that retains the verbal memory and visuospatial component that retains the visual and spatial aspects of the objects (spatial short-term memory).

Long-term Memory
The short-term memory is converted to long-term memory mainly by three ways:
1. By repeating the process of learning frequently.
2. By adding more that one sensory modality to the process of learning, for example writing and at the same time also hearing a newly acquired acknowledge.
3. By associating the process of a particular learning with a meaningful emotional event.

The process of permanent storage of memory is called consolidation:
1. Hippocampus plays an important role in consolidating memory, which is reinforced by an emotional state that is associated with the learning or the experience.
2. The medial temporal lobe is important for long-term declarative memory formation, especially the hippocampal and parahippocampal cortices. However, the hippocampus is not required for subsequent retrieval of long-term memory.
3. Long-term memories are stored in various parts of the neocortex. Various components of long-term memory reside in concerned cortical regions. For example, visual and auditory parts of memories are located in visual and auditory cortex respectively. Therefore, once long-term memories are established, they can readily be recalled by association with similar events (visual, auditory, olfactory or somatosensory) later in life.

Intercortical Transfer of Memory
Memory is transferred from one cortical hemisphere to the other through the corpus callosum and the commissural fibers (anterior and posterior). If these fibers are sectioned, then the no memory transfer occurs. If an animal (cat or monkey) is shown and taught some task
with one eye (other eye blindfolded), and then the blindfold is transferred to the other eye, the animal remembers the task and can perform it. But, if the optic chiasm and corpus callosum is cut, it can not perform the task as it can not remember what has been learned with the other eye. This is called split brain animal.

**Neural Mechanisms of Learning**

1. Prefrontal cortex is critical for coordinating the process of learning and memory.
2. The cerebral cortex processes information related to learning and communicates them to the limbic structures.
3. The prefrontal cortex gathers sensory information from the somatosensory, visual and auditory cortices.
4. **Prefrontal cortex** integrates inputs related to language and mathematical ability in the light of previously acquired learning.
5. The prefrontal cortex is considered as the **site of working memory**. New experiences are processed in the prefrontal cortex. The processed information is then transmitted to the hippocampus.
6. **Consolidation of information occurs in hippocampus over several hours into a lasting form.** Then, the learned experience is **stored in the association cortices**, from where it can be retrieved whenever needed.

**Role of Cholinergic Neurons in Memory**

1. **Acetylcholine is the major transmitter** in learning and memory, and other cognitive function.
2. Cholinergic neurons that are present plentiful in basal forebrain region project heavily to the hippocampus and different parts of cerebral cortex. The cell bodies of these cholinergic neurons are highly concentrated in basal forebrain nuclei especially in the nucleus basalis of Meynert and the nucleus accumbens.
3. Cholinergic fibers are also many projecting neurons from brainstem reticular formation (mainly from pedunculopontine nucleus) to the thalamus and spinal cord. More than 90% of projections from brainstem to thalamic nuclei are cholinergic.
4. Loss of cortical and subcortical cholinergic neurons, especially in the basal forebrain region is associated with **dementia**, an impairment of memory, abstract thinking, and judgment.

**Brain Areas for Integration of Various forms of Memory**

1. **Explicit or declarative memory** (facts and events):
   - Medial temporal lobe—
     - Short-term memory : Hippocampus
     - Working memory : Prefrontal cortex
     - Long-term memory : Various parts of neocortex
   2. **Implicit or nondeclarative memory**
     - Priming : Neocortex
     - Procedural memory : Striatum

**Biochemical Basis**

Changes underlying learning is the repeated transmission of impulses along neural circuits that results in permanent changes in the concerned neurons. One important consequence is the **new protein formation** in the nerve cells. **Increased RNA synthesis** in response to learning has been well documented. Recently it has been suggested that **activation of specific gene** is responsible for learning.

**Stangeness and Familiarity**

Stimulation of some parts of the temporal lobes causes change in interpretation of one’s surroundings such as the subject feels strange in a familiar place or familiarity with the new events. Such of strangeness or familiarity helps the normal individual to adjust to different environments. But, inappropriate feeling of familiarity with new events or surroundings is clinically known as the **déjà vu phenomenon**, (a French word, which means ‘already seen’. This phenomenon may occur in normal individuals. However, this usually occurs as an aura that precedes the onset of temporal lobe epilepsy.

**APPLIED PHYSIOLOGY**

**Amnesia**

Amnesia means **impairment of memory**. There are **two forms** of amnesia: retrograde amnesia and anterograde amnesia.

**Retrograde Amnesia**

1. Loss of memory for events that just precede the head injury or the disease is called retrograde amnesia.
2. Usually, the loss occurs only for short-term memory.
3. Retrograde amnesia occurs commonly in head injury in which patient develops concussion.

**Anterograde Amnesia**

1. Inability to recall the memory or to form new memories after the event (head injury, mental shock or disease) is called anterograde amnesia.
2. Anterograde amnesia also follows head injury but the duration covered by the amnesia usually shortens with time.

**Dr Brenda Milner**, who observed on a patient of Dr Wilder Penfield in the mid-1950s, first reported anterograde amnesia. The patient had undergone bilateral medial temporal lobectomy for the treatment of severe epilepsy and after the surgery he was unable to form any new declarative memory. Dr Milner was quite surprised to
observe that the patient could learn a difficult task, performed better with repeated trials and retained the skill over time. However, he could not remember ever having performed the task before:

1. Different areas of the cortex are responsible for learning and memory. Therefore, the degree of amnesic deficit is proportional to the amount of the cortex removed in injury or involved in the disease process, and the nature of amnesia depends on the part of cortex involved. This indicates the importance of cortex (cortical mechanism) in learning and memory.

2. Cholinergic synapses are mainly involved in this process. Therefore, amnesia can be produced in animals by injecting synaptic depolarizing blocker or disopropyl fluorophosphates, an anti-cholinesterase drug.

**Dementia**

1. Dementia is a syndrome consisting of several intellectual abilities.
2. The deficits occur for many cognitive functions including learning and memory.
3. It occurs in many conditions that affect cortical functions.
4. The commonest is the senile dementia.
5. Pathological dementia is commonly seen in neurodegenerative diseases like Alzheimer’s disease.
6. However, drug induced dementia and alcoholic dementias are not uncommon.

**Alzheimer’s Disease**

Alzheimer’s disease is the common degenerative disease of the brain characterized mainly by premature and progressive dementia.

**Etiology**

1. There is severe loss of cholinergic neurons projecting from basal forebrain to neocortex, amygdala and hippocampus. Especially, fibers projecting from nucleus basalis of Meynert (substantia innominata) are severely affected.
2. Cerebral atrophy mainly involves frontal, temporal and parietal lobes. Pronounced neuronal loss occurs in hippocampus, entorhinal cortex, parahippocampalgyri and subiculum.

**Pathology**

Three pathologic features are characteristic of the disease:

1. Presence of neurofibrillary “tangles” in the nerve cell cytoplasm is the cytopathologic hallmark of the disease. These tangles are fiber-like strands composed of hyperphosphorylated form of microtubular protein “tau”. They appear like pairs of helical filaments.
2. Other characteristic feature is the appearance of neurtic plaques scattered throughout the cerebral cortex. The plaques contain amyloid protein (amyloid β protein or Aβ protein) as the central core surrounded by degenerating nerve terminals.
3. Granulovacular degeneration of neurons, especially in pyramidal layer of hippocampus.

Normally, amyloid precursor protein (APP) is secreted from nerve cells. APP is hydrolyzed by the enzyme γ-secretase to form Aβ protein. This protein is hydrolyzed at three different sites by α-secretase, β-secretase, and γ-secretase, respectively. If APP is hydrolyzed by α-secretase, nontoxic peptide products are produced. But, if APP is hydrolyzed by β-secretase and γ-secretase, polypeptides with 40 to 42 amino acids are produced, that are toxic in nature. The most toxic among them is Aβ1-42.

1. These toxic polypeptides form extracellular aggregates that can bind to AMPA receptors.
2. They also bind to Ca²⁺ ion channels and increase Ca²⁺ influx.
3. They also induce inflammatory responses and produce intracellular tangles.
4. Eventually, the affected cells die.
5. Excessive and abnormal hydrolysis of APP results in more production of Aβ proteins that form neurtic plaques.
6. Neurtic plaques induce inflammatory reactions, tangle formation, oxidative damage and neuronal degeneration (especially cholinergic neurons).

**Features**

1. Usually the patient is above 50 years.
2. Progressive development of forgetfulness is the major symptom.
3. The disease starts with loss of short-term memory and later followed by loss of other cognitive functions.
4. Dysnomia (forgetting words especially names), visuospatial disorientation, and paranoia and other personality changes usually occur.

**Treatment**

1. Cerebral vasodilators, stimulants, and high dose of vitamin B, C, E have beneficial effects.
2. Trials of oral physostigmine, choline, lecithin and cholinergic precursor and agonists have yielded some results.
## CHAPTER SUMMARY

### Key Concepts
1. Learning is the acquisition of information or knowledge by experience and memory is retention and storage of learned information and experiences.
2. Hippocampus, prefrontal cortex and medial temporal lobe are involved in retention of explicit memory, which is associated with consciousness.
3. Implicit memory, which is not associated with awareness, important in developing skills and reflexes. The neural areas involved are neocortex, striatum, amygdala, cerebellum and reflex pathways.
4. Serotonergic fibers, cholinergic fibers and calcium play important role in learning and memory.

### Important to Know (Must Read)
1. 'Describe the mechanisms of learning and memory' may come as a **Long Question**.
2. Conditioned reflexes, Neural mechanisms of learning and memory, Molecular mechanisms of learning and memory may come as **Short Questions**.
3. In **Viva**, examiner may ask... Define learning, Define memory, Classify memory, What is short-term memory, What is long-term memory, What is priming, What is habituation, What is sensitization, Define conditioned reflexes, Name the types of conditioned reflexes, What is classical conditioning, What is operant conditioning, What are the brain areas involved in learning and memory, What are the chemicals/neurotransmitters in learning and memory.
Learning Objectives

On completion of study of this chapter, the student will be able to:
1. Name the speech areas in the brain.
2. Trace the pathway for speech and understand the theory of speech production.
3. Classify speech and language disorders and understand the physiological basis of these abnormalities.

As they are the best ways of expressing oneself, speech and language functions are fundamental to human civilization. The ability to communicate by language both in writing and speaking is the special skill.

1. Human beings are also capable of expressing their efficiency and emotions without using speech, like presenting oneself through the quality of work one does (the work language) or conveying the message through the physical changes (the body language) or through emotional feelings (the expressive language).
2. Thus, language is not limited to the expression only through speech or writing.
3. However, generally, it can be stated that language is the means of communicating one's thought through spoken words or in writings, and is also the medium for all delicate interpersonal transactions.

It is more complex to understand the physiology of language because of the intricate mechanisms in the brain that integrate this unique function. Most of our present knowledge of language processing is based on the clinical data by analyzing patients with aphasias that develop following diseases affecting the cerebral cortex, and cerebral damage due to brain injury, or neurosurgery.

Language and Speech Areas in the Brain

There are four main areas in the brain that play important role in the processing of language and speech. These four areas are collectively known language zone that are present around the Sylvain fissure (therefore, also called perisylvian areas). Two are called receptive areas and two are called executive areas (Fig. 139.1). All these areas are located in association cortex, adjacent to cortical areas that are essential in language formation.

Scientists contributed

Paul Broca (1824–1880) was a French physician, anatomist and anthropologist, best known for his research on Broca’s area, a region of the frontal lobe that has been named after him. His work revealed that the brains of patients suffering from aphasia contained lesions in a particular part of the cortex, in the left frontal region. This was the first anatomical proof of the localization of brain function.

Carl or Karl Wernicke (1848–1905) was a German physician, anatomist, psychiatrist and neuropathologist, noticed that not all language deficits were the result of damage to Broca’s area. He found that damage to the left posterior, superior temporal gyrus resulted in deficits in language comprehension. This region is now referred to as Wernicke’s area, and the associated syndrome is known as Wernicke’s aphasia (receptive aphasia) for his discovery.

Receptive Areas

The receptive areas are also called sensory speech areas as they receive and process the sensory information for speech. They are Wernicke’s area (area 22) that subserves the perception of spoken language and the angular gyrus...
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**Wernicke's Area**
The area is so named as it was described by Carl Wernicke in 1874.
1. Wernicke's area is located in the upper part of the temporal lobe, the area that is in the parietal-temporal-occipital association cortex.
2. This is the major association area for processing sensory information from the somatic sensory, visual, and auditory cortices.
3. Wernicke's area is essential for the comprehension, recognition, and construction of words and language.
4. Patients with lesion in Wernicke's area may speak, but the words they frame and put together will have no meaning.

**Angular Gyrus (Area 39)**
This is the second receptive area that occupies the angular gyrus in the inferior parietal lobule anterior to the visual receptive area. This is also called Dejerine's area. This subserves the perception of written language.

**Executive or Expressive Areas**
The executive areas are also called motor speech areas as they execute the expression of speech. They are Broca's area (area 44 and 45) and Exner writing area.

**Broca's Area**
Broca's area is concerned with motor aspects of speech, hence called motor speech area (area 44 and 45). This area was described by Paul Broca in 1865.
1. It is present in the frontal lobe, which is located in the prefrontal association cortex, adjacent to the motor cortex.
2. Broca's area regulates functions of the muscles of the lips, tongue, pharynx and larynx.
3. Patients with lesion of Broca's area are capable of comprehending a spoken or written word but they are not able to say the word.

**Exner Writing Area**
Exner writing area is located in the posterior part of the frontal lobe (though some disagreement exists for this concept). The visually perceived words are given expression in writing through this area. Thus, there are two parallel systems for speech. One is for understanding the spoken words and producing speech, and the other for understanding written word and producing writing.

**Arcuate Fasciculus**
The sensory and motor areas are intricately connected with each other. Especially, a rich network of nerve fiber, the arcuate fasciculus, which passes through the isthmus of temporal lobe and posterior end of the sylvian fissure, connects Wernicke's area with Broca's area. This fasciculus coordinates the understanding and execution of speech and the language skills. Broca's area is further connected with lower rolandic cortex by short association fibers that in turn innervate the speech apparatus (muscles of mouth, tongue and throat).

**THEORY OF SPEECH PRODUCTION**
Angular gyrus receives visual and auditory inputs and makes preliminary processing of these information.
1. Angular gyrus projects to Wernicke's area, which is concerned with the comprehension of visual and auditory information.
2. Wernicke's area projects to Broca's area through arcuate fasciculus, which further processes information received from Wernicke's area into elaborate process of vocalization (programming of articulation).
3. Broca's area projects to motor cortex that brings about the motor activities of speech apparatus, which finally produces speech (Fig. 139.2).

Language function is closely associated with the concept of cerebral dominance. It is highly lateralized function of the brain residing in the left hemisphere in right handed persons. This dominance is observed also in left-handed individuals (for details see next chapter).

**DISORDERS OF SPEECH AND LANGUAGE**
Speech and language disorders may be divided into four categories.
1. Aphasia or dysphasia: Loss or impairment of production and/or comprehension of spoken or written language due to an acquired lesion of the brain is called aphasia.
2. Disturbances of speech secondary to other global disease: Disturbances of speech or language with diseases that globally affect higher mental function like dementia, in which speech and language functions are impaired as part of the general loss. For example,
in Alzheimer’s disease gradual loss of all aspects of language function occurs without any specific aphasia. In this category, commonly, palilalia (extreme perseveration) and echolalia (patient repeats the words he hears like a parrot) are observed.

3. **Dysarthria**: Defect in articulation of speech with intact mental function and comprehension of spoken and written language. This occurs purely due to disorder of muscles of articulation, which may be due to flaccid or spastic paralysis.

4. **Aphonia**: Loss of voice due to disorder of larynx or its innervation.

## Types of Aphasia

Aphasias are broadly divided into four categories: Motor aphasia, sensory aphasia, global aphasia and dissociative language syndromes.

### Motor Aphasia (Broca’s Aphasia)

This is also called nonfluent aphasia or expressive aphasia.

1. This occurs in disease processes that affect Broca’s area.
2. The primary deficit is in the language output or speech production, with relative preservation of comprehension. In its mildest form, motor speech deficit manifests as poverty of speech (cortical dysarthria) with preservation of comprehension and ability to write.
3. In advanced form, there is complete loss of power of speaking.
4. There is no paralysis of speech apparatus as patient can chew, swallow, clear the throat, cry or even vocalize without word.
5. Usually, lower part of face, and arm of the right side are weak.

### Sensory Aphasia (Wernicke’s Aphasia)

This is also called fluent aphasia or receptive aphasia. This occurs in disease processes that affect Wernicke’s area. The syndrome comprises of two main features:

1. Impairment of comprehension of speech, and
2. Relatively fluent, but paraphasic speech (use of malformed and inappropriate words).

The impairment of comprehension is basically an inability to differentiate word elements or phonemes both spoken and written, which indicates the involvement of auditory and visual association areas. Defect in language is manifested by inability to repeat written or spoken word. Despite fluency in speech, the language is remarkably devoid of speech. They also cannot understand of what is said to them. The motor apparatus required for expression of language remains intact. Written letters are often combined into meaningless words.

### Global Aphasia

This occurs due to destruction of large part of language zones involving both Broca’s and Wernicke’s areas.

1. The lesion usually occurs due to occlusion of the left internal carotid artery or middle cerebral artery.
2. All aspects of speech and language are affected.
3. At best they may say few simple words, but characteristically they fail to carry out a series of simple commands or name objects. They cannot read, write or repeat what is said to them.
4. Usually this syndrome is associated with varying degrees of right hemiplegia, hemianesthesia, and homonymous hemianopia.

### Dissociative Language Syndromes

Dissociative language syndromes refer to language deficit that do not result from lesion of cortical language areas, but from disruption of pathways joining them. Included in this category are: conduction aphasia, pure word deafness, pure word blindness, pure word mutism, anomic aphasia and transcortical aphasia.

### Conduction Aphasia

Occurs due to separation of auditory and motor language areas without damaging these areas.

1. Characteristically, repetition is severely affected for both single words and nonwords in the face of preserved comprehension.
2. There is fluency and paraphasia in self initiated speech as seen Wernicke’s aphasia.
3. Writing is invariably impaired.

### Pure Word Deafness

This is also called auditory verbal agnosia, is characterized by impairment of auditory comprehension and repetition and an inability to write following dictation.
1. Spontaneous writing and ability to comprehend written language are preserved, that differentiated it from Wernicke’s aphasia.
2. They may declare that they may not hear, *audiometry discloses no hearing defect*.
3. Usually the lesion is bilateral in superior temporal gyrus.

**Pure Word Blindness**

This is also called visual verbal agnosia, is characterized by inability to read aloud, to understand written script and to name colors. Therefore, this is also called visual verbal color anomia.

1. Understanding spoken language, repetition of what is heard, writing spontaneously and to dictation, and conversation are all intact.
2. The striking feature is that capacity to write fluently is retained, but he cannot read what has been written.
3. This called alexia without agraphia.
4. Lesion usually affects left visual cortex geniculocalcarine tract and the connection of the visual cortex of the dominant hemisphere with the language areas.

**Pure Word Mutism**

The loss of capacity to speak while perfectly retaining the ability to write, to understand spoken words, to read silently with comprehension, and to repeat spoken words is called pure word mutism or aphemia. This is also called pure motor aphasia of Déjerine. This occurs due vascular lesion or localized injury to the dominant frontal lobe.

**Anomic Aphasia**

In anomic aphasia there is no difficulty with speech or understanding the auditory information, but there is difficulty in understanding written language or picture.

1. This occurs due to lesion of angular gyrus in categorical hemisphere without affecting language areas.
2. The visual information is not transmitted to Wernicke’s area.

**Transcortical Aphasia**

Isolation of language areas due to hypoxic injury as occurs in carbon monoxide poisoning or prolonged hypotension results in this syndrome.

1. Either the motor speech area or the sensory speech area is isolated from rest of the cortex in the same hemisphere.
2. Accordingly it results in transcortical motor aphasia (inability to initiate conversational speech with preservation of comprehension and repetition) or transcortical sensory aphasia (deficit auditory and visual word, but speech remain fluent and repetition is remarkably preserved).

## Other Language Disorders

### Agraphias

Agraphia is the *loss of previously possessed ability to communicate through writing*.

1. Though writing is an integral component of language function, many people speak without learning to read or write. Therefore, if someone has already learned writing, and then he loses this ability, agraphia is said to be present.
2. Pure agraphia is very rare.
3. There are different types of agraphias: aphasic agraphia, constructional agraphia, apraxic agraphia and linguistic agraphia (beyond the scope to discuss here).

### Disorder of Articulation

Impairment of articulation is called dysarthria. Articulation consists of contraction of pharynx, palate, tongue and lips that alter the vocal sounds.

1. In pure dysarthria, there is no abnormality of cortical language mechanisms.
2. They understand perfectly whatever is heard, and have no difficulty in reading and writing.
3. It is commonly due to neuromuscular problems.

### Disorder of Phonation

Aphonia is impairment of phonation. It occurs due to paralysis of vocal cords. Paralysis of respiratory movements can also produce aphonia because insufficient air is provided for phonation.
Association Cortex, Cerebral Asymmetry, Lobes of the Brain, and Cortical Plasticity

Learning Objectives
On completion of study of this chapter, the student will be able to:
1. Name the association cortical areas and list the functions of neocortex.
2. Understand the concept and importance of cerebral asymmetry (dominance)
3. Give the functions and dysfunctions of four lobes of the brain.
4. Appreciate cortical plasticity.

ASSOCIATION CORTEX (NEOCORTEX)
In evolution, the part of the brain that has grown maximally is the association cortex. As phylogenetically, it is the newest part of the cortex, it is also called neocortex. The association areas of the cortex are different from other cortical areas like sensory or motor cortices, as they yield no sensory evoked potentials or movements when electrically stimulated. However, they yield electrical responses to a variety of sensory stimuli.
1. They are named association cortex because originally they were thought to be associated with integration of sensory information from somatosensory cortex and relay the integrated information to the motor cortex. But, later, it was discovered that neocortex is the seat of all higher functions.
2. Neocortex is absent in fish, amphibians and reptiles, rudimentary in birds, poorly developed in non-human mammals and most developed in humans.
3. Intellect, personality, language and speech are mainly the functions of neocortex.
4. Learning and memory are also to a greater extent integrated in neocortex.
5. Though the brains of porpoise, elephant and the whale are larger than the brain of humans, the ratio of brain weight and body weight in human beings is much more than any other species. This indicates the enormous growth of cerebral cortex in human beings.

Fig. 140.1: Cortical association areas.

Functional Organization
There are four association cortical areas (Fig. 140.1):
Prefrontal association cortex, parietal-occipital-temporal association cortex, temporal association cortex and limbic association cortex.

Frontal or Prefrontal Association Cortex
This consists of rostral part of cerebral hemisphere in front of the premotor area.
Neurophysiology

Section 11: Neurophysiology

**Parietal-Temporal-Occipital Association Cortex**

This extends between the somatosensory and visual cortices above, and posterior portion of temporal cortex below.

**Temporal Association Cortex**

This extends from the lower portion of the temporal lobe to the limbic system.

**Limbic Association Cortex**

The cortical areas associated with limbic system are included in limbic association cortex.

**Feedback Control System of Neocortex**

The association cortices are part of the six-layered cortical areas of gray matter that spreads in the cerebral hemispheres (Fig. 140.2). The connections within the neocortex are a complex network of neurons. Like other cortical areas, such as motor cortex, there are pyramidal and nonpyramidal cells in association cortices.

1. A complex feedback control system exists within the neocortex.
2. The descending axons of pyramidal cell send collaterals that project via association neurons back to the dendrites of the cells from which they originate (Fig. 140.3). This forms the anatomical basis for feedback control.
3. The other collaterals connect the adjoining cells.
4. The dendrites of cells in the deeper layers receive inputs from thalamus, reticular formation, and other cortical areas.
5. Afferents from thalamus terminate mainly in layer IV of the cortex.

6. The neurons and their connections in the cortex have enormous ability to adapt, called cortical plasticity.

**Neocortical Functions**

Human being is more skilled in language and speech, and higher cognitive functions that include the ability to analyze a situation with proper reasoning and interact with the environment accordingly. Neocortex is highly developed in humans to integrate these higher intellectual functions.

**Language Functions**

The functions of language and speech in humans, i.e. to understand the spoken and written words and to express ideas in speech and writing are mainly the function of association cortex located in the perisylvian region or language areas (for details, refer previous chapter).

1. However, the language function is dependent more on one cerebral hemisphere than on the other. The hemisphere, which is concerned with language, is called as dominant hemisphere, and the other hemisphere as non-dominant hemisphere.
2. Due to its categorization in language function, the dominant hemisphere is better referred to as categorical hemisphere, and non-dominant hemisphere is known as representational hemisphere (for details, see below in Cerebral Asymmetry).

**Other Neocortical Functions**

Other higher functions include cognition, reasoning, recognition of faces, calculation and navigation.

**Recognition of Faces**

Recognition of faces is an important function as it is essential for social and emotional interaction. This is the function of representational hemisphere, which is usually the right hemisphere (described below).

1. Integration and storage of information regarding recognition of faces occur in the right inferior temporal...
lobe, that receives visual inputs from objects, particularly the visual impression of faces.
2. Lesion in this area results in prosopagnosia, the inability to recognize faces.
3. They can recognize known people by their voices, but not by seeing their faces.

Calculation
Two brain regions are involved in arithmetic calculations: (1) inferior part of the left frontal lobe, the area concerned with actual calculations, and (2) areas around the intraparietal sulci of the parietal lobes of both sides, areas concerned with memorizing numbers and finger counting. Lesion of frontal lobe results in acalculia (impairment of mathematical ability).

Navigation
Navigation means sense of direction-findings.
1. This is a special spatial skill. It is more developed in man than in woman. Men usually try to find out the directions themselves when they are lost, whereas women do seek others help for the same.
2. Right side hippocampus, which is concerned with learning, and the right caudate nucleus, which is concerned with movement are the areas involved in navigation. The actual sites and mechanisms for cognition and reasoning are not known.

Cerebral Asymmetry (Cerebral Dominance)

Concept of Cerebral Asymmetry

Two hemispheres serving discrete functions have been referred to as cerebral asymmetry. Especially, the functions of language and speech in humans are more localized in one cerebral hemisphere than in the other.
1. The hemisphere, which is concerned with language functions, has traditionally been called dominant hemisphere, and the opposite hemisphere as non-dominant hemisphere.
2. Rather, one hemisphere is categorized for language functions. However, this does not mean that the non-dominant hemisphere is less developed. Due to this categorization in language function, the dominant hemisphere is better referred to as categorical hemisphere.
3. The non-dominant hemisphere is rather focused in functions concerned more with visuospatial relations, i.e. recognizing faces, drawing pictures, identifying objects by their form, recognizing musical themes, etc. Therefore, non-dominant hemisphere is better referred to as non-categorical or representational hemisphere.
4. Thus, the categorical hemisphere, which is usually the left hemisphere, is specialized for the verbal and sequential processing of higher functions and the representational hemisphere, which is usually the right hemisphere, is specialized in nonverbal, nonmathematical and non-sequential processing of higher functions, mainly in visuospatial tasks such as observing faces.
5. Also, categorical hemisphere mediates mathematical learning and representational hemisphere mediates musical perception.

It is not only the language and visuospatial functions that exhibit preference for their location in one hemisphere, but also the other functions. For example, volitional control of movement is integrated more in left precentral motor cortex. Asymmetry exists also for cognitive and other higher functions in the cerebral cortex. This was observed by surgical section of corpus callosum (the split brain preparation), which was first undertaken to prevent spread of activation of opposite cortex during an epileptic fit. Studies in subjects with congenital absence of corpus callosum have also revealed the same. Roger Sperry, the founder of the split-brain preparation, noted in 1950s that “intellectual left hemisphere is highly evolved and a relatively retarded right hemisphere generally lack higher cognitive function”.

Relation with Handedness

Specialization of hemisphere is linked to handedness.
1. About 90% of human populations are right-handed. In 96% of right-handed individuals, the left hemisphere is the categorical or dominant hemisphere (only in 4%, the right hemisphere is dominant).
2. In left-handed individuals also the left hemisphere is the categorical hemisphere in 70% and right hemisphere is dominant only in 15% with no clear lateralization in rest 15%.
3. Therefore, the left hemisphere is often regarded as the dominant hemisphere (specialized in language functions) and the right hemisphere as nondominant hemisphere (specialized in visuospatial functions).
4. Thus, broadly, it is stated that the left hemisphere is meant for vocalization (speech) and right hemisphere for recognition of faces.
5. It has also been observed that language disorders are more common in left-handers, though they are more talented in spatial functions like music and art.

Anatomical and Biochemical Basis

The functional differences between the two hemispheres may be correlated with their anatomical variations.
1. The planum temporale, an area in the superior temporal gyrus involved in language-related auditory processing, is significantly larger in the left hemisphere.
2. It has also been confirmed by imaging studies that the upper surface of the left temporal lobe, the area primarily concerned with speech and language functions is larger in right-handed individuals.
3. In patients, the reduction in size of left superior temporal gyrus is correlated with the degree of disorders of thoughts and language.
4. There is also a biochemical differences between the two hemispheres, though the neurochemical basis of language and other cognitive functions is not clearly known. The dopamine concentration is high in the nigrostriatal pathway on the left side of basal ganglia in right-handed individuals.

**Physiological Significance**

The functional asymmetry perhaps is the ingenious way of economizing the brain tissue. By distributing important functions separately to two hemispheres, possibly nature has effectively doubled the capabilities of the brain for its given size.

**FUNCTIONS OF DIFFERENT LOBES OF THE BRAIN**

Each hemisphere of the brain consists of four lobes: Parietal lobe, temporal lobe, occipital lobe and frontal lobe (Fig. 140.4).

**Parietal Lobe**

**Location and Divisions**

Parietal lobe lies posterior to the central sulcus and rostrodorsal to lateral sulcus (sylvian fissure). It is divided into two regions:
1. The anterior region that mainly contains primary somatosensory cortex (SI), i.e. the Brodmann’s area 3, 1, and 2.
2. The posterior region that contains other sensory areas, which is considered to be the association sensory cortex. This includes the area 5 and 7 in the upper part, and area 39 and 40 in the lower part (Fig. 140.5).

**Afferent Connections**

Parietal association cortex is mainly involved in processing and integration of sensory informations. However, each area has separate connections and functions.
1. Areas 3, 1 and 2 constitute the primary sensory cortex (SI). It receives inputs from all sensory modalities through thalamus.
2. Area 5 receives inputs from cortex and thalamus. The cortical inputs come from SI, and thalamic inputs come from nucleus lateralis posterior. Cortical inputs provide somatosensory and vestibular informations.
3. **Area 7** also receives inputs from cortex and thalamus. The **cortical inputs** come from prestriate visual cortex, and **thalamic inputs** come from the pulvinar area. Cortical inputs provide somatosensory and vestibular informations.

4. **Inferior part of the parietal lobe** (area 39 and 40) receives inputs from superior temporal gyrus, prefrontal cortex (area 8, 45 and 46) and cingulate cortex (area 23 and 24).

### Efferent Connections

Unlike discrete afferent connections, efferent fibers have **common and mostly overlapping destinations** in the cortex, basal ganglia and subthalamus. Area 5 mainly projects to premotor and motor cortices that are involved in generation of somatic movements, area 7 projects to parahippocampal gyrus and subcortical areas that are destined for limbic structures.

### Functions

1. **Area 3, 1 and 2** are meant for **perception of sensations**, especially the cortical sensations (stereognosis, tactile localization and two-point discrimination), fine touch, proprioception and vibration (for details, refer Chapter 107, the ‘Sensory Cortex’).

2. **Area 5** is more involved in **processing of somatosensory information to produce movement**.

3. **Area 7** primarily processes **visual information** in order to produce not only movement, but also **arousal, attention and emotion**.

### Effects of Lesions

Lesion of parietal lobe results in following syndromes:

**Hemineglect**

Patient neglects **contralateral parts of the body and contralateral portion of the external world**. In extreme cases, they do not dress half their body, eat only from half of the plate or even read half of each page.

**Language Defects**

Patients with a left parietal lesion have **difficulty with the second half but not the first half of words**, both in writing and speaking. Some patients with parieto-occipital lesions write only with consonants and omit vowels.

**Motor Defect**

The most striking defect is inability to reach accurately for objects. **Lesion of area 5 causes deficit in tactile reaching**, and **lesion of area 7 causes deficit in visual reaching**.

**Sensory Deficits**

Loss of all cortical sensations resulting in astereognosis, and loss of tactile localization and two-point discrimination, and severe impairment of fine touch, vibration and proprioception, if SI is damaged. Pain, temperature and crude touch are least affected. If areas 5 and 7 are also involved, impairment occurs in learning and retention of tasks.

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**Deficit in Spatial Perception**

Motor and sensory deficits in posterior parietal lobe lesion (mainly area 5 and 7) are produced secondary to a defect in spatial perception. Brain receives sensory information but unable to integrate for spatial orientation that results in both inaccurate reaching and wrong interpretation of tactile cues. There will be **difficulty in dealing with three-dimensional space**. Bilateral parietal lesion causes **spatial disorientation** (patient can not find his way even in familiar surrounding).

**Attentional Deficit**

Motor and sensory deficits in posterior parietal lobe lesion may also be due to a defect in attention. Like hemineglect, there will be hemi-inattention. They ignore stimuli from contralateral side.

**Construcional Apraxia**

Bilateral parietal lesion, there is inability to copy an object or even a simple design. This is called constructional apraxia (apraxia means inability to perform organized movements in the absence of paralysis). In bilateral lesion, also there will be abolition of visual placing, though tactile placing will be retained.

**Temporal Lobe**

### Divisions and Functions

Temporal lobe is divided into **three regions**: Superior temporal gyrus, inferior temporal gyrus and mediobasal portion of temporal lobe.

**Superior Temporal Gyrus**

This consists of **Brodmann’s area 22, 41, and 42** (Fig. 140.5). This is primarily **concerned with audition** (Fig. 140.6). Areas 41 and 42 constitute primary auditory cortex.
that receives inputs from medial geniculate body (MGB) and projects to area 22, which is crucial for understanding speech. Area 22 receives input from prestriate visual areas and project to frontal lobe, parietal lobe and cingulate gyrus. Thus area 22 processes visually perceived words and symbols.

Inferior Temporal Gyrus
This consists of Brodmann’s area 20, 21, and 37. This is primarily concerned with vision. It receives input from prestriate visual areas, pulvinar area of thalamus and parietal lobe. It projects to prestriate visual areas, amygdala, entorhinal cortex and basal ganglia.

Mediobasal Portion of Temporal Lobe
It is mainly the anterior part of temporal lobe that consists of amygdala, hippocampus, other limbic structures, prepyriform cortex and other cortical areas concerned with olfaction. This part of temporal lobe is concerned with limbic functions and olfaction.

Effects of Lesions
The important deficits observed following lesion of temporal lobe are:

Prosopagnosia
This is the inability to recognize known faces, though the patient can identify people by hearing their voices. Even he may not identify his own face (seeing a mirror). This is not a perceptual deficit. The lesion involves inferior temporal lobe. Patient usually also have achromatopsia (cerebral color blindness).

Auditory Deficits
The deficit occurs mainly in auditory discrimination learning. Though, temporal lobe receives heavy inputs from MGB, the lesion in superior temporal gyrus produces deficits only in distinguishing between complex sounds (auditory tasks).

Visual Deficits
Lesion affecting inferior temporal lobe results in deficit in visual discrimination learning. Difficulty not only occurs for learning new visual discrimination tasks, but also the previously learned tasks are not remembered. Though visual perception is less affected, visual memory is significantly disturbed.

Memory Deficit
Lesion of left temporal lobe affects verbal memory and lesion of right temporal lobe affects nonverbal memory. Short-term memory depends on lateral temporal cortex. If hippocampus and amygdala are affected in temporal lobe lesion, deficit occurs in long-term memory.

Klüver-Bucy Syndrome
Removal of temporal lobe of both the sides results in Klüver-Bucy syndrome as first described by Klüver and Bucy in experimental animals. The animals are called Klüver-Bucy animals.

Frontal and Prefrontal Lobes

Divisions and Functions
The part of the cortex rostral to central sulcus (precentral sulcus) and medial to sylvian fissure is the frontal lobe. It is divided into three parts: precentral region, transitional region and prefrontal association cortex (Fig. 140.6).

Precentral Region
Precentral region contains primary motor cortex (area 4) and premotor cortex (area 6). These areas control movements, both skilled and postural.

Transitional Region
Transitional region is the area between precentral gyrus and prefrontal cortex. This includes area 44 and area 8. Area 44 is the motor speech area that controls motor activities of speech apparatus, and area 8 is the frontal eye field that controls eye movement.

Prefrontal Association Cortex (Prefrontal Lobe)
Prefrontal association cortex includes area 9–14. This is subdivided into orbital region (area 11–14 and 47), and dorsolateral region (area 9, 10, 45, and 46). Area 24 of prefrontal lobe (in cingulated gyrus) is a part of Papez circuit, which plays an important role in genesis and control of emotion.

Orbital region is connected with temporal lobe, olfactory cortex and limbic structures. The dorsolateral region receives inputs from various sensory modalities that include visual inputs from prestriate cortex and inferior
temporal gyrus, and auditory inputs from superior temporal gyrus. In fact, the prefrontal cortex provides powerful neocortical connections to basal forebrain structures including hypothalamus that are involved in control of visceral functions and emotional behaviors. Prefrontal cortex is the seat of human personality.

**Effects of Lesion**

Effects of lesions of frontal lobe are as follows:

**Motor Deficit**

Lesion of motor cortex results in **hypertonia at rest** and inability to react in term of past experience. Postural reflexes are preserved except hopping and placing reactions. Central feature of frontal lobe lesion is inability to perform a delayed response task and perseveration.

**Limbic Dysfunctions**

Limbic dysfunctions, especially emotional shallowness occurs in prefrontal lesion. There is also impairment of memory and learning capacity.

**Personality Changes**

Lack of initiative and inability to assess personal performance are striking changes in personality in frontal lobe lesion. Other features are tactlessness, change in social habits, impaired concentration, disturbance in judgment, and tendency toward irresponsible behavior.

**Prefrontal Lobotomy and Frontal Lobe Syndrome**

When the connection of thalamus with prefrontal lobe is cut (this is called prefrontal lobotomy), the major areas of frontal lobe (areas 8–12, 44–47) are disconnected. This is associated with frontal lobe syndrome, which is characterized by:

1. Difficulty in planning due to flight of ideas.
2. Sense of well-being (euphoria), and failure to understand the seriousness of others feeling.
3. Loss of recent memory with impairment of moral and social sense.
4. Loss of attention and ability to concentrate.
5. Decreased mental drive and lack of initiation.
7. May be associated with hyperphagia, and urinary and fecal incontinence.

**Occipital Lobe**

This is the area of the brain posterior to the parieto-occipital sulcus. It is mainly concerned with vision. It contains area 17 (the primary visual cortex), and 18 and 19 (the association visual cortex or higher order of visual cortex).

**CORTICAL PLASTICITY**

The basic element of the nervous system is the neuron. The neural connections are determined genetically, and once established, remain almost throughout life. However, in some special circumstances, synaptic connections can be modified in CNS, especially in the brain. This ability to modify is referred to as cortical plasticity, or more broadly the neural plasticity. But, in what situations and by what mechanism do the neurons modify?

There are three known major circumstances of neural plasticity: (1) postnatal modifications, (2) changes as a consequence to injury to the brain, and (3) the plasticity during learning or experience.

**Postnatal Development**

Postnatal modifications of neuronal connectivity occur as a result of interactions with the environment. Environmental effects affect development and alteration of many neural connections. During this early phase of development, many neural changes occur, changes in cellular, axonal and synaptic parts of the neuron. Number of synapses increases during development until maximum levels are gradually reached in early adulthood.

**Critical period:** The developmental neuropsychology exhibit an early critical period of development during which neuronal connections are sensitive or plastic to environmental influences. After this critical period, they are not sensitive to change.

**Response to Injury in Adult Brain**

In poikilothermic vertebrates, axons in the central nervous system if damaged, show enormous capacity to regenerate. The regenerated connections function nearly normal. In homeothermic vertebrates, regeneration mainly occurs in the peripheral nervous system. Following injury to central nervous system, the regeneration is incomplete. However, significant modifications do occur.

1. For example, the phenomenon of axonal sprouting that occurs following spinal transection. Axonal sprouting also occurs in other structures in central nervous system, including hippocampus, septum, cerebellum and red nucleus.
2. In some cases, the new connections have indeed formed functional connections. For example, the cerebellar projection to red nucleus is preferentially to the soma and proximal dendrites of the neurons, whereas the cortical projection to red nucleus primarily ends on distal dendrites.
3. If the cerebellar efferent fibers to red nucleus are damaged, then the cortical projection fibers sprout to establish more proximal connections at the synaptic sites that have been vacated by degeneration of the cerebellorubral fibers. These new connections increase the influence of the motor cortex on the red nucleus and contribute to the recovery of motor function. This is the best example of cortical plasticity.
4. However, not all pathways are equally capable of sprouting.

**Learning and Memory**
The capacity of certain neurons to change their behavior as a function of environmental inputs is a very important property of the nervous system. This serves the basis of learning and adaptive behaviors.

1. **Learning** refers to a process of change in behavior as a result of experience.
2. **Memory** is the storage of information of the experience-induced modification. The storage of information involves actual physical changes in neurons. Change in synaptic connections and cellular changes are the bases of learning and memory. This is another example of cortical plasticity.

### CHAPTER SUMMARY

#### Key Concepts
1. Neocortex is more specialized in language and cognitive functions.
2. Dominant (categorical) hemisphere is associated with language function, and nondominant representational hemisphere with visuospatial relations.

#### Important to Know (Must Read)
1. Usually, Long Questions are not asked from this chapter.
2. Neocortex, Cerebral asymmetry or dominance, Functions of any one lobe of the brain, Cortical plasticity may come as Short Questions.
3. In Viva, examiner may ask… What are the parts of neocortex, Neocortical functions, Concept of cerebral asymmetry, What is the meaning of dominant and nondominant hemisphere, Cerebral asymmetry or dominance, Functions of all the lobes of the brain, Effects of lesion of all the lobes of the brain, Mechanism of cortical plasticity.
Cerebrospinal fluid (CSF) is a colorless liquid that fills the ventricles of the brain and the subarachnoid space that surrounds the brain and spinal cord. CSF has many functions as follows:

1. By occupying the subarachnoid space, CSF forms a thin layer of water jacket for brain and spinal cord, which protects them from mechanical injury.
2. It also provides microenvironment for the cells in the brain.
3. CSF regulates brain metabolism.
4. As there are no lymphatic channels in brain and spinal cord, CSF removes waste products of metabolism, especially H⁺, lactate and CO₂ through its sink action.
5. However, for a neurophysician, CSF is more important for diagnosis of diseases of CNS as it reflects the nature of pathology in a wide variety of disorders of brain and spinal cord like inflammatory, infective, metabolic, neoplastic, demyelinating, etc.
6. Therefore, analysis of CSF is an essential part of investigations for many neurological disorders.

Scientist contributed

Domenico Cotugno (1736–1822), Italian physician for the first time scientifically described the clinical application of cerebrospinal fluid. He also demonstrated the aural canals and labinthine fluid, and had worked on cerebral embolism.

FUNCTIONAL ANATOMY

Ventricular System and Subarachnoid Space

Ventricular System
There are four ventricles in the brain. Two lateral ventricles, a third ventricle and a fourth ventricle.

1. Lateral ventricles are the largest ones that are located symmetrically in the cerebral hemispheres (Figs. 141.1A and B).
2. The third ventricle is located in the diencephalons, and the fourth ventricle is enclosed in the pons and medulla.
3. The two lateral ventricles communicate with the third ventricle through two independent openings called the foramina of Monro. The third ventricle communicates with fourth ventricle through a narrow passage called the aqueduct of Sylvius.
4. The fourth ventricle narrows down caudally as it enters the spinal cord that continues as the central canal in the cord.
5. Each ventricle contains a bunch of epithelial-vascular tissue known as the choroid plexus.

Subarachnoid Space
The brain and spinal cord are covered by three layers of the connective tissues, called meninges. The innermost layer, which is in close contact with the brain tissue, is called the pia mater. Next to pia mater is the arachnoid...
mater and outermost layer is the dura mater, which is in close contact with the inner wall of the skull. There are two potential spaces between these layers. The space between the pia mater and arachnoid mater is called the subarachnoid space, and the space between the arachnoid mater and the dura mater is the subdural space.

1. The subarachnoid space is enlarged at few places to form cisterns.
2. There are four major cisterns and many minor cisterns.
3. The major cisterns are: (1) Superior cistern, located dorsal to midbrain, (2) Cerebellomedullary cistern, located between cerebellum and medulla (this is a large one, therefore also called cisterna magna), (3) Pontine cistern, located near pons, and (4) Interpeduncular cistern, located at the cerebral peduncles.
4. The minor cisterns are cistern of the optic disk, cistern of lamina terminalis, cistern of corpus callosum, perimesencephalic cisterns, cistern of lateral sulcus and the lumbar cistern. The lumbar cistern is the large spinal subarachnoid space extending between L2 and S2 vertebra (Clinical Box 141.1).

FORMATIONS, CIRCULATION AND ABSORPTION OF CSF

Formation of CSF

Choroid plexuses, located in the floor of lateral, third and fourth ventricles, are the major source of CSF. However, CSF continues to be formed even after choroid plexuses are removed. The other sources of CSF are the blood vessels of subependymal regions and pia.

Rate of Formation

In an adult, the intracranial volume is about 1700 ml, of which brain measures 1200–1400 ml, CSF accounts for 70–160 ml, and blood in cerebral vessels is about 150 ml. Thus, CSF occupies less than 10% of the volume of total intracranial space. The rate of formation of CSF is about 0.35 mL/min or, 20 mL/hour or, 500 mL/day. As a whole, CSF is totally replaced four to five times daily.

Mechanism of Formation

CSF is formed by a combination of passive diffusion, active transport and facilitated diffusion.

1. Passive diffusion: The thin-walled vessels of choroid plexuses allow passive diffusion of substances from blood plasma into extracellular space surrounding choroidal cells.
2. Active transport: The choroid epithelium secretes sodium ions by active transport. This is achieved by Na⁺-K⁺ pump operating in the apical surface of the plexus that pumps Na⁺ into the ventricular cavity.
Chloride ions move into the CSF passively in response to electrical gradient generated by Na\(^+\) movement. Cl\(^-\)–K\(^+\) exchanger also exchanges chloride for K\(^+\) in the apical membrane. The sodium and chloride ions create the osmotic gradient for flow of water into the ventricles. In the basal surface, chloride is exchanged for bicarbonate, and H\(^+\) is exchanged for Na\(^+\). Secretion of bicarbonate is essential to maintain pH of brain ECF by neutralizing the acidity created by secretion of H\(^+\).

3. **Facilitated diffusion**: Penetration of substances into brain is directly proportional to their lipid solubility. Ionized compounds like hexoses and amino acids are relatively insoluble in lipid. They enter by facilitated diffusion, which is stereospecific (carrier binds only with a solute having specific configuration).

**Steps of Formation**

CSF formation occurs in two steps: ultrafiltration and active secretion.

1. **Ultrafiltration** of plasma occurs across the fenestrated capillary wall into the ECF that bathes the basal surface of epithelial cells of choroid plexus.
2. Choroidal epithelial cells transport ions and solutes into CSF, mainly by active secretion.

**Composition of CSF**

As a result of the above-mentioned transport mechanisms, osmolality and concentration of sodium ion in CSF is equal with that of plasma, whereas chloride ion concentration is higher, and potassium and bicarbonate ion concentration is lower in CSF than in plasma (Table 141.1). The concentration of glucose and protein in CSF is lower than that of plasma.

**Circulation and Absorption of CSF**

**Circulation of CSF**

Harvey Cushing described circulation of CSF as the third circulation in the body (first is blood and second is lymphatic).

1. The CSF is mainly formed in the lateral ventricle, from where it flows downward into the third ventricle through foramen of Monro, and from the third ventricle into the fourth ventricle though aqueduct of Sylvius.
2. Finally, CSF comes out of fourth ventricle through the foramen of Magendie and Luschka to enter the subarachnoid space.
3. In the subarachnoid space, CSF moves upward toward the cerebral hemispheres and downward toward the spinal cord (Fig. 141.3).
4. Thus, obstruction of foramen of Monro results in distension of lateral ventricles, occlusion of aqueduct of Sylvius causes distension of third ventricle, and blockage of foramina of Magendie and Luschka initially distends fourth ventricle and later the entire ventricular system is distended.

**Absorption of CSF**

Absorption of CSF occurs through the arachnoid villi. Arachnoid villi project into the subdural venous sinuses. The main factor that facilitates the movement of the fluid is the oncotic pressure (colloid osmotic pressure of plasma, which is 25 mm Hg) that is higher than that of CSF because CSF has very less proteins. The other factor promoting this mechanism is the hydrostatic pressures of CSF, which is about 0.5–5 mm Hg higher than that in the subdural venous sinuses.

**CSF Pressure**

In recumbent position, the normal intracranial pressure (ICP), which reflects the CSF pressure as measured by lumbar puncture is normally about 8 mm Hg or 110 mm H\(_2\)O.

1. However, in the standing posture, ICP is less (may be close to zero) and CSF pressure is more as the column...
of CSF added incrementally to the pressure in the lumbar subarachnoid space. Therefore, CSF pressure is recorded in recumbent posture.

2. It is important to note that in normal conditions, the major determinant of CSF pressure is the cerebral venous pressure, not the resistance to CSF outflow. CSF pressure is in equilibrium with capillary or prevenous pressure.

3. Increase in arterial pressure has little effect on capillary pressure due to autoregulation and, therefore, does not significantly increase CSF pressure.

4. In contrast, increase in venous pressure exerts immediate effect on CSF pressure by increasing pressure in intracerebral veins, venules and dural sinuses.

5. This forms the physiological basis for Queckenstedt test (see below). CSF pressure increases in any space occupying lesion of skull cavity.

**Functions of CSF**

1. **Protection from mechanical injury**: A major function of CSF is to protect brain from mechanical injury. Brain floats in CSF, and, therefore, the weight of the brain is decreased to about 50 g from its actual weight of 1400 g due to the effect of buoyancy. The buoyancy is due to the difference in specific gravity of brain (1.040) and CSF (1.007). Due to higher specific gravity, brain floats freely in CSF rather than resting heavily on the skull box. Thus, the risk of routine acceleration-deceleration injuries is eliminated and also the impact of major injuries is greatly diminished (Clinical Box 141.2).

2. **Provides microenvironment for brain cells**: Brain is metabolically fragile. Neurons in the brain are highly sensitive to changes in oxygen, glucose, pH, temperature, etc. in their external environment. However, the CSF ensures constancy in the external environment of neurons. CSF accomplishes this by buffering the changes in blood on one side with the brain interstitial fluid on the other.

3. **Role in homeostasis**: CSF is indirectly involved in regulation of respiration, blood pressure, water intake and visceral function by bringing about the chemical changes like hydrogen ion concentration (pH), osmolality, etc. in cerebral interstitial fluid. The changes in blood PO₂, PCO₂ and pH are transmitted to chemosensitive respiratory neurons and central chemoreceptors via CSF for appropriate homeostatic responses.

4. **Removal of proteins and waste products**: There are no lymphatic channels in brain and spinal cord. CSF removes proteins and waste products of metabolism, especially H⁺, lactate and CO₂ through its sink action. In the brain, small amount of protein that leaks into the interstitial fluid is drained by the CSF and returned to the blood stream. Thus, the CSF serves the function of lymphatics in brain.

**Clinical Box 141.2**

**Contrecoup phenomenon**: When the buoyancy effect of CSF is not adequate to protect, the brain gets injured following a hit. Surprisingly, the brain injury occurs on the opposite side of hit. This is called as the contrecoup injury. This happens because skull moves with the head along the direction of hit but the brain lags behind slightly due to its inertia. A vacuum is created between the brain and the skull. When skull comes to rest, the side of the brain opposite the blow hits the skull due to the combined effect of inertia and vacuum.

- Provides microenvironment for brain cells: Brain is metabolically fragile. Neurons in the brain are highly sensitive to changes in oxygen, glucose, pH, temperature, etc. in their external environment. However, the CSF ensures constancy in the external environment of neurons. CSF accomplishes this by buffering the changes in blood on one side with the brain interstitial fluid on the other.

- Role in homeostasis: CSF is indirectly involved in regulation of respiration, blood pressure, water intake and visceral function by bringing about the chemical changes like hydrogen ion concentration (pH), osmolality, etc. in cerebral interstitial fluid. The changes in blood PO₂, PCO₂ and pH are transmitted to chemosensitive respiratory neurons and central chemoreceptors via CSF for appropriate homeostatic responses.

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**Applied Physiology**

**Lumbar Puncture**

Sampling of CSF by lumbar puncture (LP) is an important diagnostic tool in neurology. Composition (cells, protein, glucose, etc.) color and pressure of CSF provide an important information about the nature of brain pathology. In adults, LP is performed by inserting a needle preferably through the interspace between L₁ and L₂ or, L₂ and L₃ into the spinal subarachnoid space, below the end of the spinal cord.
polymorphs are more, in tubercular meningitis lymphocytes are more (cob-web appearance due to increased fibrin clot) and in viral meningitis moderate increase occurs in lymphocytes (but no decrease in glucose). The culture of CSF confirms the nature of the infection. Cerebral hemorrhage: CSF is bloody or yellow in appearance (with red cells) and protein is raised. Encephalitis: CSF is usually clear. Lymphocyte and protein are high. Brain tumor: CSF is clear. It may be associated with mild increase in lymphocyte and protein. Poliomyelitis: Clear and colorless CSF. Significant increase in lymphocytes occurs with moderate increase in protein. Brain abscess: Clear or slightly turbid CSF. Normal lymphocyte count (0–5 cells). Mild increase in protein may occur. Spinal cord tumor: Clear or yellow CSF. Significant increase in protein may be a feature.

Queckenstedt Test
During lumbar puncture, if the needle is connected to a manometer it records CSF pressure. If the patient is lying on his side, the pressure ranges from 100 to 180 cm H₂O or 7 to 13 mm Hg. Queckenstedt test is performed to demonstrate spinal subarachnoid block, if present. For this test, the patient in lying posture (on his side), the jugular veins are pressed to see the pattern of rise in CSF pressure. Compression of jugular vein causes an immediate rise in intracranial CSF pressure, which is rapidly transmitted to the lumbar subarachnoid space, unless there is spinal subarachnoid block. Normally, the rise is rapid up to 100 to 200 mm H₂O, and the pressure returns to normal within 10 seconds following release of compression. If the spinal subarachnoid block is present, the rise and fall are slow or absent.

Hydrocephalus
Increase in the volume of CSF resulting in enlargement of cerebral ventricles is called hydrocephalus. Intracranial CSF pressure is usually high. Hydrocephalus is usually divided into two categories: communicating and noncommunicating.

Communicating Hydrocephalus
Hydrocephalus due to impaired absorption of CSF is called communicating type, because the communication between the ventricles and subarachnoid space is intact. 1. This is also called normal pressure hydrocephalus, which is a misnomer as the ICP is often intermittently elevated. 2. Communicating hydrocephalus occurs due to damage to arachnoid villi, which commonly happens following infection or inflammation of meninges.

Non-communicating Hydrocephalus
Hydrocephalus occurs due to obstruction of the foramen that drain CSF.

1. The ICP is usually very high in this type of hydrocephalus. Therefore, this is also called obstructive hydrocephalus or high-pressure hydrocephalus. 2. The manifestation depends on the site of obstruction. 3. Obstruction of foramen of Monro results in distension of lateral ventricles, obstruction of aqueduct of Sylvius causes distension of third and lateral ventricles, and obstruction of foramina of Magendie and Luschka initially distends fourth ventricle but later enlarges entire ventricular system. 4. However, the usual site of obstruction is aqueduct of Sylvius.

Effects of Increased CSF Pressure
The cranium is a rigid cavity. Therefore, increase in any one of the three elements in cranial cavity, i.e. brain tissue, blood and CSF occurs at the expense of the other two. This is called the Monro-Kellie doctrine. The chronic rise in CSF pressure compresses and damages the brain.

1. Any space-occupying lesion of cranial cavity like brain tumor increases ICP. Raised ICP/CSF pressure can be detected during ophthalmoscopic examination of the fundus of the eye. The usual finding is the papilledema, and engorgement and edema around the optic disk. This occurs because subarachnoid space extends along the optic nerve. 2. When the CSF pressure is raised, the pressure in the optic extension is also raised that compresses the optic nerve and blood vessels. Especially, venous compression decreases venous drainage from the optic disc. This causes venous and capillary engorgement around the disk. The increased capillary pressure causes papilledema (Clinical Box 141.3).

Clinical Importance
If the ICP/CSF pressure is already raised, lumbar puncture should be avoided as it may be life-threatening.

1. This is, because, CSF comes out rapidly when it is under high pressure through lumbar puncture needle, which suddenly releases pressure in cranial cavity. 2. This may suck cerebellum and medulla into the foramen magnum. 3. The herniation of cerebellum and medulla, the phenomenon also called as coning, damages the vital centers (cardiovascular and respiratory centers) in the medulla leading to sudden cardio-respiratory arrest.

Clinical Box 141.3

Fundus examination is a must in increased ICP: Whenever increased ICP is suspected, fundus examination must be performed to ensure that no papilledema is present before the lumbar puncture is performed. Otherwise, coning may cause sudden cardiorespiratory depression.
A special barrier that exists between the blood and the brain tissue is the blood-brain barrier (BBB). This was first demonstrated by Paul Ehrlich in 1885. He injected aniline dyes i.v. and found that almost all tissues of the body except the brain and spinal cord are stained. A similar barrier exists between the blood and CSF, the blood CSF barrier. Neurons are most sensitive to harmful substances that circulate in blood. However, BBB protects the neurons in the brain from these harmful agents. The BBB selectively allows small molecules, uncharged molecules and lipid soluble substances to enter the brain with ease, whereas it does not allow large molecules like proteins, water-soluble substances and substances bound to plasma proteins.

**Anatomical Basis of BBB**

BBB is formed by two special structures (Fig. 141.4):

1. In the endothelium of capillaries of the brain, cells are joined by tight junctions. This significantly decreases the permeability of the capillaries.
2. The capillaries are surrounded by the foot processes of astroglia. These glial processes form a complete sheath around the capillaries.

The blood CSF barrier resides in the choroid plexus. The capillaries of the choroid plexuses also have tight junctions between the endothelial cells. Therefore, they are very selective and they have low permeability that allows only selected substances to be secreted into the CSF.

**Circumventricular Organs**

Normally, BBB is deficient in some regions of the brain that are collectively called as circumventricular organs (Fig. 141.5). Thus, they remain outside BBB. The important circumventricular organs are:

1. Organum vasculosum of lamina terminalis (OVLT)
2. Subfornical organ (SFO)
3. Area postrema in medulla oblongata
4. Posterior pituitary and median eminence (ventral part) of hypothalamus
5. The pineal gland

**Functions of BBB**

1. Neurons depend on a normal concentration of various ions in the fluid bathing them, especially Na⁺, K⁺, Ca²⁺, H⁺ and Mg²⁺. Alteration in these ion concentrations in ECF of brain tissue results in severe consequences. BBB maintains constancy of these ions in brain fluids.
2. Many toxins either produced endogenously or administered exogenously circulate in blood. These toxins are harmful to the neurons that are highly sensitive. BBB protects neurons from these harmful substances.
3. BBB prevents escape of neurotransmitters from brain into circulation.
4. Disruption of BBB helps in identifying the location and extent of lesions in the brain.
5. BBB influences drug permeability into the brain.

**Clinical Importance**

**Kernicterus**

BBB is not fully developed during infancy and early childhood. Bilirubin has special affinity for basal ganglia. Therefore, if there is hyperbilirubinemia during infancy, as occurs in hemolytic diseases of newborn like erythroblastosis fetalis, free bilirubin enters the brain and deposited in basal ganglia. The condition is known as kernicterus.

**Brain Tumor**

BBB is absent at the site of a brain tumor. This happens because of angiogenesis in tumors. New capillaries that develop in the tumor area do not have contact with astrocytes. This anatomical defect aids in diagnosis of the
tumor. For diagnosis of brain tumor, brain is scanned for radioactivity following injection of a radioactive substance like radioiodinated serum albumin. As the BBB is deficient at the site of brain tumor, radioactivity appears only in tumor area. This helps in confirming the diagnosis and locating the tumor.

**Infection and Injury**

BBB breaks down in the regions of infection or injury. Therefore, antibiotics that do not cross BBB in normal individuals penetrate brain tissue easily in patients with infection or inflammation. This helps in appropriate use of medicines in treatment of the disease. This is the nature’s own defense mechanism that whenever natural integrity of brain is interfered by infection the repair process is also automatically triggered by making access to the medicine that heals it.

**Use of Hypertonic Solution**

BBB is temporarily weakened by exposing the cerebral capillaries to hypertonic solutions. The hypertonic fluid causes shrinking of the endothelial cells, which in turn makes the tight junctions leaky (less tight). This property of BBB is exploited in treatment of brain diseases. Normally, many drugs do not cross BBB easily. Therefore, just before administration of drugs that are aimed to penetrate brain tissue, a hypertonic solution is injected into the carotid artery. This weakens the BBB and helps in achieving proper concentration of the drug in brain.

**CSF Brain Barrier**

There is no CSF brain barrier because the ependymal cells that line the ventricles and the pia mater are highly permeable. Therefore, when a drug or an anesthetic agent is injected into the CSF by lumbar puncture can easily reach the brain and spinal cord.

**CHAPTER SUMMARY**

**Key Concepts**

1. CSF provides mechanical and metabolic protection to the brain.
2. Examination of SCF provides vital clue regarding the nature of infection or the disease in the brain.

**Important to Know (Must Read)**

1. Describe the mechanism of formation, route of circulation, functions and clinical application of CSF may come as Long Questions.
2. CSE, Mechanism of CSF formation, Functions of CSF, Blood-brain barrier, Blood-CSF barrier may come as Short Questions.
3. In Viva, examiner may ask… What is the composition and functions of CSF, How is CSF formed and circulated, Structure and importance of blood-brain barrier and blood-CSF barrier, What is normal CSF pressure, What are the effects of raised CSF, Name the structures outside BBB.
Special Senses

**Part A: Vision**
142. Functional Anatomy of Eye
143. Image-forming Mechanism
144. Photoreceptor Mechanism
145. Visual Pathway and Visual Cortex
146. Visual Acuity, Visual Field, Light and Dark Adaptations, and Visual Reflexes
147. Color Vision
148. Movements of the Eye

**Part B: Hearing**
149. Functional Anatomy and Functions of the Ear
150. Auditory Pathways
151. Mechanism of Hearing
152. Hearing Defects and Hearing Tests

**Part C: Smell and Taste**
153. Physiology of Smell
154. Physiology of Taste
“I am the inviolable Ecstasy;
Those who have looked on me, shall grieve no more.
The eyes that live in night shall see my form”

Sri Aurobindo (in ‘SAVITRI’)
Eyes are one of the most essential sense organs gifted by the nature to the living species. For the animals to get food, escape from the predator, be aware of far and near objects and find a mate, eyes play a very vital role. In human beings, eyes are not only a means to behold the beauty of the creation, but it helps in recognizing faces of the loved ones that strengthens the emotional and social bond. Vision is possible in presence of light. Accordingly, the eye has two major parts:

i. An **optical system** that helps to **focus and form an image on the receptor cells** when light rays being reflected from an object fall on the eye; and

ii. A **neural system** that **transmits the optical signal in the form of action potentials along the optic nerve to the visual cortex** to be perceived as visual sensation.

**FUNCTIONAL ANATOMY**

The eyeball is a fluid filled spherical organ with a diameter of 24 mm. The optical systems are present in its anterior part and the visual receptors are placed at the posterior surface, from which the optic nerve arises and proceeds towards the occipital cortex. Each eyeball is surrounded by a cushion of fat and is placed within a bony cavity called orbit. A cross section through the human eye (Fig. 142.1) shows that the eyeball consists of three concentric layers housing the optical system:

1. **The outermost layer** is transparent in its anterior part called **cornea** and the rest opaque area is known as **sclera**.

2. The **middle** vascular and pigmented uveal tract forms **choroid** and **ciliary body** in its posterior part and **iris** in its anterior part.

3. The **innermost layer** is called **retina**, which contains the photoreceptors in association with a neuronal network that is absent in the anterior segment.

The interior of the eyeball is divided into **three spaces**, **anterior chamber**, **posterior chamber** and the **posterior**
cavity. The fluid present in the anterior cavity (in the anterior and posterior chambers) is called aqueous humor and the posterior cavity contains vitreous humor.

Sclera and Cornea

Sclera

As we look at the eye, part of the sclera is seen as white area surrounding central dark region.
1. The sclera is a white tough avascular fibrous coat composed of collagen fibers with little elastic tissue in adult.
2. It provides protection for the contents of the eyeball.
3. The extraocular muscles regulating the eyeball movements in different directions are inserted on the sclera.
4. It is covered by a thin layer of mucous membrane called bulbar conjunctiva. The mucous membrane extends to the inner surface of the eyelids and is known as palpebral conjunctiva.
5. Inflammation of the conjunctiva is called conjunctivitis that occurs due to infection or allergy with symptoms of redness of the eyes and discharge.

Cornea

The anterior one-sixth of the sclera, which is transparent and more convex is called cornea. It is supplied by the sensory nerve endings of the trigeminal nerve. Light rays enter the eyeball through the cornea. The curvature of the cornea contributes to most of the refractive power of the eye. The sclero-corneal junction is known as limbus. The cornea is an avascular tissue (Application Box 142.1) composed of five layers. From anterior to posterior, they are: epithelium, Bowman’s membrane, stroma, Descemet’s membrane and endothelium (Fig. 142.2):

i. Epithelium: Anteriorly, the cornea is covered by a stratified squamous epithelium that is thin, transparent and continuous with the bulbar conjunctiva. The epithelium is highly proliferative in nature. If damaged, it can regenerate and heal rapidly. The complete turnover of the epithelium occurs in about a week.

ii. Bowman’s membrane: This is the basement membrane of the epithelium. If damaged, the Bowman’s membrane heals with a scar resulting in blurred vision.

iii. Stroma or substantia propria: It consists of highly structured thin collagen fibrils in a mucopolysaccharide matrix. The fibrils are of equal diameter, spaced at regular intervals and form a hexagonal lattice that confers transparency to the cornea. The stroma constitutes 90 percent of the corneal thickness.

iv. Descemet’s membrane: It is a thin elastic membrane lining the posterior part of stroma. The Descemet’s membrane is resistant to infection and can regenerate if damaged.

v. Endothelium: It consists of a single layer of leaky endothelial cells and covers the posterior surface of Descemet’s membrane. The endothelium prevents the excess hydration of the cornea. It removes the electrolytes and water flows passively out of the stroma.

Application Box 142.1

Corneal transplant is usually successful: Inflammation of the cornea is known as keratitis that may result in corneal ulcer. Corneal opacities may occur due to infection or injury. In these cases if the vision is grossly impaired, corneal transplantation shows encouraging result. As the cornea is avascular, blood-borne antibodies cannot reach the grafted tissue to cause transplant rejection. Therefore, it is one of the most common and most successful transplant operations.

Lacrimal Gland:

1. The lacrimal gland present at the lateral corner of the eye secretes a complex liquid known as tear that keeps the cornea moistened. When the eyes are open, this allows oxygen to diffuse from the atmosphere to the corneal cells, as they do not have blood supply.
2. The tears flush away the foreign particles and their lysozymes and antibodies prevent infection.
3. It is drained through the naso-lacrimal duct located at the medial corner of the eye.
4. In the absence of emotional or external stimuli, blinking helps in spreading of the film of tear over cornea and keep it moistened. Some amount of water from the tears evaporates through the corneal epithelium to air.
5. Partial dehydration of the cornea is necessary for its transparency but a totally dry cornea soon loses its transparency and a hydrated cornea becomes chalky white. Thus, precise regulation of the stromal water content is essential for the clarity of vision.

Choroid, Ciliary Body and Iris

Choroid

1. The choroid lines the inner aspect of sclera. It contains many blood vessels that provide nourishment to the structures of the eye. The choroidal blood vessels increase in size from within outwards.
2. The membrane of Bruch lines the inner side of the choroid and separates it from the retina. Beneath this membrane, the choroidal blood vessels divide to form capillaries that supply nutrition to the outer portion of the retina by diffusion. The space between the choroid and sclera is called supra- or epichoroidal space.

3. The blood vessels of choroid are bound together by connective tissue containing pigmented cells called chromatophores. In albinism, pigments may be congenitally absent in the choroid.

4. The sensory supply to the choroid is from the trigeminal nerve fibers and autonomic neurons have vasomotor function.

5. Inflammation of the choroid is known as choroiditis that invariably involves the retina causing chorioretinitis.

Ciliary Body

The ciliary body is present between the iris and the choroid. It is a triangular structure with its base directed forwards and medially and its apex is continuous with the choroid posteriorly. The iris arises from the middle of the base and projects medially.

**Parts of Ciliary Body:** The ciliary body consists of two parts: the ciliary muscle on the outer side and the ciliary processes at the inner side:

i. The ciliary muscle is made up of circular and radial multiunit smooth muscle fibers that are supplied by the ciliary ganglion, which in turn gets activated by the Edinger Westphal nucleus of the oculomotor nerve.

ii. The ciliary body attaches to the peripheral ends of the zonule (suspensory ligaments) that hold the lens in position (see Fig. 142.1). The contraction of the ciliary muscles pulls the zonule medially causing relaxation of the ligaments and the lens becomes more convex.

iii. From the inner aspect of the ciliary body several fine projections arise, called ciliary processes that are lined by ciliary epithelium and contain many capillaries interspersed with connective tissues.

iv. The ciliary processes secrete aqueous humor. The ciliary body extends backwards up to ora serrata, the point where the retina begins.

v. The inflammation of the ciliary body is known as cyclitis that invariably involves the iris causing iridocyclitis.

Iris

1. Projecting from the ciliary body, iris is a thin, pigmented, circular, contractile diaphragm that hangs over the anterior surface of the lens.

2. The iris has a central aperture, the pupil, through which light rays enter into the eye.

3. The iris along with the anterior margin of the lens separates the anterior from the posterior chamber of the eyeball.

4. Inflammation of iris is called iritis.

5. From anterior to posterior, the iris shows three layers. They are: (i) endothelium, (ii) stroma and muscles and (iii) epithelium.
increases. The sympathetic fibers from the cervical sympathetic chain supply the radial muscles (dilator pupillae) that are arranged radially near the root of the iris. Sympathetic stimulation produces contraction of radial muscles that causes pupillary dilation (mydriasis) letting more amount of light in.

iii. Epithelium: The posterior surface of the iris is covered by a double layer of pigmented epithelium.

Intrinsic Muscles of Eye

The ciliary and iris muscles are known as the intrinsic muscles of the eye.

Aqueous Humor, Lens and Vitreous Humors

Aqueous Humor

1. The aqueous humor is a protein-free clear fluid from which the lens and cornea derive their metabolic requirements.
2. In the ciliary processes, it is continuously formed from plasma by the mechanism of ultrafiltration, diffusion and active transport and a complete turnover occurs in about an hour. Its composition is similar to that of plasma, but lacks in proteins and has higher concentrations of ascorbic acid.
3. The ciliary processes form aqueous humor and pour it into the posterior chamber, from where it flows through the pupil into the anterior chamber (Fig. 142.4).

Posterior Chamber: It is a triangular space enclosed anteriorly by the back of the iris and posteriorly by the anterior surface of the lens. Laterally, its base is formed by the ciliary body. Medially the apex is situated at the point where the pupillary margin comes in contact with the lens.

Anterior Chamber: It is bounded anteriorly by the posterior surface of the cornea; and posteriorly by the anterior surface of the iris and the anterior surface of the lens that is not covered by the iris.

4. Both these chambers are filled with aqueous humor. The aqueous pressure is about 15–18 mm Hg higher than the intracranial pressure. This pressure helps to maintain the shape of the eye and keeps the vitreous humor pressurized.
5. From the anterior chamber, the aqueous humor gets absorbed by the spongy meshwork of trabecular tissue and is drained into the canal of Schlemm, a venous channel at the angle of anterior chamber (junction between the iris and the cornea).
6. Decreased absorption of aqueous humor leading to rise in intraocular pressure can occur due to reduced permeability through the trabecular tissue (open-angle glaucoma) or due to obstruction of the passage by anterior displacement of iris (closed-angle glaucoma).

The Lens

1. The lens is a biconvex transparent avascular highly elastic structure held in place behind the iris and in front of vitreous body by the suspensory ligaments.
2. The main function of the lens is to converge the light rays and focus them on the retina.
3. The crystalline lens develops from the ectoderm. It is enclosed in a thin tough transparent elastic capsule, formed from the secretion of epithelial cells.
4. It is composed of transparent collagen fibrils, which are arranged in layers like that of an onion. The cells of the lens are rich in the protein α-crystalline that increase the density and enhance the focusing power of the lens.
5. The glucose present in the aqueous humor (60 mg%) is the principal source of energy for the lens. The capsule of the lens is semipermeable that allows the nutrients to diffuse through it. The lens gets its oxygen supply from the aqueous humor that contains dissolved oxygen.
6. At rest, the lens ligaments are stretched and the anterior surface is less convex than the posterior one.
7. When the eye tries to focus, the radial fibers of the ciliary muscles contract and pull the ciliary body forward. This decreases the tension in the suspensory ligaments and relaxes the lens capsule causing the anterior surface to bulge forward and become more convex, thereby increasing the refractive power of the lens. This helps to focus objects over a wide range, from 10 cm to as far as 6 m.
8. In young people, the lens contributes about 17 diopters to the total refractive power of the eye. In children, the lens can have a greater curvature and a greater refractive power. With advancing age, the lens starts to lose its high water content, becomes tougher and less elastic. This results in decreased convexity of the lens following relaxation of the suspensory ligaments. The lens also becomes gradually less transparent with age.
9. Development of any degree of opacity in the lens or its capsule is known as cataract. It usually occurs with aging (degenerative or senile cataract) or secondary...
to diseases like diabetes mellitus. Complete opacity of the lens is known as mature cataract and before that it is called immature cataract. Absence of the lens in the eye (from its normal position) is known as aphakia, as occurs due to operative removal of the lens following cataract or due to dislocation of the lens.

The Vitreous Humor
1. The vitreous humor or vitreous body is a transparent avascular tissue of gelatinous consistency that occupies the posterior cavity of the eye. The portion of the vitreous present behind the posterior surface of the lens, acts as a cushion for the lens.
2. This jelly-like mass contains loose collagenous fibers and vitrein, a highly hygroscopic protein that is formed during early embryonic life and is not replaced thereafter.
3. In the fetal life, the hyaloid artery runs anteroposteriorly in the middle of the vitreous and nourishes the vitreous as well as the fibrovascular sheath of the lens. In the later months of intra-uterine life, the artery gets obliterated and shortly after birth it disappears leaving an empty narrow channel known as hyaloid canal. Persistent hyaloid artery is a congenital anomaly that causes defective vision.
4. The avascular vitreous may show degenerative changes in old age and high myopia, in which cases, it loses its gel like consistency and gets liquefied.

Light traverses several media in the eye to reach the retina: The incident light rays travel from air to pass through several transparent media before it reaches the retina; they are:
1. A thin film of tears, 
2. Cornea, 
3. Aqueous humor, 
4. Lens and 
5. Vitreous humor.

The Retina
1. The retina is a thin transparent membrane containing the photoreceptors cells (rods and cones) and many-layered neuronal network that takes part in initial stages of image processing.
2. When light rays are focused on the retina, it stimulates the photoreceptors, which send the signal to the next order of neurons. The chain of neurons at various levels in the optic pathway process the signal, which when arrives at the visual cortex generates the visual sensation.
3. The retina surrounds the vitreous in its inner surface and is covered by the choroid externally. It extends forwards to the anterior end of the choroid where its termination is known as ora serrata.
4. The retina consists of i) outer pigmented epithelial layer that lies in close proximity to the choroid and ii) inner neural layer that is confined to the posterior three quarters of the retina.

Pigment Epithelium
The pigment epithelium is rich in melanocytes and is continuous with the epithelium of the iris. It has the following functions:
1. Prevents scattering of light: The photoreceptor layer of retina is present next to the pigment epithelium. Some of the light rays do not strike the photoreceptors as they travel in the gap between two receptors. They strike the underlying pigment epithelium. The opaque melanin pigments absorb light rays (photons) that are not first captured by photoreceptors, preventing their reflection back to the retina. Thus, the pigment epithelium averts the blurring of visual images and preserves their sharpness and clarity. Absence of melanin in albinism causes visual problems (Application Box 142.2)
2. Phagocytosis: The pigment epithelial cells phagocytose fragments of membrane that are continuously shed from the outer segments of the photoreceptors. So, the pigment epithelium helps in the renewal process of photoreceptors by removing the degenerated membrane. The sloughed and degraded photoreceptor discs engulfed by the pigment epithelium are known as phagosomes.
3. Storage of vitamin A: The pigment epithelium stores large quantities of vitamin A, which is required for the synthesis of visual pigment rhodopsin, a protein present abundantly in rods.

Application Box 142.2
Visual problems in albinism: In albinism, melanin pigment is congenitally absent in all parts of the body, including the eye. In albinos, lack of melanin in the pigment epithelium as well as in the choroid causes nyctalopia, photophobia and defective vision. They perceive a higher light intensity compared to a normal person, and are advised to wear sunglasses to reduce the intensity.

Neural Layers
The retina is very thin (about 200 µm thick in humans) sheet of tissue that shows ten different layers (Fig.142.5). From outer to inner, they are:
1. Layer of pigment epithelium
2. Layer of rods and cones
3. External limiting membrane
4. Outer nuclear layer
5. Outer plexiform layer
6. Inner nuclear layer
7. Inner plexiform layer
8. Layer of ganglion cells
9. Layer of nerve fibers
10. Internal limiting membrane.
   i. The visual receptors (rods and cones) synapse with bipolar cells, which in turn synapse with ganglion cells. In each eye, there are about 120 million rods, 6 million cones and only 1 million ganglion cells.
ii. The photoreceptors converge on bipolar cells and bipolar cells converge on ganglion cells; *the functional convergence is more than 100:1*.

iii. **Horizontal cells** are interneurons present in the outer plexiform layer, where they send out processes and interconnect adjacent receptor cells as well as give input to the bipolar cells.

iv. **Amacrine cells** are interneurons present in the inner plexiform layer, where they send out processes and interconnect adjacent ganglion cells.

v. The horizontal and amacrine cells form both presynaptic and postsynaptic connections. The interneurons have only dendrites but no axons. The retinal neurons also form gap junctions with the adjacent neurons.

vi. Interspersed between the neural elements are present the supporting glial cells, called the **Müller cells** that have a nutritive function. The processes of these cells form the internal limiting membrane that separates the retina from the vitreous and an external limiting membrane in the receptor layer.

vii. The nerve fibers of the ganglion cells congregate to form the optic nerve that come out of the eyeball through the optic disk.

viii. When the neural layer gets separated from the pigment layer, the condition is called **retinal detachment**. The patient suffers from blindness and is treated by rejoining the layers by laser surgery or cryosurgery (Application Box 142.3)

ix. In premature babies, if oxygen is administered at high concentration (more than 40 percent) as a life saving measure, there occurs vascular proliferation from the peripheral retina causing retrolental opacity, a condition known as **retro-lental fibroplasia**.

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**Application Box 142.3**

**Retinal detachment cause blindness:** The central retinal artery and veins enter the eyeball with the optic nerve and supply the bipolar and ganglion cells present in the inner layers of retina. The rods and cones present in the outer layers of retina derive their nourishment from the choroidal capillaries by diffusion. Therefore, rods and cones suffer from hypoxic injury and vision is affected in retinal detachment.

**Light traverses several layers in the retina to reach the photoreceptors:** The incident light rays pass through the layer of blood vessels, ganglion cell axons and neurons of retina before it strikes the photoreceptors (rods and cones); the only exception is the fovea centralis, where light rays reach directly. Passage of light across different layers of retina does not affect the visual image quality much as the retina is quite thin and the layers are very transparent.

**Fovea Centralis**

If a line is drawn straight along the visual axis, at the posterior pole of the eye, a small yellowish spot of about 1–2 mm in diameter is present, called the **macula lutea** (*in Latin* macula = spot; lutea = yellow). This area is specialized for sharp colour vision. Its central part has a small depression of about 0.4 mm in diameter, called the **fovea centralis** (fovea = shallow pit). The **fovea is the most sensitive part of the retina having maximum visual acuity** because of the following reasons:

1. Its layer of photoreceptors contains large number of tightly packed small sized cones only.
2. The other neural layers of retina are displaced laterally to the side of the fovea, so that light rays directly reach the exposed photoreceptors.
3. In the fovea region, the ratio of cone cell to ganglion cell is 1:1, i.e. a cone cell synapses with a single bipolar cell, which in turn synapses with only one ganglion cell. Thus, the receptive field of a ganglion cell is smallest in the fovea region and it increases toward periphery of the retina.

4. Unlike at other regions of the retina, the ganglion cell axons, the central artery of retina and central retinal vein pass at the sides of macula, not over it. The macula gets its nutrition from the choroidal blood vessels by diffusion, as retinal blood vessels are absent in the macular region.

**Optic Disk**

1. The optic disk is located about 3 mm medial to and slightly above the posterior pole of the eye.

2. It is an area in the retina devoid of the photoreceptors. Therefore, while mapping the peripheral field of vision, a blind spot is observed at the temporal visual field.

3. The ganglion cell fibers and the central retinal veins come out of the eye and the central artery of retina enter into the eye through the optic disk.

4. In ophthalmoscopy it looks white in colour, as it contains bundles of myelinated nerve fibers.

**Fundus**

1. This is the posterior portion of the interior of the eye as seen through an ophthalmoscope. The normal fundus gives a red back ground owing to the presence of choroidal blood vessels.

2. The microcirculation of the eye, i.e. the branches of the central artery and vein that ramify and spread over the superficial layers of retina can be directly viewed through the lens with the help of an ophthalmoscope.

3. The fundus is the only region in the body where arterioles are readily visible. Therefore, this window is useful for the diagnosis of ocular diseases as well as conditions not directly related to ocular functions.

4. The progress of several systemic diseases like diabetes mellitus, hypertension and other diseases that affect the vessel wall can be assessed by examining the changes in retinal blood vessels. In diabetes mellitus and hypertension, degeneration and exudation occur that appear as opacities against the transparent background. In atherosclerosis, the narrowing of the vessel lumen is observed.

**Intraocular Pressure**

1. The intraocular pressure (IOP) is 10–20 mm Hg.

2. The volume of lens, aqueous, vitreous, blood inside the vessels and the elasticity of the three layers contribute to the development of this pressure.

3. It maintains the shape of the eyeball. The IOP keeps the retina pressed against the choroid so that retinal surface is even for the formation of a clear image.

4. IOP is measured by tonometry. The variation in IOP mainly depends on the amount of aqueous humor present at any given moment.

5. Increased IOP causes glaucoma and in long-term leads to degeneration of the retina.

### CHAPTER SUMMARY

**Key Concepts**

From outside to inside of the orbital cavity, eye has three layers: Sclera, choroid and retina. The protective, avascular sclera is modified to form cornea and the vascular choroid forms iris in the anterior segment of the eyeball. The retina contains the photoreceptors, the rods and cones and the neural layers that finally form the optic nerve. Optic disk is the area for the passage of optic nerve and blood vessels, is devoid of photoreceptors and is seen as blind spot in visual field. Fovea centralis is the area with highest visual acuity in the retina. The lens helps to project the image on the retina by changing its curvature, the ciliary muscle and the suspensory ligaments contribute in this regard. The aqueous humor fills the anterior and posterior chambers present in front of the lens, The vitreous humor fills the cavity posterior to lens. Increased intraocular pressure causes glaucoma.

**Important to Know (Must Read)**

1. In examination, Long Questions are usually not asked from this chapter.

2. Layers of retina, Aqueous humor may be asked as Short Questions in exam.

3. In Viva, examiner may ask… Name the layers of the eye, Name the layers of the retina, What is fovea centralis, What is blind spot, What is glaucoma.
When light rays from an object fall on the eye, the image of the object is focused on the retina by the following three mechanisms:
1. Refraction of light rays by cornea and lens
2. Ciliary muscle activity-causing accommodation of lens
3. Change in pupil size by iris muscles.

**REFRACTION OF LIGHT RAYS**

When light rays traveling through a transparent medium pass into a second transparent medium with a different density (e.g. air to water), they bend at the interface. This change of direction of light rays at the junction between the two interfaces is called refraction. The degree and direction of bending depend on the following factors.

i. The **angle** at which the ray strikes the surface of the second medium (curvature of the interface). If the ray strikes at an angle of 90°, there is no bending and the ray passes straight. Oblique rays bend more sharply. If the surface of the next medium is curved, based on the type (concave or convex) and degree of curvature (greater the radius of curvature, more is the bending), parallel rays of light bend in different angles and travel in the new medium in different directions.

ii. **Difference in density** between the two mediums. When the rays pass from a less dense to a dense medium (e.g. air to water), bending is toward the midline.

iii. **Wavelength** of light. Light rays of longer wavelength get refracted more than lights of shorter wavelength. The visible light has wavelengths ranging from 400 nm to 750 nm.

**Refractive Index (RI)**

The ratio of speed of light in a given medium to that in air is called the refractive index of that medium. Light travels rapidly in a less dense medium (e.g. air) and slowly in a denser medium (e.g. lens). The refractive index (RI) of air being taken as 1, the RI of other media is as follows: cornea (1.38), aqueous humor (1.33) lens (1.38) and vitreous humor (1.33).

**Principles of Optics**

1. The light rays coming from a distant source of light (more than 6 m) are assumed to be parallel and the rays coming from a distance of less than 6 m are diverging rays.
2. A convex/positive lens has increasingly more bending power (from center to periphery) and brings parallel light rays to a common point, called **focal point** (Fig. 143.1A). A converging lens can form a real image. The lens present in the eye is a biconvex lens.
3. The line joining the centers of the two spheres parts of which form the lens surfaces is called the **principal axis** of the lens. The optical center of the lens is a point on the principal axis through which light rays pass without being deviated. When parallel rays of light strike the surface of the lens, they converge to meet at a point behind the lens called the **principal focus**. The distance between the optical center of a lens and the principal focus is called the **focal distance**. In case of a biconcave lens, the light rays striking the surface of the lens diverge and they appear to diverge from the principal focus situated in front of the lens (Fig. 143.1B).
Chapter 143: Image-forming Mechanism

Refractive Power

1. The refractive power of a lens is expressed in diopters (D), which is the reciprocal of the focal distance of a lens in meters. A lens with a focal length of one meter has a refractive power of 1/1 = 1 D. Similarly, lenses with focal lengths of 2 m, 0.5 m and 0.25 m have refractive powers of 0.5 D (1/2), 2 D (1/0.5), and 4 D (1/0.25) respectively. In other words, the greater the lens curvature, the greater is its refractive power; here, the principal focus is nearer and the focal distance is smaller.

2. As light rays enter the eye, they get refracted by the cornea. The light rays are further refracted at both surfaces of the lens so that they come into exact focus on the retina. The eye has a total refractive power of 60 D; out of which 43 D is contributed by the cornea and only 17 D by lens.

3. The cornea has the greatest refractive power because the change in density between the two media is maximum in it, i.e., 1 to 1.33. When the head is immersed in water, the refractive power contributed by the cornea becomes zero because water has the same refractive index as cornea.

Reduced Eye

1. The process of refraction is explained with the help of a schematic eye or reduced eye (Fig. 143.2). The path of light rays and the image formed on the retina is diagrammatically represented by assuming that all refractions occur at the anterior surface of the cornea, between a medium with RI 1.0 (air) and a medium with RI 1.33 (water).

2. The nodal point (optical center of the eye) is situated 7 mm behind the anterior surface of the cornea and coincides with the junction of the middle and posterior third of the lens. The light rays from an object that pass through the nodal point do not get refracted, whereas other rays get refracted at the anterior surface of the cornea and are brought to focus on the retina. As the eye is about 24 mm in length, the distance of the nodal point from the retina (focal length) is about 24 – 7 = 17 mm (0.017 m). The refractive power of the schematic eye will be 1 divided by its focal length in meters i.e. 1/0.017 = 59 D. This value is same with the refractive power of the normal human eye, when measured with X-ray.

3. As shown in the diagram, the light rays coming from the object AB, get refracted and form an image A'B' on the retina. The rays arising from B pass through the nodal point (N) without any refraction; ANB and A'NB' are similar triangles. The angle subtended by the object AB at the nodal point (< ANB) is known as the visual angle. The size of the retinal image can be calculated if, the height of the object and its distance from the eye (observer) is known.

\[
\text{Size of object} = \text{distance of object from nodal point} \\
\text{Size of image} = \text{distance of image from nodal point}
\]

If the object is 1 meter high and 10 meters away from the eye, the size of the image will be

\[
\frac{1 \times 1000 \, \text{mm}}{17 \, \text{mm}} = \frac{10 \times 1000 \, \text{mm}}{17 \, \text{mm}}
\]

\[
\text{Size of image} = \frac{17\, \text{mm}}{10} = 1.7 \, \text{mm}
\]

Retinal Image

1. The emergent rays from the posterior surface of the lens form a real, inverted and much smaller image on the retina. The image is upside down (Fig. 143.2).

2. Also, there is left to right reversal of the image, i.e. light rays from the left side of an object strikes the right side of the retina, and from right side of an object strikes the left side of the retina. That means a tree will be focused as branches down and base up; a man lying down with head on left side will be focused as man with head on right side. But actually we do not see an inverted world because neural processing helps the brain to perceive visual images with upright appearance. This phenomenon is inherent to us since birth.
Section 12: Special Senses

ACCOMMODATION

Basically, to be able to see an object clearly, the object should be present at the focal point of the focusing apparatus. Like a camera, the eye can focus on objects at varying distances by changing its point of focus.

1. As the cornea is a fixed structure whose curvature does not change, its refractive power is constant in air medium.
2. The ability of the eye to alter its focal distance is due to the malleable lens that can change its curvature of the anterior surface by contraction of the ciliary muscles (the radius of curvature changes from 12 mm to 6 mm).
3. The curvature of the posterior surface of lens does not change much (6 mm at rest to 5 mm in full accommodation).
4. In a normal resting eye, the ciliary muscles are relaxed, the lens is stretched and the eye is focused on distant objects at more than 6 m. To focus an object present within 6 m, the eye has to increase its refractive power, a process called accommodation, in which the curvature of the lens is increased (Fig. 143.3).
5. During accommodation, the ciliary muscle contracts, pulls the ciliary body forward and medially toward the lens. The suspensory ligaments relax, the tension exerted on the lens capsule decreases and the elastic lens becomes more convex.

Near Point

It is defined as the nearest point to the eye, at which an object can be clearly seen. At this point, accommodation is exerted to its maximum. The ciliary muscle has contracted to its maximum and the suspensory ligaments have maximally relaxed. If an object is kept at a distance less than this, it appears blurred and can be visible clearly with the help of a convex lens. The maximum accommodation ability of the eye decreases throughout life, and so the near point recedes, slowly during the initial 35–40 years of life and then rapidly afterwards (Table 143.1).

This gradual loss of accommodation is due to progressive loss of elasticity of the lens. With advancing age, the lens becomes harder that results in decreased ability of the lens to increase its curvature in response to the contraction of the ciliary muscles.

Near Response

When an individual changes his gaze from a distant object to a near object, three changes occur in his eye. They are:

i. Increase in curvature of the anterior surface of the lens (accommodation)
ii. Convergence of the visual axes (as the eyeballs rotate medially); more near the object, greater the inward rotation.
iii. Constriction of the pupil.

SIZE OF THE PUPIL

1. In bright light, the pupil constricts to a minimum diameter of 2 mm and in dark, it dilates to about 8 mm. As the area of a circle depends upon the square of its radius, the pupil can change its area to 16-fold in conditions of extreme illumination.
2. The small size of pupil decreases spherical and chromatic aberration by eliminating the peripheral rays and increases the depth of focus.
3. The optimum vision is achieved with a pupillary diameter of 2 to 3 mm.

Chromatic Aberration: As light travels in a medium, its velocity depends on its wavelength. Lights with longer wavelength (e.g. red color light) get refracted more and lights with shorter wavelength (e.g. violet color light) get refracted less. Thus, lights of different colors get focused at different points on the retina. This is known as chromatic aberration.

Spherical Aberration: Spherical aberration occurs due to non-uniform refractive index of the lens at its center and the periphery. Light rays that pass through the lens near its periphery get refracted more than the rays that enter through its central portion. Thus, lights of a single wavelength get focused at different points on the retina. This is known as spherical aberration.
DEFECTS OF THE IMAGE FORMING MECHANISM

1. The refractive condition of a normal eye is known as emmetropia and the eye is known as an emmetropic eye, in which the parallel rays of light from a distant object are focused on the retina without accommodation (Fig. 143.4A).

2. If the refractive state differs from the normal, it is known as ametropia, in which parallel rays are focused in front or behind the retina resulting in a blurred image. If this occurs in children, one of the images is suppressed, causing loss of vision in one eye. This is called amblyopia ex anopsia. Ametropia is of three types: myopia, hypermetropia and astigmatism. The refractive errors of the eye may be due to abnormality in the following.
   i. Axial length of the eye
   ii. Refractive power
      − Curvature of the surface of the cornea or the lens,
      − Refractive indices of the media,
      − Position of the lens.

Myopia (Short-sightedness)

1. It is a refractive error in which parallel rays of light from a distant object are brought into a focus in front of the retina when the accommodation is at rest (Fig. 143.4B).

2. The cause of myopia can be genetic or acquired. Congenitally, the eyeball may be elongated, i.e. the anteroposterior diameter of the eye is increased. Myopia is acquired, when increased duration of close works over years like reading, working on computer or sewing, etc., continuously activates the accommodation process, especially the lens becomes more convex.

3. Myopia can be corrected by using spectacle with biconcave lens that diverges the incident rays before they strike the cornea (Fig. 143.4C).

Hypermetropia (Far-sightedness)

1. It is a refractive error in which parallel rays of light from a distant object are brought into a focus behind the retina when the accommodation is at rest (Fig. 143.4D).

2. It occurs mainly due to decreased anteroposterior diameter of the eye.

3. It can be corrected by using spectacles with biconvex lens that converges the incident rays before they strike the cornea (Fig. 143.4E). The near point is farther than normal, so the patient needs the biconvex lens (reading glass) at an earlier age.

Astigmatism

1. It is a refractive error in which parallel rays of light from a distant object cannot converge to a point focus on the retina due to unequal refraction at different meridians.

2. The corneal surface is not perfectly spherical; therefore, the curvature of one meridian differs from the other. Accordingly, the light rays are focused at different points on the retina causing blurring of image. It can also occur if the lens curvature is not uniform or if the lens is pushed out of alignment.

3. It is corrected by using a cylindrical lens.
Presbyopia

1. This is a physiological phenomenon seen in all persons at about the age of 40 years in which the near point of vision recedes beyond the normal reading or working distance due to progressive loss of plasticity of the lens.
2. As the near point recedes beyond the point at which one is used to do close work or read ordinary prints, which is about 28 cm from the eye, the person has difficulty in reading, writing and performing close work (e.g. threading a needle).
3. It is corrected by using a convex lens for near works, usually prescribed in the form of bifocal lenses, so that when the person wants to change his focus from a near to distant object, each time he need not take off the glasses.

CHAPTER SUMMARY

Key Concepts

When light rays from an object fall on the eye, the image of the object is focused on the retina by three mechanisms:
1. Refraction of light rays by cornea and lens
2. Ciliary muscle activity-causing accommodation of lens
3. Change in pupil size by iris muscles.

A distant object is brought into focus by the following three mechanisms:
 i. Increase in curvature of the anterior surface of the lens (accommodation)
 ii. Convergence of the visual axes (as the eyeballs rotate medially); more near the object, greater the inward rotation.
 iii. Constriction of the pupil.

The refractive condition of a normal eye is known as emmetropia. The refractive errors are mainly myopia (short-sightedness), corrected by a biconcave lens; hypermetropia (far-sightedness), corrected by a biconvex lens; astigmatism, corrected by a cylindrical lens; and presbyopia corrected by a biconcave lens:

Important to Know (Must Read)

1. In examination, Long Questions are usually not asked from this chapter.
2. Refractive errors, Accommodation, Near point may be asked as Short Questions in exam.
3. In Viva, examiner may ask… Name the mechanisms of image formation, Name the refractive power of the eye, What is near point, What is near response, What is the defect in myopia, how is it corrected, What is the defect in hypermetropia, how is it corrected, What is the defect in astigmatism, how is it corrected, What is the defect in presbyopia, how is it corrected.
**CHAPTER 144**

*Photoreceptor Mechanism*

**LEARNING OBJECTIVES**

On completion of study of this chapter, the student **WILL** be able to:

1. Understand the structure of rods, cones and photopigments, and their functions.
2. Describe the mechanism of phototransduction.
3. Understand the importance of photoreceptor potential.

**STRUCTURE OF PHOTORECEPTORS**

The rods and cones are the specially structured neurons that act as receptors for the light stimulus reaching in the form of photons. They convert the light energy to chemical energy that gets transmitted to the bipolar cells as synaptic potential. Each cell has two segments: the outer segment is a modified cilium that contains the photopigments; the inner segment contains the nucleus and numerous mitochondria that provide energy for the phototransduction process. At the base, it forms a synaptic terminal that releases neurotransmitter substances when light strikes the eye. The rods are numerous in the extrafoveal portion of the retina, whereas the fovea contains only cones.

**Rods**

1. The rod cell is so named because of its long, slender, and cylindrical appearance (Fig. 144.1).
2. Its outer segment contains numerous disks composed of cell membrane in which the molecules of the photopigment rhodopsin are embedded. The disks are separated from the cell membrane. The double membrane disks are flattened and piled up on each other in a regular manner like a stack of coins.
3. From the upper part of the outer segment, old disks are regularly shed and removed by phagocytosis by the pigment epithelium, and replaced with new disks synthesized at the inner edge of the outer segment. This renewal is a continuous and rapid process, at a rate of about 30 disks per day.
4. The rod cell synapses with several rod bipolar cells.

**Cones**

1. Cones have a broad inner segment and a tapered outer segment that gives them a conical appearance (Figs. 144.1A and B). The foveal cones are smaller and thinner than the extrafoveal cones.
2. The outer segment is formed by infoldings of the cell membrane. The membrane invaginations look similar to the rod disks, but they are not separated from the cell membrane, and their size increases from tip to the...
inner edge of the outer segment. The photopigments are present on these membrane infoldings.

3. The renewal process in cones is more diffuse and occurs at multiple sites in its outer segment.

**Photopigments**

There are four different photosensitive compounds present in the retina, one in the rods and three in the cones. The photopigment is made up of retinene, the actual light-sensitive component of the photopigments and opsin, an integral membrane protein. The retinine (chromophore molecule) is the same in each of the photopigments but the opsin differs in each of the four pigments. The retinenes are also called retinals, as they are the aldehyde of vitamin A.

**Rod Pigment (Rhodopsin)**

1. The photosensitive pigment in the rods that absorbs light is called rhodopsin or visual purple. It is a G protein coupled serpentine receptor for light.
2. It is a globular protein with a molecular weight 41,000. Rhodopsin molecules are very tightly packed with a density of 30,000 molecules per square micrometer of the membrane; consequently, they make up 90% of the total protein in disk membranes.
3. It is made up of retinene1 and an opsin, called scotopsin. Opsin is a single polypeptide that spans the membrane seven times. Retinene1 remains parallel to the surface of the membrane being encircled by the opsin chain.
4. The peak spectral sensitivity for rhodopsin is 500 nm.

**Cone Pigments**

1. Three types of cone pigments are present in the retina. The pigments differ in the wavelength of light that optimally excites them.
2. The peak spectral sensitivity for the red-sensitive pigment is 560 nm; for the green-sensitive pigment is 530 nm; and for the blue-sensitive pigment, it is about 420 nm.
3. Each cone cell contains one of the three cone pigments; accordingly, there are red, green and blue cones. Each pigment contains retinene1 and an opsin that has seven membrane spanning domain like scotopsin; but structures of opsins and the way they bind to retinene1 differs.

**Melanopsin**

This pigment is present in some photoreceptors that do not contain rhodopsin or the cone pigments. The receptors converge on to specific ganglion cells, whose fibers travel in the optic nerve and project to the suprachiasmatic nuclei and pretectal nuclei. These nuclei are concerned with the regulation of circadian rhythms and pupillary light reflexes. In the absence of the gene for melanopsin, or in damage to the receptors or to the pathways, the above responses are abolished.

**MECHANISM OF PHOTOTRANSDUCTION**

When light strikes the eye, a hyperpolarizing potential is generated in the rods and cones, known as photoreceptor potential. The conversion of the light energy to neural signal by the visual pigments in the photoreceptors is known as phototransduction. Light induces a conformational change in the photopigment and the associated G protein that alters membrane potential by lowering the cGMP level. The change in membrane potential at the synaptic terminal modulates the discharge of the neurotransmitter glutamate and thus, initiates a response in the next order of neuron. The mechanism of phototransduction can be better appreciated by understanding the processes taking place in the absence of light.

**Processes Occurring in the Dark**

1. The Na+ channels on the outer segment membrane of the photoreceptors are cGMP-dependent. In the absence of light, a constitutively active guanylyl cyclase hydrolyses GTP to cGMP, increasing the concentration of cGMP in the cytoplasm.
2. The elevated level of cGMP maintains the cGMP-gated Na+ channels in the open state, allowing Na+ influx. Inside the rod cell Na+ flows from the outer segment toward the inner segment along its concentration gradient, as the cytoplasmic Na+ concentration of the inner segment is maintained at a lower level by the action of Na+-K+ ATPase.
3. Outside the cell, sodium flows from the inner segment to the outer segment along a favorable gradient (Fig. 144.2). Thus, in the outer segment, continuous inflow of sodium is maintained producing a decrease in membrane potential that in turn keeps the voltage-gated calcium channels open in the synaptic terminal.
4. The calcium-mediated exocytosis of synaptic vesicles results in a steady release of the neurotransmitter glutamate. Thus, the photoreceptor cell is depolarized at rest.

**Genesis of Photoreceptor Potential**

**In Rods**

1. In the dark, the retinene1 in the rhodopsin remains in a less stable form, known as 11-cis-retinal.
2. When light strikes the eye, it absorbs a photon and gets isomerized to all-trans-retinal, which is a more stable form. This isomerization induces the opsin to undergo a series of rapid conformational changes and
converts rhodopsin to **metarhodopsin II**, which in turn activates an associated G protein, called $G_{t1}$ or *transducin*. During the activation process, GDP is exchanged for GTP, causing separation of $\alpha$ subunit from $\beta, \gamma$ subunit.

3. Transducin stimulates **cGMP phosphodiesterase** that converts cGMP to 5’GMP (Fig. 144.2). The decreased cGMP level in the cytoplasm leads to closure of cGMP-gated Na$^+$ channels. This produces a hyperpolarizing membrane potential as compared to the potential in the dark, resulting in decreased neurotransmitter release at the terminal synapse. Thus, the photoreceptor becomes hyperpolarized on stimulation.

4. The phototransduction pathway gets amplified during the cascade of reactions increasing the response several fold. The absorption of 1 photon stimulates 1 metarhodopsin molecule that activates 700 transducins causing hydrolysis 1400 cGMP molecules.

**In Cones**

The mechanism of phototransduction in cones is similar to that in the rods except that the **G protein** is $G_{t2}$, which stimulates the enzyme cGMP phosphodiesterase.

**Restoration of the Resting State**

1. Soon after isomerization, all-trans-retinal is separated from opsin. As a result, the reddish color imparted to opsin by the photopigment disappears and opsin becomes pale yellow. Hence, the process is called *bleaching*.

2. The all-trans-retinal moves to the pigment epithelium, where the enzyme **retinal isomerase** converts it into **11-cis-retinal**. The photopigment then gets translocated to the outer segment of the rods to recombine with opsin, replenishing the rhodopsin store (Fig. 144.3). It takes a few minutes for one regeneration cycle to get completed.

3. Following activation of phosphodiesterase by the G protein, the intrinsic GTPase activity of the $\alpha$ subunit hydrolyzes GTP to GDP, terminating the action of G protein. The stimulating activity of transducin comes...
Section 12: Special Senses

CHAPTER SUMMARY

Key Concepts

1. Rhodopsin or visual purple is a G protein coupled serpentine receptor for light, peak spectral sensitivity for rhodopsin is 500 nm. There are three cone pigments, peak spectral sensitivity for the red-sensitive pigment is 560 nm; for the green-sensitive pigment is 530 nm; and for the blue-sensitive pigment, it is about 420 nm.

2. When light strikes the retina, 11-cis-retinal is converted to all-trans-retinal, conformational change in rhodopsin forming metarhodopsin II, activation of transducin and closure of cGMP-gated Na\(^+\) channels leading to hyperpolarization, response in bipolar cells and action potential in the optic nerve.

Important to Know (Must Read)

1. In examination, Long Questions are usually not asked from this chapter.

2. Mechanism of phototransduction may come as Short Questions.

3. In Viva, examiner may ask… Name the photoreceptors and say their functions, Steps of Mechanism of phototransduction.

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to an end by its binding to \(\beta\)-arrestin, a cytosolic protein. The enzyme rhodopsin kinase causes phosphorylation of light-activated rhodopsin and thus, deactivates it.

4. The cGMP-gated channel present in the photoreceptor membrane is a nonselective cation channel, through which Na\(^+\) and Ca\(^{2+}\) enter into the cell. When light strikes the eye, the channels close and the concentration of Na\(^+\) and Ca\(^{2+}\) decreases in the cell. The reduced Ca\(^{2+}\) concentration stimulates guanylyl cyclase and inhibits phosphodiesterase, resulting in raised cGMP level. Thus, cytoplasmic cGMP concentration is maintained by the activity of guanylyl cyclase and phosphodiesterase, both of which are finely regulated by cytoplasmic Ca\(^{2+}\) concentration.

5. In the dark, Ca\(^{2+}\) influx exerts an inhibitory effect on guanylyl cyclase, so that cGMP concentration does not become very high. Ca\(^{2+}\) is removed from the outer segment by a Na\(^+\)-Ca\(^{2+}\) exchanger. Thus in the resting state, the photoreceptors are partially depolarized and increased amount of neurotransmitters are released at the synaptic endings. The mechanism of phototransduction is summarized in Figure 144.4.
1. The visual sensations arise in the rods and cones (end organs), from which they are transmitted to the bipolar cells that convey the sensation to the ganglion cells.

2. From the retina, the fibers of the ganglion cells congregate to form the optic nerve as they come out through the optic disc. Each optic nerve carries about 1 million fibers from each retina. The optic nerve travel to the optic chiasm, where about half the fibers of each eye cross over to the opposite side. After the chiasm, fibers travel as optic tract to the lateral geniculate body (LGB).

3. Fibers from the temporal half of retina do not cross the midline and travel in the optic tract of the same side. Fibers from the nasal half of retina decussate at the chiasm and travel in the optic tract of the opposite side (Figs. 145.1A to D). Thus, each optic tract contains fibers arising from temporal half of retina of the same side and nasal half of the retina of the opposite side. In other words, left optic tract contains fibers from the left half of the retina (right visual field) and right optic tract contains fibers from the right half of the retina (left visual field). The macular fibers decussate in a similar manner at the chiasm, i.e. nasal half of the fibers decussate at the chiasm and temporal half of macular fibers travel uncrossed in the optic tract.

4. The direct and crossed fibers in the optic tract pass to the alternating lamina of the LGB and end there. From the neurons of the LGB, the axons project to the ipsilateral primary visual cortex or V1 (Brodmann's area 17), located on the sides of the calcarine fissure on the medial aspect of the occipital lobe of the brain. The fibers carrying visual impulses from the LGB to the calcarine cortex are called geniculocalcarine tract. As these fibers spread out from LGB, they are known as optic radiation. Since V1 is also known as striate cortex, these fibers are named as geniculostriate tract.

5. From the LGB, macular fibers project to the posterior pole of the occipital lobe. The effects of lesion at various levels of the visual pathway are shown in the Figure 145.1A to D and are described as ‘visual field defects’ in chapter 146.

6. There are about one million fibers in the optic nerve, two million fibers in the geniculocalcarine tract and 1000 million fibers in the visual cortex; a good example of divergence.

7. The optic nerve fibers project to several other areas of the brain like association visual cortex, superior colliculus, pretectal nucleus, and suprachiasmatic nucleus of hypothalamus. These fiber systems participate in regulation of other functions like circadian rhythm associated with vision.
**Processing of the Visual Signal**

**Responses of the Retinal Neurons**

**Photoreceptors**
1. The threshold for rods is lower than that for cones. Therefore, rods are more sensitive (can respond to a single photon of light). The response is proportionate to the stimulus intensity, but after reaching a maximum, the response does not increase. Thus, rods are receptors for dim light and detect absolute illumination at lower range of stimulus intensity.
2. The cones require high level of illumination for activation and then, their response becomes proportionate to the stimulus intensity. So, the cones are receptors for bright light and they detect changes in stimulus intensity.
3. Thus, the human eye can operate over a wide range of light intensities. However, the response of rods (sharp onset, slow offset) is slower than that of cones (sharp onset, sharp offset).
4. When a light stimulus is placed in the receptive field of a photoreceptor, it produces a hyperpolarizing response that is transmitted to the bipolar cell.

**Bipolar Cell**
1. Bipolar cells link the photoreceptors to ganglion cells.
2. Some of the bipolar cells show a depolarizing potential that is excitatory to the ganglion cells, whereas other cells show a hyperpolarizing potential that is inhibitory.
3. Based on their morphology and function, the bipolar cells have been classified into 12 types. The cells show on-center and off-center response like the ganglion cells, as described below.

**Horizontal Cell**
1. The photoreceptors also give input to the horizontal cells, which respond with a hyperpolarizing membrane potential.
2. With their branching dendrites, they link rods and cones to other photoreceptors. Also, the dendrites of the horizontal cells spread laterally and produce inhibition of the bipolar cells on which they synapse.

**Amacrine Cell**
1. They exhibit a depolarizing response.
2. The amacrine cells spread laterally and synapse on the ganglion cells to which they provide excitatory input.
3. They are classified into 29 types based on their connections with the ganglion cells in the inner plexiform layer. Thus in the retina, the bipolar cells, horizontal cells and amacrine cells comprise the neural network layer, in

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**Figs. 145.1A to D**: Effects of lesions at various levels of visual pathway. (A) lesion of right optic nerve produces blindness in right eye; (B) lesion of optic chiasm produces bitemporal hemianopia; (C) lesion of right optic tract produces left homonymous hemianopia; (D) lesion of right geniculocalcarine tract produces left homonymous hemianopia with macular sparing.
which considerable processing of visual information takes place. The cells form gap junctions with the adjacent cells, so that signals spread in the form of electrotonic potentials rather than propagated action potentials.

**Ganglion Cells**

1. They integrate the information received from bipolar and the amacrine cells and respond with a depolarizing potential that propagates along the axon (optic nerve). In the resting state, the ganglion cell has a discharge rate of 20–50 spikes/s.
2. The response shown by the ganglion cells is of two types and accordingly, there are two types of cells: (i) on-center ganglion cells and (ii) off-center ganglion cells.
3. **Receptive field** of a ganglion cell is the area of the visual field in which presence of a light stimulus modulates the discharge pattern of a ganglion cell. The central retinal neurons have smaller receptive fields, whereas peripheral retinal neurons have larger receptive fields.
4. The on-center ganglion cell increases its spike rate in response to a central small circular light stimulus (Fig. 145.2) in its receptive field (on-response) and when an annulus of light around the center is turned on, its spike rate decreases (off-response).
5. In the off-center ganglion cell, spike rate decreases when the central stimulus is turned-on and its spike rate increases in response to the surround illumination.
6. Thus, the on-center ganglion cell is stimulated in response to an excitatory center with an inhibitory surround, whereas an off-center ganglion cell is stimulated in response to an inhibitory center with an excitatory surround. Due to this center-surround antagonism, the ganglion cells have been called as contrast detectors.
7. During the off-response of the on-center ganglion cell, the annulus of light activates a ring of photoreceptors that stimulates the horizontal cells (present on both sides of the receptor circle) on which they synapse. The horizontal cells in turn inhibit the nearby photoreceptors by lateral inhibitory inputs. In this manner, the centrally present photoreceptors get inhibited by the surrounding horizontal cells. Thus, decrease in activity of the neighboring cells by stimulation of a specific cell is known as afferent or lateral inhibition. This is an important component of information processing during which, signal transmission through a particular pathway is allowed, whereas, propagation of action potential through the adjacent pathways is inhibited. Examples of afferent inhibitions are common in spinal cord. In the retina, this improves the sharpness at the periphery of an image and increases contrast.
8. Based on size, there are two types of ganglion cells present in the retina: large ganglion cells (magno or M cells), and small ganglion cells (parvo, or P cells). Axons of the M ganglion cells project to the magnocellular layer of the LGB; and the P ganglion cells project to the parvocellular layer and interlaminar region of the LGB. The function of M cells is to add potentials from different kinds of cones and transmit the integrated response in the form of action potential. The M cells convey information regarding movement and stereopsis to the LGB. The P cells analyze and subtract response from one type of cone from response from another. They send the information regarding color, texture and shape of an object to the LGB.

### The Important Features of Retinal Neurons:

1. The photoreceptors get hyperpolarized on activation.
2. **Input to the retina is received** by the photoreceptors and the ganglion cells are the output cells.
3. **Processing of visual signals** mainly occurs in the neuronal network layer.
4. The responses of the cells of the neuronal network layer are integrated in the ganglion cell layer. Ganglion cells generate the action potentials.
5. The photoreceptors and the ganglion cells are spontaneously active in the resting state, so that, any change in visual stimulus brings in a response in them. The visual information (largely from the rods) converges on the ganglion cells. This decreases the contrast of an image, but improves the sensitivity.
In The LGB

Organization and Topography

1. Most of the fibers of the optic tract terminate in LGB of thalamus. The LGB is divided into six well-defined layers numbered 1 to 6 from ventral to dorsal direction. The ventrally placed layers 1 and 2 have large cells and accordingly are called magnocellular that receive input from the M ganglion cells (Fig. 145.3). Layers 3, 4, 5, 6 are called parvocellular as they contain small cells and receive input from the P ganglion cells. The interlaminar region of the LGB receives inputs from the P ganglion cells.

2. Each LGB receives half of the inputs from both retina through the optic tract and the inputs from each eye project distinctly to specific layer of LGB. Inputs from nasal retina of contralateral eye projects to layers 1, 4 and 6; and inputs from temporal retina of ipsilateral eye project to layers 2, 3 and 5.

3. There is a precise topographic map of the retina in each layer of the LGB, i.e. for each point of retina that is excited; there is a specific part of the LGB that receives the potential and responds to it. Thus, the right half (temporal half) of right retina is represented thrice in the right LGB; once in layer 2, again in layer 3, and once more in layer 5. Similarly, the right half (nasal half) of left retina is represented thrice in the right LGB; once in layer 1, once in layer 4, once in layer 6. Along a perpendicular line through the layers of LGB, all the cells have the same visual field (Fig. 145.3). Moreover, this point-for-point representation follows an orderly pattern. When a visual stimulus activates a group of photoreceptors that are adjacent to each other, the photoreceptors excite the adjacent cells in the LGB. The fibers from the upper part of the retina project to the medial half of LGB, and fibers from the lower part of the retina project to the lateral half of LGB.

4. The macular area (central retina) projects to the posterior LGB, whereas the peripheral retina projects more anteriorly. The macular area has the highest representation in the LGB compared to the peripheral retina. The neurons of LGB respond to a point stimulus. When the stimulus is a bar of light, they respond to each point of the bar.

5. Apart from the inputs from retina, LGB receives maximum inputs from the visual cortex and other brain areas. The visual cortex inputs provide necessary feedback regarding perception of orientation and movement of an object. The output from LGB goes to visual cortex as geniculostriate fibers. From the cell bodies in layers 1 and 2, the fibers form the magnocellular pathway that is concerned with detection of movement, depth and flicker. The fibers form the cell bodies in layers 3-6, carries the impulses regarding color, texture, shape and finer detail. Axons that originate from the interlaminar region of the LGB carry the impulses for color vision.

VISUAL CORTEX

Primary Visual Cortex

Organization and Topography

1. The primary visual cortex (V1) is the Brodmann’s area 17, located on the sides of the calcarine fissure. It is also known as the visual-sensory area as it receives the sensory information regarding vision.

2. The fibers from the LGB project to the visual cortex in a topographic manner. The geniculocalcarine tract fibers mainly terminate on the medial part of occipital cortex, above and below the calcarine fissure.
The fibers from the medial part of the LGB representing upper quadrants of both retinas travel in the dorsal part of the optic radiation and project to the upper lip of the calcarine fissure. The fibers from the lateral part of the LGB representing the lower quadrants of both retinas form the ventral part of the optic radiation and project to the lower lip of the calcarine fissure.

3. The cortical area devoted to receive afferents from macula is much greater than other areas. **Thus macula has a much larger cortical representation than peripheral retina.** In the visual cortex, the fibers from LGB carrying macular afferents terminate more posteriorly in the calcarine fissure and the fibers from LGB carrying impulses from peripheral retina terminate anteriorly in the calcarine fissure (Fig. 145.4).

4. Like other cortical areas, the visual cortex is divided into six parallel layers, numbered 1–6. The magnocellular and parvocellular fibers from the LGB project to the **deeper layer 4C** of the visual cortex. **This layer receives information regarding movement, location, orientation, texture, shape and color of an object.** The fibers from the interlaminar region of the LGB project to the superficial layers 2, 3 of the visual cortex. The cells of **layers 2,3** are rich in the enzyme cytochrome oxidase and form clusters, known as **blobs** that receive information regarding color of an object. The cells from layer 4 also project to the neurons in layers 2 and 3. The neurons that have their cell bodies in layers 2 and 3, project to the other cortical regions and to the neurons in layer 5. The neurons that have their cell bodies in deeper layer 5, project to the superior colliculus, pulvinar and other brainstem nuclei. The neurons in layer 6 projects to the LGB.

**Responses**

1. Like the bipolar cells, ganglion cells and cells of LGB, the cells of visual cortex exhibit **on-center and off-center responses.** But unlike these cells that respond to a point stimulus, the neurons of visual cortex respond to a linear stimulus like lines, edges or bars of light. Thus, the stimulus can be a bar of light against dark background or a dark bar against a light background.

2. The neurons of layer 4 of the visual cortex respond to a stimulus that is positioned at any angle i.e. they have no preferred orientation. But, the cells in other layers are responsive to the orientation of the bar stimulus. Based on this, the cells are classified into simple, complex and hypercomplex cells.

**Simple Cell**

i. A simple cell responds best to a particular orientation of the bar stimulus.

ii. When the stimulus is rotated as little as 10° from the precise position, the response decreases.

**Complex Cells**

i. The complex cells show a low resting discharge rate, whereas the simple cells discharge only on stimulation.
ii. The complex cells respond best when a bar stimulus moves laterally without change in its orientation. Thus, the complex cells are concerned with movement and velocity of the stimulus and less with its central location.

Hypercomplex Cells

i. There are also hypercomplex cells that respond best to a moving bar with a precise orientation but also with a defined length.

ii. Some respond better to a stimulus having a specific angle (for example, a L-shaped or tongue-shaped stimulus) or having two borders.

3. Thus, the visual cortex receives information regarding orientation, movement, depth, velocity as well as color of an object and help in depth perception and stereopsis (binocular depth perception).

4. Usually, the simple cells project to the complex cells and the hypercomplex cells receive inputs from the complex cells. So, the receptive fields of the simple cells are smaller than those of the complex cells and receptive fields of the hypercomplex cells are larger than those of the complex cells.

5. When a microelectrode is inserted perpendicularly into the visual cortex, the cells that come across have the same orientation (orientation column). The adjacent column of cells differ 5°-10° in their orientation in an organized manner. Thus, the simple and complex cells are called feature detectors.

6. All cortical neurons have their receptive fields in the contralateral visual field. Each neuron of the layer 4 receives input from only one eye. Also, the cells receiving input from one eye alternate with the cells receiving input from the other eye. When radioactive dye is injected into one eye, the dye reaches visual cortex by axoplasmic transport. The cortical picture presents a typical banded pattern. These alternate columns of cells are known as ocular dominance columns. Many of the simple and complex cells of the visual cortex receive input from both the eyes. Thus the visual cortex integrates inputs from both the eyes. The effects of lesions of visual cortex are described under visual field defects.

7. The primary visual cortex (V1) projects to various cortical areas. Area V8 receives the information about color vision; area V3A and M1v are concerned with movement of an object. The LO area is for recognition of larger objects, whereas the functions of area V4v and V7 are not known. The V2, V3 and VP area receive inputs from larger visual fields and are involved in continuous processing of the information.

Other Visual Areas

Visual Association Areas

1. Area 18 and 19 are the visual association areas.

2. They are located on the lateral part of the occipital cortex.

3. They receive visual information from primary visual cortex and are concerned with interpretation of the visual stimulus.

4. They help in appreciating the finer attributes of the objects like perception of form, texture, shape, depth, location and orientation of the object.

5. Lesions of the association areas produce impairment of these higher visual functions.

Frontal Eye Field

1. The frontal eye field (Area 8) is present in the frontal gyrus.

2. It controls saccadic eye movements. Convergence, divergence and near response is influenced by activity in an area anterior to the frontal eye fields.

Superior Colliculi

1. The superior colliculus is divided into superficial and deep layers.

2. The superficial layers receive inputs from the optic tract fibers and visual cortex and in turn project to various thalamic nuclei like anterior thalamic nucleus, pulvinar and lateral geniculate body.

3. The neurons of the superficial layer receive inputs from both the eyes and they discharge in response to a stimulus moving quickly in a particular direction.

4. Thus, the superior colliculi coordinate simultaneous bilateral eye movements, like saccades and convergence and keep the eyeball in focus.

5. The deep layers of superior colliculi receive visual inputs from its superficial layers, inputs from the auditory fibers and somatosensory inputs from different body parts.

6. Also the neurons of the deep layers regulate the head and eye position. The sensory stimuli are integrated in the deep layers for various reflex activities that involve appropriate head movement and change in eye opposition.

Pretectal Nucleus

1. Fibers from the optic tract project to the pretectal nucleus and cause activation of the Edinger-Westphal nucleus.

2. These fibers mediate pupillary reflexes and are involved in the regulation of visual fixation.

Suprachiasmatic Nucleus

1. It is a small nucleus in the medial hypothalamus, located just above the optic chiasm.

2. It receives fibers from the optic nerve and synchronizes sleep-wake cycle, secretion of hormones like cortisol, and other circadian rhythms with light-dark cycle.

3. Information from the visual receptors regarding light intensity during diurnal cycle gets transmitted to the suprachiasmatic nucleus and entrains its biological clock activity.
CHAPTER SUMMARY

**Key Concepts**

1. Lesions at a particular level of visual pathway leads to specific visual field defects that helps to assess the site of lesion.
2. The brain areas activated by visual stimuli are area 17, LGB of thalamus, area 18, 19, area 8, superior colliculi, pretectal nucleus, suprachiasmatic nucleus, pulvinar and anterior nucleus of thalamus. Apart from these areas, some other areas are also activated, such as parts of inferior temporal cortex, posteroinferior parietal cortex, amygdale, caudate nucleus, putamen and claustrum.

**Important to Know (Must Read)**

1. In examination, **Long Questions** are usually not asked from this chapter.
2. Effect of lesions at various levels of visual pathway is asked as a **Short Question**.
3. In **Viva**, examiner may ask... Name the areas or pathways for processing of visual signal, Macular sparing, What is the on-center and off-center response of the ganglion cells, What are important features of retinal neurons, Cortical visual areas.
VISUAL ACUITY

Visual acuity is defined as the ability of the eye to identify two closely placed points as two distinct points. The shortest distance, by which two objects can be separated and still be visualized as two different objects, is known as the minimum separable distance. Visual acuity expresses the resolving power of the eye, that is, the extent to which the eye can perceive the details and contours of an object. Visual acuity is the function of the cones. There are different tests for testing visual acuity for distant vision and near vision.

Factors Affecting Visual Acuity

Optical Factors
These factors mainly determine the degree of visual acuity. They include the structures that take part in the image forming mechanisms of the eye and help in focusing an object, such as:
- Curvature of the cornea,
- Curvature of the lens,
- Plasticity of the lens, and
- Condition of the ciliary muscle and lens ligaments.

Retinal Factors
Visual acuity depends on the functional status of the cones. As the cones are concentrated more at the fovea, visual acuity is highest at the fovea and decreases toward the periphery.

Stimulus Factors
Various features of an object affect the visual acuity, such as:
- Size of the object,
- Distance of the object from eye,
- Color of the object,
- Contrast between the object and the background,
- Shape of its borders,
- Brightness of the object, and
- Duration for which the object remains in view.

Visual acuity increases as the size of the object increases and distance of the object from eye decreases. Thus, visual acuity is directly proportional to the visual angle. Visual acuity is more for white object compared to any colored object. But the contrast between the object and the background plays a more important role than the color per se.

Tests for Visual Acuity

Test for Distant Vision
1. Visual acuity is tested with the help of Snellen’s chart that contains rows of black capital letters against a white background. The rows are numbered as 60, 36, 24, 18, 12, 9, 6 and 5 from top to bottom; and the size of the letters in each row gradually decreases.
2. The number below each row depicts the distance in meters from which the letters can be read by a normal eye. The subject is asked to read the chart with each eye separately from a distance of 6 meters. The number denoting the lowest row, which he is able to read is noted.

3. Visual acuity is calculated by using the formula \( d/D \), where \( d \) is the distance at which letters are read and \( D \) is the distance at which letters can be read by a normal eye. For example, if a person can read by his right eye the letters designated by the number 18, the visual acuity for right eye will be \( 6/18 \). The normal visual acuity is \( 6/6 \) for each eye.

**Principle:** The letters of the Snellen’s chart are designed in such a way that the width of each point of a letter subtends an angle of 1 minute at the nodal angle (Fig. 146.1). Thus, for any closely placed two points, the minimum separable distance should draw an angle of 1 minute at the nodal angle.

**Test for Near Vision**

Near point of vision is tested by Jaeger’s chart, which the subject is asked to read from ordinary reading distance. The chart contains paragraphs of different letter sizes (fonts).

**VISUAL FIELD**

1. The area of the external world that can be seen when the gaze is fixed is known as the visual field of that eye (Fig. 146.2).

2. The visual field is limited on the medial side by the bridge of the nose and superiorly by the roof of the orbit, so that the field is not circular.

3. The visual field plotted with one eye is known as **monocular visual field**. The visual field of both eyes overlap in the center and is called **binocular visual field**. An object present in the binocular visual field can be seen by both eyes simultaneously.

4. Each visual field is divided into a **nasal** (medial) and a **temporal** (lateral) portion. For each eye, light rays from an object situated in the nasal field stimulate the temporal retina and light rays from an object situated in the temporal field stimulate the nasal retina.

5. Also, light rays from objects located at the upper part of the visual field stimulate the lower part of retina and those from the lower part of the visual field stimulate the upper part of retina.

**Binocular Vision**

When a person looks at an object with both eyes, the light rays coming from the object stimulate both the retinas. The action potentials transmitted by the neural pathways from both retinas get fused at the visual cortex, so that the brain perceives them as single image. Thus, viewing an object as a single one with both eyes is called **binocular vision**.

**Corresponding Points**

1. The two points on the retinas on which the light rays from an object fall simultaneously to make binocular vision possible, are known as **corresponding points**. The foveas and all points lying at the same distance and in the same direction from the foveas are corresponding points.

2. For a distant object, the eyeballs move symmetrically and for a near object, the eyeballs converge to allow the light rays to fall on corresponding points.

3. When light rays fall on non-corresponding points, double vision (diplopia) results. For example, while looking at an object if you slightly push one eyeball out of the line, diplopia occurs. When the light rays from any object do not fall on the corresponding points for a prolonged period, especially in children below the age of 6 years, diplopia is not seen as one of the images get suppressed (suppression scotoma). This is a cortical phenomenon. This suppression does not develop in adults. It should be detected and treated early; otherwise there occurs permanent loss of visual acuity in the eye, in which the image is suppressed. Diplopia also occurs when the medial rectus or lateral rectus muscles are damaged or over-activated.

**Mapping of Visual Field**

This is done to diagnose visual field defects.

**For peripheral field:**

1. The peripheral visual field is mapped by an instrument called perimeter and the process is known as **perimetry**, in which each eye is mapped separately.
2. The perimeter chart paper has circles called isopters around the central point, drawn at 10° intervals across the radius from 0° to 90°. There are radial lines called meridians that join the periphery at 10° intervals.
3. During the procedure one eye is covered, while the test eye is fixed on a central fixation point. A small contrast target is moved slowly from the periphery to the center in selected meridians along the arc of the instrument, completing the full circle. The spot, where the target becomes first visible is marked in the chart paper.

**For central field:**
1. The central visual field comprises of the area within 30° from the central fixation point. It is mapped with Bjerrum's screen.
2. The area where the target is not seen is designated as blind spot or scotoma. The scotoma area is mapped by noting the spot where the object disappeared to the point, where again it reappeared. The physiological blind spot due to optic disc is observed on the temporal visual field.

**Visual Field Defects**

Blindness of visual field is known as anopia. Hemianopia means half blindness, which can be homonymous (same side of both visual fields) or heteronymous.
1. **Lesion of optic nerve** cuts off impulse transmission from that eye and produces blindness in that visual field (Refer Fig. 145.1A, Chapter 145).
2. **Lesion of optic chiasm** damages the nasal fibers from both eyes and produces bitemporal (heteronymous) hemianopia (Refer Fig. 145.1B). This usually occurs in tumors of anterior pituitary, especially of the growth hormone secreting somatotrophs, in which the enlarged pituitary presses upon the optic chiasm and produces bitemporal hemianopia, a feature of acromegaly.
3. **Lesion of one side temporal fibers** produces blindness of that side nasal field. For example, lesion of right side temporal fibers produces right nasal hemianopia. **Lesions of both side temporal fibers** produce binaural hemianopia.
4. **Lesion of optic tract** damages ipsilateral temporal fibers and contralateral nasal fibers resulting in homonymous hemianopia. Lesion of left optic tract produces right homonymous hemianopia; and lesion of right optic tract produces left homonymous hemianopia (Fig. 145.1C).
5. **Lesion of geniculocalcarine tract** damages ipsilateral temporal fibers and contralateral nasal fibers resulting in homonymous hemianopia with macular sparing as macular fibers travel dorsal to the optic radiation and escape the damage (Fig. 145.1D). The visual field defects are similar to that of optic tract.
6. **Lesion of occipital cortex** damages ipsilateral temporal fibers and contralateral nasal fibers with macular sparing. It produces scotoma (blind patches) in the homonymous visual fields, which are usually quadratic in nature (upper or lower quadrant of either half of visual field). The macular fibers are spared unless the damage is widespread because i) the macular fibers project separately in the visual cortex, and ii) they have a much larger representation compared to rest of the retina.

**VISUAL ADAPTATIONS**

The human eye has the ability to discriminate and identify objects under a wide range of illumination. However, when light intensity changes dramatically, the visual system requires some time for adaptation of the eye to respond optimally. The visual system employs several mechanisms that work together in extreme conditions of illumination and help the eye to adapt to achieve optimum efficacy in that situation. The processes involved in visual adaptation can occur in the pupil, in the retina (chemical and neural), or higher up in the nervous system. **Regulation of pupillary size by the iris can vary light intensity by about 16-fold**. Visual adaptation is of two types: light adaptation, and dark adaptation.

**Light Adaptation**

When a person suddenly moves from a dimly lighted area to broad daylight, initially the light seems very bright and uncomfortable and the image appears blurred. The visual system activates several mechanisms to adjust to the bright light so that vision improves after some time. This is known as light adaptation. **Light adaptation takes about 5 minutes**. As an immediate reaction to sudden bright light, the pupil constricts and the amount of light entering the retina is reduced.

**Mechanisms of Light Adaptation**

There are two mechanisms of light adaptation: neural and chemical.

**Neural Adaptation**

1. Following a light stimulus, the sensitivity of retinal photoreceptors decreases that manifests in the form of decreased burst of activity to a constant stimulus.
2. The horizontal cells may have a feedback inhibitory effect upon the photoreceptors. Besides, with the increase in light intensity, the amplitude of receptor response does not rise proportionately. This mechanism plays a greater role in quickly bringing down the sensitivity of the photoreceptors, so that light adaptation is mainly a neural phenomenon.

**Chemical Adaptation**

1. In bright light, rhodopsin is rapidly converted to all-trans-retinal and opsin. Due to this bleaching, rod responsiveness decreases considerably as rhodopsin becomes less available. As the rods fail to respond further to bright light, cones come into play. Thus, with increased light intensity, rods become deactivated and cones get stimulated.
2. When rhodopsin level falls by 7 percent, vision is taken over by the cones. The image appears less bright as cones are less sensitive to light than the rods.

3. Moreover, in the hyperpolarized rods, there is reduced Ca\(^{2+}\) influx that removes the inhibitory effect on guanylyl cyclase. As a result, the cGMP level is restored that increases the sensitivity of photoreceptors after some time.

4. Chemical mechanisms have a lesser role in light adaptation.

**Dark Adaptation**

1. If a person stays for some time in bright sunlight and then moves into a dimly lighted room, he experiences a temporary blindness that improves after a few minutes. During this time his eye adjusts to the low levels of illumination, a phenomenon known as dark adaptation.

2. **Maximum dark adaptation occurs in about 20 minutes.**

3. In dim light, the pupil dilates to allow the maximum possible light through the eye to have optimal vision.

**Mechanisms of Dark Adaptation**

There are two mechanisms of light adaptation: neural and chemical.

**Neural Adaptation**

1. The first phase of adaptation occurs due to decrease in threshold of the cones that occurs within 5–10 minutes. This increases retinal sensitivity to 100 times.

2. In the second phase of adaptation, the rod threshold falls reaching its maximum in about 20 minutes (Fig. 146.3) and the retinal sensitivity increases by 1000–10,000 times.

3. As the rods are more sensitive than the cones, in the low levels of illumination of the darkened room, visual capacity depends mainly on the rods.

**Chemical Adaptation**

1. In the dark, there occurs regeneration of the photopigment rhodopsin. During the exposure to bright light, due to conversion of rhodopsin to all-trans-retinal and opsin, no rhodopsin is available that can be activated by light. Hence, rods become insensitive to light.

2. **Rods cannot respond completely again until rhodopsin is restored to its resting state.** This process requires several minutes, during which the level of rhodopsin rises increasing the sensitivity of the rods proportionately.

3. In the retina, the rod concentration is more in an area that is about 15-20 degrees from the fovea. The dark-adapted retina is most sensitive to light in this region. As a whole, the dark-adapted retina is 10,000 times more sensitive than the light adapted one.

4. Vitamin A is required for the synthesis of retinine. So, vitamin A deficiency adversely affects the dark adaptation ability, the condition known as night blindness, in which the person has impaired vision in the evening and dim light.

The time taken to accomplish full dark adaptation depends on the intensity, duration and wavelength of light to which the eyes were exposed when the person was in bright light. If the light was very bright or if the eyes were exposed for longer period, the dark adaptation requires longer time. Longer wavelength of light like red color stimulates the cones to a fair extent, but rods are relatively insensitive to it. So a person previously exposed to red light becomes rapidly dark-adapted. The aircraft pilots and radiologists who work in dim light are advised to wear red goggles when they are in bright light, so that the rods are less bleached and their sensitivity is maintained. When they enter the dimly lit room, they do not have to wait for 20 minutes as rods are soon dark-adapted.

**VISUAL REFLEXES**

**Light Reflex**

1. When light is focused on one eye, the pupils of both eyes constrict; this is called light reflex. The pupillary constriction occurring in the stimulated eye is called direct light reflex and in the other eye is called consensual light reflex (Flowchart 146.1).

2. The latent period of the response is about 0.2–0.5 sec.

3. **Afferent pathway:** The first order neurons from the retinal ganglion cells project to the pre-tectal nucleus; from here the second order neurons start and proceed to the mid-brain Edinger-Westphal nucleus.

4. **Efferent pathway:** The parasympathetic fibers of the oculomotor nerve form the first order neurons that originate from mid-brain Edinger-Westphal nucleus and project to the ciliary ganglion. The second order neurons start from here and innervate the sphincter pupillae.

**Accommodation Reflex**

1. The pupils of both eyes constrict on looking at a near object; this is called accommodation reflex (Flowchart 146.2).
Afferent pathway: The first order neurons from the retinal ganglion cells project to the LGB; from here, the second order neurons start and project to the area 17, from where the third order neurons start and proceed to the mid-brain Edinger-Westphal nucleus.

Efferent pathway: From the striate cortex via the occipito-mesencephalic pathway, impulse travels to the oculomotor nerve nucleus and then to the sphincter pupillae, ciliary muscle and medial rectus.

Argyll Robertson Pupil

1. In neurological lesions in the pretectal-superior colliculi region of the mesencephalon, the fibers mediating light reflex are damaged.
2. This is the classical pupillary abnormality in neurosyphilis; but it is not seen now days, because syphilis can be effectively treated at its early stage.
3. Here the light reflexes (both direct and consensual) are lost, but accommodation reflex is present (ARP: accommodation reflex present, to remember) because the fibers for accommodation reflex take a different course.

CHAPTER SUMMARY

Key Concepts
1. Lesions at a particular level of visual pathway leads to specific visual field defects that helps to assess the site of lesion.
2. The brain areas activated by visual stimuli are area 17, LGB of thalamus, area 18, 19, area 8, superior colliculi, pretectal nucleus, suprachiasmatic nucleus, pulvinar and anterior nucleus of thalamus. Apart from these areas, some other areas are also activated, such as parts of inferior temporal cortex, posteroiinferior parietal cortex, amygdale, caudate nucleus, putamen and claustrum.

Important to Know (Must Read)
1. In examination, Long Questions are usually not asked from this chapter.
2. Visual acuity and factors affecting it, Visual field, Light adaptation, Dark adaptation, Light reflex and consensual Light reflex, Argyll Robertson Pupil may be asked as Short Questions.
3. In Viva, examiner may ask… Define Visual acuity and say the factors affecting it, Test for distant vision, Test for near vision, Define Visual field, What are the corresponding points, Light adaptation and its mechanisms, Dark adaptation and its mechanisms, Pathway of Light reflex and consensual Light reflex, Pathway of accommodation reflex, Argyll Robertson Pupil.
The human eye is sensitive to the light rays in the visible spectrum. There are seven colors in the visible spectrum, called spectral colors. They are violet, indigo, blue, green, yellow, orange and red (VIBGYOR), with wavelengths ranging from 400–750 nm. Human eye does not respond to electromagnetic radiations beyond the visible spectrum, for e.g. ultraviolet (< 400 nm) and infrared (> 750 nm) rays. Some animals like bats can see infrared rays. The colors produced by mixing two or more spectral colors are known as extra-spectral colors like brown or purple. Human beings have the ability to identify more than 100 different colors.

Characteristics of Color

There are three characteristics of color: intensity, hue and saturation. In abnormalities of color vision, one or more of these characteristics are decreased.

Primary Colors

There are three primary colors: red, green and blue. Any color can be produced by mixing various proportions of these three colors. The primary colors when mixed in equal proportions produce white color.

Complementary Color

Each color has a complementary color. When any color is mixed with its complementary color, the mixture appears white. For example, purple and green, orange and blue, yellow and dark blue are complementary colors.

Effect of Illumination

The color perceived depends not only on the wavelength but also other factors like illumination. When the spectral colors are seen in dim light, the brightest part of the spectrum is green, as the rods are most sensitive to the green light. When viewed in high illumination, for e.g. in daylight, the brightest part of the spectrum is yellow, as the cones have maximum sensitivity in the yellow region. Thus, the brightest part of the spectrum shifts to left during scotopic vision and shifts to right during photopic vision. This shift of the maximum sensitivity to the visible spectrum with change in illumination is known as Purkinje shift phenomenon.

Effect of Background Color

The color perceived by the eye depends on the background color. For example, green letters against a green background is hardly visible, whereas they are seen as green against yellow or red background.

Effect of Intensity

For the same wavelength of light, when the intensity of the color is increased or decreased, different shades of the color are perceived.
Cones are receptors for color vision. The information about color sensation is processed at different levels of the visual pathway. The color perceived by a person is determined by the type, number and degree of cone stimulation as well as the number and pattern of action potentials in the neural pathways. There are two theories of color vision; pigment theory and opponent theory.

THEORIES OF COLOR VISION

Pigment Theory

This theory is also known as Young-Helmholtz theory as it was proposed in 1801 by Thomas Young and later modified by Hermann von Helmholtz. It is also known as Retinal or Trichromatic theory.

1. There are the three types of cones and each has a pigment with a different absorbance spectrum. The red-sensitive pigment absorbs light maximally in the yellow portion of the spectrum, the green-sensitive pigment absorbs maximally in the green portion and the blue-sensitive pigment absorbs maximally in the blue-violet portion.

2. Traditionally, the cones and cone pigments are called red, green and blue respectively. Now, they are called L (long-wavelength), M (middle-wavelength) and S (short-wavelength) as they show optimal sensitivity to lights of wavelengths of 560 nm, 530 nm, and 420 nm respectively.

3. As evident from the graph (Fig. 147.1), each type of cone pigment can absorb a wide range of wavelengths. Also, a particular color (light of a fixed wavelength) produces different levels of stimulation in various cones.

4. The visual cortex compares the relative frequency of action potentials in the activated cone pathways and deciphers the wavelength and thereby, makes out the color. For example, a green monochromatic light with a wavelength of 530 nm produces excitation of the red, green and blue cones at a ratio of 31:67:36, and interpreted as green.

5. The change in intensity is perceived by appreciating the change in wavelength. Stimulation of only one type of cones does not encode any color perception. At least two types of cones are required for color vision.

6. According to the Young-Helmholtz theory, the color vision is due to presence of three types of cones each maximally sensitive to one of the three primary colors. Therefore, normal color vision depends on the integrity of the cones. Absence or malfunction of one or more than one type of cone cells lead to different forms of color blindness.

7. The fovea mediates color vision as it contains densely packed cones. However, fovea contains only M and L types of cones. Thus, it can distinguish fine spatial details of a colored object, but its capacity to discriminate different colors is less compared to the extra-foveal regions. As already described above, the color perception is altered by the illumination, background color and intensity of the light.

8. The gene for human rhodopsin is located on chromosome 3. The gene for the blue-sensitive cone pigment is located on chromosome 7. The genes for the red-sensitve and the green-sensitive cone pigments are arranged in tandem on the q arm of the X-chromosome.

9. Humans, apes and old world monkeys are trichromats; i.e., they have all the three cone systems. Many mammals are dichromats; i.e., they have only two cone pigments, a blue-sensitive and a red-sensitive pigment.

Opponent Theory

This theory is also known as Hering’s theory of color vision as it was proposed by Hering. It is based on his observation that there is no greenish-red or bluish-yellow color. The red and green colors oppose each other, and the blue and yellow colors are opposed. The ganglion cells, the cells of the LGB and the neurons of the visual cortex show color-opponent property.

Single-opponent Property

1. The ganglion cells and the cells of LGB are single-opponent type. There are two subtypes.

2. Some cells are excited when a red stimulus is present in the center of the receptive field, and inhibited when a green stimulus is present in the periphery of the receptive field. Also, these cells are inhibited by a green center and excited by a red periphery.

3. The other type of cells shows increased discharge rate to a green center and the discharge rate decreases in
response to a red periphery. They are also activated by a green periphery and inhibited by a red center.

**Double-opponent Property**

1. The cells of the visual cortex are **double-opponent type**. They are of two types.
2. One type of cells are excited by a red center with green periphery; and inhibited by a green center with red periphery.
3. The other type of cells are excited by a green center with red periphery; and inhibited by a red center with green periphery.

**Transmission and Encoding of Color Signals**

1. The P ganglion cells receive information regarding color from the cone cells and transmit to the parvocellular layer of the LGB.
2. Processing of impulses start at the ganglion cell level where inputs from different types of cones are added or input from one type of cone is subtracted from the input from another cone.
3. Impulses from the LGB are transmitted to the deep layer 4 C of V1 and blob region of the visual cortex through specific neural pathways. The blob region of visual cortex contains clusters of cells that have high concentration of the enzyme cytochrome oxidase.
4. A red-green pathway transmits the subtracted inputs of the L- and M-cone responses; a blue-yellow pathway signals the differences between the S-cone and the sum of L- and M-cone responses, and a luminance pathway that transmits the added inputs of the L- and M-cone responses. Information from the deep layer 4 C of V1 and the blobs is transmitted to the V8 region of the visual cortex.

**COLOR BLINDNESS**

Total loss of color vision is known as achromatopsia. In partial loss of color vision, the inability to distinguish certain colors is termed as color blindness. It affects 8% males and 0.4% females. The most common type is red-green color blindness. Color blindness is described with the help of the following terms.

**Use of prefix:** Prefixes like prot-, deuter- and trit- are used to describe the red-, blue- and green- cone system defects respectively.
Use of suffix: The suffix **anomaly** means color weakness and the suffix **anopia** means color blindness.

**Classification**

Color blindness is classified in the following manner:

**I. Trichromats**

This is the most common type. About 6% of affected males are trichromats. These are individuals with all three-cone systems, but one of the cone systems is defective. Thus, the ability of the individual to recognize one of the primary colors is decreased. Accordingly, trichromats can have one of the following defects:
- Deuteranomaly (most common)
- Protanomaly
- Tritanomaly

**II. Dichromats**

About 2% of affected males are dichromats. These are individuals with two-cone systems and the third cone system is absent. Thus, a dichromats can have any of the following:
- Deuteranopia (most common)
- Protanopia
- Tritanopia

**III. Monochromats**

These are individuals with only one cone system present. Thus, they cannot appreciate any color, because minimum two cone systems are required to appreciate a color. They see only black and white and shades of gray.

**Etiology**

The cause can be inherited (genetic) or acquired, due to lesion or drugs.

**Genetic**

1. Color blindness is inherited as an X-linked recessive disorder. As males contain XY chromosomes in their germ cells, they develop color blindness if the X chromosome contains the defective gene. On the other hand, the females develop color blindness only when both the X-chromosomes are defective, which is rare.
2. The female having one defective X chromosome remains symptom free, but transmits the disease to half of her sons. Thus, females are the carriers and males are the sufferers.
3. All the daughters of a colorblind person become carriers and half of their sons suffer from the disease. Thus, color blindness manifests in every second-generation males.
4. Deuteranomaly and protanomaly are the most common defects. Tritanomaly and tritanopia are rare and occur equally in both males and females.

**Lesion**

Following lesion of the striate cortex (area V1), the individual suffers from complete loss of color vision (achromatopsia).

**Drugs**

Drugs like sildenafil (Viagra) inhibits the enzyme phosphodiesterase. Thus, individuals develop transient blue-green color weakness till the effect of the drug lasts.

**Tests for Color Blindness**

Color vision is tested by the following ways.

**Ishihara Chart**

1. It is the most routinely used test for color blindness in clinics.
2. It consists of lithographic plates in which numerals or pathways are drawn in colored spots against a background of spots of different colors and sizes (Fig. 147.4).
3. The subject reads the number and traces the pathway. The colors of the numerals or tracings and the background colors are arranged in such a way that a colorblind person reads them differently or traces a different pathway from a normal person.

**Edridge-Green Lantern Test**

In this test, the light from a lantern is shown through different colored glass pieces fixed on a rotation disc and the subject is asked to name the colors.

**Holmgren’s Wool-matching Test**

The subject is asked to perform a series of color matching from a collection of wools of different colors.

Test for color blindness is usually done as part of health check up for entering into professions like driving, flying, traffic services, railways, armed forces and medicine. When there is loss of visual acuity, color vision is also affected. But in conditions like bilateral lesions of the inferomedial occipital region or optic neuritis, color vision is more affected.
## CHAPTER SUMMARY

### Key Concepts
1. There are two theories of color vision: Retinal (Young-Helmholtz) theory and Neural (Opponent) theory.
2. Color blindness is classified into trichromats, dichromats, monochromats.
3. Color blindness is tested by Ishihara chart, Edridge-Green Lantern test, Holmgren’s wool matching test.

### Important to Know (Must Read)
1. ‘Describe the theories of color vision’ may come as a Long Question.
2. Color blindness may be asked as Short Questions.
3. In Viva, examiner may ask… Name the theories of color vision, What are the types and causes of color blindness, What are the tests of color blindness.
EXTRAOCULAR MUSCLES

The extraocular muscles (EOM) originate on the bone of the orbit and insert on the sclera. They are lateral rectus, medial rectus, superior oblique, inferior oblique, superior rectus, and inferior rectus (Fig. 148.1).

Nerve Supply of EOM

The lateral rectus muscle is supplied by the abducent nerve and the superior oblique by the trochlear nerve (LR\textsubscript{6}, SO\textsubscript{4}). The oculomotor nerve innervates rest of the extraocular muscles.

Action of EOM

Normally, the eye can move 50° medially, 30° upwards and 50° downwards. When the eyeball is placed temporally (abducted position), the superior rectus causes elevation and the inferior rectus causes depression of the eyeball. When the eyeball moves nasally (adducted position), the superior and inferior oblique elevate and depress it respectively. In the mid-position of the eyeball, the lateral and medial recti cause lateral and medial movement of the eyeball respectively (Fig. 148.2). The actions of the various extraocular muscles are shown in the Table 148.1.

Cardinal Movements

The vertical and horizontal movements of the eyeball made from the mid-position of the gaze are called \textbf{cardinal movement}.

**Fig. 148.1:** Extraocular muscles.

**Fig. 148.2:** Action of the various extraocular muscles.
Chapter 148: Movements of the Eye

Conjugate Movement
When a person looks at an object, the eyeballs move symmetrically and the visual axes of both eyes meet at one point. This is known as conjugate movement of the eyes. It is possible due to integration of the afferent inputs of the III, IV and VI cranial nerves at the level of the brainstem.

Types of Ocular Movements
Various types of movements occur in the eyeball that helps to visualize the object clearly by controlling the extraocular muscles. The eye movements depend on an intact visual cortex. There are four types of eye movements: saccades, smooth pursuit movements, vergence and vestibular movements. The first three movements are described in relation to a stationary head.

Saccades
These are rapid, jerky movements that occur when the gaze shifts from one object to the other. Their function is to keep the new object in focus by changing the orientation of the eyeball. Thus, they prevent the adaptation of neurons in the visual pathway and reduce the strain on the extraocular muscles by bringing out the change. When the gaze is fixed on an object for longer period, the extraocular muscles remain contracted to maintain the position of the eyeball and this may lead to muscle fatigue. These movements are regulated in the frontal cortex and superior colliculi.

Smooth Pursuit Movements
These are the tracking movements of the eyes as they follow moving objects. These movements are integrated in the cerebellum.

Saccades and pursuit movements are commonly experienced while looking out of the window of a moving train. As one looks at an object, initially, pursuit movement follows the object and the eyeball rotates sideways till its maximum. When the object cannot be viewed anymore, the eyeball rapidly moves to the earlier position to fix the gaze on a new object.

Vergence
These movements occur when an object comes near or moves far from the eye. For example, if an object comes near in the midline, both the eyeballs turn medially (convergence), and if it goes away, both eyeballs rotate laterally (divergence). So, for a single eyeball, when an object moves closer in the nasal field of vision, convergence occurs; and when it moves closer in the temporal field of vision, divergence occurs.

Vestibular Movements
When the head moves, to keep the object in focus, the eyeball moves in response to stimuli arriving from the semicircular canals. Vestibular movements occur while standing/sitting on a unsteady platform (e.g. a ship) and looking at an object.

Nystagmus
The involuntary, rhythmic, oscillatory movement of the eyeball is known as nystagmus.

Table 148.1: Innervation, function and effect of paralysis of external ocular muscles.

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Innervation</th>
<th>Function</th>
<th>Effect of paralysis</th>
</tr>
</thead>
</table>
| Lateral rectus  | Abducent (VI cranial nerve) | Outward movement of the eyeball (Abduction) | Due to the unopposed action of the healthy medial rectus muscle, the following features are seen:  
  i. Inability to move the eye outward  
  ii. Diplopia (on trying to look in outward direction)  
  iii. Convergent squint |
| Superior oblique| Trochlear (IV cranial nerve) | Downward movement of the adducted eyeball | i. Inability to move the adducted eye downwards  
  ii. Diplopia (while looking down from mid position of gaze) |
| Medial rectus   | Oculomotor (III cranial nerve) (Adduction) | Inward movement of the eyeball and IV cranial nerves, there are outward and downward deviations of the eyeball | In lesion of oculomotor nerve:  
  • Due to unopposed action of VI  
  • Paralysis of individual muscle is rare. However, if it occurs, the action of the affected muscle is lost  
  The other features of oculomotor nerve palsy are:  
  • Ptosis (drooping of eyelid)  
  • Dilated and fixed pupil  
  • Loss of accommodation (as the oculomotor nerve supplies eyelid, constrictor pupil and ciliary muscles) |
| Superior rectus | Oculomotor (III cranial nerve) | Upward movement of the abducted eyeball |  |
| Inferior rectus | Oculomotor (III cranial nerve) | Downward movement of the abducted eyeball |  |
| Inferior oblique| Oculomotor (III cranial nerve) | Upward movement of the adducted eyeball |  |
producing minute oscillations at a rate of 30-80 cycles per second. This is called **physiological nystagmus**.

2. Due to these small tremor-like movements, the image constantly moves over a small area of the retina. As the same neuron does not get stimulated continuously, the adaptation of neurons in the visual pathway is prevented.

3. Though the stimulus first activates the photoreceptors, it is the neurons that get adapted earlier than the photoreceptors. If the image falls on the same spot of the retina, the neural discharge gradually decreases and the object disappears from view.

4. Thus, the physiological nystagmus helps the eyes to see the object clearly for a longer duration. As the oscillations help to fix the gaze on an object, they are also called **fixation movements**.

When the eyes follow a rapidly moving object, **optokinetic nystagmus** is seen. The eyes slowly follow the object and then quickly come back to the initial position of gaze by a rapid saccade.

### Pathological Nystagmus

In some pathological conditions, these oscillatory movements become noticeable. Nystagmus can be due to disorders of vestibular system or lesion of the neural pathways controlling the ocular movements.

### Abnormalities of Eye Movements

Abnormalities of eye movements are summarized in Table 148.1. In case of paralysis of eye muscles, abnormalities like diplopia, strabismus and loss of accommodation occur.

### Depth Perception

1. The two eyes are positioned slightly away from each other. So, each eye receives a little different image of the same object compared to the other.

2. Fusion of both the images at the level of visual cortex helps to view the object as a single one, but the difference helps in depth perception (stereoscopic vision).

3. The depth perception ability is greatly reduced in case of blindness of one eye.

### Electro-oculography

1. It records the effect of light and dark adaptation on the retinal resting potential generated at the pigment epithelium.

2. The eyeballs move laterally for placing electrodes at the inner and outer canthi.

3. The retinal resting potential is 6 mV across the pigment epithelium with the receptors side being electrically positive and the choroidal side electrically negative.

4. EOG is useful in the diagnosis of retinal dystrophies and degenerative diseases like retinitis pigmentosa.

### Electoretinography

1. Recording of electrical activities of the retina by stimulating it with a flash of light is called **electoretinography**.

2. With fully dilated pupil and following application of local anesthetic, it is performed by placing contact lens electrode (recording electrode) on the cornea, reference electrode on the skin of the forehead, and reference electrode over the earlobe.

3. Electoretinogram (ERG) has an initial rapid downward negative deflection a-wave that appears after a latent period, followed by a bigger upward positive deflection b-wave of short duration, and following this, a prolonged small amplitude positive c-wave (Fig. 148.3).

4. The a-wave originates from the retinal photoreceptors; the b-wave originates from the bipolar cells and the c-wave originates from the pigment epithelium.

5. ERG assesses the functional integrity of the retina. It gives information about the rods located at the periphery of the retina and their connections. It is helpful in the diagnosis of retinal detachment, retinal dystrophy, vitamin A deficiency, etc.

### Visual Evoked Potentials

1. It is the recording of electrical activities generated in the visual pathway from retina to the visual cortex in response to light or pattern stimulation of the eye.

2. It is done by placing scalp electrodes over the occipital cortex. A chain of waves are produced at various latencies, out of which P100 is a large positive potential that occurs after 100 milliseconds after application of the stimulus.

3. Abnormalities in the latency and amplitude of various waveforms are helpful in the diagnosis of various diseases, like optic neuritis, optic atrophy, refractive errors, color blindness, etc.
CHAPTER SUMMARY

**Key Concepts**

1. Nerve supply of the extraocular muscles: Supraoblique by Trochlear nerve, Lateral rectus by Abducent nerve (SO4 LR6), others by Oculomotor nerve.
2. Abnormality of eye ball movement leads to diplopia.
3. The involuntary, rhythmical, oscillatory movement of the eyeball is known as **nystagmus**.

**Important to Know (Must Read)**

1. In examination, **Long Questions** are usually not asked from this chapter.
2. Actions of extraocular muscles, Nystagmus may come as **Short Questions**.
3. In **Viva**, examiner may ask… Name the eyeball movements and the cranial nerves for that, What is physiological and pathological nystagmus, What are visual evoked potentials, What is electroretinography.
The ear contains receptors called **hair cells** for two important sensations: **hearing** and **equilibrium**. Anatomically, the ear is divided into three parts: the **external (outer) ear**, the **middle ear** and the **internal (inner) ear**. The inner ear houses both the **organ for hearing** (the auditory apparatus or cochlea) and the **organ for equilibrium** (the vestibular apparatus). The external ear, middle ear and the cochlea of the inner ear are components of the hearing system.

The vestibular apparatus consisting of the saccule, utricle and three semicircular canals is concerned with the main maintenance of body equilibrium, detail mechanism of which is discussed in last chapters of motor physiology.

**EXTERNAL EAR**

It consists of the auricle (**pinna**) and external auditory canal. The small portion of cartilage in front of the opening of the auditory canal is known as **tragus**. The external ear collects sound waves and channels them inward.

**Pinna**

The pinna is a flap of elastic cartilage covered by skin. In humans, the pinna is fixed, but in many animals, it is movable, and can be oriented in the direction of sound. The rim of the auricle is called the **helix** and the lower soft tissue portion is called the **lobule**.

The pinna has the following functions:

1. It **funnels the sound waves** into the auditory canal to reach the tympanic membrane.
2. It helps to establish the **direction of sound waves**, i.e. to know whether they are from the front or the back or from the above or below.

**EXTERNAL AUDITORY CANAL**

1. This canal (meatus) is a 2.5 cm long tube in the temporal bone extending from the auricle to the eardrum (Fig. 149.1).
2. It **conducts the sound waves** to the **middle ear**. The external one-third of the canal is cartilaginous and the internal two-third is bony.
3. The canal contains sebaceous glands that secrete **cerumen** (earwax) and fine hairs that line its wall. The oily cerumen and the hairs prevent entry of dust and foreign particles into the ear. Excessive production of sebum along with lack of proper cleaning of the external meatus leads to hardening and **impaction of the wax** that blocks the passage and produces conduction deafness. The **pinna and the external auditory canal together produce a 15 dB increase in the sound intensity** due to their characteristic shape.

**MIDDLE EAR**

1. The middle ear (tympanic cavity) is a rectangular air-filled compartment in the temporal bone (Fig. 149.2).
2. It is lined by epithelial cells and has **four walls**, a floor and a roof. The **anterior wall** opens to the exterior via the eustachian tube at the level of the nasopharynx.
3. The posterior wall communicates with the mastoid air cells (air cavities in the mastoid process of the temporal bone) through a space called the tympanic antrum. When infections of the middle ear spread to the mastoid air cells, they cause mastoiditis.

4. The medial wall contains two small membrane-covered openings (the oval window and the round window) and separates the middle ear from the internal ear.

5. The lateral wall is formed by the tympanic membrane that separates the external ear from the middle ear.

6. The bony floor separates the middle ear from the jugular fossa.

7. The roof of the middle ear is formed by tegment tympany that separates it from the middle cranial fossa.

8. The middle ear contains three bony ossicles and two ossicular muscles.

**Tympanic Membrane**

1. Tympanic membrane (eardrum) is a thin elastic semitransparent conical membrane about 50–90 mm² in surface area, with its apex (called umbo) toward the middle ear.

2. It is made up of collagen and elastic fibers. Its inner surface is lined by mucous membrane and its outer surface is covered by skin. Inflammation of the tympanic membrane is known as tympanitis.

**Functions**

1. The eardrum receives the sound waves arriving from the external ear. When sound waves strike the tympanic membrane, it vibrates almost at the same frequency as that of the sound waves. Thus, it is extremely sensitive to the pressure changes and acts as a resonator.

2. The vibrations of the membrane get transmitted to the tiny bone (malleus) attached to it. It stops vibrating soon after the cessation of the sound waves; therefore, it critically dampens the sound waves.

3. Though thin, it stands as a mechanical barrier and prevents the entry of foreign particles, dust and secretions from the outer ear to the middle ear.

**The Auditory Ossicles**

1. These are three small bones, the malleus (hammer), the incus (anvil), and the stapes (stirrup). They extend across the middle ear cavity, one joined to the other (Fig. 149.2).

2. The handle of the malleus (manubrium) is attached to the internal surface of the tympanic membrane. The malleus articulates with the body of the incus that, in turn, articulates with the head of the stapes.

3. The footplate of the stapes is attached to the oval window (fenestra vestibuli). The pressure waves travel...
through the **ossicular chain** and produce **vibrations of the footplate of stapes**, which then transmits the vibrations to the perilymph of the inner ear through **oval window**.

4. In a disease called **otosclerosis**, there is bony fixation of the stapes to the walls of the fenestra vestibuli, resulting in **severe deafness due to loss of the vibrating capacity of the stapes**.

5. The **round window** is present below the oval window and is enclosed by a flexible membrane called the secondary tympanic membrane.

### Ossicular Muscles

Stapedius and tensor tympani are **two tiny skeletal muscles** attached to the **stapes** and the **malleus** respectively.

**Functions**

1. The **tensor tympani** muscle pulls the handle of the malleus inwards, thereby increasing tension of the tympanic membrane. This makes the membrane more responsive to the sound waves.

2. The stapedius is the smallest skeletal muscle in the body. In **response to loud noise**, it contracts and pulls the footplate of stapes out from the oval window, dampening the vibrations transmitted to the inner ear (Application Box 149.1).

3. In paralysis of the stapedius muscle, the patient is abnormally sensitive to loud noises, known as **hyperacusis**.

**Application Box 149.1**

**Tympanic reflex:** Also called **acoustic reflex**, this is a protective reflex that decreases the damage to the inner ear from loud sound. In response to a prolonged loud noise, the tensor tympani and the stapedius contract reflexively (simultaneously in both the ears), causing the outward movement of the malleus and the inward movement of the stapes. This makes the ossicular chain much closer to each other and articulation sites very tight. As a result, the vibration transmitted through the ossicles to the inner ear is reduced, decreasing the degree of stimulation of the receptors (hair cells) present in the inner ear. Thus, the reflex checks over-stimulation of hair cells and prevents the damage to the cochlea. The reflex does not protect the ear from abrupt and brief loud sound like gunshot or bomb blast, as the activation time for the reflex is 40–160 ms.

The middle ear muscles also **contract in response to low-frequency sound waves**. This helps to filter the low-frequency waves and augments the transmission of waves of 1000–2000 Hz (the range of voice communication). The **masking of most of the low-frequency environmental noise** allows the human voices to be audible inspite of noise in the environment. The muscles are active prior to and during vocalization. This minimizes hearing of one’s own voice. They contract before and during any movement that may stimulate the ossicles-like chewing, swallowing, yawning, walking and gross bodily activities and, thereby, suppress the sounds produced by these movements. Contraction of tensor tympani and stapedius related to speech and movements are not reflex responses as they contract prior to these events.

To summarize, the **middle ear muscles** have the following functions. They:

1. Prevent the damage to the cochlea.
2. Improve sound transmission to the cochlea in the range of voice communication while decreasing responses to frequencies above and below this range.
3. Suppress the self-generated sounds.

### Eustachian Tube

This connects the middle ear with the pharynx and equalizes the air pressures on both sides of the tympanic membrane. This helps the membrane vibrate at the same frequency as the sound waves. The pharyngeal end of the tube is normally closed by a valve-like mechanism, but muscle movements open it during swallowing, yawning or sneezing, and the pressure in the middle ear equilibrates with the atmospheric (pharyngeal cavity) pressure (Application Box 149.2).

**Application Box 149.2**

**Eustachian tube dysfunctions:** Altered pressure on either side of the tympanic membrane can stretch it and cause pain. For e.g. **during rapid ascent in an airplane**, the fall in atmospheric pressure causes the tympanic membrane to be pushed out by the higher pressure in the middle ear. The middle ear pressure does not fall due to the closed eustachian tube. **During diving in water or rapid descent** in a plane, the tympanic membrane is pushed in due to increased outside pressure. If the tube is blocked due to common cold, the air in the middle ear is gradually absorbed and the tympanic membrane is sucked in, causing pain and loss of hearing. The discomfort and pain in the ear due to pressure difference are relieved by swallowing saliva or air.

### Functions of the Middle Ear

1. Receives sound waves from the external ear by the tympanic membrane.
2. Transmission of sound waves to the inner ear through the tympanic membrane and the ossicular chain.
3. Amplification of sound waves: The force of sound waves that strike the tympanic membrane increases several times as it reaches the footplate of stapes and oval window. This occurs due to the following two factors:
   
   i. The surface area of the tympanic membrane (50 mm²) is much larger than that of the oval window (3 mm²), the ratio being 17:1. The total force of a sound wave exerted on the tympanic membrane is transmitted to the oval window. But due to the decrease in the surface area, the pressure (force per unit area) increases 17 times.
   
   ii. The ossicular chain acts as a lever while transmitting the vibration of sound waves. As the handle of malleus is 1.3 times longer than the long process of incus, there is a lever ratio of 1.3:1 that enhances the force 1.3 times.

Thus, the total amplification of sound waves occurs about 17 × 1.3 = 22 times. In this manner, the middle ear performs the job of **impedance matching** (Application Box 149.3).
4. Equalization of air pressure through the eustachian tube.

5. Protects against prolonged, loud sound by activation of tympanic reflex.

**Application Box 149.3**

**Impedance matching:** When sound waves travel from air to water, about 99.9% of the sound energy gets reflected away from the surface of water. So, the conduction of sound from air to fluid meets with considerable **acoustic impedance** (resistance). If the sound waves were to strike the oval window directly (without traversing through the tympanic membrane and the ossicular chain), only 0.5% of the sound energy would have reached the endolymph in the inner ear. The **amplification process helps to compensate for the loss.** This is known as **impedance matching** and the middle ear is, therefore, often called an **impedance-matching device.**

### INNER EAR

The internal (inner) ear is situated in the petrous part of the temporal bone. It is called **labyrinth** due to presence of complicated series of canals. Structurally, it is divided into an outer bony labyrinth that houses an inner membranous labyrinth. The **bony labyrinth** contains a fluid called **perilymph** that surrounds the membranous labyrinth. The composition of perilymph is similar to that of cerebrospinal fluid. The **membranous labyrinth** is a series of fluid-filled membranous tubes and sacs, which mimic the shape of bony labyrinth. The fluid present in the membranous labyrinth is called **endolymph** that is rich in K⁺ and resembles intracellular fluid. The membranous labyrinth consists of the auditory apparatus (cochlea) and the vestibular apparatus (the saccule, utricle and the semicircular canals). The function of cochlea is transduction of the sound energy into action potentials in the cochlear nerve fiber. The vestibular apparatus is described under motor physiology.

### Cochlea

The cochlea (meaning **snail’s shell**) is a snail shaped fluid-filled coiled tube. It has an outer bony spiral canal that houses an inner membranous cochlear duct.

**Fig. 149.3:** Membranous labyrinth.

**Bony Cochlea**

1. The bony cochlea is a part of the bony labyrinth. In humans, the cochlea is 35 mm in length and makes two-and-three-fourth (2¾) turns around a central bony core called the **modiolus.**

2. Cochlear vessels and nerves traverse through the base of modiolus. Around the modiolus, a thin plate of bone called **spiral lamina** gives attachment to the basilar membrane.

**Membranous Cochlea**

1. The membranous cochlea (cochlear duct) is like a tube that follows the spiral shape of bony cochlea. It is **filled with endolymph** and is part of the membranous labyrinth (Fig. 149.3).

2. In the cross-sectional view of the cochlea, the **Reissner’s membrane** and the **basilar membrane** divide the cochlea into three fluid-filled compartments known as **scala vestibuli**, **scala media**, and **scala tympani** (Fig. 149.4).

   i. **Scala vestibule:** Scala vestibuli is the gap above the Reissner’s membrane, **filled with perilymph.** At the base of the cochlea, it is separated from the middle ear by the oval window. At the apex of the cochlea, it is continuous with scala tympani through a small opening called **helicotrema.**

   ii. **Scala media:** Scala media is the compartment between Reissner’s and basilar membranes. The space is **filled with endolymph** that is secreted by a layer of fibrous vascular tissue known as **stria vascularis** present along the lateral wall of scala media. The stria vascularis pumps K⁺ into and Na⁺ out of the scala media as its cells have a high concentration of Na⁺-K⁺ ATPase. So, the endolymph is rich in potassium. The cochlear duct is triangular in cross section. The Reissner’s membrane, basilar membrane and the stria vascularis form the three walls of the cochlear duct. The basilar membrane...
houses the organ of Corti that locates the receptor cells for hearing. The two membranes join at the apex of the cochlear duct, leaving a narrow gap between the membranous and bony cochlea, known as helicotrema.

**iii. Scala tympani:** Scala tympani is the space below the basilar membrane, filled with perilymph. At the base of the cochlea, it is separated from the middle ear by the round window, which is enclosed by a flexible membrane called the secondary tympanic membrane.

**The Organ of Corti**

The Organ of Corti or the spiral organ is the specialized structure located on the basilar membrane containing the receptors for hearing, the hair cells (Fig. 149.5). It consists of hair cells, the associated nerve terminals, supporting cells, tunnel of Corti, basilar membrane, tectorial membrane and reticular lamina.

1. When sound waves reach the organ of Corti, it acts as a transducer to convert the mechanical form of energy into action potentials in the cochlear nerve (detailed mechanism is described in the chapter 151).

2. There are two (inner and outer) groups of hair cells situated on the basilar membrane, separated by the rods of Corti.

3. The triangular gap between the rods of Corti is known as the tunnel of Corti.

4. The inner hair cells (closer to the modiolus) are about 3500 in human cochlea. They form a single row and extend the entire length of cochlea.

5. Lateral to the tunnel of Corti, there are present three rows of outer hair cells, about 20,000 in number.

6. The hair cells are surrounded by the supporting or sustentacular cells. The apical ends of the hair cells contain cilia (hair) that pass through the reticular lamina supported by the rods of Corti.

7. The cilia of the outer hair cells project into a thin flexible gelatinous tectorial membrane that covers the rows of hair cells, whereas the cilia of the inner hair cells do not touch the membrane. The viscous membrane contains collagen and glycoprotein and is firmly attached only along its medial edge.

**Hair Cell**

**Structure**

1. The structures of inner and outer hair cells are anatomically similar, but the outer hair cell is longer than the inner hair cell.

2. The hair cell is a cup-shaped structure with fine cilia (hairs) at its apical end (Fig. 149.6), and its basal end makes synaptic connections with the afferent fibers.

3. Unlike the hair cells in vestibular apparatus, there is no kinocilium in the cochlear hair cells and the cilia are known as stereocilia.

4. The height of stereocilia is about 4 to 10 µm and they are 0.2 to 0.8 µm in diameter with a narrow base. The bases of the stereocilia are inserted into the apical part of the hair cell.

5. Each stereocilium has a core of cross-linked and closely packed actin filaments that are surrounded by myosin molecules. There are about 50–100 stereocilia present at the apical surface of the hair cell.

6. The tips of cilia are linked to each other by minute strands called tip-links. Mechanically sensitive cation channels (transduction channels) are present at the junction of cilia with the tip-links. The tip-links help the bundle of stereocilia move as a unit.

7. Along the basilar membrane, the size of inner hair cells does not change, but the size of outer hair cells gradually increases from the base to the apex of the cochlea.

8. The stereocilia of inner hair cells (about 50 in number) are present in three parallel rows, whereas that of outer hair cells (about 100 in number) are present in three rows in a W-shaped formation.

9. The height of the stereocilia gradually increases toward the lateral edge of the hair cell; therefore, the row of stereocilia toward the stria vascularis is the tallest.

10. The tips form a slope, the lower end being towards the modiolus. The tips of stereocilia of outer hair cells are embedded in the tectorial membrane, whereas those of inner hair cells are just short of contact with the tectorial membrane. Along the basilar membrane, the height of stereocilia progressively increases from the base toward the apex of cochlea.

**Innervations of Hair Cells**

1. Each nerve fiber forms synapse with only one hair cell, but a single hair cell gets synaptic connection from 8–30 nerve fibers.

2. The inner hair cells are the actual receptor for hearing. Though they constitute only 15–20% of the hair cell population, they receive about 90–95% of the afferent nerve endings.
3. On the other hand, only 5–10% of the afferent neurons innervate the bases of the outer hair cells.

4. The axons of the afferent nerves form the auditory (cochlear) division of the VIII cranial and project to the brainstem.

5. The efferent neurons (olivocochlear axons) arise from the superior olivary complex in the brainstem. They mainly give projections to the bases of the outer hair cells and form few synapses with the afferent axons of the inner hair cells.

**Fig. 149.6:** Structure of cochlear hair cell at rest; Depolarization of hair cell occurs as stereocilia bend towards the longest stereocilium; Repolarization of hair cell occurs as stereocilia bend away from the longest stereocilium.

**CHAPTER SUMMARY**

**Key Concepts**

1. The external ear directs the sound waves to the tympanic membrane, the middle ear transmits and amplifies the sound waves, the inner ear is for sound perception

2. Impedance matching, equalization of air pressure and protection by tympanic reflex are the other functions of the middle ear.

3. The inner rows of hair cells present in the organ of Corti, in the cochlea of inner ear are the receptors for sound perception.

**Important to Know (Must Read)**

1. In the examination, Long Questions are usually not asked from this chapter.

2. Functions of the middle ear, organ of Corti, Cochlea, Hair cell may come as Short Questions.

3. In Viva, examiner may ask… Name the functions of middle ear, What is impedance matching, What is tympanic reflex, Structure of cochlea, Functions of hair cells.
The auditory pathways in general have five orders of neurons and the neuronal arrangement is a complex one

1. **First-order neurons** (cochlear division of VIIIth cranial nerve) start from the hair cells and project to the ipsilateral dorsal and ventral cochlear nuclei of the medulla oblongata. The cochlear nerves are bipolar neurons whose cell bodies are present in the spiral ganglion located in the modiolus (Fig. 150.1).

2. **Second-order neurons** start from the cochlear nuclei and project to the superior olivary nuclei (bilaterally) and to the nuclei of trapezoid body (contra-laterally). Some of the fibers from the dorsal cochlear nuclei (second order neurons) ascend contra-laterally in the lateral lemniscus to the inferior colliculi.

3. **Third-order neurons** from the superior olivary nuclei ascend bilaterally in the lateral lemniscus to the inferior colliculi. Fibers from trapezoid body ascend ipsilaterally in the lateral lemniscus to the inferior colliculi. Some of the fibers from the superior olivary nuclei (third order neurons) project to the medial geniculate body. Fibers in the lateral lemniscus give collaterals to the reticular nuclei and cerebellum.

4. **Fourth-order neurons** project bilaterally from the inferior colliculi to the MGB of the thalamus.

5. **Fifth-order neurons** start from the MGB and terminate in the primary auditory cortex (areas 41 and 42) situated in the superior temporal gyrus (Fig. 150.2).

6. Characteristically, the middle order neurons in the auditory pathway give extensive bilateral projection. This helps to preserve the auditory capacity even if one of the centers is affected.

7. Moreover, the fibers do not project to single nucleus, rather to **multiple higher centers**. For example, all the second order neurons do not project only to the superior olivary nuclei. This is in contrast to the specific nature of projection of the fibers of the visual pathway.

8. In the auditory pathway, the neurons while ascending up give collaterals to the reticular nuclei and cerebellum. Thus, the body becomes alert and adjusts its posture to protect itself from the source of sound stimulus that may be harmful.

9. There is an orderly **tonotopic organization** of the fibers in the auditory pathway. That means each fiber responds to a given tone and arrangement of fibers is based on the tone of sound. The systematic pattern of fibers is evident from the first order neurons and right up to the primary auditory cortex.

**Dorsal and Ventral Cochlear Nuclei**

The innermost part of the cochlear nuclei receives inputs from neurons responding to high tone that originates from the basal part of cochlea. The peripheral part of the cochlear nuclei receives inputs from neurons responding to low tone that originates from the apical part of cochlea.

**Superior Olivary Complex**

The superior olivary complex is the first nucleus in the auditory pathway that integrates binaural stimuli, which means auditory signals from both ears converge on single neurons. It receives information from the trapezoid body for
comparing timing of auditory stimuli arriving at both ears. All these contribute to the localization of the source of sound. Superior olivary complex receives the ascending sensory fibers and acts as the auditory relay center. It projects bilaterally to the inferior colliculus. It also gives rise to the efferent fibers called olivocochlear bundle.

**Inferior Colliculus and MGB**

The inferior colliculus is divided into a central nucleus (situated in its core) and a peripheral region that has pericentral and external nuclei. The core neurons are tonotopically organized from superficial to deep layers for low to high frequency sounds.

**Peripheral Part:** The peripheral part receives somatosensory information from the medial lemniscus and spinothalamic tract and visual input from the superior colliculus. Inferior colliculus projects to midbrain reticular formation and periaqueductal gray, thus mediates the arousal and attention to auditory stimuli. It projects to the vermal part of the cerebellum that contributes to maintenance of posture in response to sound. For example, startling reaction to noise or dancing to music.

**Central nucleus:** The central nucleus projects to laminated part of MGB, which projects to deep layers of auditory cortex. The peripheral nuclei project to nonlaminated part of MGB, which projects to superficial layers of auditory cortex. MGB and auditory cortex mediate conscious perception of sound.

**Auditory Cortical Areas**

**Primary Auditory Cortex**

1. The primary auditory cortex (Brodmann’s area 41) is located in the sylvian fissure, in the superior portion of the temporal lobe.
2. The auditory map of cochlea has a point for point representation in the cortex of the animals. In the primary auditory cortex of humans, high tones are represented posteromedially and low tones are represented in its anterolateral part.
3. Similar to the visual cortex, the auditory cortex has isofrequency columns. When an electrode enters vertically, it comes across neurons all of which code for the same frequency.

**Other Auditory Cortical Areas**

There are other auditory cortices as well as auditory association areas that integrate auditory inputs with motor speech area (words to be spoken after listening), with visual areas (words to be written after listening), with motor areas (playing an instrument after listening to the music) and so on. These cortical areas show distinct hemispheric specialization.

1. Usually the left hemisphere is concerned with the analytical aspect whereas the right hemisphere processes the subjective or abstract features of the auditory signals. Thus, they exhibit marked cortical asymmetry while processing the auditory inputs.
2. Besides, the auditory areas show great cortical plasticity. Based on the modified inputs arriving in the auditory cortices, the size and extent of the different areas change by the learning and experience of the individual. It is a known phenomenon that people who become blind at an early age have a greater acuity of hearing; they can better localize the source of a sound stimulus than people with normal vision. Also, trained musicians who can better perceive and differentiate various tones have larger auditory areas responding to tone.

Brodmann’s area 22: Brodmann’s area 22 processes auditory signals related to speech. In the right hemisphere, it processes the intensity, pitch and melody of speech. In the left side, it helps in understanding the words that is being spoken.

Planum temporale: Planum temporale, a part of the posterior superior temporal gyrus processes auditory signals related to language. This area is larger in the left hemisphere than in the right cerebral hemisphere. However, the left side planum temporale in musicians is bigger than that in a normal person.

**Efferent Pathways**

(Descending Fibers from Auditory Cortex)

**Pathways**

The efferent fibers originating from auditory cortex project to the medial geniculate body, inferior colliculus and cerebellum via pons. The fibers from inferior colliculus project to the superior olive and cochlear nuclei. The efferent fibers arising from the superior olivary complex are known as the olivocochlear bundle. These fibers descend down and innervate the outer group of hair cells of the ipsilateral as well as contralateral organ of Corti. The olivocochlear fibers are cholinergic that secrete acetylcholine at their nerve endings that hyperpolarizes them.

**Functions**

1. Stimulation of these efferent fibers hyperpolarizes the outer hair cells and elongates them, whereas inhibition of the efferent fibers depolarizes the outer hair cells and shortens them.
2. The flexibility is due to presence of a membrane protein called prestin, a motor protein in the outer hair cell membrane.
3. The olivocochlear fibers modulate the transmission of nerve impulses in the afferent fibers. This enhances the pitch discrimination ability and increases the amplitude and clarity of sound waves.
4. The efferent modulation also augments the capacity to distinguish sounds in the presence of noise and protect the inner hair cell by dampening their responses to loud music.
5. The olivocochlear fibers also give projection to the cochlear nucleus, where they influence the transmission of impulses from first order to second order neurons. The descending fibers from the higher nuclei also influence the ascending fibers.

**CHAPTER SUMMARY**

**Key Concepts**

1. The auditory pathway starts from the hair cells in the cochlea and the fibers travel as auditory nerve.
2. The action potentials travel to auditory cortex through cochlear nuclei and medial geniculate body.
3. Brodmann’s area 41 is the primary auditory cortex.

**Important to Know (Must Read)**

1. In examination, Long Questions are usually not asked from this chapter.
2. Auditory pathways may come as Short Questions.
3. In Viva, examiner may ask… Name the auditory pathways, auditory cortical areas.
CHAPTER 151
Mechanism of Hearing

Learning Objectives
On completion of study of this chapter, the student **WILL** be able to:
1. Mention the properties of sound waves.
2. Understand the process of transmission of sound waves in the middle and inner ear.
3. Explain the transduction mechanism of sound waves.
4. Describe the mechanism of hearing.
5. List the differences between the cochlear microphonics and action potentials.

Sound Waves
Sound waves are alternate compression and rarefaction of molecules that strike the tympanic membrane to produce the sensation of sound. The waves originate from a vibrating source (like vocal cord, tuning fork, etc.) and travel through an elastic medium, like air or water.

**Speed**
1. Sound waves travel in the air at a speed of 340 m/s at 20 °C at sea level.
2. The speed is **more in hot environment and high altitude**.
3. The speed decreases with decrease in temperature.
4. Sound waves travel with a **much greater speed in water** and the speed is **more in seawater than fresh water** (in fresh water, the sound waves travel with a speed of 1400 m/s).

**Frequency**
1. The frequency of sound waves is expressed as the **number of waves per second**. Its unit is **Hertz** (1 Hz = 1 cycle per second). The audible range of frequency for human ear is 20 to 20,000 Hz, but the ear is more sensitive to sounds with frequencies of 1000–4000 Hz.
2. The frequency is also expressed as tone or pitch. A sinusoidal sound wave having one frequency is called a **pure tone**; for example, 50 Hz or 100 Hz.
3. A **complex sound** wave contains more than one frequency; they are perceived as musical sounds if regularly repeated and as noise when there is no regular pattern. Speech is a complex sound.

**Pitch**
1. The **subjective sensation produced by the frequency of sound** is known as **pitch**. The **greater the frequency, the higher is the pitch**.
2. During conversation, the male and female pitches are about 120 Hz and 250 Hz respectively. Therefore, **female voice is shriller** and is audible from a greater distance.
3. A person can differentiate about 2000 pitches and this capacity can improve with training. **Pitch discrimination is best between 1000–3000 Hz**. The human ear can sense a difference in frequency of **as little as 3 Hz** in this range.

**Wavelength**
The **distance between the two adjacent peaks** is known as the wavelength of sound. It is inversely related to frequency.

**Loudness**
1. The **amplitude of sound waves** determines the loudness or intensity of the sound. The **greater the amplitude, the louder is the sound**.
2. Frequency also affects loudness. So, the hearing threshold varies with frequency. Sound intensity is measured in units called **decibels (dB)**.

3. The sound intensity in **bel** is the logarithm of the ratio of the intensity of the sound heard and the standard sound (1 dB = 0.1 bel). So,

\[
\text{Number of decibel} = 10 \log \left( \frac{\text{Intensity of the sound heard}}{\text{Intensity of standard sound}} \right)
\]

As this is in logarithmic scale, a sound of 0 decibel means the intensity is equal to that of the standard. Each 10 dB interval corresponds to a 10-fold increase in sound intensity.

4. The human audibility curve ranges from 0–100 dB and the human ear is more sensitive to intensity of about 60 dB at a frequency of 1000–4000 Hz.

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### Hearing Threshold

The threshold of hearing is 0 dB at 1000 Hz for a normal young adult in a noise-free environment. The sound is felt as well as heard at a level of 140 dB. The decibels scales for common sounds are given in figure 151.2 as the intensity of sound is proportionate to the square of sound pressure, 0 dB is equal to \(2 \times 10^3\) dynes/cm\(^2\) (hearing threshold).

- 140 dB Pain and damage to the ear
- 120 dB Uncomfortable to the ear
- 80 dB Shouting
- 60 dB Normal conversation
- 20 dB Whispering
- 0 dB Hearing threshold

### Masking

The hearing threshold for a given sound increases in the presence of background sounds or noise. This phenomenon is known as **masking**.

#### Mechanism of Masking

1. As the background sounds stimulate the auditory receptors and nerve fibers, they are in a relative or absolute refractory state when the given sound arrives. So, the **sensitivity of the auditory apparatus to a given tone decreases in the presence of other sounds**.

2. The degree to which a given sound can be masked **depends on the frequency of the masking sound**. Low-frequency sounds mask high-frequency sounds more effectively. A fairly broad-spectrum noise (**white noise**) serves as a useful mask for a wide range of test frequencies.

This is a common experience in the factory areas where sound due to machineries makes it difficult for two people to talk in a normal voice. Even in so-called calm environment, some amount of auditory stimuli is present, which exerts a masking effect on the ears. Therefore, in **conduction deafness, sound is better heard in the defective ear**. The hearing threshold increases in a soundproof room.

### Transmission of Sound Waves

#### Transmission through Outer and Middle Ear

Sound waves travel in the external ear canal to the tympanic membrane. In the middle ear, the vibrations of the tympanic membrane get transmitted through the chain of ossicular bones, become amplified and reach the footplate of stapes (Described in detail in chapter 149).

- **Ossicular conduction**: Sound waves are transmitted from the external ear to the internal ear **primarily through tympanic membrane and the ossicular chain** causing vibration of the footplate of stapes. This is known as **ossicular conduction**.

- **Air conduction**: From the tympanic membrane, sound waves are also transmitted **through the air of the middle ear cavity** and vibrate the secondary tympanic membrane to reach the perilymph of the scala tympani. This is known as **air conduction**. The force of vibration is much reduced as it arrives at the surface of perilymph. Moreover, the stronger vibration reaching through oval window is a greater stimulus for the hair cells. So, **air conduction has a very negligible role in normal hearing**.

- **Bone conduction**: From the environment, sound waves get **mechanically transmitted through the bones** and reach the inner ear. Bone conduction is better appreciated when a vibrating tuning fork is placed on the bone of the skull, especially the mastoid process. It is used to diagnose hearing loss due to defective transmission of sound waves through the external and middle ear.

#### Transmission through the Inner Ear

1. Sound waves are transmitted from the footplate of stapes to the fluid of the inner ear. The stapes moves inward and outward, causing similar movement of the membrane of the oval window.

2. The perilymph is incompressible as the cochlea is encased in a rigid bone. When the stapes moves inward, the **pressure in the perilymph of scala vestibuli increases**.

3. This depresses the **Reissner’s membrane** that, in turn, produces depression of the **basilar membrane**. The pressure wave is then transmitted to the perilymph of the scala tympani and finally produces **outward movement of the round window into the middle ear** (Fig. 151.1).

4. When the stapes moves outward, there occurs upward bulging of basilar membrane and the round window is pulled inward. The up and down movements of the basilar membrane produce **upward and downward movements of the organ of Corti**.

### Transduction of Sound Waves

The mechanical energy (pressure waves) of sound is converted into receptor potentials in the hair cell and finally gets conducted as action potentials in the auditory pathways.
Resting State of Hair Cell

In the resting state, two potentials are recorded from the ear.

i. Resting membrane potential of the hair cell

The basolateral resting membrane potential of the hair cell is \(-60\) mV. It is due to the continuous efflux of \(K^+\) across its basolateral membrane.

ii. Endocochlear potential

The basolateral surface of the hair cell is bathed in perilymph, which has a low \(K^+\) concentration and zero potential (an electrically neutral fluid like ECF).

The apical surface of the hair cell containing the stereocilia is bathed in endolymph, which has a high \(K^+\) concentration and relative to perilymph has a potential of \(+80\) mV (endolymphatic or endo-cochlear potential).

4. The presence of tight junctions between the supporting cells prevents any leakage of perilymph into the scala media. Thus, the potential difference across the apical membrane is \(140\) mV (\(+80\) mV – \((-60\) mV)).

5. This large electrical gradient favors the cation influx, especially \(K^+\) through the transduction channels. In the absence of any auditory stimulus, the hair cell remains at rest, with the stereocilia straight up, without any deflection.

6. In the resting state, however, about \(15\%\) of the cation channels are open, allowing a steady leak of \(K^+\) into the cell.

Genesis of Receptor Potential in Hair Cells

Process of Depolarization

The hair cell is a mechanoreceptor, which is very sensitive to the direction and degree of movement of cilia. The appropriate stimulus for hair cell is the lateral bending of cilia. A deflection of stereocilia of as little as \(0.5\) nm generates a response in the hair cell.

1. When the shorter stereocilia bend toward the taller stereocilia (toward the stria vascularis), the cation channels open, allowing entry of \(K^+\) (mostly) and \(Ca^{2+}\) into the hair cell (Fig. 149.6).

2. The resulting depolarization causes opening of voltage-gated \(Ca^{2+}\) channels at the base of the hair cell. Increased cytoplasmic calcium concentration causes movement and fusion of vesicles with the synaptic specialization at the base of the hair cell.

3. This results in release of glutamate into the synaptic cleft, producing action potentials in the afferent nerve fibers. The number of action potentials increases with the increased release of neurotransmitter.

4. Following depolarization, a molecular motor made up of myosin drags the channel to the resting state and relaxes the stretched tip-links.

5. The resting membrane potential is restored by diffusion of \(K^+\) along the electrochemical gradient through the leaky \(K^+\) channels at the basolateral membrane.

Process of Repolarization

When the stereocilia are pushed away from the taller stereocilia (toward the modiolus), the apical transduction channels close, leading to hyperpolarization (Fig. 149.6).

There occurs no change in membrane potential when the stereocilia bend at right angles, and the maximum change takes place when they are positioned at \(45^\circ\)-angle (midway). Thus, the hair cell acts as a fine transducer in which depolarization or hyperpolarization depends on the direction of movement of hairs; and the magnitude of change in membrane potential depends on the distance they travel toward or away from the mid-position.

Role of Cochlea

When the sound waves arrive at the inner ear, the basilar membrane, the hair cells and the tectorial membrane act in an integrated fashion to convert the mechanical form of energy into appropriate action potentials for the precise perception of sound. The cochlea also encodes the frequency and amplitude of sound waves that is further processed in the auditory pathway higher up in the cortex. Thus, the cochlea acts as a fine transducer as well as a frequency analyzer.

Special Features of Basilar Membrane

The structure and property of basilar membrane are not the same throughout its length.

1. Although the cochlea as a whole becomes narrower toward the apex, the basilar membrane is narrow at the base and wider toward the apex (Fig. 151.2). Also, it is stiff at the base and becomes gradually elastic toward the apex. Its stiffness decreases 100-fold from base to apex.
2. These mechanical characteristics enable the membrane to respond differently to the wide range of sound frequencies along its length. When sound wave of a given pitch arrives, only hair cells situated at a particular location along the membrane are maximally stimulated as maximum bowing of basilar membrane occurs at that area and a fixed set of afferent fibers discharge.

3. Thus, each successive spot along the basilar membrane is most sensitive to a slightly different frequency. As there occurs encoding of specific tones at specific places, this is known as place coding or place theory in the auditory system.

4. The basilar membrane is the first site for pitch discrimination. The hair cells toward the apical region maximally respond to low frequency sound waves and those toward the basal region maximally respond to high frequency sound waves. Thus, different frequencies can be mapped in a serial manner along the length of the basilar membrane. This orderly representation of tone is maintained in the cochlear neurons as well as in the neurons of the auditory pathway. This is called the tonotopic organization.

5. The basilar membrane also responds to different intensities of sound waves. Loud sound causes greater displacement of the basilar membrane that generates increased number of action potentials in the cochlear nerve.

Fig. 151.2: High-frequency sound waves cause maximal displacement of the basilar membrane near its base and low-frequency sound waves cause maximal displacement at the apical end, near the helicotrema.

Fig. 151.3: Traveling wave. The orange and red lines represent the waves at two instants of time respectively.

Traveling Wave

As the stapes moves in and out, it generates a series of traveling waves in the perilymph of scala vestibuli. The organ of Corti including the basilar membrane vibrates in accordance with the undulation created in the perilymph, i.e. it distorts in the form of a traveling wave. As the wave travels along the basilar membrane from its base to apex, it attains maximum amplitude at a point depending on its frequency and then falls rapidly (Fig. 151.3).

1. High-frequency sounds cause maximal displacement of the membrane near its base, near the oval window and low-frequency sounds generate waves that attain maximum amplitude at its apical end, near the helicotrema (Fig. 151.2).

2. Thus, the perception of different tones by the brain depends upon the type of wave generated in the cochlear fluid and the basilar membrane. This phenomenon is also known as traveling wave hypothesis.

3. In 1960, Georg von Békésy formulated the traveling wave hypothesis and place theory of hearing by carrying out experiments on cadaver ears for which he was awarded Nobel Prize. He performed his experiments by placing silver grains on the basilar membrane and observing their movement in response to vibrations at the oval window with simple instruments like a microscope and a stroboscopy.

The Shearing Force Bends Cilia

The upper parts of the hair cells are tightly held by the reticular lamina and the tips of the hairs of the outer hair cells are embedded in the tectorial membrane. So, the basilar membrane and the tectorial membrane are joined by the cilia of the outer hair cells.

1. When the stapes moves, the pressure gradient tends to deform both the membranes in the same direction. But, as the two membranes are hinged at different axes on the modiolus and the tectorial membrane is attached only at one border, the pressure gradient creates a lateral shearing force that causes bending of the cilia.
2. The hairs of the inner hair cells do not touch the tectorial membrane, but they bend by the movement of endolymph between the inner hair cells and the tectorial membrane.

3. When the basilar membrane moves up, the stereocilia bend away from the modiolus, and the hair cells depolarize.

4. When the basilar membrane moves down, the stereocilia bend towards the modiolus, and the hair cells repolarize.

**Cochlear Potentials**

In addition to the resting potentials recorded from the ear (endolymphatic potential and resting membrane potential of the hair cell), three dynamic potentials are seen when an auditory stimulus arrives at the cochlea. These are cochlear microphonic (CM), summation potentials (SP) and nerve action potentials (AP). These potentials can be detected by placing electrodes on the round window or in areas near the cochlea and the procedure is known as electrocochleography.

**Cochlear Microphonics**

1. CM is the electrical response that represents the summed intracellular potentials of many hair cells.
2. It is generated instantaneously due to the large current flow across the apical surface of the hair cells along a favorable electrical gradient.
3. Each and every auditory stimulus produces CM and it follows the pattern of vibration of the basilar membrane precisely. So, CM is an oscillatory event that has the same frequency as the auditory stimulus.
4. It is proposed to originate primarily from the outer hair cells since it almost disappears after lesions that destroy the outer hair cells but spare the inner hair cells. The differences between CM and action potentials are listed in Table 151.1.

**Summation of potentials:** This dynamic cochlear potential can be a positive summation potential (+SP) or negative summation potential (−SP). The bending of cilia may cause +SP and −SP may have a neural origin. Positive SP can be masked, whereas negative SP cannot be masked.

**Localization of the Source of Sound**

A normal individual can determine the direction of the source of incoming sound waves quite accurately. This helps the body to be on the alert and prepare itself to react appropriately to the situation. Various factors are involved in the localization of the source of sound.

**Difference in Time of Arrival of Stimulus in Two Ears**

This is an important factor in localizing the source of sound waves when the stimulus is of low frequency and originates in the horizontal plane. The difference in time between the arrivals of the stimulus in two ears helps to decide the direction of sound.

1. When a stimulus is situated at equal distance from both ears of a listener, it reaches both ears simultaneously.
2. When the stimulus originates off to one side, the ear on that side receives the sound earlier.
3. When the stimulus frequency is below 3000 Hz, the brain can detect a difference in time of as little as 20 µs. This is possible due to the remarkable response time of hair cells.
4. The hair cells can sense repetitive movement of up to 100,000 times per second.

**Difference in Intensity that Reaches the Two Ears**

In the horizontal plane, the stimulus intensity is perceived more in the ear that is nearer to the stimulus. When the stimulus frequency is above 3000 Hz, this factor plays a greater role in sound localization.

**Role of Pinna**

Many animals like cat and deer can turn their pinna to focus hearing in a particular direction without changing head position. In humans, this capacity is absent. Sound wave enters the auditory canal both directly and after being reflected by the pinna and the sound that we hear is a combination of the two. The shape of the pinna/tragalus tends to accentuate or attenuate certain sound frequencies depending on the angle at which sound waves approach the ear. Thus, the pinna is essential in humans for sound localization in the vertical plane.
tal plane, as the pinna is curved little forward, the sound coming from the back differs in quality from the sound that comes from the front.

The time of arrival of stimulus and its intensity decides the pattern of action potentials from each ear. This causes maximal or minimal activation of neurons in the auditory centers that receive inputs from both ears. As the superior olivary nucleus is the first station to receive bilateral input, it is the first center for localization of the source of sound. The auditory cortex finally compares the input from both ears and determines the direction of sound. In lesions of auditory cortex, sound localization is very much affected.

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<th>CHAPTER SUMMARY</th>
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<tr>
<td><strong>Key Concepts</strong></td>
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<tr>
<td>1. The hair cells of cochlea convert the mechanical form of energy of sound into action potential in the auditory nerve.</td>
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<tr>
<td>2. Bending of stereocilia toward kinocilium produces depolarization and bending of stereocilia away from kinocilium produces repolarization in the hair cell.</td>
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<td>3. The basilar membrane acts as a pitch discriminator.</td>
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<td>4. Cochlear microphonic is the electrical response that represents the summated intracellular potentials of many hair cells.</td>
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<td><strong>Important to Know (Must Read)</strong></td>
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<tr>
<td>1. ‘Describe the transduction of sound waves in the cochlea’ may come as a <strong>Long Question</strong>.</td>
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<td>2. Masking, Genesis of receptor potential in the hair cells, Travelling wave hypothesis may come as <strong>Short Questions</strong>.</td>
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<td>3. In <strong>Viva</strong>, examiner may ask… What is pitch of sound, What is hearing threshold, What is masking, What is travelling wave hypothesis, How localization of sound wave is possible.</td>
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Hearing Defects and Hearing Tests

LEARNING OBJECTIVES
On completion of study of this chapter, the student WILL be able to:
1. Classify hearing defects and give their causes.
2. Give the physiological basis of assessment of hearing defects.
4. Name the hearing tests and describe the procedure of each test.
5. Understand the principle of audiometry and BAEP.

HEARING DEFECTS

Deafness

Deafness is divided into the following types: conductive deafness and sensorineural deafness. When both conductive and sensorineural deafness are present, it is known as mixed deafness.

Conductive Deafness

Conduction deafness occurs due to impaired transmission or amplification of sound to the cochlea due to disease of the external or middle ear. The sound waves are conducted to the cochlea by the skull bones (bone conduction) and produce vibration of the basilar membrane. So, the hearing loss is usually partial. The common causes are:

1. **Blockade of external auditory canal**: This occurs as a result of impaction of wax, or foreign bodies. Less common causes are atresia or tumor of the auditory canal.
2. **Thickening of the tympanic membrane** following repeated middle ear infections. Sometimes perforation may occur due to infection or trauma.
3. **Acute and chronic otitis media**
4. **Immobility of the ossicles**. This may occur as a result of adhesions due to repeated middle ear infections or bony ankylosis. When the stapes is fixed to the oval window in an abnormally rigid manner, it is known as otosclerosis.
5. **Destruction of the auditory ossicles**.
6. **Eustachian tube obstruction** leads to absorption of the air from the middle ear cavity by the lining mucosa. This results in negative intratympanic pressure producing a retracted tympanic membrane.

Sensorineural Deafness

This occurs as a result of diseases of the cochlea or of the 8th cranial nerve. The degree of hearing impairment depends on the extent to which the structures are affected. When deafness occurs due to lesion of the cochlear nuclei or their central connections, it is known as central deafness. It occurs rarely and is associated with marked neurological deficits.

The common causes of sensorineural deafness are:

1. **Toxic degeneration of the hair cells** caused by chronic treatment with drugs such as aminoglycoside antibiotics (streptomycin, kanamycin, gentamycin). These drugs block the mechanosensitive channels in the stereocilia of hair cells, subsequently leading to their degeneration. They also affect the hair cells of the vestibular apparatus, so nerve deafness along with abnormal vestibular functions occurs. Some other drugs like salicylates, quinine, cytotoxic drugs and certain diuretics also produce deafness.
2. **Senile degeneration of the hair cells**: There occurs gradual cumulative loss of hair cells and neurons due to effect of aging. This is known as presbycusis. It is seen in about one-third of population over 75.
Hearing aids are useful to restore the hearing capacity to some extent in cases of deafness due to partial decrease in functions of middle ear or cochlea. Treatment of Deafness

Mixed Deafness

Mixed deafness can be due to trauma, infection or tumor that affect both middle ear and inner ear. Deafness due to genetic mutations is classified into syndromic (when it is associated with abnormalities in other systems) and nonsyndromic (when deafness is the only abnormality). The mutations can occur in the following proteins and cause nonsyndromic deafness.

- **Connexon 26**, which helps in normal recycling of $K^+$ through supporting cells.
- **Myosin-VIa**, which is associated with actin in the hair cell processes.
- **Myosin-Ib**, which causes movement of the cation channels at the tip links.
- **Myosin-VI**, which plays a role in the formation of the cilia.
- **α-tectorin**, which is the major protein in tectorial membrane.

Mutations in the following proteins cause syndromic deafness.

- **Sulfate transport protein**: Its mutation results in Pendred’s syndrome (deafness and goiter).
- **KVLQT1**, a $K^+$ channel protein that is present in the stria vascularis and maintains the high $K^+$ concentration of the endolympm and a normal QT interval in the heart. Its mutation produces long QT syndrome (deafness and a longer QT interval that predisposes to ventricular arrhythmias)
- **Barttin**, the mutation of which causes deafness and renal manifestation of Bartter’s syndrome.

**Tinnitus**: It is the perception of sound in the absence of any sound in the environment. It may be buzzing, roaring or ringing type and can be pulsatile. It is usually associated with conductive or sensorineural deafness.

**Weber’s Test**

Normally, environmental noise is transmitted to the inner ear by ossicular conduction along with the sound the person listens to, and tends to mask it; but it has no effect on sound conducted through the bone of the skull.

1. If the base of a vibrating tuning fork is placed at the center of the forehead, or the vortex of the skull, a normal person will hear it equally in both ears, but
a person with conduction deafness will hear it louder in the diseased ear, because the environmental noise does not reach the inner ear of that side.
2. On the other hand, the subject with nerve deafness hears the sound louder in the normal ear.

Schwabach’s Test
This test employs the same principle as the Weber’s test. The bone conduction of the patient is compared with that of a normal person. If the bone conduction of the patient is better than normal, he has conduction deafness, but if his bone conduction is less than normal, he has nerve deafness.

Audiometry
The assessment of hearing using electronic equipments is known as audiometric test. The tests can be subjective (results are based on patient’s answer) and objective.

Pure tone audiometry: This is one of the most generally used subjective tests for assessing hearing. The machine is called audiometer and the recording is called audiogram. This test is useful to detect or rule out conduction deafness.
1. The audiometer emits pure tones of various frequencies, which the subject listens to through the earphones (for air conduction) or by an electronic vibrator fixed to the mastoid process (for bone conduction).
2. The test is performed in a soundproof room and each ear is tested separately beginning with the better ear. A total of 6-10 different tones with frequencies ranging from 250 to 8000 are tested and the threshold decibels against each tone are plotted for each ear.
3. When hearing loss is present, the non-test ear is masked by sounds of broad-spectrum to have proper evaluation.
4. During the bone conduction test, the non-test ear is masked with some narrow band of noise centered on the test frequency as vibrations on any part of the skull easily get heard at the untested ear.

Other subjective hearing tests are speech audiometry (measures the patient’s ability to recognize and repeat correctly lists of words that are presented to him), alternate binaural loudness balance test, loudness discomfort level test and tone decay test. The objective hearing tests are impedance measurements (by tympanometry and intra-aural reflex measurements) and evoked responses (electrocochleography and brainstem auditory evoked responses). Electrocochleography measures the earliest evoked potentials generated in the cochlea and the auditory nerve, such as cochlear microphonic, summation potentials and nerve action potentials from the cochlear neuron. It is useful to diagnose Meniere’s disease, where an elevation of summation potentials to action potentials is seen.

Brainstem Auditory Evoked Responses (BAER)
The potentials recorded from the auditory pathway in response to a brief auditory stimulation are known as BAERs. As the stimulus travels from the cochlea to higher up, it generates action potentials in all the nerve fibers. BAERs assesses functional status of the auditory pathway up to the mid brain.
1. The waveforms of BAEP are named I, II, III, IV and V.
2. Wave I originates from peripheral portion of eighth cranial nerve adjacent to the cochlea.
3. Wave II arises from cochlear nucleus. Action potentials in the superior olivary nucleus generate wave III.
4. Wave IV arises from lateral lemniscus.
5. Wave V originates from inferior colliculi. Absence or reduced amplitude of a waveform indicates lesion of the area that gives rise to that wave.
6. BAEP is useful in assessing hearing loss in infants and small children. It is also useful in localizing brainstem lesions, diagnosing tumors at various levels of the auditory pathway, coma, brain death and strokes affecting the brainstem.
7. Otoacoustic emissions differentiate sensory from neural hearing loss (Application Box 152.1).

Application Box 152.1
Otoacoustic emissions: These are inaudible sounds that result from the self-induced vibrations of the outer hair cells. The emissions can be spontaneous or evoked in response to an auditory stimulus. It can be measured with microphones inserted into the external auditory canal. It is used to assess auditory threshold and to discriminate sensory from neural hearing loss. Detection of oto-acoustic emissions indicates the intactness of the outer hair cells and is a rapid screening method for hearing impairment in newborns.

CHAPTER SUMMARY

Key Concepts
1. Deafness can be sensorineural, conductive or mixed deafness.
2. Deafness is tested with the help of a tuning fork by Rinne, Weber, and Svabach tests.

Important to Know (Must Read)
1. In examination, Long Questions are usually not asked from this chapter.
2. Sensorineural deafness, Conductive deafness, Mixed deafness, Tests for hearing may come as Short Questions.
3. In Viva, examiner may ask... What is sensorineural deafness, What is conductive deafness, What is mixed deafness, What are the tests of hearing, What is Audiometry, What is brainstem auditory evoked potential.
The sense of smell is known as olfaction. It is classified under visceral senses because of its close association with gastrointestinal functions. The receptors for smell are chemoreceptors located in the olfactory mucosa of the nasal epithelium. In animals, this primitive sense is well developed. It is useful for them to locate their mates, friends or foes, and for the identification of territory. In humans, it increases the quality of food by recognizing its flavor; enhances social and emotional interactions by odors of perfumes, deodorants and fragrance of flowers and enriches memory. It also has some protective function in guiding us to avoid stale food or to go away from the polluted environment. The olfactory system is the only sensory system that does not relay in the thalamus on its way to the cortex. And, the olfactory system has asymmetric cortical representation.

**SITE OF OLFACTION**

**Olfactory Epithelium**

The olfactory receptor cells are located in a specialized area of the nasal mucosa lining the roof of the nasal cavity called the olfactory epithelium or the olfactory mucus membrane.

1. It is a patch of yellowish-pigmented mucus membrane extending laterally to the superior concha and medially to the upper third of the median septum (Fig. 153.1).

2. It occupies an area of about 2.5 sq.cm in each nostril in humans and is covered by mucus produced by the Bowman's glands.

3. The area is large in animals like dogs in which the sense of smell is pretty well developed (*macrosmatic* animals). Human beings are *microsmatic*.

4. The olfactory epithelium contains 10–20 million receptor cells interspersed between supporting cells and progenitor cells. The progenitor cells are stem cells that form new receptor cells by mitosis.

During quiet breathing, the mainstream of inspired air does not reach the olfactory epithelium as it lies above, but some air does move up by convection current and reach the olfactory epithelium. The mucus layer dissolves...
the chemical particles and helps in their transport to reach the cilia of the receptor. Sniffing, or deep breathing channels the air to the olfactory mucus membrane. Sniffing is a semireflex response evoked spontaneously by a novel odor.

**Vomeronasal Organ**

In rodents and other mammals, another patch of olfactory mucus membrane is present along the nasal septum, called vomeronasal organ.

1. The organ is considered necessary for successful mating behavior as it helps in recognition of odors that act as pheromones.
2. During the mating season of an animal, hormone like substances called pheromone is secreted from its body that attracts the mating partner.
3. The pheromone of each species has a specific odor that is identified by the mating partner of the same species.
4. Axons from the vomeronasal organ project to the accessory olfactory bulb and from there primarily to areas in the amygdala and hypothalamus. These areas are concerned with reproduction and feeding behavior.

**Olfactory Receptor Cell**

1. The olfactory receptor cell is a bipolar neuron. It has a short thick dendrite with an expanded end called an olfactory rod that gives off 10–20 cilia (Fig. 153.2).
2. Each cilium is about 2 µm long and 0.1 µm in diameter. The cilia are unmyelinated and project into the mucus where they interact with odorants dissolved in the mucus.

Unlike other neurons in the body, the olfactory neurons (also the taste receptor cells) are unique, as they are regenerated. The olfactory neurons are replaced in about 60 days as basal cells proliferate to form new receptor cells. A growth factor called bone morphogenic protein (BMP) exerts an inhibitory effect on this renewal process. Another unique feature of olfactory mucosa is that it is one place in the body where the nervous system is exposed to the external environment.

**OLFACTORY PATHWAYS**

**Olfactory Nerves**

The axons from the olfactory receptor cells gather into about twenty bundles known as olfactory nerves, which pierce the cribriform plate of the ethmoid bone and enter the olfactory bulbs.

**Olfactory Bulb**

Above the cribriform plate of the ethmoid bone, and below the orbital surface of the frontal lobe on each side, the portion of the brain that projects forward is known as the olfactory bulb.

**Olfactory Glomeruli**

These are globular formations present in the olfactory bulb, where olfactory receptor cells synapse with the tufted cells and mitral cells.

1. As many first order neurons converge on the few second order neurons, the high convergence ratio increases the olfactory sensitivity.
2. The periglomerular cells inhibit adjacent glomeruli and the granule cells inhibit mitral and tufted cells. The later is an example of feedback inhibition, i.e. the mitral and tufted cells stimulate granule cells (the neurotransmitter released is glutamate) and the granule cells in turn cause inhibition of mitral and tufted cells by releasing GABA.

**Olfactory Tract**

The axons of mitral and tufted cells extend to the olfactory cortical areas as olfactory tract.

**Cortical Olfactory Areas**

1. While entering to the brain, the fibers of the olfactory tract divide into lateral and medial olfactory stria. The fibers terminate in the apical dendrites of the pyramidal cells of the olfactory cortex.
2. The lateral olfactory stria projects to the piriform cortex, amygdala, entorhinal cortex, anterior olfactory...
nucleus and olfactory tubercle. Fibers from these areas project to the orbitofrontal cortex via thalamus, frontal cortex, hippocampus, hypothalamus and contralateral olfactory bulb (Fig. 153.3). Sniffing activates the piriform cortex.

3. The amygdala evokes emotional responses associated with smell. The entorhinal cortex is concerned with olfactory memories.

4. The lateral and anterior orbitofrontal cortex of the frontal lobe is concerned with conscious perception and differentiation of odors. However, olfaction is asymmetrically represented in the orbitofrontal cortex as right side olfactory cortex is bigger than the left. In lesion of orbitofrontal cortex, the capacity to discriminate odors is lost.

5. The medial olfactory stria projects to the septum and hypothalamus that mediates various autonomic responses associated with smell.

6. Olfactory fibers also project to the dorsal motor nucleus of vagus that mediates parasympathetic responses to smell (e.g. secretion of saliva, gastric juice and pancreatic juice during the cephalic phase).

**Fig. 153.3:** Olfactory pathway. From olfactory bulb, axons of the tufted cells and mitral cells travel in the lateral olfactory stria, which project to the anterior olfactory nucleus, olfactory tubercle, piriform cortex, entorhinal cortex and amygdala. The axons of the mitral cells from the accessory olfactory bulb form the medial olfactory stria and project to the amygdala. Information from these areas except the anterior olfactory nucleus travel to the orbitofrontal cortex via thalamus, frontal cortex, hippocampus and hypothalamus. The anterior olfactory nucleus projects to the contralateral olfactory bulb.

**Physiology of Olfaction**

**Mechanism of Olfaction**

**Role of Mucus and Odorant-Binding Protein**

1. Olfactory receptors are activated only when the odoriferous substances are dissolved in the thin layer of mucus covering the olfactory epithelium and come in contact with the cilia of the olfactory receptors.

2. Generally, odoriferous substances are small molecules with a few carbon atoms. The water-soluble odorants dissolve in the mucus overlying the olfactory epithelium to reach the cilia of the olfactory receptors.

3. The lipid-soluble odorants bind to an Odorant-Binding protein to get transported in the hydrophilic mucus and reach the receptors. The protein is secreted into the mucus by the lateral nasal gland.

**Olfactory Transduction**

There are about 1000 different odorant receptors and all of them are coupled to heterotrimeric G proteins.

1. When the odorant binds to the receptor, the G protein is activated that leads to opening of cation channels causing influx of calcium (major mechanism), activation of adenylyl cyclase-cAMP system, where the G protein is called G<sub>olf</sub>, and stimulation of phospholipase C-IP<sub>3</sub>, DAG system.

2. Activation of one of these systems produces depolarization of the olfactory receptor cells leading to generation of action potentials along the olfactory nerve fiber.

Electrophysiological studies show that there is a continuous background firing of impulses through the olfactory tract, and the introduction of odors increases the frequency of these impulses. The dendrites of the periglomerular cells and granule cells spread laterally and produce inhibition of the olfactory glomeruli on which they
Chapter 153: Physiology of Smell

The physiology of smell involves the sense of smell, which is transmitted through olfactory receptors in the nasal cavity. These receptors detect various odors and convert them into neural signals that are sent to the brain. The process of olfaction is complex and involves multiple steps, such as the binding of odorants to receptors, signal transduction, and neural processing.

Factors Affecting Olfaction
1. **Type of odorant stimulus**: If the odorant chemicals are volatile, they are easily transported by air into the nose and are quickly recognized. A strong odor has a high lipid or water solubility.
2. **Threshold of olfactory receptors**: The olfactory receptors are extremely sensitive to minute concentrations of some of the odoriferous substances. For example, methyl mercaptan, which gives garlic its odor, can be smelt at concentrations as low as 0.000,000,4 mg per liter of air. Usually, women are more sensitive to smell than men and their sensitivity to odors increases further at the time of ovulation. The threshold for olfaction increases with advancing age, both for male and female.
3. **Intensity of stimuli**: Humans can discriminate over 10,000 different odors. But their ability to distinguish changes in intensity of odors is less significant. The change in concentration of odors must be over 30 percent to be perceived, compared with a 1 percent change in the visual system.
4. **Olfactory adaptation**: When the olfactory receptors are exposed to a continuous stimulus, they adapt rapidly. It is well known that when somebody enters a scented room, he becomes aware of the odor, but after some time, the perception of the odor declines and ceases over time. Adaptation occurs due to activation of cyclic nucleotide-gated (CNG) ion channels by calcium-calmodulin complex. When CNG A4 is knocked out, adaptation is slowed.

Reflexes Associated with Olfaction
1. **Sniffing** at the arrival of a new odor.
2. **Salivation, or gastric secretion, and pancreatic secretion** at the smell of food.
3. **Sneezing, lacrimation** and, in extreme case, **respiratory inhibition** in response to irritant odors. Chemicals like chlorine, ammonia, menthol and peppermint stimulate the naked nerve endings of trigeminal pain fibers that are present in the olfactory mucus membrane. Thus, these odors produce unpleasant and burning sensation.
4. **Vomiting** in response to a foul smell.

**CHAPTER SUMMARY**

**Key Concepts**
1. Olfactory receptor cell is a bipolar neuron. Mucus and odorant binding protein help to mediate olfaction.
2. Olfaction is transmitted to olfactory tubercle, olfactory glomerulus, amygdala, entorhinal cortex, piriform cortex, hippocampus, hypothalamus, thalamus, frontal cortex and orbitofrontal cortex.

**Important to Know (Must Read)**
1. In examination, **Long Questions** are usually not asked from this chapter.
2. Olfactory pathways, Cortical olfactory areas, Vomeronasal organ, Mechanism of olfaction may come as **Short Questions**.
3. In **Viva**, examiner may ask… What are the olfactory areas, Olfactory pathways, Vomeronasal organ, Factors affecting olfaction, Reflexes associated with olfaction.
The sense of taste is known as **gustation**. Like smell, it is classified under **visceral senses** and the gustatory receptors are **chemoreceptors**. **Flavor** of a food gives an intricate, rich and pleasurable sensory experience by amalgamating many sensory inputs. The sensations that contribute to flavor and make a food enjoyable are sight, smell, taste, temperature and texture; out of them, taste and smell are the most important components. Taste is uniquely associated with ingestion. A pleasant taste increases appetite and stimulates the secretion of digestive juices, whereas a disagreeable taste may serve as a warning of danger and the food is rejected. This **primitive sense** is more developed in animals and adds to their survival. Taste plays a role in maintaining the nutritional balance. For example, adrenalectomized rat show preference for saline and patients with adrenal insufficiency or salt-deprivation exhibit salt preference. When the taste afferents are sectioned, the animals eat and drink indiscriminately. So, taste is important for a normal ingestive behavior. Furthermore, taste is the basis of many associative learning processes.

**Receptor Organs (Taste Buds)**

The receptor organs for taste are called taste buds that house the chemoreceptors responsible for taste. They respond to gustatory stimuli (**tastants**) and mediate the taste sensation. There are a total of about 10,000 taste buds. The number decreases with advancing age.

### Location

The **taste buds** are located mainly on the tops of the numerous **fungiform papillae** and along the sides of the less numerous **circumvallate** and **foliate papillae** of the tongue. A few taste buds are present in the mucosa of the palate, epiglottis, pharynx, larynx and esophagus.

1. A papilla is a small, elevated projection on the dorsum of tongue.
2. The small fungiform papillae are located at the **tip** and along the **edges** of the anterior two thirds of the tongue.
3. Each papilla contains up to five taste buds at its top.
4. The circumvallate and foliate papillae are large and each contains up to 100 taste buds along its sides.
5. In the posterior third of the tongue, the circumvallate papillae are located in the walls of a V-shaped trough and the foliate papillae are located at the edge of the V.
6. The dorsum of the tongue is covered by small conical threadlike **filiform** papillae that do not contain taste buds. Also, there are no taste buds in the under surface of the tongue.

### Structure

The taste buds are oval shaped multicellular structures about 70 µm high and 50 µm in diameter. The following types of cells are present in a taste bud: **basal cells**, **supporting** (sustentacular) cells and the **gustatory receptor** cells (Fig.154.1).
Chapter 154: Physiology of Taste

1. The gustatory receptor cells or taste cells are of three types: dark cells, light cells and intermediate cells, also known as type I, type II and type III cells respectively.

2. The cells lie mostly buried in the surface of the tongue; the chemicals reach the receptor cells through an opening in the apical surface of the taste bud called the taste pore (gustatory pore).

3. The supporting cells do not have any afferent synaptic connections.

4. The basal cells are formed from the epithelial cells surrounding the taste bud. They are the undifferentiated stem cells from which new receptor cells are constantly formed by differentiation and proliferation replacing the old receptor cells.

Gustatory Receptor Cell

1. The taste receptor cell is a modified epithelial cell. The receptor cell is elongated and extends full length of the taste bud.

2. At its apical end, a single, hair like microvillus (gustatory hair) projects to the external surface through the taste pore, where it comes in contact with the gustatory stimuli or tastant.

3. The microvilli increase the surface area of the receptor cells. The receptor cells are the most numerous taste bud cells (50–75%).

4. A taste bud contains about 50–150 taste receptor cells that are surrounded by the supporting cells.

5. The apical regions of the receptor cells and the supporting cells are joined by tight junctions so that only the microvilli come in contact with the fluids in the oral cavity. Unlike the olfactory system, the gustatory receptor cells are not primary afferent neurons. At their basal end, the receptor cells form synapse with the nerve endings of primary sensory axons (first order neuron). Each taste bud is innervated by 50 afferent fibers and each afferent fiber receives input from about five taste buds.

6. The receptor cells have a life span of about 10 days. Like the olfactory receptors they degenerate and are continuously replenished by new receptor cells from the basal cells. In this process, the synaptic connections of the old cell also degenerate and the new ones are formed. Moreover, it has been observed that sectioning the afferent nerve fiber leads to degeneration of the taste buds supplied by it; and when the nerve regenerates, new taste buds are formed near the nerve endings. It is believed that the nerve terminals release growth factors and exert a trophic effect on the taste buds, as well as confer on them chemical specificity.

Innervation

1. The fungiform papillae receive sensory innervation from chorda tympani, a branch of the facial nerve.

2. The circumvallate and foliate papillae are innervated by sensory fibers from the glossopharyngeal nerve.

3. The taste buds in the palate are supplied by the greater superficial petrosal branch of the facial nerve, whereas the taste buds in other parts are innervated by the vagus nerve.

PHYSIOLOGY OF GUSTATION

Taste Pathways

1. The chorda tympani branch of the facial nerve and the glossopharyngeal nerve carry sensations from the taste buds in the anterior two-third and posterior one-third of the tongue respectively.

2. The taste sensations from other areas travel in the vagus nerve.

3. The afferent fibers (first order neurons) enter the medulla oblongata and terminate in the nucleus tractus solitarius (Fig. 154.2).

4. From there, the second-order neurons arise and ascend in the ipsilateral medial lemniscus to terminate in the ventral posteromedial (VPM) nucleus of thalamus.

5. From the thalamus, the third-order neurons arise and travel in the thalamic radiation to project to the face and tongue areas of the ipsilateral primary sensory cortex (foot plate of the post-central gyrus) Flowchart 154.1).

6. The third-order neurons also project to the anterior insular cortex and frontal operculum; these areas in turn project to lateral orbitofrontal cortex.

7. While ascending up in the medial lemniscus, the second-order axons give branches to the reticular formation, the limbic system, hypothalamus and the locus ceruleus.

Type of Tastes

Traditionally, four basic taste sensations have been described: sweet, salty, sour and bitter. Recently, a fifth type called umami (delicious in Japanese) has also been included...
in the list of basic tastes. The umami taste is pleasant and sweet but differs from standard sweet taste. The different sub-modalities of taste sensations are believed to arise from various combinations of these primary taste sensations blended with simultaneous stimulation of touch, temperature and pain receptors in the oral and lingual mucosa, as well as olfactory sensations.

It was used to be thought that the receptors for the four basic tastes are located at specific areas on the surface of the tongue, producing regional differences in the sensitivities to the various taste sensations. So, the idea was that the tip of the tongue is sensitive to sweet; anterior edges to salt, the sides to sour and the back of tongue is sensitive to bitter taste. However, the current concept is that there are no such regional specificities; all tastes are sensed from all parts of the tongue and adjacent areas containing taste buds.

**Mechanisms of Gustatory Transduction**

The receptors for taste are chemoreceptors that respond to chemicals arriving on the lingual epithelium. A gustatory stimulus produces depolarization of the receptor cell.

1. In the oral cavity, when the chemicals released from dissolved food particles come in contact with the microvilli, they bind to specific receptors and activate them.
2. There occurs a graded depolarization of the receptor cell, defined as its receptor potential.
3. The depolarization when adequate opens voltage-gated calcium channels causing Ca\(^{++}\) influx.
4. The raised intracellular Ca\(^{++}\) level results in exocytosis of the neurotransmitter glutamate from the basal end of the receptor cell into the synaptic cleft.
5. Glutamate promotes generation of action potentials in the sensory axons. Thus, the process of generation of action potentials by the gustatory stimuli acting on the taste buds is known as gustatory transduction.

Most of the receptors respond to more than one of the primary tastes; only they have differences in sensitivity to the various tastants. The chemicals are of diverse structure, and the receptors have multiple mechanisms and second messenger systems to signal the different taste modalities. Many of the chemicals act by stimulating the gustatory G protein, gustducin. Usually, depolarization of a receptor occurs either due to increase in membrane permeability to Na\(^{+}\), or closure of K\(^{+}\) channels, or increase in Ca\(^{++}\) concentration.

Recently, a protein that binds the tastants molecules has been cloned. It is secreted by Ebner’s glands. The protein concentrates the tastants molecules and transports them to the membranes of the microvilli.

**Salty Taste**

The salty taste is elicited by Na\(^{+}\), present most commonly in NaCl, the table salt.

1. The salt-sensitive receptors have epithelial Na\(^{+}\) channels (ENaC) in their membrane, which allows Na\(^{+}\) entry when extracellular Na\(^{+}\) concentration increases.
2. The increased intracellular Na\(^{+}\) level results in depolarization of the receptor cell. It has been found that following application of the Na\(^{+}\) channel-blocking diuretic amiloride on the tongue, the ability to taste salt is
not completely abolished.
3. This indicates that apart from the ENaC-mediated mechanism, there is some other mechanism for depolarization.
4. All inorganic anions taste salty, but the cation may have varied effect. Potassium salts are bitter and salty; lead and beryllium salts taste sweet and mercuroxic salts taste metallic.
5. Lysyltaurine is a more potent salty agent than NaCl.

**Sour Taste**
The sour taste is triggered by hydrogen ions, the adequate stimulus being a pH of less than 4 of the solution.
1. Hydrogen ions block the K⁺ channels in the apical cell membrane.
2. The decreased K⁺ permeability produces depolarization of the receptor cell.
3. The ENaCs also allow entry of H⁺ and the inward positive current leads to depolarization.
4. The sour taste may also be due to stimulation of HCN, a hyperpolarization-activated cyclic nucleotide-gated cation channel receptor and other receptors.
5. All acids are sour and the intensity of perception depends on the degree of dissociation of the acid (i.e. the number of free hydrogen ions). However, some organic acids give mixed sensations. Citric acid is sweet and sour, and picric acid is bitter and sour.

**Umami Taste**
1. The umami taste is produced by glutamate, which is present in purine 5-ribonucleotides such as inositol monophosphate (IMP) and guanosine monophosphate (GMP) in the diet.
2. Also, glutamate is present as a flavor-enhancer in monosodium glutamate (MSG), a food additive used extensively in Asian cooking.
3. Glutamate binds to and activates a cation channel that permits Na⁺ and Ca²⁺ entry causing depolarization of the receptor cell.
4. Glutamate also activates a metabotropic glutamate receptor, mGluR4 resulting in a decrease in cAMP level. However, further mechanism of depolarization following receptor stimulation is not clear.

**Bitter Taste**
The bitter taste is elicited by a variety of unrelated substances, many of which are poisons and the bitter taste serves as a protective warning to avoid them. As per the large variety of chemicals, the receptors and the mechanisms of their activation are also many.
1. Many of the bitter compounds act by binding to receptors coupled to G protein and produce depolarization by activating the G protein-mediated second messenger cascades. The number of receptors linked to G protein is as many as 24 in humans.
2. Strychnine binds to a receptor of T2R family linked to the G protein gustducin and stimulate phosphodiesterase. The resultant decrease in the level of cAMP produces depolarization. Some compounds activate G protein linked to another class of receptor and activate phospholipase C to increase the formation of IP3 and DAG that trigger the release of Ca²⁺ from the endoplasmic reticulum. The raised intracellular Ca²⁺ concentration leads to depolarization and release of neurotransmitter. The mechanism of action of the bitter tasting amino acid leucine is G protein-mediated.
3. Quinine is the yardstick for bitterness. It acts by direct stimulation of the G protein bypassing the receptor. Some compounds produce inhibition of the phospholipase that metabolizes cGMP. Substances like morphine, nicotine and caffeine taste bitter. Many non-alkaloids taste bitter e.g. urea, magnesium, potassium, calcium and ammonium salts. Calcium binds directly to K⁺ channels and block them leading to depolarization.

**Sweet Taste**
Sweet substances act by the G protein gustducin. About 20% of taste cells express G protein-coupled receptors of T1R family. Some of them also express the G protein, named gustducin.
1. The stimulation of G protein-coupled receptor activates membrane adenylyl cyclase and increases cAMP level.
2. The raised intracellular cAMP activates protein kinase A that promotes phosphorylation of K⁺ channels on the basolateral membranes.
3. This results in closure of K⁺ channels and depolarization of the cell.
4. Some sweet substances increase intracellular IP₃ level and promote Ca²⁺ release from the endoplasmic reticulum, thereby cause depolarization.

Most sweet substances are organic; specially all sugars tend to elicit a sweet taste (e.g. glucose, sucrose, fructose). Sucrose is taken as a standard for sweet substances. Several non-sugar substances also taste sweet, e.g. salts of lead and beryllium, chloroform, aspartame and saccharin. As salts of lead are sweet, ingestion of toxic amount of these salts can happen. The α-amino acids are sweet, but polypeptides are often bitter. The proteins thaumatin and monellin are much more sweeter.

**Encoding of Gustatory Stimuli**
An adequate gustatory stimulus depolarizes the receptor cell and generates action potentials in the afferent nerve fibers. Most of the receptor cells respond to more than one of the primary taste stimuli. Each afferent fiber forms synapse with a number of receptor cells. Thus, the afferent neuron receives information regarding a variety of taste stimuli. However, each afferent neuron fires optimally in response to one of the five primary taste stimuli. The neurons in gustatory cortex are stimulated in response to the
pattern of action potentials in the afferent neuron. Thus, the encoding of the gustatory sensation is not a simple labeled-line, chemical sensory system.

**Taste Thresholds and Intensity Discrimination**

**Taste Thresholds**

1. The lowest concentration of a gustatory stimulus to which the taste buds respond by depolarization is known as the threshold concentration of that substance. The threshold concentrations for different types of tastants vary (Table 154.1). The thresholds of bitter compounds are usually low.
2. As most of these are poisons, taste buds detect them even at a very low concentration and warn us from further ingestion.
3. Generally, women are less sensitive to sour taste and more sensitive to sweet and salt taste as their threshold for these sensations vary with men.
4. The artificial sweetener saccharin (threshold concentration –23 µmol/L) is 67 times sweeter than sucrose. The proteins thaumatin and monellin (threshold concentration 0.01 µmol/L) are 100,000 times sweeter than sucrose.

**Intensity Discrimination**

As in olfaction, the ability of humans to discriminate differences in the intensity of tastes is poor. A 30% change in the concentration of the substance being tasted is required before an intensity difference is perceived. Humans can discriminate 4000–10,000 tastants.

**Factors Influencing Taste Sensation**

1. **Age:** The taste sensation decreases after the age of 50. The number of taste buds starts decreasing due to the enhanced degeneration process.
2. **Gender:** Generally, women are less sensitive to sour taste and more sensitive to sweet and salt taste.
3. **Concentration of the tastants:** The intensity of the taste sensation depends on the concentration of the tastants solution. A concentrated solution elicits a stronger taste sensation than a diluted one.
4. **Area of stimulation:** The intensity of the taste sensation depends on the number of taste buds stimulated. When a tastants solution is applied to a small area of the tongue, it produces a weaker sensation. On application of the same solution to larger area of tongue produces a more intense sensation. This is due to activation of a greater number of afferent fibers.
5. **Temperature of the tastants:** Increased temperature over some ranges tends to enhance the perceived taste sensitivity, especially for cooked food and spicy food. For example, perception of sweet taste is increased when the food is not warm.
6. **Duration of stimulation:** The taste receptors show a slow but definite adaptation. If a tastant solution is applied to one area of the tongue for a longer duration, the intensity of taste sensation gradually decreases. This occurs due to decrease in discharge of afferent nerve fibers over time.
7. **Effect of other tastants:** When two or more stimuli are applied simultaneously or one after the other, one may affect the perception of the other. This is due to facilitation or blockade at receptor level. For example, sweet taste enhances with little bit of salt, salty taste can be reduced by mixing with sour and sour taste decreases when taken with sugar. Also, when a bitter stimulus is applied before any other tastants, the bitter taste lingers and dominates over other tastes.
8. **Effects of taste modifying substances:** A plant protein known as miraculin changes the taste of acids from sour to sweet. Gymnemic acid when applied to the tongue abolishes the sensation of sweet, but does not affect other taste sensations.
9. **Effects of drugs:** Some drugs containing sulfhydryl groups, such as captopril and penicillamine tend to cause temporary loss of taste.
10. **Nutrient deficiencies:** The preference for salty food arises in salt deficiency and calorie deficiency produces preference for sweet food.
11. **Culture and habit:** As the food habit is different in various cultures, the brought up and habit of a person also affects his taste.
12. **After-affects:** If illness occurs following ingestion of a new food, aversion develops toward that food. This may be due to structural and functional alterations in higher order and cortical neurons, a central phenomenon.

**APPLIED PHYSIOLOGY**

**Ageusia and Hypogeusia**

Absence of the sense of taste is known as ageusia, and diminished taste sensitivity is known as hypogeusia. These conditions can occur due to the following causes:

- **Xerostomia** (dryness of mouth): Due to lack of salivary secretion, the chemicals in food are not dissolved in saliva and can not reach the receptor cell. In Sjögren's...
syndrome, an autoimmune inflammatory disease, there is progressive loss of salivary glands.

- **Drugs** like captopril, cisplatin and penicillamine
- Tobacco use for a prolonged period
- Deficiency of vitamin B₃ or zinc
- **Damage to receptor cells** as occurs in old age or due to radiation therapy and herpes virus
- **Injury to gustatory pathway neurons**: Due to trauma or neoplasm, stroke, or systemic diseases like diabetes mellitus and hypothyroidism.

**Selective taste blindness**: Some persons show insensitivity to the taste of certain substance, whereas perception of other tastants remains unaltered. This is known as **selective taste blindness**. For example, some individuals do not perceive the taste of the bitter tasting chemical phenyl thiocarbamide, but the taste sensations of sweet, sour, salt and other bitter stimuli are normal. The condition is inherited as an autosomal recessive trait and may be due to lack of formation of a particular receptor protein.

**Dysgeusia**

Distorted sense of taste is known as **dysgeusia or parageusia**, in which the perception of taste is different from what is being presented (for e.g. a salty food may taste bitter), or there is perception of a taste without any tastants in the oral cavity (hallucination of taste). Dysgeusia is a feature of **temporal lobe epilepsy**.

**Following adrenalectomy** in man, there is an increased sensitivity to taste sensation.

**Tests for Taste Sensation**

The tests are done by serial application of different types of taste solution over the surface of a protruded tongue. The person is asked to indicate the type of taste perceived by showing a card and the tongue is thoroughly rinsed after application of each taste solution.

**Reflexes Associated with Taste**

On arrival of the taste molecules at the vicinity of the taste buds, there occurs reflex stimulation of salivary secretion sometimes called **taste-salivary reflex**. The afferent goes via the Vth, IXth, and Xth cranial nerves to the nucleus tractus solitarius, which project to the superior and inferior salivary nuclei of the facial and glossopharyngeal nerves. The efferent fibers from there innervate the salivary glands.

Presence of food in the mouth also stimulates gastric and pancreatic secretion by activating vagal efferent fibers, thereby prepares the alimentary tract for the digestion of the food if swallowed. These reflexes are the basis of the **conditioning reflexes** described by Pavlov and others.
155. Structure and Functions of the Skin
156. Regulation of Body Temperature and Acclimatization to Hot and Cold Environments
157. Physiology of Exercise and Sports Science
158. Principles of Acid-Base Homeostasis
159. Regulation of Volume, Composition and Osmolality of Body Fluid Compartments
160. Physiology of Growth and Development
161. Physiology of Nutrition
162. Physiology of Aging and Oxidative Stress, Prevention of Aging and Physiology of Yoga
“The Spirit shall look out through Matter’s gaze
And Matter shall reveal the Spirit’s face.

........The Spirit shall take up the human play
This earthly life become the life divine”

Sri Aurobindo (in ‘SAVITRI’)
The skin is the outermost layer of the body that serves as protective cover for the body.

1. It acts as a partition between the internal and external environments of the body.
2. With all its appendages, skin is also called as an integument. For its many secretory and synthetic functions, it is also called as an organ of the body.
3. As it covers the whole body part, it is considered as the largest organ of the body.
4. The total weight of the skin is about 3.5 kg.
5. Skin is in close contact with the external environment. Therefore, skin is immediately affected by the changes in the environment.
6. Skin plays an important role in providing nonspecific immunity to the body.

Layers of the Skin

The skin is divided into two main layers: epidermis and dermis.

Epidermis

The epidermis is formed by stratified squamous epithelium. It consists of five layers (Fig. 155.1). From superficial to deep, they are as follows:

i. Stratum corneum
ii. Stratum lucidum
1. Nuclei are almost absent in many cells.
2. They are continuously shed off from the skin.
3. It is particularly thick in palm and sole, where the skin is thicker than other parts of the body.

**Stratum Lucidum**

Stratum lucidum appears homogenous throughout.
1. It is composed of flattened cells that contain eleidin, a protein, which is the precursor of keratin.
2. These cells degenerate and migrate superficially to form the stratum corneum.

**Stratum Granulosum**

Stratum granulosum layer contains cells that are rich in granules.
1. The granules are formed by keratohyalin.
2. This layer consists of multiple layers of rhomboidal cells.

**Stratum Spinosum**

Stratum spinosum is formed by multiple layers of polyhedral cells, called prickle cells due to presence of spine-like processes on their surface.
1. These cells migrate toward the surface forming the three superficial layers to it, one after another.
2. Langerhans cells that participate in defense mechanism are present in this layer.

**Stratum Germinativum**

Stratum germinativum is the deepest layer of epidermis.
1. This is also called stratum basale.
2. Cells are arranged in a single layer on a basement membrane. Active mitosis occurs in the cells of this layer and the cells gradually shift through the above four layers toward the surface.
3. **Melanocytes**, the cells that synthesize and secrete melanin are present in this layer of the skin. Melanocytes have melanosomes and melanin granules that are located mainly in the cytoplasmic or dendritic processes (Fig. 155.2).

4. Tyrosine plays an important role in **melanin synthesis in melanocytes** (Application Box 155.1). Tyrosinase converts tyrosine into 3,4-dihydroxyphenylalanine (DOPA), which is converted into melanin through a series of biochemical reactions.

5. Melanin is stored in the vesicles formed by Golgi complex in melanocytes and secreted by exocytosis.

**Dermis**

Dermis is the inner layer of the skin. Many glands, hair follicles and few smooth muscles along with fibroblasts and histiocytes are present in the dermis. Dermis is composed of two layers: a superficial papillary layer and a deep reticular layer.

**Papillary Layer**

The papillary layer is a compact layer that forms papillae. Papillae from this layer project into the epidermis. The papillae contain blood vessels, nerve endings and lymphatics.

**Application Box 155.1**

**Skin Color:** Color of the skin depends mainly on the quantity of melanin formed by the melanocytes in the stratum germinativum layer of the skin. Melanin makes the skin dark. Exposure to sunlight facilitates melanin formation. Hence, individuals remaining indoor during most part of their work usually have skin that is less dark. Also, the amount of blood flow through the skin determines its color. More the blood flow more pink or red is the skin. Decreased blood flow makes the skin pale. Other factors that contribute to the color of the skin are the quantity of carotene, reduced Hb, and bilirubin deposited in the skin. Genetic factor is the important determinant of skin color.

**Reticular Layer**

The reticular layer consists of fat and loose areolar tissue impregnated with reticular and elastic fibers. This layer merges with the subcutaneous layer of fat and binds the skin with deeper structures.

**Glands in the Skin**

The glands in the skin are mainly the sweat glands and sebaceous glands (Fig. 155.3). Sweat glands are present all over the skin except in ear drum, lips and glans penis and are of two types: merocrine and apocrine.

**Merocrine Glands**

The merocrine glands are known as eccrine glands. They are present in the skin all over the body.
1. They are simple tubular glands.
2. The tubules of the gland are very long and coiled.
3. These glands form the usual sweat glands in the skin, and are usually present in a large number.
4. Thus, sweat is formed mainly from the eccrine glands.
5. Following injury, cells from these glands also contribute to regeneration of the skin.
Apocrine Glands
The apocrine glands are present on mons pubis, axilla, circumcumanal area and areola of breasts.
1. The ceruminous and mammary glands belong to this category of cutaneous glands.
2. The secretion from these glands starts after puberty and impart characteristic odor to the body.

Sebaceous Glands
Sebaceous glands are small pear-shaped alveolar glands (Fig. 155.3).
1. They open at the hair follicles or directly to the skin.
2. Obstruction of secretion from these glands is responsible for acne formation, which is seen at puberty due to increased activity of dehydroepiandrosterone.

Skin Blood Supply
Blood supply of skin varies between 1 to 150 ml/100g of the tissue. On average, it is about 13 ml/100 g of tissue, which accounts for about 460 ml/min through the entire cutaneous circulation. Details of arrangement of cutaneous blood vessels and regulation of cutaneous blood flow are described in “Cutaneous Circulation” in Cardiovascular system.

Skin Appendages
Skin appendages include hairs and nails.

Hairs
Types of Hair
Different types of hairs are seen in fetus and in postnatal life. On the body of the fetus, the hairs are soft that are called lanugo hairs. After birth, two types of hairs are seen: vellus and terminal hairs.

Vellus hairs: The vellus hairs are soft hairs found on the body of infants and children.
Terminal hairs: The terminal hairs grow during puberty and replace the vellus hairs of childhood. However, vellus hairs persist on the face of adult females.

Parts of the Hair
Hair is a keratinized thread developed from epidermis. It has a shaft that projects outside the skin, a root that remains within the skin and a deeper part called hair bulb, which is embedded in the hair follicle (Fig. 155.4). It is indented below by the connective tissue papilla.

Structure of the Hair
The hair is composed of medulla, cortex and the cuticle (Fig. 155.4).

The medulla: The medulla is the central part of the hair. It is formed of cubical cells at the root that are cornified toward the shaft.
The cortex: The cortex is formed of cells as in medulla.
The cuticle: It is the outermost layer, is formed of a single layer of cell.

The hair follicle consists of an epithelial root sheath, which is continuous with the stratum germinativum. A connective tissue sheath is present around the follicle. The erector pilorum muscle is attached to the follicle. Contraction of this muscle causes erection of hair during sympathetic stimulation. Sebaceous glands also open to the hair follicle.

Nails
Nails are modified skin at the tip of digits. Nail consists of a root, a body and the free margin. The root is continuous with the stratum germinativum layer of the epidermis. In fact, constant proliferation of cells from stratum germinativum, continuous growth of nail occurs. These cells in the nail become flat and keratinized.

Fig. 155.3: Skin appendages. Note the hair follicles (3), erector spinae muscle (6) and sebaceous (7) and sweat glands (5). 1: Epidermis; 2: Dermis; 4: Hair.

Fig. 155.4: Parts of the hair.
FUNCTIONS OF THE SKIN

1. **Body protection**: Skin is the outer covering that forms protective layer on the body. It provides *mechanical protection against infection*. Therefore, *loss of skin in burns leads to severe sepsis*. Skin is also impervious to many substances in the air and water. Thus, the body is protected from toxic materials in the environment.

2. **Temperature regulation**: Secretion of sweat contributes significantly to temperature regulation. It causes *evaporation of water* from the skin. Skin also allows *insensible perspiration*, which helps in temperature regulation. *Sebum*, the secretion of sebaceous glands makes the skin greasy that prevent the skin from drying.

3. **Sensory organs**: There are many sensory receptors in the skin (*exteroceptors*) that help to collect information from the environment. Hair follicles are rich in such receptors. Therefore, hairs are more sensitive sensory structures. All types of receptors such as pain, touch, temperature, etc. are present in skin.

4. **Vitamin synthesis**: In exposure to sunlight, skin synthesizes 7-dehydrocholesterol, the precursor of vitamin D₃.

5. **Contributes to central blood volume**: Cutaneous vascular bed accommodates a good quantity of blood volume. Hence, constriction of blood vessels in the skin contributes to central blood reservoir, from where blood can be diverted to vital organs at the time of need. In circulatory shock, blood is diverted from cutaneous and splanchnic bed to cerebral and coronary circulations to perfusion of brain and heart respectively. This is achieved by sympathetic vasoconstriction, an immediate compensatory mechanism activated by shock. Also, cutaneous vasoconstriction or dilation plays an important role in temperature regulation (for details, refer the next chapter).

6. **Water and electrolyte balance**: Sweat secreted from the skin contains water and salt. Hence, excessive sweating leads to dehydration and sodium deficiency. Hormones like aldosterone affect water and electrolyte excretion from skin. Sweat also acts as a medium for excretion of waste materials from the body.

7. **Protection from UV rays**: Skin protects the body against the damaging effects of ultraviolet (UV) rays of sunlight. Melanin pigment in the skin helps in this process.

8. **Immunity**: Skin plays a crucial role in nonspecific defense of the body. Sebum secreted from sebaceous glands is acidic and contains microbicidal agents. Clasmatocytes in the skin are cells of mononuclear phagocyte system that trap and kill organism. Skin is the *natural dressing of the body*. Therefore, loss of skin as occurs in burns or injury allows pathogenic bacteria to enter the body, which produces septicemia if uncontrolled.

9. **Route for drug administration**: Many lipid soluble drugs like steroids and fat-soluble vitamins are applied over the skin. *Topical ointments* are applied on the skin as their absorption is poor from the skin, hence they act locally. However, drugs are used as *subcutaneous implants* when their release into circulation is required at a lower rate over a period of months or years. Estrogen-progesterone contraceptive implants are examples.

10. **Diagnosis of diseases**: Apart from skin diseases, many diagnostic signs of systemic diseases are recorded from the skin surface. For example, typical skin changes that occur in systemic lupus erythematosus. *Degree of anemia, jaundice and cyanosis* are detected from skin and mucus membrane.

11. **Beauty of the Body**: The beauty of the body primarily depends on the health of the skin. Prettiness of an individual not only lies in his or her complexion and construction of the body, but also on the physical condition of the skin that makes the body surface shining and attractive. An unhealthy skin imparts poor appearance to the body, irrespective of its color. Therefore, health of the skin is the real wealth of the being.

### CHAPTER SUMMARY

**Key Concepts**

1. Skin is the protective layer of the body. Therefore, in burns, organisms enter the body and produce septic shock.
2. Vitamin D formation takes place in skin.

**Important to Know (Must Read)**

1. Usually, *Long Questions* are not asked from this chapter.
2. Functions of skin, Glands in skin, Melanocytes, may come as *Short Questions*.
3. In *Viva*, examiner may ask… What are the layers of skin, What are the glands present in skin and their function, Types of hairs and their function, Functions of skin.
Regulation of internal body temperature provides an opportunity for higher creatures to lead a life independent from the environmental conditions. In addition, human beings are especially equipped to protect themselves from extreme weathers by changing their bodily clothing and altering the physical environments. Few animals are also blessed with extra protection from extreme conditions by natural physical adaptations. For example, an animal in Greenland acclimatizes to extreme cold by growing a thick and hairy skin that insulates its body from external freezing temperature.

Types of Animals

Based on the ability to regulate body temperature, animals are broadly divided into two categories: homeothermic and poikilothermic.

Homeothermic Animals

Animals that are capable of regulating their internal body temperature are called homeothermic animals.

Poikilothermic Animals

Animals that are not capable of regulating their internal body temperature are called poikilothermic animals.

Components of Temperature Regulation

As chemical and metabolic reactions of the body are temperature sensitive, most physiological activities depend on internal body temperature.
Body temperature is affected by following factors:
1. In homeotherms, temperature regulating mechanisms create an optimal internal environment for body functions to continue smoothly.
2. Also, an effective thermoregulatory system prevents body from damaging consequences of pathological alterations in body temperature.
3. Such a system works on the principle of visceral reflex mechanisms.
4. Therefore, the components of a temperature controlling system consist of a general reflex mechanism, such as: 1. Sensor (thermal sensor), 2. Afferent pathway, 3. Integrating center (s), 4. Efferent pathway, and 5. Effector organs.
5. Effector organs such as skeletal muscle, skin and sweat glands control heat mainly by generating and transferring heat from the body.

Factors Influencing Body Temperature

Normal body temperature and its recording: The normal body temperature of an adult is approximately 37°C, which corresponds to 98.6°F. However, the temperature varies from 36.6°C to 37.2°C or 98°F to 99°F (Table 156.1):
1. Though, axillary temperature is recorded in clinics by using a clinical thermometer, it is ideal to record temperature orally (sublingually). Normally, axillary temperature is 0.5°C less than sublingual temperature.
2. In infants and children, rectal temperature is recorded. The temperature recorded from body surface is called shell temperature, which varies between 31°C to 34°C on different parts of the body and at different times. This temperature is significantly less than the inner body temperature and is easily affected by environmental conditions.
3. The temperature recorded from inner body structures is called core temperature, which is about 37°C. The core temperature is not easily affected by environmental conditions.
4. Rectal, vaginal and esophageal recordings of temperature represent core temperature. However, recording of temperature through these routes is not convenient, and therefore, oral temperature recording is preferred clinically, which is more or less close to inner body temperature.

Table 156.1: Normal and abnormal body temperatures.

<table>
<thead>
<tr>
<th></th>
<th>Centigrade</th>
<th>Fahrenheit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>36.6–37.2°C</td>
<td>98–99°F</td>
</tr>
<tr>
<td>Subnormal</td>
<td>&lt; 36.6°C</td>
<td>&lt; 98°F</td>
</tr>
<tr>
<td>Febrile (pyrexia)</td>
<td>&gt; 37.2°C</td>
<td>&gt; 99°F</td>
</tr>
<tr>
<td>Hyperpyrexia</td>
<td>&gt; 41.6°C</td>
<td>&gt; 107°F</td>
</tr>
<tr>
<td>Hypothermia</td>
<td>&lt; 35°C</td>
<td>&lt; 95°F</td>
</tr>
</tbody>
</table>

Fig. 156.1: Diurnal variation in body temperature.

Time of the Day
In human beings, body temperature is maintained by a circadian 24-h cycle, known as body-temperature rhythm that varies approximately by 0.8°C.
1. The body temperature is usually lowest in the early morning between 3 to 6 am and maximum in the evening between 4 to 6 pm (Fig. 156.1).
2. This circadian rhythmicity is an intrinsic mechanism and is independent of the sleep-wakefulness cycle.

State of Physical Activity
Increased physical activity generates excess heat by increasing rate of metabolism. Temperature is elevated during physical activity and remains elevated during recovery period. This increased body heat triggers appropriate heat-loss mechanisms.

Age
Newborns, infants and elderly people are less capable of maintaining body temperature especially in an altered state of environmental condition.
1. Sweating and shivering are poorly developed in newborns. Therefore, they fail to regulate body temperature and behave like a poikilotherm.
2. The surface-to-mass ratio is also high in infant and children, which render them susceptible to wide fluctuations in core temperature on exposure to extreme weathers.
3. Elderly people have decreased metabolic rate, reduced muscle mass, atrophied sweat gland, decreased cardiovascular reserve to dissipate heat and impaired ability to sense heat and cold.
4. Therefore, they are also subjected to greater fluctuations in core temperature on exposure to external challenges for their reduced ability to generate and dissipate heat.
Gender

In women during their reproductive life, under the influence of progesterone, body temperature increases by about 0.5°C following ovulation (in the post-ovulatory phase of the menstrual cycle). This is called rise in basal body temperature, which is an indicator of ovulation. Females are less influenced than males by external temperature due to the presence of more subcutaneous fat in them.

State of Sympathetic Activity

People with higher basal sympathetic tone, irrespective of other factors have higher body temperature due to increased catecholamines that increase body metabolism. Therefore, individuals with sympathetic personality have higher body temperature than parasympathetic personality.

Body Size

Heat generation and dissipation depend on the ratio of body mass to body surface area. Therefore, in smaller animals, body heat is more than in larger animals, and so also in human beings.

Type of Meal

Food rich in protein generates more heat due to higher SDA (specific dynamic action) of proteins. Animal proteins produce more heat than plant proteins.

Environmental Conditions

Environmental temperature and humidity and state of atmospheric circulation of air influence body temperature by altering evaporation of heat from the body.

Types of Clothing

Nature of clothing influences body temperature. Usually, silken and linen clothing increase body temperature by preventing heat loss from the body. Cotton clothing has opposite effect. Therefore, generally it is advised to wear cotton dresses in summer and silk and linen dresses in winter.

Sleep

During sleep, body temperature is less due to central as well as peripheral mechanisms. In sleep, heat production is less due to decreased sympathetic and physical activities, and also due to decreased sensitivity of the thermostat.

Emotion and Excitement

Emotional reactions and excitements increase body temperature.

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PRINCIPLES OF HEAT GENERATION AND TRANSFER

Heat Generation

Rate of Heat Production

In normal individuals, rate of heat production varies between 80 kcal/h at rest to 600 kcal/h during exercise. This is intimately associated with the rate of metabolism that generates heat.

1. The resting metabolic rate (RMR) provides basal energy required for maintaining the functions of the cells at rest such as active transport of ions in the tissues, cardiac pump activity and activity of respiratory muscles.
2. Any extramuscular activity, voluntary such as walking or involuntary such as shivering adds to the overall metabolic heat production. Increased process of digestion and absorption following a meal is the example of state of increased metabolism.
3. Many hormones such as thyroxine, norepinephrine, epinephrine and glucagon increase the rate of cellular metabolism.

Rate of heat generation: In resting conditions, body’s energy requirement is low.

1. Therefore, the rate of heat production is less, which is about 80 kcal/h.
2. Conversely, during exercise, the rate of heat generation is more, which is proportionate to the intensity of exercise.
3. This may be as high as 600 kcal/h as occurs in severe exercise such as fast walking for about one hour.
4. The thermal load in physical exercise increases the core temperature of the body by about 1°C for every 10 minutes of exercise if heat loss is not activated adequately.
5. Usually, the body increases the rate of heat dissipation to match its heat generation.

Heat Transfer

Heat transfer from the tissue to the circulating blood depends on four factors: the rate of energy production, the temperature of the tissue, temperature of the incoming blood to the tissue and the amount of blood flowing through the tissue.

1. The rate of O₂ consumption by the tissue is a measure of metabolic rate of the tissue. On average, 1.5 to 2 mL of O₂ per minute is consumed by one kg of muscle tissue. The principal source of heat in the body is the skeletal muscle, which can increase its rate of heat production to more than 100-fold.
2. However, the remarkable increase in tissue temperature in exercise is relatively short-lived for following two reasons:
- The increase in muscle temperature reverses the temperature gradient between muscle and the blood perfusing it. Therefore, after few minutes of exercise, heat flows from muscle to blood.
- Dilation of blood vessel of skeletal muscle decreases vascular resistance in muscle. This, plus increased cardiac output rapidly increase blood flow through the exercising muscle, which is proportionate to the intensity of exercise. Blood flow increases up to 30-fold, which accounts for the major transfer of heat from active muscle to blood. However, as exercise continues, muscle temperature increases to a new steady-state level. This causes continuous transfer of more heat from exercising muscle to blood. Thus, it keeps increasing the core temperature of the body as warm venous blood leaves active muscles and enters the body core.

### Transfer of Heat from Body Core to the Skin

The excess of heat carried away from active tissue to the blood, temporarily increases core temperature. Body prevents the core from overheating by transferring heat to the surface:

1. Heat is transferred from core to the skin, which is relatively cool. Skin is the largest organ in the body. Most of the heat generated in the body flows to the skin by convection in the blood. The blood flow to the skin can increase markedly due to thermogenic vasodilation.
2. Therefore, under physiological conditions, alteration in cutaneous blood flow is the primary determinant of heat flow from body core to body surface (skin). Hence, the capacity to restrict blood flow to the body surface is an essential defense mechanism against cooling of the body in hypothermia.
3. Similarly, the ability to promote skin blood flow is an essential defense against hyperthermia.
4. Some heat is also transferred from the body core by the evaporation of water from the respiratory tract, which is proportional to the rate of pulmonary ventilation.

### Heat Loss from Skin to the Environment

Transfer of heat from skin to environment occurs by radiation, conduction, convection, and evaporation (see below).

### Clothing Limits Heat Transfer

Clothing insulates the body from environment and impairs heat transfer between body core and the environment. However, with light clothing, heat is also transferred from a warmer body to a cooler environment by means of radiation, conduction, convection and evaporation that take place from the clothing surface rather than directly from the skin. Hence, more clothing to cover larger area of skin and use of thicker clothing are major ways of protecting body temperature in cold atmosphere.

### HEAT BALANCE

This is the balance between heat preservation and heat dissipation. It maintains body temperature within its narrow normal range. Disturbance in this balance leads to abnormal body temperature.

### Heat Preservation

Preservation of heat in the body depends on heat production in the body and heat gain from the environment.

### Mechanisms of Heat Production

At rest, heat production depends on basal metabolic activity, muscular activity, specific dynamic action of food and nonshivering thermogenesis.

#### Basal Metabolic Activity

The rate of basal metabolism is the major cause of heat production in the body. In this process, food substrates are oxidized to produce energy:

1. One gram of carbohydrate, protein and fat yield about 4, 4, and 9 calories respectively. The metabolic energy is utilized in transport of membrane pumps, energy-requiring chemical reactions and muscular activity. However, most of the metabolic energy used in these processes is converted into heat in the body. This is called metabolic heat.
2. At rest, the metabolic rate is about 70 to 80 kcal/h. About 70% of energy production occurs in the core of the body, especially by the viscera in the thorax, abdomen and pelvis, and the brain, although the visceral structures constitute about only 36% of the total body mass.
3. Though muscle and skin contribute to about 56% of body mass, their contribution to heat production is only 18% (Table 156.2).
4. However, during physical exercise, 90% of heat is produced by muscular activity.

#### Muscular Activity

As noted above, enormous quantity of heat is produced by skeletal muscle during exercise:

1. However, also at rest, the basal muscular activity to some extent contributes to heat production. This is mainly by the activity of respiratory muscle, cardiac muscle and basal tone of skeletal muscle.
2. On exposure to cold, skeletal muscle activity increases that increases heat production by several fold. This is called shivering thermogenesis.

### SDA of Food and Digestion of Food

During digestion and assimilation of food, the energy produced is converted into heat.
Chapter 156: Regulation of Body Temperature and Acclimatization to Hot and Cold Environments

1. The metabolic rate and thus, the heat production increases by about 15% after a meal. This is called specific dynamic action (SDA) of food or thermic effect of food, which lasts for several hours after food intake.

2. SDA is maximum for protein. Therefore, ingestion of protein-rich food has more thermic effect.

3. Also, during digestion and absorption of food from GI tract, increased intestinal motility and secretion increases heat production.

4. The act of eating increases heat production due to increased muscular activity of chewing and swallowing.

Nonshivering Thermogenesis

Hormones such as catecholamines and thyroxine increase body metabolism. Therefore, these hormones contribute to basal heat production. This is called nonshivering thermogenesis. Excess secretion of these hormones increases body temperature.

Mechanisms of Heat Gain from the Environment

From environment, body gains heat if environmental temperature is more than body temperature, and vice versa when environmental temperature is less. Heat gain from environment occurs by three mechanisms: radiation, conduction and convection. Heat gain also occurs by means of ingestion and ventilation.

Radiation

Heat transfer by radiation occurs between the skin and solid bodies in the environment. The infrared portion of the electromagnetic energy spectrum carries radiation energy. Therefore, infrared cameras can detect warm bodies at night.

Conduction

Heat transfer by conduction occurs when the body touches a solid material of different temperature. For example, body lying on hot sand conducts heat from the sand.

Convection

Transfer of heat into the body from a hot fluid or air is called convection. For example, when body is immersed in warm or hot water, heat is conducted into the body.

Ingestion and Ventilation

Ingestion of hot food and drinks (tea, coffee) allows heat to enter the body. Breathing hot air from external environment as in hot climate, increases body temperature.

Heat Dissipation

Heat is lost from body through skin (cutaneous heat loss), lungs (pulmonary heat loss) and excretory organs (excretory heat loss). From skin, heat is dissipated into the atmosphere by means of radiation, conduction, convection and evaporation.

Cutaneous Heat Loss

From skin, heat is dissipated into the atmosphere by means of radiation, conduction, convection and evaporation.

Radiation

Heat transfer by radiation occurs between two objects having difference in their temperature. In winter, the radiant heat loss from the body to low radiant temperature of the environment accounts for substantial loss of heat from the body.

1. Therefore, sudden feeling of chill on exposure to cold weather occurs due to abrupt fall in skin temperature owing to radiant heat loss.

2. Radiation of heat from the body accounts for about 50% of heat loss from the body (Table 156.3) when the body is at rest in a neutral thermal indoor environment (the environment in which air temperature, air flow and humidity to the naked body does not lead to shivering or sweating).

3. The loss of heat by radiation increases with decreased environmental temperature.

Conduction

Conduction transfers heat from the body when the body touches a solid material of different temperature. For example, placing an ice pack on the body surface causes heat loss by conduction. However, clothing causes minimal heat loss.

Convection

Heat transfer by convection occurs when body is exposed to a fluid or air with temperature different from the body. The convective heat loss is proportional to the difference in temperature.
between skin and ambient temperature. **Exposure to cold air or water** causes convective heat loss from the body (Application Box 156.1).

**Application Box 156.1**

**Hypothermia killed people of Titanic disaster:** When the body is immersed in water, heat exchange essentially occurs by convection. Radiation and evaporation do not contribute to heat loss in this condition. As conductivity and thermal capacity of water are high, the heat-transfer in water is 100 times greater than that in air. Immersion of body in chilled water leads to speedy loss of heat from the body leading to rapid hypothermia. Therefore, it was believed that the most of the deaths in the historic Titanic shipwreck disaster were due to hypothermia rather than drowning.

**Evaporation**

Dissipation of all of the extra heat during exercise can occur by evaporating sweat from the skin surface. The rate of evaporation does not depend on the temperature gradient, rather proportionate to the water vapor-pressure gradient between skin and environment. Evaporation from skin accounts for loss of about 600 cal (20% of heat) from the body. Evaporation occurs in two forms: sweating and perspiration (Flowchart 156.2).

**Sweating**

Evaporation of 1 g of water from the skin removes about 0.6 kcal from the body.
1. As the sweat glands of the body release about 30 g (approximately 30 mL) of fluid per minute or 2 liters/h to the skin surface, **evaporation can remove 18 kcal/min** from the body.
2. Thus, almost all of the heat produced during severe exercise could be removed by evaporation under normal conditions by sweating.
3. However, the efficiency of heat transfer from the skin to the environment depends on many physiological and environmental factors such as humidity and skin temperature.
4. **When humidity is high**, the water vapor-pressure gradient between skin and environment becomes low. This decreases evaporation and therefore, facilitates accumulation of excess heat produced during exercise. Hence, humidity impairs exercise performance.
5. **When humidity is low**, as in desert conditions, heat loss by evaporation occurs easily, even in the presence of high ambient temperature.

**Perspiration**

Perspiration is **insensible water loss**. Diffusion of fluid from the body to epidermis occurs continuously, which is evaporated from the body surface, even in the absence of external sweating. This is called perspiration. It is not sensed by the body as sweating does not occur. It usually does not depend on external conditions (Application Box 156.2). It accounts for loss of about 400 kcal heat per day.

**Application Box 156.2**

**Heat exhaustion is common in athlete:** It is now possible to predict the amount of heat loss from the body with knowledge of the transfer coefficients, and the gradients of temperature and water vapor-pressure between the skin and environment. This is especially important for an athlete, as dissipating the thermal load is essential for prolonging exercise. A physiotherapist and a sports physician must understand these basic principles to treat thermal illnesses. In **heat exhaustion** as occurs in excessive heat exposure, core temperature may be as high as 39°C as body cannot dissipate the heat load. This leads to **dehydration** that reduces sweating, and **hypovolemia** that reduces blood flow from muscle to core of the body and from there to the skin. Heat exhaustion is common in athletes. In extreme cases, it may lead to **heat stroke**, in which core temperature may be 41°C or more, due to impaired thermoregulatory mechanisms.
**Pulmonary Heat Loss**

Pulmonary heat loss occurs in **three ways**: evaporation of water in expired air, warming of inspired air and panting (see Flowchart 156.2).

**Evaporation of Water in Expired Air**

About 300 ml of water is lost from lungs in expired air. This is because **water is evaporated in expired air**. Also the expired air is warm and the **heat is lost in expired air**. All these account for about loss of 200 kcal of heat. In animals, especially in dogs, it is the primary mechanism of heat loss.

**Warming of Inspired Air**

Air breathed in from atmosphere is cooler than the body temperature. Therefore, in the air passage warming of inspired air occurs. In this process, 2% of heat is lost from the body.

**Panting**

Panting is the **rapid and shallow breathing through the mouth cavity**. It is typically seen in dog and few other mammals. As mouth cavity is kept open in such breathing, evaporation of water increases from the mouth. Thus, heat is lost in this process. However, normally panting does not occur in humans.

**Excretory Heat Loss**

Any substance (fluid, semisolid or solid) lost from the body is warm. Stool coming out of large intestine and urine coming out of urinary tract are warm as they come out of the inner core of the body. In this process of removal of excretory products, heat is lost from the body.

**Balance Between Production and Loss of Heat**

Balance between heat loss and heat production, **keeps the core temperature constant**. Heat is mainly **produced by metabolism**:

1. Metabolic heat moves from the tissue to the core, then from core to the skin, and finally from skin to the environment.
2. **Increase in metabolism or interference of transfer of heat** at any part of this heat dissipation system leads to **increase in core temperature**.
3. When metabolic production of heat equals heat loss, the **heat storage is nil** and the core temperature is constant.
4. **Core temperature rises** when the heat storage is positive, as occurs in exercise, prolonged immersion in warm water, and excessive heat exposure.

**Thermal Sensors**

Thermal sensors are **thermoreceptors** that are located in **two crucial structures** in the body: the skin and the hypothalamus. They respond to changes in local temperature. These thermosensitive sensors are **free nerve endings** that are distributed over the skin of the entire body surface. The cutaneous thermoreceptors inform body about thermal condition of the external environment. There are also specialized sensors in the preoptic area of hypothalamus (hypothalamic thermoreceptors) that inform CNS about the body’s internal thermal condition.

**Cutaneous Thermoreceptors**

The cutaneous thermoreceptors are present in the skin and mucus membrane. Few thermoreceptors are also located in visceral organs. They sense change in ambient temperature. There are **two distinct thermoreceptors**: warmth and cold receptors.

**Warmth Receptors**

Warmth receptors constitute only about 10% of thermoreceptors. They are **stimulated at higher temperature**. When local temperature increases to 44–46°C, warmth receptors increase their firing rate steadily.

**Cold Receptors**

About 90% of thermoreceptors in skin are cold receptors. They typically increase their firing rate when local temperature decreases from approximately 40°C to 24°C.
1. Two types of receptor responses are observed in thermoreceptors: static and dynamic.
   - When temperature change is sustained, a stable change occurs in the sensor’s firing rate. This is called tonic or static response.
   - When change in temperature is a temporary one, the response of thermoreceptors becomes phasic or dynamic.
2. Cutaneous sensors provide information about immediate change in ambient temperature to hypothalamic thermoregulatory center. Thus, for the body, they serve as early-monitoring and warning systems for rapidly changing environmental temperature.
3. Thermoreceptors also provide information to the cerebral cortex about conscious perception of the thermal environment and the degree of thermal comfort.

**Hypothalamic Thermoreceptors**

Hypothalamus plays an important role in temperature regulation. About 10% of hypothalamic neurons exhibit an increased activity in response to change in local temperature:
1. Hypothalamic thermoreceptors play a crucial role in conditions in which rate of heat production and dissipation changes dramatically as happens during exercise.
2. Failure on the part of hypothalamus to detect such changes and mediate such responses leads to rapid alteration in core temperature resulting in grievous consequences.
3. In hypothalamus, unlike that in the skin, warmth receptors greatly outnumber cold receptors.

**Hypothalamic Temperature Regulating Neurons**

Hypothalamic neurons that are involved in regulation of body temperature are broadly divided into three categories: sensor neurons, heat-loss neurons and heat production neurons.

**Sensor Neurons**

These are hypothalamic thermoreceptors that are located in anterior hypothalamus. As described above, warmth sensitive cells are more than the cold sensitive cells. They collect information about temperature from both peripheral and central thermoreceptors.

**Heat-loss Neurons**

Neurons that on activation induce loss of heat are located in anterior hypothalamus.
1. Therefore, anterior hypothalamus is called heat loss center.
2. On stimulation, this center produces vasodilation and sweating that promote heat loss.

3. Lesion of this center produces hyperthermia, especially on exposure to hot environment.
4. Preoptic area is also classified under heat loss center.

**Heat-production Neurons**

The neurons that on activation activate mechanisms that prevent loss of heat and produce internal heat are located in posterior hypothalamus.
1. Therefore, posterior hypothalamus is called heat production center.
2. On activation, it produces cutaneous vasoconstriction and shivering that increase body temperature.
3. Lesion of this center leads to hypothermia, especially on exposure to cold environment.

**Role of Hypothalamic Centers**

The hypothalamic temperature regulating centers integrate information collected from thermoreceptors and direct alterations in efferent systems to modify rate of heat transfer.
1. Change in the temperature of the environment close to the skin alters both the tonic and the phasic components of thermoreceptor activity.
2. The impulses generated in cutaneous thermoreceptors ascend up in the sensory pathway in the spinal cord. Through collaterals from the ascending pathways information reach hypothalamus.
3. Hypothalamus integrates thermal information received from skin and from other parts of the body including the hypothalamus.
4. Hypothalamus, compares the prevailing thermal situation with the standardized set of thermal condition of the body, and accordingly directs appropriate command signal in the efferent pathway to alter the rate of the heat transfer from the body by either generating and preserving the heat, or by dissipating the heat.
5. For its major role in regulation of body temperature, hypothalamus is designated as thermostat of the body.
6. This should also be noted that thermosensitivity of hypothalamus is 8–10 times greater the thermosensitivity of skin.

**Nature of Responses**

The responses are both reflexive and feedback in nature. Reflex responses occur mostly to cutaneous receptor stimulation and feedback responses occur mostly to change in core temperature.

**Reflex Responses**

The cutaneous sensors provide information about ambient temperature that affects rate of body’s heat transfer:
1. These are the reflex responses to change in skin temperature, which may be anticipatory in nature.
2. These anticipatory responses to the stimulation of skin receptors are important components of an effective
thermoregulatory system as relying on central receptors alone is not adequate to overcome body’s thermal inertia.

**Feedback Responses**

Thermoregulatory responses to changes in internal body temperature, as occur during exercise, are mainly negative feedback in nature.

1. These effects modify the rate of heat transfer that restores the core temperature to normal level.
2. The feedback responses are also essential components of an effective thermoregulatory system as they minimize change in core temperature and consequent changes in metabolic activity.

**Mechanism of Hypothalamic Integration**

The major effector mechanisms for temperature regulation are located in hypothalamus. This hypothalamic thermostat has a set point for body temperature:

1. The set point is set with a narrow range of temperature with average set temperature of 98.6°F or 37°C.
2. However, the so called “set point temperature” has no physio-anatomical basis though it reflects the effective integrated response of the regulatory system.
3. Any deviation from this set temperature is received by the thermostat as an error signal.
4. The hypothalamic integration center receives and interprets the change in the firing rate from either cutaneous or hypothalamic cold or warmth receptors as an error signal that would otherwise lead to body heating or cooling, if appropriate responses are not activated.

5. Thermal error signal influences hypothalamic integration centers to generate appropriate thermal effector signals (Flowchart 156.3).

**Responses**

Responses activated by thermal effector signals are mediated by various autonomic, somatic and endocrine mechanisms. The target effector organs mainly include cutaneous blood vessels, sweat glands and skeletal muscles. These responses may be broadly grouped into two categories: responses activated by heat and responses activated by cold (Table 156.4).

**Responses Activated by Heat**

On exposure to hot environment, body temperature increases. Body tries to reduce its temperature by activating the mechanisms that promote heat loss and the mechanisms that reduce heat production.

**Responses that Promote Heat Loss**

Three major responses promote heat loss from the body: cutaneous vasodilation, sweating and increased breathing.

**Cutaneous Vasodilation**

On exposure to heat, the major mechanism activated for heat dissipation is cutaneous vasodilation:

1. Skin blood flow increases up to ten-fold above the resting level. By increasing flow of blood from deeper parts of the body to the skin (the body surface), core heat is transferred to the surface.
2. This is called heat flow.
3. Heat flow to the skin is the primary mechanism of heat dissipation to the environment.
4. Thus, by adjusting the caliber of cutaneous arterioles, body facilitates blood flow, and therefore heat flow, from the core to the skin surface, the principal site of heat dissipation.

Mechanisms: Cutaneous blood flow is controlled by sympathetic nervous system.
- Active vasodilation in cutaneous vascular bed is achieved by sympathetic inhibition (neural vasodilation).
- Impulses from heat-loss center of hypothalamus inhibit sympathetic discharge to the cutaneous blood vessels.
- Vasodilation also occurs due to locally released kinins from sweat glands (chemical vasodilation) and direct effect of heat on blood vessel (thermogenic vasodilation).

Sweating
In moderate heat load, the primary mechanism of heat transfer is by increasing cutaneous blood flow.
1. When heat load is adequately large, the autonomic response activates the eccrine sweat glands to secrete sweat onto the skin surface. This promotes evaporation from skin surface that facilitates heat loss.
2. The secretory segment of the sweat gland is innervated by cholinergic sympathetic fibers. The activity of sympathetic fibers to sweat gland is increased by hypothalamic drive.
3. Sweating is almost nil in cold weather, which may reach 700 mL/h or more in a very hot weather. Associated loss of salt from the body causes sodium depletion (Application Box 156.3).

Increased Respiration
In hot weather, respiration is stimulated. Normally, in the process of breathing, hot air comes out of the lung that removes body heat to the environment.
1. Respiratory stimulation occurs due to both sympathetic and thermogenic activation. Therefore, expired air is felt warmer than the atmospheric air.
2. Panting as occurs in animals like dogs also facilitates heat loss from the oral cavity.

Application Box 156.3
Sweating causes salt and water loss: Excess sweating as occurs in hot and humid environments or during exercise, causes loss of water and salt. At its maximum rate, loss of water in sweat may be more than one liter per hour. Though concentration of salt is variable in sweat, sweat is always hypotonic to plasma. Therefore, sweating always increases osmolality of plasma. The NaCl concentration remains high following sweating until the lost water is replaced. The hypernatremic dehydration predisposes to heatstroke (see below).

Responses that Reduce Heat Production
Heat production is reduced by loss of appetite and reduced physical activity.

Decreased Appetite
In a hot environment, anorexia develops. Decreased food intake decreases the amount of heat produced by metabolism and SDA of food.

Decreased Physical Activity
In extreme hot weather, physical activities are reduced. The individual loses interest to work and develops lethargy. Decreased muscular activity decreases heat production.

Responses Activated by Cold
On exposure to cold environment, body temperature decreases. Body tries to elevate its temperature by activating the mechanisms that promote heat gain. Heat is gained by processes that promote heat production and the processes that reduce heat loss.

Responses that Promote Heat Production
Heat generation is increased by shivering and nonshivering thermogenesis, increased physical activity and increased intakes.

Shivering Thermogenesis
On immediate exposure to cold, shivering is initiated in responses to impulses from posterior hypothalamus. Shivering is an immediate thermogenic mechanism in severe cold. Shivering is involuntary rhythmic contractions of skeletal muscle. It increases metabolic rate by three to four times of that of basal level.

Nonshivering Thermogenesis
Increased cellular metabolism associated with uncoupling of oxidative phosphorylation increases body temperature. This is called nonshivering thermogenesis or chemical thermogenesis.
1. This occurs mainly due to secretion of catecholamines in response to sympathetic stimulation.
2. The quantity of brown fat in the body determines the level of chemical thermogenesis. This is because the cells of brown fat are rich in mitochondria that carry out uncoupled oxidation.
3. Also, brown fat has rich sympathetic innervation. In infants and children, brown fat is present in consider-ably large amount than in adults. Therefore, nonshivering thermogenesis is the major mechanism of increasing body temperature in infants and young children, as they do not have well developed shivering mechanism.
4. In adults, nonshivering thermogenesis contributes to about only 10% of thermogenesis. Chemical thermogenesis also occurs due to increased thyroxine secretion.
5. In chronic exposure to cold, TSH secretion from hypothalamus increases that causes thyroid hyperplasia and increased thyroxine secretion. Thyroxine promotes body metabolism and heat production.
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**Increased Physical Activity**
Apart from shivering, physical activities like rubbing the palm increase in cold. *Increased muscular activity* increases body heat.

**Increased Appetite**
Cold stimulates appetite. *Increased food intake* increases heat production by increasing metabolism and SDA of food.

**Responses that Decrease Heat Loss**
Heat loss from the body is decreased by cutaneous vasoconstriction, piloerection and change in body posture.

**Cutaneous Vasoconstriction**
The immediate response to cold exposure is cutaneous vasoconstriction:
1. Cutaneous vasoconstriction is mediated by sympathetic nerves in response to discharge from posterior hypothalamus.
2. Vasoconstriction in skin elicits reduction in cutaneous blood flow to about half the resting rate of cutaneous blood flow.

**Change in Body’s Attitude**
In severe cold, body flexes maximally (curling up of the body), especially in sleep and in lying posture. This reduces the surface area of the body for heat loss.

**Piloerection**
Cold induces contraction of piloerector muscle at the hair root that causes piloerection.
1. The skin becomes like goose skin and entraps a layer of air in the hairs.
2. This acts as an insulator and reduces the transfer of heat from the body.

**Role of Training**
Temperature regulation occurs by two drives: cutaneous drive and hypothalamic drive.
1. *Cutaneous drive* is the skin blood flow and other factors like sweating modulate effector response in response to change in core temperature.
2. Core temperature changes the sensitivity of hypothalamus, the hypothalamic drive.
3. Cutaneous drive is controlled by the hypothalamic drive.
   Under normal conditions, peripheral responses increase linearly with an increase in hypothalamic drive. Increase in skin temperature has two effects: shifts the temperature threshold downward, and increases the sensitivity of the peripheral response to particular level of hypothalamic drive. Training produces similar effects in an athlete, i.e., *increases heat loss for a given hypothalamic drive*.
   When skin temperature is lowered, the temperature threshold is shifted upward and sensitivity of peripheral responses to hypothalamic drive is also decreased. Dehydration that limits the ability to sweat has similar effects.

### ACCLIMATIZATION TO TEMPERATURE CHANGES IN ENVIRONMENT

**Heat Acclimatization**
Physiological changes that occur due to prolonged or repeated exposure to a particular stressful environment are called acclimatization. Acclimatization to heat occurs when the individual has repeated exposure to heat or when he performs regular strenuous exercise that increases body temperature and activates heat loss mechanisms. In fact, *acclimatization is greater* when the individual has combined chronic exposure to heat and exercise. Usually, heat tolerance develops within one week to 10 days of exposure.

**Systemic Changes**
Acclimatization responses include cardiovascular changes, and changes in sweating, body temperature and fluid and electrolyte balance.

**Cardiovascular Adaptations**
Cardiovascular change is mainly the *reduction in heart rate*. The reduction in heart rate occurs to the extent that maintains minimum level of activity. Usually, this occurs within a week’s time.

**Changes in Sweating**
Changes in sweating occur more slowly. The *core temperature threshold for sweating is reduced*. Therefore, sweating occurs earlier and at a lower core temperature:
1. *Sweating rate becomes high* for a given level of core temperature as sensitivity of sweat glands increases to cholinergic stimulation.
2. Thus, sweat rate becomes high and the higher sweat rate remains for sustained period.

**Changes in Core and Skin Temperatures**
The changes in sweating reduce the level of core and skin temperature during exercise.
1. This enables the individual to *perform exercise for a longer duration*.
2. The *core temperature threshold for cutaneous vasodilation and sweating is reduced*, which maintains adequate transfer of heat from the body core to the skin.

**Changes in Fluid and Electrolyte Balance**
After the initial acute water and sodium loss due to profuse sweating, the plasma volume is restored, which then slowly increases. However, after one or two week’s time, the volume change disappears.
1. Sweat glands conserve sodium by secreting sweat with less sodium. All these effects are mediated through increased aldosterone secretion, which occurs due to initial hyponatremia.
2. Sweat gland response to aldosterone is slower than the response of kidney, and aldosterone escape phenomenon as seen in kidney tubule does not occur in sweat glands.
3. Thus, sweat glands keep on preserving body sodium substantially till acclimatization continues. In unacclimatized person, continuation of excess sweating results in water and electrolyte loss.

**Consequence of Heat Acclimatization**
The major consequence of heat acclimatization is due to the persistence of sodium-retaining property of sweat glands.
1. Therefore, sweat contains less salt irrespective of its amount secreted. Hence, loss of a given volume of sweat does not result in proportionate loss in salt from the body.
2. This leads to smaller decreases in ECF volume for a particular volume of sweat loss.
3. However, effects of heat acclimatization vanish rapidly when the individual is lastingly shifted from the hot environment or when he is not exposed to such environments repeatedly.

**Cold Acclimatization**
The cold acclimatization depends on the nature, degree and duration of the cold exposure. The changes mainly occur in blood vessels, metabolism and tissue temperature.

**Cardiovascular Changes**
Two important changes that occur on exposure to severe cold for a longer duration are cold induced vasodilation and the Lewis-Hunting response.

**Cold-induced Vasodilation**
When skin temperature is below 15°C for a longer duration, cold-induced vasodilation occurs, which increases cutaneous blood flow:
1. This is observed mainly in areas rich in arteriovenous anastomoses as in hands and feet.
2. The mechanism of this vasodilation is not exactly known, but it may be due to the inhibitory effect of cold on the vascular smooth muscle.
3. The degree of vasodilation response is not adequate in individuals who have less experience of previous exposures to cold.

**Lewis-Hunting Response**
On repeated exposure to cold, cold-induced vasodilation begins early and the degree of response becomes more. This produces greater cutaneous blood flow:
1. In addition, vasoconstriction alternating with vasodilation occurs in succession.
2. This rhythmic pattern of vasoconstriction-vasodilation resulting in decreased and increased blood flow is called the Lewis-Hunting response.
3. It is typically seen in individuals those who regularly dip their body parts in cold water like fishermen working in rivers in winter season.
4. This response that intermittently increases blood flow to the part protects the part from cold injury.

**Metabolic Changes**
Though, generally it is believed that human beings acclimatize to cold environment by increasing their metabolic rate, reports of experimental studies in humans and animals do not reveal such findings.
1. Instead, reports indicate that on chronic cold exposure, additional decrease occurs in core temperature and also a fall is seen in temperature threshold for shivering, and a decline in metabolic response.
2. These changes are aimed at preserving metabolic energy, which may be helpful during prolonged stay in a cold environment.

**Decreased Heat Conductance**
The conductance of heat from inner core of the body to skin surface is lowered in repeated exposure to cold.
1. This causes tissue insulation in cold acclimatization.
2. This is another factor that contributes to decreased metabolic response in cold acclimatization.
3. The increased tissue insulation is not related to the degree of subcutaneous fat as it is also seen in very lean subjects.
4. The exact mechanism of tissue insulation is not known, but, it may be due to enhanced countercurrent heat exchange in the acclimatized subjects.

**APPLIED PHYSIOLOGY**

**Fever**
Fever or pyrexia is the increase in body temperature above normal range. It is the common feature of most of the diseases.

**Etiology**
Etiology of fever may be broadly classified into infectious and noninfectious conditions. However, sometimes cause of the fever can not be established, which is designated as pyrexia of unknown origin (PUO).

- **Infectious conditions**: Infections by pathogenic organisms are the common causes of fever. Infection may be caused by bacteria, viruses, fungi, protozoa, etc.
- **Noninfectious conditions**: Noninfectious conditions include trauma, inflammatory diseases like rheumatoid arthritis, immunologically-mediated disorders, tumors, etc.
Pyrogens

Whatever may be the etiology, pyrexia is caused by various pyrogens. Pyrogens are chemical substances that increase body temperature. Pyrogens are classified into two categories: exogenous and endogenous.

Exogenous pyrogens: Exogenous pyrogens are substances that enter the body from outside. Most of the exogenous pyrogens are of microbial origin. The whole or part of infecting organism or the toxins released from the organism can act as pyrogens. The typical example is the endotoxin of gram-negative bacteria, which is lipopolysaccharide.

Endogenous pyrogens: Endogenous pyrogens are produced in the body. Chemicals released from infected tissue, traumatic tissue, inflamed tissue or inflammatory cells constitute endogenous pyrogens. Usually, exogenous pyrogens on entry into the body activate cells like monocytes and macrophages to release endogenous pyrogens that stimulate hypothalamus to produce fever. Examples of endogenous pyrogens are prostaglandins, interleukins, tumor necrosis factor, etc. IL1 is a potent endogenous pyrogen.

Pathogenesis of Fever

Toxins released from infecting organism or traumatized tissue stimulate the inflammatory cells like monocytes and macrophages to release cytokines, the endogenous pyrogens.

1. Cytokines act on organum vasculosum of lamina terminalis (OVLT) which in turn stimulate preoptic area to increase the local production of prostaglandins especially prostaglandin E2 (PGE2).

2. There are four types of prostaglandin receptors in hypothalamus: EP1, EP2, EP3 and EP4. EP3 mainly mediates the effects of PGE2. PGE2 alters the firing rate of the central thermoregulatory controller, raising the thermostat set point (Flowchart 156.4).

3. Raised hypothalamic set point activates the mechanisms that stimulate heat production and inhibit heat loss (Clinical Box 156.1).

Clinical Box 156.1

PGE2 inhibitors reduce fever: Endogenous pyrogens produce fever by increasing the local synthesis and release of prostaglandin E2 in hypothalamus. Therefore, aspirin and many other nonsteroidal anti-inflammatory drugs decrease body temperature by inhibiting hypothalamic production of prostaglandins.

Effects of Fever

Fever is considered as the defence mechanism of the body against the disease. It eliminates the disease process by following mechanisms.

1. Fever kills the organism by its thermogenic effects. For example, high fever in malaria kills malarial parasites. Also, fever is bacteriostatic.

2. Fever induces enzymatic activity that in turn kills the invading organism.

3. Fever enhances production of antibodies that kill the microorganisms.

However, fever for a longer duration is harmful to the body as it produces negative nitrogen balance and alkalosis. Very high pyrexia causes unconsciousness by suppressing brain centers.

Conditions of Heat Stress

Heat stress is the condition of excessive hyperthermia in which impact of heat on the body becomes acute and severe. Such a situation occurs when the heat-transport power of the environment overwhelms thermoregulatory competence of the body. Factors that cause derangement in the rate of heat dissipation or production or transfers excess heat from the environment into the body that body can not assimilate lead to progressive hyperthermia resulting in heat stress. As in humans, core-temperature is regulated within a fairly narrow range; the impact of heat stress often becomes life-threatening.
The common condition that leads to heat stress is the prolonged exposure to an environment, which is extremely hot and humid. Especially, when physical activity that increases the rate of heat-production is increased in such a condition, severe hyperthermia ensues. Evaporation from the skin becomes an important mechanism for heat dissipation in such conditions. But, this is impaired by a higher humidity of the environment that reduces the skin-to-environment gradient for water-vapor pressure, which reduces evaporation. Thus, very hot and humid weather predisposes to progressive hyperthermia and consequent heat stress. Loose clothing helps in preventing heat stress (Clinical Box 156.2)

The common consequences of heat stress are: heat syncope, heat exhaustion and heat stroke.

Clinical Box 156.2

Loose clothing protects from radiative hyperthermia: Without prior adaptive capabilities, exposure to heat of the scorching sun as in arid deserts or the heat of large furnaces leads to heat stress. Radiation of heat from environment to the body is the major mechanism of severe hyperthermia in such conditions, which is called radiative hyperthermia. The most effective protection against such radiative hyperthermia is to cover the skin with loose white clothing, which screens the radiation of heat from the environment and at the same time allows movement of air underneath the clothing that maintains loss of heat by evaporation and convection.

Heat Syncope

Heat syncope is a state of circulatory failure that occurs due to excessive heat. The major pathophysiologic dysfunction is due to venous pooling of blood that decreases venous return. Venodilation occurs as a compensatory increase in skin blood flow in prolonged exposure to heat. The consequent decrease in end-diastolic volume lowers cardiac output and blood pressure.

In severe form, it may cause loss of consciousness. As thermoregulatory mechanisms are intact, usually core temperature is not raised. Skin mostly remains wet and cool. In addition to transferring patient from hot environment immediately, treatment aims at improving the diastolic filling of the heart.

Heat Exhaustion

Heat exhaustion is a common condition of heat stress. It occurs due to imbalance in cardiovascular homeostasis in a hot environment. This is also called heat collapse.

1. Collapse is usually precipitated during exercise.
2. Decreased diastolic filling of ventricle is the primary pathophysiologic mechanism of heat exhaustion.
3. However, exact mechanism of decreased venous return is not known, though hypovolemia is proposed to be the major contributing factor.
4. Hypotension activates baroreceptors reflex and therefore prevents fainting.

Features of heat exhaustion include nausea, vomiting, tachycardia, weakness, confusion, headache, ataxia and vertigo. The patient sweats profusely. In severe cases, dilatation of pupil may develop and patient may faint.

Fluid replacement and rest usually improves the condition. Salt intake helps. Though, usually body temperature is normal or slightly elevated in heat exhaustion, hyperthermia and dehydration frequently lead to heatstroke. Hence, it is better to keep the patient in a cool environment.

Heat Stroke

Heat stroke is the most perilous disorder of heat stress. It is characterized by high core temperature, neurological deficits and loss of consciousness. Convulsion is the frequent association.

There are two forms of heatstroke: classical and exertional. 1. For classical heatstroke, the major cause is a very high environmental heat that supercedes thermoregulatory mechanisms of the body.
2. For exertional heatstroke, the major cause is accelerated production of metabolic heat. Usually, exertion in a high ambient temperature predisposes to heatstroke.

The exact pathophysiologic mechanism of heatstroke is not known. Though hyperthermia is the major culprit, state of physical exercise is an important contributor. A triggering factor could be release of endotoxin from bacterial flora which may occur due to intestinal ischemia that allows the toxins to enter circulation and activate inflammatory responses.

Treatment Should be Prompt

Damage to kidney, liver and skeletal muscle are usual complications. In heatstroke, patient rapidly becomes comatose. The skin is dry and body temperature is usually above 106°F. In fact, it is a medical emergency and requires prompt treatment.

1. Immediate therapy includes rapid reduction in body temperature, which is effectively accomplished by immersion of the body in cold water.
2. Prompt hydration and maintenance of a patent airway prevents complications to develop.

Malignant Hyperthermia

Malignant hyperthermia is a rare condition of very high increase in body temperature that usually occurs on exposure to anesthetics or depolarizing neuromuscular blockers.

1. Halothane anesthesia is known to cause in susceptible individuals.
2. There is a genetic predisposition.
3. The plasma calcium concentration rises abnormally that severely activates myosin ATPase activity.
4. The consequent hyperactivity and hypermetabolic processes rapidly increase core temperature. Treatment is by immediate use of dantrolene sodium that decreases the release of calcium from the sarcoplasmic reticulum.
Hypothermia

The common cause of hypothermia is prolonged exposure to severely cold environment or prolonged immersion in cold water.

1. Hypothermia occurs earlier and in more severe form in cold water than in cold air. Hypothermia in infants and elderly people is severe than in adults.
2. Peripheral vasoconstriction and shivering do not prevent hypothermia during prolonged exposure in cold water for water’s high thermal conductivity.
3. However, a thick layer of insulating fat decreases loss of heat to the water.
4. Therefore, swimmers in cold water apply a thick layer of grease on the skin surface before swimming.

When body temperature is less than 25°C, ill effects of hypothermia develop. Decreased body metabolism, enzymatic activity, slowing of heart and respiration, and slowing reflex activities are features of hypothermia. If very severe, loss of consciousness occurs, which may further lead to death.

CHAPTER SUMMARY

**Key Concepts**

1. Body temperature is a vital clinical sign. Hence, temperature regulation is vital process of the body.
2. Though skin plays important role in temperature regulation, hypothalamus (the thermostat) controls all the mechanisms (both central and peripheral).
3. Heat stroke should be treated immediately.

**Important to Know (Must Read)**

1. ’Describe the mechanism of regulation of body temperature’ may be a Long Question.
3. In Viva, examiner may ask… What is the normal body temperature, Name the actors affecting body temperature, What are the mechanisms of heat generation and transfer, What is heat balance, What are the mechanisms of heat dissipation, Role of hypothalamus in temp. regulation, What are hypothalamic thermoreceptors, Heat acclimatization, Cold acclimatization, What is heat stress, What is heat syncope, What is heat exhaustion, What is heat stroke, What is malignant hyperthermia.
Chapter 157

Physiology of Exercise and Sports Science

Learning Objectives

On completion of study of this chapter, the student will be able to:
1. Classify the degrees of exercise.
2. List the factors affecting exercise.
3. Describe the physiological changes in exercise.
4. Appreciate the importance of aerobic training.
5. List the benefits of exercise.

General Aspects

Exercise physiology is the study of the physico-chemical processes in the body that allow conversion of chemical energy into mechanical work and the changes in the organ systems in response to the effects of the work.

1. Exercise is basically the repetition of contraction-relaxation processes of skeletal muscles that require adequate energy. Continued skeletal muscle activity utilizes energy that depends on the rate of nutrients and \( \text{O}_2 \) supply to the exercising muscles.
2. Physical exercise is a common form of acute stress encountered during everyday life. On exposure to such an acute bout of stress, the body makes rapid and integrated adjustments in functions of body organ-systems to meet its metabolic and thermal need.
3. Slower adaptations occur in physiological changes in the body to the repeated exposures to physical exercise stress, which forms the basis for health improvement in any physical training.
4. The study of exercise physiology makes us understand the physiological processes responsible for building the energy for skeletal muscle activities during exercise and the acute effects of exercise on body systems and the adaptive changes in body functions by the stress of physical exercise.

Types of Exercises

Physiological changes during exercise depend on the type of exercise. Exercises have traditionally been classified into two categories: isotonic and isometric.

Isotonic Exercise

Exercise that involves isotonic muscle contraction is categorized as isotonic exercise. In isotonic muscle contraction, muscle length changes; therefore, the external work is done. Hence, this is also called dynamic exercise. Walking, jumping and jogging are the examples of isotonic exercises.

Isometric Exercise

This type of exercise involves isometric muscle contractions. As in isometric contraction, muscle length remains same, no external work is done. Hence, this is also called static exercise. Trying to lift a heavy weight and pushing against a wall are examples of isometric exercise.

Degrees of Exercise

Exercise has been graded into mild, moderate, severe and very severe depending on level of heart rate increase, rate of oxygen consumption and the amount of work done.
Mild Exercise
In mild exercise, the rise in heart rate is about 25%. That means, if the pre-exercise basal heart rate is 80/min, it increases to about 100/min. The oxygen consumption is 0.5 L/min to 1 L/min and the work done is 150–350 watts.

Moderate Exercise
In moderate exercise, the rise in heart rate is about 50%. Heart rate increases from its pre-exercise value of 80/min to about 120/min. The oxygen consumption is 1 L/min to 2 L per min and the work done is 350–550 watts.

Severe Exercise
Heart rate rises to about 80%. That means, if the pre-exercise basal heart is 80, it increases to 144. The oxygen consumption is 1.8 L/min to 2.5 L/min. and, the work done is 550–700 watts.

Very Severe Exercise
In very severe exercise, the rise in heart rate is close to 100%, which reaches to about 160/min. The oxygen consumption is more than 2.5 L per min. and, the work done is usually more than 700 watts.

Factors affecting Muscle Work
In physical exercise, it is the skeletal muscle that works. The degree and duration of exercise (muscle work) depends on the following factors:
1. Type of muscle contraction
2. Size of the muscle
3. Type of muscle fibers
4. Strength of muscle
5. Motor units
6. Ability to sustain fatigue
7. Level of training
8. Environmental conditions

Types of Muscle Contraction
1. In isometric contraction, the muscle does not shorten, whereas in isotonic contraction, muscle shortening occurs. Isotonic contractions are of two types: concentric and eccentric. In concentric type of isotonic contraction, muscle shortens while exerting a force, e.g. during climbing up stairs.
2. In eccentric contraction, lengthening of muscle occurs while resisting an external load, e.g. during walking down stairs.

Size of the Muscle
As the exercise is performed by skeletal muscles, it is the muscle mass that contributes significantly to the quantity and quality of exercise. Also, mass of the muscle depends on the quantum of the work the muscle performs, and the regularity with which exercise is performed by the individual. The type of exercise also contributes to muscle mass. Thus, muscle mass and exercise are reciprocally related.

Type of Muscle Fibers
There are two types of skeletal muscle fibers: type I and type II. Type I fiber is slow-twitch or oxidative type, and type II fibers are fast-twitch or glycolytic type.
1. Generally, type II fibers shorten rapidly than type I fibers and the velocity of shortening depends on myosin ATPase activity.
2. In concentric type of muscle contractions, the velocity of shortening declines rapidly with increasing load. That means, when the load is more, muscle shortens slowly.
3. Type I muscle fibers have lower velocity of shortening at a given load.
4. Practice of aerobic (isotonic or free-hand type) exercises slowly increases number of type I or oxidative type muscles and isometric exercises increase type II muscles in the body.

Strength of Muscle
Strength of the muscle is a single most important factor for rate and duration of exercise. Vice versa, muscle strength increases by exercise.

Role of Motor Units
A motor unit consists of a single motor nerve and all the muscle fibers innervated by it (refer to Fig. 28.6; Chapter 28). A motor unit is a functional unit of muscle contraction as all muscle fibers in a motor unit contract at the same time in response to discharge of the motor neuron of the unit.
1. The muscles involved in skilled activities have more innervation ratio and, therefore, have less number of muscle fibers per motor unit, whereas muscles involved in regulation of posture have more number of muscle fibers per motor unit and less innervation ratio.
2. Usually, all muscle fibers of a particular motor unit are of same type (either oxidative or glycolytic). Intensity of muscle contraction can be increased by increasing the number of motor units that fire simultaneously. This is achieved by recruitment of motor units.
3. When discharge to a motor neuron is of low intensity, it activates relatively smaller motor units that usually consist of type I (slow-twitch) fibers.
4. With increased discharge to the motor neuron, larger motor units are recruited. As the larger motor units contain more of type II (fast-twitch) muscle fibers, with increased intensity of discharge, the force generated during contraction increases proportionately.
5. Thus, recruitment of additional motor units increases the velocity of muscle contraction.
6. This pattern of recruitment of motor units in which smaller motor units are recruited first (at lower intensity of discharge) and larger motor units later is known as the size principle (Application Box 157.1).

**Application Box 157.1**

Applications of size principle: Size principle has two important applications: (1) With increased effort for work, force of contraction increases in a graded fashion with gradual recruitment of more motor units. (2) Sustaining mild to moderate tension for a longer duration is easier than to sustain maximal tension. This is, because, lower tension involves slow-twitch muscle fibers and higher tension involves fast-twitch muscle fibers. Contraction of fast twitch fibers causes rapid depletion of glycogen (the fuel of muscle) and, therefore, it becomes difficult to maintain maximal muscle contraction.

### Ability to Sustain Fatigue

Fatigue is the inability to maintain force generated by muscle. Fatigue occurs due to central and peripheral causes. Central causes include failure of transmission of information from motor cortex or other cortical areas to muscle. However, central defects for fatigue are rare.

1. **Peripheral causes** are impaired neuromuscular transmission, defect in generation of action potential in the muscle fiber, decreased excitation-contraction coupling, and decreased availability of O₂ or glycogen to meet the energy requirement that impair the ability to generate force.
2. Fatigue developed during isometric exercise like weightlifting is different from that of isotonic exercise such as jogging.

### Fatigue in Isometric Exercise

Fatigue in isometric contraction is of high-frequency type. In maximal effort of isometric exercise, all muscle fibers including fast-twitch fibers are recruited rapidly. In fast-twitch or glycolytic muscle fibers, fatigue occurs within seconds to minutes, which is due to:

1. Immediate decrease in glycogen
2. Decline in pH locally in muscle, and
3. Impaired conduction of action potentials along the muscle fiber due to accumulation of K⁺ in the T-tubular system that comes from ECF.

However, this high-frequency fatigue lasts for a short duration. Therefore, the muscle can generate another isometric contraction following the previous one. However, if contraction occurs in quick succession, the maximum isometric tension developed decreases gradually. Training has little effect on high frequency fatigue. For example, weightlifting can enhance muscle strength but cannot provide resistance to fatigue.

### Fatigue in Isotonic Exercise

Fatigue in isotonic contraction is of low-frequency type.

1. This occurs during repeated isotonic muscle contractions that mainly recruit slow-twitch or oxidative fibers.
2. The frequency of motor-neuron discharge to cause fatigue is much less than the frequency required for maximal isometric contraction.
3. Fatigue develops slowly over a period of many minutes to hours, which occurs due to impaired excitation-contraction coupling following depletion of the intracellular Ca²⁺.
4. Training for aerobic exercises imparts resistance to low-frequency fatigue and, therefore, promotes exercise endurance, especially when moderate effort is repeatedly applied to perform the exercise.
5. Thus, training enhances performance and decreases fatigability.

#### Level of Training

Appropriate training promotes exercise performance and impairs fatigability. Aerobic training enhances isotonic exercise endurance (for details, see below).

#### Environmental Conditions

Environmental temperature and humidity are two important conditions that influence exercise. Hot and humid environment decreases physical performance.

1. As such, body temperature increases during exercise that activates heat dissipation mechanism by mainly promoting cutaneous vasodilation and sweating.
2. The rate of heat production in the body increases in proportion to the intensity of exercise.
3. To transfer heat from the body to the environment, skin blood flow and sweating increase proportionately. The evaporative heat loss is thus increased.
4. In increased ambient humidity, evaporative loss is impaired, which further increases body heat (for details, see the previous chapter) and impairs exercise performance.
5. Physical training decreases the degree of hyperthermia induced by exercise by first reducing the threshold for sweating so that sweating begins earlier and later by increasing sweating sensitivity so that more sweating is produced at a given temperature level.
6. Thus, training enhances evaporative heat loss. The resulting dehydration caused by evaporative heat loss should be managed by fluid replacement during prolonged exercise.

### Energy Source in Exercise

During physical activity, chemical energy is converted to mechanical work. The energy is derived from muscle metabolism. The rate of metabolism in skeletal muscle is low at rest. The energy sources in exercise are:

1. ATP
2. Phosphocreatine
3. Fatty acids
4. Glycogen
5. Lactic acid
ATP and Phosphocreatine

For physical activity to occur energy is required, which comes from **breakdown of ATP and regeneration of ATP from phosphocreatine**.
1. During exercise, metabolisms in skeletal muscle increase enormously that are meant to supply energy for repeated contraction and relaxation of the muscles.
2. The normal reserve of high-energy compounds in muscles is not adequate to sustain muscle activity for more than few seconds.
3. Hence, muscle regenerates ATP from other sources.

**Aerobic Exercise**

During aerobic exercise, ATP regeneration is made possible by the oxidation of glucose, free fatty acids and ketone bodies.
1. Therefore, ATP regeneration to sustain light muscular activity is adequate in type I or oxidative type of muscle fibers.
2. This is achieved by delivery of sufficient O₂ and glucose to the skeletal muscle.

**Aerobic Conditions**

During anerobic conditions, glycolysis and phosphocreatine are the limited sources of ATP.
1. Phosphocreatine in muscle can regenerate ATP by the creatine phosphokinase reaction that transfers the high-energy phosphate from phosphocreatine to ATP.
2. Type I and II muscle fibers can regenerate ATP to some extent by glycolysis.
3. However, glycolysis is insufficient in generating ATP in comparison to aerobic regeneration of ATP.
4. Also, glycolysis accumulates protons in muscle cell that decreases intracellular pH and inhibits force generation. Hence, anaerobic metabolism impairs muscle activity.

**Triglycerides and Fatty Acids**

Though storage of O₂ in the body is negligible, sufficient reserve of fuel is available to sustain exercise for a longer duration. The stored energy is derived from triglycerides in the adipose tissue and intramuscular stores of fatty acids. In prolonged exercise, free fatty acids (FFA) account for approximately 60% of total supply of fuel to the muscle.
1. Catecholamines secreted early in exercise stimulates hormone-sensitive lipase in adipocytes, which converts triglycerides to free fatty acids.
2. When exercise is prolonged, increased secretion of growth hormone mobilizes more FFA. FFA is oxidized by exercising muscle, and increased availability of fatty acids decreases the demand on muscle glycogenolysis and glucose oxidation.

**Muscle and Liver Glycogens**

Muscle glycogen is another source of energy. Normally, 300 to 400 g of glycogen can be stored in muscles, which at the time of need can provide about 1500 kcal of energy.
1. Glycogen is also stored in liver, which can accommodate about 100 g of glycogen that can supply about 400 kcal.
2. However, hepatic glycogen is of little use for acute muscular contractions as it is not made available immediately for the muscle cells.
3. Nevertheless, as exercise continues, glycogen is released from liver for utilization by the exercising muscle.

**Glucose**

Glucose utilized by muscle during exercise decreases blood glucose level, which is balanced by release of glucose from liver.
1. In anaerobic exercise, carbohydrates breakdown to form acetate that enters blood to reach liver, where it is converted to glucose through gluconeogenesis. Glucose released from liver finally reaches muscle. This completes the Cori cycle.
2. Thus, end products of anaerobic metabolism released from muscle are converted to fuel (glucose) by liver that muscle can reuse.
3. Hepatic release of glucose is mainly due to activation of glycogenolysis. Gluconeogenesis from lactic acid and alanine also contributes, especially when exercise is prolonged.
4. Thus, immediate sources of fuel are ATP and phosphocreatine that are available in the muscle.
5. The next immediate fuel source is glycogen stored in the muscle. Glycogenolysis that liberates glucose is also a source of energy.
6. Lactic acid released from muscle also supplies energy. In prolonged exercise, liver glycogen that releases glucose into blood by glycogenolysis becomes the fuel source for the exercising muscle (Application Box 157.2).
7. When exercise reaches its steady state, the oxidation of glucose released from muscle and liver glycogen accounts for about 50% of the energy supply, and rest of energy source is the oxidation of fatty acid.

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**Application Box 157.2**

Carbohydrate diet prior to exercise post postpones fatigue in runners:

The time of onset of fatigue during exercise largely depends on the amount of glycogen stored in the skeletal muscles, especially in muscles of leg prior to beginning of exercise. In a normal well built individual, muscle glycogen storage averages about 15 to 18 g/kg of muscle, which is reduced to about 3 g/kg of muscle after a marathon running. To avoid early onset of fatigue due to muscle glycogen depletion, the runner *should have more muscle glycogen at the beginning of running*. As diet is the main source of this glycogen, consumption of carbohydrate-rich diet few days or at least a day prior to the competition will increase resting muscle glycogen and delays the onset of fatigue.
PHYSIOLOGICAL CHANGES IN EXERCISE

The major changes in exercises are change in oxygen uptake, cardiovascular changes, respiratory changes, and changes in tissue. All these changes are primarily meant to increase oxygen supply to the tissue and to promote oxygen utilization by the tissue. Oxidation of fuel is required for supply of energy during exercise.

**O₂ Uptake During Exercise**

**VO₂max**

The O₂ consumption by the whole body during rest is about 250 ml/min. It increases to 15–20 times during maximal exercise (Table 157.1).

1. The maximum amount of O₂ that can be consumed by an individual is called as maximal O₂ consumption or VO₂max. The average VO₂max in an adult is about 3 L/min and in athlete about 5 L/min. This is an important physiological indicator of an individual’s capacity to carry out aerobic exercise as it represents highest attainable rate of aerobic metabolism during performance.

2. In a normal individual, usually it is limited by the rate of increase in cardiac output rather than increase in ventilatory capacity or diffusion capacity.

3. However, the ability of the tissue to extract and utilize O₂ and the size of total muscle mass contribute to it. It reaches its peak during young adulthood and then declines slowly with advancement of age.

**Phases of Muscular Exercise**

Based on O₂ uptake, the entire period of a physical exercise is divided into three phases: adaptation phase, steady phase and recovery phase.

**Adaptation Phase**

This is the early part (first 2–5 minutes) of muscular exercise during which oxygen consumption increases linearly to reach its VO₂max level.

1. However, the O₂ consumption in this stage is much less than its demand for the body.

2. Therefore, oxygen deficit occurs at the beginning of exercise and continues throughout exercise. Hence, energy supply above VO₂max is fulfilled by the anaerobic pathway.

**Table 157.1: Oxygen consumption by whole body at different level of activities.**

<table>
<thead>
<tr>
<th>Degree of activity</th>
<th>O₂ consumption (L/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td>0.25</td>
</tr>
<tr>
<td>Slow walking</td>
<td>0.8</td>
</tr>
<tr>
<td>Fast walking</td>
<td>1.5</td>
</tr>
<tr>
<td>Prolonged jogging</td>
<td>2</td>
</tr>
<tr>
<td>Fast running</td>
<td>3</td>
</tr>
</tbody>
</table>

**Steady Phase**

This phase of exercise is characterized by plateau state of VO₂max, which indicates that O₂ consumption is at its maximum and steady state. However, excess energy requirement in the presence of oxygen deficit is met with by anaerobic pathway like breakdown of creatine phosphate and muscle glycogen.

1. Due to anaerobic metabolism, lactic acid level rises sharply in blood decreasing the blood pH. Acid in blood is buffered by the bicarbonate buffer and in the process more CO₂ is produced that causes hyperventilation.

2. Therefore, the energy supply by anaerobic metabolism is limited by his capacity to withstand metabolic acidosis produced by the accumulation of lactic acid.

3. Trained athletes produce less quantity of lactic acid for any given level of work and have also the greater ability to tolerate for lactic acidosis.

**Recovery Phase**

This refers to the period following completion or termination of exercise during which extra amount of O₂ is consumed. The extra amount of O₂ consumed during recovery phase is called oxygen debt.

1. The degree of O₂ debt is proportionate to the extent to which energy demand during exercise exceeds the capacity of aerobic synthesis of energy store.

2. The O₂ debt is meant to remove the excess lactate produced by anaerobic metabolism, to replenish the ATP and phosphorylcreatine store, to replace the O₂ that comes from myoglobin.

**Respiratory Changes**

The primary objective of respiratory changes is to facilitate O₂ consumption of the body to meet the metabolic need during exercise. Also, hyperventilation removes excess of CO₂ produced during exercise. Therefore, arterial pO₂ and pCO₂ are maintained almost at the normal level even during the strenuous exercise. The major respiratory changes are increased ventilation and increased O₂ uptake by the lungs.

**Increase in Ventilation**

The major respiratory change in exercise is increase in ventilation. The increased ventilation matches the metabolic demand of exercise and ventilation may increase to about twenty-fold.

1. The ventilation increases almost linearly with the increase in intensity of exercise (Fig. 157.1) until the anaerobic threshold is reached.

2. Anaerobic threshold is the degree of exercise that produces an elevation of blood lactate levels that results from a shift from aerobic to anaerobic metabolism.

3. The anaerobic threshold is also called lactate threshold, as lactate concentration rises gradually with
exercise intensity. Lactic acidosis stimulates the ventilation further and, therefore, the rise in ventilation becomes supralinear.

4. In athletes, lactate threshold occurs at higher level than in non-athletes. The anaerobic threshold occurs at approximately 60% of the maximal exercise level, irrespective of the physical fitness of the individual.

5. Above the anaerobic threshold, the increase in ventilation is proportionate to the increase in O2 consumption as lactic acid produced imparts additional respiratory drive.

6. As removal of CO2 exceeds its production that occurs due to additional hyperventilation, arterial pCO2 falls significantly below the resting level during strenuous exercise.

Increase in Rate and Depth of Respiration

Increase in ventilation occurs due to increased both rate and depth of respiration.

1. At rest in adults, the rate of respiration is 12–18 /min and the tidal volume is about 500 ml. Therefore, the minute ventilation (rate × tidal volume) is 6 to 7 L/min. During exercise, both rate and depth of respiration increase.

2. In mild to moderate exercise, with increase in intensity of exercise, the tidal volume (depth of respiration) increases rapidly with the increase in rate of respiration. However, tidal volume increases to its limited maximum value beyond which ventilation is largely increased by increase in the rate of respiration when vigorous exercise is continued for a longer period.

3. In severe exercise like running fast for an hour, minute ventilation increases to 80–100 L/min as rate of respiration may increase to about 40/min and the tidal volume to 2.5 L (Table 157.2).

4. However, even during most strenuous exercise, minute ventilation remains below the maximum breathing capacity of and individual, which is normally 100–120 L/min.

Mechanisms and Effects of Increased Ventilation

Exact mechanism for increased ventilation is still not clearly known. However, the effects are possibly mediated through neural, chemical and thermal mechanisms.

1. Ventilation increases abruptly at the beginning of the exercise, and then followed by a brief pause, again increases gradually to reach a steady state as the exercise is continued.

2. The initial rise is mediated via neural mechanism and gradual increase in the later phase is mediated by chemical mechanism.

Neural Mechanisms

Neural mechanism plays an important role at the beginning of exercise.

1. Ventilation increases abruptly at the beginning of the exercise due to psychic stimuli originating in the limbic system.

2. Also, impulses originating from proprioceptors (especially from receptors in the muscle, joints and ligaments) contribute.

3. Neural factors also contribute to anticipatory hyperventilation before the onset of exercise.

Chemical Mechanisms

Humoral mechanism contributes to the gradual increase in ventilation in the later phase of exercise. The arterial pO2, pCO2, pH and K+ play role in this process.

1. Though the alveolar and arterial pO2 are maintained at normal levels throughout exercise and in different degrees of exercise, and arterial pCO2 and pH are maintained in mild to moderate exercise (Table 157.3), it is believed that these chemical factors play role in exercise hyperventilation.

2. However, the exact mechanism by which they stimulate ventilation is not exactly known. It is suggested that accentuations of the normal oscillations in pO2 and pCO2, synchronous with respiration stimulate the carotid body chemoreceptors that in turn stimulate ventilation.

Arterial pO2

Alveolar and arterial pO2 remain almost normal throughout the period of exercise. Inspite of rapid utilization of O2 by exercising muscle, arterial pO2 is maintained due to proportionate increase in ventilation.
In the later stage in strenuous exercise, lactic acidosis due to anaerobic metabolism stimulates ventilation and maintains arterial pO\(_2\).

Though pO\(_2\) remains constant in exercise (Fig. 157.2), oxygen is believed to play some role in inducing ventilation as experimentally it is observed that increase in ventilation is less in magnitude while breathing air than while breathing 100% oxygen.

Also, increase in ventilation is proportionate to increase in oxygen consumption.

**Arterial pCO\(_2\)**

Despite increase in production of CO\(_2\) from metabolically active tissue in exercise, the arterial pCO\(_2\) remains normal in mild to moderate exercise. This is because hyperventilation removes extra CO\(_2\) produced by muscle metabolism.

1. However, ventilation is stimulated due to increase in chemoreceptor sensitivity to CO\(_2\) or to fluctuation in CO\(_2\).
2. When exercise continues beyond lactate threshold, further stimulation of ventilation removes more CO\(_2\) than the amount of CO\(_2\) produced.
3. Therefore, in strenuous exercise pCO\(_2\) falls (Fig. 157.2). Inspite of decline in pCO\(_2\), ventilation is stimulated by fall in pH (Application Box 157.3).

**Arterial pH**

Lactic acidosis is produced due to excess anaerobic metabolism in severe exercise. With continuation of strenuous exercise, excess of lactic acid produced is buffered and in the process of buffering, CO\(_2\) is formed. This extra CO\(_2\) further stimulates ventilation.

1. Thus, with increased production of lactic acid, the generation of CO\(_2\) is more and therefore, there is proportionate increase in ventilation. Due to increased ventilation, accumulation of CO\(_2\) in the blood is prevented.
2. Consequently, the buffering of the acid is called isocapnic buffering, as CO\(_2\) change relatively little. However, further continuation of exercise beyond lactate threshold (exhaustion of lactate buffering) decreases blood pH due to accumulation of more lactic acid. This heralds the onset of metabolic acidosis.

3. Overload of lactic acid causes supralinear stimulation of ventilation (see above).

4. Stimulation of ventilation occurs via activation of peripheral chemoreceptors in carotid and aortic bodies. During this period of exercise, arterial pCO\(_2\) falls significantly secondary to extrahyperventilation (Fig. 157.2).

5. However, the decline in arterial pCO\(_2\) induced by excessive ventilation becomes the physiological mechanism for respiratory compensation of metabolic acidosis (for details, refer the chapter “Acid-Base Balance”).

**Plasma K\(^+\) Level**

During moderate to severe exercise, K\(^+\) is released from exercising skeletal muscles. Increased plasma K\(^+\) stimulates peripheral chemoreceptors that aids to hyperventilation induced by other chemical factors.

**Thermogenic Mechanism**

Exercising muscles generate heat and increase body temperature. Increased temperature in sustained exercise contributes to stimulation of ventilation.

**Application Box 157.3**

Ventilation-perfusion ratio: As oxygen is utilized rapidly by actively exercising muscles, the pO\(_2\) of venous blood is grossly reduced. Hence, blood returning to lungs is severely depleted of oxygen. The decreased pulmonary arterial pO\(_2\) is another stimulus for stimulation of ventilation. Though pulmonary arterial blood flow increases during exercise, the increase in ventilation is more than the increase in blood flow. Therefore, ventilation-perfusion ratio rises from 0.8 at rest to about 4 in severe exercise.
Increase in O$_2$ Uptake

Hyperventilation in exercise increases oxygen uptake in the lungs. This helps to maintain arterial oxygenation. Maximal O$_2$ uptake may be about 20 times the resting O$_2$ uptake. In severe exercise, oxygen uptake is raised from 250 ml/min at rest to about 4 L/min.

Mechanisms of Increased Oxygen Uptake

1. Increased alveolar-to-arterial gradient of pO$_2$: In exercise, skeletal muscles extract more O$_2$ from the arterial blood and therefore O$_2$ content of the venous blood reaching the lungs is decreased by about five times (to about 3 ml/100 ml of blood from its resting value of about 15 ml/100 ml). This further increases the pO$_2$ gradient from the alveolar air to pulmonary capillary blood. Hence, O$_2$ uptake in the lungs increases radically.

2. Increased perfusion of lungs: Increased cardiac output in exercise increases pulmonary blood flow to approximately six times. As more blood passes through the lungs per unit time, more quantity of O$_2$ is automatically picked up by the lungs.

3. Increased diffusion capacity of alveolo-capillary membrane: Increased pulmonary blood flow causes opening of several capillaries in the lungs that are normally closed at rest. This promotes better alveolar perfusion. Opening up of more capillaries increases the surface area available for diffusion in the presence of more oxygen that has already been made available due to increased ventilation. Thus, diffusion capacity of the lungs increases to about three times. This raises the rate of oxygen diffusion from 21 ml/min/mm Hg at rest to about 65 ml/min/mm Hg during exercise.

Importance of Maximal O$_2$ Uptake

The VO$_{2\text{max}}$ (maximal oxygen uptake by the lungs) is an index of the functional capacity of an individual to sustain exercise.

1. The VO$_{2\text{max}}$ in a moderately active adult male is 35 to 40 ml O$_2$/min/kg body weight.

2. In a strong athlete, VO$_{2\text{max}}$ may be as high as 80 to 90 ml O$_2$/min/kg body weight, which is about 20-fold increase of their resting VO$_2$.

3. Therefore, while assessing athletic potential of an individual, it is imperative to measure the VO$_{2\text{max}}$ along with measurement of other work efficiencies.

4. In chronic lung and heart diseases, VO$_{2\text{max}}$ may be as low as 10 ml O$_2$/min/kg body weight. At high-altitude VO$_{2\text{max}}$ is decreased.

O$_2$ Store of the Body

The O$_2$ store of the body is only about one liter. This mainly represents the O$_2$ bound to hemoglobin.

1. This much of O$_2$ can maximally support a mild exercise for one minute, a moderate exercise for 30 seconds and a heavy exercise for 15 seconds, and maximal exercise for less than 5 seconds.

2. Therefore, for the exercise to continue for more than 15 to 30 seconds, the body must transport O$_2$ from the atmosphere to the mitochondria in the muscle at a rate proportionate to the O$_2$ utilization by the exercising muscle.

3. In exercise, increased alveolar ventilation promotes oxygenation of blood and increased cardiac output ensures adequate supply of oxygenated blood to the muscles.

Factors that Limit O$_2$ Uptake by Muscles

Five major processes are involved in the transport of O$_2$ from the atmosphere to the mitochondria in exercising muscle. Therefore, alteration in any of these mechanisms by physiological or pathological processes could retard final O$_2$ uptake by the exercising tissue. These steps are:

1. Pulmonary ventilation that facilitates O$_2$ delivery to the alveoli in lungs.

2. Pulmonary perfusion that promotes pick-up of O$_2$ from the alveoli.

3. Ventilation-perfusion ratio contributing to oxygenation (O$_2$ content) of arterial blood.

4. Dynamism of circulation that delivers O$_2$ from lungs to the muscle.

5. Extraction of O$_2$ from blood by the exercising muscle.

The final muscle oxidative metabolism depends on the adequacy of muscle blood flow, O$_2$ delivery to muscle, the pO$_2$ gradient between blood and muscle mitochondria, and aerobic metabolic capacity of the muscle cells.

Cardiovascular Changes

The primary aim of cardiovascular changes in exercise is to supply adequate oxygenated blood to exercising muscle at a rate that meets their metabolic demand. This is achieved by following cardiovascular responses:

1. Increase in the cardiac output

2. Increase in the skeletal muscle blood flow

3. Redistribution of blood flow in the body

4. Changes in blood pressure

5. Changes in blood volume

The cardiovascular changes in exercise depend on the severity and the type of exercise. The changes described below are the cardiovascular responses in isometric or aerobic exercise. A note on cardiovascular changes during isometric exercise will be given at the end of this section.

Increase in Cardiac Output

Ability of the cardiovascular system to transport O$_2$ to the exercising muscle determines VVO$_{2\text{max}}$.

1. The increase in cardiac output that determines the rate of O$_2$ delivery to the tissue is the limiting step in oxygen extraction.
1. During exercise, the cardiac output increases from its resting level of 5 L/min to about 25 L/min. In athletes, the maximal increase in cardiac output is greater than in nonathletes.

2. During a strenuous exercise, in strong athletes, cardiac output can increase up to 35–50 L/min. The increase in cardiac output is proportionate to increase in oxygen consumption.

3. Increase in cardiac output occurs due to increase in both heart rate and stroke volume (Table 157.4).

### Table 157.4: The increase in heart rate (HR), stroke volume (SV) and cardiac output (CO) in different degrees of physical activities, in a middle-aged.

<table>
<thead>
<tr>
<th>HR/min</th>
<th>SV (ml)</th>
<th>CO (L/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td>75</td>
<td>70</td>
</tr>
<tr>
<td>Slow walking</td>
<td>100</td>
<td>85</td>
</tr>
<tr>
<td>Fast walking</td>
<td>130</td>
<td>100</td>
</tr>
<tr>
<td>Prolonged jogging</td>
<td>150</td>
<td>110</td>
</tr>
<tr>
<td>Fast running</td>
<td>190</td>
<td>120</td>
</tr>
</tbody>
</table>

4. Increase in cardiac output occurs due to increase in heart rate and stroke volume (Table 157.4).

### Increase in Heart Rate

The heart at rest is called basal heart rate (BHR). BHR is a good index of parasympathetic activity (vagal tone). However, BHR is a poor index of endurance fitness, as BHR is influenced by genetic and other factors too.

1. Heart rate increases linearly with the severity and duration of exercise.

2. The maximal heart rate (maximum increase in heart rate) achieved is determined by the age of the subject.

3. This is also called target heart rate (THR) that forms the basis of treadmill test while assessing cardiac status of an individual.

4. The approximate THR in an adult aged 40 years is 190. THR decreases with age. However, THR also depends on the basal heart rate, physical fitness, and gender of an individual. Increase in heart rate after exercise takes time to return to normal (Clinical Box 157.1)

### Clinical Box 157.1

**Heart rate normalizes slowly:** After the cessation of exercise, tachycardia continues for a longer period than the persistence in increased blood pressure. The heart rate takes considerable time to return to resting levels as the hormonal effects and body temperature take time to normalize.

Tachycardia in exercise occurs due to following mechanisms:

1. **Increased sympathetic discharge:** The increase in heart rate occurs even before exercise begins, which is called anticipatory tachycardia. This occurs due to psychic stimuli that originate from limbic system and activates sympathetic discharge via hypothalamus. During exercise, increase in motor activity produces tachycardia by stimulating sympathetic system.

2. **Muscle-heart reflex:** Peripheral reflexes that originate from the exercising muscles and joints increase the heart rate, which is known as muscle-heart reflex. This causes tachycardia as soon as the exercise begins.

3. **Hormonal mechanisms:** Release of catecholamines from adrenal medulla in response to sympathetic stimulation and plasma chemical changes contribute to increase in heart rate.

4. **Thermogenic stimulation:** When exercise is continued, heat is produced from exercising muscles. The increased body temperature stimulates pacemaker activity and increases heart rate.

### Increase in Stroke Volume

The stroke volume increases simultaneously along with heart rate. In isometric exercise both heart rate and stroke volume increase, but increase in stroke volume is much less. In isotonic exercise, the increase in stroke volume is more than the increase in heart rate. In athletes, increase in stroke volume is the major mechanism to increase cardiac output (Application Box 157.4). Stroke volume increases by following mechanisms:

1. **Increased sympathetic discharge:** Increased sympathetic activity increases myocardial contractility that increases stroke volume.

2. **Increased EDV:** End-diastolic volume (EDV) increases due to increased venous return. Venous return increases due to increased activities of skeletal muscle pump, thoracic pump, and abdominal pump. Also, sympathetic venoconstriction increases venous return. Increased venous return increases stroke volume by Frank-Starling mechanism.

### Application Box 157.4

Athletes increase CO by increasing SV: The vagal tone is high in strong athletes. They increase a target cardiac output (CO) by mainly increasing the stroke volume, whereas nontrained individuals increases cardiac output mainly by increasing heart rate. Thus, at any level of cardiac output athletes have lower heart rate than nonathletes.

### Increased Skeletal Muscle Blood Flow

At rest, skeletal muscle blood flow is 2–4 ml/100 gm of tissue/min, which accounts for about 16% of total cardiac output as the total muscle blood flow is approximately 850 ml/min.

1. During strenuous exercise, the skeletal muscle blood flow can increase to about 50–80 ml/100 gm/min, about 20 times the resting level.

2. This primarily occurs due to arteriolar dilation and opening of capillaries. In skeletal muscle at rest, many capillaries are closed and blood flow occurs mainly via thoroughfare vessels.

3. During exercise, opening of capillaries not only increases blood flow but also increases surface area for gas exchange.
Mechanisms that increase Muscle Blood Flow

Skeletal muscle blood flow increases by three mechanisms: autonomic, metabolic and hormonal factors.

Autonomic Control

Sympathetic innervation of skeletal muscle is cholinergic, which is called sympathetic vasodilator system. These fibers heavily terminate on arterioles and cause arteriolar dilation on activation. This increases muscle blood flow during exercise.

Metabolic Control

In prolonged exercise, accumulation of metabolites in the exercising muscles causes dilation of their blood vessel. The metabolites are carbon dioxide, lactic acids, potassium ions, decreased local oxygen content, accumulation of hydrogen ions in the tissue, and local rise in temperature. Metabolites cause dilation of arterioles and precapillary sphincters. The metabolic vasodilation maintains blood flow during recovery from exercise.

Humoral Control

The skeletal blood vessels contain both α adrenergic and β receptors respectively. These two receptors counter each other in their action and therefore, may not contribute significantly to the increase in skeletal blood flow during exercise. However, some authors do believe that adrenaline through the β-receptor mediated vasodilation contribute to increase in muscle blood flow before, during and after the exercise.

Blood flow through non-exercising muscles also increases during exercise though the flow is less than in exercising muscles.

Redistribution of Blood Flow

Increased skeletal muscle blood flow is not only due to increased cardiac output but also to redistribution of blood flow. Sympathetic vasoconstriction in visceral and cutaneous vascular bed diverts enough blood to the exercising skeletal muscles. However, vasodilation in coronary and cerebral circulation maintains blood flow to these two crucial vital organs.

Visceral Blood Flow

Sympathetic discharge to visceral structures causes constriction of blood vessels in visceral organs. This significantly contributes to divert blood flow to skeletal muscles. Renal blood vessels also constrict that decreases renal blood flow to about 80% in severe exercise. Therefore, prolonged and severe exercise may impair kidney functions. Hence, proteinuria may occur in strenuous exercise.

Cutaneous Blood Flow

Cutaneous blood flow is also decreased due to sympathetic cutaneous vasoconstriction. This also contributes to divert blood flow to exercising muscles. However, in prolonged exercise, increased body temperature causes thermogenic vasodilation to promote dissipation of heat generated during exercise.

Cerebral Blood Flow

Though sympathetic vasoconstriction occurs in cerebral vessels, the degree of vasoconstriction is less, and metabolic vasodilation immediately following the increased sympathetic activity maintains cerebral blood flow.

Coronary Blood Flow

During exercise, sympathetic vasodilation occurs in coronary blood vessels due to presence of more number of β receptors in coronary arteries. Also, metabolic factors contribute. Increased coronary blood flow is nature’s autoregulatory process to supply adequate nutrient and oxygen to the cardiac muscle to maintain increased cardiac output throughout the period of exercise.

Adipose Tissue Blood Flow

Blood flow to adipose tissue increases during exercise. This helps in lipolysis to mobilize fatty acids from triglyceride stores in fat depots to the exercising muscles.

Blood Pressure Changes

Systolic Blood Pressure

Systolic pressure always increases in exercise proportionate to increase in cardiac output (Fig. 157.3). This occurs due to sympathetic induced cardio-acceleration.

Diastolic Blood Pressure

Change in diastolic pressure depends on the degree of exercise. Diastolic pressure depends on peripheral resistance, which primarily depends on the diameter of blood vessel.

1. In mild to moderate exercise, vasoconstriction induced by sympathetic activation increases diastolic pressure.
2. In moderate to severe exercise, vasodilation decreases peripheral resistance, which in turns decreases diastolic pressure.
3. Vasodilation occurs in exercise by three mechanisms: sympathetic cholinergic vasodilation of skeletal blood vessels, metabolic vasodilation and thermogenic vasodilation (see above).

Mean Blood Pressure

Mean pressure increases in mild to moderate exercise that helps in promoting blood flow to skeletal muscle through dilated vessels. In severe and continued exercise, mean pressure gradually falls as diastolic pressure falls. However, in isometric exercise mean pressure continued to increase throughout the period of exercise (Fig. 157.4).
Pulse Pressure

Pulse pressure becomes very wide as systolic pressure increases and diastolic pressure decreases. Increased pulse pressure helps in promoting perfusion of skeletal muscles.

In Pulmonary Circulation

Systolic pressure increases to 25–30 mm Hg from its resting 15–20 mm Hg in severe exercise. Diastolic blood pressure may rise from 5–8 mm Hg at rest to 8–10 mm Hg. Pulmonary blood flow increases.

Cardiovascular Changes in Isometric Exercise

1. **Heart rate increases** as soon as exercise begins and continues to increase throughout exercise. Tachycardia at the beginning of exercise is mainly due to vagal withdrawal, though sympathetic stimulation contributes to tachycardia in the later part of exercise.

2. However, **increase in stroke volume is relatively less** as vagal inhibition is the major means of cardiac changes.

3. **Systolic and diastolic pressures increase sharply**.

4. Mean pressure **remains elevated throughout** the entire period of exercise (Fig. 157.4). As exercising muscle remains tonically contracted during isometric exercise, skeletal muscle blood flow is grossly reduced due to mechanical vasocompression (Application Box 157.5).

**Application Box 157.5**

Isometric exercise is harmful for hypertensive patients: As blood pressure remains elevated throughout the entire period of isometric exercise, people suffering from hypertension and vascular anomalies should not perform these exercises. Also, people with heart diseases should not practice isometric exercises as they put maximum stress on the heart. However, isotonic exercise is advised for the management of hypertension.

**Change in Blood Volume**

Efficiency of maintaining arterial blood pressure during exercise depends on effective blood volume, which depends on total blood volume and venomotor tone that determine venous return to the heart. Effective **circulating volume falls** during prolonged exercise, especially when exercise is performed in hot environment. In strenuous exercise, blood volume decreases 15–20% that causes hemoconcentration. Blood volume decreases due to four factors:

1. Increased hydrostatic pressure increases capillary filtration that facilitates escape of fluid from vascular compartment into extravascular space.

2. Accumulation of osmotically active metabolites in the tissue reverses the normal filtration gradient. Thus, water moves into the interstitial tissue space.

3. Increased sweating and evaporation from skin during exercise contribute to fall in effective blood volume. The rate of water loss from the skin may be up to 30 mL/min in severe exercise.
4. In prolonged exercise, **redistribution of blood volume** to the skin to increase cutaneous blood flow for heat transfer from the body decreases effective blood volume. Cutaneous vasodilation is an effective means of dissipation of heat from the body generated during exercise.

   Hemococoncentration due to decreased blood volume **increases blood viscosity** (Clinical Box 157.2). However, oxygen-carrying capacity increases as relative red cell concentration becomes more.

**Clinical Box 157.2**

**Ingesting salt with water facilitates recovery:** Dehydration due to exercise should be treated by water containing salt. The most effective means of restoring body fluid volume following severe exercise is to ingest NaCl with water volume. Successful rehydration occurs effectively and early, when water is ingested with salt. Rapid recovery of fluid volume provides a better ability to regulate body temperature in exercise-collaps.

**Other Changes**

### Changes in the Tissue

The changes at the tissue level are aimed at extracting large amount of O₂ from blood, utilizing O₂ in the exercising tissue and facilitating removal of CO₂ from the tissues to the blood. These objectives are achieved by the following mechanisms:

1. **Increased blood flow:** Increased blood flow to skeletal muscles supplies more O₂ to the tissue. **Opening up of muscle capillaries** increase perfusion and surface for exchange of gasses between blood and the muscle.

2. **Increased pO₂ gradient:** As exercising muscle rapidly utilize O₂, the gradient of O₂ between capillary blood and muscle tissue is increased. This facilitates O₂ extraction from capillary blood into the tissue fluid.

3. **Increased oxidative metabolism:** The muscle metabolism is increased by **activation of oxidative enzymes**.

4. **Right shift of O₂-Hb dissociation curve:** Due to increased tissue metabolism, CO₂ accumulate in the tissue, temperature increases locally and red blood cell **2,3-DPG** increases. All these factors shift O₂-Hb dissociation curve to the right, which causes **three-fold increase in O₂ extraction** from blood.

### Endocrinal Responses

During exercise, many hormones are secreted in large quantity. Not only do these hormones mediate acute physiological effects of exercise, but also participate in the long-term adaptation to exercise.

**Catecholamines**

Secretion of catecholamines from adrenal medulla increases in severe exercise. These hormones promote **availability of fuels to the exercising muscles by mobilizing fatty acids** from adipose tissue and glucose from liver.

**Glucagon**

Secretion of glucagon is increased in prolonged exercise. By facilitating **glycogenolysis**, it mobilizes glucose from glycogen and by **promoting lipolysis**, it mobilizes fatty acids from the adipose tissue.

**Antidiuretic Hormone**

Secretion of ADH is increased during exercise. ADH helps in **water retention by increasing water reabsorption from kidney.** This compensates for water loss from the body that occurs in the form of sweating during exercise for dissipation of heat.

**ACTH and Cortisol**

ACTH secretion is increased during strenuous exercise. This increases glucocorticoid secretion and helps in **mobilizing fat**. Cortisol reduces the impact of exercise stress.

**Endorphin**

Secretion of endorphin increases greatly during exercise. This **reduces the exercise stress**, and **alleviates post-exercise pain**.

### AEROBIC TRAINING

The primary aim of training is to increase the resistance to fatigue. Therefore, this is also called **endurance training**.

1. Aerobic training increases VO₂max and promotes elimination of excess heat produced during exercise. By increasing VO₂max, the rate of ATP production increases more aerobically.

2. When VO₂ is less than 50% of the maximum, essentially all of the net energy release in active muscle is aerobic.

3. Thus, O₂ demand and the metabolic load of muscle are met without disturbing acid-base balance. Increase in VO₂max raises the threshold of exercise intensity for lactic acid production.

4. Thus, training alters both the O₂ transport and O₂-acceptance systems.

5. Training has minimal effects on respiratory responses and more effects on cardiovascular responses.

### Systemic Effects

**Respiratory Effects**

**Effects on O₂ Delivery and Extraction**

Aerobic training **improves both O₂ delivery and extraction.** There is large increase in VVO₂max with training. Though there is no evidence to suggest **increase in Hb concentration** in physical training, O₂-carrying capacity of blood increases with training. Also, tissue extraction of O₂ is marginally high in training.
Effects on Cardiovascular Responses

On Cardiac Output and Plasma Volume

Training increases stroke volume and decreases maximal heart rate.

1. The net effect is greater increase in maximal cardiac output. Therefore, athletes achieve a target increase in cardiac output by increasing stroke volume.

2. Increase in stroke volume is mainly due to increased myocardial contractility, which occurs due to elevation in key oxidative enzymes in the mitochondria of type I muscle fibers.

3. Also, expansion of the plasma compartment occurs in training that increases the preload and, therefore, increases ventricular filling.

4. As heart is more efficient in increasing stroke volume than heart rate, higher stroke volume reduces the myocardial metabolic load for any level of physical activity.

5. Red blood cell volume increases with aerobic training. However, greater expansion of plasma-volume reduces the hemoglobin content resulting in physiological sports anemia.

6. The increased blood volume promotes the ability to achieve greater cutaneous blood flow in unfavorable conditions like hot environment.

Other Effects

On O\textsubscript{2} Diffusion into Muscle

With training, O\textsubscript{2} extraction from blood to tissue increases. This is because trained muscles accommodate more blood flow as they have more new capillaries.

1. Increased capillary density increases greater surface area for O\textsubscript{2} diffusion and reduces the diffusion distance for O\textsubscript{2} between the capillary blood and membrane of muscle cells.

2. This also increases the transit time of blood to pass through the muscle capillaries that allows more time for O\textsubscript{2} extraction.

3. Training also increases total skeletal-muscle blood flow.

Effects on Mitochondrial Enzymes

Oxidative enzyme activity in the muscle mitochondria is enhanced in training.

1. The mRNA number and activity increase in muscle cell that increases transcription of muscle proteins.

2. The enzymes activated by training are succinate dehydrogenase, NADH dehydrogenase and cytochrome oxidase.

3. This provides an increased ability to oxidize free fatty acids and other fuels.

Effect on Insulin Sensitivity

Aerobic training increases tissue sensitivity to insulin. This promotes glucose transport into the muscle. This helps in sparing muscle glycogen.

Advantages of Training

Aerobic training improves performance of athletes and sportspersons.

1. This is because the endurance activity facilitates muscle oxidative capacity. Regular training is essential not only for maintaining physical fitness of sportspersons but also for armymen and police personnel.

2. Also, in general population, health of individuals practicing regular physical training remains always better than untrained individuals.

3. It also slows the process of aging. Regular physical exercise promotes psychological health.

4. This is attributed to increased secretion of endorphins in response to exercise that is known to alleviate mental stress and produce a sense of well-being.

5. Thus, regular physical training is the key to a healthier and happier life.

Therapeutic Benefits of Regular Exercise

Practice of regular exercise is an important component of management of many diseases.

1. Practice of regular exercise is known to reduce insulin resistance. Tissue sensitivity to insulin and glucose tolerance improves with exercise. Exercise increases the number of insulin receptors and promotes activity of glucose transporters. Hence, exercise therapy is very useful in the treatment of diabetes mellitus.

2. Regular exercise is known to decrease sympathetic tone. Therefore, exercise like morning walk is the best mode of treatment of hypertension.

3. Exercise improves coronary perfusion and causes neovascularization of myocardium. Therefore, regular practice of slow and mild exercise (without exerting much pressure on heart) helps patients suffering from chronic myocardial infarction.

4. Exercise, if practiced regularly, decreases body fat mass. Especially, brisk walking in the early morning is very effective in this regard. Physical activities like walking, running, swimming, dancing and jogging are known to induce energy expenditure that in the long run is very effective in reducing obesity (Table 157.5).

<table>
<thead>
<tr>
<th>Table: 157.5: Energy expenditure of various exercises.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exercise</td>
</tr>
<tr>
<td>---------------------------</td>
</tr>
<tr>
<td>Active standing</td>
</tr>
<tr>
<td>Cooking</td>
</tr>
<tr>
<td>Making bed</td>
</tr>
<tr>
<td>Slow walking (3 km/h)</td>
</tr>
<tr>
<td>Fast walking (6 km/h)</td>
</tr>
<tr>
<td>Dancing</td>
</tr>
<tr>
<td>Active gardening</td>
</tr>
<tr>
<td>Climbing stairs</td>
</tr>
<tr>
<td>Prolonged jogging</td>
</tr>
<tr>
<td>Swimming (50 m/min)</td>
</tr>
<tr>
<td>Running (12 km/h)</td>
</tr>
</tbody>
</table>
Therefore, this is the best way to check obesity and obesity-induced disorders.

5. Exercise prevents osteoporosis. Regulated exercise program stimulates osteoblastic activity, facilitates bone mineral metabolism and preserves the geometry. Immobilization and inactivity stimulate osteoclastic activity and produce bone loss. Specific exercises are prescribed for treatment of osteoarthritis as they improve muscle strength and joint stability.

6. Exercise prevents osteoporosis. Regulated exercise program stimulates osteoblastic activity, facilitates bone mineral metabolism and preserves the geometry.

CHAPTER SUMMARY

**Key Concepts**

1. Regular practice of exercise improves tissue oxygenation, tissue metabolic and oxidative capacity and decreases insulin resistance.
2. Exercise improves autonomic balance (increases vagal and decreases sympathetic activity).

**Important to Know (Must Read)**

1. 'Describe the respiratory and cardiovascular changes in a moderate isotonic exercise' may be a Long Question.
2. Types of exercise, Factors affecting muscle work, Energy sources in exercise, VO\(_{2\text{max}}\). Respiratory changes in exercise, CV changes in exercise, Physiologic effects of training may come as Short Questions.
3. In Viva, examiner may ask... What are the types of exercise, Grades of exercise, Name the factors affecting muscle work, What are the energy sources in exercise, What is VO\(_{2\text{max}}\) and what is its significance, What are the respiratory changes in exercise, What is lactate threshold and what is its importance, CV changes in exercise and tissue changes in exercise, What is size principle, Physiologic effects of training, What are the benefits of exercise.
The pH of body fluids is maintained within a narrow range as blood pH is maintained between 7.35 and 7.45. This is due to fine adjustment between production of acids and bases and their disposal from the body. The balance is due to integrated functions of buffer systems that are mainly executed by lungs and kidneys. Therefore, pulmonary and renal dysfunctions invariably lead to acid-base abnormalities. In the present chapter, we give a brief outline of acid-base physiology that helps to understand their role in various body functions and genesis of disorders of acid-base homeostasis. However, for details of acid-base homeostasis, a text book of biochemistry may be referred.

### GENERAL CONCEPTS

#### Definitions

**The pH:** The pH of a solution is defined as negative logarithm of the hydrogen ion concentration in the solution.

**The pK and K_a:** The pK is defined as negative logarithm of the ionization constant of an acid (K_a) in a solution. The pK is the pH at which the concentration of acid and its conjugate bases are in equal proportion for a buffer. The lower the pK, the stronger is the acid.

**The acid and alkali:** Acid is defined as a substance that adds H^+ to the body fluids and alkali or base is defined as a substance that removes H^+ from the body fluids.

**The Buffer:** A pH buffer of a solution is defined as a system of weak acid and its salt conjugate base or a weak base and its conjugate acid that minimizes the change in the pH of the solution within a narrow range when a strong acid or base is added to it.

#### Concept of Acid-Base Balance

Acids and bases are generated continuously in the body. There are also mechanisms that buffer (trap and neutralize) the acids and bases. Hence, the balance between acid and base is accomplished by maintaining the equilibrium between their production in the body and removal from the body.

1. Acid base balance is achieved through the coordinated functions of kidneys and lungs.
2. Various metabolisms in the body add acids to the body fluids.
3. Also, skeletal muscles add acid to the body during exercise in anaerobic conditions.
4. The various types of acids produced in our body are carbonic acid, sulfuric acid, phosphoric acid, lactic acid etc. Volatile vs. Nonvolatile acids: In adults, acid or base added to the body fluids is determined primarily by the diet.

1. In a normal diet, approximately 15 to 20 mole of acid is added daily, which is converted into carbon dioxide. Therefore, this acid is called volatile acid. The CO_2 produced by metabolism is removed through expired air. Therefore, the effect of CO_2 on body and especially on acid base balance is well coordinated.
2. However, the normal process of metabolism such as oxidation of sulfur containing amino acids, incomplete oxidation of energy substrates (lactic acid by glycolysis, ketoacid by triglyceride beta oxidation etc) and oxidation of these intermediates (gluconeogenesis of lactate, ketone body oxidation etc) all together produces large amount of acids that cannot be removed by lungs. Therefore, these acids are called as **nonvolatile acid**.

3. The nonvolatile acid is handled by kidney. Typically, a diet rich in proteins such as meat, fish and milk produces acid more than base.

**pH-Buffer Systems**

The pH of a fluid system is protected and stabilized by action of buffers. A pH buffer in a solution is the one that minimizes the alteration in pH produced when an acid or a base is added to the solution. A buffer is mixture of weak acid and a salt of its conjugate base or a weak base and the salt of its conjugate acid. The efficiency of buffer system is explained by the **Henderson-Hasselbalch equation** (described in 1908), which is:

\[
HA \leftrightarrow H^+ + A^-
\]

According to Henderson-Hasselbalch equation, in a buffer system, the undissociated acid (HA) is in equilibrium when its concentration of anion equals the concentration of cations. At equilibrium, the rate of dissociation of an acid to form \( H^+ + A^- \) and the rate of association to from \( H^+ \) and \( A^- \) from HA are equal. The **dissociation constant** (\( K_a \)) or equilibrium constant is the product of cations and anions divided by undissociated acid, i.e.,

\[
K_a = \frac{H^+ \times A^-}{HA}
\]

The strength of an acid is **determined by the level of** \( K_a \). Higher the dissociation constant, stronger is the acid as it is more ionized. This is because the magnitude of acidity depends on concentration of free \( H^+ \) in the solution. The examples of strong acids are hydrochloric acid, phosphoric acid, nitric acid and sulfuric acid. Weak acids have low \( K_a \) and ionize slowly. Examples of weak acids are lactic acid, and carbonic acid. Whenever, an acid of higher strength than HA is added to the system, the \( H^+ \) concentration is raised.

The value of a buffer is expressed in terms of \( \beta \) units. **One \( \beta \) unit** is the amount of base or acid required to bring the change of pH by about one unit. If the concentration of acid and base are equal for a buffer then its pH is equal to its pK. A good buffer resists the changes in pH in the interval of ± 1 pH unit of its pK. That is why the pK value of a good buffer should be near to body pH value. For effective buffering, the ratio of acid to base should be 1:10 or base to acid be 10:1. Buffers are more effective at higher concentrations. For example, a 10 mmol/L buffer solution is far more effective compared to 1 mmol/L of the same buffer solution.

There are **four buffer systems** in our body:

1. **Bicarbonate-carbonic acid buffer system**
2. **Plasma protein buffer system**
3. **Phosphate buffer system**
4. **Hemoglobin buffer system**

   **The first three act mainly in the extra-cellular fluid** and the **last two and first one act in the intracellular fluid**, especially in red cells.

**Bicarbonate-Carbonic Acid Buffer System**

This is the most important buffer system of the plasma. It is also a buffer in red cells. This buffer is the main buffer system in plasma. Though its pK is 6.1 and ratio of base to acid in plasma is 20:1, it is an effective plasma buffer due to its high concentration (>20 mmol/L). Also this system works effectively in conjunction with lungs to retain or eliminate acid in terms of \( CO_2 \) whenever required. **Buffering by bicarbonate system depends on**:

1. Removal or retention of acid via lungs in the form of carbon dioxide.
2. Titration of nonvolatile acids by the bicarbonates in the renal tubules.

In plasma, the partial pressure of carbon dioxide is relatively constant so that it can be expelled or retained in body via lungs. The \( HCO_3^- \text{+} H_2CO_3 \) buffer system easily generates or assimilates it and therefore resists the change in the pH.

**Plasma Protein Buffer System**

This accounts for 95% of non-bicarbonate buffers in plasma. The albumin component of plasma protein acts as the buffer. The **histidine residue** of albumin is responsible for its buffering action. As the pK value of histidine is 7.3, albumin (each albumin molecule contains 16 histidine residues) acts as an excellent buffer at physiological pH of blood. For each molecule of bicarbonate ion generated, one molecule of non-bicarbonate buffer disappears. Therefore, **non-bicarbonate buffer is essential to maintain the bicarbonate concentration**.

**Phosphate Buffer System**

Phosphate buffer system has both organic and inorganic components.

**Organic Component**

The **organic phosphate is the major buffer system** in the intracellular fluid. It consists of AMP, ADP, ATP, and 2,3-DPG. As 2,3-DPG is more in red cells, it accounts for about 16% of the non-bicarbonate buffer system in RBC.

**Inorganic Component**

Inorganic component of the phosphate buffer consists of **disodium hydrogen phosphate** \((Na_2HPO_4)\) and **sodium dihydrogen phosphate** \((NaH_2PO_4)\) with a ratio of 4:1. The
pK value is 6.8. Though, it represents only 5% of the non-bicarbonate buffer system in plasma, in the intracellular fluid its buffering contribution is appreciable. Phosphate buffer plays important role especially in the renal tubule in acidification of urine.

**Hemoglobin Buffer System**

This is the non-bicarbonate buffer that plays a major buffering role in red cells. Out of total buffering load in red cells, it accounts for approximately 85%, and rest 15% is by 2,3-DPG, which is a phosphate buffer. The histidine residue in Hb is responsible for its buffering action. In lungs, with oxygenation of Hb, the H⁺ ion is liberated and buffering capacity of Hb is decreased due to the decrease in pK value. However, when O₂ is released from oxyhemoglobin complex in the tissue, Hb binds to H⁺ and forms HHb, a weaker acid.

**REGULATION OF ACID-BASE BALANCE**

Two major mechanisms in the body regulate acid-base balance: respiratory mechanism and renal mechanism.

**Respiratory Mechanism**

The respiratory mechanism is mainly operated by change in the rate and depth of respiration in response to the inputs from central and peripheral chemoreceptors.

1. Chemoreceptors respond to acid base imbalance immediately.
2. During acidosis, bicarbonate ions are sequestered. The carbonate salt formed from it gets converted into carbon dioxide. Thus, pCO₂ increases in plasma. The decrease in plasma pH or the increase in pCO₂ is sensed by peripheral chemoreceptors.
3. The central receptors sense change in pH of CSF.
4. Chemoreceptor stimulation in turn stimulates respiratory center that produces hyperventilation and subsequent expulsion of excess CO₂. This decreases pCO₂, and fall in pCO₂ is rapidly equilibrated across blood brain barrier.
5. The CSF pH increases. Till CSF pH returns to normal it takes time for the respiration to become normal.

**Renal Mechanism**

Kidneys maintain acid base balance by reabsorbing the filtered HCO₃⁻ and by excreting the non-volatile acid produced by metabolism. For effective acid base homeostasis, the amount of net acid excretion should equal to the amount of non-volatile acid production.

Normally, the pH of glomerular filtrate is 7.4, which is same as that of plasma. Nevertheless, pH of urine is acidic, ranging from 4.5 to 8.2 (average 6.4). The kidneys regulate excretion of nonvolatile metabolic acids and retention of bases. Thus, in the process of acidification urine, kidney plays an important role in the acid-base balance in our body. Acidification of urine is primarily due to secretion of H⁺ into the tubular fluid. However, kidneys cannot excrete free acids; rather while excreting H⁺ with other anions, they excrete the salts of free acids (urinary buffers). Kidneys achieve these processes by following tubular mechanisms that we describe briefly here (for details, refer Chapter 65 ‘Acidification of Urine’).

1. Exchange of H⁺ with Na⁺
2. Excretion of ammonium
3. Excretion of H₂PO₄⁻
4. Excretion of other acids
5. Reclamation of bicarbonate

**Exchange of H⁺ with Na⁺**

In proximal tubule, Na⁺-H⁺ exchangers are located in the apical membrane of epithelial cells that secrete H⁺ into the tubular fluid in exchange of reabsorption of Na⁺. Dissociation of H₂CO₃ in the cytoplasm of epithelial cells is the source of H⁺ for reabsorption of sodium. The Na⁺-K⁺ ATPase on the basolateral membrane extrudes Na⁺ from renal tubular cell into interstitial fluid and maintains Na⁺ gradient. The H⁺ binds to NH₃ or HPO₄²⁻ in the tubular fluid and gets excreted in the urine. The excretion of H⁺ in urine as H₂PO₄⁻ accounts for about 90% of the total titratable acidity in urine. This acid load increases when protein intake is high. Action of HPO₄²⁻ is more in the DCT and collecting tubules whereas action of ammonia is more in proximal and distal tubules.

**Excretion of Ammonium**

Transamination of amino acids into glutamate transfers ammonia in our body. Tubular cells in kidney are rich in glutaminase that breaks glutamine to glutamate and ammonia. Glutamate dehydrogenase converts glutamate into α-ketoglutarate and NH₄⁺. Ammonia diffuses from cells into tubular fluid and binds with H⁺, where acidic pH favors formation of ammonium ion. NH₄⁺ binds with various anions like phosphate, chloride, sulfate etc and excreted in urine, which accounts for about 60% of the total acid excretion. In acidosis, excretion of acid as NH₄⁺ is increased to a greater extent.

**Excretion of H₂PO₄⁻**

H⁺ is secreted into tubular fluid binds to H₂PO₄⁻ to form H₂PO₄⁻. Phosphate concentration in tubular fluid increases with high intake of protein and acidaemia. As acid is excreted along with its buffer without causing change in the urinary pH, it allows more of H⁺ to be secreted in exchange of Na⁺. Normally, this accounts for about 30 mmol of H⁺ excretion per day in urine.

**Excretion of Other Acids**

Acetone, acetoacetic acid and β-hydroxybutyric acid are excreted in unionized form in urine whenever their load is increased in plasma. However, stronger acids like sulfuric acid, hydrochloric and phosphoric acids are deionized to give H⁺ ion that in turn is buffered and excreted in urine.
Reclamation of Bicarbonate

New bicarbonate is formed in exchange for acids in the tubular fluid. This depends on the acidic load in the tubular fluid. Also, bicarbonate is filtered by the glomerular filtering membrane and reabsorbed from the tubular fluid. About 80% of the total filtered bicarbonate is reabsorbed in PCT, 15% in thick ascending limb of Loop of Henle and 5% in the collecting ducts. When bicarbonate reabsorption is decreased, acidosis develops. Bicarbonate reabsorption depends on plasma bicarbonate level. Increased plasma bicarbonate to more than 26 mmol/L increases its filtration and makes urine alkaline. When plasma bicarbonate is below 26 mmol/L, all filtered bicarbonate is absorbed in exchange of acid and urine becomes acidic. Usually, 90% of the filtered bicarbonate is reclaimed in PCT. The degree of bicarbonate reclaimed is proportionate to the amount of Na\(^+\) reabsorbed.

Formation of New Bicarbonate

Formation of new bicarbonate is the generation of bicarbonate ion in the tubule, which occurs in two ways: excretion of titratable acids and excretion of ammonium ion.

Excretion of Titrable Acids

The H\(^+\) ion secreted into tubular fluid is excreted after being titrated by the urinary buffers, which is called as titratable acid. This depends on the pH of tubular fluid and the buffering capacity of the fluid. Though the major tubular buffer is HPO\(_4^{2-}\), other buffers are creatinine, citrate and various organic solutes. Let us see the example of titratable acid HPO\(_4^{2-}\). For phosphate buffer, H\(_2\)PO\(_4^{2-}\) exists as Na\(_2\)HPO\(_4\), the sodium salt of phosphate in the tubular lumen. When sodium is displaced, it enters tubular cell with bicarbonate ions whereas NaH\(_2\)PO\(_4\) gets excreted in the urine. Thus, for each mmol of H\(^+\) excreted as a titrable acid, one mmol of bicarbonate ion is added to the plasma.

Excretion of Ammonium Ion

Glutamine metabolism produces one molecule of ammonia (NH\(_3\)) and one molecule of ammonium ion (NH\(_4^+\)). Ammonia being lipid soluble diffuses into tubular lumen and binds to acid to form ammonium ion, which is excreted in the urine. In this process of regeneration, bicarbonate is freed from H\(^+\). The HCO\(_3^-\) enters the blood as new HCO\(_3^-\). The NH\(_4^+\) enters the tubular fluid. When H\(^+\) secretion in collecting duct is decreased, net acid excretion by the kidney is decreased. This decreases supply of new HCO\(_3^-\) to systemic circulation.

Acidosis occurs as a result of increased addition of acid or decreased removal of acid or an increased loss in base.

Alkalosis occurs as a result of increased addition of base or decreased removal of base or an increased loss of acid. Alkalosis represents elevated arterial pH, and an increased pCO\(_2\) that occurs due to the subsequent compensatory alveolar hypoventilation to reduce the pH. Usually, alkalosis is due to failure of kidneys to excrete bicarbonate at their normal capacity.

Abnormalities of acid-base balance are typically classified into four groups.
1. Metabolic acidosis
2. Respiratory acidosis
3. Metabolic alkalosis
4. Respiratory alkalosis

Metabolic Acidosis

Metabolic acidosis is the condition of primary bicarbonate deficit that results from excessive removal of bicarbonate into urine in order to buffer the extra acids of the body.

Etiopathogenesis

Excess acid in body occurs due to three major reasons:
1. Increased acid production: Increased production of organic acids like acetooxid acid, β-hydroxybutyric acid as occurs in diabetic ketoacidosis; increased production of lactic acid as occurs in lactic acidosis; and other conditions such as methanol toxicity, salicylate toxicity, etc.
2. Reduced acid elimination: Reduction in the elimination of acids such as in renal failure, renal tubular acidosis etc. in such conditions, the accumulated acid has to be neutralized in body for which body bicarbonate gets exhausted resulting in acidosis.
3. Excessive loss of bicarbonate: Decreased reclamation of bicarbonate from the renal tubules due to its decreased reabsorption, excessive loss of bicarbonate from duodenal fluid as occurs in acute diarrhea, pancreatitis, intestinal fistula etc., lead to acidosis.

Respiratory Compensation

With metabolic acidosis, ratio of HCO\(_3^-\) concentration to pCO\(_2\) decreases. The resulting fall in pH stimulates respiratory compensatory mechanism and hyperventilation occurs. This results in a drop in pCO\(_2\) and pH is restored (Table 158.1). When the respiratory compensatory mechanism is in full swing, the pCO\(_2\) falls by 1 to 3 mM of Hg for every mM fall in HCO\(_3^-\).

Renal Compensation

Renal compensation to metabolic acidosis causes increased acid excretion, ammonia formation and reclamation of bicarbonate ions.
Respiratory Acidosis

Respiratory acidosis is a condition where there is decreased excretion of carbon dioxide resulting in increased pCO$_2$.

Etiopathogenesis

Respiratory acidosis occurs in conditions that depress respiratory centers, affects respiratory apparatus, and in some other causes.

1. **Conditions that depress respiratory centers:** Drugs such as narcotics and barbiturate, CNS infections like encephalitis, meningitis, and coma due to cerebrovascular damage.

2. **Conditions that affect respiratory apparatus:** The common examples are chronic obstructive pulmonary disease, severe asthma, adult respiratory syndrome, chest wall deformity.

3. **Other causes:** Respiratory acidosis also occurs in extreme obesity, sleep apnea syndrome, abdominal distention as in severe ascites (as in severe ascites), etc.

Respiratory acidosis is an imperative situation and body immediately employs the buffer systems to bring down increased level of carbon dioxide. The buffers decrease the pCO$_2$ and raise the level of bicarbonate ions.

$$\text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{H}^+ + \text{HCO}_3^-$$

As the pCO$_2$ is more, the production of bicarbonate is more. The acid released in the form of H$^+$ is buffered largely by the hemoglobin and the protein buffers. In the early phase this could cause a transient metabolic alkalosis due to overproduction of bicarbonate ion within a short span of time.

Renal Compensation

The renal mechanism causes increased body acid excretion, ammonia formation and reclamation of bicarbonate ions. However it gets activated in 6 to 12 hours and reaches its peak action in 2 to 3 days.

Respiratory Compensation

Increased pCO$_2$ stimulates the respiratory centers that cause hyperventilation. It leads to increase in respiratory rate and depth, which is not possible if the respiratory defect itself is the reason for respiratory acidosis.

With the expulsion of carbon dioxide from the system, ratio of HCO$_3^-$ to pCO$_2$ increases and pH returns to normal.

Metabolic Alkalosis

Metabolic alkalosis is a condition of primary bicarbonate excess. It is associated with ratio of HCO$_3^-$ to pCO$_2$ more than 20. Rise in pH is associated with binding of free calcium ions to body proteins and other anions. This leads to increased neuromuscular excitability and at a pH above 7.56, tetany may result even at normal calcium concentration in serum.

Etiopathogenesis

Metabolic alkalosis can be categorized into three types: saline responsive alkalosis, saline resistant alkalosis and exogenous base-load alkalosis.

Saline Responsive Alkalosis

In saline responsive alkalosis the urinary chlorine level is less than 10 mM/L. It could be associated with normal or raised blood pressure.

Normotensive Saline Responsive Alkalosis

Examples are excess vomiting (loss of acid), pyloric or duodenal obstructions, prolonged administration of diuretics (diuretic abuse), post-hypercapnia, cystic fibrosis, post-bicarbonate therapy of organic acidosis etc.

The vomiting and gastric aspirations are associated with hypovolemia (due to ECF contraction) and loss of sodium, chloride and hydrogen ions. The hypovolemia stimulates kidneys to activate renin-angiotensin-aldosterone system. Both hypovolemia and stimulation of aldosterone leads to a decrease in GFR and enhanced renal
retention of bicarbonates. During vomiting, the bicarbonate enters plasma in exchange of the chloride ions lost and causes alkalosis. Correction with simple sodium chloride in the form of saline is sufficient to reverse these situations.

**Hypertensive Saline Responsive Alkalosis**

Example is Liddle syndrome.

Liddle syndrome is an autosomal dominant disease that is associated with low levels of renin and aldosterone due to defective internalization and degradation of receptors for ENAC (epithelial sodium channel). This leads to persistent sodium absorption, hypertension, hypokalemia and alkalosis.

**Saline Unresponsive (Resistant) Alkalosis**

The urinary chlorine level is greater than equal to 10 mM/L. It could be associated with normal or raised blood pressure.

Normotensive saline unresponsive alkalosis are Mg²⁺ deficiency, K⁺ deficiency, diuretics, Bartter syndrome etc.

Hypertensive saline unresponsive alkalosis are primary aldosteronism, Cushing syndrome, renal artery stenosis etc.

Alkalosis due to K⁺ deficiency is saline resistant and correction of this deficiency alone leads to correction of alkalosis. Bartter syndrome is an autosomal recessive syndrome that is associated with defective chloride absorption and hence volume depletion and activation of renin angiotensin system.

**Exogenous Base-Load Alkalosis**

Chronic alkali infusion causes this, which is mild in normal kidney function but worsens in patients with chronic renal insufficiency. The milk alkali syndrome is due to excessive ingestion of milk and antacids.

**Respiratory Alkalosis**

Excess elimination of acid in the form of carbon dioxide via the respiratory route causing alkalosis is called respiratory alkalosis. This causes a decrease in $pCO_2$ and hence an increase in the ratio of $HCO_3^-$ to $pCO_2$.

**Etiopathogenesis**

There etiological factors of respiratory alkalosis can be classified into 3 types: pulmonary, non pulmonary and others.

- **Pulmonary factors**: Diseases like asthma, pneumonia, and congestive cardiac failure etc cause respiratory alkalosis.
- **Non-pulmonary factors**: Hypoxia (in acute conditions as in high altitude, anemia), hyperthyroidism, infections (such as meningitis, encephalitis, septicemia etc), hyperpyrexia (febrile state) and drugs (salicylates, catecholamines, progesterone etc).
- **Others**: Hyperventilation as occurs in indiscreet usage of ventilator.

**Compensation**

Compensation to respiratory alkalosis occurs in two ways.

If the alkalosis is a temporary, then compensation occurs by the buffers of red cells and tissue buffers that consume the bicarbonate ions.

If alkalosis is prolonged, the renal compensatory mechanisms get activated. Kidney responds to respiratory alkalosis in the same way as it reacts to metabolic alkalosis. Thus, there is an increased excretion of alkali in the form of bicarbonate, decreased elimination of acids, ammonia and potassium and increased chloride retention.

If alkalosis is severe, ketone bodies are formed due to decreased usage of the carbohydrates leading to ketosis and ketonuria.
Chapter 159

Regulation of Volume, Composition and Osmolality of Body Fluid Compartments

Learning Objectives

On completion of study of this chapter, the student WILL be able to:
1. Understand the mechanisms of water balance of the body.
2. Understand the control of NaCl concentration and osmolality of ECF.
3. Appreciate the physiological basis of iso-osmotic, hypo-osmotic and hyperosmotic dehydration and volume expansion.

General Concept

The regulation of volume, composition and osmolality of the body fluid is closely associated with control of water and electrolyte balance of the body as body water content reflects volume of body fluid compartments and concentration of electrolytes influence composition and osmolality of body fluids. Kidneys play important role in regulation of ECF volume. ECF volume is primarily dependant on Na⁺ homeostasis, which contributes significantly to the regulation of plasma osmolality. Thus, control of volume, composition and osmolality are major functions of kidney that is interdependent physiological phenomena.

Exchange Between the Compartments

Various ions move across the cell membrane between ICF and ECF. Change in volume and ionic composition of one compartment therefore affects the volume and composition of the other.
1. Depending on the rate and degree of transfer of ions across the membrane, concentration and electrical gradients are created on both sides of the membrane.
2. The electrochemical gradients create osmotic imbalance in the fluid compartments. Water moves along the osmotic gradient to maintain osmotic equilibrium.
3. Exchange of fluid between the plasma and the interstitial compartment across the capillary wall driven by Starling forces is the best example of such phenomena.
4. Osmolality of ECF and ICF are equal at steady state. As cell membrane is highly permeable to water, change in the osmolality in either ECF or ICF results in the rapid water movement between these compartments to achieve the osmotic neutrality. Usually, movement of NaCl determines osmolality and volume of fluid compartments.

Normally, volumes of different body compartments can be calculated by following formula.
- Total body water = 0.6 × Body weight,
- Intracellular fluid volume = 0.4 × Body weight,
- Extracellular fluid volume = 0.2 × Body weight,
- Interstitial fluid volume = 0.75 × ECF, and
- Plasma volume = 0.25 × ECF

Control of Body Fluid Volume

Control of volume of body fluids is directly linked with water balance of the body. Therefore, before discussing regulation of body fluid volumes, first let us understand the mechanism of water balance. Normally, water balance is achieved by matching the water gain with water loss.

Water Gain

Body gains water from two sources: exogenous and endogenous.
Exogenous Water Gain

Exogenous water gain occurs mainly by two processes: drinking of water and water ingested with food. The exogenous water intake normally ranges from 2 to 10 liters per day.

Water Drinking

Drinking of water is the major form of water gain. It ranges from 0.5 to 8 liters/day depending mainly upon the environmental conditions. Fluid drinking is more in hot and humid weather, and less in cold climate. However, social and personal habits considerably influence this. Water intake is mainly controlled by the thirst centers in the brain. Change in plasma volume and osmolality influences thirst center and control water ingestion.

Ingestion of Water in Food

Water is a major constituent of foodstuffs. Water is present in all food preparations and fruits. Though water content of food is highly variable, it is present in high quantity in liquid preparations such as soup, dal, rasam, sambar, mohr, fish curry, vegetable curries etc. However, social habits and personal preferences contribute to it. On average, water ingested in food (in breakfast, lunch, snacks and dinner) varies from 1 to 5 liters per day.

Endogenous Water Gain

Water is one of the products of metabolism (other products are ATP, CO₂ and temperature). During the process of tissue oxidation, water is continuously produced in the body and this endogenous production of water accounts for 300–500 ml of water added per day to the body fluids.

Water Loss

Water loss from the body occurs mainly in four forms: urine, feces, sweat and as insensible loss.

Loss in Urine

Urinary loss of water is a major form of water output from the body. About one liter of water is excreted from the body in urine per day. However, this varies with liquid intake, environmental conditions and salt intake. Kidney plays important role in water balance by altering water excretion in urine, which primarily depends on the concentration of ADH in plasma. ADH contributes to urine volume that may vary from 0.5 to 18 L/day. This independently determines urine osmolality, which varies from 50 to 1200 mOsm/kg H₂O with the corresponding change in urine volume. Osmolality of tubular fluid also decides water output in urine. Increased tubular fluid osmolality as occurs in diabetes mellitus increases urinary water loss.

Fecal Loss

Normally, about 200 ml of water per day is excreted in feces. The ingested water is mostly absorbed in the intestine.

Increased absorption of water leads to constipation and decreased absorption causes diarrhea. In diarrhea, water is also lost in vomiting.

Loss in Sweat

Water is routinely lost from the body in the form of sweat. Sweating is greatly influenced by environmental temperature and the level of physical activity. The rate of sweat production per day is about 0.1 L at ambient temperature of 23°C, 1.5 L at 38°C and 5 L at 45°C. Exercise in hot weather increases water loss in sweating.

Insensible Water Loss

In addition to loss in sweating, water is continuously evaporated from the skin surface. Water is also continuously evaporated from respiratory passage in breathing. These losses constitute the insensible water loss.

Positive and Negative Water Balance

The net water gain or loss leads to alteration in many physiological functions as it produces increase or decrease in volume of fluid compartments and accordingly produces dilution or concentration of body fluids.

Positive water balance occurs when water gain is more than its loss. This causes hemodilution, therefore, decreases in osmolality of plasma, decreases red cell count and produces hyponatremia.

Negative water balance occurs when water loss is more than its gain. This leads to dehydration and causes hemoconcentration. It increases plasma osmolality. Due to hemoconcentration, there is relative increase in red cell count.

Factors Influencing Water Balance

Water intake and output are controlled mainly by two factors: plasma osmolality and blood volume.

Plasma Osmolality

Plasma osmolality is an important factor for water balance. Alteration in osmolality is detected by osmoreceptors in hypothalamus that stimulates thirst. Increased plasma osmolality is also a primary stimulus for ADH secretion.

Factors that Affect Plasma Osmolality: Plasma osmolality depends on the presence of osmotically active molecules in plasma, especially NaCl, proteins and glucose. Among them, most effective in influencing osmolality is NaCl. However, abnormal increase in protein and glucose in plasma also exerts considerable osmotic effect.

Blood Volume

Alteration in blood volume is detected by volume receptors in atria that send impulses to hypothalamus and pituitary to secrete ADH. However, increased osmolality is a better stimulus than hypovolemia for stimulation of thirst and ADH secretion.
Factors that Affect Blood Volume: Blood volume depends on the plasma volume and the hematocrit. Increase in water content of plasma causes plasma expansion and increase in cell count, especially red cell count increases blood volume. Conversely, in opposite conditions, blood volume decreases.

Effective Circulating Volume

Effective circulatory volume (ECV) is the blood volume in the arterial system that causes effective perfusion of the tissues. This is also called effective blood volume. This excludes the stagnated blood present in some visceral organs and inactive muscles, which does not participate in perfusion. The pressure in the circulation that helps in this perfusion is called effective perfusion pressure. Approximately 20% of blood volume forms the ECV. The ECV is monitored by the volume sensors located entirely within the vascular tree.

1. In physiological conditions, ECV reflects the ECF volume and arterial volume, and ECV contributes to cardiac output and arterial pressure. Change in volume of ECF, changes vascular volume and therefore, changes ECV, cardiac output and arterial pressure.

2. In pathological conditions, in which expansion of ECF is the major pathophysiological change of the disease as occur in congestive heart failure, nephrotic syndrome and hepatic cirrhosis, the ECV does not completely depend on ECF volume. In such conditions, inspite of expansion of ECF volume, ECV may remain normal or even reduced. Kidneys sense change in ECV not ECF, therefore, decrease in ECV causes renal retention of Na⁺ and H₂O that further aggravates fluid accumulation and edema formation. ECV and Na⁺ balance are closely linked. Therefore, retention of Na⁺ causes expansion of ECV and depletion of Na⁺ contraction of ECV.

Volume Sensors

Volume sensors or ECV sensors are volume receptors located in the vascular tree that respond to stretch of the vessel wall. These are basically the vascular baroreceptors that are generally classified into two categories: low-pressure baroreceptors and high pressure baroreceptors.

Low-pressure volume receptors: are located in low pressure side of circulation, especially in atria, and pulmonary vasculature.

High-pressure volume receptors: are present in high-pressure compartment of circulation. These receptors include the baroreceptors present in carotid sinus, aortic arch and left ventricle.

Volume sensors are also present in liver (hepatic volume receptors), brain (central nervous system Na⁺ sensors) and in JG apparatus.

Effects of Stimulation of Volume Receptors

Low-pressure volume receptors respond mainly to fullness of vascular tree, i.e. vascular volume. Afferents from these receptors travel in vagus nerve and terminate in nucleus tractus solitarius (NTS) in the medulla. Fibers also contact ADH secreting neurons in posterior pituitary. Therefore, stimulation of these receptors leads to:
1. Sympathetic activation and
2. ADH release.

High-pressure volume receptors respond primarily to the arterial pressure. Afferents from these receptors from carotid sinus and aortic arch travel in 9th and 10th cranial nerves to terminate in NTS in medulla. They inhibit sympathetic discharge and activate vagal output.

Stimulation of receptors of JG apparatus activates the renin-angiotensin mechanism. Activation of hepatic sensors, regulate gastrointestinal Na⁺ absorption and stimulation of CNS Na⁺ receptors, may be located in the hypothalamus regulate renal sympathetic nerve activity.

Control of NaCl Concentration in ECF

The major ionic constituents of ECF are Na⁺ and Cl⁻. These two ions contribute considerably to ECF volume and therefore, they determine plasma volume. Kidneys play important role in the regulation of ECF and plasma volumes by controlling transfer of Na⁺ and Cl⁻ between tubular fluid and interstitial fluid. The volume sensors in JG apparatus generate appropriate signals in response to changes in ECV that first influence GFR and then renal excretion of NaCl and water.

Control of Renal NaCl Excretion

Renal NaCl excretion is controlled by both neural and hormonal mechanisms.

Neural Mechanism

Stimulation of renal sympathetic nerve, in response to activation of volume receptors leads to decrease in NaCl excretion by three mechanisms:
1. Reduction in GFR: Sympathetically induced vasoconstriction of afferent arterioles decreases GFR. This decreases filtered load of Na⁺ to the tubule. Thus, urinary Na⁺ excretion is reduced by the glomerular tubular balance.
2. Increased Na⁺ reabsorption: Sympathetic stimulation directly increases reabsorption of Na⁺ from proximal tubule.
3. Increased renin release: Sympathetic stimulation increases renin secretion from JG cells that activate RAS, which in turn promotes formation of angiotensin II and aldosterone. Both these hormones increase reabsorption of Na⁺ and Cl⁻ from renal tubule.

Hormonal Mechanism

Hormonal mechanisms controlling NaCl concentration are mainly three: renin-angiotensin-aldosterone axis, ANP and ADH (for details, refer “Regulation of blood pressure”).
Regulation in Different Condition of ECF

Regulation of Na⁺ Excretion in Euvolemia

During euvolemic (normal blood volume) Na⁺ excretion is adjusted by reabsorption of NaCl in the tubule. Aldosterone is the primary regulator of Na⁺ reabsorption from kidney. Till kidneys are normal and aldosterone secretion is normal, any level of dietary intake of NaCl is adjusted to maintain normal blood volume.

Regulation of Na⁺ Excretion in Hypervolemia

Volume expansion initiates responses that increase sodium excretion from kidney. These mechanisms decrease absorption of NaCl from kidney and therefore, decrease ECF volume.
1. Decreased renal sympathetic activity,
2. Decreased activity of renin-angiotensin-aldosterone system, and
3. Increased ANP secretion.

Regulation of the Na⁺ Excretion in Hypovolemia

Hypovolemia as occurs in excessive sweating, vomiting and diarrhea decrease in ECV, which is detected by low-pressure volume sensors that initiate following responses:
1. Increased renal sympathetic discharge,
2. Activation of renin-angiotensin-aldosterone system and
3. Increased ANP secretion.

Body Fluid Osmolality

Normally, osmolality of ECF and ICF are at equilibrium, inspite of a marked difference in the composition of electrolytes in these two compartments, Na⁺ being the main cation of ECF and K⁺ being the principal action in the ICF. The osmotic equilibrium between the ECF and ICF is maintained by easy transfer of water between these compartments. It is easy to measure plasma osmolality, which therefore, indirectly reflects the osmolality of ECF and ICF.

Normal plasma osmolality ranges between 280 to 295 mOsm/kg H₂O. Sodium and chloride contributes to about 90% of plasma osmolality. Out of total 295 mOsm/kg H₂O, about 270 mOsm is contributed by NaCl, and rest 20 mOsm is contributed by urea, glucose, protein and other ions like K⁺, Ca²⁺, etc. Whenever, Na⁺ moves from one compartment to the other, Cl⁻ also moves along with it to maintain electroneutrality. Therefore, it is mainly the Na⁺ that contributes to osmolality.

The total body osmolality is proportional to the total body sodium content plus total body potassium content divided by the total body water. Therefore, osmolality change in the body fluid occurs when there is change in water content and electrolyte content, NaCl and K⁺. In other words, water balance in the body is most important determinant of the body fluid osmolality.

Control of Body Fluid Osmolality

The tonicity (osmolality) of the ECF primarily depends on electrolyte and water contents of the compartment. The change in osmolality is detected by osmoreceptors that on activation provide appropriate signal to influence secretion of ADH and water intake that buffer the tonicity.

The osmoreceptors are located in the anterior hypothalamus. They detect change in osmolality of plasma. When osmolality of plasma is high, stimulation of osmoreceptors leads to:
1. Increase in ADH release that increases water reabsorption by the kidneys, and
2. Activation of thirst centers that increase water intake.

When osmolality of plasma is low, vasopressin secretion is decreased and the thirst centers are inhibited.

Tonicity of Fluid

1. Isotonic fluid is the fluid having the same osmolality as that of plasma. 0.9% NaCl solution, 5% glucose, 10% mannitol and 20% urea are considered to be isotonic. These solutions do not change the volume of the body cells.

2. Hypotonic fluid is the fluid having osmolality less than that of plasma. Red cells swell when placed in these solutions and eventually burst (hemolysis).

3. Hypertonic fluid is the fluid having osmolality more than that of plasma. Red cells shrink and undergo creation when placed in these solutions.

Disorders of Fluid Volume

Disturbances of fluid volume result in either dehydration or overhydration. However, based on osmolality of the body fluid they are categorized into six varities:

1. Iso-osmotic dehydration
2. Hypo-osmotic dehydration
3. Hyperosmotic dehydration
4. Iso-osmotic volume expansion
5. Hypo-osmotic volume expansion
6. Hyperosmotic volume expansion

Iso-osmotic Dehydration

Iso-osmotic dehydration occurs in conditions of loss of isotonic fluid from the body as commonly occurs in diarrhea, vomiting and hemorrhage. Usually, ECF osmolality does not change as osmotic effect for shift of water between the ECF and ICF compartments does not exist. The effects of iso-osmotic volume contraction are:

1. Decrease in ECF and plasma volumes.
2. Concentration of protein in plasma is increased because of loss of ECF. This relatively increases oncotic pressure that keeps fluid in the plasma. Therefore, normally plasma volume is less reduced in comparison to loss of interstitial fluid.
3. Hematocrit is increased due to hemoconcentration. However, size and shape of red cells remain unchanged as osmolality is not affected.
4. Arterial blood pressure decreases as the total blood volume is decreased.

**Compensatory Mechanisms**

Decreased blood volume stimulates ADH release, which stimulates water reabsorption from kidney. Hypovolemia that decreases stretch of atria due to decreased venous return inhibits secretion of ANP, the natriuretic hormone. Therefore, natriuresis and diuresis are decreased. Renin-angiotensin system is activated. Thirst is also stimulated.

**Hypo-osmotic Dehydration**

Loss of salt in excess of water causes hypo-osmotic volume contraction. This usually results from adrenocortical insufficiency associated with renal loss of NaCl. Aspiration of gastric secretions may cause hypertonic fluid loss from the body. The effects of hypo-osmotic dehydration are:

1. Plasma volume decreases due to dehydration.
2. Osmolality of ECF and plasma is decreased due to excess loss of solutes or NaCl.
3. Water shifts from ECF to ICF until osmolality equals in both compartments.
4. In severe salt deficiency, the plasma volume reduces sufficiently and cellular overhydration occurs. Swelling of cells of thirst center inhibits thirst. Consequently, dehydration is accentuated.
5. Red cells swell due to endosmosis.
6. Arterial blood pressure decreases due to reduced ECF volume.

**Compensatory Mechanisms**

In spite of decreased ECF volume, thirst is absent. However, appetite for salt intake is stimulated. The salt appetite that stimulates the individual to consume a large amount of NaCl helps in restoring ECF osmolality. When ECF osmolality returns to normal, water shifts from ICF to ECF causing partial restoration of ECF volume. Restoration of plasma concentration of NaCl stimulates thirst, which contributes to restore the remaining deficit of ECF volume.

**Hyperosmotic Dehydration**

Hyperosmotic volume contraction occurs when loss of water is more than the loss of solutes from the body. This typically occurs in reduced water intake for a longer duration, diabetes insipidus (pure water diuresis), excessive sweating (sweat is hypotonic to plasma) and alcoholism. The effects of hyperosmotic dehydration are:

1. Decrease in ECF and plasma volumes due to loss of water.
2. Increase in osmolality of ECF and plasma due to pure or relatively more water loss.
3. Due to osmotic effects, water shifts from ICF to ECF. This decreases ICF volume and causes intracellular dehydration.
4. Protein concentration of plasma increases due to decrease in ECF volume and water loss.
5. Hematocrit increases due to decreased plasma content. In severe condition red cell may shrink.
6. Decreased blood volume decreases arterial pressure.

**Physiological Corrections**

Increased osmolality of blood stimulates the hypothalamic osmoreceptors. Increased ADH secretion compensates by increasing water absorption from kidney that dilutes osmolality. Decreased blood volume stimulates sympathetic system that activates renin-angiotensin system. Angiotensin increases water reabsorption from kidney, stimulates thirst, causes vasoconstriction and increases aldosterone formation. All these effects aim at restoring the plasma volume and osmolality to normal level.

**Iso-osmotic Volume Expansion**

Iso-osmotic volume expansion usually occurs when isotonic fluids are infused for more than the required amount. ECF osmolality does not change in iso-osmotic expansion. Therefore, water does not shift between the ECF and ICF compartments. The effects of iso-osmotic volume expansion are as follows:

1. ECF and plasma volumes increase.
2. Plasma protein concentration decreases due to hemodilution that inhibits ADH secretion and promotes water excretion.

**Hypo-osmotic Volume Expansion**

When water gain exceeds loss, hypo-osmotic volume expansion occurs. This occurs due to hemodilution that decreases osmolality of plasma. Usually, it occurs in excessive infusion of hypotonic saline, ingestion of large volume of water, colonic wash with plain water or hypotonic fluid, and syndrome of inappropriate ADH secretion (SIADH). Effects of hypo-osmotic volume expansion are:

1. Volume of ECF and plasma increases due to increased water content in the fluid compartment.
2. Osmolality of ECF and plasma decreases due to water retention.
3. Water is transferred from ECF to ICF as the filtration gradients are reversed. ICF osmolality decreases initially.
4. Concentration of plasma protein and hematocrit decreases due to hemodilution.
5. Red cells swell due to endosmosis.
6. Arterial pressure increases due to increased blood volume.

**Compensatory Mechanisms**

The decreased osmolality is detected by osmoreceptors that activate appropriate signals to induce water diuresis that decreases plasma volume and increases plasma osmolality to normal. Increased plasma volume activates vascular high-pressure receptors and restores blood pressure.

**Hyper-osmotic Volume Expansion**

Excessive administration of hyperosmotic saline causes hyperosmotic volume expansion. This occurs rarely. The effects of hyperosmotic overhydration are:

1. The volume of ECF and plasma is more.
2. Osmolality of plasma and ECF is more. Therefore, water moves from ICF into ECF till osmolality is equilibrated. This causes cellular dehydration.
3. Protein content in plasma and red cell count decrease due to increased plasma volume.
4. Arterial pressure increases due to expansion of ECF compartment.

**Compensatory Mechanisms**

Increased plasma and ECF volumes increase secretion of ANP that produces natriuresis and diuresis. This restores plasma volume and osmolality. Also, plasma volume expansion inhibits ADH secretion that causes diuresis.
The terms growth and development are closely and mutually dependent. Generally, growth refers to increase in the physical size, as the infant grows into childhood and then into adulthood, whereas development refers to the maturation, which is the improvement in the capacity of the organ systems and body parts. Therefore, these terms are usually used together to denote the entire process of maturation, both in quality and magnitude.

**Patterns of Growth**

Though there is a usual pattern of growth for the whole body in general, different parts of the body do not follow a uniform pattern of growth. Especially, growth pattern is different for gonadal, neural and lymphoid tissues. Accordingly, the growth curves are different for these different body tissues.

**General Growth Curve**

General growth is the growth of the body as a whole, which include increase in height and weight, and growth of the musculoskeletal, cardiovascular, respiratory, gastrointestinal, endocrine and excretory systems. Though the growth rate is different at different stages of life, there are **two periods of rapid growth**. The period of rapid growth is called growth spurt. The two general growth spurts are **infantile** (during infancy) and **pubertal** (during late puberty) spurts.

**Infantile Growth Spurt**: Height and weight are good indices of growth in infants and children. After few days of initial weight-loss following birth, the neonate gradually starts gaining weight. During infancy, birth weight is almost doubled at sixth month and increased to approximately three times at one year of age. In post-natal life, the height gain is maximal during first two years of life, which is about 25 cm per year. Then, height gain almost decreases speedily to 5–10 cm per year for next 8–10 years (Fig. 160.1).

**Pubertal Growth Spurt**: Full pubertal development ranges between 12–18 years of life. Weight gain is rapid during this period, which occurs at about 3 to 4 kg gain per year between 12 and 18 years of age. During puberty, rapid growth occurs in the musculoskeletal system. Therefore, both height and weight increase during the period. Height gain increases from its prepubertal 5 cm/year to about 10 cm per year during puberty. Growth spurt appears 2 to 3 years earlier in girls. Cessation of height-gain occurs immediately after puberty due to closure of epiphysis induced by sex hormones.

**Neural Growth Curve**

Neural growth includes the growth of brain, spinal cord, and eye.

1. These organs grow rapidly during infancy.
2. In first year of postnatal life, the brain attains about 70% of the adult size (Fig. 160.2). Rest 30% growth occurs in next 3 to 5 years to attain fully developed size by 5th to 6th year of life.
3. The head circumference increases proportionately in first year and then in next 5 years of age.

**Gonadal Growth Curve**

The reproductive organs, especially gonads and accessory sex organs remain rudimentary throughout childhood. They start growing at puberty and rapidly between 14 to 18 years of age.

**Lymphoid Growth Curve**

Lymphoid tissues grow very rapidly during infancy and childhood. Lymphoid organs include lymph nodes, spleen, tonsils, adenoids, thymus and lymphatic tissues of the intestine.

1. At puberty, these organs undergo partial involution.
2. The tonsils and adenoids attain maximum size at the age of 6–10 years.
3. An ENT surgeon should have this physiological knowledge of growth rate of tonsils at childhood and should not be tempted to remove these structures surgically just because of their big size.

**REGULATION OF GROWTH**

Patterns of growth and development vary between races and communities. However, the important factors affecting growth are: hormonal, nutritional, social, environmental and genetic factors.

**Hormonal Factors**

The important hormones which affect the growth and development are: Growth hormone, insulin-like growth factor, thyroxine, gonadal hormones and insulin.

**Growth Hormone**

Growth hormone plays an important role in the growth and development of the individual at various stages of life (for details, refer “Growth Hormone”). Especially, during childhood, it determines the height and frame of the body. Its deficiency leads to severe growth retardation, which is known as pituitary dwarfism.

**Insulin-like Growth Factor-I**

Many physiological activities of growth hormone are mediated through insulin-like growth factor-I (IGF-1), secreted from liver. It is also called as somatomedin-C. Somatomedin C promotes protein synthesis, visceral growth, epiphyseal growth and musculoskeletal development.

**Thyroxine**

Thyroxine has direct effects on growth and development (for details, refer “Thyroid Gland”). It also stimulates secretion of GH and IGF-1. Therefore, deficiency of thyroxine at childhood leads to thyroid dwarf or cretinism.

**Gonadal Hormones**

Main gonadal hormones are testosterone in male and estrogen in female. They control growth and maturation during pubertal and adolescent periods. They have many anabolic actions. Their deficiency produces retardation of growth spurt during puberty.

**Insulin**

Insulin is an anabolic hormone and promotes growth of tissues (for details, refer “Endocrine Pancreas”). Therefore, growth retardation is the hallmark of diabetic children.

**Nutritional Factors**

Growth of tissues depends on the supply and utilization of all ingredients of nutrition. Proteins, fats, carbohydrates, vitamins, minerals and trace elements are essential components of balanced diet and contribute to growth and development in various ways.
1. Though proteins are the building blocks for tissues, others are cementing substance. Therefore, for optimal growth, all are equally important.

2. **Nutritional requirement is therefore more during active periods of growth.** Nutritional deficiency during childhood is the commonest cause of growth retardation in developing countries.

3. In children, growth becomes faster after a period of food deprivation (Application Box 160.1).

**Environmental Factors**

Environmental temperature and humidity, light-dark cycle and altitude have considerable effects on growth development. The people living in temperate zone have small height and less built than people living at normal temperature and sunlight.

**Social Factors**

Social factors play important role in growth and development during childhood. Not only physical growth, but also psychological growth takes place during the formative years of development. **Proper love and care** from parents and relatives are very essential for mental development of a child. **Harmony in the family** is very crucial in this development. Quarrels between parents, divorce, family disturbances and economic deficiency are causes of psychological abnormalities in children.

**Genetic Factors**

Irrespective of other factors, genetic inheritance is the major determinant of height and body constitution.

**Application Box 160.1**

*Catch-up growth:* In children, following a period of illnesses or starvation, the rate of growth becomes faster than normal. This accelerated growth aims at catching up the level of growth prior to illness. Therefore, it is called catch-up growth. However, the exact mechanism of catch-up growth is not known.

**BEHAVIORAL DEVELOPMENT**

Behavioral development of a child depends on environmental, social and genetic factors. The child learns from the environment and gathers experiences during the process of learning. Age and physical health contribute to the psychological growth. Therefore, both physical and environmental factors are essential behavioral attainment. The details of milestone of developmental process can be studied from a pediatric book.

**Aspects of Behavioral Development**

Behavioral development can be divided into four aspects: motor, language, psychosocial and adaptive behaviors.

**Motor Behavior**

Motor behavior is the behavioral response that involves motor activities like change in posture, standing, walking, etc. In infants, it starts with grasp reflex, and gets improved slowly with ability to walk and run (Table 160.1).

**Language Behavior**

Language is the best form of communication. It manifests with facial expression, phonation, vocalization of words, and formation of phrases or sentences. This needs coordinated functioning of speech apparatus with neural signals originating in speech areas. Speech is a **mixture of sensory and motor behavior.** It is greatly modified by social influences.

**Psychosocial Behavior**

Psychosocial development depends mainly on social and cultural environments and neurological maturation of the child. Emotional responses to environmental stimuli, control of bowel and bladder habits, proper sleeping, taking bath, ability to feed oneself and develop self-dependence are important milestones of psychosocial development.

**Adaptive Behavior**

Ability to adjust with different environmental situations includes not only psychosocial development, but also smoother sensory-motor coordination like hand-eye co-ordination that help to perform and protect in various adverse situations.

**Measurement of Psychological Development**

Mental and psychological developments are measured by developmental and intelligence quotients.

**Developmental Quotient**

Developmental quotient (DQ) is the **measure of developmental parameters with respect to age.** Development of
adaptive and psychological behaviors compared with age of the child.

$$DQ = \frac{\text{Maturity age}}{\text{Chronological age}} \times 100$$

**Intelligence Quotient**

Intelligence quotient (IQ) measures the intellectual abilities of a child, which requires some degree of maturity of the mind. Usually, such mental maturity starts growing after the age of five. Therefore, IQ can be tested only after this age, whereas DQ can be tested at any age.

$$IQ = \frac{\text{Mental age}}{\text{Chronological age}} \times 100$$

**DEVELOPMENTAL DEFICIENCIES**

Developmental deficiencies at childhood lead to dwarfism.

**Dwarfism**

Dwarfism is the short stature in comparison to the age of the individual. It can occur due to deficiency of growth hormone, growth hormone releasing hormone, IGF-1, growth hormone receptors, thyroid hormone and insulin.

**Pituitary Dwarf**

Deficient secretion of GH or GHRH early in life leads to pituitary dwarf. Mental development remains apparently normal, but reproductive functions are severely impaired.

**Thyroid Dwarf**

Deficiency of thyroid hormone at childhood causes cretinism. Mental development is grossly impaired though reproductive functions are apparently normal (for details, refer ‘Hypothyroidism’ in chapter 46).

**Laron Dwarfism**

This is also called growth hormone insensitivity. This occurs due to unresponsiveness of GH receptors to GH, due to mutation of GH receptor gene. The plasma level of GH is usually normal or may be elevated, and IGF-1 and IGF binding protein (IGFBP) are distinctly reduced. May be, deficiency of IGF-1 also has the same picture.

**Gonadal Dwarfism**

In gonadal dysgenesis as occurs in individuals with chromosomal pattern XO, dwarfism is a prominent feature.

**Metabolic Dwarfism**

Different metabolic and bone diseases produce dwarfism. Achondroplasia, an autosomal dominant condition, is the most common form of pathological dwarfism.

**Psychosocial Dwarfism**

When children are chronically ignored, mistreated and abused, they fail to develop and have short stature. This is also called Kasper Hauser syndrome.

**Constitutional Dwarfism**

Hereditary and constitutional dwarfsms are common forms of dwarfism.
Nutrition related diseases are common among all the diseases. Chronic malnutrition (both overnutrition and undernutrition) affects body systems. The proper nutrition provides strength while improper nutrition invites disease and death.

The **objectives of good nutrition** are to:
1. Restore and provide energy
2. Maintain and control physiological processes of the body
3. Promote growth and development
4. Repair the tissue damages
5. Prevent degeneration of the tissues
6. Minimize the impact of diseases on the body
7. Maintain immunity and fight infection.

**Dietary requirements:** In general, a standard diet for an adult should contain 50% carbohydrate, 20% fat, 20% protein and 10% fibers. With advancement of age, the fat and carbohydrate intake should be reduced and the fiber intake should be increased.
1. The total caloric intake mainly depends on the individual’s ability to use and store energy and on the level of physical activity. In general, men require more calories per kg of body weight than women. **Calorie is supplied by carbohydrate and fat.**
2. In addition, lipids are primary component of the cell membrane and intracellular signal transduction pathways (such as diacylglycerol and inositol triphosphate). Fats also enhance satiety, help in the absorption of vitamins from intestine, prevent tissue degeneration and protect against some malignancies.
3. Therefore, the present trend of low-fat intake to prevent arteriosclerosis-induced diseases and diabetes should not be vigorous to the extent of causing cell degeneration.
4. Though, the daily protein requirement in adult humans is approximately 0.8 g/kg body weight, it is more in growing children, in adults following major surgeries, athletes, during pregnancy and lactation, and during recovery from diseases.
5. Therefore, the diet should contain all essential amino acids and minimum proteins that are required for tissue maintenance and repair. Proteins also play key role in host defense mechanisms.
6. Vitamins and minerals are not energy sources, but are necessary for enzymatic reactions, growth and metabolisms.

**ESSENTIAL NUTRIENTS**

The essential nutrients in balanced diet are carbohydrates, proteins, fats, vitamins and minerals. Carbohydrates provide energy, proteins build the body and give strength, lipids provide energy and control growth, vitamins regulate growth and metabolism and minerals promote tissue repair and growth.
Carbohydrates

The carbohydrates are the major source of energy. Complete breakdown of 1 gm of carbohydrate yields 4 kcal. When carbohydrate is consumed in excess, obesity develops due to conversion of carbohydrate into fat in the body.

Types of Carbohydrates

There are three classes of carbohydrates: monosaccharides, disaccharides and polysaccharides.

Monosaccharides

The monosaccharides are the major carbohydrates. There are two types of monosaccharides: hexoses and pentoses. Hexoses contain six-carbon chain, and are the main monosaccharides. The examples are glucose, fructose and galactose. Pentoses contain five-carbon chain and examples are ribose and xylose.

Disaccharides

The disaccharides are sucrose (glucose plus fructose), lactose (glucose plus galactose) and maltose (glucose plus glucose). Sucrose is present in the sugar and lactose is present in milk. Deficiency of lactase causes lactose intolerance, in which the undigested lactose produces watery diarrhea. The condition manifests in early childhood.

Polysaccharides

The polysaccharides are starch and glycogen. There are many units of glucose arranged in a complex way.

1. Starch is present in plants and contains two types of fibers: crude fiber and dietary fiber. Fibers are usually indigestible. Crude fiber consists mainly of cellulose and forms the structural framework of plant cells.
2. Dietary fibers are soluble and insoluble types.
3. Soluble fibers bind with bile acids and reduce the absorption of cholesterol from intestine. Therefore, adequate fibers in diet help in decreasing fat content of the body and retard development of atherosclerosis. They also dissolve in water to form gel that slows the passage of digested food in large intestine, thus enhancing the rate of absorption.
4. Insoluble fibers contain cellulose, hemicellulose and lignins. They facilitate water absorption from intestine and aid to the volume of feces. Therefore, fibers enhance bowel emptying and are used in the treatment of constipation.

Glycogen is the polysaccharide of glucose found in animals. Its branching is less complex than starch. It has only storage property, whereas starch acts both as a store house and structural support in plants.

Sources and Functions of Carbohydrates

Major source of dietary carbohydrates is plants. Main carbohydrate is starch. They are also available as lactose in milk, and as glycogen in meat. The sources and functions of carbohydrates are summarized in Table 161.1.

Effects of Deficiency or Excess

Carbohydrate is the major source of calorie. The total requirement depends on the level of physical activity. In India, carbohydrates constitute about 80% of diet. Deficiency of carbohydrates results in deficiency of its metabolites that are needed for breakdown of fats into CO₂ and water. The increased fatty acids form ketone bodies and ketosis develops. Decreased dietary fiber content has been suggested to produce carcinoma of the colon and diverticulosis. Excess carbohydrate intake causes obesity.

Lipids

Types of Lipids

The main lipids in the diet are triglycerides. Some amount of cholesterol is also present. Triglyceride is the glycerol attached to three molecules of fatty acids. The different triglycerides differ in structure depending on the degree of saturation, the length of the carbon chain and the presence or absence of essential fatty acids.

Table 161.1: Sources and functions of carbohydrates.

<table>
<thead>
<tr>
<th>Carbohydrates</th>
<th>Sources</th>
<th>Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monosaccharides</td>
<td>Glucose: Sugar, rice, bread, fruits, vegetables, honey, etc.</td>
<td>Final form for tissue utilization</td>
</tr>
<tr>
<td></td>
<td>Fructose: Fruits and honey</td>
<td>Converted to glucose by the liver</td>
</tr>
<tr>
<td></td>
<td>Galactose: Milk lactose</td>
<td>Converted to glucose by liver</td>
</tr>
<tr>
<td>Disaccharides</td>
<td>Sucrose: Sugar cane, pineapple</td>
<td>Converted to glucose by the liver</td>
</tr>
<tr>
<td></td>
<td>Lactose: Milk</td>
<td>Converted to glucose by the liver</td>
</tr>
<tr>
<td></td>
<td>Maltose: Germinating seeds</td>
<td>Converted to glucose by the liver</td>
</tr>
<tr>
<td>Polysaccharides</td>
<td>Starch: Plants, rice, potato, wheat, cassava, corn</td>
<td>Converted to glucose by the liver</td>
</tr>
<tr>
<td>Fibers</td>
<td>Soluble fibers: Fruits, legume, grains</td>
<td>Increase the time of absorption of food and Decrease absorption of cholesterol from intestine</td>
</tr>
<tr>
<td></td>
<td>Insoluble fibers: Vegetables, wheat bran</td>
<td>Increase passage of intestinal content, prevent</td>
</tr>
</tbody>
</table>
Degree of Saturation
A fatty acid is saturated when every carbon atom in it has two hydrogen atoms attached. When in the carbon chain of fatty acids two adjacent carbon atoms are attached with a double bond as each C does not have a hydrogen atom to connect with, they are called mono-unsaturated fatty acids (MUFA). When two or more double bonds occur in a fatty acid, they are called polyunsaturated fatty acids (PUFA). Fatty acids containing five or six double bonds are said to be highly unsaturated fatty acids (HUFAs). PUFA is better than MUFA as MUFA may not retard atherosclerosis and hence it decreases membrane plasticity.

Length of the Carbon Chain
Fatty acids in biological systems usually contain an even number of carbon atoms, commonly between fourteen and twenty-four. Fatty acids with twelve carbon atoms in the chain or less are described as short chain fatty acids, and those with fourteen or more are termed as long chain fatty acids.

Presence of Essential Fatty Acids
Essential fatty acids are the highly unsaturated. They are linoleic acid, linolenic acid and arachidonic acid. They are essential for the synthesis of eicosanoids like thromboxane, leukotrienes, prostacyclin and prostaglandins. There are involved in various physiological processes of the body like conception, induction of labor, abortion, immunity, inflammation and maintenance of vascular tone. As arachidonic acid can be synthesized in the body from linoleic and linolenic acids, it is a semi-essential fatty acid.

Cholesterol
Cholesterol is a sterol that forms steroid hormones and vitamin D. Cholesterol is a component of cell membranes. It is formed in the liver from acetyl-CoA.

Functions of Dietary Fats
1. Lipids provide energy. Breakdown of 1 gm of fat yields 9.0 calories, whereas carbohydrates and proteins provide only 4.0 calories each.
2. Lipids are the essential component of cell membrane. They maintain membrane fluidity and integrity.
3. Vitamins A, D, E, and K are soluble in fat. Thus, fat transports these vitamins for their absorption from intestine.
4. Palatability of food depends on quantity and quality of fat in it.
5. Gastric emptying greatly depends on the fat content of food eaten. More the fat, slower is the emptying.
6. Essential fatty acids are essential for formation of many eicosanoid compounds like prostaglandins, leukotrienes etc.
7. Cholesterol is the primary source of steroid hormones.
8. Subcutaneous fat acts as an insulator that plays important role in temperature regulation. It prevents transfer of heat between the body and the environment.
10. During starvation, fatty acids converted to ketone bodies supply energy. Thus, more the fat content, better is the ability to sustain food-deprivation.
11. Exert a cushioning effect and protect the internal organs.

Daily Requirements and Sources
Dietary fat should provide 25–35% calorie, and adequate essential fatty acids (especially linoleic acid). Average Indian diet contains approximately 25% fat. Americans eat 40–45% and Africans consume about 10% fat in diet.

Diseases Associated with Excess Fat
Consumption of excess fat leads to obesity. Acceleration of atherosclerosis, coronary heart disease (CHD), stroke, breast and prostate cancer, hypertension and diabetes are common obesity associated disorders.
1. Serum cholesterol levels below 210 mg/dL is considered normal. 211–240 mg/dL is high normal and above this is abnormal. Though, only about 10 per cent of the total plasma cholesterol is derived from diet, rest comes from liver. Excess carbohydrate is converted to fat in the body.
2. High LDL, VLDL and TG are harmful, whereas high HDL is good for health. High HDL protects from heart disease and retards the process of atherosclerosis.
3. Regular physical activities, practice of yoga, cheerful mind and consumption of fish and vegetables in diet increase HDL and decrease VLDL.
4. Mild to moderate alcohol consumption, especially red wine has been proved to be effective in increasing plasma HDL and reducing the incidence of coronary artery disease.
5. Decreased calorie intake, decreased intake of saturated fat and increased consumption of PUFA is helpful in this regard.

### Proteins

#### Structure of Proteins

Proteins are made up of amino acids. Usually, proteins contain about 16% nitrogen. Therefore, the food protein content, or the amount of protein loss in urine can be derived from estimating the nitrogen content in food or in urine. This is done by multiplying the nitrogen content with factor 6.25, i.e. 16 in 100 parts. The proteins (amino acid chains) have two ends, the carboxy terminal that attaches a carboxyl group (COOH), and amino terminal that attaches an amino group (NH₂), a hydrogen atom, and a group or radical (indicated by R) which is different for various amino acids.

#### Classification of Amino Acids

There are twenty amino acids that form different proteins. Some are essential and some are nonessential amino acids (Table 161.2).

#### Functions of Proteins

Proteins are building blocks of the body. They help in formation of and maintenance of tissues. They are integral part of cell membrane. The major functions can be listed as follows.

1. **Growth and development**: Proteins are essential components of cell membrane and membrane of intracellular organelles. Especially, they are major parts in muscles and bones. They are necessary for the maintenance and repair of tissues.
2. **Synthetic functions**: Proteins are needed for formation of peptide hormones, hemoglobin and clotting factors. All enzymes are proteins.
3. **Maintenance of pH**: The plasma proteins and hemoglobin are important buffers.
4. **Transport functions**: Lipids and carbohydrates are transported as lipoproteins and glycoproteins. Many hormones and vitamins are present in plasma bound to proteins, for example thyroxine binding globulin for thyroid hormones, transcortin for cortisol and retinol binding protein for vitamin A.
5. **Immunity functions**: Antibodies are immunoglobulins. They are primary elements of humoral immunity.
6. **Maintenance of osmotic pressure and Filtration in Capillaries**: The oncotic pressure (osmotic pressure of plasma proteins) contributes considerably to filtration of fluid across the capillary wall.
7. **Storage**: Proteins act as storage source for various substances. For example, iron is stored in ferritin, a storage protein.
8. **Receptors**: Proteins act as receptors on various cells and tissues of the body.
9. **Buffer**: The albumin act as an excellent buffer in plasma due to its high histidine content which has its pK value nearer to plasma pH.
10. **Coagulation**: The prothrombin and fibrinogen molecules when activated help in blood coagulation.
11. **Enzymes**: The enzymes in our body are structurally proteins. These help in normal functioning of the system.

#### Daily Requirement and Sources

The minimum daily intake of protein should be 1 g/kg body weight. The requirement is more in infants, children and adolescents and during pregnancy, lactation and recovery from diseases.

Protein is derived from both animals and plants. The main animal sources are meat, eggs, milk, poultry products and fish. Major plant sources are nuts, beans, peas, cereals (maize and wheat) and dal. In general, animal proteins contain all the essential amino acids and therefore are called grade I proteins. Plant proteins have limited amounts essential amino acids, and are referred to as grade II proteins. Fish protein has been found to be antiatherosclerotic. Protein deficiency occurs in kwashiorkor and marasmus, the two types of malnutrition.

#### VITAMINS

Nomenclature for ‘Vitamins’ was coined by Casimir Funk in 1912 for their ‘vital’ roles in the body. Though vitamins are not energy sources they play important role in metabolism. They act as cofactors in many enzymatic reactions such as decarboxylation, carboxylation, etc. They act as oxidizing and reducing agents in oxido-reductive processes. Vitamins decrease the risk of various cancers.

### Table 161.2: Essential and non-essential amino acids.

<table>
<thead>
<tr>
<th>Essential</th>
<th>Non-Essential</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>Aspartic acid</td>
</tr>
<tr>
<td>Leucine</td>
<td>Proline</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>Alanine</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>Glycine</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>Glutamic acid</td>
</tr>
<tr>
<td>Methionine</td>
<td>Serine</td>
</tr>
<tr>
<td>Valine</td>
<td>Tyrosine</td>
</tr>
<tr>
<td>Threonine</td>
<td>Cysteine</td>
</tr>
<tr>
<td>Histidine</td>
<td>Asparagine</td>
</tr>
<tr>
<td>Arginine</td>
<td>Glutamine</td>
</tr>
</tbody>
</table>

1. Growth and development: Proteins are essential components of cell membrane and membrane of intracellular organelles. Especially, they are major parts in muscles and bones. They are necessary for the maintenance and repair of tissues.
Antioxidant vitamins are β-carotene, vitamins C and E. Adequate vitamin E intake lowers the risk for coronary artery disease.

**Classification**: Vitamins are broadly classified into two types: water-soluble and fat-soluble (Table 161.3).

The clinical presentation of vitamin deficiency is related to the corresponding function of the vitamin. However, in generalized malnutrition, multiple deficiencies can result leading to complex clinical presentation. It should be noted that the functions of vitamins are mostly intracellular. Hence, their plasma concentration does not necessarily reflect the intracellular concentration and hence their functional availability. On the other hand in deficiency state the decreased plasma level is an earlier indicator than tissue levels.

Vitamin deficiency can arise due to:
1. Inadequate intake (with normal requirement)
2. Impaired absorption
3. Impaired metabolism
4. Increased requirement (as in adolescence, pregnancy, lactation)
5. Increased loss

**Vitamin A**

Vitamin A functions in the body in three forms: retinol (alcohol form), retinal (aldehydes form) and retinoic acid (acid form).

**Sources of Vitamin A**

Animal sources: Rich sources are liver, egg yolk, fish, fish liver oils (cod liver oil, halibut liver oil) and cream of milk.

<table>
<thead>
<tr>
<th>A. Water-soluble vitamins</th>
<th>B. Fat-soluble Vitamins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiamine (Vitamin B&lt;sub&gt;1&lt;/sub&gt;, aneurine, anti-beriberi factor)</td>
<td>Vitamin A (Retinol, retinol, retinoic acid)</td>
</tr>
<tr>
<td>Riboflavin (Vitamin B&lt;sub&gt;2&lt;/sub&gt;, lactoflavin, hepatoflavin)</td>
<td>Vitamin D (Antirachitic factor, cholecalciferol, ergocalciferol, calcitriol)</td>
</tr>
<tr>
<td>Niacin (Vitamin B&lt;sub&gt;3&lt;/sub&gt;, nicotinic acid, nicotinamide)</td>
<td>Vitamin E (Tocopherol, antisterility factor)</td>
</tr>
<tr>
<td>Pantothenic acid (Vitamin B&lt;sub&gt;5&lt;/sub&gt;, pantothenol, pantotheine)</td>
<td>Vitamin K (Antihemorrhagic factor)</td>
</tr>
<tr>
<td>Pyridoxine (Vitamin B&lt;sub&gt;6&lt;/sub&gt;, pyridoxal, pyridoxamine)</td>
<td>Cobalmin (Vitamin B&lt;sub&gt;12&lt;/sub&gt;, cyanocobalamin, anti-pernicious anemia factor)</td>
</tr>
<tr>
<td>Folic acid (folacin, pteroylglutamic acid)</td>
<td>Biotin (Vitamin H, anti-egg white injury factor)</td>
</tr>
<tr>
<td>Ascorbic acid (Vitamin C, antiscorbutic factor)</td>
<td></td>
</tr>
</tbody>
</table>

by their antioxidant property. Antioxidant vitamins are β-carotene, vitamins C and E. Adequate vitamin E intake lowers the risk for coronary artery disease.

**Plant sources**: Main plant sources are carrots, spinach, tomatoes, broccoli, green peas, mangoes, ripe papaya and sweet potato. The main plant source of vitamin A is carotenoid, which is the precursor of this vitamin.

**Daily Requirement**

Daily requirement of vitamin A in adults is 750–900 mg of retinol. In lactating mothers, requirement is 50 per cent more. 1 IU is 0.3 µg of retinol and 0.6 µg of β-carotene. Adult male and female require 3000 IU per day.

**Functions of Vitamin A**

Vitamin A plays an essential role in vision, epithelial cell function, bone formation, reproduction and prevention of stress.

**Role in Vision**

Retinal is required in the formation of rhodopsin and for the phototransduction mechanism. Rhodopsin is the pigment found in rods and is essential for dim-light vision. Retinol is oxidized to retinal, which combines with opsin to form rhodopsin. Vitamin A is also part of the opsins in the cones. When retinol is low, the regeneration of retinal is less, and this impairs the dim light vision and dark adaptation. The condition is known as night blindness, which is an early sign of vitamin A deficiency.

**Epithelial Cell Function**

It is required for normal synthesis of mucopolysaccharide and growth of epithelial tissue. Deficiency of vitamin A causes keratinization of epithelial cells. The cells lose their cilia and fail to secrete mucus. This suggests that Vitamin A maintains the integrity of epithelium.

**On Bone and Teeth Formation**

Vitamin A deficiency prevents bone growth. Remodeling of bone and osteoblastic activity are impaired. Enlargement of skull is restricted, which in infants and children fails to accommodate the growing brain.

**On Growth**

Experimentally it has been seen that diets deficient in vitamin A produce growth failure. It promotes cell division and differentiation.

**On Reproduction**

Vitamin A deficiency impairs spermatogenesis. In females, abortion occurs in severe vitamin A deficiency. Failure to conceive is common in vitamin A deficiency.

**Prevention of Stress Dysfunctions**

Beta carotene, the precursor of vitamin A prevents the impact of stress on body. During stress, free oxygen radicals are produced in large quantity. Beta carotene and vitamin A trap and neutralize free radicals. Thus, they act as antioxidants (Application Box 161.1).
On Immunity

Vitamin A has recently been observed to promote cellular and humoral immunity.

Application Box 161.1

**β-carotene is anticancer:** β-carotene is a potent antioxidant. One of the mechanisms of cancer is the oxidative damage of tissues. By preventing the effects of oxidants, vitamin A prevents malignancy. It also inhibits expression of receptors on cancer cells.

Vitamin A Dysfunctions

**Vitamin A Deficiency**

The early and common feature of vitamin A deficiency is **night blindness**, also called **nyctalopia**. In this disease there is impairment in dark adaptation and failure to see in dim light. Bitot’s spots appear on the conjunctiva and conjunctiva becomes dry and rough (xerosis of conjunctiva or **xerophthalmia**). The cornea becomes dry and opaque and finally degenerates (**keratomalacia**).

**Vitamin A Toxicity**

Excess of vitamin A causes headache, drowsiness, nausea, dry skin, hair loss, and diarrhea. In infants, loss of appetite, weight loss, scaly dermatitis and bone pain and in women, menstrual irregularities occur.

Vitamin D

The commonly available form of vitamin D are D₂ and D₃ otherwise known as **cholecalciferol**. Vitamin D is formed from its two precursors: provitamin D₃ or **ergosterol** found in plants and provitamin D₃ or 7-dehydrocholesterol found in the skin. The biologically active form of vitamin D is calcitriol (1,25-dihydroxycholecalciferol) formed first in liver and then finally in kidney (for details, refer “Calcium Metabolism”).

**Sources and Daily Requirement**

Cholecalciferol is richly available in fish liver oil. Egg yolk, margarine, lard, butter and cheese also contain good amount of the vitamin. 7-dehydrocholesterol is formed from cholesterol in the intestinal mucosa and then is transferred to skin where it gets activated by UV rays.

The daily requirement is 100 IU in adults (1 IU = 2.5 µg vitamin D₃). Infants and children, and pregnant and lactating mothers need about 220 IU per day. Breast milk contains relatively low amount of vitamin D and hence premature infants (this vitamin is transported across placenta mainly in the last trimester of pregnancy) are at risk of vitamin D deficiency.

**Functions of Vitamin D**

Vitamin D plays an important role in calcium metabolism (for details, refer “Regulation of Calcium Metabolism”).

Vitamin D Dysfunctions

**Deficiency of Vitamin D:** Deficiency of vitamin D produces rickets in children and osteomalacia in adults (for details, refer the chapter “Regulation of Calcium Metabolism”). **Hypervitaminosis D:** Excess of vitamin D in the body produces anorexia, lassitude, constipation and polyuria. In chronic hypervitaminosis, **urinary lithiasis** and **metastatic calcification** occurs. Metastatic calcification is deposition of excess calcium in kidney, lung, muscle, blood vessels and gastric mucosa.

Vitamin E

Vitamin E is known as **tocopherol**. There are four different types of tocopherols: α, β, γ, and δ. The δ tocopherol is the most active form.

**Sources of Vitamin E**

Vegetable oils, such as soybean, corn, coconut, peanut, and cottonseed oil are the main dietary sources. Eggs and fruits and green leafy vegetables also contain vitamin E. The recommended daily intake of tocopherol is about 10 mg in men and 8 mg in women.

- For children: 10 – 15 IU per day
- For adults: 20 – 25 IU per day
1 mg of α tocopherol is 1.49 IU of vitamin E.

**Functions of Vitamin E**

Vitamin E is the **most potent antioxidant**. Particularly in cell membranes it protects the unsaturated fatty acids against free radical attack. It also traps and inactivates free radicals produced in the body. Therefore, it prevents degeneration of the body. It also maintains integrity of red cell membrane.

**Effects of Deficiency**

Vitamin E deficiency leads to hemolysis. In infants, retinal damage and retrolental fibroplasia occur. Decreased immunity, degeneration and increased vulnerability to malignancies are common.

Vitamin K

Vitamin K is a group of substances known as **quinones**. It was discovered and named by Dam, a Danish scientist. He described its role in blood coagulation (in Danish coagulation is written as 'koagulation'; hence the vitamin is named as ‘K’)

**Source and Daily Requirement**

Vitamin K is present **both in plants and animals**. In plants it is present as phylloquinone in association with chlorophyll in dark green leafy vegetables. In animals, it is present in menaquinones in milk, eggs and meat. It is also formed
by bacteria in the intestine. Especially, Lactobacillus bifidus synthesizes this vitamin. The synthesis is hindered by antibiotics. The total daily dietary intake is estimated at 200–500 mg. It is not stored in the body in appreciable quantity.

Functions
Vitamin K helps in physiological activity of four clotting factors: Factor II, VII, IX, and X by γ carboxylation of their glutamate residue. These are called vitamin K dependent clotting factors. Therefore, deficiency of vitamin K causes bleeding disorder.

Vitamin C
Vitamin C is ascorbic acid. It was initially isolated from lemon juice, oranges and cabbage. It was detected for its deficiency effects that caused death from scurvy. Therefore it is also called antiscorbutic vitamin.

Structure
Naturally occurring vitamin is L-ascorbic acid. It is a six-carbon molecule that closely resembles glucose. In lower mammals, it is formed from glucose via uronic acid pathway. In human beings, monkey and guinea pigs the key enzymes for conversion of ketogulonolactone to ascorbic acid is absent. Therefore, its supply in diet is essential to prevent its deficiency. It is absorbed in the jejunum and upper ileum by simple diffusion or by co-transport with sodium.

Dietary Sources and Daily Requirements
The major sources of vitamin C are citrus fruits and vegetables. Guava, amla and lemon are richest source of ascorbic acid. Tomato, green chilly, green leafy vegetables, plantains, nomads, milk and bananas contain considerable amount of the vitamin.

A daily intake of about 100 mg is considered adequate to prevent scurvy.

- Infants : 30 mg per day
- Children : 50 mg per day
- Adolescence : 75 mg per day
- Adults : 100 mg per day
- Pregnant and Lactating women : 150 mg per day

Functions
Ascorbic acid plays important role in many metabolic and protective activities:

1. It hastens oxidation-reduction reactions. It is sensitive to reversible oxidation. It serves as H⁺ transport agent.
2. It plays a role in collagen synthesis. In collagen synthesis, it facilitates hydroxylation of proline and lysine in collagen. As collagen is the vital protein in all connective tissues that binds cells together, deficiency of vitamin C causes hemorrhages in areas exposed to mechanical stress. Collagen forms bone matrix, and is an important component of cartilage, teeth, and scar tissue. Therefore, these structures suffer heavily in scurvy. The gums become spongy and bleed easily. The bones become weak and deformed. The formation of enamel in teeth is impaired. Healing of wounds is delayed.
3. In the CNS, synthesis of two neurotransmitters depends on vitamin C. These are conversion of tyrosine to noradrenaline, and tryptophan to serotonin.
4. Activity of fibroblast and osteoblasts requires vitamin C. Therefore, bone formation and growth of fibrous tissue is seriously impaired in vitamin C deficiency.
5. It acts as an antioxidant due to its reducing property. It converts ferric iron to ferrous iron in the stomach. This prevents the ferrous oxidation. It also facilitates absorption of iron from GI tract and its transport in blood.
6. It also converts folic acid to the folinic acid, the active form in which folic acid is absorbed. It stimulates absorption of calcium.
7. It promotes formation of ferritin in the tissue.
8. It helps in synthesis of carnitine, which transports fatty acids into cells. It also activates degradation of cholesterol to bile acids and the detoxification of drugs in the liver cells. Therefore, vitamin C prevents hypercholesterolemia.
9. Vitamin C in high dose acts as an antioxidant. In high doses it cures common cold.
10. It acts as a coenzyme for cathepsin and hepatic esterases.

Deficiency of Vitamin C
Vitamin C deficiency results in scurvy. The main defect in scurvy is failure to deposit intercellular cement substance. Therefore, capillaries become fragile. Petechial, subcutaneous, subperiosteal and internal hemorrhages are common. Gums are swollen, spongy and red and bleed with little pressure. Due to poor osteoid activity, bones become weak and readily fracture. Hypochromic microcytic anemia occurs due to iron deficiency. Wound healing is delayed.

Thiamine (Vitamin B₁)
This vitamin is known since early 19th century for its role in prevention of beriberi. Therefore, it is commonly called antiberiberi factor.

Sources and Daily Requirements
It is present in both plant and animal foods. Richest plant source is outer germ or bran layer of rice. Other sources are peas, beans, whole cereal grains, bran, nuts, etc. Animal source is liver, meat egg and milk.
Daily requirement is 1 mg for adults, 0.3 mg for infants and 1.3 mg for adolescent, 1.4 mg in pregnancy and 1.5 mg during lactation.

**Functions of Thiamine**
Thiamine plays crucial role in carbohydrate metabolism. Therefore, persons eating more carbohydrates require more thiamine. Thiamine acts as a coenzyme for decarboxylation reactions. As thiamine pyrophosphate, it acts as prosthetic group for many enzymes such as pyruvate dehydrogenase, α-ketoglutarate dehydrogenase and transketolase.

1. **Oxidative decarboxylation:** Pyruvate dehydrogenase catalyzes the decarboxylation of pyruvic acid to acetyl-coA. Also, α-ketoglutarate dehydrogenase catalyses the oxidative decarboxylation of α-ketoglutaric acid to succinyl-coA.

2. **Transketolation:** Transketolase acts in the pentose phosphate pathway to transfer two-carbon units from one sugar unit to another.

**Thiamine Deficiency (Beriberi)**
The deficiency of thiamine results in beriberi. It manifests in two major forms: dry beriberi (without edema) and wet beriberi (with edema). **Wet beriberi predominantly affects cardiovascular system** that usually manifests as tachycardia, wide pulse pressure and cardiomegaly. **Dry beriberi predominantly affects nervous system** and produces Wernicke’s encephalopathy and Korsakoff’s psychosis. Thiamine deficiency may manifest in infants whose mothers are deficient in the vitamin.

**Wet Beriberi:** Thiamine deficiency leads to accumulations of pyruvic acid and α-ketoglutarate as they are not metabolized, especially after a carbohydrate meal. They are metabolic vasodilators. They produce widespread vasodilation, and cause cardiac failure. Both these factors facilitate the genesis of edema, for which the condition is called wet beriberi.

**Dry Beriberi:** Beriberi commonly affects neuronal tissues as neurons primarily derive energy from glucose metabolism. Therefore, common presentation of beriberi is neurological symptoms. Demyelination of both sensory and motor fibers cause peripheral neuropathy. This may cause total anesthesis, or paresthesia. Motor feature are paralysis of extremity muscles leading to foot drop and wrist drop. This is called ‘dry beriberi’ as edema is absent.

Chronic alcoholics develop thiamine deficiency in the brain due to a poor diet and that manifests as Wernicke’s encephalopathy and Korsakoff’s psychosis. In **Wernicke’s encephalopathy**, symmetrical paralysis of extrinsic muscles occur that cause symmetrical ophthalmoplegia. Nystagmus and ataxia are common. In **Korsakoff’s psychosis**, memory is grossly impaired. The patient cannot remember even the recent events. **Wernicke’s encephalopathy** responds rapidly to thiamine supplementation.

**Infantile Beriberi:** Infantile beriberi occurs due to deficiency of thiamine in maternal breast milk. In acute form, infant develops heart failure and breathlessness, and may die if remains untreated. In chronic form, neurological features develop and the characteristic feature in infants is aphonia, in which no sound is heard when baby cries. Diarrhea, vomiting and convulsions are common.

**Riboflavin (Vitamin B₂)**
Riboflavin consists of ribose and flavin molecules. It is the precursor of the coenzyme FAD (flavin adenine dinucleotide and FMN (flavin adenine mononucleotide). These coenzymes are part of flavoproteins.

**Sources and Daily Requirements**
Riboflavin is present in both plants and animal products. Rich sources are whole grains, dry beans, peas, germinating seeds, milk, meat, fish, eggs and green vegetables. Daily requirement in adult is about 1.5 mg.

**Function of Riboflavin**
Riboflavin helps in oxidative phosphorylation. During oxidative phosphorylation, H⁺ is released during the flavoprotein–cytochrome respiratory chain activity to combine with oxygen and form water. It facilitates conversion of vitamin B₂ and folic acid to their coenzymes that are required for DNA synthesis. Therefore, riboflavin influences cell division and growth.

**Effect of Deficiency**
Riboflavin deficiency causes angular stomatitis, cheilosis and cracks on the lips (lips are red, sore and dry) and glossitis. It also causes seborrheic dermatitis.

**Niacin (Nicotinic Acid)**
Niacin was discovered in 1937 by Elvehjem, as pellagra preventing factor. Presently, pellagra is still found in corn-eating countries like Romania, Yugoslavia and Egypt, as niacin in corn or maize binds to protein and its free form is not available.

**Sources and Daily Requirements**
Niacin is present in both animal and plant food. Liver, kidney, red meat, fish, legumes, groundnuts, and cereals (bran and germ), and green vegetables are rich sources. **Eggs are rich in tryptophan that is converted to niacin in the body.** In adults, its daily requirement as niacin is 17–20 mg. Part of this requirement is met by endogenous synthesis from tryptophan.

**Functions of Niacin**
Niacin is part of the coenzymes NAD (nicotinamide adenine dinucleotide) NADP (NAD-phosphate). NAD is required in
the catabolism of carbohydrates, fats, proteins and alcohol that releases energy. NADP acts as cofactor for enzymes dehydrogenase that removes hydrogen during the synthesis of fat. In high dose, it prevents hyperlipidemia.

**Effects of Deficiency**

Deficiency of niacin causes **pellagra**. The major features of pellagra are: dermatitis (erythematous skin rash leading to desquamation), diarrhea, dementia (loss of memory). These three features are conventionally called ‘three Ds’. The affected skin is dry, hyperpigmented, scaly, and cracked.

**Pyridoxine (Vitamin $B_6$)**

Human beings cannot synthesize vitamin B6; therefore, they need dietary supplementation. It is unique among water-soluble vitamins in that it is stored in large quantities in muscles.

### Dietary Sources

Pyridoxine is present in both plants and animal foods. Yeast, rice polishing, germinal portion of seeds, whole grain cereals, bananas, potatoes, egg-yolk, liver, kidney muscle and fish are rich sources. The recommended daily intake of pyridoxine is about 2.0mg.

### Functions of Vitamin $B_6$

Pyridoxal phosphat e is an essential coenzyme for many biological reactions.

1. **It acts as a cotransaminase**: In protein metabolism, during transamination, deamination and decarboxylation reactions, as in the formation of serotonin from tryptophan and noradrenaline from tyrosine it acts as a coenzyme. It also acts as a **coenzyme in the synthesis of mRNA** and formation of heme of hemoglobin.
2. It serves as a coenzyme for the conversion of muscle and liver glycogen into glycogen phosphate.
3. It takes part in transulfuration reaction that involves transfer of $\text{--SH}$ group as occurs during formation of homoserine from homocysteine.
4. It acts as a **coenzyme for kynureninase**, which converts 3-OH-kynurenine to 3-OH-anthranilic acid that in turn forms nicotinic acid. Therefore, in pyridoxine deficiency niacin synthesis from tryptophan does not occur. In such condition, level of kynurenine and 3-OH-kynurenine increase in blood and are converted into xanthurenic acid, which is excreted in urine. Thus, increased level of xanthurenic acid in urine is an index of pyridoxine deficiency.
5. In lipid metabolism, it serves as a coenzyme for the conversion of linoleic acid into arachidonic acid. It also acts as factor during synthesis of cholesterol.
6. It acts as **coenzyme for the synthesis of various neurotransmitters** like norepinephrine, dopamine, histamine, etc.

**Deficiency of Pyridoxine**

In infants, deficiency causes hyperirritability and convulsions. In adults, deficiency causes hypochromic anemia, weakness, nervousness, irritability and insomnia (Clinical Box 161.1).

**Clinical Box 161.1**

INH therapy requires high dose of niacin: Tryptophan is converted into niacin in the body, which requires the presence of three other vitamins, namely, thiamine, pyridoxine, and riboflavin. Pyridoxine is important in this action. Isoniazid, an antitubercular antagonizes the action of pyridoxine and therefore, precipitates deficiency of niacin. Therefore, in the treatment of tuberculosis with INH, vitamin B complex tablets are routinely prescribed to prevent the development of pellagra.

**Pantothenic Acid**

Pantothenic acid is derived from β-alanine and pantoic acid. The active form of pantothenic acid is coenzyme A. It occurs mostly as acetyl co-A in the body.

### Functions

Acetyl CoA is a common substrate for release of energy from carbohydrates, fats and proteins. It has many other functions.

1. It combines with oxaloacetate to form citric acid that initiates TCA cycle.
2. It is used for acetylcholine formation.
3. It is used for cholesterol synthesis.
4. It is used for ketone body formation.
5. Along with malonyl CoA, it is utilized for synthesis and elongation of fatty acids.

### Sources and Daily Requirements

Pantothenic acid is present in all types of foods. However, richest sources include kidney, liver, meat, fish, chicken, whole grain cereals and skimmed milk. Daily requirement of pantothenic is:

- **In adults**: 5 to 12 mg
- **In children**: 4 to 5 mg
- **In infants**: 1 to 2 mg

### Effects of Deficiency

As it is present in all food stuffs, its deficiency is usually not reported. However, deficiency occurs along with malnutrition.

**Folic Acid**

Chemically, folic acid is pteroyl glutamic acid. It is present in all green leafy vegetables, liver, meat, fish and kidney. Daily requirement is about 400 µg. The active form of vitamin is tetrahydrofolate. Folate is first acted upon by vitamin C to form dihydrofolate which in turn forms tetrahydrofolate. During these procedures NADP is formed. Folinic acid, one of the active form of folic acid
causes maturation of red cells by facilitating DNA synthesis. Deficiency of folic acid leads to megaloblastic anemia (for details, refer “Erythropoiesis”). FIGLU (formimino-glutamic acid) excretion in urine is an index of folic acid deficiency. Folic acid is vital for purine and pyrimidine (and hence nucleic acid) synthesis. Folate supplement during pregnancy reduces the risk of neural tube defect in newborn.

**Vitamin B<sub>12</sub>**

Vitamin B<sub>12</sub> is also called cyanocobalamine or extrinsic factor of Castle. Normal serum level of the vitamin is 0.008 to 0.42 µg/dl. It is present mainly in animal food, especially in liver and meat. Its main function is to bring maturation of red cells (for details, refer “Erythropoiesis”). Dietary deficiency of this vitamin is rare (except in strict vegetarians: vegans) as considerable amount is stored in liver. Deficiency of vitamin B<sub>12</sub> leads to megaloblastic anemia. Intrinsic factor from stomach helps in absorption of Vitamin B<sub>12</sub>. Therefore, gastric atrophy, chronic gastritis and gastrectomy cause pernicious anemia, which is special type of megaloblastic anemia that occurs due to stomach diseases. Methyl malonic aciduria is sensitive index of Vitamin B<sub>12</sub> deficiency.

**MINERALS AND ELECTROLYTES**

Minerals are also nutrients. More than twenty minerals have been considered as essential nutrients. In general, animal foods are a better source of minerals than plant foods. In cereals and legumes, the minerals are concentrated in the outermost layers. Therefore, refining of cereals leads to considerable loss of minerals. The minerals that are important in body functions are calcium, phosphorus, iron, iodine, zinc, copper and selenium. Deficiency of minerals occurs due to the same general reasons as vitamin deficiency.

**Calcium and Phosphorus**

Calcium is adequately present in milk and milk products. Phosphorous is present in high concentration in protein-rich food. Calcium is essential for formation of bones and teeth, blood clotting, neuromuscular excitability and activation of several enzymes in the body. ATP, ADP and AMP contain phosphorus. Phosphate groups also form part of DNA and RNA molecules. Phosphorylation of enzymes is essential for action of many hormones. (for details of calcium and phosphorous, refer Chapter 61).

**Iron**

An adult has about 4 g irons, two thirds of which exists in the form of hemoglobin and the remaining is present in myoglobin, cytochromes, transferrin, ferritin, and storage iron as hemosiderin.

**Sources**

Iron is present in meat, liver, fish, green leafy vegetables, potatoes, legumes and fruits. Milk is a poor source of iron. Cooking in iron utensils contributes to significant amounts of iron in the diet.

**Functions**

Iron is an important component of hemoglobin and myoglobin. Iron is essential in oxidation-reduction reactions. It is present in cytochromes and acts as an electron carrier. It is also involved in antibody production and synthesis of purines that are a components of DNA and RNA.

**Requirements**

The ICMR recommendation in adult man is 28 mg iron per day, in non-pregnant woman 30 mg per day, and in pregnant woman 38 mg per day. In India, chronic intestinal blood loss due to hookworm infestation demands more iron intake.

**Deficiency**

The commonest deficiency among minerals is that of iron which is common even in affluent society, particularly in women in their reproductive years. Iron deficiency leads to a microcytic hypochromic anemia.

**Iodine**

The iodine content of water and food greatly depends on the iodine content of the soil in the region. In general, in hilly areas there is iodine-deficient soil. Therefore, iodized-salt is prescribed for individuals in hilly areas. Daily intake of 10 g iodized salt supplies 150 micrograms of iodine. Iodine is essential for synthesis of thyroid hormones. Daily requirement of iodine is 1 µg/kg body weight. Iodine deficiency leads to hypothyroidism, which manifests as thyroid swellings, known as goiter (For details of iodine metabolism, refer Chapter 57).

**Zinc**

An adult contains about 2g of zinc, of which about 75% is present in bones. Skin, hairs and testes contain considerable amount of zinc. Circulating zinc is concentrated in red cells.

**Sources**

Flesh foods and sea foods are good sources of zinc. Though cereals and legumes contain good amount of zinc, zinc absorption from these sources is limited by presence of phytic acid.

**Functions**

Zinc is part of metallo-enzymes that include carbonic anhydrase, carboxypeptidase, alkaline phosphatase, DNA polymerase and RNA polymerase, etc. It is also a cofactor
in the synthesis of collagen. Zinc is essential for normal growth in children, reproductive function, wound healing, and for sense of taste and smell.

**Daily Requirements**

For adults, daily requirement is 15 mg. The requirement is more in growing children, pregnant woman and lactating mother.

**Deficiency**

Zinc deficiency is characterized by poor growth and sexual development. Deficiency also causes poor wound healing, loss of appetite, diminished taste and smell sensations and several skin disorders. Zinc deficiency is precipitated by iron supplements as both of them share common intestinal transport mechanisms. In acrodermatitis enteropathica there is inherited defect of intestinal zinc absorption.

---

**Selenium**

Selenium is required in very small amounts. Its content in diet depends on the selenium content of soil.

**Sources**

If the soil has adequate selenium, food will have adequate quantity of it. Selenium is present in both plant and animal foods. Refining of grains and boiling of vegetables reduce selenium content in food.

**Functions**

Selenium is an important component of the antioxidant system of the body. It is present in glutathione peroxidase, the enzyme that prevents lipid peroxidation.

**Requirements**

The daily requirement is 1 µg/kg body weight.

**Deficiency**

Deficiency of selenium is usually seen in low intake and due to long term parenteral nutrition. Selenium deficiency may possibly predispose to some cancers. Selenium deficiency may also cause Keshan disease, in which cardiac muscle degeneration is an important feature.

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**RECOMMENDED DIETARY ALLOWANCES**

The recommended dietary allowance (RDA) of a nutrient is the quantity, which meets the known nutritional needs of a normal healthy population. RDAs depend on age, gender, body weight, climate, physiological states especially in women (pregnancy, lactation etc), and physical activity of the individual (Table 161.4). Therefore, RDA varies from regions to regions and countries to countries. Various illnesses affect the RDA. RDA helps in framing balanced diet for a population.

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**Balanced Diet**

A balanced diet is a satisfactory diet that provides adequate quantities of all essential nutrients. It depends mainly on the age, sex, body weight and level of physical activity of the individual. It should provide energy intake, and desirable minimum protein intake on the basis of RDA. For example, for a 50 kg moderately active woman, the diet should provide 2225 kcal and at least 50 g protein everyday. As 50g protein provides 200 kcal; the remaining 2025 kcal should be obtained from carbohydrates and fats. The diet should also provide at least the RDA of vitamins and minerals (Table 161.4).

This can generally be achieved by:

1. A mixture of cereals and pulses as the major source of energy. This ensures adequate calorie and protein. If the grains are not highly refined, it also ensures adequate fiber intake.
2. Vegetables and fruits should be about 400g per day per person. This ensures adequate vitamin and mineral intake.
3. Some milk and milk products should be included in the diet.
4. For children and pregnant women, some nonvegetarian food like fish, egg or meat should be included.

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**DISORDERS OF NUTRITION**

**Malnutrition**

Malnutrition is common in the developing world. Poor socioeconomic status, infectious diseases, parasitic diseases, lack of personal hygiene, unsafe drinking water and lack of medical facilities and improper or inadequate nutrition are the etiological factors for malnutrition. Decreased food intake can be due to various reasons such as: decreased food availability, poor dietary habits, food faddism and emotional factors, etc.

Malnutrition is common in children, and occurs in two forms: marasmus and kwashiorkor. The total starvation causing depletion of calorie leads to marasmus in children. Sometimes calorie is adequate but food may be grossly deficient in proteins and vitamins; the condition known as kwashiorkor in children. Accordingly the condition is called protein-energy malnutrition. Usually, when edema is present, is called kwashiorkor, and when edema is absent, is called marasmus.

**Scientists contributed**

Christiaan Eijkman (1858–1930), pioneered in studying the effects of dietary deficiency and malnutrition. He shared Nobel Prize in Physiology and Medicine with G. Hopkins in 1929.
**Kwashiorkor**

Cicely Williams described this condition in 1993. Kwashiorkor occurs in children when the food is devoid of proteins and vitamins, but fills the stomach. So the energy supply is adequate; however, growth and repair of the body are severely restricted.

**Clinical Features**

The striking features are **pitting edema, muscle wasting, anemia, skin changes, and apathy**. The edema begins in the feet and then becomes generalized. Edema is due to decrease in plasma protein concentration that lowers the oncotic pressure. The liver is often enlarged as a result of fatty infiltration that causes bulging of abdomen. Anemia is common due to protein deficiency. Diarrhea, malabsorption are common due to decreased digestive enzymes particularly in the pancreas and the intestinal secretions. Appetite for food is poor. The growth of brain and viscera is impaired. The child becomes weak and apathetic.

**Biochemical Changes**

Plasma Proteins concentration falls in plasma. The concentrations of plasma transferrin (the iron-transport protein) and retinol-binding protein (the transporter for vitamin A) also decrease. Plasma insulin concentration is low or normal, cortisol and growth hormone concentrations are elevated, and thyroid hormones are either normal or raised.

**Treatment**

The primary treatment is adequate and proper supply of nutrition. Restoration of body proteins by feeding with skimmed milk (fat-free) mixed with casein (the milk protein), a vegetable oil and sugar (to increase the energy content) is the hallmark of initial treatment. As the child recovers, locally available foods rich in proteins, fruits and vegetables are introduced. Antibiotics and drugs for intestinal worms should be advocated. Blood transfusion or intra-muscular injections of iron should be considered if anemia is severe.

**Marasmus**

Marasmus is due to starvation in children. The food intake is low not only in proteins but also in total calorie. The clinical features include grossly emaciated infant, thin skin and bones, absence of edema and no skin or hair changes. The body weight is below 60 percent of the standard weight for the age. Treatment includes feeding a standard diet rich in all ingredients.

**Obesity**

Obesity is a complex disorder of appetite regulation and energy metabolism. Genes that predispose to obesity in humans and animals have already been identified indicating the importance of genetic factor in the development of this disorder. Obesity has become so common in developed and developing nations that presently it replaces undernutrition and infectious diseases as the most significant threat.

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**Table 161.4: Recommended dietary allowance (RDA) for Indians as finalized by ICMR, 1990.**

<table>
<thead>
<tr>
<th>Categories</th>
<th>Particulars or age</th>
<th>Body Weight (kg)</th>
<th>Energy (Kcal/d)</th>
<th>Protein (g/d)</th>
<th>Calcium (mg/d)</th>
<th>Iron (mg/d)</th>
<th>Vitamin A (mg/d)</th>
<th>Vitamin C (mg/d)</th>
<th>Folic Acid (mg/d)</th>
<th>Vitamin B&lt;sub&gt;12&lt;/sub&gt; (mg/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult man</td>
<td>Moderate work</td>
<td>60</td>
<td>2875</td>
<td>60</td>
<td>400</td>
<td>28</td>
<td>600</td>
<td>40</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>Woman</td>
<td>Moderate work</td>
<td>50</td>
<td>2225</td>
<td>50</td>
<td>400</td>
<td>30</td>
<td>600</td>
<td>40</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>Pregnant</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2525</td>
<td>65</td>
<td>1000</td>
<td>38</td>
<td>600</td>
<td>40</td>
</tr>
<tr>
<td>Lactation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2775</td>
<td>75</td>
<td>1000</td>
<td>30</td>
<td>950</td>
<td>80</td>
</tr>
<tr>
<td>Infant</td>
<td>0–6 months</td>
<td>5.4</td>
<td>108 kcal/kg</td>
<td>2.05g/kg</td>
<td>500</td>
<td>350</td>
<td>25</td>
<td>25</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6–12 months</td>
<td>8.6</td>
<td>98 kcal/kg</td>
<td>1.65 g/kg</td>
<td>500</td>
<td>350</td>
<td>25</td>
<td>25</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Children</td>
<td>1–3 years</td>
<td>12.2</td>
<td>1240</td>
<td>22</td>
<td>400</td>
<td>400</td>
<td>40</td>
<td>40</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4–6 years</td>
<td>19.0</td>
<td>1690</td>
<td>30</td>
<td>400</td>
<td>400</td>
<td>40</td>
<td>40</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7–9 years</td>
<td>26.9</td>
<td>1950</td>
<td>41</td>
<td>400</td>
<td>26</td>
<td>600</td>
<td>40</td>
<td>60</td>
<td>1</td>
</tr>
<tr>
<td>Boys</td>
<td>10–12 years</td>
<td>35.4</td>
<td>2190</td>
<td>54</td>
<td>600</td>
<td>19</td>
<td>600</td>
<td>40</td>
<td>70</td>
<td>1</td>
</tr>
<tr>
<td>Girls</td>
<td>10–12 years</td>
<td>31.5</td>
<td>1970</td>
<td>57</td>
<td>600</td>
<td>34</td>
<td>600</td>
<td>40</td>
<td>70</td>
<td>1</td>
</tr>
<tr>
<td>Boys</td>
<td>13–15 years</td>
<td>47.8</td>
<td>2450</td>
<td>70</td>
<td>600</td>
<td>28</td>
<td>600</td>
<td>40</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>Girls</td>
<td>13–15 years</td>
<td>46.7</td>
<td>2060</td>
<td>65</td>
<td>600</td>
<td>41</td>
<td>600</td>
<td>40</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>Boys</td>
<td>16–18 years</td>
<td>57.1</td>
<td>2640</td>
<td>78</td>
<td>500</td>
<td>30</td>
<td>600</td>
<td>40</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>Girls</td>
<td>16–18 years</td>
<td>49.9</td>
<td>2060</td>
<td>63</td>
<td>500</td>
<td>50</td>
<td>600</td>
<td>40</td>
<td>100</td>
<td>1</td>
</tr>
</tbody>
</table>
contributor to ill health. In particular, obesity is associated with type-II diabetes mellitus, coronary heart diseases, hypertension, certain form of cancer and sleep-breathing problems. Therefore, there is an urgency to control weight gain in all populations at all age groups. Few decades before, obesity was considered as an indication of wealth and health or maximally as a cosmetic problem. But, now it has become an epidemic that threatens global wellbeing even in growing children and adolescents. Recent research has shown that the number as well as the size of adipocytes in obese people is more than people with normal body weight. Weight loss in an obese adult can reduce the size but not the number of adipocytes. Hence, these adults do retain the tendency to put on weight faster than normal individuals. Therefore, prevention as well as early intervention is crucial to fight obesity.

**Etiopathogenesis**

At present, the epidemic of obesity is due to combination of genetic susceptibility, easy availability of high-energy foods, and decreased requirement for physical activity in the modern society. Secondary obesity occurs in various diseases like hypothyroidism, Cushing syndrome, etc. Understanding the neural mechanism of feeding behavior focuses light on the management of primary obesity.

**Neural Mechanisms**

Obesity is primarily a disorder of feeding behavior. There are many mechanisms of regulation of feeding behavior. Neural mechanism is the most central among them. Feeding is categorized under the ‘behavioral functions’ of the body for its subjection to control by activities that are normally influenced by behavioral changes. Therefore, food intake regulation is a complex phenomenon like that of control of other behavioral activities of human beings. Food intake is primarily controlled by hypothalamic centers. There are two established group of hypothalamic nuclei that influence feeding: the lateral hypothalamus (LH), known as feeding center and the ventromedial hypothalamus (VMH) known as satiety center. Stimulation of LH increases food intake and stimulation of VMH brings satiation (causes cessation of feeding). Normally, VMH inhibits LH to stop feeding when satiation is achieved (refer Fig. 49.14; chapter 49). However, the neurotransmitter systems that elicit these responses are not yet clearly known.

Recently, role of extrahypothalamic centers on feeding has been investigated. Limbic system is known as the center of the neural circuitry controlling behaviors. Therefore, it has been suggested that the behavioral aspect of feeding is controlled by mesolimbic structures that project to hypothalamic nuclei. Septum, amygdala, nucleus accumbens and caudate are important components of mesolimbic structures. From our laboratory it has been reported that lesion of basolateral amygdala (BLA), nucleus septal lateralis (NSL) leads to increase in food intake and body weight indicating that normally these brain areas inhibit ingestive behaviours, and lesion of nucleus accumbens and caudate results in decrease in food and water intake and body weight indicating that normally they stimulate ingestive behaviors. Reciprocal connections exist between hypothalamus and these limbic structures, especially the connections are strong between hypothalamic feeding areas and septal nuclei. Therefore, it is suggested that the projections from these structures modify the activities of hypothalamic feeding areas. We propose that the behavioral aspects of feeding are controlled by these limbic influences. Future research on this aspect will reveal the better understanding of mechanism of body weight gain and the physiological basis of intervention for control obesity.

**Measurement and Grades of Obesity**

There are many ways of determining obesity, but commonly employed methods are estimation of body mass index (BMI), waist circumference, skin fold thickness and bioimpedance. However, obesity determination by BMI is a better index as it encompasses correction for age and sex. BMI within 17–24.9 is considered normal and healthy. Obesity is defined as BMI more than 30. BMI within 25–29.9 is overweight. BMI within 30–34.9 is mild obesity, within 34–39.9 is moderate obesity & BMI more than 40 is considered severe obesity.

**Physiological Basis of Management**

Physiologically, obesity is the excess weight gain for the age and gender. Weight of an individual depends on the balance between one’s energy intake and energy expenditure irrespective of genetic, socio-cultural and environmental predispositions. Therefore, the obese individual should understand the importance of restricting the intake of energy and facilitating the expenditure of the same. Controlling intake and expenditure are both equally essential in the management of obesity. Expenditure is expedited by practice of regular physical exercises. Recently, people have become conscious of the role of physical exercise in maintaining good health. However, unless intake is also controlled simultaneously, a greater reduction in body weight cannot be achieved. To restrict food intake needs the conscious effort and will, which is the most difficult task for human being as it requires determination to overcome the habit and ravenousness of excess eating. Therefore, controlling obesity by regulating behavioral aspect of food intake is a challenge to the obesity research.
Chapter 162

Physiology of Aging and Oxidative Stress, Prevention of Aging and Physiology of Yoga

**Learning Objectives**

On completion of study of this chapter, the student **WILL** be able to:

1. Understand the theories of aging.
2. List physiological changes in geriatric age.
3. Understand the mechanism of oxidative degeneration of the body.
4. Appreciate the physiological processes that prevent aging.
5. Understand the physiology of yoga.

**Aging**

**Theories of Aging**

The biological changes of aging are clearer than the mechanisms that mediate it. The common changes include:

1. Chromosomal abnormalities
2. Increased DNA cross-linking
3. Increased frequency of single-strand breaks in DNA
4. Decrease in DNA methylation
5. Loss of DNA telomeric sequences.
6. Increased posttranslational changes in proteins.
7. Deamidation, oxidation, and nonenzymatic glycation of proteins.
8. Deformation in mitochondrial structure

Aging is **multifactorial**. There are many theories of aging that are not completely compatible in their explanation. However, they can be broadly divided into two categories: genetic theories and random damage theories.

**Genetic Theories of Aging**

Genetic theories of aging are based on **programmed senescence**, which means that at the time of birth, the time of aging and death has been predetermined. That means the aging is preprogrammed by a biological time table. Three genetic theories have been developed recently:

**Mutation Theory**

The first one is the mutation theory. Usually animals die much before completion of their expected life span due to adverse effects of natural forces. Therefore, this theory suggests the importance of mutations that retard long survival. The mutations pile up in the genes that are transferred from generations to generations.

**Theory of Pleiotropic Antagonism**

The second one is the theory of pleiotropic antagonism, which proposes that aging is caused by the harmful effects of genes. These unfavorable genetic effects are preserved for the purpose of survival and these properties are imparted prior to reproduction.

**Ecological Theory**

The ecological theory applies to the ecological conditions where external hazards are less. In such favorable environments, the mutation is such that the aging process is retarded to allow the animal to reproduce and protect the offsprings.
Random Damage Theories
The random damage theories explain that the aging is due to the loss of balance between ongoing damage and repair. The continuous tissue damage in the body occurs due to constant production of free radicals, and by the processes of oxidation and glycation. However, the damage is immediately repaired by regenerating an anti-stress mechanisms. With advancement of age, especially after the age of 40 years, capacity to repair cells, tissues and organs decline. Regenerative senescence was also suggested to be due to the arrest of the cell cycles at the G1/S phase. This is the phase in cell cycle at which synthesis of DNA starts. Thus, DNA synthesis is impaired.

Role of Cell Replication
In recent times, the length of telomeric DNA has also been associated to cell replication. Telomeric DNA is present at the ends of chromosomes. Telomeric DNA has following important functions:
1. Prevents chromosomal instability.
2. Slows chromosomal fragmentation and rearrangement.
3. Anchors chromosomes to nuclear matrix.
4. Acts as a buffer between coding regions of DNA and the ends of the chromosomes.
5. It is also necessary for cell divisions.

There are about a total of 2000 base pairs of the telomere. During each cell division, approximately 50 base pairs are lost. Thus, cell division decreases length of telomeric DNA. Telomeric shortening causes loss of gene accessibility. Gene accessibility is necessary to repair the cell damage that occurs continuously by metabolic processes. Thus, telomeric shortening slows the repair process. Along with cytoplasmic factors that mediate arrest of DNA synthesis, telomeric shortening limits the ability of the cell to divide, which prevent replacement of cells that are lost by apoptosis.

In the past, many mechanisms have been postulated for aging. These are: somatic mutation theory which postulates that aging occurs due to cumulative spontaneous mutations, the error catastrophe theory which proposes that aging results from errors in the synthesis of proteins critical to the synthesis of genetic material, and the intrinsic mutagenesis theory which hypothesizes that aging is due to the intrinsic DNA rearrangements. However, none of these theories have satisfied the physiological bases of aging. However, calorie intake and free radicals are known to play important role in aging.

Role of Calorie Intake
Strongly implicated in aging is calorie consumption. Increased calorie intake after the age of 40 has been proved to facilitate aging. It has been observed that calorie restriction delays the onset of aging. The calorie restriction by about 30% of the total calorie intake has been documented to have following effects:
1. Increase in average life expectancy and maximum life span.
2. Delay in onset of age-associated diseases.
3. Decrease in deterioration of physiologic processes like immune responsiveness, glucose metabolism, muscle atrophy etc.
4. Influence on gene expression, protein turnover and cross-linking.

The impact of general calorie restriction is different from specific dietary restriction like restriction for fat etc. However, calorie restriction must be supplemented with vitamins and antioxidants to prevent aging.

Role of Free Radicals and Oxidative Stress
Due to metabolism, free radicals like peroxide and hydroxyl radicals are continuously produced in the body. These radicals damage the cells and tissues by the process of oxidation. The free radicals damage DNA and proteins and cause peroxidation of lipids in the membranes. This is called oxidative stress. However, simultaneously a scavenging system of antioxidants also exists in the body that neutralizes the toxic effects of free radicals. Antioxidants include glutathione, vitamin E, vitamin A and vitamin C. Oxidative stress becomes more when production of oxidants is more than the generation of antioxidants (for details of free radicals, see below). Oxidative stress promotes aging.

Atherosclerosis
Atherosclerosis is the process of narrowing of lumen of blood vessels due to deposition of lipid materials on the inner endothelial lining of blood vessels. Atherosclerosis is the physiological process that starts almost at infancy. In childhood, atherosclerosis starts in aorta and larger arteries. As age advances, atherosclerosis starts in smaller arteries and arterioles. Due to atherosclerosis, blood supply to organs and tissues of the body decreases that causes structural and functional degenerations. This is the natural process of degeneration (aging) and death. However, the process of degeneration is facilitated by accelerated atherosclerosis in which fibrofatty plaques are deposited in greater amount in the blood vessel lumen and hyperplasia of smooth muscle of blood vessels occur. This causes premature aging and death.

The predisposing factors for accelerated atherosclerosis are:
1. Hyperlipidemia (hypercholesterolemia, hypertriglycerideremia)
2. Obesity
3. Diabetes mellitus
4. Hypertension
5. Smoking
6. Excess eating (increased calorie intake) after 40 years of age
7. Chronic stress
8. Lack of exercise
**Table 162.1: Physiological changes in geriatric age groups.**

<table>
<thead>
<tr>
<th>Organ system</th>
<th>Physiological changes</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>General</td>
<td>Increased fat mass of the body</td>
<td>Proneness for atherosclerotic diseases</td>
</tr>
<tr>
<td></td>
<td>Decreased body water content</td>
<td>Susceptibility to rapid dehydration</td>
</tr>
<tr>
<td>Respiratory</td>
<td>Decreased compliance of chest wall and lung parenchyma</td>
<td>Decreased ventilation and decreased pO₂</td>
</tr>
<tr>
<td></td>
<td>Decreased activity of cough reflex</td>
<td>Easy aspiration</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>Decreased arterial compliance</td>
<td>Increased systolic pressure</td>
</tr>
<tr>
<td></td>
<td>Decreased β receptor sensitivity</td>
<td>Decreased cardiac output</td>
</tr>
<tr>
<td></td>
<td>Decreased SA nodal activity</td>
<td>Decreased heart rate</td>
</tr>
<tr>
<td></td>
<td>Decreased baroreceptor sensitivity</td>
<td>Impaired blood pressure regulation, especially in response to standing</td>
</tr>
<tr>
<td>GI system</td>
<td>Decreased hepatic function</td>
<td>Impaired intermediary metabolisms</td>
</tr>
<tr>
<td></td>
<td>Decreased gastric acidity</td>
<td>Impaired digestion</td>
</tr>
<tr>
<td></td>
<td>Decreased colonic motility</td>
<td>Constipation</td>
</tr>
<tr>
<td>Hematologic</td>
<td>Decreased bone marrow activity</td>
<td>Anemia</td>
</tr>
<tr>
<td>Immune system</td>
<td>Decreased T cell function</td>
<td>Decreased defenses</td>
</tr>
<tr>
<td></td>
<td>Increased autoantibodies</td>
<td>False-positive rheumatoid factor, antinuclear autoimmune disease</td>
</tr>
<tr>
<td>Renal</td>
<td>Decreased GFR</td>
<td>Impaired excretion of waste products, increased serum creatinine</td>
</tr>
<tr>
<td></td>
<td>Impaired capacity for urine concentration and dilution</td>
<td>Decreased ability to excrete salt or fluid load</td>
</tr>
<tr>
<td>Endocrine</td>
<td>Impaired insulin secretion</td>
<td>Increased glucose level in response to illness</td>
</tr>
<tr>
<td></td>
<td>Decreased thyroxine secretion and thyroxine clearance</td>
<td>Decreased body metabolism</td>
</tr>
<tr>
<td></td>
<td>Increased ADH release and decreased aldosterone secretion</td>
<td>Impaired Na⁺ and water excretion</td>
</tr>
<tr>
<td></td>
<td>Decreased testosterone secretion</td>
<td>Impotence</td>
</tr>
<tr>
<td></td>
<td>Vitamin D activation</td>
<td>Osteopenia, easy fractures</td>
</tr>
<tr>
<td>Nervous system</td>
<td>General brain atrophy</td>
<td>Forgetfulness, confusion</td>
</tr>
<tr>
<td></td>
<td>Decreased catecholamine secretion</td>
<td>Psychological gloominess</td>
</tr>
<tr>
<td></td>
<td>Decreased brain dopaminergic synthesis</td>
<td>Stiff gait and postures</td>
</tr>
<tr>
<td></td>
<td>Decreased righting reflexes</td>
<td>On walking body sways, may even fall</td>
</tr>
<tr>
<td></td>
<td>Decreased stage 4 sleep</td>
<td>Get up very early morning, insomnia, sleep apnea</td>
</tr>
<tr>
<td></td>
<td>Impaired thermal regulation</td>
<td>Decreased body temperature</td>
</tr>
<tr>
<td>Musculoskeletal</td>
<td>Decreased muscle mass</td>
<td>Weakness</td>
</tr>
<tr>
<td></td>
<td>Decreased bone density</td>
<td>Osteopenia, easy fracture</td>
</tr>
<tr>
<td>Genitourinary</td>
<td>Atrophy of vaginal mucosa</td>
<td>Dyspareunia, bacterial infections</td>
</tr>
<tr>
<td></td>
<td>In males, prostrate enlargement</td>
<td>Urinary incontinence</td>
</tr>
<tr>
<td>Eyes and ears</td>
<td>Presbyopia</td>
<td>Decreased accommodation</td>
</tr>
<tr>
<td></td>
<td>Opacification of lens</td>
<td>Decreased vision</td>
</tr>
<tr>
<td></td>
<td>Decreased sound conduction</td>
<td>Decreased audition</td>
</tr>
</tbody>
</table>

**Geriatric Changes**

Though there is no clear cut demarcation of age to say that the individual has entered into geriatric age group, usually age more than 60 years is considered for the purpose. The body physiology changes in geriatric age group. The increase in blood pressure, osteoporotic changes in bones, decreased digestive functions, decreased immunity, decreased audiovisual acuity, loss of memory and decreased sensory and motor responses are common physiological changes in old age (Table 162.1). Increased incidence of diabetes, hypertension, malignancies and GI dysfunctions are common in elderly.

**OXIDATIVE STRESS**

**Free Radicals**

Oxygen, which is essential for survival of all living organisms, is the cause of degeneration and death as the products of oxidative metabolism are potentially toxic. Superoxide anions (O₂⁻) formed during metabolism, at high concentration are harmful to tissues.

**Formation of Free Radicals**

When molecular oxygen in tissues is univalently reduced, produces superoxide radical. This is because the molecular oxygen is paramagnetic and contains two unpaired elec-
trons (e⁻) that reside in separate orbitals. When oxygen molecule takes up one electron by universal reduction, it becomes superoxide anion $O_2^-$. Superoxide anion is highly reactive and toxic to cell membranes.

**Other Free Radicals**

Superoxide anion ($O_2^-$) captures other electrons to form hydrogen peroxide, $H_2O_2$, which is also harmful. $H_2O_2$ further react with superoxide anion, in presence of $Fe^{++}$ (ferrous ion) to form hydroxyl radical ($OH^-$), and singlet oxygen ($O_2$) (Application Box 162.1). The reaction is called Haber’s reaction or Haber-Weiss-Fenton’s reaction.

$$\text{H}_2\text{O}_2 + \text{Fe}^{2+} \rightarrow \text{OH}^- + \text{O}_2 + \text{OH}^-$$

**Application Box 162.1**

**Ceruloplasmin as an antioxidant**: Ceruloplasmin acts as ferroxidase that converts ferrous iron ($Fe^{2+}$) to ferric iron ($Fe^{3+}$). Therefore, in the absence of $Fe^{3+}$, hydroxyl radical cannot be formed. Thus, it halts Haber’s reaction that prevents formation of highly reactive free radicals. Hence, ceruloplasmin serves as antioxidant.

Superoxide anion can accept $H^+$ and from hydroperoxy radical, which can also act as an oxidant.

Thus, superoxide anion in tissues, form free radicals like hydroperoxy radical, hydroxyl free radical and hydrogen peroxide. All these free radicals are very reactive and toxic to biological membranes.

Nitric oxide reacts with $O_2^-$ to produce peroxynitrite ($\text{ONOO}^-$), which is degraded to form the highly reactive $\text{OH}^-$ radical. Other free radicals produced in the body are $\text{CCl}_3^-$ and free halogen radical like $\text{Cl}^-$ formed from $\text{CCl}_4$.$O_2^-$ Formation in Metabolic Pathways:

Various cytosolic oxidations produce $O_2^-$ in different ways:
1. $O_2^-$ is formed by oxidative deamination by L-amino acid oxidase.
2. It is formed during univalent oxidations of $O_2$ in the respiratory chain.
3. $O_2^-$ is formed during methemoglobin formation.
4. $O_2^-$ is formed during cytosolic hydroxylations of steroids, drugs, and xenobiotics, by Cyt-P450 or Cyt-P448 system.
5. Free radicals are produced in tissues on exposure to ionizing radiations.

**Scavengers of Free Radicals**

**Superoxide Dismutase**

Superoxide dismutase is present in both cytosol and mitochondria of most aerobic tissues. It destroys the $O_2^-$. $2O_2^- + 2H^+ \xrightarrow{\text{Superoxide dismutase}} \text{H}_2\text{O}_2 + \text{O}_2$

**Catalase**:

Catalase destroys $H_2O_2$ in the tissues to $O_2$.

$$2\text{H}_2\text{O}_2 + \text{H}_2\text{O} \xrightarrow{\text{Catalase}} 2\text{H}_2\text{O} + \text{O}_2$$

**Glutathione Peroxidase**

This is a selenium-containing enzyme which destroys $H_2O_2$ with the help of reduced glutathione (G-SH). It is present in cytosol and mitochondria.

**Ferricytochrome**

$O_2^-$ can be oxidized to $O_2$ by ferricytochrome.

**Endogenous Ceruloplasmin**

It halts Haber’s reaction (see above)

**Effects of Free Radicals**

Free radicals are highly reactive. They form lipid peroxides and lipoxides. These radicals constitute a threat to the integrity of biomembranes. The free hydroxyl radical is most reactive and an extremely potent oxidant.

**Lipid Peroxidation**

Conversion of membrane lipids and unsaturated fatty acids to lipid peroxides and lipoxides is called lipid peroxidation. It is a chain reaction initiated by free radicals. Lipid peroxidation has potentially devastating effects.

A free radical with unpaired electrons takes away hydrogen from methylene group of polysaturated fatty acids and converts it into a free fatty acid radical which binds with $O_2$ to give fatty acid-peroxy radical, that in turn changes to fatty acid-hydroperoxide, a toxic agent.

**Anti-Oxidants**

Anti-oxidants reduce the process of lipid peroxidation. Naturally occurring antioxidants include:

1. **Lipid soluble antioxidants**
   - Vit E (tocopherols)
   - β-Carotene: is an antioxidant at low $pO_2$
   - Lycopene
   - Chelators of metal ions such as DTPA (diethylene triamine penta acetate), and EDTA.

2. **Water soluble antioxidants**
   - Vitamin C (Ascorbic acid)
   - Urates
   - Ceruloplasmin

**Clinical Significance of Free Radicals**

**Role in Aging**

Free radicals promote aging and facilitate certain diseases like diabetes mellitus, atherosclerosis, cancer, etc.

**Neonatal Oxygen Radical Diseases**

When preterm baby is exposed to free oxygen radicals during vigorous oxygen therapy, following diseases occur:

1. Bronchopulmonary dysplasia
2. Retinopathy
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3. Necrotizing enterocolitis
4. Periventricular leukomalacia
5. Patent ductus arteriosus
6. Intracranial hemorrhage

**Rheumatoid Arthritis**
Free radicals released by the neutrophils aggravate diseases like rheumatoid arthritis. Drugs like corticosteroids and NSAIDs interfere with the formation of free radicals and provide relief.

**Atherosclerosis and Thrombosis**
Free radicals released by the endothelial cells of blood vessels oxidize the LDL particles deposited under the endothelial cells. LDL oxidation increases lipid peroxidation that in turn increases the thrombo-hydroperoxides. This increases thromboxane production, which decreases the prostacyclin/thromboxane ratio and leads to thrombosis.

**Chronic Granulomatous Diseases**
In chronic granulomatous diseases like tuberculosis, phagocytic function of macrophages has been found to be defective and there is increased susceptibility to microbial and fungal infection.

**Role in Phagocytosis**
\(H_2O_2\) is used in killing microorganisms in phagocytosis. During inflammation, \(O_2^-\) uptake is increased greatly, which is called oxygen burst. Also, production and utilization of \(H_2O_2\) occurs as an early event in phagocytosis. Utilization of glucose by HMP-shunt increases in phagocytosis. NADPH produced by this pathway can react with \(O_2\) to produce superoxide anion (\(O_2^-\)), which can subsequently produce \(H_2O_2\) also.

**PREVENTION OF AGING**
Aging is a natural process. Though aging cannot be completely prevented, its onset and progress can be delayed and its impact on the body can be reduced. Physiologically, the processes that can achieve this objective are:
1. Calorie restriction
2. Balanced diet
3. Regular physical exercise
4. Mental and physical relaxations
5. No smoking
6. Avoidance of excess of sex, alcohol and politics
7. Practice of asanas and breathing exercises
8. Spiritual life

**Calorie Restriction**
Calorie restriction has been proved to delay the process of aging. After the age of 40 years, one has to cut down the calorie intake by 30 to 40%. Excess of calorie in the body is converted to fat that promotes the process of atherosclerosis.

**Balanced Diet**
Balanced diet for elderly people is different from that of children and adults. Not only calorie intake is restricted for old age, the diet should contain more fiber and less fat. Total fiber content should be minimum 20% of the total food. Fibers decrease fat absorption from intestine and protects from colorectal malignancies. The protein content should be moderate and vitamins should be adequate. Water intake should be always more.

**Physical Exercise**
Regular physical exercise is known to reduce obesity, hyperlipidemia and atherosclerosis. Physical exercise improves blood flow to organs and the aerobic capacity of the individual. There is still no conclusive evidence to document that exercise prevents aging or not. However, exercise certainly improves work capacity as assessed from maximum oxygen uptake. Physical exercise also improves cardiac performance and reduces musculoskeletal disability. Physical exercise is also reported to prevent age related decline in resting metabolic rate.

**Mental and Physical Relaxations**
Mind should be in a state of relaxation to reduce the load of stress. Nowadays, life is stressful and stress is unavoidable. Stress is there in all spheres of life, such as stress of work, of study and of family, stress of achievements and acquiring wealth, and so on. One should realize that being a human being one cannot achieve everything. One should try to progress, but not by depleting his mind and body. Presently, life has become hectic due to the increased demand of establishing at all stages of life. One should have time to relax the mind and body. To ensure that, one can follow the following schedule in routine life:
1. Take a light dinner (stomach should not be heavy before you sleep).
2. Sleep early in the night (ideally one should sleep by 10 pm, not after 11 pm).
3. Get up early in the morning (one should get up by 5 am, not after 6 am).
4. Take a light morning walk.
5. Look at the rising sun, and changing sky colors when sun rises.
6. Perform some light relaxing asanas and sahaja paranayama (for details, see below).
7. Be sincere and honest in your duties.
8. In the evening, look at the setting sun and the sky.
9. Look at the sky, sea, rivers and green fields, flowers in the garden and try to feel the vastness of the nature. Stretch your mind with these vast natures.

Look at the flowers, animals, forests and vast water. If one does not have such opportunities, one can sometimes avail them from television by watching channels like national geography, animal planets etc.
1. Do not think ill of others.
2. Concentrate on works that help you to progress, do not work beyond your capacity.
3. Do not follow a tight schedule every day.

**No Smoking**

Smoking is known to induce atherosclerosis and bronchopulmonary diseases. Oral and bronchogenic malignancies are common in smokers. Smoking is a known risk factor for diabetes, hypertension and heart diseases. Smoking should be totally abandoned. Smoking inhibits immunity.

**Avoidance of Excess of Alcohol, Sex and Politics**

Mild to moderate alcohol intake (especially red and port wines) is recently proved to be good for health. It improves myocardial performance. It increases plasma level of HDL and decreases VLDL, LDL and TG. Therefore, it inhibits atherosclerosis. However, excess consumption of alcohol is harmful. Alcohol is rich in calorie. Therefore, if taken in large amount, it gets converted into fat in the body. Alcohol intake in large amount also inhibits appetite. It induces alcoholic cirrhosis of liver, myocardial depression and brain degeneration. Hence, if at all, one should consume alcohol, but alcohol should not consume him.

Sex is known to reduce stress. However, illicit sex and indulgence in sex are harmful to mind and body. One should learn to convert sexual energy into higher vital energy that refines lower natures and kindles the opening of higher forces in the being.

No politics please. Do not be involved in bad-politics. Nowadays politics means to attain supremacy by hook or crook. Practice of such politics creates bad vibrations in the being and promotes degeneration not only in personal life but also in social life. This is detrimental to a progressive and harmonious life and the society. Also, one should not play politics in routine life. Keep away from it as much as possible.

**Practice of Asanas and Breathing Exercises**

Recently, the interest for practice of asanas and breathing exercises has increased in general public for health substantial health benefits acquired by these practices. However, if practiced with proper yogic attitude, asanas become yogasanas and breathing exercises become pranayamas. There are different types of yogasanas and pranayamas (for details, see below in “Physiology of Yoga”). If learned properly and practiced appropriately, they improve quality of life enormously. The life span is prolonged by these yogic practices.

**Spiritual Life**

There is a need to know the difference between a religious and a spiritual life. Religious life is the practice of a sect of faith and belief, living a fixed way of life prescribed by the religion and following the dogmas of the religion. A spiritual life is a disciplined and progressive way of living based on complete faith on Almighty and aims at attaining higher perfections. There is a global need to come out of religious narrowness and to practice a higher spiritual living. Recently, there have been a lot of discussions on practice of yoga in various fora. People generally think that yoga means practice of asanas and pranayamas. Thus, many people believe that they are practicing yoga through these techniques and few even declare themselves as yoga teachers and yogis as they are just capable of teaching asanas and pranayamas. We have come across such so called yoga teachers and practitioners those who preach and teach yogasanas, conduct seminars, symposia and workshops on yoga but fail to implement discipline in their own personal life. Any personal failure and family discord raises their level of stress. This is the present state of yoga in our society. There is a need to learn the real yoga and practice it in its proper essence.

Yoga aims at complete union with the Divine and manifestation of Divine qualities in human life, which can be attained by an ardent aspiration for a divine life, sincere effort for rejection of lower movements of the being and complete surrender to the Supreme Divine. One should have realization that the Divine guides, Divine protects and the Divine executes, and we have to just become the true instruments of Him so that slowly He manifests through us. Not only yogasanas and pranayamas should be performed in this spirit of realizing the Divine, but also in all works or karmas (be it study, work in the office or work at home or any form of work) of life should have this spiritual touch. Such realizations through study, works and experiences lead to gradual and complete fulfillments of spiritual life free from fear and stress, anxiety and ambitions, desires and petty satisfactions. Such a practice not only prolongs the life, but also improves the quality of life (for details, see below “Physiology of Yoga”).

**Physiology of Yoga**

Yoga is the complete union with the Divine and manifestation of Divine qualities in human life. In other words, it is a conscious effort to attain and lead the Divine life. That means yoga is the evolution of human consciousness into divine consciousness. **Swami Vivekananda** says “Yoga is a means of compressing one’s evolution into a single life, or even a shorter time than that”. In the entire world, India is the land of spiritual knowledge and the leader of spiritual progress of the earth. Therefore, The Mother of Sri Aurobindo Ashram, Pondicherry has said “Oh India, the Land of Spiritual light and knowledge, wake up to your true mission in the world, and show the way to union and harmony.” It is true that without realization of this spiritual knowledge, world union and harmony will not be possible. There are several schools of yoga in India. However, the four are most popular and widely accepted. These are:
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1. Hatha yoga
2. Raja yoga
3. Tantric yoga
4. Integral yoga

Hatha Yoga

Hatha yoga includes practice of asanas, pranayamas and kriyas (for details, refer a standard yoga book). It mostly aims at perfection of the body. A true hathayogi not only achieves a healthy body and a greater longevity but also gains mastery over functions of the body. However, in general, it neglects perfection of the mind. Therefore, hatha yoga by large attains improvement of physical health without refinement of the mind.

Raja Yoga

Raja yoga basically aims at perfection of the mind. A controlled and quietened mind is the basic need for practice of any yoga. Patanjali, the master of raja yoga, has advocated yoga into eight limbs. Therefore, Patanjali’s yoga is popularly known as Ashtanga yoga. The eight limbs are: yama, niyama, asana, pranayama, pratyahara, dharana, dhyana and samadhi.

Yama: Yama consists of five principles. These are, ahimsa (nonviolence), satya (stick to truth only), asteya (non-stealing), brahmacharya (celibacy), aparigraha (non-receiving, or not being greedy)

Niyama: Niyama also consists of five principles: shaucha (cleanliness, purification of body and mind), santosh (be satisfied with what you get), tapas (austerities, or disciplining our movements), swadhyaya (study of spiritual scriptures) and ishwarpriyadhan (surrender to the Divine).

Asana: Practice of different body postures that are steady and comfortable. There are different asanas, and practice of each asana has the benefit. Like meditation is usually performed in padmasana, and relaxation of the body is performed in savasana. Refer a standard book on asanas for details, as it is beyond the scope of this book to discuss them.

Pranayama: Control of movement of prana, the vital energy. This is performed by practice of controlled breathing exercises. Different pranayamas have different benefits. In general, they decrease sympathetic activity and improve parasympathetic activity.

Pratyahara: Pratyahara is withdrawal of senses to free the mind. It is mainly the material withdrawal, the withdrawal from physical enjoyment.

Dharana: Dharana is the concentration of the mind on a particular point, which may be an object, a sound or an idea.

Dhyana: Dhyana is the meditation, in which mind is allowed to flow towards the point of concentrations.

Samadhi: This is the state of super-consciousness. The individual enters into a transcendent consciousness, and withdraws from external objects.

Tantra Yoga

The tantra yoga is based on the principles that all creation is Divine manifestation and all worldly activities are Divine play. It does not reject the world as an illusion but considers it as a manifestation of the Divine. Therefore, one should wholeheartedly participate in the cosmic play and enjoy them without attachment. However, enjoyment has the danger of falling in the trap of attachment as the modern man is not purified internally. Therefore, this yoga was not accepted widely by others.

Integral Yoga

The Yoga of the Gita

The Gita taught us the triple path of work, knowledge and devotion as a synthesis of yoga. The yoga through knowledge, or jnana yoga, the yoga through devotion, or bhakti yoga, and the yoga through works, or karma yoga are the triple paths of yoga.

Integral Yoga

Integral yoga is the yoga of Sri Aurobindo. It is the synthesis of all the yogas described above, but it goes beyond all of them. It accepts the central principle of triple path of yoga of the Gita, but does not stop at salvation. It proposes the transformation of the being as a whole; the mental transformation, the vital transformation and finally the physical transformation. Therefore, this is also called yoga of transformation.

Integral yoga of Sri Aurobindo is based on the concept of evolution, according to which if life has evolved from matter and mind has evolved from life, then mind is bound to be evolved into a state of supermind. Therefore, though man (mental being) is presently the highest creation of the nature in the evolutionary process, he is not the final being. Man does not mark the end of evolution. The present human consciousness is likely to evolve into a higher consciousness, what Sri Aurobindo says Supramental Consciousness. Therefore, his yoga is also called the Supramental Yoga.

1. Man practices this yoga by three fundamental means: aspiration for higher spiritual life, rejection of lower qualities from his nature and surrender to the Divine. However, in this yoga of transformation, human endeavor is not adequate to transform the physical part of the being. Therefore, Sri Aurobindo descended the Supramental Force from the supracosmic level to the earth to transform human nature and the body.

2. The Supramental Force is the force of the Divine Mother. Therefore, this yoga is also called the Yoga of the Divine Mother. One who can open to the Mother, and totally surrender to the Mother, will finally with Her force, grace and protection be able to ascend in the ladder of evolution to reach the state of Supramental...
Consciousness. It is the Divine Force that gradually transforms the being.

3. **Surrender to the Divine** is the first and foremost condition in this yoga. The true surrender brings complete trust on the Divine, and also the spiritual experiences.

4. Without spiritual experience, no yoga is possible. Yoga is never done by giving speeches, conducting seminars and workshops and by mechanically practicing asanas and pranayamas. Rather true yogis do not speak much; they become example to others through their actions and change others through their vibrations.

5. Surrender gives the confidence that the Divine takes care of everything, and therefore there is no need to worry for anything. Hence, what is required from man is to **surrender to the Supreme** and become a sincere worker of the Divine. This knowledge Lord Krishna taught to Arjuna in the midst of Mahabharata (Kurukshetra) War. He told.... “Sarba Dharma Parihstajya Mamekam Saranam Braja”, that means ‘Oh Arjuna, abandon everything, and just surrender to me’, .... rest all I shall deliver. A truly surrendered life makes the life stressless and progressive and brings the true happiness. To lead this stress-free and happy life is the best way to prevent aging.

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**CHAPTER SUMMARY**

**HOW TO REMAIN YOUNG?**

*The Message of The Mother of Sri Aurobindo Ashram, Pondicherry, India*

It is not the number of years you have lived that makes you grow old. You become old when you stop progressing.

As soon as you feel you have done what you had to do, as soon as you think you know what you ought to know, as soon as you want to sit and enjoy the results of your effort, with the feeling you have worked enough in life, then at once you become old and begin to decline.

When, on the contrary, you are convinced that what you know is nothing compared to all which remains to be known, when you feel that what you have done is just the starting-point of what remains to be done, when you see the future like an attractive sun shining with the innumerable possibilities yet to be achieved, then you are young, however many are the years you have passed upon earth, young and rich with all the realizations of tomorrow.

And if you do not want your body to fail you, avoid wasting your energies in useless agitation. Whatever you do, do it in a quiet and composed poise. In peace and silence is the greatest strength.
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