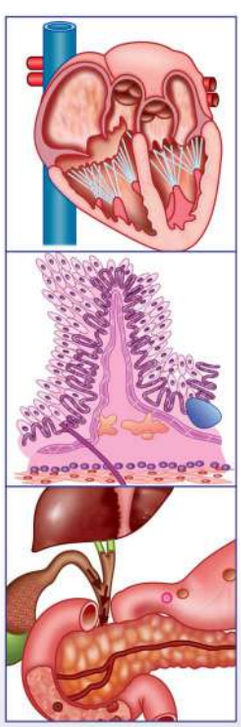
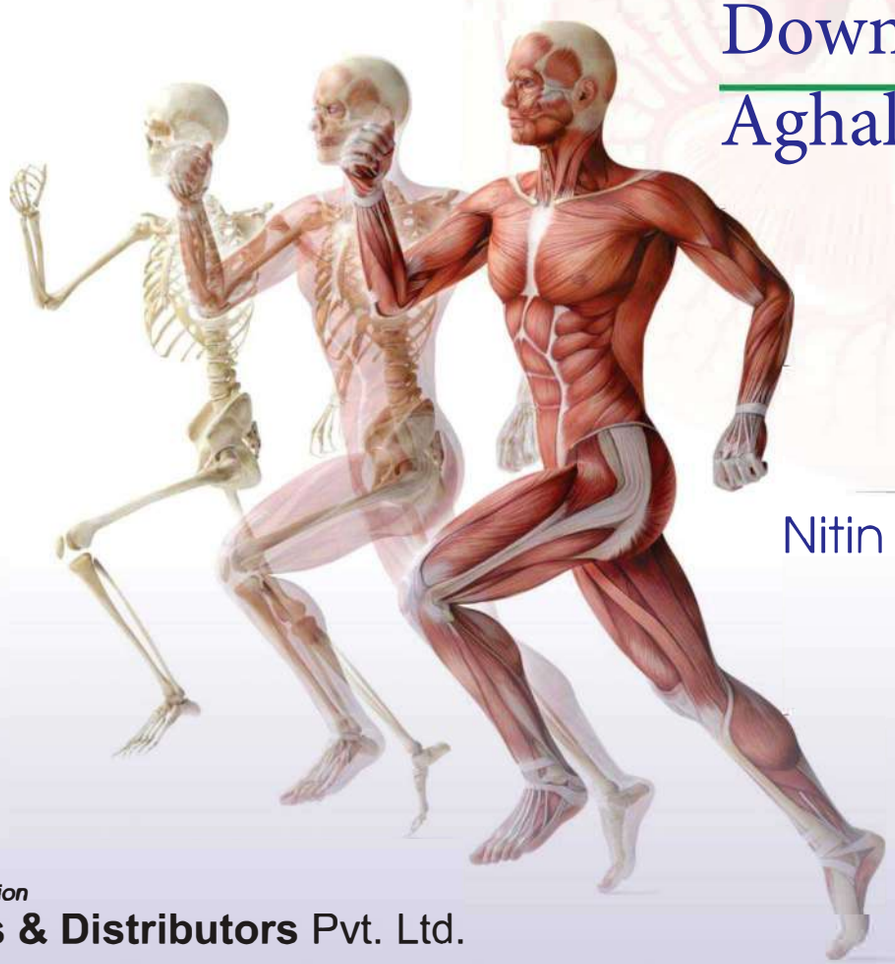
Volume 1

CC Chatterjee's

Human Physiology





Editor
Nitin Ashok John

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CC Chatterjee's

Volume 1

Human Physiology

Twelfth Edition

Contents of Volumes 1 and 2

Volume 1

Volume 2

CC Chatterjee's

Volume 1

Human Physiology

Twelfth Edition

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eISBN: 978-93-881-7802-0

Twelfth eBook Edition: 2018

Published by Satish Kumar Jain and produced by Varun Jain for
CBS Publishers & Distributors Pvt. Ltd.

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Preface to the Twelfth Edition

It gives me immense pleasure in writing the Preface to the twelfth edition of CC Chatterjee's *Human Physiology*. This book has been very popular and widely read from its first edition which was published in 1951. Dr CC Chatterjee, a doyen in the field of physiology, was a dedicated academician, devoted teacher, author par excellence, a noble friend, philosopher and a guide to his colleagues and students. He was an enthusiastic physiologist who strived with greatest zeal to give the best integrative knowledge of basic medical sciences, especially that of physiology, to his students.

I express my special gratitude and sincere thanks to Dr Surrinder H Singh, *Ex*-Professor and Head, Department of Physiology, Lady Hardinge Medical College, New Delhi, a renowned teacher of physiology, who provided regular review inputs for updating the book from time to time, and for her devotion in reading the manuscript thoroughly and providing valuable feedback so that no part of the information is left uncovered by oversight.

I express my sincere thanks to my colleagues Dr Neelam Mishra, Professor and Head, Department of Physiology, Government Medical College, Nagpur; Dr MS Phatak, Professor and Head, Department of Physiology, Indira Gandhi Government Medical College, Nagpur; Dr Geeta Kurhade, Senior Lecturer, Department of Physiology, University of West Indies; Dr SV Umadevi and Dr D Niraimathi, Associate Professors, Department of Physiology, Indira Gandhi Medical College and Research Institute, Puducherry; Dr Rakhee Tirpude, Associate Professor, Department of Physiology, NKP Salve Institute of Medical Sciences and LMH, Nagpur, and Dr Sanjay Andrew Rajaratnam, Professor and Head, Department of Physiology, Chettinad Hospital and Research Institute, Chennai, for their valuable suggestions.

As Prof AM Seligman, Dr Barbasa R Betty and Dr Davenport permitted the inclusion of the reference of illustration in the earlier reprint edition and as these are included in this edition too, I extend my gratitude to them. I am also thankful to CBS representatives Mr Ajay (Karnataka), Mr Sarvanan and Mr Jyoti (Chennai) and Mr Ajay Shrivas (Nagpur) for providing constant feedback from various faculty members all over the country for contents to be included in the book and this was immensely helpful.

The twelfth edition of CC Chatterjee's *Human Physiology* is especially designed for undergraduate and postgraduate students of medicine, paramedical sciences and allied health sciences, and will help them in excelling in their examinations and professional career as well.

The key features of this book are the simple language and comprehensiveness which have remained unchanged ever since the first edition. All the topics of physiology are correlated with anatomy, biochemistry, pathophysiology and applied physiology for a thorough integrated learning of the functional aspects of human body. Recent advances have been included to give better insight to understanding the physiological principles. Clinical case scenarios are included to help students in learning of physiological basis of clinical signs and symptoms. Moreover, this book retains the ideas, thought process, knowledge, lucidity and comprehensiveness, original diagrams and intellectual concepts of the doyen physiologist Dr Chandi Charan Chatterjee whose contribution to physiology will always be remembered in the times to come.

In spite of all the untiring efforts, any mistakes or omissions left unknowingly may please be excused, while valuable suggestions are welcome from faculty and students for future printings and editions of the book.

I wish to acknowledge and give special thanks to Mr SK Jain, Chairman and MD, Mr Varun Jain, Director and Mr YN Arjuna, Senior Vice President—Publishing and Publicity for their suggestions and eagerness to make this twelfth edition colourful and informative so that the text is updated with advancements in medical sciences to this day.

I am thankful to Mrs Ritu Chawla AGM—Production, Mr Vikrant Sharma DTP operator, Mrs Baljeet Kaur, Mr Sanjay Chauhan, Mr Neeraj Prasad, Graphic designers, Mr Ananda Mohanty Proofreader, and all publishing team of CBS Publishers & Distributors, New Delhi, for their excellent inputs in shaping the book to its present form.

And last but not the least, I am thankful to my wife Dr Jyoti and my son Joshua for all their support and encouragement.

Nitin Ashok John
Editor

Preface to the First Edition

At the outset, I would like to pay my humble regards to my revered teacher, Dr Charubrata Ray, MB, BSc, from whose lips I learnt how to 'read' and 'think' Physiology. A quiet unassuming man, a scholar with an inborn spirit of research, a teacher of rare genius—teaching thousands of students throughout his life without the least material interest of his own—Dr Ray represents that long-forgotten school of 'Indian Gurus' with whom teaching was a creed and not a profession. In teaching he sprouts wings. Seldom a teacher could have claimed to have so many students and seldom could he command so much respect from them. There are thousands today who take his name with grateful reverence. May he livelong and lead us with his kindly light.

For the last few decades, physiology has been making so rapid progress that it is being increasingly difficult for the average students to manage the subject within the limited period fixed by the universities. Owing to this reason, they are compelled to go in for 'notes', 'synopses', 'made easier' and such other short-cut devices which somehow enable them to squeeze through the examinations but fail to give them a comprehensive knowledge of the subject as a whole. This state of affairs is cutting at the root of medical education and is likely to undermine the standard medical graduates. What is required today is a textbook of reasonable size, including the essentials of histology, biochemistry and biophysics which will give the student a bird's-eye view of the whole subject and at the same time enable him to pass the examination with credit. This book is an attempt in that line.

It has been drawn up to meet the requirements of the preclinical medical students of the different Indian and foreign universities mainly. Advanced and post-graduate students will certainly derive some help from it but should not depend on this book alone. I have no hesitation to say that a good deal of attention has been paid to assure success in examinations. Each system has been divided into a number of problems in such a way that they are usually set or likely to be set as questions by various examining bodies. At the beginning of each system a few introductory lines have been added in which the fundamental principles of that system have been discussed. The students are advised to read these portions carefully and thoroughly to have a better grasp of the subject.

I have tried to avoid as much of the applied aspects as possible because it is my experience that a book meant for the pre-clinical students, should not contain much of applied discussions. The beginner only gets confused and tries to cram up the unnecessary applied details, leaving aside those portions more essential for him. The little 'applied' necessary for them should best be left to the teachers.

18th July, 1951

CC Chatterjee

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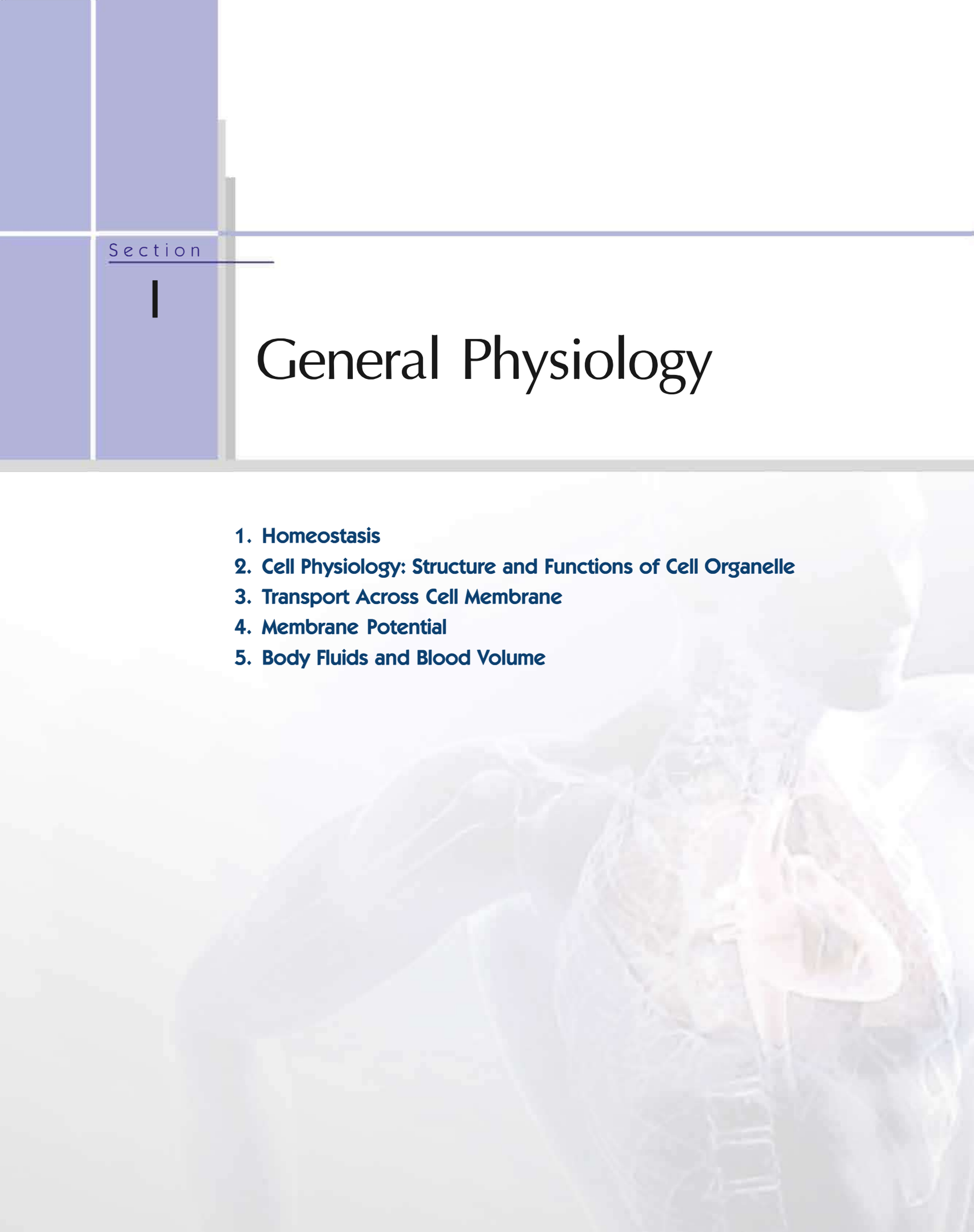
Section

I

General Physiology



Chapter

1

Homeostasis

INTRODUCTION

French Physiologist Claude Bernard in nineteenth century advocated that the 'stability of the internal environment (the *milieu intérieur*) is the condition for the free and independent life'. The organism's ability to keep a constant internal environment was termed as homeostasis by Walter Bradford Cannon an American Physiologist in twentieth century. Canon explained the concept of maintenance of constancy in internal environment.

The composition of ions, electrolytes, water, etc. varies between intracellular fluid and extracellular fluid. The extracellular fluid contains more amount of nutrients such as glucose, fatty acid, amino acids and ions such as sodium, chloride, bicarbonate while intracellular fluid contains higher amount of potassium, phosphate and magnesium. Inorder to keep a stable internal environment there is constant adjustments of chemical composites of extracellular and intracellular content which is aided by various transport mechanisms. Homeostasis is a state of dynamic equilibrium rather than a constant, unchanging state.

All systems of human body participate in maintenance of homeostasis. The physiological well-being of human body is maintained by the harmonious functional interaction between various systems.

Claude Bernard
(1813–1878)

Walter Bradford Cannon
(1871–1945)

Role of Various Systems of Body in Homeostasis

1. **Skeletal and muscular system:** The skeletal and muscular system is responsible for the movements of the human body. The locomotion of entire body is based on presence of bones to which muscles are attached. Movements are affected by muscle. They help in movement of limbs and locomotion. Locomotion aids in movement of individual to meet the need of food which provides nutrition to the human body and aids in homeostasis.
2. **Kidney:** The kidney plays vital role in whole body homeostasis. It maintains the extracellular fluid volume, electrolyte concentration, acid–base balance, removes waste product from the blood and also produces hormones erythropoietin which stimulates red blood cell production, renin which regulates blood pressure and calcitriol which promotes the renal reabsorption of phosphate and intestinal absorption of calcium.
3. **Cardiovascular system:** Heart pumps the blood in circulation and maintains the systemic circulation and this aids in delivering O_2 and nutrients to the various tissue and organ of the body.
4. **Respiratory system:** The main respiratory function of lung is to deliver oxygen to tissue and remove carbon dioxide from the body.
5. **Endocrine system:** It regulates the metabolism, growth and development, sleep, emotions, mood, sexual function, reproduction, stress response, tissue functions among many other important body functions via feedback mechanisms. Most of the mechanisms of the endocrine system are negative feedback. For example, adrenocortical releasing hormone (ACTH) and thyrotropin releasing hormone (TRH) are controlled by negative feedback mechanisms.

6. **Reproductive system:** It has a little role in homeostasis. The reproductive system relates in creation of progeny. Sex hormones like testosterone influence muscular growth. Estrogen, produced by the ovaries in females, is important for bone growth. Therefore, estrogen deficiency leads to impaired bone development.
7. **Digestive system:** It controls the absorption, and digestion of carbohydrates, lipids, proteins, vitamins and nutrient along the gastrointestinal tract.
8. **Special senses:** Vision, hearing, taste and smell also participate in homeostasis. Example: Vision and hearing aids in motor and sensory response to a stimuli while smell and taste promotes food and water intake.
9. **Nervous system:** It consists of central or somatic nervous system and autonomic nervous system. *Central nervous system controls important body functions such as* regulation of muscle tone, maintenance of posture and equilibrium, planning and programming of movements, co-ordination for movements, expression of emotions, learning, memory, speech, etc. *Central nervous system perceives sensory information and also co-ordinates motor response.* Autonomic nervous system controls sympathetic and parasympathetic functions.

FEEDBACK HOMEOSTASIS REGULATIONS

The homeostasis regulation involves the receptor, the control centre and the effector. The stimulus produces change in variable. The receptor receives information regarding the changing environment and conveys it to control system via afferent pathway. The control centre receives and processes the information obtained from the receptor. The information is send along the efferent pathway to the effector. Then the effector responds to the instruction of the control centre by either enhancing or opposing the stimulus. This is a process in continuity which restores and maintains homeostasis.

Hormones released by endocrine system regulate the activities of body cells. Hormones are released into circulation in response to stimulus. The stimulus may either decrease or increase the amount of hormone secretion. This self-adjusting mechanism is called feedback homeostasis regulation mechanism. The feedback mechanisms are of two types: Positive feedback mechanism or negative feedback mechanism.

When the response to a stimulus increases the original stimulus, it is known as **positive feedback mechanism** while when the response to a stimulus reduces the original stimulus, it is the **negative feedback mechanism**.

Examples of Negative Feedback Mechanism

1. **Thermoregulation:** The core temperature range of a human body is between 36.1°C and 37.8°C, with average of 37°C and this is considered to be the normal body temperature in an adult. As the body temperature increases, the sensory receptors detect and send the information to the hypothalamus. The hypothalamus responds to the stimuli by initiating vasodilatation and sweating (sweat glands in the skin are stimulated via cholinergic sympathetic nerve) and this decreases the body temperature. When core body temperature decreases, there is vasoconstrictor response via sympathetic nerve stimulation, shivering, along with adaptive change via psychological response (wearing warm clothes and curling up in the bed which reduces the surface area (skin) and this aids in preventing heat loss. Increase in metabolic rate, thermogenesis and shivering response helps to overcome hypothermic effects.

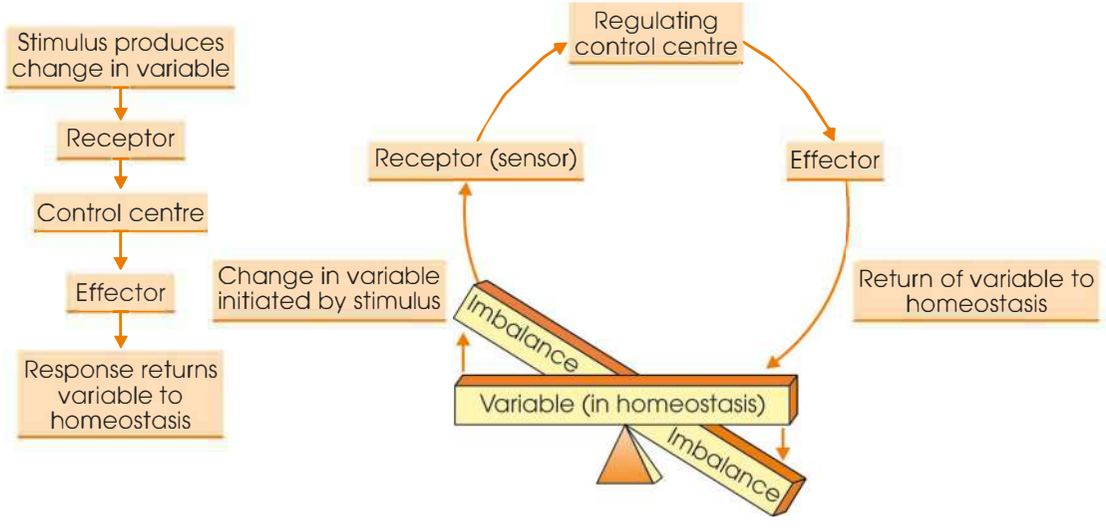


Fig. 1.1: Homeostasis control mechanism

2. **Insulin mediated control of blood glucose** level is an example of negative feedback. Blood glucose concentration increases after meals (stimulus). This releases insulin from pancreas, and it transports glucose from the blood into tissues (the response). Blood glucose concentrations then lowers down which decreases the secretion of insulin into the blood.

Insulin mediated control of blood glucose: Example of negative feedback mechanism

Example: Negative feedback mechanism—control of blood glucose level

Blood glucose level increases → insulin secreted from pancreas → Increases glucose uptake by cells (except brain) → glucose converted to glycogen in liver → blood glucose level decreases → insulin release from pancreas is inhibited.

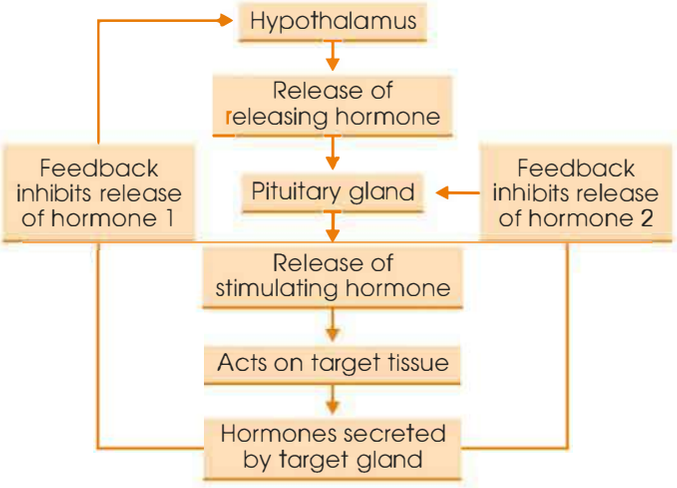


Fig. 1.2: Pituitary hormones and negative feedback mechanism

3. **Pituitary hormone release:** Release of releasing hormone from hypothalamus and stimulating hormone from pituitary gland is by negative feedback mechanism. The hormone then work on target gland to produced desired effect. Adrenocortical releasing hormone (ACTH), thyrotropin releasing hormone (TRH), etc. are released by negative feedback mechanism.
   Example: **Increase level of T_3, T_4 hormone** decreases the secretion of thyrotropin releasing hormone (TRH) from hypothalamus and thyroid stimulating hormone (TSH) from pituitary directly and also via TRH thereby decreasing the T_3, T_4 hormone level and *vice versa*.
4. **Increase concentration of carbon dioxide** in the blood, stimulates the chemoreceptors; which further stimulates the respiratory centre to increases the rate and depth of breathing. The increased ventilation removes more carbon dioxide and CO_2 level comes down.

Example of Positive Feedback Mechanism

Positive feedback mechanism brings over further increase in variable in response to initial increase in variable.

1. **Clotting cascade:** When the blood vessel gets damaged, platelets adhere to the injured site and release chemicals which further attract more platelets. The platelets continue to pile up and initiate clotting cascade. The clotting factor which is activated further acts as enzyme to activate the other clotting factors until a clot is formed.

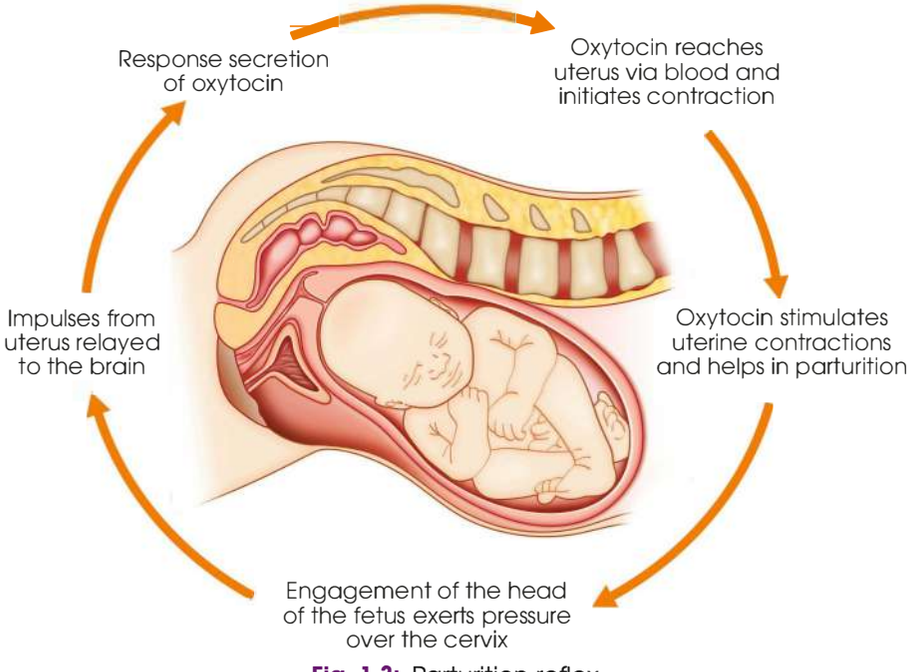


Fig. 1.3: Parturition reflex

2. **Parturition reflex:** At the full term of pregnancy at end of the third trimester the head of the fetus gets engaged and exerts pressure over the cervix and the sensory information of mechanical stretch of the cervix is relayed to the paraventricular and supraoptic nuclei of the hypothalamus which further increase the secretion of oxytocin from the posterior pituitary. Oxytocin acts on the myometrium which stimulates uterine contractions, and this in turn further increases pressure on the cervix until the fetus is delivered.
3. **Hodgkin's cycle:** Opening of set of sodium channel in a membrane (nerve, neuron, etc.) further activates opening of more sodium channel leading to depolarization.

Regulatory factor (R): It is a measure of accuracy of regulation system that leads to some residual change in the controlled variable, but is to a lesser extent. Thus, reaching to state of perfect harmony of physiological balance; may not be attainable.

R = Change with regulation/change without regulation.

Gain of control system

The degree of effectiveness with which a control system maintains constant condition is determined by the gain of negative feedback. The control system is not 100% efficient; some error always remain:

Gain = Correction/error

Examples

Calculation of gain:

1. The systolic blood pressure in a subject is 120 mm of Hg under physiological condition. The BP becomes 100 mm with regulation and 60 mm without regulation. What is the gain of the regulatory system?

   The error is 20 mm; correction is 40 mm.

   Thus, gain 40/20 = 2
2. A cold exposure which is expected to bring the body temperature down to 20°C actually brings it down only to 36.5°C.

   Thus, the observed change is only 0.5°C. The expected change without regulation is 17°C and correction is 16.5°C.

   Thus, gain 16.5°C/0.5°C = 33

   The gain of the system is 33.

Internal Factors Influencing Homeostasis

Genetics: Genetic predisposition leads development of certain genetic disorders and diseases.

External Factors Influencing Homeostasis

Lifestyle modification such as balanced diet and regular physical activities also aid in maintenance of homeostasis. Diet devoid of iron may lead to anaemia while balance diet will restore iron level. The regular physical activity improves mental and physical well-being, increases muscular mass and stability, and increases the ability of the cardiovascular system to deliver oxygen to the tissues.

EXAM-ORIENTED QUESTIONS

Essay

1. Define homeostasis. Enlist the various body systems involved in homeostasis. Discuss positive and negative feedback mechanism.

Short Notes

1. Describe positive feedback mechanism citing two examples.
2. Describe negative feedback mechanism citing two examples.

Chapter

2

Cell Physiology: Structure and Functions of Cell Organelle

INTRODUCTION

The living substance of plants and animals is described by the general term, *protoplasm*, which is bounded by a delicate membrane and contains various microscopic and sub-microscopic structures. The smallest unit of protoplasm, capable of carrying out independent existence, is the cell (Fig. 2.1). The word *cell* (L. *cella*—a storeroom, a chamber) was first introduced in the biology by Robert Hooke (1635–1703).

Robert Hooke
(1635–1703)

The cell is the structural and functional unit of the living matter and is capable of carrying on the processes of life independently. The *tissue* which form the body consists entirely of cell and of extracellular materials elaborated by cells.

Furthermore, growth, reproduction and continued responsiveness to stimuli are the characteristics of cells and not of their parts. In unicellular organism like *Amoeba*, a single cell can perform all these physiological functions, but in multicellular organism different cells have got various activities and accordingly these structures have been changed according to its functions. These functional differentiations are inevitable in a multicellular organism; and for this reason different kinds of cells become dependent upon one another.

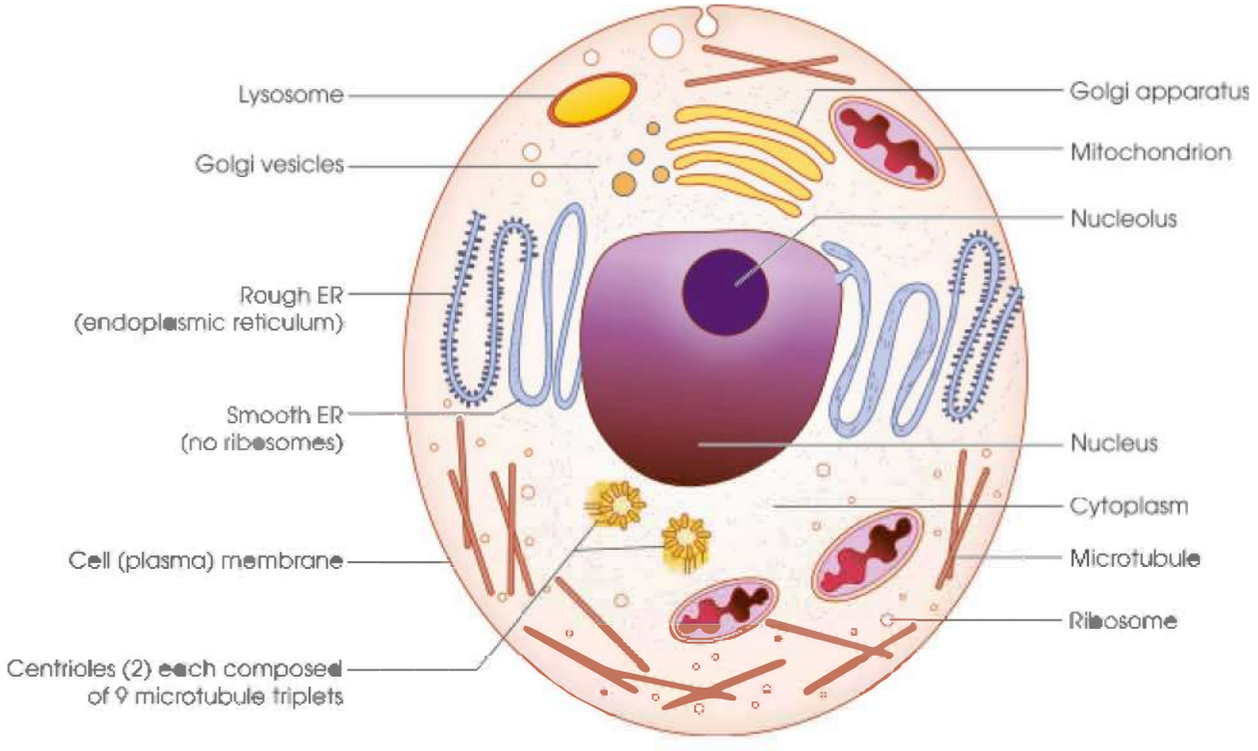


Fig. 2.1: Composite structure of a cell based on what is seen under light microscopes (diagrammatic representation)

These functional differentiations of the cells in multicellular organism are mostly dependent upon the specific properties of the protoplasm with which a particular cell is constituted. In the unicellular organism, the protoplasm of the single cell has got the basic properties *like irritability, conductivity, contractility, absorption, assimilation, excretion, secretion, growth and reproduction*. Thus in the unicellular organism, a single cell is capable of multiple functions. But in the multicellular organisms, all these properties of protoplasm are divided and delegated to specific cells and their protoplasm's as well. So for the particular functions of a cell, certain physical equipment capable of expressing their functions must be present in the protoplasm of those cells.

The protoplasm which bears the specific physical equipment of a particular cell unit can be divided into two major organizations:

1. ***Nucleus***, composed of *nucleoplasm* (karyoplasm), which controls the activities of a cell.
2. ***Cytoplasm*** (Gr. Kytos—hollow (cell) + plasma—thing formed) surrounding the protoplasmic nucleus, which carries out such activities.

In 1833, Brown recognized a conspicuous spherical body, *nucleus*, in the interior of plant cell and subsequently it was also found in all animal cells.

The nucleus contains chromosomes which harbour the genetic materials and typically one or more ***nucleoli***, which are concerned with the synthesis of proteins.

Robert Brown
1773–1858

Organelles: Nucleus and cytoplasm contain a number of components of characteristic form and staining properties. These components belong to organelles (organoids) and inclusions (paraplasm). The organelles are small internal organs of the cells, which are concerned as organised units of living substance possessing important specific functions in cell metabolism. The nature and number of organelles determine the volume and functions of the cytoplasm. The cytoskeleton is composed of microtubules, intermediate filaments and microfilaments.

Inclusion bodies: The ***inclusions*** are lifeless (non-protoplasmic) accumulations of metabolites, e.g. protein, lipoids (fatty, phospholipid and steroidal compounds) and carbohydrate, crystals, pigments, secreting droplets, etc. in addition, the nucleus and cytoplasm contain an apparently amorphous protoplasm which serves as a ground or matrix where organelles lie.

Syncytium: At some places, the cell membrane is incomplete and the protoplasm of the adjoining cells runs together, such as in liver, umbilical cord (Wharton's jelly), etc. Such a mass of undifferentiated protoplasm with scattered nuclei is called syncytium or plasmodium.

CELL STRUCTURE

In multicellular organs, the cells are not of same size and shape only due to the presence of differentiation of functions. But there are certain structural characteristic features which are common to them all. Each cell can be broadly divided into two principal units (Fig. 2.2):

1. Cytoplasm
2. Nucleus.

I. CYTOPLASM

The cytoplasm is the protoplasm which surrounds the nucleus and is bounded peripherally by the cell membrane.

Characteristic Features

1. Cytoplasm may be homogeneous, vacuolated, granular, reticular or fibrillar.
2. The ground substance of the protoplasm, hyaloplasm, contains a number of bodies and structures, vacuoles, and so on and also a number of very tiny particles which undergo active movement—Brownian movement.
3. The cytoplasm is capable of performing different kinds of work directed by the nucleus. As cytoplasm is specialized for performing special functions, the appearance as well as the protoplasmic constituents; are also changed from cell to cell. Hence, certain groups of cells will be identified by their nuclei and also by the appearance and the amount of cytoplasm. In the light microscope, the cytoplasm can be classified into two groups (Flowchart 2.1):
   a. Cytoplasmic organelles
   b. Cytoplasmic inclusions.

Membranous Organelles

Plasma Membrane

The plasma membrane or plasmalemma or cell membrane is the outer covering of the cell and is a flexible, responsive and dynamic structure. This membrane isolates the individual cell from its neighbours and takes part in the maintenance of the internal environment by active transport of ions and nutrients.

1. Under the light microscope, the membrane is thin and invisible, and sometimes the limits of a cell may be distinguished because the cell membrane is folded to form a cuticular or brush border or because mucoprotein or other cellular secretion is present to coat the membrane.

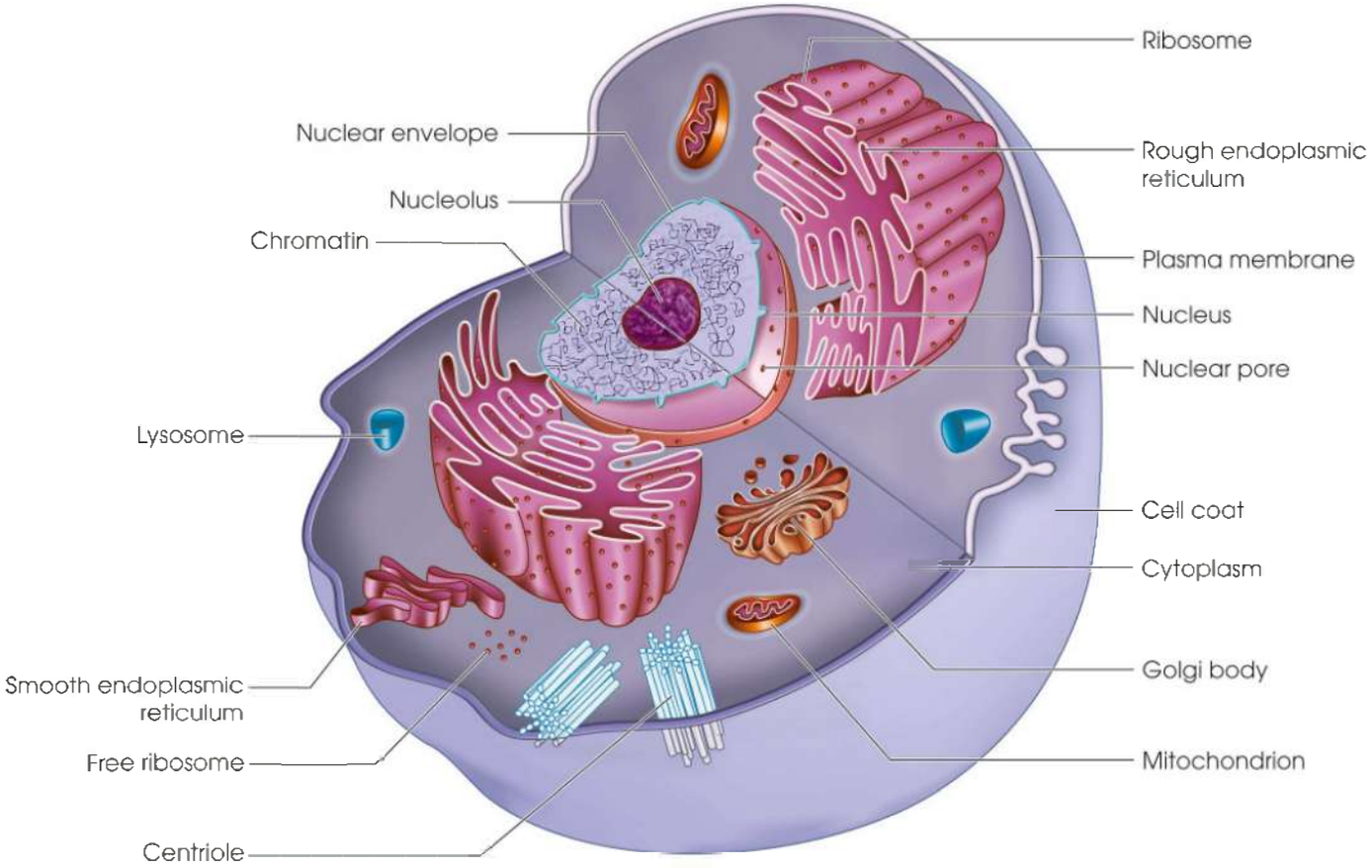
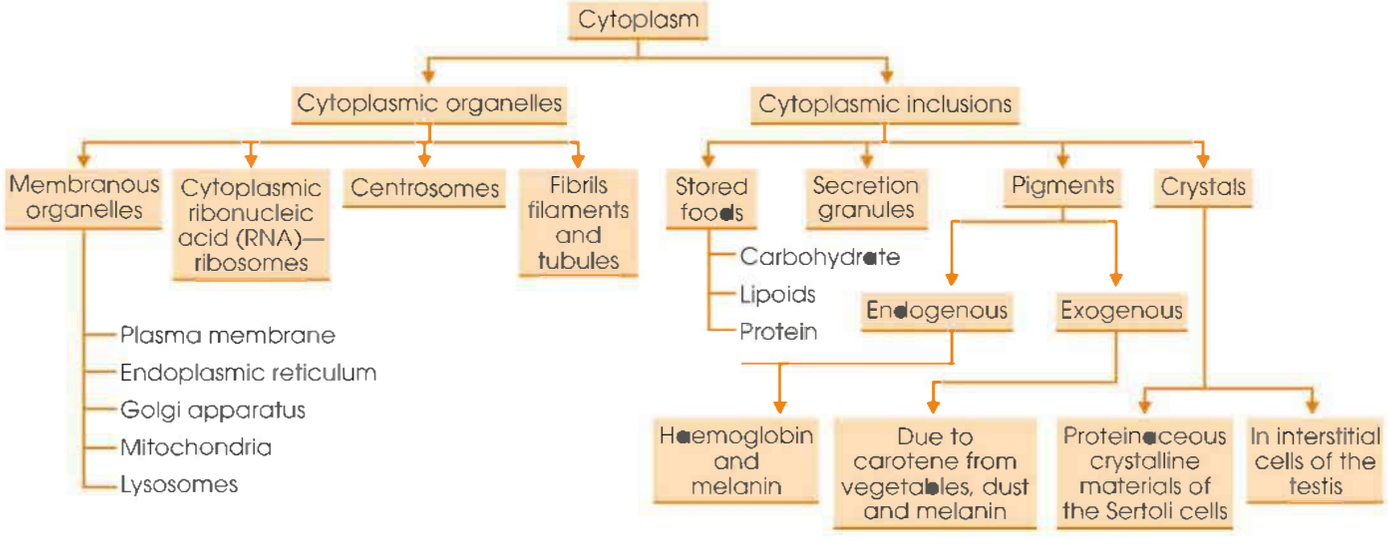


Fig. 2.2: Structure of animal cell

Flowchart 2.1: Classification of cytoplasm under light microscope



2. Cell membrane are 7.5–10 nm and is a trilaminar (triple-layered) structure. This basic trilaminar structure of all cell membrane is generally described as *unit membrane*. At high magnification with electron microscope, the cell membrane consists of double (bimolecular) layer of lipid molecules (light-stained), which are sandwiched within the two densely stained protein layers.
3. Lipid layer is mostly phospholipid of which the head end contains the water-soluble and positively charged phosphate group (polar or hydrophilic) while the tail end contains the water-insoluble and negatively charged lipid group (non-polar or hydrophobic).
4. Phospholipid molecules of the lipid layer are arranged in two rows where the hydrophobic ends

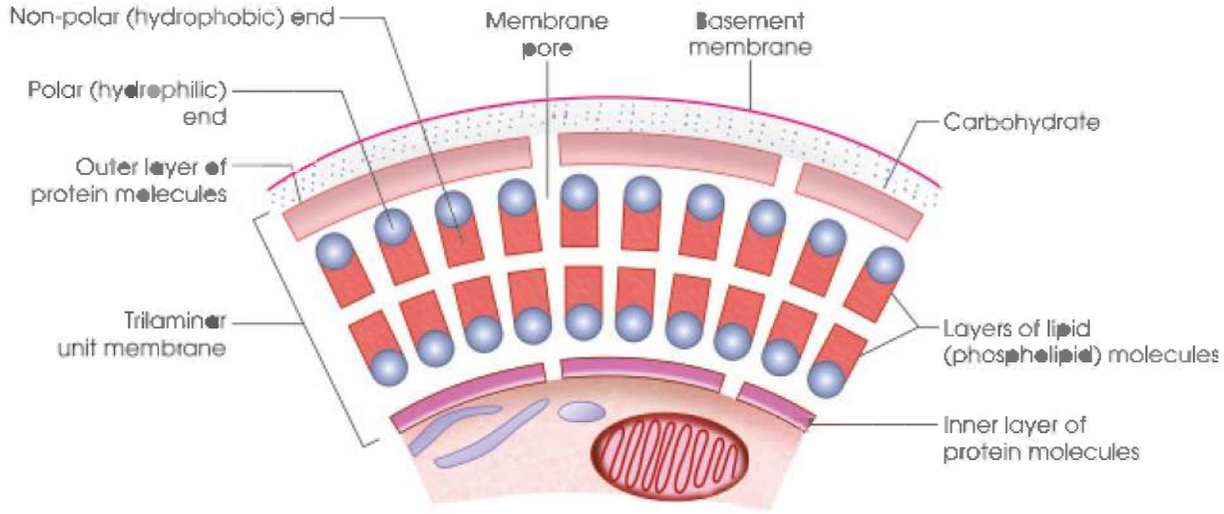


Fig. 2.3: Structure of cell membrane as seen under electron microscope (diagrammatic representation)

line up side by side in same row but abutting on the hydrophobic ends of the other row (Fig. 2.3). Thus, the non-polar groups of lipid molecules face each other but the protein molecules that form the inner and outer layers of the unit membrane are adsorbed on the polar groups.

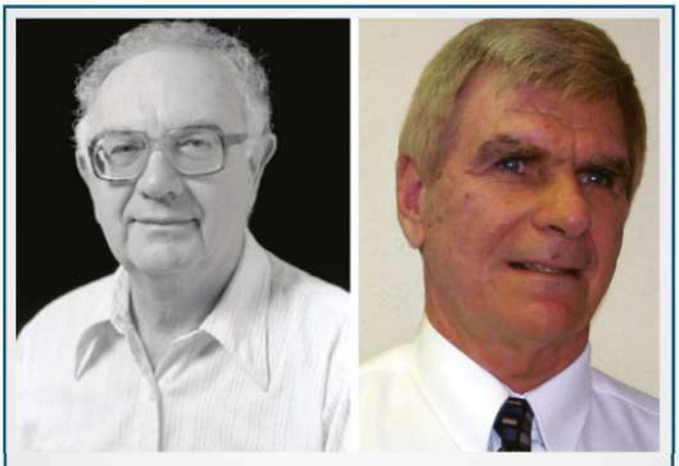
Cell Membrane

Structural detail of plasma membrane was proposed in 1935 by Hugh Davson and James Danielli. They proposed that lipid membranes are layers composed by proteins and lipids and the membrane has pore-like structures which allow specific permeability for certain molecules.

The Fluid Mosaic Model

SJ Singer and GL Nicolson proposed the Fluid Mosaic Model in 1972.

1. Cell membrane comprises approximately 55% of proteins, 25% phospholipids, 13% cholesterol, 4% of other lipids and around 3% carbohydrates.



SJ Singer and GL Nicolson

2. The fluid mosaic model (Fig. 2.4) states that cell membranes are composed of a phospholipid bilayer with admixed protein molecules freely floating around it. It is called fluid because individual phospholipids and proteins move side-to-side within the layer like it is a liquid; and is termed mosaic because of the topographic pattern produced by the scattered protein molecules.
3. The lipid bilayer consists of phospholipid molecules. The fatty acid portion of phospholipid is hydrophobic and faces the interior of the membrane while phosphate end is hydrophilic in nature and this faces the exterior of the cell to the ECF on one side and the ICF on the other side. Cholesterol which is lipid in nature and part of the cell membrane determines the fluidity of the membrane.
4. The proteins of cell membrane are glycoprotein and lipoprotein. The glycoprotein acts as receptors for hormones and neurotransmitters while lipoprotein functions as ion channels and enzymes. (a) Some proteins in the membrane are called intrinsic protein as they completely span the bilayer while others are extrinsic protein and are partly embedded in the bilayer. (b) Some of the intrinsic proteins are channel proteins which allow the movement of molecules that are normally too large to pass through the membrane by forming a tube-like structure that spans through the whole membrane while other intrinsic proteins are transport proteins (carrier proteins) which use energy in the form of ATP to actively move substances across the membrane. (c) Integral proteins also act as receptors for water-soluble chemical such as peptide and hormone. Peripheral proteins act as enzymes and coenzymes in order to carry out metabolic reactions.
5. The membrane carbohydrates are in form of glycoprotein or glycolipid. The glycol part of

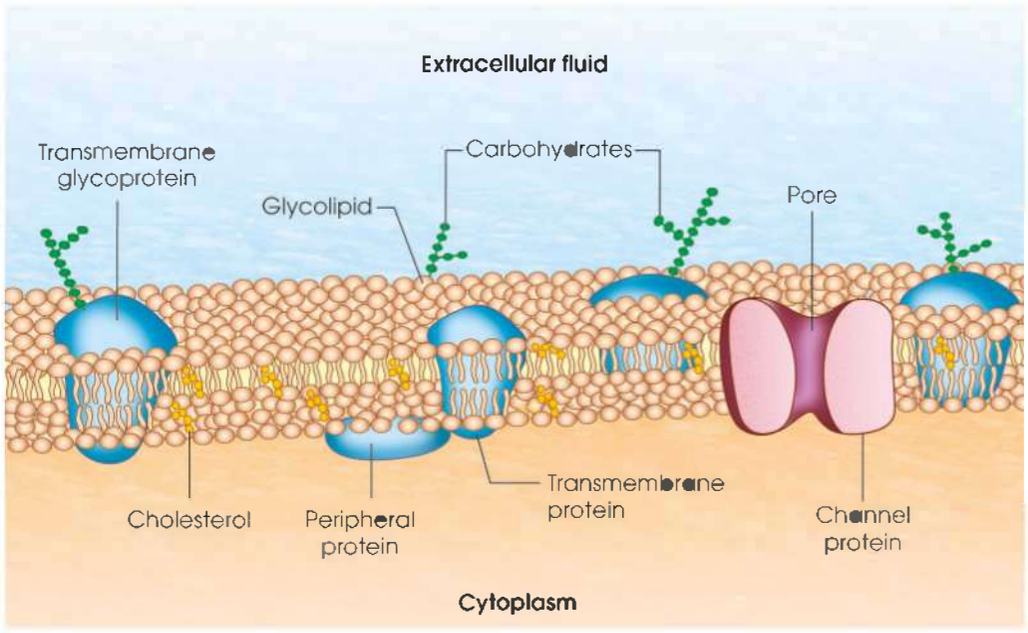


Fig. 2.4: Fluid mosaic model: Structure of cell membrane

carbohydrate molecules protrude to outside of the cell. (a) Proteoglycans which are carbohydrate substance ***bind*** to small protein core and are loosely attached to the cell membrane. (b) The **lose** carbohydrate coat over cell membrane forms the glycocalyx. (c) Both glycolipids and glycoproteins can act as cell receptor sites. Hormones may bind to them, as may drugs, to instigate a response within the cell. They are also involved in cell signalling in the immune system.

Discovery of cell membrane through time frame line

1895: Ernest Overton was first to hypothesized that cell membranes are made out of lipids.

1925: The phospholipids bilayer had already been proposed by Gorter and Grendel in 1925. Evert Gorter and François Grendel observed that the red blood cell membranes are formed by a fatty layer which is two molecules thick and thus they described the bilipid nature of the cell membrane.

1935: Hugh Davson and James Danielli suggested a model for the cell membrane describing it as a lipid layer surrounded by protein layers on either side.

1957: J. David Robertson, based on electron microscopy studies stated that all membranes in the cell, i.e. plasma and organelle membranes have similar structure: A bilayer of phospholipids with monolayers of proteins on either side and therewith established the "Unit Membrane Hypothesis".

1972: SJ Singer and GL Nicolson proposed the Fluid Mosaic Model explained the structure and thermodynamics of cell membranes which was supported by experimental evidence.

Functions of Plasma Membrane

- ***Transport:*** It facilitates the transport of materials across it. It is selectively permeable to certain substance and helps transports of substances needed for survival. The various transport mechanisms are:
  a. *Diffusion* (oxygen, carbon dioxide, small molecules, etc.) and passive osmosis (water).
  b. *Transmembrane protein channels and transporters* Aquaporins for water transport, ion channels for sodium and potassium transport, etc.
  c. *Endocytosis:* It is a process by which cell absorbs molecules by engulfing them: Example: Pinocytosis: Small vesicles, by a process of pinocytosis (drinking by cells), encircle and carry fluid within it across the membrane. By the pinocytic process fluid of smaller molecules (0.01–2.0 µm) can be engulfed in Fig. 2.5. The inducer can increase greatly the process of pinocytosis and phagocytosis: The phagocytosis (eating by cells) is similar to engulfing of solid materials by the amoeba.
  d. *Exocytosis* in which cells removed undigested products brought in by endocytosis, or to secrete enzymes and hormones or to excrete substances outside the cell.

In addition to transport, the cell membrane:

- Helps in the protection of cell. It surrounds cytoplasm of cell and forms a physical barrier between intracellular component and extracellular compartment.
- It anchors to the cytoskeleton to the extracellular matrix and thereby provide shape to the cell and maintains its structural integrity.
- Receives stimuli from the outside. The protein component of cell membrane acts as ligand receptors. The cell membrane contain receptor site for some hormones, immune proteins and neurotransmitters thus the cell recognizes and process these signals.
- Takes in food and excretes waste products.

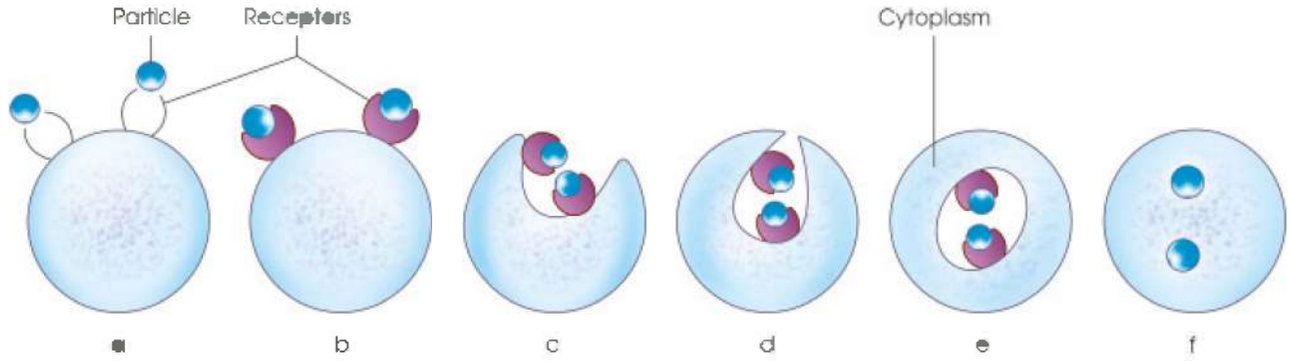


Fig. 2.5: Different phases of pinocytosis; the particle in solution approaching the cell surface (a and b) and gradually being drunk (c to f)

Note

When broken, it is quickly regenerated from the cytoplasm possibly with the help of surface tension.

- They aid in cell recognition (identifiers).
  Example: Glycoproteins (e.g. major histocompatibility complex, ABO blood group antigens). The surface protein markers which are embedded in the cell identify the cells, thus helps neighbouring cell to communicate with each other.
- The proteins in cell membrane act as enzymes and catalyze reactions and thus involved in metabolic process.

Endoplasmic Reticulum (Ergastoplasm)

These consist of network of canals (tubules) and vesicles (cisternae). These are three-dimensional and bounded by membrane of about 80 Å in thickness. The elements of the endoplasmic reticulum may connect intermittently with the plasma membrane at one hand and on the other hand with the outer nuclear membrane. Two types of endoplasmic reticulum have been recognized:

1. ***Rough-surfaced endoplasmic reticulum:*** This reticulum is studded with osmiophilic granules—the ribosomes lying in rows in contact with the membranes of the endoplasmic reticulum (Fig. 2.6). The roughness of the membrane is due to the presence of these granules—Palade granules.
2. ***Smooth-surfaced endoplasmic reticulum:*** This type of endoplasmic reticulum does not possess osmiophilic granules—the ribosomes at the outer border of the membrane. This is why it is smooth.

Functions of Endoplasmic Reticulum

1. As the smooth-surfaced endoplasmic reticulum is very abundant in the interstitial (Leydig) cells of the testis and in cells of the corpus luteum, this reticulum is concerned with the synthesis of steroid hormones.
2. In the parietal cells of the gastric mucosa, it is concerned with secretion of hydrochloric acid.
3. In the skeletal muscle, it (sarcoplasmic reticulum) is concerned in some way with binding of the Ca^{++} ions and also plays role in conducting impulses in the substances of muscle cells.
4. In the liver cells both types of reticula are concerned with the synthesis of protein and carbohydrate.

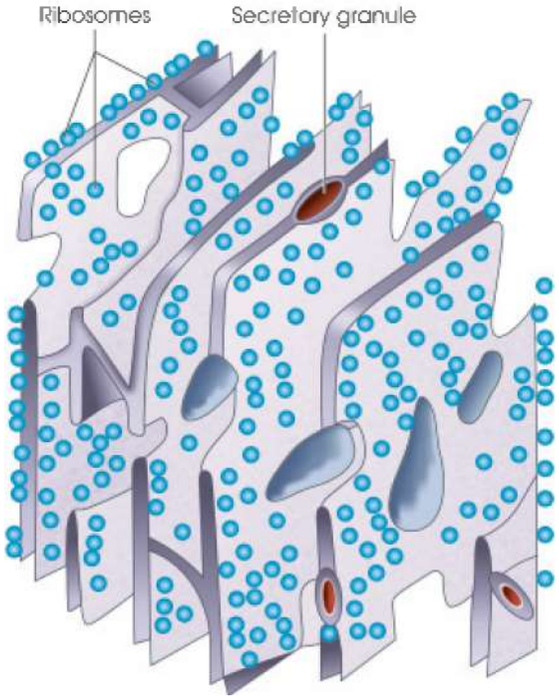


Fig. 2.6: Three-dimensional structural representation of endoplasmic reticulum (rough) showing embedded ribosomes and secretory granules on the wall (diagrammatic representation)

Microsome and Microsomal Fractions

Different fractions of cells can be obtained by centrifugation. After removing the nuclei and mitochondria, one such cell fraction obtained by centrifugation at about 20,000 to 100,000 g* was known as microsomal fractions and was erroneously known to be a separate type of cell organelle. But in electron microscopic

Note

*g = 981 cm per sec per sec = (981 cm sec^{-2})

studies of this microsomal fraction reveal that this is nothing but a chief composition of rough-surfaced and smooth-surfaced membranous vesicles of the endoplasmic reticulum. In the intact fractions the ribosomes are adherent to the rough-surfaced vesicles. Smooth-surfaced vesicles are also present in some portions of microsomal fractions. So there is nothing to believe that the microsome or microsomal fractions have got a discrete and independent structure in the cytoplasm.

Golgi Apparatus (Golgi Complex)

Camillo Golgi
1843–1926

The Golgi apparatus was first discovered in 1898 by Camillo Golgi.

The structure (Fig. 2.7) looks like a network of fine threads (Golgi network) or irregular granular material. It is usually located near the nucleus, and in the gland cells found between the nucleus and apex of the cell. Following main structures can be observed in Golgi apparatus under electron microscope.

1. *Flattened (distended) vesicles:* These are the most prominent vesicles in the Golgi apparatus. In a longitudinal section it looks like tubules arranged in stack. The peripheral portion of each vesicle becomes distended with its content and then the distended portion becomes separated from the vesicle to constitute the ovoids to round-shaped secretory vesicles.
2. *Secretory vesicles:* Under electron microscope the secretory vesicles are not normally visible until these are budded off from the distended peripheral end of flattened vesicles. These vesicles become electron dense only after being distended with their contents—the protein material. Recently it is known that protein material after being synthesised in the rough surface (granular) of the endoplasmic reticulum (vide ribosomes) is stored in the flattened vesicle and the secretory vesicle as well. The contents of the secretory vesicles are ultimately discharged at its cell surface as zymogen granules. Protein materials, being synthesised in the ribosome of the endoplasmic reticulum, are not transferred to the flattened vesicles directly but through a via medium—the microvesicles of the Golgi apparatus.
3. *Microvesicles:* These are small in size and their diameters are about 40 micron. Microvesicles have been considered to be the carrier systems of the protein molecules synthesised in the rough surface of the endoplasmic reticulum to the flattened vesicles. From autoradiograhic studies Caro and Palade have described that protein molecules, after being synthesised, first appear in the microvesicles and then in the flattened vesicles or the secretory vesicles. In certain cells these are numerous in between the region of the rough-surfaced endoplasmic reticulum and the flattened vesicle.

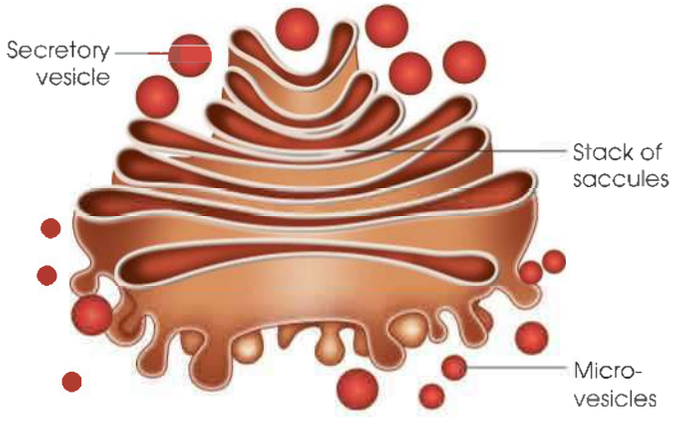


Fig. 2.7: Structure of Golgi apparatus (diagrammatic representation)

Functions

1. It is probably concerned with synthetic process of the cell, specially secretions. The secretory substance, being synthesised by the endoplasmic reticulum, passes to the Golgi apparatus which possibly modifies the products of synthesis by concentrating and chemically altering it to some extent.
2. In addition, Golgi apparatus independently synthesises polysaccharide part of glucoprotein secretion.

MITOCHONDRIA

These are relatively solid bodies, granular, rod-shaped or filamentous in form and remain scattered throughout the cytoplasm (dimensions varying from 0.5 to 5.0 microns). In humans, mitochondria mDNA is inherited solely from the mother.

Characteristic Features

1. They are surrounded by a trilaminar double membrane, the inner one of which remains folded and forms a number of partitions, the cristae mitochondriales. These cristae may be *complete*, *septate* or *incomplete*.
2. Numerous projecting particles known as elementary particles are present on the inner mitochondrial membrane and cristae. The fluid of the intramitochondrial space is called *matrix*. The matrix may contain small dense granules. It has been postulated that most of the enzymes of the mitochondria are present on the elementary particles, the coenzymes

in the matrix, and inorganic ions like calcium and magnesium in the granules (Fig. 2.8).

3. The number and size of mitochondria give an indication of the energy requirements of a particular cell. They are more numerous and longer in the young and active cells. The sites where Krebs cycle enzymes (*cyclophorase system*) are present in high concentration like liver, kidney (Fig. 2.9) and heart; possess mitochondria in a larger amount. They are comparatively few in skeletal muscle fibres where the enzymes are also less. Mitochondria are comparatively rare in cancer cells which derive their energy from anaerobic glycolysis.
4. For light microscopy, mitochondria can be stained supravitally with Janus Green B. Sometimes they remain grouped together at one pole of the cell. Chemically they contain about 40% of fat (neutral fat, phospholipids and cholesterol) and two different kinds of proteins. Ribonucleic acid (RNA) remains in combination with protein as ribonucleoprotein (RNP).

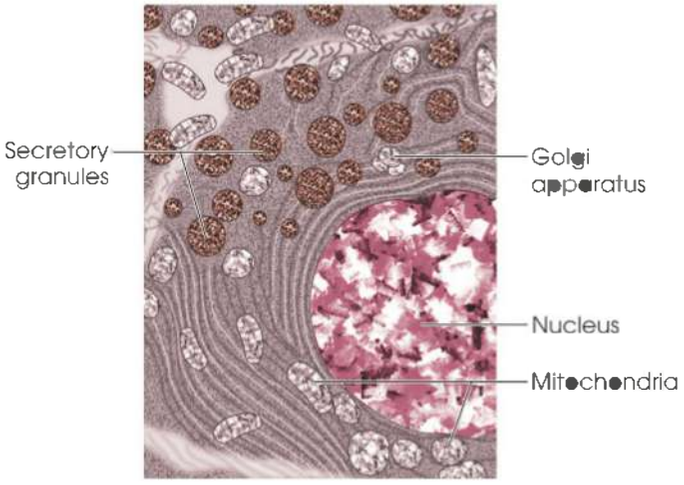


Fig. 2.8: Electron micrographic representation of the basal portion of the renal tubular epithelium (rat) embedded on basement lamina showing part of the nucleus, Golgi apparatus, numerous mitochondria

Functions

1. **Oxidative phosphorylation and ATP formation:** All the enzymes of the citric acid cycle with the exceptions of certain dehydrogenase are present in the fluid content of the mitochondria. The acetic acid—the breakdown products of pyruvic acids, fatty acids and also of amino acids are fed into the mill of Krebs cycle enzymes of mitochondria. In presence of oxygen, the Krebs cycle runs within the mitochondria with the catalyzing help of another set of enzymes—*respiratory enzymes*. These are *flavoprotein* enzymes and *cytochrome*, and present in the inner membrane of the mitochondria. These respiratory enzymes use certain products of Krebs cycle as substrate. These enzymes present in the mitochondria help in oxidative phosphorylation and are the site for formation of adenosine triphosphate (ATP) which is the high energy-producing substance in the cell. The mitochondria supply 95% of cell's energy and are called *powerhouse* or *power plant* of the cell.
2. **Protein synthesis:** Recently it has been studied that the mitochondria also possess some amount of deoxyribonucleic acid (DNA). There is also some indications that RNA is also synthesised in association with DNA and this RNA helps in synthesising certain amount of protein in the mitochondria.

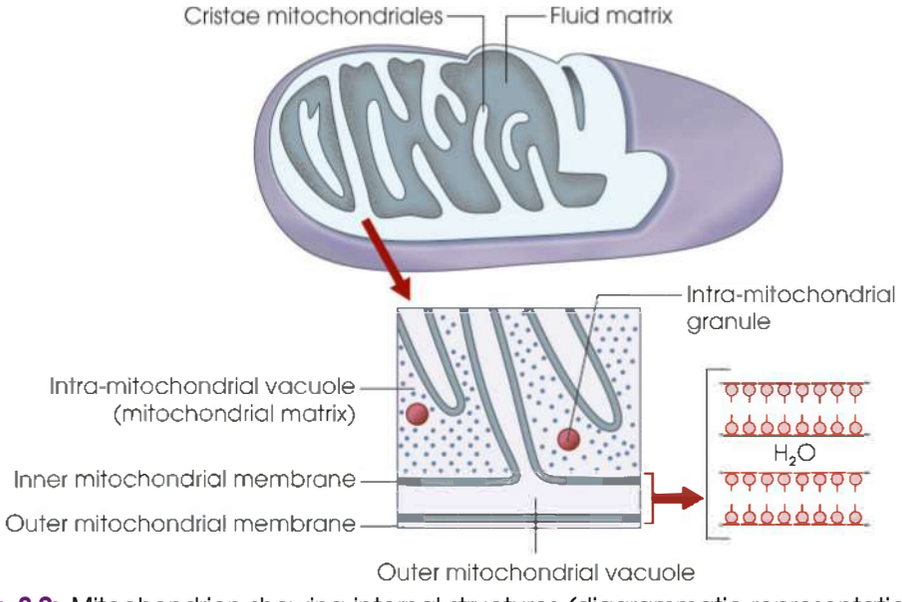


Fig. 2.9: Mitochondrion showing internal structures (diagrammatic representation)

Leber optic atrophy also called **Leber's hereditary optic neuropathy** (LHON) is a mitochondria inherited degeneration of retinal ganglion cells and its axons leading to acute or sub-acute loss of central vision. **Leber optic atrophy** was first described by the German Ophthalmologist in 1871 by Theodor Leber (1840–1917).

Theodor Leber

LYSOSOMES

The lysosome has been discovered and recognised as a separate cytoplasmic organelle. In 1955, Christian de Duve and his association first postulated this existence.

Christian de Duve
1917–2013

Characteristic Features

1. Its size varies from 0.25 micron to 0.50 micron.
2. These are membranous vesicles having a spherical and bag-like structure and are filled with hydrolytic enzymes capable of demolishing large molecules (protein, carbohydrate, lipids and nucleic acids) into fragments which may then be oxidised by the mitochondria.
3. The lysozymes are present in all animal cells except in the *erythrocytes*. Certain leucocytes contain a specific type of granules which are considered to be lysosomes.
4. The enzymes of lysosomes are potent enough to digest its own cellular contents in which it inhabits but in ordinary conditions it is not so happened. Under certain conditions it may digest its own cellular content and for this reason it is sometimes described dramatically as *suicide bag*.
5. Lysosomal enzymes, like other proteins, are synthesised by the ribosomes of granular endoplasmic reticulum of the same cell and are transported to the Golgi apparatus in the form of microvesicles for storage. The stored enzymes are ultimately budded off from the stack of *Golgi saccules* and developed into primary or inactive lysosomes.
6. Primary lysosome develops into active or secondary lysosome (autolysosome) only during intracellular digestion. The fusion of primary lysosome with the particle brought into the surface of the cell (phagosome) or the intracellular material gives rise to *secondary* or *active lysosome*.
7. Lysosomes that digest the degenerated mitochondria or other intracellular structures are specifically described as *cytolysosome*.
8. Lysosome enzyme do not digest own cytoplasm because enzymes are acid hydrolase and function at acidic pH. Hence, if the lysosomes break it unables to destroy the organelles efficiently at cell pH.

Functions

1. **Digestion:** The general function of the lysosome is the intracellular digestion and for this reason it is sometimes described as *digestive apparatus* of the cell (Fig. 2.10). When a particulate or food substance comes in contact with cell surface, the substance is engulfed by the cell membrane forming a membranous vesicle with engulfed particle. The lysosome thus meets with the particle and is fused. *Hydrolysing enzymes* of the lysosome thus digest the food particle. However, this is happened in some kinds of cells, because in such cells the particulate is not utilised by the cell without the help of lysosomes.
2. **Cell necrosis or autolysis:** When the cell is damaged, the lysosomal digestive enzymes are released and digest off cellular elements. In case of degeneration of its own cellular organelles, such as mitochondria and ribosomes during acute anoxia, the lysosomes then digest them only to maintain the energy requirement of the cell.
3. Phagocytosis is also a remarkable function of lysosome. Leucocytes contain lysosome like granules which destroy the ingested bacteria. During this process, the blood cells themselves are destroyed along with the bacteria. For this phagocytic (phagocytotic) functions, certain cells contain (macrophages and polymorphonuclear leucocytes) recognisable lysosomes. In damaged cells, the number of lysosomes; are increased greatly. Thus, it is clear that lysosome stores safely a quite good number of destructive enzymes within the cells only to remove the foreign material through phagocytosis. It has been postulated that the lysosome brings about death of cells only to make space for the new cells. Systemic growth of cells; are made through the planned death of cells through the lysosomal activity.
4. **Stimulus for cell division:** The rupture of lysosome is the stimulus for cell division and alteration of the behaviour of lysosome may be one of the causes of cancerous growth. Furthermore acrosome of the spermatozoa appears to be a large lysosome.

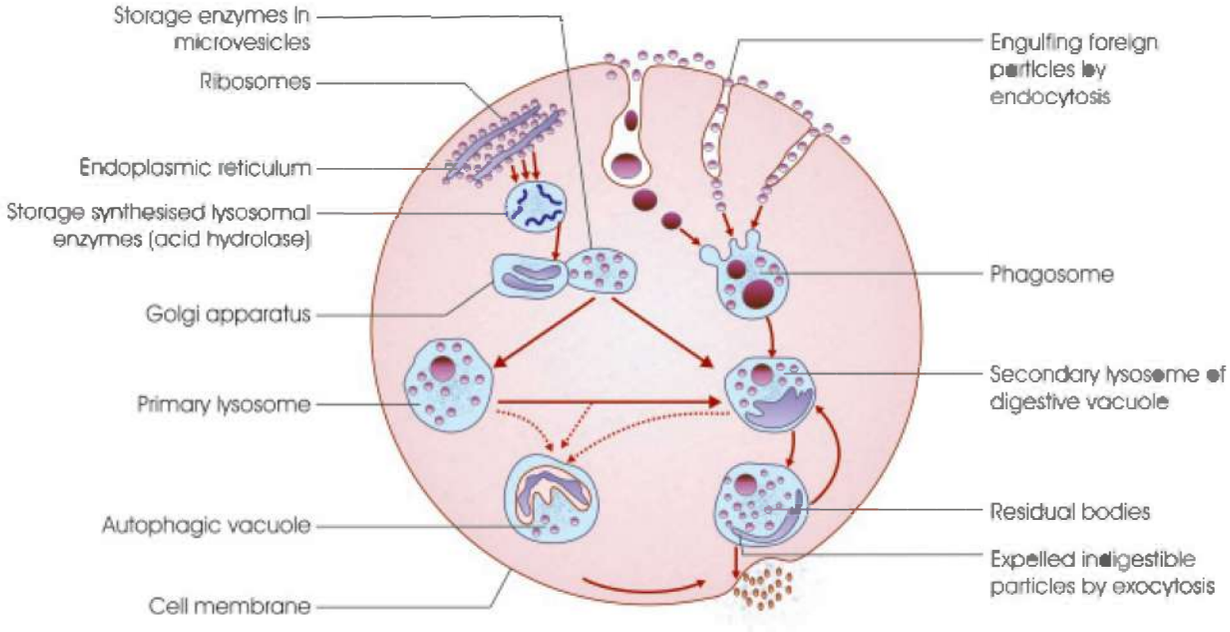


Fig. 2.10: Digestive function of lysosomes (diagrammatic representation)

Lysosomal storage diseases: These are inherited metabolic disorders that lead to accumulation of various toxic materials in the cells as a result of enzyme deficiencies. There are around 50 lysosomal storage disorders affecting different parts of the body, including the brain, skin, heart, skeleton and central nervous system. The few common lysosomal storage disorders are:

Aspartylglucosaminuria: The patient presents with recurrent history of infections, hernias and diarrhoea. The other noted features seen are enlargement of the liver (hepatomegaly) and enlarged tongue (macroglossia).

Cystinosis: The excessive storage of the amino acid cystine in all cells of the body results in marked growth retardation, impaired kidney function and increased sensitivity to light.

Gaucher disease types I, II, and III: Gaucher diseases are of three distinct types; types I, II and III). The most affected individuals have type I Gaucher disease and presents with signs and symptoms of bruising, chronic fatigue, and hepatosplenomegaly. Gaucher disease type II occurs in newborns and infants and these patients develop neurological symptoms such as difficulty swallowing, involuntary muscle spasms and the loss of previously acquired motor skills. Gaucher disease type III occurs the first decade of life. The patient presents with complains of inability to coordinate voluntary movements and muscle spasms of the arms, legs or entire body.

Niemann-Pick disease types A/B, C1 and C2: It is a group of inherited disorders related to fat metabolism. Certain characteristics common to all types include enlargement of the liver and spleen. The children suffering from Niemann-Pick disease type A or C may experience. Liver failure without neurological symptoms, low muscle tone, delayed motor development commencing before age 2, progressive live failure which begins in infant life of seizures.

RIBOSOMES OR CLAUDE'S PARTICLES

They are ribonucleoprotein in nature and are found scattered throughout the cytoplasm either singly or in groups (*polyribosomes or polysomes*) and range in size from 100 to 150 Å in diameter. They are so rich in RNA that they may contain as much as 60% of total RNA in the entire cell. These ribonucleoproteins are concerned with protein synthesis and their presence gives the membrane a strong basophilia. Cells responsible for the secretion of proteins have an abundance of granular reticulum.

Functions: Being attached to the rough-surfaced endoplasmic reticulum ribosomes (Fig. 2.6) synthesise protein and the canals of the reticulum work as passageways through which proteins move on way to Golgi apparatus. So ribosomes are *protein factories*.

CENTROSOME

It consists of another specialised part of clear cytoplasm, the *centrosphere*, containing in its interior two or more deeply staining particles—the *centriole* (generally arranged in pairs, i.e. *diplosome*) lying close to the nucleus in the resting cell.

Characteristic Features

1. Electron microscopy has revealed that the centriole is an empty cylinder which is 3 to 5 micron long and the compact walls of centrioles are made of thin parallel nine tubular structures longitudinally arranged. Each tubule consists, in turn, of three subunits or *triplets*.

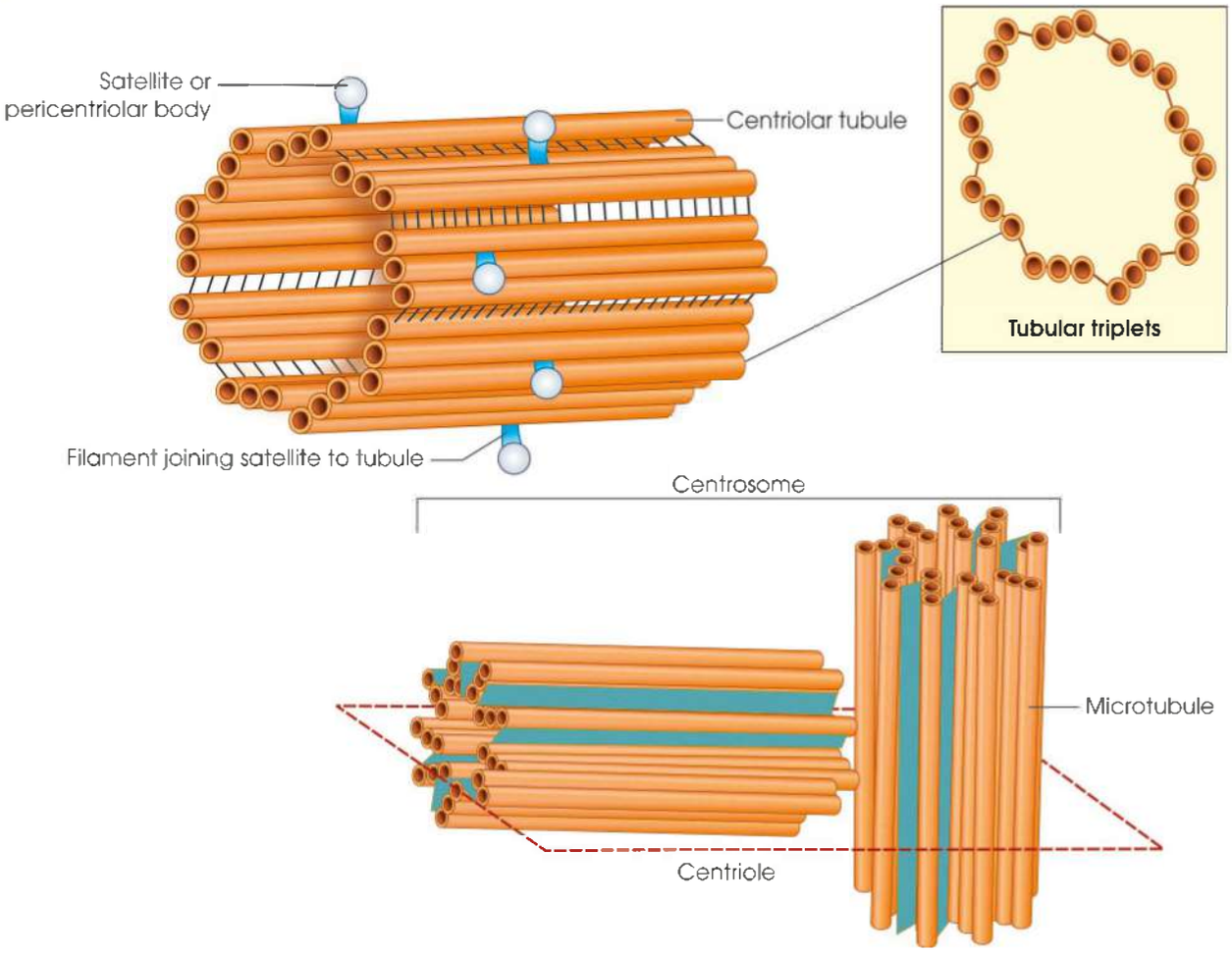


Fig. 2.11: Arrangement of centriolar tubule, nine in number, each tubule consisting of three centriolar subunits in right-sided inset (diagrammatic representation)

2. The *pericentriolar bodies* or *satellites* which are round in shape are attached to centriolar tubules by a filament of chromatid (Fig. 2.11).
3. *Centriole is closely related to spindle formation during mitosis* (*normal cell division*) and also in sustaining other fibrillar structure like cilia and flagella. At the beginning of cell division, the centriole and centrosome divide.
4. A system of radiating lines, made up of microtubules, then grows out from each of the two newly formed centrioles and the whole structure, due to its star-like shape, is called aster. The two asters grow in size and repel each other till they occupy the opposite poles of the elongated cell. The diverging fibres from the two asters meet at the equator of the cell and form the achromatic spindle.
5. Along the fibres of this spindle; half the number of chromosomes—formed by the breaking up of the nucleus in the mean time—are drawn towards each aster and then the cell divides. Each daughter cell thus carries one aster and half the number of chromosomes.
6. After division the radiating lines (microtubules) of the astral system disappear leaving the centrioles and the centrosome only. Nerve cells have got no centriole and are incapable of reproduction.

Functions: Centrioles control polarisation of spindle fibres and play some part in their formation.

Plasmosin: It is a constant and characteristic constituent of cytoplasm. It consists of elongated protein particles rich in deoxyribonucleoprotein. They join up lengthwise and form the so-called intracellular fibrils, e.g. tonofibrils in the epithelial cells, myofibrils in the muscles, and neurofibrils in the nerves.

Vacuoles: They are demonstrated by placing living cells in a dilute neutral red dye solution. Some amount of lipid materials are often found around the vacuoles.

Nissl bodies (granules): They are found in nerve cells.

II. NUCLEUS

The nucleus (Fig. 2.12) is generally a round body occupying the centre of the cell. Its shape, size, position and number vary. The nucleus may contain many lobes.

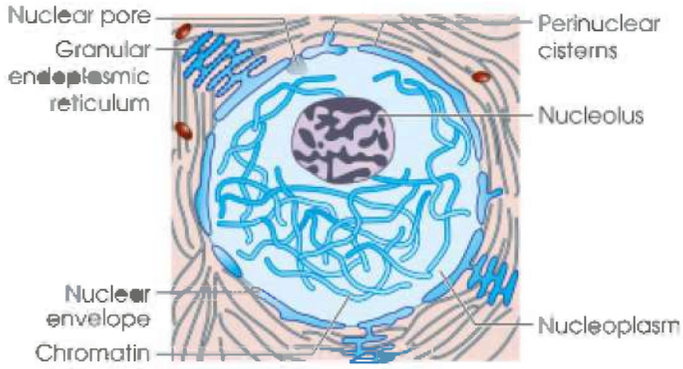


Fig. 2.12: Structure of nucleus

Usually most mature cells possess a nucleus, but there are certain larger cells in the body which may contain more than one nucleus. If the nucleus is removed, the cell dies.

Characteristic Features

1. The nuclear material differs from the cytoplasm in several respects. First, it is more opaque to the ultra-violet rays. Secondly, it shows many selective staining reactions but usually takes basic stain, while cytoplasm may take neutral, basic or acid stain. Thirdly, the nucleus is very rich in deoxyribonucleic acid (DNA), while cytoplasm is rich in ribonucleic acid (RNA).
2. The nuclear membrane is also a unit membrane. Surrounding the nucleus, there is a lipoprotein nuclear envelope. This envelope is double-layered and the spaces between two folds are known as perinuclear cisterns.
3. In the apparently quite permeable membrane of the nucleus, pores (areas of discontinuity) of about 6 micron diameter are closed by a thin homogeneous membrane which permits passage of molecules from the nucleus to the cytoplasm (Fig. 2.13). This indicates connecting link between the genes and ribosomes, the site of cytoplasmic protein synthesis.

Nucleolus

Inside a nucleus there is usually single or may be from two to five smaller bodies known as nucleolus or nucleoli which lie among nuclear sap (karyoplasm) and among the pale-staining karyoplasm chromatin granules lie.

Characteristic Features

1. The nucleolus comprises the irregular network or rows of fine granules, nucleolonema as seen in EM. The nucleolus loses its identity during cell division. The nucleolus contains still smaller nucleus known as nucleololus or nucleolinus or nucleolonucleus.
2. The nucleus is responsible for the synthesis of messenger RNA (mRNA) which carries the genetic information in code through the pores in the nucleus.

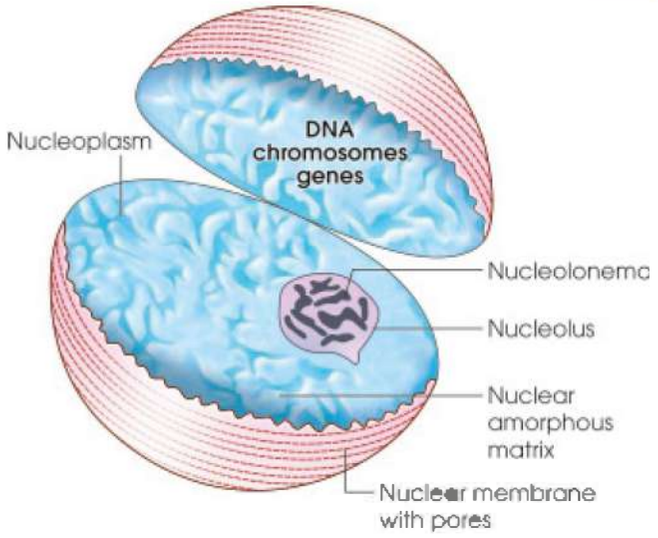


Fig. 2.13: Diagram shows chromosomes extending through amorphous matrix and innermost limit of cytoplasm and porous nuclear membrane

Recently it has been studied that mRNA is formed in the strands of DNA within the nucleus and actually the strands of DNA (Fig. 2.14) direct the synthesis of specific mRNA.

3. The mRNA thus formed in the nucleus comes out of the nucleus for carrying the DNA-message to the protein-synthesising centre (ribosome) of the cytoplasm. Here it is attached to the ribosome and stretched out (Fig. 2.15) on its surface to direct the protein synthesis.
4. The amino acid sequences in the protein are determined by the transfer or soluble RNA (tRNA or sRNA) which recognises the code for the amino acids; the tRNA is carrying to the particular spot of the ribosomal surface where the mRNA is already attached.
5. Main function of tRNA is to transfer the specific amino acid to the template of mRNA for correct amino acid sequence. There are 20 specific tRNA for 20 specific amino acids. With the help of these tRNA the protein is synthesised with proper sequences at the template of the mRNA and are stretched on the surface of the ribosome.
6. After completion of protein synthesis, the protein molecules become detached from ribosomal particles and pass into the canal of the endoplasmic reticulum. From here it passes into the Golgi complex.

Structure of Nucleus

According to staining reactions, two types of nucleoli are found. Those taking basic stain are called karyosome, and those taking acid stain, plasmosome. The body of the nucleus is made up of a fine network of a particular substance, called linin. The meshes of this network are filled up with clear protoplasm—the nucleoplasm (karyoplasm, karyolymph or nuclear sap).

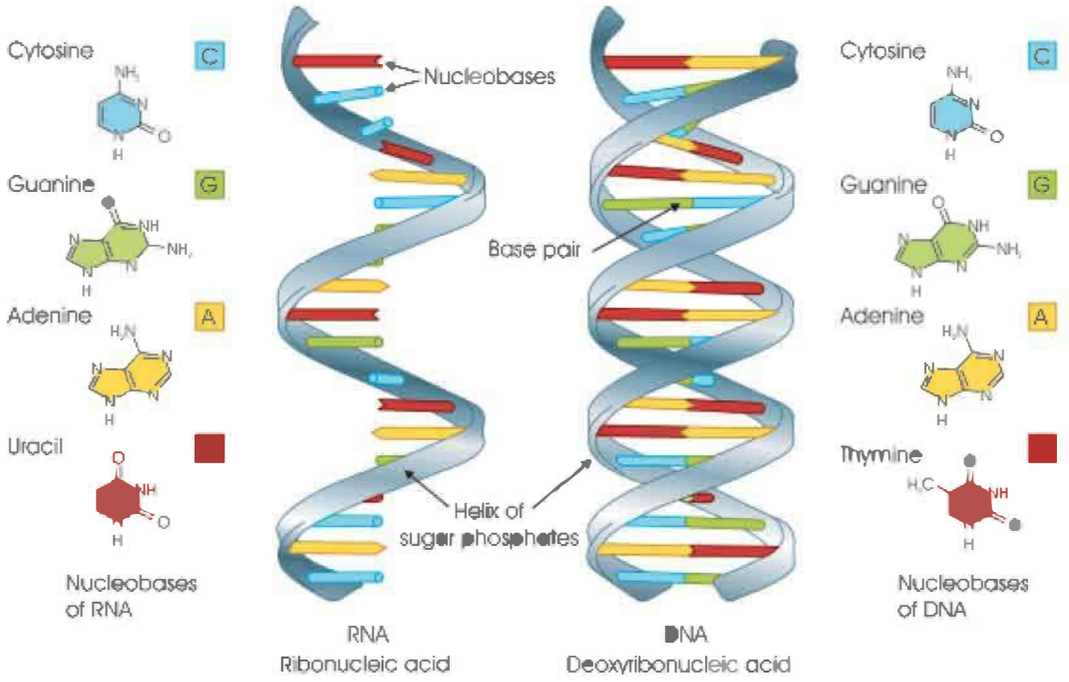


Fig. 2.14: Structural difference of helical arrangement of DNA strand and RNA strand having joints with their corresponding nitrogenous bases (diagrammatic representation)

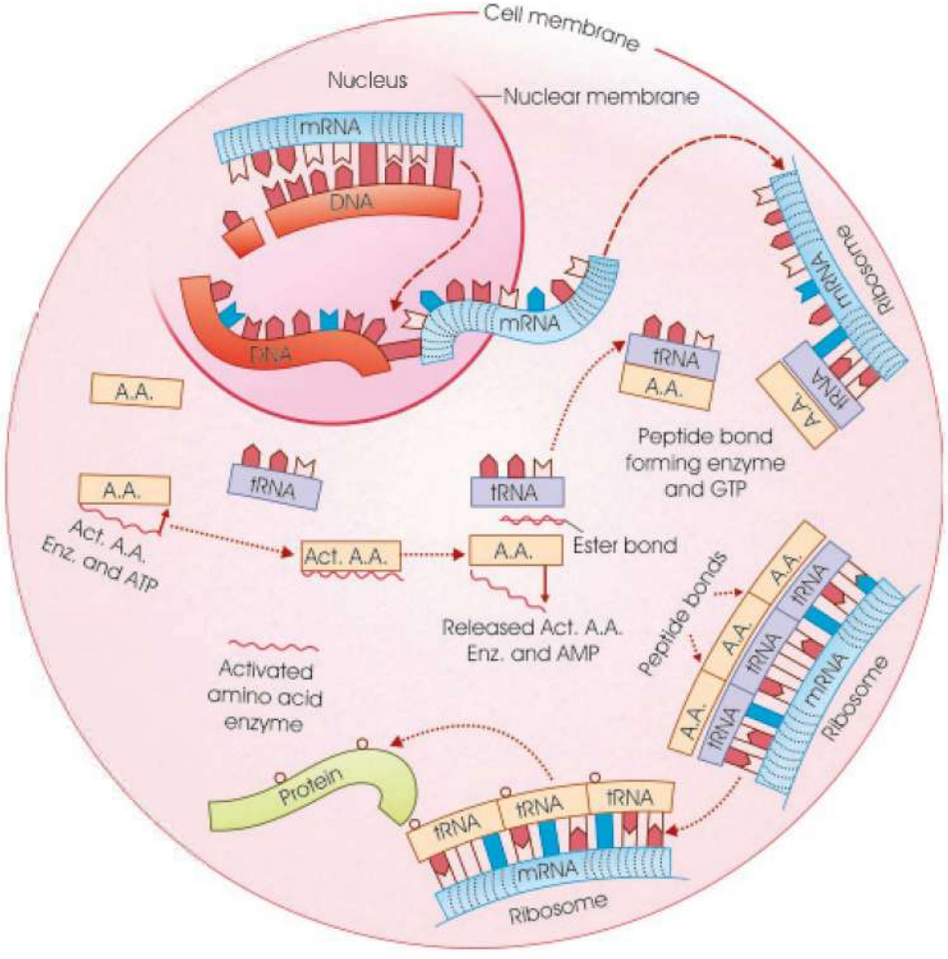


Fig. 2.15: Diagrammatic representation of the functions of DNA, mRNA and tRNA in protein synthesis
A.A.—amino acid; Act. A.A. Enz.—activated amino acid enzyme; Act. A.A.—activated amino acid; ATP—adenosine triphosphate, AMP—adenosine monophosphate; GTP—guanosine triphosphate

In unstained specimens nothing more can be seen. But in stained specimen, numerous particles of blue-staining materials of irregular shape but smaller than nucleoli are found in the nucleus. This material is generally described as chromatin.

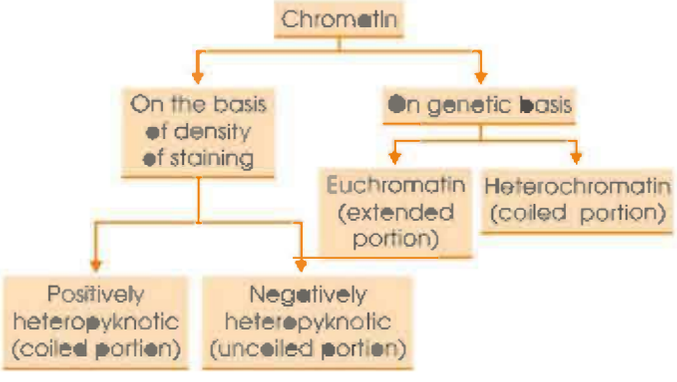
CHROMATIN

Chromatin contains different genes which determine the heredity of the cell, and again the reassembly of different chromatins form chromosome, it likely would invite different accidents. There is likely chance that the genes belonging to one chromosome would have been incorporated into another chromosome.

Characteristic Features

1. Chromatins seen in interphase nucleus are densely stained scattered portions of chromosomes and these are visible in the microscope. This visibility of the chromosome mainly depends upon the coiling and uncoiling of the chromosomes.
2. During cell division the chromosomes become tightly coiled and this coiled chromosomes or the coiling portions of the chromosomes are stained deeply. But following cell division or in interphase stage the coiled chromosomes become uncoiled all over its length but some portions still remain coiled. These tightly coiled portions become visible as granules or granular mass in the interphase nuclei.
3. Chromatin can thus be described as heteropyknotic, because of having one or two densities. On the basis of density in staining, the chromatin can be grouped into two types (Flowchart 2.2). The coiled portions of the chromosomes are called the positively heteropyknotic and the uncoiled (expanded) portion is called the negatively heteropyknotic.
4. But on the genetic basis, there is another terminology for chromatin. The extended portion of the chromosome is genetically active and is called euchromatin and the coiled portion (positively heteropyknotic) is genetically inactive and is termed heterochromatin (Fig. 2.16).

Flowchart 2.2: Classification of chromatin



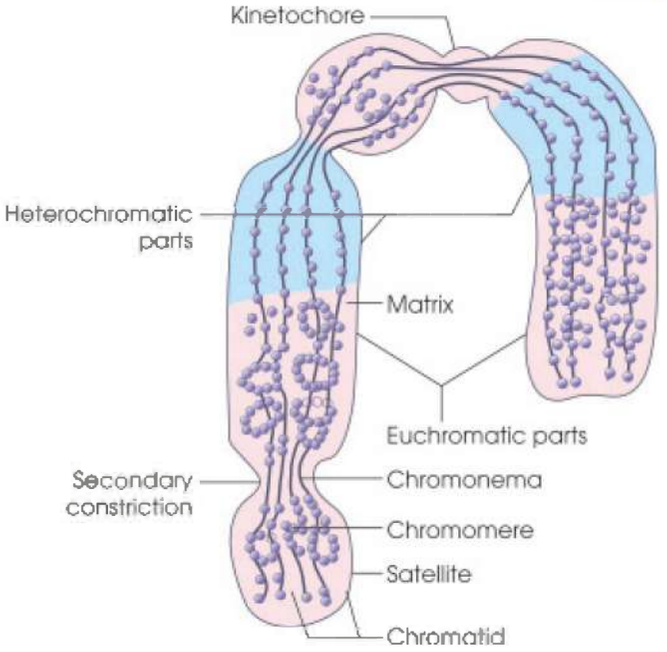


Fig. 2.16: Electron microscopic structure of chromosome (diagrammatic representation)

5. The euchromatin is actively engaged in the synthesis of specific messenger RNA (mRNA). It is believed that the heterochromatic portion is concentrated with DNA and RNA while the euchromatic region contains DNA and histone.

Chromosomes

Thus from the preceding section it can be emphasised that the chromosome is present as individual bodies in the interphase as well as in the mitosis. The predominant component in the chromosome is DNA molecule.

The genes are located in chromosome of the nucleus and can be called the discrete unit of transmission of hereditary character, because it is the specific locus or spot on a chromosome carrying the genetic material or information for a specific character. The gene is a part of the DNA molecule.

Structures of Chromosomes

Characteristic Features

1. Each chromosome consists of one to four coiled threads called *chromonema* and also contains juxtaposed minute particles known as *chromomeres* (Fig. 1.15) which are rich in DNA.
2. Most of the chromosomes possess usually two constrictions—*primary* (*kinetochore*) and *secondary*.
3. Chromosomes having terminal or almost terminal kinetochore are called *acrocentric chromosomes*.
4. While chromosomes having unequal arms and almost centrally situated kinetochore are known as *metacentric chromosomes*.

5. Chromosomes possessing secondary constriction in addition to primary one are called submetacentric chromosomes (Fig. 2.17).

Autosomes and sex chromosomes

- In human being, there are 46 chromosomes, arranged in pairs, in the nucleus of each cell. In each individual somatic cell nucleus, there are 22 pairs of somatic chromosomes, also called autosomes, which are homologous and concerned with the transmission of ordinary hereditary characteristics and the remaining pair is concerned with the determination of sex.
- In the female, the sex chromosomes consist of a pair of identical large X chromosomes, whereas in the male, the pair consists of an X chromosome and a Y chromosome which is small and has influence on sex determination.
- One of the X chromosomes, present in the female, is tightly coiled and can be seen under microscope in the nuclei of squamous epithelial cells and neutrophil granulocytes. This X chromosome may also be present in sexually abnormal cases.
- On the other hand, in females germ cells (gametes) have 22 autosomes and one X chromosome; in males it may be 22 autosomes and either an X or a Y chromosome.
- It is these chromosomes which determine the specific characteristics of the cell and it is through them than that the hereditary qualities pass from one generation to the other.

Cell life

- Nucleus is the most essential part of the cell. Upon it depends; the power of morphological and chemical synthesis.
- The non-nucleated fragment of the cell quickly dies; because it possesses no power of synthesis and regeneration. While that fragment of a cell which contains the nucleus, rapidly re-synthesize the lost parts and carries on normal life. If the nucleus of a cell is punctured, the cell dies.
- From the above facts—it seems that whole cell life appears to be divided up into a number of functional compartments and that each one of the so-called organoids possibly acts as the presiding officer of a particular department of cell life.

GENE EXPRESSION AND REGULATION

The functional unit of DNA is recognized as a gene. Cellular functions are genetically influences and it is the gene expression which determines the variation in cellular activities amongst the various cells of our bodies.

The different proteins which are synthesized in varied tissues of the body mainly depend on the gene expression. The transcription (synthesis of RNA from DNA) and translation (genetic message which is

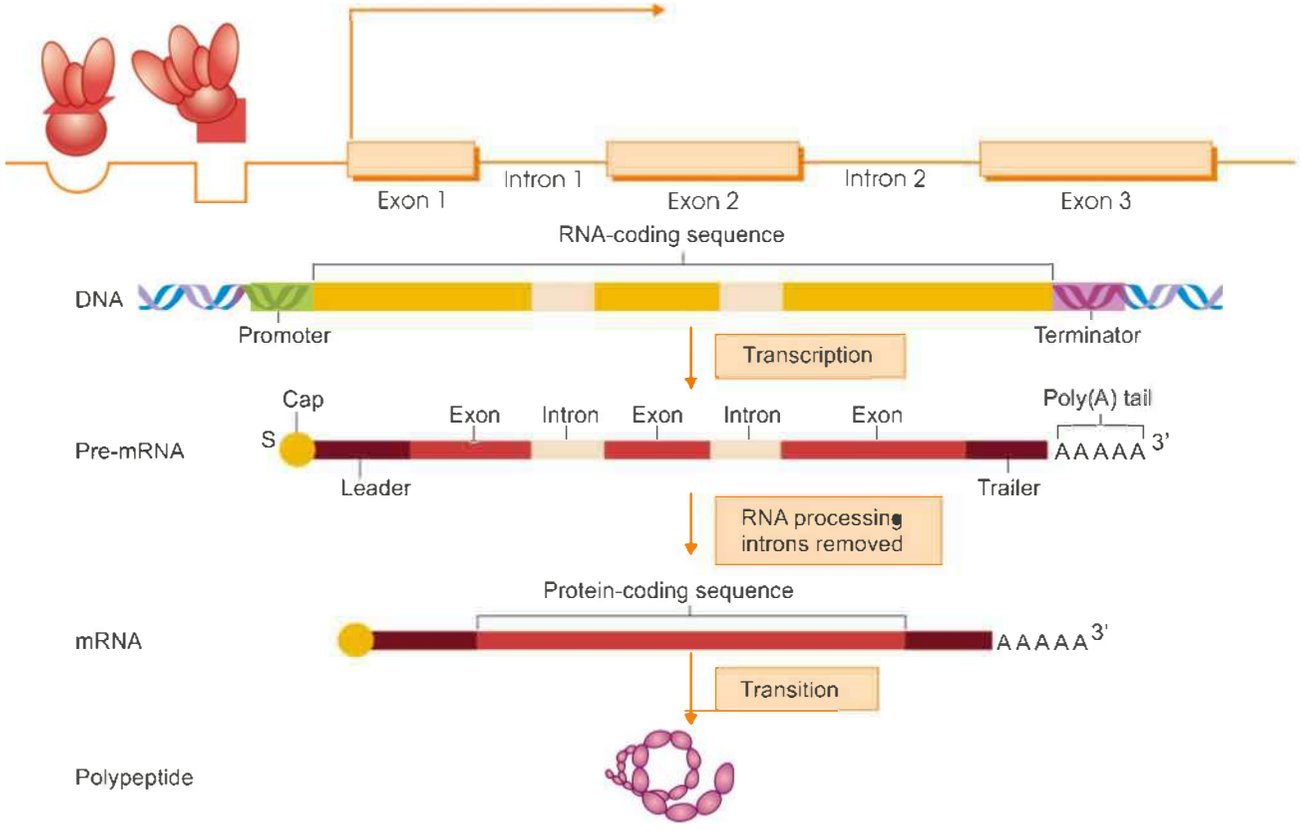


Fig. 2.17: Formation of mRNA by introns splicing

received from DNA by mRNA is converted into chain of polypeptide with typical amino acid sequence) process involved in synthesis of proteins has been explained under heading nucleolus and nucleus and also depicted in Fig. 2.15 earlier. The gene unit is composed of exons, introns and regulatory DNA sequences.

1. **Exons:** These are the coding sequence of DNA which code for formation of RNA.
2. **Introns:** These are the DNA coding sequence.
3. Regulatory DNA sequence contains repressor, promoter and operator.

There is sequence of nucleotides on the DNA strand immediately ahead of the gene to the transcribed and is called promotor. The RNA polymerase which has an appropriate complementary structure which identifies or recognizes the promotor and gets attached to the promotor. It is the recognition site where the RNA polymerases attaches to area which is transcription start site. As RNA polymerases attaches; the promoter sequence proceeds along exons and introns. Operator lies between the promoter and exons and introns and occupies around ten nucleotide space. There is a repressor nucleotide sequence after the promoter region and identified as 5′ region. This region codes for repressor protein that if get attached to operator shall prevent the RNA polymerase from moving ahead towards the structural gene.

Recombinant DNA technology: The factors influencing gene regulation are regulation through mRNA, gene rearrangement, regulation through transcription factors and gene amplification. Recent advances in genetic engineering has build up the recombinant DNA technology today which is being used for synthesis of hormones (growth hormone, erythropoietin, etc.), gene therapy, lab diagnosis of HIV/AIDS virus, DNA finger-printing for identifying the persons in forensic labs, etc. Moreover, the amplifying target DNA sequence helps in sex identification, diagnosis of AIDS, etc.

Heredity: Heredity, according to Oxford Dictionary, is the property by virtue of which offspring inherit the nature and characteristics of parents and ancestors. G Mendel (1865) postulated that chromosomes contain the hereditary characters, which are now known to be ultra-microscopic DNA particles.

Genes and Genetic Diseases

Genes are ultra-microscopic DNA particles present inside the chromosomes, which are carried from one generation to other, carrying the hereditary characters along with it. Depending upon the effectiveness of the gene to influence the character of the offspring; the genes from the parents might be shown prominently in the offspring, in which case it is called dominant gene. It is also possible for the characters not to be exposed, and then the gene is named as recessive gene. If one of the gene is dominant and the other recessive, the character of the dominant gene is indicated in the offspring.

Some of the hereditary diseases are transmitted from parents to offspring due to dominant gene. Here at least one of the parents has the particular disease. The diseases inherited are, certain types of retinitis pigmentosa, sickle cell anaemia, night blindness, a number of metabolic disorders (pentosuria, lipomatosis), etc. There are some diseases which may be carried over to the offspring through recessive gene. Here the parents are apparently healthy but simply act as carriers. Children born of closely related parents have a greater chance of these diseases to be transmitted to them. Diseases transmitted, are albinism, hepatolenticular degeneration, certain metabolic disorders (alcaptonuria, cystinuria, fructosuria), etc. Some of the hereditary diseases may be due to pathologic characteristics of one of the sex chromosomes, only the male sex usually suffer from some hereditary diseases like haemophilia, colour blindness, night blindness (nyctalopia), etc. The female acts as carriers.

Applied physiology: Example of sex-linked inheritance of haemophilia. In this disease which is due to hereditary disorder, the blood does not coagulate extravascularly. This disease is transmitted by 'sex-linked' transmission. The haemophilic gene is linked with recessive X chromosome in female which only acts as carrier of the disease but does not suffer from it. This is due to the other X chromosome in female being dominant. If any female carrier (xX) marries a normal male (XY), some of their male offspring (xY) will suffer from the disease and again some of the female offspring (xX) will act as carrier in the first generation (Fig. 2.18A). In case of marriage of male sufferer (xY) with a normal female (XX) male offspring of the first generation will have no abnormalities (XY). But the female offspring will be the carrier (xX) (Fig. 2.18B). In case of marriage between female carrier (xX) and male sufferer (xY), a certain percentage of both male and female offspring the first generation will suffer from the disease and some of the female will be carrier only. According to Mendel's law of heredity dominant gene always characterizes over the recessive sex-lined gene. The female carrier (xX) does not suffer only for having dominant gene 'X' from the male. On the same ground the female may suffer when the individual possesses the recessive sex-linked gene (xx) (Fig. 2.18C).

CYTOPLASMIC INCLUSION

The cytoplasmic inclusions are not the living metabolic machinery of the body but are certain structures present in the cytoplasm of the cells.

These are:

1. Stored foods
2. Secretion granules
3. Pigments crystals

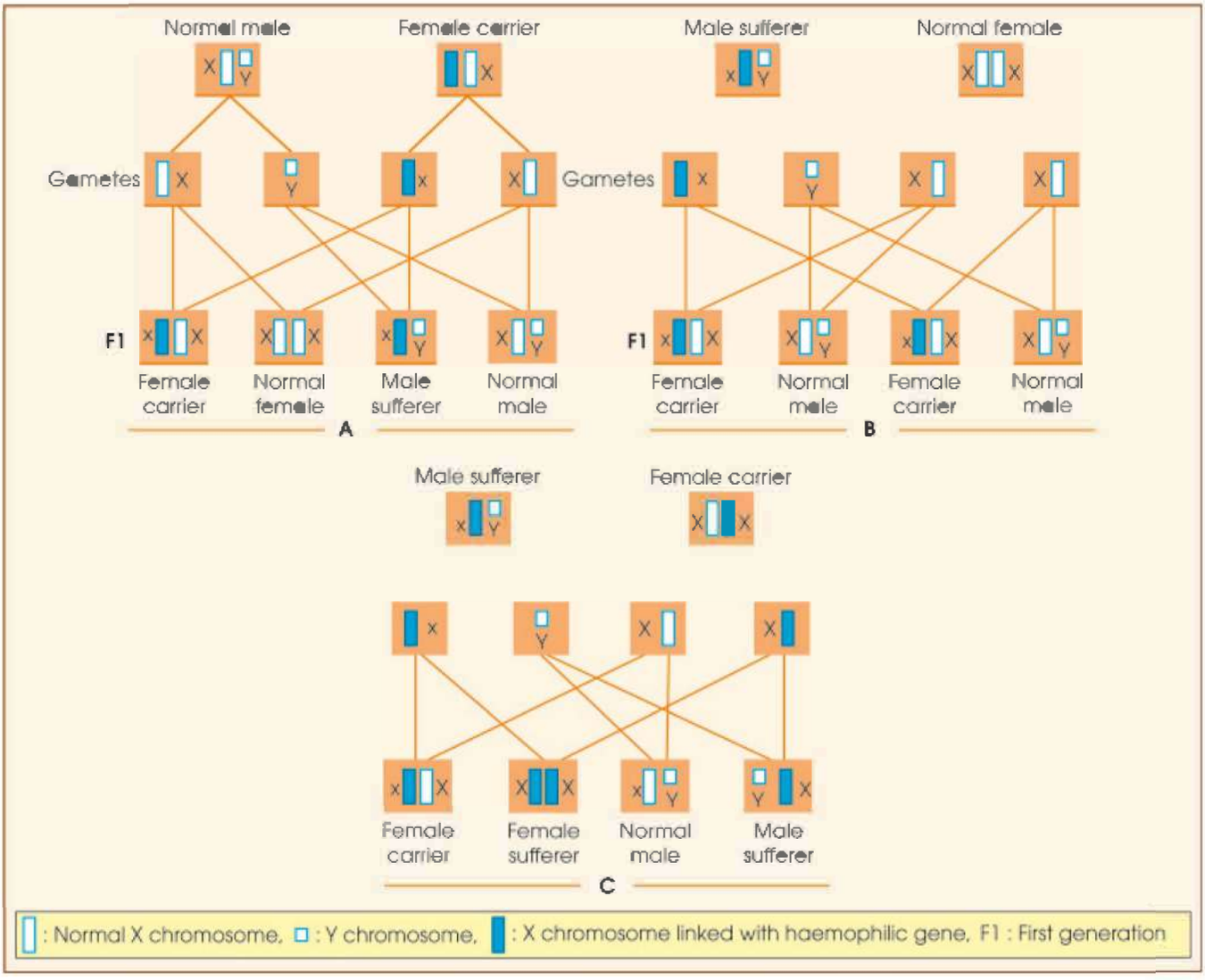


Fig. 2.18A to C: Schematic representation of transmission of haemophilia by union between female carrier and normal male (A), between male sufferer and normal female (B) and between male sufferer and female carrier (C) transmission of haemophilia by union between male sufferer and female carrier

1. **Stored foods:** A healthy subject can withstand starvation: For weeks, only because of his stored foods in the cytoplasm. Every cell for its metabolic functions requires fuel and this fuel is always supplied from external source. When this external source fails to supply, the internal food source—stored foods in the cytoplasm thus maintain it for a certain period. These stored foods are the protein, carbohydrate and fat which are present as inclusions in certain cells.

   Carbohydrate: It is absorbed from the intestine in the form of monosaccharide and stored in the cytoplasm of animal cells as macromolecules—glycogen. It is stored as such particularly in the liver cells and also in other cells too.

   Fat: It is mostly stored in connective tissue fat cells and it may also accumulate in the liver cells under certain conditions of dietary deficiency.

   Protein: It is rarely stored as cytoplasmic inclusions and cells at a certain stage consume their own cytoplasm as a stored food. Reserve of protein mainly exists in the matrix.
2. **Secretion granules:** Digestive enzymes and other fluid materials are synthesised from raw materials brought in the cytoplasm through the blood and tissue fluid. These materials remain in the cytoplasm as small globules or droplets of fluid and usually precipitated in the form of granules during fixation. These granules can be stained successfully with special histochemical techniques.
3. **Pigments:** There are certain pigments in the cytoplasm. In order to be visible these pigments do not require any dye for staining. These pigments are present as cytoplasmic inclusion in the cell. They may be classified into two groups: Endogenous type and exogenous type.

Endogenous Pigments

These pigments are those which are synthesized within the body. These are as follows:

Haemoglobin and its derivatives: Haemoglobin is the iron containing pigment of the red blood corpuscles (RBC). In normal condition the lifespan of red cells averages about 120 days, as they are destroyed by phagocytosis. The haemoglobin of these cells is broken down into *haemosiderin* (iron containing pigments) and *haematoidin* (non-iron containing pigments).

a. Haemosiderin is disposed in the cytoplasm of phagocytes as granules or an irregular mass. Normally it is also present in certain amount in phagocytes of spleen, liver, bone marrow, and the quantity is increased during rapid destruction of RBC in diseased state. A green pigment, biliverdin, is the breakdown product of haemoglobin. On reduction biliverdin gives rise to a yellow-brown pigment, bilirubin.
b. Haematoidin is a breakdown product of haemoglobin during destruction of RBC and is identical to bilirubin. Bilirubin does not contain iron and is very soluble. For this reason it is dissolved in the blood and is not stored in the cells and thus is continuously removed from the liver cells into the bile.

Melanin: This is a brown-to-black pigment present in the skin, eye, etc. This pigment is absent in the albino. The dark colour of the Negroes is due to the presence of this pigment in a large amount. This pigment is present in the cytoplasm of cells as granules or cluster of granules. It is tyrosine derivative and its concentration in the cells alters during derangement of tyrosine metabolism.

Exogenous Pigments

These are the yellowish pigments: Carotene and lipochrome

1. Carotene pigments sometimes accumulate in the cells when excess carrot is consumed. Sometimes it may look like jaundice as the carotene pigment gives a yellow colour of the skin and the body fluid.
2. Lipochrome is present in the vegetables. Besides these, certain dusts like carbons and minerals like lead and silver are also present. Lipochrome is also a yellow pigment present in certain cells particularly in older ones. Its quantity mostly depends upon the wear and tear of cells. Furthermore the quantity of lipochrome in the cells is mostly dependent upon the activity of cells. It is present in the liver, muscle and cardiac cells. Recently it is described that lipochrome is present in the lysosome and is sometimes referred to as lipofuscin. It is soluble in fat solvent and present in several types of cells already described.
3. *Dust:* Coal dust may be deposited in the body through inspired air. Pigmentation may occur in the system which may not be so harmful.
4. *Minerals:* Certain minerals like silver or lead taken through mouth may produce pigmentation in the body. Gray pigmentation of the body may occur due to taking excessive silver as medicine. Similarly excessive lead may produce lime in the gums.

Crystals: Certain cells such as Sertoli and interstitial cells of the testes contain certain proteinaceous crystalline materials. In significance is quite unknown.

CELL DIVISION AND NUCLEAR DIVISION

This process can best be studied by using the drug colchicine which arrest nuclear division at metaphase. The division of one cell into two daughter cells is the basis of the continuity of life and underlies the complexity of metazoan organisms. Mitosis (indirect cell division), amitosis (direct cell division) and meiosis are considered as some of several types of existing cell division.

Cell division may be separated into:

1. *Karyokinesis:* Division of nuclear material starts with doubling of the chromosome in parent nucleus, followed by distribution between two daughter nuclei in equal proportion.
2. *Cytokinesis:* Cytoplasmic division in which each of two daughter cells receives one of the daughter nuclei. In a few cell types, karyokinesis may occur without cytokinesis.

Mitosis (Fig. 2.19)

During growth or regeneration of tissue, cells divide by mitosis. Each chromosome reproduces itself by splitting lengthwise, so that two identical portions (or chromatids) pass to the daughter cells and grow into mature chromosomes identical with those in the parent cell. Normal cell division or mitosis is divided usually into four stages—*prophase*, *metaphase*, *anaphase* and *telophase*. The interval between each cell division which is comparatively long is called *interphase*. The process of mitosis together with interphase period constitutes the cell cycle. The cycle is extremely short in the case of intestinal epithelium and very long in adult hepatic cell.

Prophase

During the stage of prophase each chromosome looks as a pair of long entwined filamentous structures throughout the nucleus due to coiling of DNA molecules. They gradually shorten and thicken and become darker. The two centrioles with microtubules forming asters move to the opposite side. Spindle (group of tubules) becomes visible. At the end of prophase nuclear membrane and nucleolus disappear. The chromosomes separate from each other and become prominent.

Metaphase

In the stage of metaphase, the chromosomes assemble at the equator of spindle fibres. Chromosomes appear

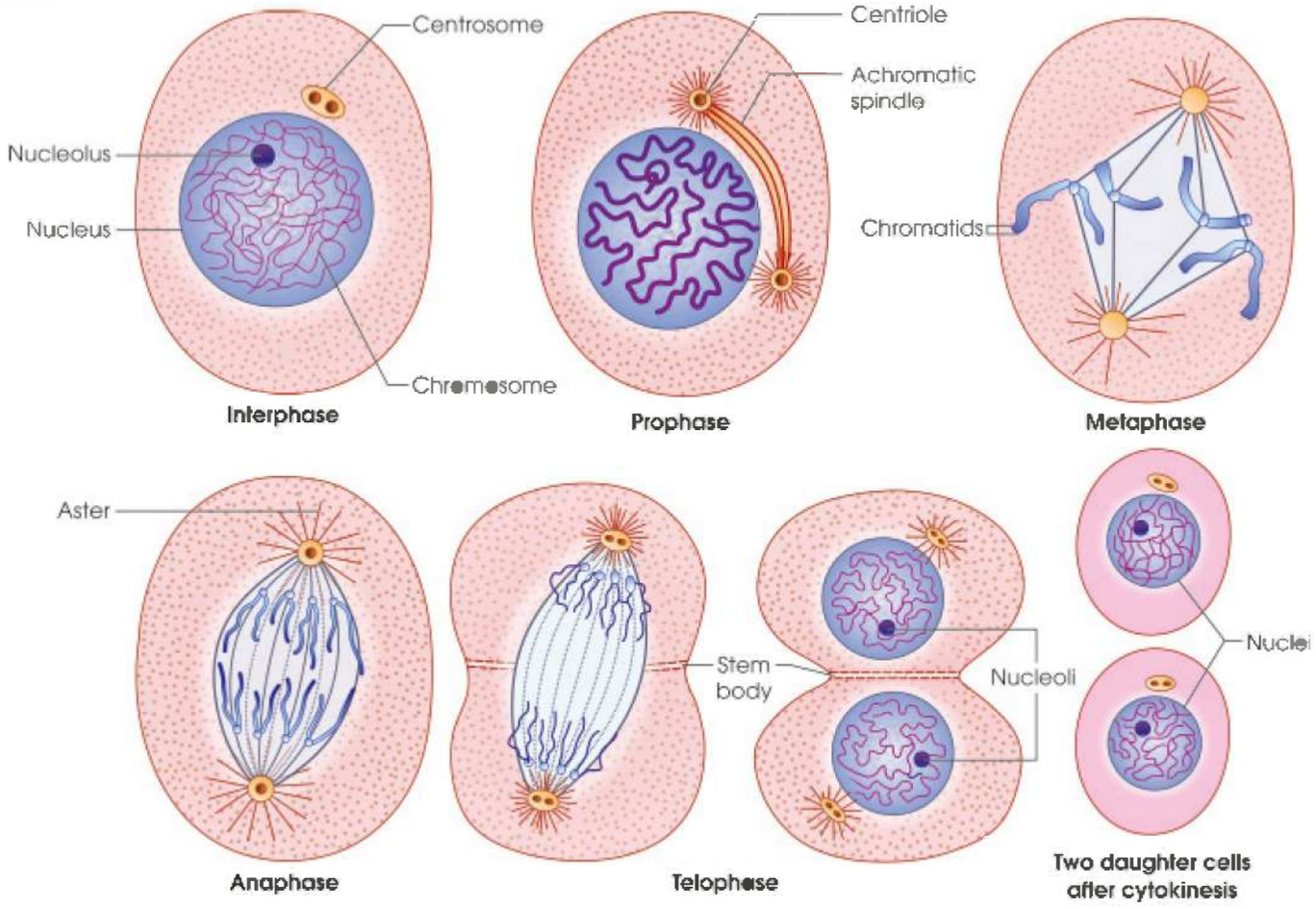


Fig. 2.19: Mitotic cell division showing the changes in the centrosomes, centrioles, chromatids and nucleus of a cell (diagrammatic representation)

as if suspended by microtubules (spindle filaments) in a single plane midway between two asters. Two chromatids are formed from each chromosome by longitudinal splitting.

Anaphase

During anaphase the separated chromatids which form the chromosome of the daughter cell move towards the opposite poles of the cell. Newly separated chromatids now are new chromosomes.

Telophase

In the stage of telophase, a gradually elongating nuclear membrane due to uncoiling of DNA molecules is formed around two groups of chromosomes. Spindle fibres disappear. Formation of nucleolus completes the process of cell division and two identical daughter cells after cytokinesis are usually formed from one parent cell. The different cytoplasmic organoids of the parent cell are distributed between the two daughter cells.

Interphase

This is a period between end of telophase of one cell division and beginning of prophase of next division. Chromosomes become elongated and too thin to be visible as such but chromatin granules are visible. Each chromosome duplicates (duplication of DNA molecules) forming two chromatids attached at centromere. Two newly formed cells grow.

Meiosis

Meiosis is the heterotypical process of the formation of germ cells, or sexual reproduction. This process occurs during spermatogenesis and oogenesis and starts at the stage when the primary spermatocytes and oocytes divide to form secondary spermatocytes and oocytes respectively. By the process of meiosis or reduction division each male or female germ cell has in it 22 autosomes and either an X or a Y chromosome (only in sperms). In the course of fertilisation the male germ cell (spermatozoon) and female germ cell (ovum) unite and the numbers of chromosomes are restored to the original 23 pairs. When the sperm and ovum unite, the resultant cell (zygote) possesses a full (diploid) complement of chromosomes, one half from the female parent and another half from the male. This process of meiosis is essential to prevent the otherwise doubling of the number of chromosomes which would have taken place by the usual process of mitosis.

Amitosis

It is a direct process of cell division and under which the nucleus of the cell first constricts in the middle to divide into two nuclei. After that the cytoplasm divides in the centre into two units and each cytoplasmic unit contains one nucleus. Thus from one cell, two daughter cells containing nucleus are formed.

LIFE CYCLE OF CELLS

Two major types of cell may be recognized:
1. Somatic cells
2. Gonadal cells
   - Somatic cells are the diverse cells which make up the somatic structure of the body being fated to die with or before the individual they constitute. A somatic cell begins its span of life as one of the daughter cells of a mitotic division. The frequency of mitotic division and the consequent production of daughter cells vary with the cell type.
   - Gonadal cells are gametes (i.e. spermatocytes and oocytes) capable of uniting sexually to form a new individual.

FORMATION OF TISSUE, ORGAN AND SYSTEM

The cells in the body of the multicellular animals vary in structure and function. It is seen that one variety of cell performs one kind of work and constitutes one type of tissue.

Tissue

Tissue may be defined as an aggregate of same type of cells combined by sub-serving the same general function independently and united by varying amounts of intercellular substance (e.g. blood, bones, cartilage, muscle, nervous tissues, etc.).

Organ

An organ is a group of more commonly two or more tissues, which basically function independently in some instances, in particular patterns to form larger functional units (e.g. blood vessels, kidney, skin, glands, etc.).

Organ System

When several organs function inter-relatedly they form organ system (e.g. respiratory system composing the nose, larynx, trachea and lungs; urinary system comprising kidney, urethra and ureters). This arrangement is based upon the principle of division of labour and is an essential requirement for coordinated living.

1. One group of cells is set apart for one function and will specialise for that job. During the initial stages of embryological development the cells are all similar. As development proceeds, one group of cells takes up one particular work, and in order to perform the work in the best possible way, it undergoes the necessary change in structure and mode of life. This process of adaptation of a cell, for the purpose of doing a particular function, is called differentiation or specialisation.
2. The fertilised ovum (zygote) divides at first into two cells, these again into two more, and so on until a large mass of embryonic cells is formed. The first few cells are believed to be totipotent, which means that, each one of them has got the potency of creating a total individual. The first evidence of differentiation is found in this cellular mass, where the cells become arranged in three distinct layers, known as the ectoderm, the mesoderm and the entoderm. The following tissues, in general, are derived from the three germ layers, described below.

Ectoderm: Epithelium of mouth, nose, anterior surface of cornea, external auditory canal, skin, hair, urethra, etc. and also some glands like sweat (or sudoriferous), sebaceous (or oil) and mammary glands, the endocrine system (pituitary, adrenal medulla and pineal gland), brain and cranial motor nerves.

Mesoderm: Connective tissue including blood and bone marrow cells, the three different kinds of muscle in general, lymphatic organs, endothelium of blood vessels, epithelium of urinogenital tracts (e.g. urethra) and the adrenal cortex.

Entoderm: Epithelium of the digestive and respiratory tract, bladder, thyroid, parathyroid and thymus.

Cancer cells malignant neoplasm

The genetic structures of the normal tissue cell and the cancer cell (malignant neoplasm) are different. These differences between them are quantitative rather than qualitative. These concern their functional potentialities and behaviour rather than their requirements and constitution. The similarity between normal tissue and cancerous tissue is that there is no magic bullet which seeks out the unwanted cancerous structure and leaves uninjured the normal tissue and organ from which the neoplasm has arisen. This neoplasm disrespects normal growth limits and does not obey the feedback mechanism which normally controls the cellular growth and reproduction. The cancer cells are not controlled in such a way, and so they can grow and proliferate without any limits. These cells compete with normal cells for the available nutrients. When the number of cancer cells increase to a great extent, they draw all the nutrition. As a result the normal cells suffer from lack of it and death ensures. For example, the cells of normal epidermis combine to form a tissue whereas the cancer cells which arise in the precursors of white blood cells (leukaemia) are individual. Due to their rapid mobility, the cancer cells spread throughout the body and do not combine to form a tissue. When the overgrowth of tissue proceeds without any regard to the surrounding tissues or the requirement of organism as a whole, this excessive usually progressive and apparently purposeless process is known as neoplasm or new growth.

By adding suffix oma, simple or benign neoplasm of epithelial tissues from the surface epithelium can be described as papilloma; that of epithelial tissues from the grandular epithelium adenoma that of fibrous tissue, fibroma; that of muscle, myoma; that of adipose (fatty) tissue; lipoma and malignant or cancerous neoplasmas are carcinoma from epithelial tissue and sarcoma from connective tissue.

SPECIAL STRUCTURAL FEATURES OF CELLS

Cell Junctions (Fig. 2.20)

It is the connection between the neighbouring cells or the contact between the cell and extracellular matrix. The cell junctions are classified into three types: Occluding junction, communicating junction and anchoring junction.

1. **Occluding junctions:** These are cell-to-cell junction that seals cells together in an epithelium. Example, tight junction.

   Tight junctions: They are also called as occluding junctions or zonulae occludens. They are the closely associated areas of two cells whose membranes fuse forming an impermeable barrier to fluid. They provide strength and stability to the cell. They are seen in the wall of renal tubules, choroid plexus and along apical margin of intestinal mucosa.

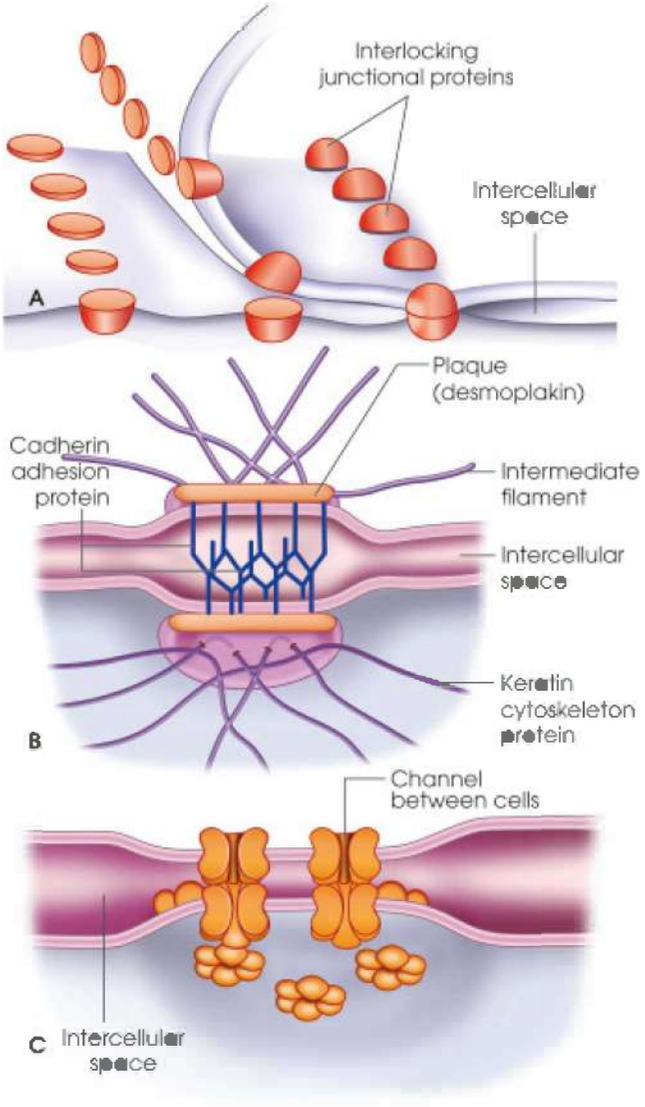


Fig. 2.20A to C: Cell junctions: (A) Tight junction; (B) Desmosome, (C) Gap junction

2. ***Anchoring* junctions:** These are desmosome (connects intermediate filament of one cell with other cells), hemidesmosome and anchoring junction. Desmosomes are also known as macula adherens and is a cell structure specialized for cell-to-cell adhesion. The cell adhesion proteins of the desmosome are members of the cadherin family. The hemidesmosomes look like half-desmosomes that attach cells to the underlying basal lamina. The hemidesmosomes use desmopenetrin cell adhesion proteins which are members of integrin family. The adherens junction exhibit there nature of anchor through their cytoplasmic actin filaments.
3. **Communicating junctions:** Cell junctions which permit the intercellular exchange of substance are called communicating junctions. These junctions permit the movement of ions and molecules from one cell to another cell. The two types of communicating junction are gap junction and chemical synapse.

   Gap junctions: These are low resistance intercellular junction that allow passage of ions and smaller molecules between the cells. It is present in heart, basal part of epithelial cell of intestinal mucosa, etc. The junctional unit is connexons which are array of protein (contains 6 connexins sub-unit). The connexons of one cell align with connexon of other cells. The intercellular space narrows from 25 to 3 nm at gap junction. They act as channel allowing passage the substance having molecular weight less than 1000, aid in exchange of chemical messenger between cells and are responsible for rapid propagation of action potential from one cell to another cell.

   Chemical synapse: It is the junction between a nerve fiber and a muscle fiber or between two nerve fibres; and through which signals get transmitted by the release of chemical transmitter.

   Cell adhesion molecules (CAMs): These are proteins which are present on cell membrane and are involved in binding of one cell to another or with extracellular matrix (Fig. 2.21). These proteins act as transmembrane receptor and consist of three domains:
   1. Extracellular domain (binding domain)—that interacts either with CAMs of the extracellular matrix or other CAMs of the same kind.
   2. Intracellular domain (cytoplasmic domain)—which interacts with cytoskeleton.
   3. Transmembrane domain

   These cell adhesion molecules can be divided into 4 major families: Cadherin superfamily, selectins, imunoglobulin superfamily and the integrins.

Cadherins are calcium dependent homophilic glycoprotein and are concentrated at intermediate cell junctions and it links actin filament network via specific linking proteins catenins.

Selectins: They are family of heterophilic cell adhesion molecules which bind fucosylated carbohydrate. The

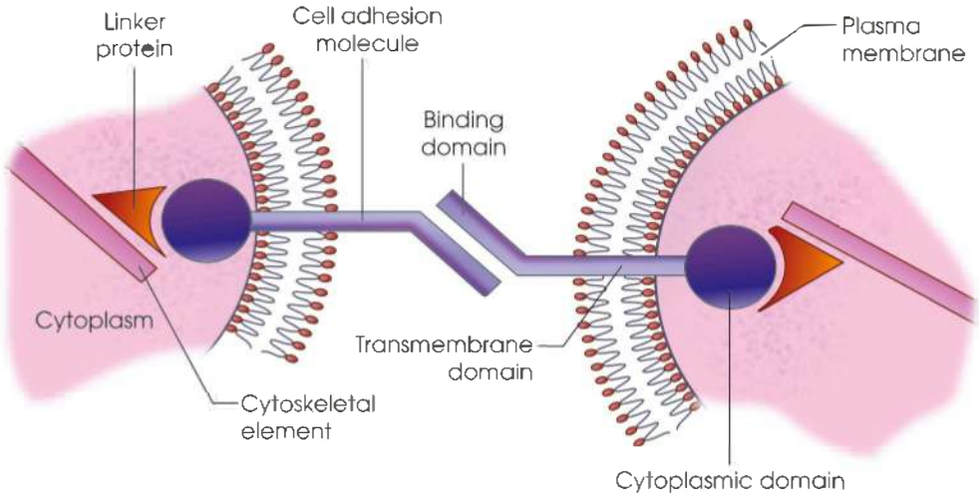


Fig. 2.21: Domain of cell adhesion molecules

three family members of selectins family are E-selectin (endothelial), P-selectin (platelet) and L-selectin (leukocyte). The P-selectin glycoprotein ligand-1 (PSGL-1) is expressed on all white blood cells.

Immunoglobulin superfamily molecules: They consist of more than 25 molecules. Few of the important immunoglobulin super family molecule are Intracellular adhesion molecule 1(ICAM1; CD54), intercellular adhesion molecule 2 (ICAM2), platelet endothelial cell adhesion molecule 1 (PECAM 1; CD31), vascular cell adhesion molecule 1 (VCAM1; CD106) and mucosal addressing cell adhesion molecule 1 (MAdCAM1).

Integrins: There are twenty different heterodimeric combinations of integrins (having fifteen different α and eight different β subunits) at cell surfaces. They bind epithelial and muscle cells to laminin in the basal lamina, allow white blood cells and fibroblast to adhere to fibronectin and collagen as they move and also allow platelets to stick to exposed collagen in a damaged blood vessel.

Roles of Cell Adhesion Molecules

1. They promote cell to cell and cell to matrix interactions.
2. They play critical role in many normal biological processes.
   Examples: Embryonic cell migration, immune system functions, wound healing.
3. They participate in intracellular signaling pathways (primarily for cell death/survival, secretion, etc.).

Molecular Motors

These are the biological molecular machine which has a vital role in movement in living organisms. Few examples of biologically active motor molecules are:

1. **Cytoskeleton motors:** These are myosin, kinesin and dynein. Myosin aids in intracellular cargo transport and muscular contraction. Kinesin moves the cargo inside the cell along the microtubules away from the nucleus while dynein transports cargo along microtubules towards the cell nucleus.
2. **Polymerisation motors:** Few polymerisation known molecular motors are actin and dynamin. Actin polymerisation using ATP which generates forces and can be used for propulsion. Dynamin separate clathrin buds from the plasma membrane using GTP.
3. **Nucleic acid motors:** RNA polymerase, DNA polymerase and helicases. The RNA polymerase transcribes RNA from the DNA template, DNA polymerase turns single-stranded DNA into double-stranded DNA and helicases separates double strands of nucleic acids before the transcription or replication.

Apoptosis

It is a programmed cell death. The apoptosis is genetically influence and dead cells are removed by phagocytosis. The resorption of the tail of tadpole during metamorphosis into a frog occurs by apoptosis.

Few examples of apoptosis in human are

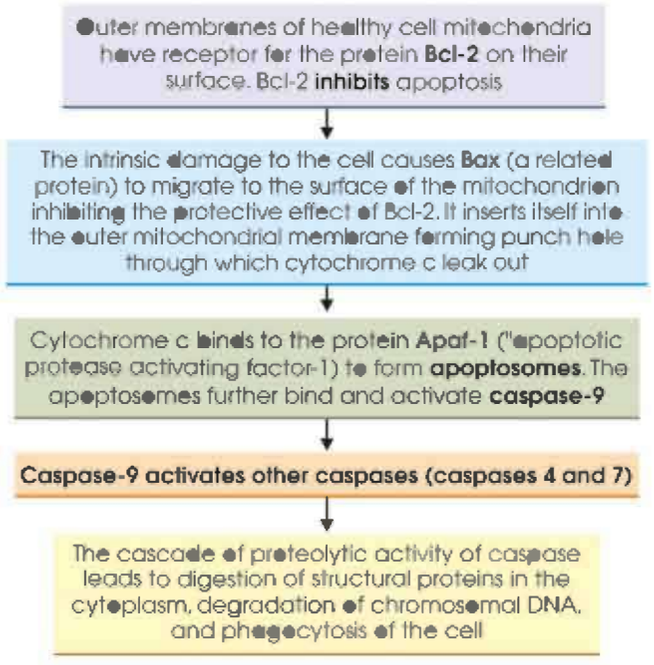
- The removal of tissue web formation between fingers and toes of the foetus.
- Degeneration of neurons.
- The periodic sloughing off of endometrium at time of menstruation.
- Removal of clones of immune cells which are not appropriate.
- Regression of duct system during time of sex differentiation in foetus.

Mechanisms of Apoptosis

Cell commits suicide by apoptosis due to signals arising within the cell; or signal triggered by death activators like tissue necrosis factor-α and lymphotoxin or by reactive oxygen species.

Intrinsic Mechanism of Apoptosis (Flowchart 2.3)

Flowchart 2.3: Mechanism of apoptosis



Apoptosis Triggered by External Signals

The Fas (First apoptosis signal) and TNF receptor are integral membrane proteins. The FasL (Fas ligand) and TNF respectively are complementary death activator and they transmit a signal to the cytoplasm that leads to activation of caspase 8. The caspase 8 initiates a cascade of caspase activation leading to phagocytosis of the cell.

Applied Physiology

The genetic defects in apoptosis may be seen a mutation in the gene for Fas. It produces autoimmune lymphoproliferative syndrome (ALPS). The features of ALPS include; accumulation of lymphocytes in the spleen and lymph nodes (due to which they get enlarged), appearance of clones that are auto reactive producing autoimmune disorders as haemolytic anaemia and thrombocytopenia.

EXAM-ORIENTED QUESTIONS

Essay

1. Describe the structure and functions of cell membrane.
2. Enlist the organelles in cell. Describe the structure and functions of any two organelles.
3. Describe the structure and functions of mitochondria, endoplasmic reticulum and Golgi apparatus.

Short Notes

1. Fluid mosaic model
2. Structure of nucleus
3. Functions of nucleus
4. Functions of mitochondria
5. Functions of lysosomes
6. Lysosomal storage disease
7. Cell division
8. Mitosis and meiosis
9. Cell adhesion molecules
10. Molecular motor
11. Intercellular cell junction
12. Apoptosis

RECENT ADVANCES: STEM CELL RESEARCH

Shinya Yamanaka, Japanese physician and researcher shared the 2012 Nobel Prize for Physiology or Medicine with British developmental biologist John B. Gurdon for the discovery on how the mature cells could be reprogrammed. They inserted specific genes into the nuclei of adult cells (e.g. connective tissue cells), and this process resulted in the reversion of cells from an adult state to a pluripotent state. As pluripotent cells, these cells regain the capacity to differentiate into any cell type of the body. The reverted cells became known as induced pluripotent stem (iPS) cells.

Shinya Yamanaka
1962

Sir John Bertrand Gurdon
1933

Sir John Bertrand Gurdon is best known for his pioneering research in nuclear transplantation and cloning. Gurdon's recent research is centered towards analyzing intercellular signaling factors involved in cell differentiation, and explores and investigates the mechanisms involved in reprogramming the nucleus in various transplantation experiments. Gurdon has been verifying the role of histone variants, and demethylation of the transplanted DNA in cell reprogramming.

REFERENCE

Simonsson S; Gurdon J. DNA demethylation is necessary for the epigenetic reprogramming of somatic cell nuclei. Nature Cell Biology 6, 2004: 984–990.

RECENT ADVANCES: CHROMOSOME PROTECTION BY TELOMERES

The 2009 Nobel Prize in Physiology or Medicine was awarded jointly to Elizabeth H. Blackburn, Carol W. Greider and Jack W. Szostak "for the discovery of how are the chromosomes being protected by telomeres and the enzyme telomerase".

Elizabeth H. Blackburn
1948

Carol W. Greider
1961

Jack W. Szostak
1952

The DNA molecules carrying genes are packed into chromosomes, and they are capped by the telomeres on their ends. The unique DNA sequence in the telomeres protects the chromosomes from degradation. Shortening of telomeres leads to ageing of cell. The high level of telomerase activities leads to maintenance of telomerase length and delays cell ageing. This discovery helped us to understand cell better, and stimulated the development of potential new therapies.

REFERENCES

1. Szostak JW, Blackburn EH. Cloning yeast telomeres on linear plasmid vectors. Cell 1982; 29:245–55.
2. Greider CW, Blackburn EH. Identification of a specific telomere terminal transferase activity in Tetrahymena extracts. Cell 1985; 43:405–13.
3. Greider CW, Blackburn EH. A telomeric sequence in the RNA of Tetrahymena telomerase required for telomere repeat synthesis. Nature 1989; 337:331–7.

Chapter

3

Transport Across Cell Membrane

INTRODUCTION

The membrane transport mechanisms regulate the passage of solutes such as small molecules and ions through cell membrane. The unique property of a cell membrane is that it is selectively permeable and this helps in transport of nutrients across the membrane and removal of the intracellular waste product aiding in cell growth, development and survival. The selective permeability helps in drug absorption and excretion and thus helps to combat infections. The selective membrane permeability characteristic of cell membrane also allows them to separate substances of distinct chemical nature.

Lipid bilayer helps in transport of lipid soluble substances but it is a formidable barrier to larger and more hydrophilic molecules. These are transported by aid of transport proteins. The lipid layer is permeable to fat soluble substances such as oxygen, carbon dioxide and alcohol while it is impermeable to water-soluble substance such as urea, glucose and ions. The substances move across the cell membrane by passive and active processes. The molecules in general by process of diffusion are carried across the membrane down a concentration gradient without utilisation of any source of energy or aid of a protein. The active and passive transport process require proteins transporters. The active transport is via transport protein while passive transport is carried out via channel protein. Active transport is a transport mechanism against a concentration gradient with ATP as a source of energy.

In order to understand the mechanism of transport it is necessary to be acquainted with common terms involved in transport mechanism.

Introducing to Key Terms and Concepts

Terminology

Solutions: The molecules (solutes) dissolved in a liquid (solvent) are known as solutions. All molecules in a solution are constantly in random motion, which causes mixing.

Brownian motion: All molecules have random tendency to move due to their inherent kinetic energy that is the energy of motion which is termed as Brownian motion.

Concentration: The amount of solute in a solvent.

Concentrations gradient: It is the difference between the concentration of substances between intracellular and extracellular environment. For example, concentration of sodium and chloride are low and potassium high in intracellular environment but *vice versa* in extracellular environment (high sodium and chloride and low potassium). This ionic difference creates the concentration gradient.

IMPORTANT CONCEPTS IN TRANSPORT MECHANISM

1. **Energy expenditure in transport mechanism:** The ATP pumps use energy from the hydrolysis of ATP to transport molecules against their concentration gradient.
2. **Channels:** They allow the movement of ions or small molecules down their concentration or electric potential gradients. The water channels are constitutively open (some potassium channel may be constitutively open) while other channels are either voltage-gated or ligand-gated.
3. **Transporter molecule:** A transporter moves molecules either down their concentration gradient or against their concentration gradient, depending on the type of transporter (Fig. 3.1).

Ion Channels

The ions pass through ionic channel. Many of these channels are open or voltage-gated. The rate of ion transport through the open channel is very high (often 10^6 ions per second or greater) and ions pass through channels down their electrochemical gradient. Ion channels allow only ions of a certain size and or charge to pass through them. Sodium channels are 0.5 nm in diameter and their inner surface is negatively charge

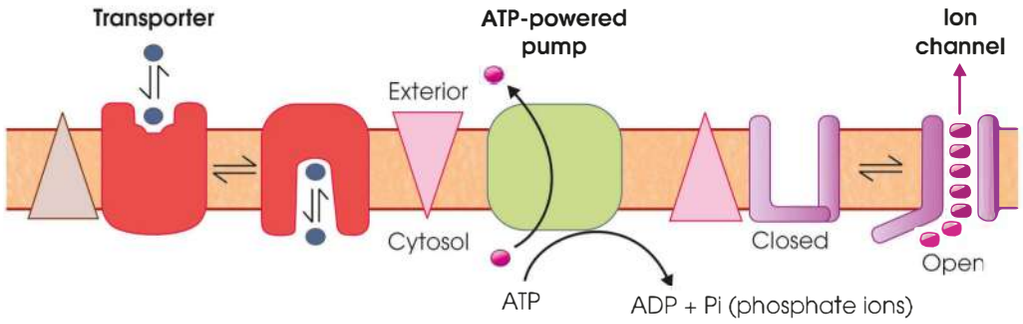


Fig. 3.1: ATP pump, ion channel and transporters

while potassium channel is 0.3 nm in diameter. The ionic channels are open and gated type. Cells tend to have some ungated K^+ and ungated Cl^- ion channel while Na^+ and Ca^{++} move through gated ion channels.

Voltage-gated: Voltage-gated ion channels that open or close in response to changes in the membrane potential. The voltage-gated Na^+, Ca^{++}, K^+, and Cl^- ion channels play an important role in functioning of nervous system and muscles.

In resting state there is negative charge on inside of the cell membrane and due to which likely the sodium gates are in closed state. The depolarization leads to decreased negativity inside of the cell membrane, which triggers the sudden opening of the sodium gate and sodium moves inward. Similarly, the potassium gates which are located inward intracellular side open when inside of the membrane gains positivity.

Ligand-gated: The ion channels which are ligand-gated; open in response to the binding of an extracellular or intracellular regulatory molecule. An important example is the acetylcholine receptor in the membrane of skeletal muscle cells. As acetylcholine binds to the receptor it causes opening of sodium channel which produces depolarization and muscular contraction.

Temperature-gated: The temperature-gated ion channels are found in sensory neurons in the skin and mucous membranes and open with increase or decrease in temperature. This leads to the sensations of warm and cold.

Mechanical-gated: Mechanical-gated channels respond to stretch. *Example:* Stretch sensitive channels are present on ventricular musculature and ion channel in hair cells of cochlea.

Types of Transport Mechanism

a. Passive transport
   1. Diffusion: Simple and facilitated diffusion
   2. Osmosis.

b. Active transport
   1. Primary active transport
   2. Secondary active transport: Sodium co-transport and counter-transport.

c. Transport of macromolecules
   1. Pinocytosis
   2. Phagocytosis

Passive Transport Mechanism

The passive transport mechanisms are diffusion and osmosis.

Diffusion: It is a passive process by which molecules moves from area of higher concentration gradient to that of lower concentration gradient and does not require energy for the process.

The diffusion is of two types: Simple diffusion and facilitated diffusion.

Simple diffusion (Figs 3.2 and 3.3)**:** The movements of molecules from area of higher concentration to that of lower concentration due to kinetic motion of the ions or molecules through the intermolecular spaces or membrane openings without the aid of any carrier protein is known as simple diffusion. It does not require any energy (ATP) for transport. Examples: The lipid-soluble compounds (alcohol, oxygen, nitrogen) diffuse through the lipid bilayer; while water-soluble substances pass through the aqueous channel (urea, glucose and ions). Diffusion also occurs through leak channel and gated channels. The leak channels are open always, e.g. K^+ channels. Gated channels are voltage-gated, ligand-gated, mechanical-gated and temperature-gated as explained earlier.

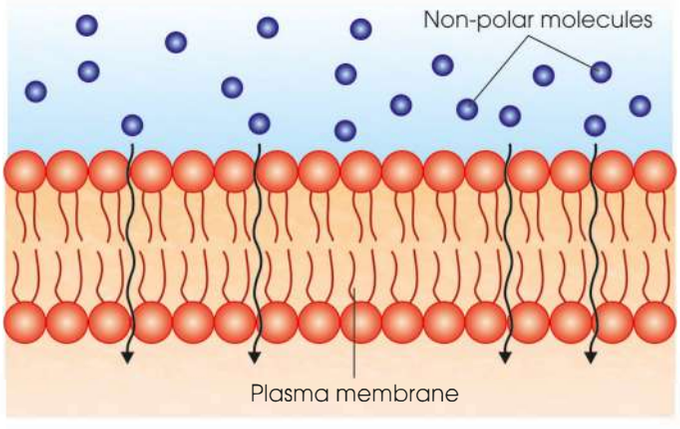


Fig. 3.2: Simple diffusion

The main factors affecting diffusion are the concentration gradients of the substances, number of channels and opening in the membrane and kinetic motion of the molecule.

The factors that affect the diffusion rate across the cell membrane are enlisted below:

1. **Thickness:** Greater the thickness of cell membrane slower will be the diffusion.
2. **Temperature:** The higher the body temperature faster is the diffusion.
3. **Size of the molecule:** Smaller the molecular size of substance rapid is the diffusion.
4. **Concentration gradient difference:** Larger the concentration gradient faster is the diffusion.
5. **Cross-sectional area of membrane:** Diffusion is directly proportional to the available cross-sectional area.
6. **Diffusion coefficient (D) of the substance:** D: Permeability X area of cross section available for transport.

Facilitated diffusion (Figs 3.3 and 3.4)**:** Carrier protein-mediated transport of substance across cell membrane is known as facilitated diffusion.

Characteristic Features

1. The substance to be transported binds to the carrier protein. The binding causes conformational change in carrier protein molecule and this aid in the transport and release of the substance to the other side of the membrane (Fig. 3.1).
2. The energy for transport is provided by the concentration gradient of the substance transported. No energy source (ATP) is required. This carrier-mediated transport of ions and organic substrates into or out of the cell down their concentration gradient passively is also called passive carrier-mediated transport.
3. The facilitated diffusion occurs faster than simple diffusion.

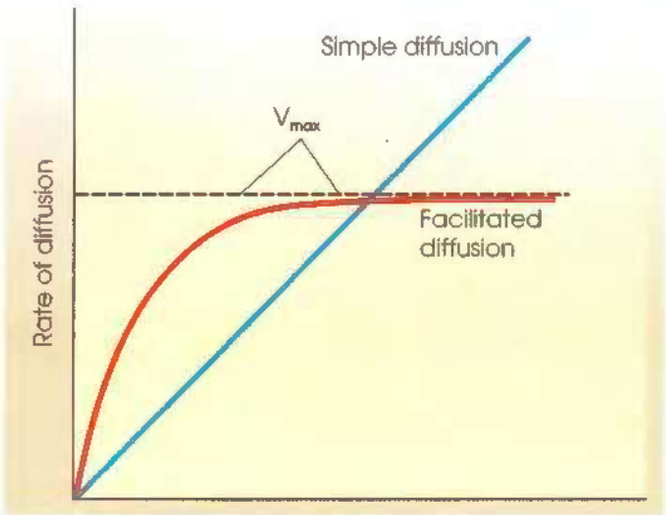


Fig. 3.3: Simple and facilitated diffusion and saturation

4. When concentration of the transported substance becomes high enough the facilitated transporter gets saturated and this limits the rate of transport.
5. If more than one substance competes to bind with common transporter, it leads to competitive inhibition.

Examples of Facilitated Diffusion

1. Insulin-mediated transport of glucose in muscle cells. When more insulin is present, more of these glucose transporters (GLUT) are added to the membrane of cells of such muscle and glucose moves into the muscle cells.
2. Transport of glucose along intestinal epithelium by glucose transporter.

Difference between passive transport and active transport is given in Table 3.1, Figs 3.1 and 3.4.

Osmosis

It is the process of movement of solvent from the solution with lower concentration of solute to high concentration of solute (Fig. 3.5). Glucose which does not freely diffuse through cell membrane is known to be osmotically

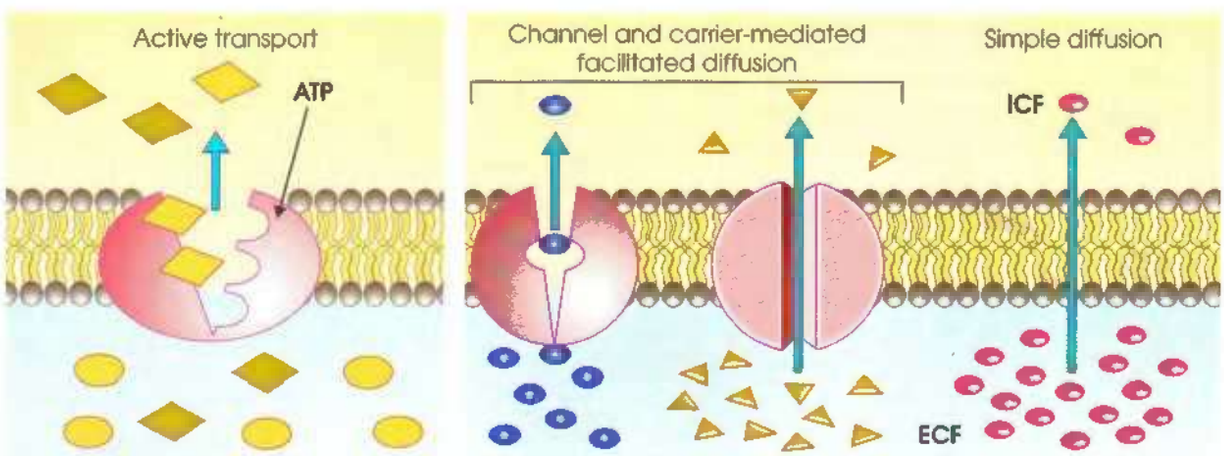


Fig. 3.4: Transport mechanisms: Active transport, channel and carrier-mediated facilitated diffusion and simple diffusion

Table 3.1: Difference between passive transport and active transport

Passive transport	*Active transport*
Simple diffusion does not require carrier protein for transport	It requires carrier protein for transport of substances
Facilitated diffusion which is a type of passive transport requires a membrane proteins called carrier protein for transport	
It does not require energy for transport	It requires ATP as source of energy for transport
In simple diffusion substances move in the direction of concentration gradient	Substances can move against concentration gradient from lower to higher concentration

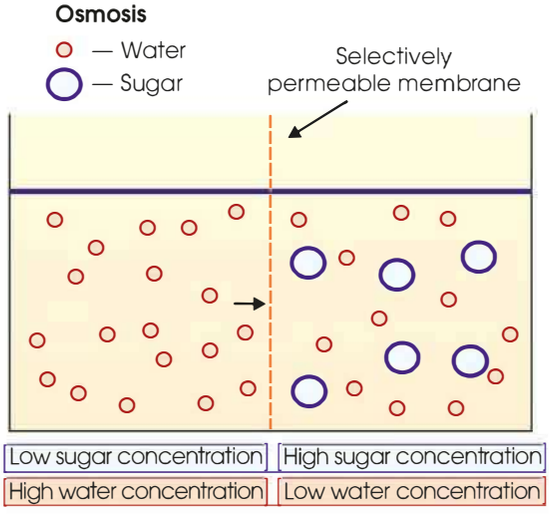


Fig. 3.5: Process of osmosis

active. The other osmotically active substances are dextran, plasma protein, NaCl. The examples of osmosis in human body are: Re-absorption of water by the distal and proximal convoluted tubules of the nephron, re-absorption of tissue fluid from venous end of the blood capillaries and absorption of water through the gastrointestinal tract (stomach, small intestine and the colon), etc.

Jean-Antoine Nollet first documented observation of osmosis in 1748.

Jean-Antoine Nollet
(19th November 1700–25th April 1770)

Osmotic pressure: It is the minimum hydrostatic pressure required to stop osmosis. Osmotic pressure is the colligative property which means that the osmotic pressure depends mainly on the molar concentration of the solute. The concentration of osmotically active substance is expressed as osmoles. Osmolarity refers to number of osmotically active substance in one litre of solution. Osmolality refers to number of osmotically active substance dissolved in one kg of water. The plasma osmolality is 290 mOsm per kg and is mainly due to sodium chloride.

Other process favouring transport mechanisms are filtration along capillary membrane which occurs due to difference in hydrostatic and oncotic pressure and solvent drag. During bulk flow of water it carries solutes along with it by solvent drag mechanism.

The co-transport mechanism is classified as uniport, antiport and symport.

1. **Antiport:** Carrier transporter transporting one substance for another. Examples: Sodium–hydrogen transport in renal tubules. Another example is the Na^+/Ca^{2+} exchanger is found in many cells and tissue in human body and helps in maintaining homeostasis. It helps in influx of Na^+ down its gradient into the cell and Ca^{2+} efflux from the cell against its gradient. Three Na^+ molecules enter the cell for every Ca^{2+} that moves out of the cell.
2. **Uniport:** Transport of a single substance by the carrier protein. Example: Transport of sodium or potassium ions through their respective channels.
3. **Symport:** Unidirectional co-transport of two or more substance from one side of cell membrane to another.

MEMBRANE TRANSPORT: ACTIVE TRANSPORT

A. Active Transport (or Primary Active Transport)

Active transport is a process in which a solute is moved against a concentration or electrochemical gradient using ATP as source of energy (Table 3.2) (Figs 3.4 and 3.6).

Thus, the key features of primary active transport are:

1. Move substrates against concentration gradient
2. Uses carrier proteins (just like carrier-mediated facilitated diffusion)
3. Requires energy, such as ATP

Table 3.2: ATP-powered primary active transporters

ATP-powered primary active transporters are of four types:

1. P-type ATPase (P-class pumps are composed of two polypeptides, α and β, and they get phosphorylated as part of the transport cycle: Sodium potassium pump, calcium pump, proton pump
2. V-ATPase: Vacuolar ATPase
3. F-ATPase: Mitochondrial ATP synthase, chloroplast ATP synthase
4. ABC (ATP binding cassette) transporter: MDR, CFTR, etc.

Examples

I. 3Na–2 K active transport pump: The three-sodium moves out of the cell against two-potassium which moves to the interior using ATP as the source of energy. The steps involved in 3 Na^+–2K^+ active transport pump are:

1. Three intracellular Na^+ ions bind to the receptor site within the pump channel.
2. ATP dissociates to ADP and Pi providing energy to the channel.
3. Using the energy transferred from the ATP, the channel reconfigures by the conformational change in protein molecule permitting the 3Na^+ ions to diffuse into extracellular fluid.
4. The exposed K^+ binding sites permit two K^+ ions to enter the channel and release the Pi.
5. Another ATP binds to its site and the channel resumes its original configuration permitting the 2K^+ ions to diffuse into the intracellular fluid.

The net effect is to transfer 3Na^+ out of the cell and 2K^+ into the cell. As long as ATP remains available, the cycle can repeat.

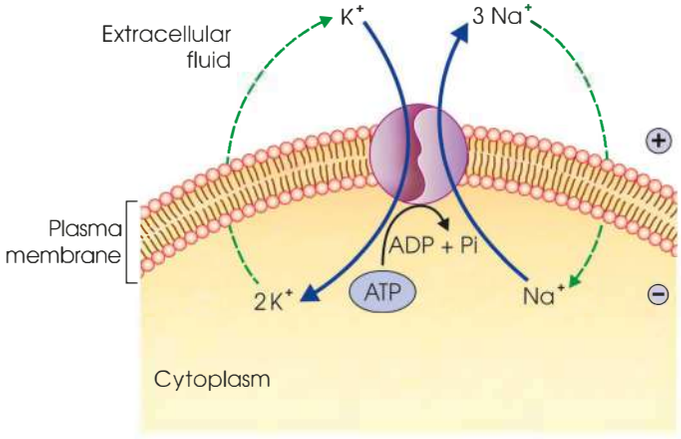


Fig. 3.6: 3 Na–2 K active transport pump

Sodium potassium ATPase pump is an electrogenic pump which creates electrical potential across the membrane and is necessary for transmitting electrical signals across the nerve and muscle. It also maintains the intracellular volume by regulating water movement across the membrane and also maintains the resting membrane potential across the cell membrane.

II. The H^+/K^+-ATPase is present in the luminal membrane of parietal cells.

a. The H^+/K^+-ATPase pump drives H^+ ions in exchange for K^+ by primary active transport into the glandular lumen.
b. The K^+ from the parietal cell circulates back to the lumen via luminal K^+ channels. One HCO_3^- enters into circulation for every H^+ ion secreted by parietal cells into lumen and is exchanged for a Cl^- ion via an anion antiporter.
c. The Cl^- ions diffuse out of the cell to the lumen via Cl^- channels. Thus, one Cl^- ion reaches the lumen for each H^+ ion secreted.

3. Ca^{2+} ATPase (Fig. 3.8) pump present in membrane of endoplasmic reticulum, sarcoplasmic reticulum and all other cell membranes with aid of calcium ATPase

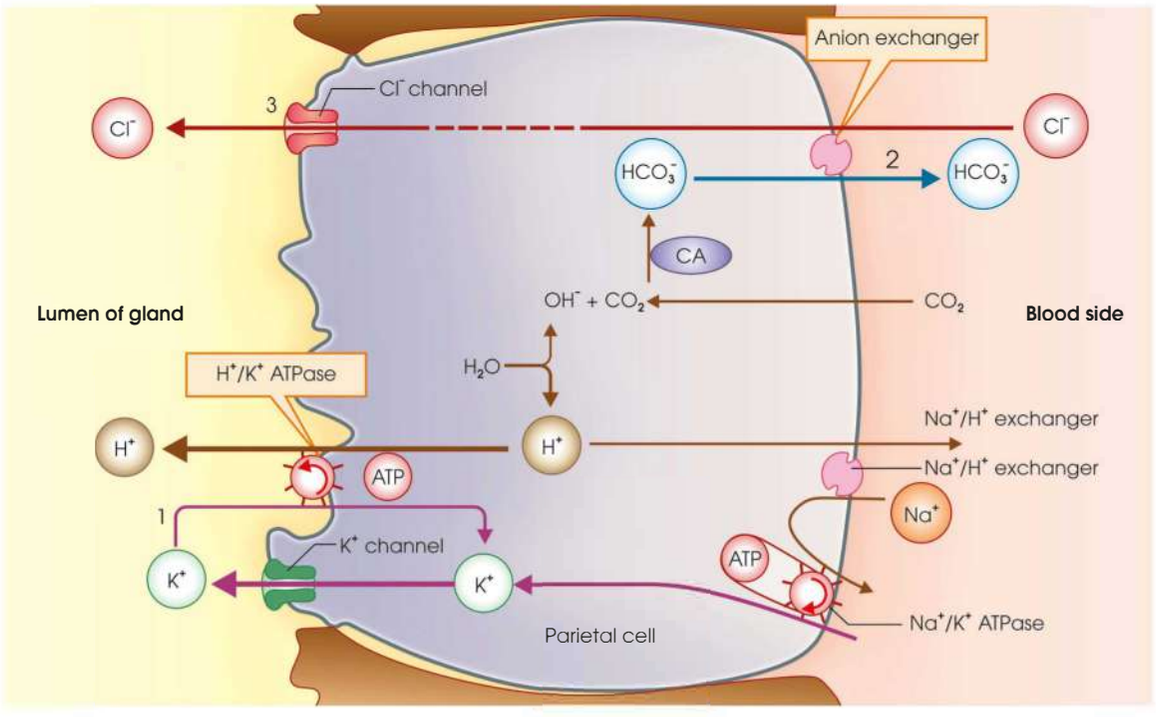


Fig. 3.7: H^+/K^+ ATPase pump secreting H^+/into glandular lumen from parietal cell

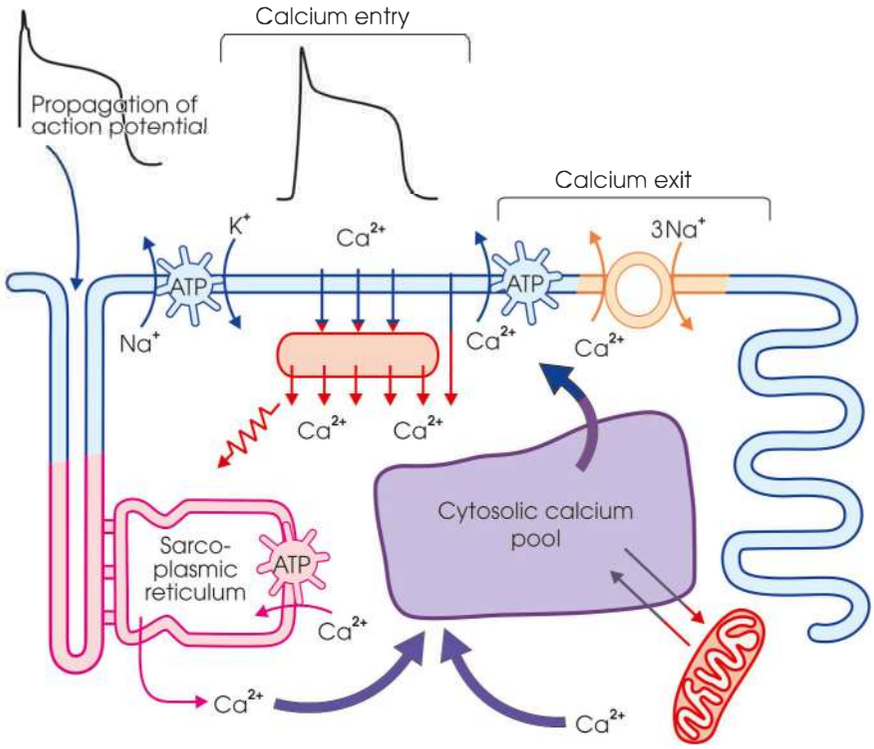


Fig. 3.8: Ca^{2+} ATPase pump in cardiac muscle

maintains concentration in the cytoplasm. An action potential in cardiac muscle stimulates the release of Ca^{2+} from the sarcoplasmic reticulum through a voltage-gated channel. The action potential induces depolarization of the cell surface and influx of Ca^{2+} result is actin–myosin activation and contraction of cardiac muscle. The cardiac muscle cells have a Na^+/Ca^{2+} antiporter and Ca^{2+} ATPase that maintain a low cytoplasmic Ca^{2+} concentration normally. During cardiac relaxation Ca^{2+} is removed from the cytoplasm by the SR calcium pump and the sarcolemmal Na^+/Ca^{2+} exchange. The sarcolemmal Na/Ca^{2+} exchange is involved in the regulation of cardiac cellular Ca^{2+} content (Fig. 3.8).

B. Secondary Active Transport

It is also known as co-transport or coupled transport mechanism. The secondary active transport use stored energy in ion gradients to actively transport molecules across membranes. The co-transport mechanism is classified as uniport, antiport and symport (Fig. 3.9).

1. **Antiport:** Carrier transporter transporting one substance for another. Examples: Sodium–hydrogen transport in renal tubules. Another example is the Na^+/Ca^{2+} exchanger is found in many cells and tissue in human body and helps in maintaining homeostasis. It helps in influx of Na^+ down its gradient into the cell and Ca^{2+} is efflux from the cell against its gradient. Three Na^+ molecules enter the cell for every Ca^{2+} that moves out of the cell.

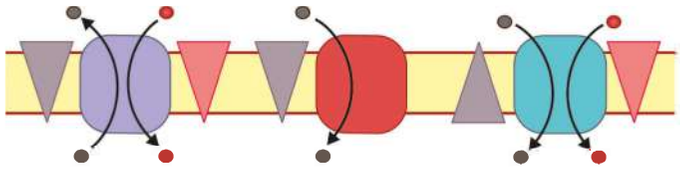


Fig. 3.9: Transport mechanisms; uniport, symport, antiport

2. **Uniport:** Transport of a single substance by the carrier protein.
   Example: Transport of sodium or potassium ions through their respective channels.
3. **Symport:** Unidirectional co-transport of two or more substance from one side of cell membrane to another.
   Examples
   a. Sodium glucose or sodium amino acid co-transport (Fig. 3.10). The Na^+/glucose co-transporter (SGLT1) is an electrogenic transporter favouring glucose, and galactose absorption in the small intestine and reabsorption of filtered glucose and galactose (SGLT2) in the proximal tubule of kidney nephrons.
   b. Na^+/I^- symporter (NIS): It is localised at the basolateral membrane of thyroid follicular cells and co-transports $2Na^+$ ions and 1 I^- ion per transport cycle. It is also found in mammary glands, where it functions to transport iodide into the lactating mother's breast milk.
   c. Na^+/phosphate co-transport (NaPi) in the apical membrane of epithelial cells of the small intestine and renal proximal tubules.

Robert K. Crane: Robert K. Crane was the first to discover flux coupling in biology by citing the sodium–glucose co-transport as the mechanism for intestinal glucose absorption.

Robert K. Crane
1919–2010

***Example*:** Intestinal absorption of glucose and sodium (and water)

1. The Na-K-ATPase pump located in the basolateral membrane is responsible for maintaining a low intracellular concentration of Na^+. The sodium potassium pump creates an ionic gradient as a result of primary active transport.
2. This results in concentration difference between luminal and intracellular Na^+; which provide energy for absorption of Na^+ and glucose into the cell with aid of the SGLUT transporter protein. This mechanism of transport is known as secondary active transport.
3. Glucose diffuses from the cell into the interstitial space through the basolateral membrane by facilitated diffusion and the sodium is removed from the cell by the Na-K-ATPase pump.
4. The osmotic absorption of water (osmosis) in interstitial fluid is due to accumulation of glucose and Na^+ in the interstitial fluid.
5. The Na^+, glucose, and H_2O which is accumulated in the interstitial space is removed by diffusion into the intestinal capillaries (simple diffusion) which then via circulatory system is carried in systemic circulation.

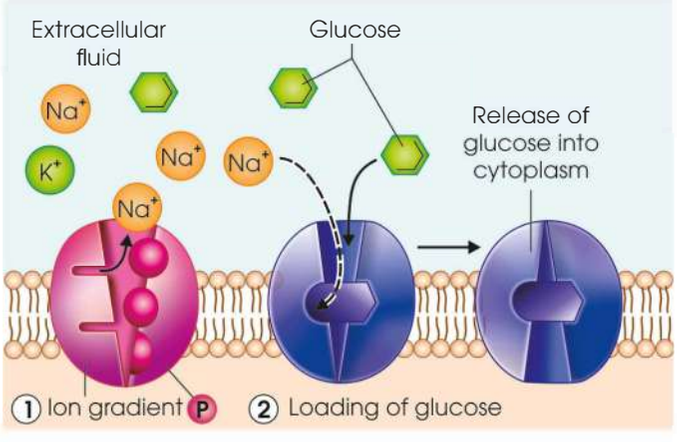


Fig. 3.10: Secondary active Glucose; sodium and glucose co-transport

Note

The secondary active transporters apart from plasma membrane are found in the membrane of synaptic vesicles in axon terminals. For example, H^+/neurotransmitter exchangers utilize proton electrochemical gradient across the vesicle membrane to drive the uphill transport of neurotransmitter into the vesicle. In all secondary active transport mechanism the electrochemical gradient of the driving force of ion is maintained by primary active transporters.

Sodium glucose and sodium amino acids are example of co-transport while sodium for hydrogen and bicarbonate for chloride in renal tubules are example of secondary active counter transport.

ABC TRANSPORTERS

These ATP-binding cassette transporters are the transmembrane proteins which exhibit ATP-binding domain on one side and ligand-binding domain on the other surface. The ATP on binding with its domain; provides the energy to pump the ligand across the membrane. Human genoneme contains around 48 genes for ABC transporters. Many of these have been causally related to diseases such as cystic fibrosis, Stargadt's disease, drug-resistant tumors, Dubin-Johnson syndrome, ataxia, progressive familiar intrahepatic cholestasis, etc. Multi-drug resistance is frequently associated with over-expression of ABC transporters especially in tumour cells. They are also expressed in the membranes of normal cells, by which they facilitate the transport of various endogenous substances.

The examples of ABC transporters are:

1. CFTR—the cystic fibrosis transmembrane conductance regulator: It is a membrane protein and chloride channels. The gene that encodes the human CFTR protein is located on chromosome 7. The mutations of the CFTR gene affects chloride ion channel functions and produces dysregulation of epithelial fluid transport in the pancreas, lung and other organs, resulting in cystic fibrosis.
2. TAP, the transporter associated with antigen processing.
3. Transporter used by liver cells; to pump the salts of bile acids out into the bile.

VESICULAR TRANSPORT MECHANISMS

Vesicular transport: The materials move into or out of the cell by means of vesicles, also called bulk transport. The various mechanisms involved are:

I. Endocytosis (clathrin-mediated)

1. Receptor-mediated endocytosis (Fig. 3.11)
2. Pinocytosis (Fig. 3.12)
3. Phagocytosis (Fig. 3.12)

II. Exocytosis

All are active processes (requiring ATP) though they are not usually referred to as "active transport".

1. **Receptor-mediated endocytosis:** The processes involved in endocytosis are:
   - The target molecules bind to receptor in cell membrane.
   - The area coated with ligands form pockets on membrane surface.

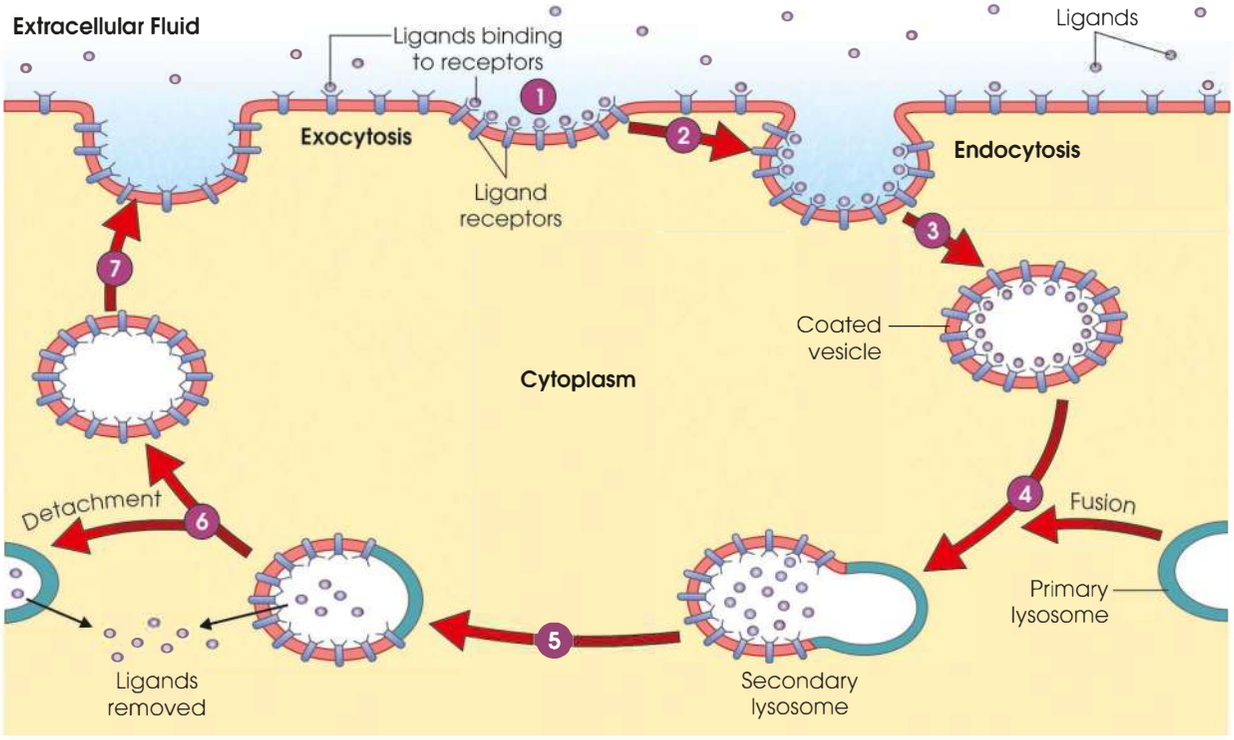


Fig. 3.11: Receptor-mediated endocytosis and exocytosis. 1. Ligand binding to receptors, 2. Area coated with ligands from pockets on membrane surface, 3. Pocket pinch to from endosomes, 4. Fusion of coated vesicles with lysosomes, 5. Removal of ligands, 6. Separation of lysosomal and endosomal membranes, 7. Fusing of endosome with cell membrane

- The pockets pinch to form endosomes which are the coated vesicle.
- Coated vesicle fuses with lysosomes.
- Molecules are removed and absorbed in cytoplasm.
- Lysosomal and endosomal membrane separates.
- The endosomes fuses with cell membrane and receptors are again available for ligand binding.

2. **Phagocytosis (cell eating):** It is the intake of particles more than about 0.5 µm in diameter by process of endocytosis. The plasma membrane fuses forward and encircles the particles near the cell surface; to form phagocytic vesicles called phagosomes. The lysosome draws near and fuses with these phagosomes and releases their digestive enzymes to digest the content in phagosomes. Phagocytosis is used for defensive purposes. The neutrophils and macrophages defend against foreign bodies such as bacteria, virus, dead cell, cellular parts and other waste matter by phagocytosis.
3. **Pinocytosis:** It is the active intake of droplets of extracellular fluid along with small particles into the cell. Pinocytosis helps in intake of important materials into cells. The macromolecule binds at the receptor site of cell membrane. The membrane invaginates and covers the macromolecule completely. The macromolecules enclose by part of the membrane forms a bubble and this pinocytic vesicle separates out from cell membrane, moves into cytoplasm. It helps to intake of ions, sugars and amino acids and also solutes such as insulin and lipoproteins in concentrated form into the cells.

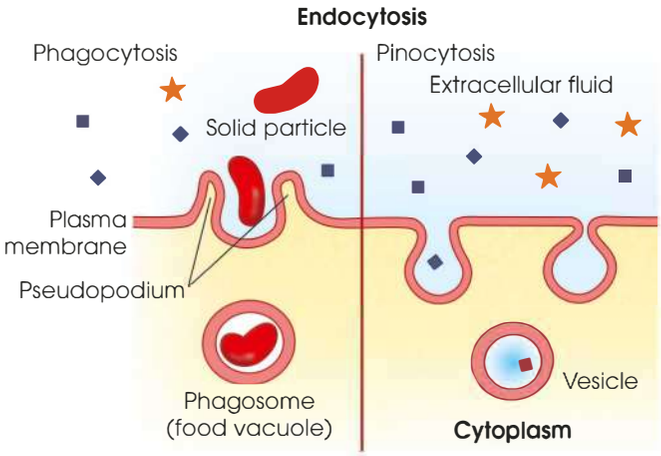


Fig. 3.12: Phagocytosis and pinocytosis

Exocytosis: It is process by which the intracellular substances are released into the surrounding tissue. The neurotransmitters at nerve ending are released by exocytosis.

The process involved in exocytosis is (Fig. 3.13):

1. *Vesicle trafficking:* It is required for the transportation of a vesicle over a moderately small distance.
2. *Vesicle tethering:* Tethering involves transport over distances of more than about half the diameter of a vesicle from a given membrane surface (>25 nm). They occur during concentrating synaptic vesicles at the synapse.

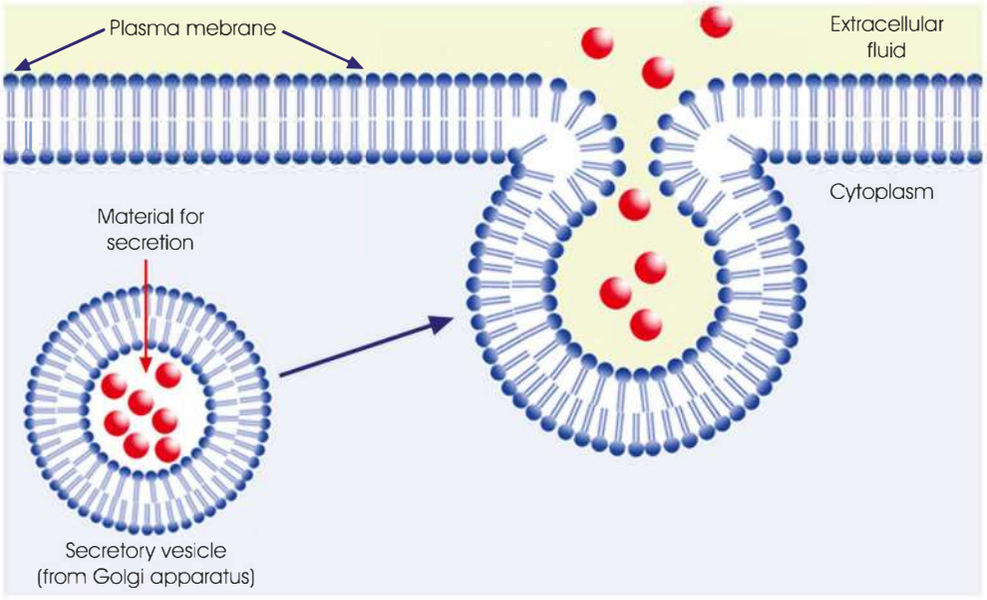


Fig. 3.13: Exocytosis

3. *Vesicle docking:* The secretory vesicles transiently dock at the cell plasma membrane, forming t-/v-SNARE complex, producing continuity between the opposing bilayer.
4. *Vesicle priming:* In neuronal exocytosis, the term priming has been used to include all of the molecular rearrangements and ATP-dependent protein and lipid modifications that take place before exocytosis.
5. *Vesicle fusion:* Transient vesicle fusion is driven by SNARE proteins, releasing vesicle contents into the extracellular space.

Transport through Cellular Sheets

In certain places in body such as intestinal epithelium, epithelium of renal tubules, epithelium of exocrine glands, the transports occur through trans-cellular sheath rather than cell membrane. The sodium and water are transported along luminal membrane by diffusion while they are actively transported via basolateral membrane into the extracellular fluid of the surrounding connective tissue and blood vessels. It is by this same mechanism substances are absorbed from glomerular filtrate into renal tubules and so also ions and nutrients into blood from intestine.

Inherited Ion-channel Diseases

1. **Chloride-channel diseases:** Cystic fibrosis and Inherited tendency to kidney stones.
2. **Potassium-channel diseases:** Long QT syndrome; a rare, inherited tendency to epileptic seizures in the newborn; and several types of inherited deafness.
3. **Sodium-channel diseases:** Inherited tendency to certain types of muscle spasms; and Liddle's syndrome (inadequate sodium transport out of the kidneys, because of a mutant sodium channel, leads to elevated osmotic pressure of the blood and hypertension).

EXAM-ORIENTED QUESTIONS

Essay

1. Describe the various transport mechanisms across cell membrane.

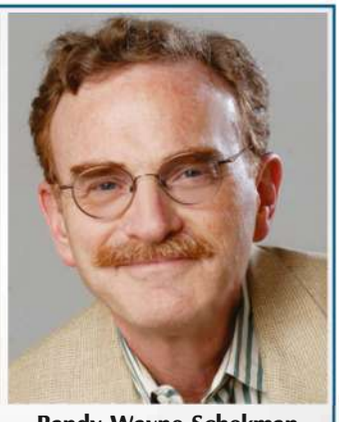
Short Notes

1. Active transport: Primary and secondary
2. Diffusion
3. Differentiate between simple and facilitated diffusion
4. Vesicular transport mechanisms
5. Transport through cellular sheets

RECENT ADVANCES: VESICULAR TRANSPORT MECHANISM

James Rothman, Randy Schekman, and Thomas Südhof were awarded the 2013 Nobel Prize in Physiology or Medicine 'for their discoveries of machinery regulating vesicle traffic, a major transport system in our cells'. The four decade long research

James Edward Rothman
1950



Randy Wayne Schekman
1948

work by these scientists got the final recognition as they successfully explained the fundamental mechanisms involved with cargo macromolecules transport between the membrane-enclosed compartments which constitute the secretory system of eukaryotic cells.

Thomas Christian Südhof
1955

The discoveries by Rothman, Schekman, and Südhof established a general paradigm for the mechanisms of vesicular transport that applies to the secretory pathway and also to other important intracellular trafficking processes such as endocytosis, retrograde transport from endosomes to the Golgi complex and from the Golgi complex to the ER, and transport to lysosomes.

The steps involved in these vesicular transports are:

1. The transport vesicle is sculpted from the donor membrane. This is achieved by the assembly of a protein coat.
2. The assembled protein coat promotes vesicle budding as it selects specific cargos for incorporation into the vesicle.
3. As vesicle scission from the donor membrane occurs it loses its protein coat and translocates along the cytoplasm.
4. Tethering factors associated with the acceptor membrane then capture the vesicle.
5. This then promote formation of complexes between vesicle SNAREs (v-SNAREs) and target SNAREs (t-SNAREs).
6. This results in e 'Zippering' of the SNAREs.
7. Finally this triggers membrane fusion, resulting in the delivery of cargo into the acceptor compartment.

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NOBEL PRIZE 2016: AUTOPHAGY

Yoshinori Ohsumi
1945

Yoshinori Ohsumi, the Japanese Cell Biologist received the 2016 Nobel Prize for Physiology and Medicine for discovering of mechanisms for autophagy. Autophagy is a fundamental process by which the cellular components get degraded and recycled. Autophagy provides fuel for energy for renewal of cellular components, and plays a vital role in cellular response to varied types of stressful conditions. It also helps in elimination of intracellular bacteria and viruses. Cells also use autophagy to eliminate the damaged proteins and organelles are eliminated by cell by the process of autophagy thus overriding the negative consequences of ageing.

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Chapter

4

Membrane Potential

INTRODUCTION

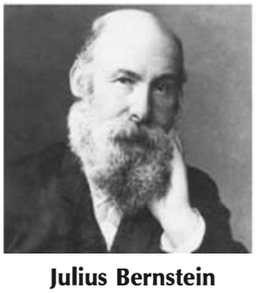
The difference between the electric potential across the cell membrane is due to ionic gradient created due to variation in ionic concentration of substances between intracellular and extracellular environment. The relatively static membrane potential of a resting quiescent cell is called the resting membrane potential (RMP). While the specific dynamic electrochemical phenomena leads to generation of graded membrane potential and action potential.

ROLE OF ION IN GENERATING MEMBRANE POTENTIAL

1. These potentials result from movements of ions across the membranes.
2. There is unequal distribution of ions on the both sides of the cell membrane. The ions move from high to low concentration by diffusion and as a result of electrical gradient which favors movement of ions away from like charge toward opposite charge.
3. Each ion which flow through channels reaches equilibrium between two forces.
4. The equilibrium potential for each ion is determined by Nernst equation.
5. Membrane potential is much depended on intracellular potassium level; as the membrane permeability to potassium is about hundred times higher than that to sodium.

Ionic Mechanisms of Resting Potentials

Julius Bernstein in early 1900 suggested that the resting potential (V_m) is equal to the potassium equilibrium potential (EK). The selectively permeability of cell membrane to different ions and ionic concentration differences (the unequal distribution of ions in the inside and outside of cells) are the key factors responsible for generation of resting potential.



Julius Bernstein
1839–1917

PHYSICO-CHEMICAL PRINCIPLES INVOLVED IN GENERATING RMP

Nernst Potential

The Nernst potential for any ion is the membrane potential at which the ion is in equilibrium, i.e. there is no net movement of the ion across the membrane. At the equilibrium potential, the chemical and electrical gradients are equal and opposite in direction.

Nernst equation

The mathematical equation to calculate equilibrium potentials for certain ions is:

$$Ei = \left(\frac{RT}{Fz}\right) \cdot \ln \frac{[X]1}{[X]2}$$

- R = Gas constant
- T = Absolute temperature (K)
- E = The potential difference across the membrane
- F = Faraday's constant (96,500 coulombs/mole)
- z = Valence of ion

As K^+ is major cation present intracellularly, its diffusion creates negativity inside the cell. Thus, it is the main contributing factor towards generation of RMP.

Goldman-Hodgkin and Katz (GHK) Equation

Nernst equation cannot be used if a membrane is permeable to two different ions. It is possible, however, to apply the Goldman-Hodgkin and Katz (GHK) Equation. The trans-membrane movements of three ions Na^+, K^+, and Cl^- contribute to the membrane potential. This equation describes the potential across a membrane that is permeable to Na^+, K^+ and Cl^-.

Goldman-Hodgkin and Katz equation

$$V_m = \frac{RT}{F} \ln \left(\frac{p_K [K^+]_O + p_{Na} [Na^+]_O + p_{Cl} [Cl^-]_i}{p_K [K^+]_i + p_{Na} [Na^+]_i + p_{Cl} [Cl^-]_O} \right)$$

- V_m is the membrane potential. This equation is used to determine the resting membrane potential in real cells, in which K^+, Na^+, and Cl^- are the major contributors to the membrane potential.
- R is the universal gas constant (8.314 $J.K^{-1}.mol^{-1}$).
- T is the temperature in Kelvin (K = °C + 273.15).
- F is the Faraday's constant (96,485 $C.mol^{-1}$).
- p_K is the membrane permeability for K^+.
- p_{Na} is the relative membrane permeability for Na^+.
- p_{Cl} is the relative membrane permeability for Cl^-.

Gibbs-Donnan Membrane Equilibrium

The development of RMP is based **on principles of Gibbs-Donnan membrane equilibrium**. The two solutions containing ions are separated by a semi-permeable membrane; at equilibrium each of the solution will be electrically neutral. There is equal and balance distribution of ions in each solution and they become electrically neutral (total quantity of cations will be equal to total quantity of anions). The large impermeable negatively charged intracellular molecules attract positively charged ions (e.g. Na^+ and K^+) and repelling negative ones (e.g. Cl^-) in process of genesis of RMP.

The resting membrane potential is produced and maintained by

1. **Membrane selectivity:** It is the difference of permeabilities between different ions.
2. **Na^+/K^+ ATPase pump:** This is the active transport mechanism involved for moving particles across a biological membrane, against the concentration gradient. The Na^+/K^+ ATPase pump creates a concentration gradient by moving 3 Na^+ out of the cell and 2 K^+ into the cell.
3. **Permeability of membrane to potassium:** RMP is created by the distribution of ions and its diffusion across the membrane. Potassium ions are important for RMP
   a. As the membrane is highly permeable to K^+, it plays an important role in generating resting potential. The outside of the cell has a low concentration of K^+ ($[K^+]_o$) than the inside of the cell which has a high concentration of K^+ ($[K^+]_i$).
   b. Positively charged K^+ thus moves by diffusion from its area of high concentration to its area of low concentration. Thus, inner surface of membrane becomes more negative. The negative charge at the inner surface of membrane attracts the positive charge K^+ ion that is moving out and tends to retain it back.
   c. Thus, the electrical force which is directed inward counterbalance the diffusion force which is directed outward.
   d. As a result equilibrium is established; and the concentration force moving K^+ outward balances the electrical force holding in it. The potential at which the equilibrium is achieved is called the Nernst equilibrium potential.
4. **Permeability of membrane to sodium ions:** At rest the membrane remains less permeable to sodium then potassium. The exit of K^+ is not balanced by entry of Na^+; therefore the interior of cell remains negative.
5. **Role of ion channels in resting membrane potential:** The protein channels are present on the cell membrane. This allows ions to diffuse passively without direct expenditure of metabolic energy especially through the open channel. These channels have selectivity for certain ions, for example sodium or potassium specific selective ion channels. All cell membranes have more permeability to K^+ than to Na^+ because they have more K^+ channels than Na^+.

RECORDING OF MEMBRANE POTENTIAL

The membrane potential can be recorded using cathode ray oscilloscope. The two of the microelectrodes are placed on surface of the nerve fibre. The electrodes are connected to the cathode ray oscilloscope. As the voltage changes a potential difference ranging between –80 mV and –40 mV (average of –60 mV of negativity inside the cell) is noted in the cathode ray oscilloscope. In the absence of any stimuli sensitization the resting potential is generally constant (Fig. 4.1).

The recording can be also carried as follows:

The tip of the recording microelectrode can be inserted inside the neuron, and the reference electrode is placed in the extracellular fluid. The electrodes when connected to a voltmeter measures the difference in charge across the membrane (in this case –70 mV inside negative). The resting membrane potential is an

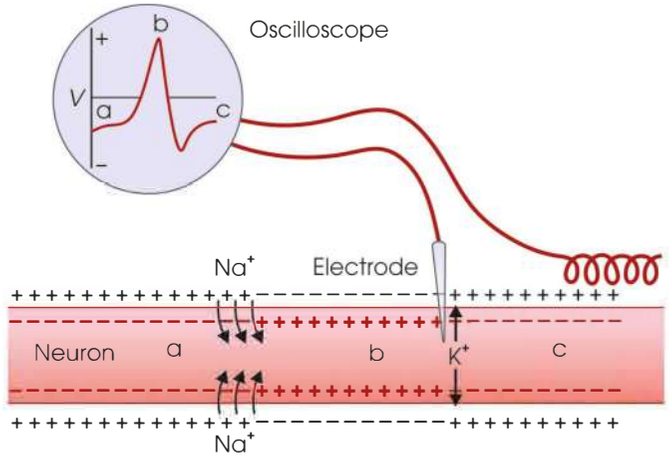


Fig. 4.1: Recording of membrane potential

electrical potential difference (voltage) that exists across the plasma membrane of an excitable cell under resting conditions.

GRADED POTENTIAL AND ACTION POTENTIAL

Introduction

The changes in the membrane potential produce electric signals in nerve cells. The ion concentration difference between intracellular and extracellular fluid gives rise to the resting membrane potential (RMP). Membrane permeability to these ions also influences the RMP. The transient changes from the RMP produce electrical signals which transmit information in nerve cells in form of action potential.

The different channel types are responsible for transmitting electrical signals over long and short distances in the nervous system:

1. Graded potentials are activated by the opening of mechanically or chemically gated channels and they travel over short distances.
2. Action potentials are generated by the opening of voltage-gated channels and travel over long distances.

Terms Associated with Membrane Potential

1. Depolarization—a decrease in the potential difference between the inside and outside of the cell.
2. Hyperpolarization—an increase in the potential difference between the inside and outside of the cell.
3. Repolarization—returning to the RMP from either direction.
4. Overshoot—when the inside of the cell becomes +ve due to the reversal of the membrane potential polarity.

GRADED POTENTIAL

The local, graded, non-propagated potentials are called receptor or generator potentials. A sub-threshold electrical stimulus does not produce a true action potential but generates electrical signals. The stimuli may be electrical, chemical, or mechanical. These stimuli produce either graded potential or action potential. The observed response is a sub-threshold response (Fig. 4.2).

The characteristics of graded potentials are:

1. It is a local effect and changes in membrane potential are confined to relatively small regions of the plasma membrane.
2. It is graded response and refers to the magnitude of the potential change. Magnitude is graded with the magnitude of the stimulus. The graded events can be depolarizing or hyperpolarizing.
3. The graded potentials are conducted with decrement and the conduction magnitude falls off further you move from the point of origin.

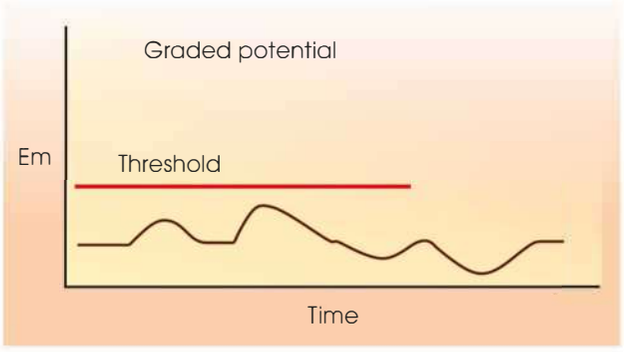


Fig. 4.2: Graded potential (below threshold)

Local Response of Graded Potential

1. Graded potentials and the local current they generate functions as signals over very short distances.
2. Graded potentials die out in 1–2 mm of the origin.
3. The charge is lost across the membrane because of leaky channels and the magnitude of the potential decreases with distance from the site of origin (charge density falls).

Types of Graded Potential

1. *Receptor* (*generator*) *potentials*: The sensory receptors respond to stimuli from thermoreceptors, mechanoreceptors, chemoreceptors, nociceptors and electromagnetic receptors. The graded potential from stimuli is called receptor potential. When graded potential reaches the threshold an action potential is generated and sensory information is sent to the spinal cord and brain.
2. *Pacemaker potential*: The specialized coronary muscle cells in the cardiac pacemaker region (SA node) have leaky ion channels. The generated graded potentials in pacemaker can potentially induce a true cardiac action potential. The graded potential is responsible for cardiac automaticity.
3. *Postsynaptic membrane potentials*: These are the graded potentials that develop on the postsynaptic membrane during synaptic transmission. When graded potentials reach threshold the action potential develop.
4. *End plate potentials* (*EPP*): These are the postsynaptic graded potential that develops at the neuromuscular junction. The postsynaptic membrane potentials are important in generation of action potential in nerve to nerve and nerve to muscle communication.
5. *Graded potentials* in the neurons travel through the neuron until they reach the trigger zone. If they depolarize the membrane above threshold voltage (about –55 mV) an action potential is triggered and it travels down the axon.

Summation of Graded Potential (Fig. 4.3)

Summation of graded potentials demonstrates a key property of neurons—postsynaptic integration. The magnitude of graded potentials are added together to

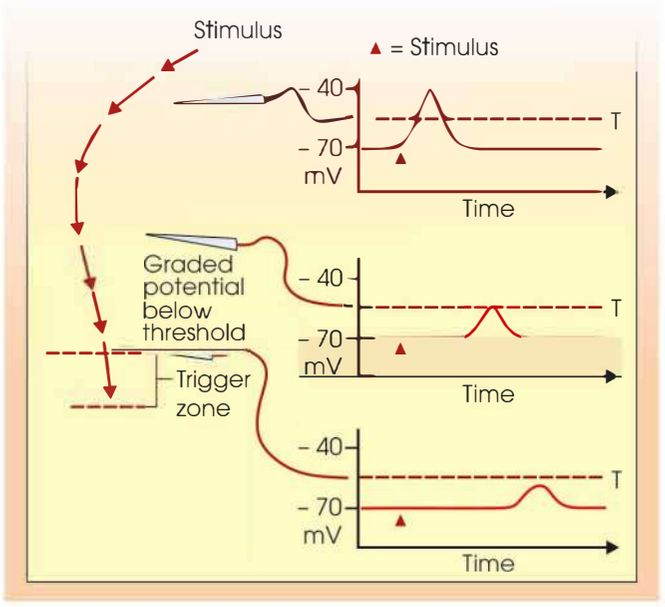


Fig. 4.3: Summation of graded potential

have a combined effect on the postsynaptic membrane leading to summation. The summation of graded potentials can be temporal summation and spatial summation.

Temporal summation occurs from the summation of graded potentials overlapping in time which initiates action potential.

Spatial summation occurs from the summation of several graded potentials from several converging neurons simultaneously.

ACTION POTENTIAL

It is the rapid and transient change in a membrane potential which occurs when nerve cell membrane is stimulated. The membrane potential changes from the resting potential-70 millivolts (neurons) to about +30 mV in a brief short period of time (few milliseconds). Action potentials are responsible for muscle contraction, hormone release, GI secretions, cardiac contractions, etc. The special senses like vision, hearing and touch are all dependent on action potentials for transmission of information to the brain. The cardiac potentials are recorded as electrocardiograph (ECG) while neuronal transmissions are recorded as electroencephalograph (EEG). The concepts of action potential generation are important in understanding the physiological functions of the body.

CHARACTERISTICS OF ACTION POTENTIAL

1. Rapid changes in ion conductance result in generation of action potential.
2. Specific voltage-gated ion channels mainly contributes towards generation of action potential.
3. Action potentials are generated on regions of cell membranes that are electrically excitable. The tissues can be stimulated mechanically, chemically or electrically. The electrical stimulus is used for experimentation purpose as the intensity and duration of stimulus can rightly be controlled.
4. Action potentials generated are of standard size and shape for a specific cell type.
5. It follows the principle of 'All-or-none law': When threshold potential is reached an action potential is generated while in case of sub-threshold potential no action potential will be generated. The threshold stimuli (graded potential) cause the generation of an action potential.
6. It follows the principle of accommodation: The threshold of stimulus should rapidly increase to its peak intensity. The slow rising strength does not produce action potential inspite of it ultimately reaching the threshold strength.
7. The time duration of the action potential is always the same for a specific tissue. Action potentials not only have a specific size and shape but they also exist within a specific time frame which averages to 1 to 5 msec.
8. Voltage inactivation: If a cell membrane is maintained at a voltage potential above threshold then the voltage-gated channels are not reset and, hence, inactivated and no action potentials can be generated.

The stages of the action potential are

1. *Resting stage:* It is the polarized stage and reflects the normal resting membrane potential (RMP). It varies with the cell type, e.g. RMP of nerve is –90 mV, pacemaker is –60 mV and skeletal muscle averages to –83 mV.
2. *Depolarization stage:* The sodium ion (Na^+) moves into the cell as the threshold for voltage-gated Na^+ channels is exceeded. The membrane potential changes from RMP to threshold (as voltage-gated sodium channel opens) and as it is reaching 0 mV; voltage-gated sodium channel fully open and further as the membrane potential reaches to + 30 mV there is inactivation of sodium channels and opening of the K^+ channels with completion of depolarization.
3. *Repolarization stage:* Potassium (K^+) ions flow out of the cell as voltage-gated K^+ channels are opened and the cell membrane potential moves back toward the resting membrane potential.
4. *Hyperpolarization:* The potential typically overshoots above the rest potential to about –90 mV. This leads to hyperpolarization.

NERVE ACTION POTENTIAL

Action Potential Generation in a Nerve Cell (Fig. 4.4)

1. **Resting potential:** The resting membrane potential in nerve cell averages around –70 mV. When a nerve cell is stimulated, the Na^+ channels open.

If the stimulus strength is adequate, the opening of the Na^+ channels is sufficient to change the membrane potential from –70 mV up to –55 mV, the action threshold is reached.

2. **Depolarization:** As the action threshold is reached more of the voltage-gated Na^+ channels open. The Na^+ influx drives and changes the membrane potential to about +30 mV. This process of change in membrane potential to positivity is called depolarization. The Na^+ channels close and the K^+ channels open. The K^+ channels are much slower to open and the depolarization gets completed.
3. **Repolarization:** The opening of K^+ channels open, the membrane begins to repolarize back toward its resting stage
4. **Hyperpolarization:** The potential typically overshoots above the rest potential to about –90 mV. This leads to hyperpolarization. The process of hyperpolarization raises the threshold for any new stimulus and prevents the neuron from receiving another stimulus during this time.
5. **Resting potential:** The Na^+/K^+ pump eventually brings the membrane back to its resting state of –70 mV from hyperpolarized state.

Ionic Basis of Action Potential

Role of voltage-gated sodium channel and potassium channel in generation of action potential

Resting state: In resting state the nerve fibre remains in polarized state and the membrane potential lies within –70 mV. The inside of the nerve is negative and the outside of the nerve is positive (Fig. 4.1). The sodium ion concentration outside the membrane is higher than that of inside the membrane. The potassium ion concentration inside the membrane is also higher than that of outside the membrane. K^+ can permeate through the membrane at resting state but the Na^+ cannot permeate. The influx of sodium leads to depolarization while efflux of potassium leads to repolarization. The voltage-gated sodium channel and potassium channel are main contributors towards generation of action potential. The action potential occurs in successive stages of depolarization, repolarization, negative after-potential and positive after-potential.

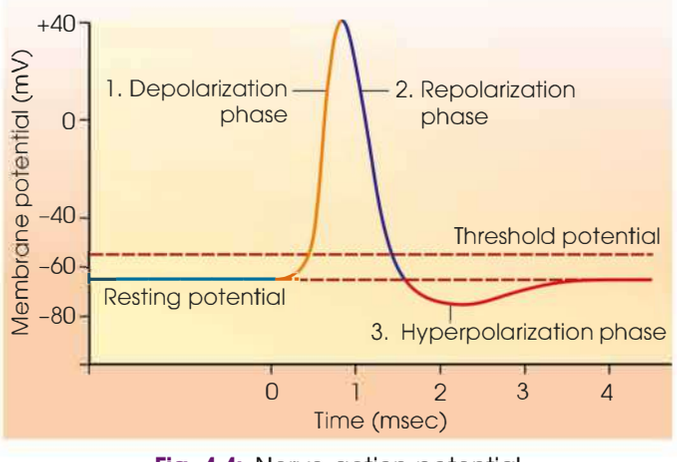


Fig. 4.4: Nerve action potential

Depolarization (excitability): Permeability of Na^+ to membrane is increased only after excitation and it is the first event of the action potential. The threshold stimulus leads to influx of sodium through leaky channels and via the opening of the voltage-gated sodium channel. The membrane potential; decreases from –70 to –55 mV.

As the depolarization proceeds further; large number of voltage-gated channel opens. So the depolarization starts with the onset of Na^+ entry and thus an increase in Na^+ conductance is taken place. The tremendous increase is Na^+ conductance during this period is known as activation of membrane produce large and sweep depolarization and the membrane potential reaches to +35 mV. Thus, the reversal of potential is caused with the development of positivity inside the membrane and negativity outside. The Na^+ sodium influx stops due to inactivation of gates of sodium channel. The sodium channel remains open for very brief period of time. Thus, this speedy closure produces auto-deactivation of the sodium channel. The voltage-gated K^+ channels fully open at +35 mV causing efflux of K^+ ions.

Repolarization: But as soon as the action potential attains the voltage approximately +35 mV, K^+ efflux out from inside the membrane. The inside of the membrane becomes negative and outside becomes positive again. This stage is the repolarization phase and K^+ conductance is increased to the maximum. But at the later period of this phase (at the termination of spike potential) K^+ conductance is slowed down. As the membrane potential reaches to iso-potential level and as it is reaching towards the resting membrane potential the inside of the membrane achieves negativity; this limits efflux of potassium ions. Thus, a few milliseconds are delayed in restoring the membrane potential. This state is known after depolarization phase-potential and is attributed to slow efflux of potassium ions. In the later phase of repolarization the sodium channel is closed and then its inactivation gate opens slowly while the K^+ channel begin to close and gradually are completely closed. Thus, as membrane reaches resting state the activation gates of sodium and potassium channel are closed while inactivation gate of sodium channel opens.

Hyperpolarization: This increased negativity inside hinder further efflux of K^+. Most of the voltage-gated K^+ channels are closed but as some of the voltage-gated K^+ channels the efflux continues and membrane potential becomes more negative producing the phase of after hyper polarization. The resting membrane potential is yet to be achieved. It is achieved by the

complete closure of voltage-gated K^+ channel. The resting ionic composition is restored by the active Na^+ K^+ *pump mechanism* (*increased activity of* Na^+ K^+ ATPase)

In this way resting normal ionic status is established.

Cardiac Action Potentials

The cardiac muscle exhibits two types of action potentials; these are fast action potential and occurs in atrial and ventricular muscles and Purkinje fibres while slow response action potential occurs in sino-atrial node and atrio-ventricular (AV) node. Cardiac action potential of atrial and ventricular muscles and Purkinje fibres has a true resting potential, a fast depolarization phase, and a prolonged plateau phase (Fig. 4.5).

The ionic conductance responsible for various phases as below:

Phase 0: Rapid depolarization and overshoot: The hundredfold opening of voltage-gated sodium channels increases the influx of Na^+ ions. The opened sodium channel further activates the opening of the other voltage-gated sodium channels by process of auto- activation. The membrane potential reaches to peak at +25 mV with positivity inside the cell. In pacemaker cells the increase in membrane voltage is mainly due to activation of L type calcium channels. The L type calcium channel activates towards end of pacemaker potential and contributes towards later stages of pacemaker potential.

Phase 1: Initial rapid repolarization: Decreased Na^+ and increased K^+ conductance. There is inactivation of the fast Na^+ channels. The sodium influx ceases. The outward transient rectifying K^+ channels opens leading to efflux of potassium ions.

Phase 2: Plateau phase: The L type calcium channels (activated by sodium flow during phase 0) activation lead to influx of calcium into the cell. The increased Ca^{++} conductance due to slow and prolonged opening of calcium channel leads to plateau phase. There is efflux of potassium ions through the slow delayed rectifier K^+ channel. The influx of calcium into the cell is balanced by the efflux of potassium to the exterior of the cell, resulting in the plateau in action potential graph recording.

Phase 3: Repolarization: This phase is produced due to increased K^+ and decreased Ca^{++} conductance. The closure of L-type Ca^{2+} channels prevents calcium influx. The outward rectifying K^+ channels open increasing potassium permeability and more potassium moves outside.

Phase 4: Resting potential: It denotes the membrane potential when the cell is not being stimulated. This phase is observed as a horizontal line in non-nodal tissue action potential. This phase is produced due to increased K^+ and decreased Na^+ and Ca^{++} conductance. The opening of the inward rectifying K^+ channels restores membrane permeability to potassium ions and thereby reinstating resting membrane potential.

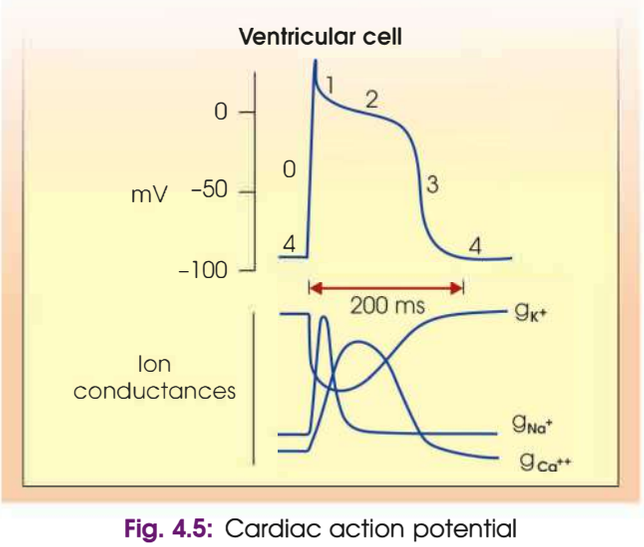


Fig. 4.5: Cardiac action potential

PROPERTY OF ACTION POTENTIAL

1. **Strength and duration of intensity:** The minimum strength of stimulus when applied for adequate time produces a response and is known as *rheobase*. The minimum duration for which a stimulus of double the strength of rheobase is applied is called *chronaxie*. Muscle fibres have faster chronaxie value then nerve.
2. **All-or-none law:** The sub-threshold stimulus fails to produce response. The threshold stimuli cause the generation of an action potential.
3. **Excitability of nerve fibre:** The excitability of nerve is reduced during action potential.

An action potential has 2 refractory periods

a. *Absolute refractory period (Fig. 4.6):* During this period, the cell is unresponsive to any further stimuli. No other action potential can be fired at this point, regardless of the strength of the

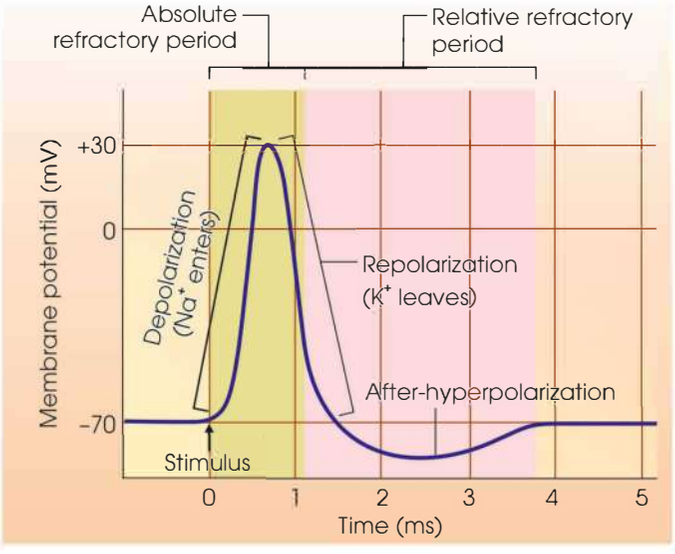


Fig. 4.6: Refractory period: Absolute and relative refractory period

stimuli. The role of the absolute refractory period is to ensure one-way propagation of action potentials and it places a limit on the rate at which a neuron can conduct impulses.

b. *Relative refractory period:* During this period, another action potential can be produced but the strength of the stimuli must be greater than normal to trigger an action potential. The role of the relative refractory period helps to limit the frequency of action potentials.

4. **Accommodation to slow depolarization:** If a slow depolarization occurs the voltage-gated channels do not respond and no action potential occurs.
5. **Conductivity:** The action potential is conducted through the length of the neuron. The wave of action potentials travel down the axon. The resistance of the membrane to current leak out of the cell and the diameter of the axon determine the speed of action potential conduction. The large diameter axons provide a low resistance to current flow within the axon and this in turn, speeds up conduction. The conduction time in myelinated nerve is faster than unmyelinated nerves. The myelin sheath prevents current leak out of the cells. The portions of the axons lacking the myelin sheath are called *nodes of Ranvier*. There is high concentration of Na^+ channels at the nodes which reinforces the depolarization to keep the amplitude of the action potential constant. The apparent leapfrogging of action potential from node to node along the axon is called *saltatory conduction*.

Applied physiology

Multiple sclerosis: There is loss of myelin sheath in the nervous system in multiple sclerosis. This slows down the conduction of action potential. These patients complain of fatigue, muscular weakness, difficulty with walking and loss of vision.

Differences between graded potential and action potential

Graded potentials	*Action potentials*
1. Magnitude varies	1. No variation: All-or-none
2. Decremental (passive spread)	2. Non-decremental (self-regenerating)
3. No refractory periods	3. Two refractory periods (absolute and relative)
4. Summation is possible	4. No summation possible
5. Trigger: NTs, hormones, etc.	5. Trigger: Threshold reached
6. Occurs at cell body (direction can vary)	6. Occurs at axon hillock (one way direction)

ION CHANNEL STUDIES

The patch clamp technique is conducted to study the single or multiple ion channels in cells. The technique helps to assess and evaluate role of ions in functioning of excitable cells such as muscle fibers, neurons, cardiomyocytes, etc. The voltage clamp technique was modified further to the patch clamp technique. The patch clamp technique was developed by Erwin Neher and Bert Sakmann in 1976. Neher and Sakmann were the first to identify the existence of specific ion channels. The Nobel Prize in Physiology or Medicine in 1991 was awarded to Neher and Sakmann for their discovery of patch clamp technique and ionic study.

Erwin Neher (1944) **Bert Sakmann** (1942)

Procedures

1. The glass micropipette called a patch pipette (Fig. 4.7) is used as a recording electrode, and a reference ground electrode is bath around the cell. A thin glass micropipette is brought in contact with the cell membrane.

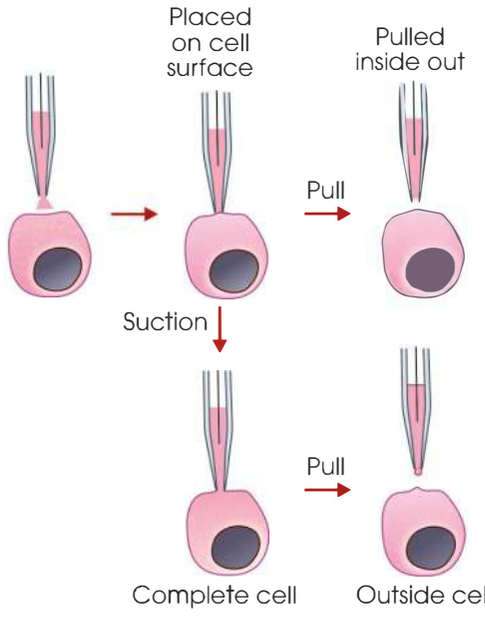


Fig. 4.7: Schematic diagram: Micropipettes are used for patch clamp

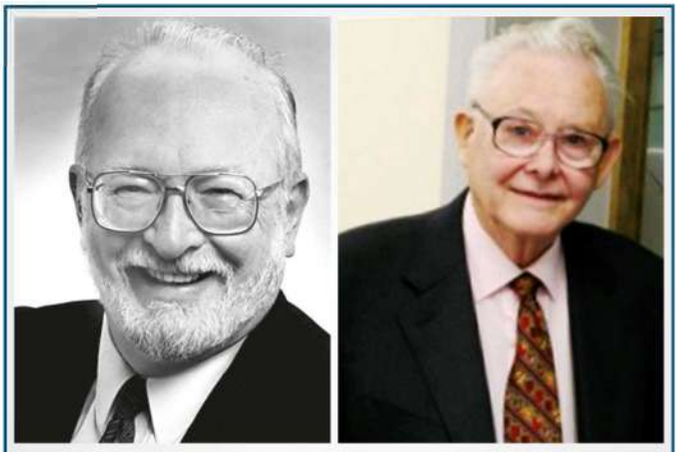
2. The membrane fragment forms a tight seal with the external orifice of the pipette. The exchange of ions between the inside of the pipette and the outside can only occur via the ion channel in the membrane fragment.
3. The portion of cell membrane is suctioned into the pipette. This creates an omega-shaped area of membrane which if appropriately formed, creates a resistance in the 10–100 gigaohms range forming a gigaohm seal.
4. Based on the study the interior of the pipette can be filled with a solution similar to that of the ionic composition of the bath solution in the case of cell-attached recording, or matching the intracellular ionic composition for whole-cell recording. The content or concentration of these solutions can be changed by adding ions or drugs to study the ion channels under varied conditions.

RECENT ADVANCES: MAGNETIC RESONANCE IMAGING IN MEDICINE

Paul Lauterbur of the University of Illinois and Sir Peter Mansfield of the University of Nottingham were awarded the 2003 Nobel Prize in Physiology or Medicine for their "discoveries concerning magnetic resonance imaging explaining the applicability of MRI in medicine". Lauterbur's advocated the use of magnetic field gradients to determine spatial localization for rapid acquisition of 2-D images.

REFERENCE

Dawson M Joan, Paul Lauterbur. The Invention of MRI, Boston: MIT Press, 2013.



Paul Christian Lauterbur 1929–2007 **Sir Peter Mansfield** 1933–2017

EXAM-ORIENTED QUESTIONS

Essay

1. Define resting membrane potential. Discuss the ionic bases of generation of resting membrane potential. Add note on recording of membrane potential.
2. Define action potential. Discuss the stages of action potential with the role of ions in the same. Discuss the properties of action potential.

Short Notes

1. Resting membrane potential
2. Action potential and its stages
3. Graded potential
4. Properties of action potential
5. Differentiate between graded potential and action potential
6. Patch clamp techniques

Chapter

5

Body Fluids and Blood Volume

INTRODUCTION

Water is the most vital and at the same time; the most abundant component of the human body. It constitutes about 70% of the total body weight and within which the major cations like sodium, potassium, calcium, hydrogen, magnesium and anions like chloride, bicarbonate and protein of the body are dissolved. Without water there would be no form of life and it forms the intracellular medium within which metabolic reactions—characteristics of living substances take place. Water-deprivation brings about death earlier than that of food-deprivation. If water is given instead of food, life may continue for several weeks by the loss of most of the body fat and 50%, of tissue protein.

TOTAL BODY WATER AND ITS DISTRIBUTION

Total body water in an average human being, weighing about 70 kg is 40 to 45 litres. In *human being* it is about 65% of the body weight in males and about 10% less in females. But the above values vary mostly with the relative degrees of leanness and fatness of the individual. In lean person, the value is higher than that of in obese person. In general, woman contains more fat than man. The total body water content can be determined most accurately by the *process of desiccation*.

In 1863, Bischoff determined the water content of an executed criminal by the method of desiccation. Mitchell and his associates (1945), Widdowson and his co-workers (1951) have also determined the water content of the human beings by direct method. The average water content in different tissues of the body has been presented in Table 5.1.

It has been observed after studying thoroughly the water content of the body in man as well as in different animal species that the total water content in man is similar to that of in other animals. Besides this, the relative distribution of water in the various organs and tissues is mostly same in man as well as in other species (Table 5.2).

The percentage of water in various tissues and the proportion of total weight of the body which each tissue represents, have been presented in Table 5.3.

Table 5.1: Average water content in various tissues of the body

Constituents	*Weight in kg*	*% of body weight*	*% of H_2O content*	*H_2O content in litres*
Body	75	–	57.68	43.26
Skeleton	11	16	22	2.5
Muscles	30	42	76	23
Fatty tissues	13	18	30	4

Table 5.2: Percentage of water in organs in relation to their body weight

Constituents	*Man*	*Dog*	*Rabbit*	*Rat*
Skeleton	22	32	21	48
Muscles	76	74	75	76
Skin	72	59	71.5	77
Heart	79	78.5	79	78
Lungs	79	79	78	82
Kidneys	83	78	79	77
Brain	75	75	78	77.5
Liver	68	74	76	74
Blood	83	83	83	81
Entire body	63	66	69	65.5

Table 5.3: Percentage of water in tissues and the proportion of total body weight

Constituents	*% of water*	*% of body weight*	*Litres of water/70 kg*
Skeleton	22	16.0	2.5
Muscles	76	42.0	22.0
Skin	72	18.0	9.0
Heart	79	0.5	0.3
Lungs	79	0.7	0.4
Kidneys	83	0.4	0.3
Brain	75	2.0	1.1
Liver	58	2.3	1.0
Blood	83	8.0	5.0
Adipose tissue	16	13.0	0.9

The water of the body can be considered to be distributed within two main compartments—the *extracellular* and the *intracellular*. The distribution of body water in different compartments has been presented schematically in Fig. 5.1. The cell membrane actually provides the boundary in between the extracellular and the intracellular compartments.

Extracellular Fluid Compartment

The extracellular fluid compartment is a compartment containing heterogenous collections of fluids and not a continuous fluid phase. Edelman and Leibman (1959) have studied thoroughly the distribution pattern of body water by dilution technique and also by tissue analysis. It is postulated that 55% (F) of water is present in the intracellular space and the rest in the extracellular space. The extracellular fluid phase can be divided into following subcompartments:

1. **Transcellular water:** 2.5% (A)
2. **Dense connective tissue and cartilage water:** 7.5% (B)
3. **Plasma water that is confined within the vascular system:** 7.5% (C)
4. **Interstitial fluid and lymph:** 20% (D)
5. **Inaccessible bone water:** 7.5% (E)

The term *transcellular* was introduced by Edelman and associates (1952) in order to designate the extracellular fluid having been separated from the other extracellular fluid by an epithelial membrane. This transcellular fluid includes: (a) Cerebrospinal fluid, (b) joint or synovial fluid, (c) intra-ocular fluid, (d) fluids of the pleural, pericardial and peritoneal cavity, (e) fluids within the ducts of the digestive gland, (f) mucous membranes of the nasorespiratory tract, gastrointestinal tract and genitalia, (g) intraluminal fluid of gastrointestinal system.

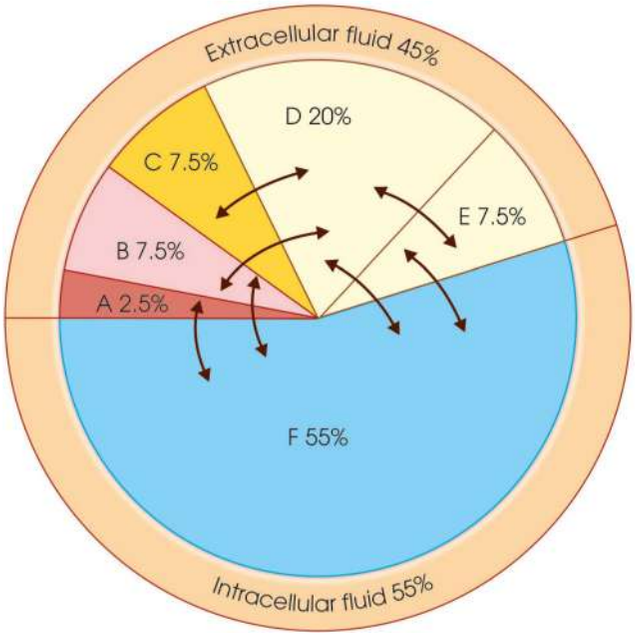


Fig. 5.1: Schematic representation of the distribution of body water in different compartments. A: Transcellular water. B: Dense connective tissue and cartilage water. C: Plasma water. D: Interstitial and lymph water. E: Inaccessible bone water. F: Intracellular water.

Intracellular Fluid Compartment

It is neither a continuous nor a homogeneous phase and represents the sum of the fluid contents of all the cells of the body. In a cell there are many anatomic subdivisions and for this reason there is a striking difference in water content and ionic composition in between the cytoplasm, nucleus, mitochondria and microsomes of various cell types. This intracellular fluid contains about 30–40% of the body weight and holds about 55% of the whole body water.

MEASUREMENT OF BODY FLUID COMPARTMENTS

Total body water and **extracellular water** can be measured by dilution technique with varying degree of precisions. Volume of water present in each compartment cannot be measured directly and thus *indirect method*—the dilution technique has been adopted for its determination.

In this technique the amount of dye used, the final concentration of the dye in the solution is made, are considered for determining the volume of distribution. For example, if a known quantity of dye—Q is taken and the final concentration is achieved as C, then the volume of distribution V will be; V = (Q/C).

If a beaker of unknown capacity is taken, then its volume can be determined by mixing uniformly a known amount of dye in the volume of water present in the beaker. If the final concentration of the dye is determined by the calorimeter then the volume capacity of the beaker can be determined. Suppose 35 mg of dye has been added and the final concentration that has been achieved to be 0.07 mg per ml, then the volume of the beaker will be:

$$\text{Volume (V)} = \frac{\text{Quantity of dye}}{\text{Concentration of dye}} = \frac{35}{0.07} = 500 \text{ ml}$$

The result will be valid only when the drug will be mixed thoroughly.

In vivo determination of body fluid compartments by the dilution principle, certain points are generally considered. The dye injected in the body must be evenly distributed and confined to the body fluid compartment to be measured. If the dye is excreted or lodged in other compartments or metabolised then those amount should be determined and subtracted from the quantity administered. So the equation will be:

$$\text{Volume of distribution} = \frac{\text{Quantity administered} - \text{Quanitity excreted}}{\text{Equilibrium concentration}}$$

5

TOTAL BODY WATER

Total body water is generally determined by using antipyrine. The antipyrine is evenly distributed throughout all the body water compartments and thus diffuses readily across the cell membrane. It is not bound to any intracellular and extracellular compartments. It is also slowly excreted and slowly metabolised.

Tritiated water (H_3O or HTO) and deuterium oxide (D_2O)—the two isotopes are often used for the determination of total body water. D_2O and H_3O are distributed in the body exactly like water. These are excreted in the urine, faeces and respiratory gases and also evaporated through the skin. As for example of using the D_2O or HTO for the measurement of total body water, suppose 100 ml of D_2O in isotonic saline solution is injected intravenously to a man of weighing about 75 kg. After an equilibrium period of 2 hours, the plasma sample is analysed and D_2O concentration is found to be 0.0023 per ml. During the period of equilibrium it is found to have a loss (through respiratory, urinary and circulatory pathways) of average 0.5% of the quantity administered. So the volume of distribution will be:

$$\text{Volume of distribution} = \frac{\text{Quantity administered} - \text{Quantity excreted}}{\text{Equilibrium concentration}}$$

$$= \frac{100 - 0.5}{0.0023} = \frac{99.5}{0.0023}$$

= 43,260 ml or total body water is 43.26 litres

So the body water will constitute

$$= \frac{43.26}{75} \times 100 = 57.68\%$$

EXTRACELLULAR FLUID VOLUME

The extracellular fluid volume is not determined so precisely only due to lack of substances that may diffuse to cross the capillary walls readily, enter the cell interstices easily but do not permeate through the cell membrane. Besides this, the substance must be non-toxic and the rate of excretion must be very low in comparison with the rate of distribution in extracellular compartment. There are no such ideal substances available but several substances that have been used are inulin, raffinose, sucrose, mannitol thiosulphate, radiosulphate, thiocyanate, radiochloride and radiosodium.

BLOOD VOLUME

Definition: The term blood volume means the total amount of blood in circulation, as well as in the blood stores.

Normal blood volume: It can be expressed in two ways: (a) *In relation to body weight:* 78 to 97 ml (average 90 ml) per kg of body weight; or about 1/11th (9%) of the total body weight. That of plasma is 50 ml per kg body wt. or 1/20th (5%) of the total body weight. (b) *In relation to body surface:* 2.5 to 4 litres (average 3.3) per sq metre of body surface. A man, weighing 70 kg, has about 5 litres of blood in his circulation.

VARIATIONS UNDER DIFFERENT PHYSIOLOGICAL CONDITIONS

1. *Age:* In infants, the blood volume is greater in proportion to the body weight but is lesser in proportion to the body surface. [The body surface of infants is proportionally larger than their body weight]. This larger volume is due to greater number of corpuscles as well as larger amount of plasma.
2. *Sex:* In males, the blood volume is 7.5% higher (per sq metre of body surface) than in females. This is due to greater number of red cells in the males. The plasma volume is same in both.
3. *Body weight and surface area (vide above)*
4. *Pregnancy:* Blood volume increases due to increase of both cells and plasma but the rise in plasma volume is much greater than the rise in cell volume. It falls after delivery.
5. *Muscular exercise:* Raises blood volume probably due to contraction of spleen.
6. *Posture:* In erect posture there is about 15% diminution of total plasma. It passes out into the tissue spaces.
7. *Blood pressure:* Rise of blood pressure lowers blood volume by pressing out more fluid into the tissue spaces. Lowered blood pressure draws in more fluid from the tissue spaces and raises the blood volume. Altitude: At higher altitude the blood volume rises, due to anoxia produced, the number of red cells increases in such conditions.
8. *Anoxia* due to any other cause will raise blood volume.
9. *Adrenaline injection:* Raises blood volume probably by splenic contraction.

METHODS OF DETERMINATION OF BLOOD VOLUME

Direct Method

1. *Welcker:* An animal is bled to death and the blood is collected. Then its blood vessels are washed out by pumping saline solution into the vessels. The saline washings are added to the already collected blood. The colour of this mixture is matched against the sample of normal

Hermann Welcker
1822–1897

Note

Defibrinated blood. When a sample of blood is constantly stirred or shaken with fine twigs of piece of wire or glass beads, the blood clots and the fibrin gradually collects upon the surface of the foreign bodies. In this way all the fibrinogen is converted to converted into fibrin in a short time and is quickly removed from the blood sample. This blood will not clot any further and remains fluid. It is composed of serum in which red and white cells remain suspended. Defibrinated blood is frequently used in physiological experiments, instead using by anti-coagulant.

blood of the same animal. From these data the volume may be calculated. In dogs, the total blood volume is 7.7% of body weight.

2. *Bischoff:* The above method was applied upon decapitated criminals. It is obvious that the direct methods have got no place in clinical medicine, for which one of the following indirect methods is used.

Indirect Method

In this method known amount of a particular substance is introduced into the blood stream. After some time a sample of blood is drawn out and the concentration of the injected substance is determined in it. From this, the degree of dilution is calculated. Total blood volume can be found out from these data. The substance to be used must have certain special qualities. It must not be toxic, must not alter blood volume, must not easily pass into the tissue spaces or be excreted, and must not be taken up by the phagocytic cells of the blood. Also it must not change its colour and chemical composition while in the body. Usually, two classes of substances are used for this purpose: 1. Dye stuff like Congo red, Evans blue, etc. 2. Radioactive substances.

Dye method: For clinical purposes the blood volume can be determined by dye method. The dye Evans blue (T-1824) which is non-toxic and escapes slowly from the blood vessels, is mostly used in present times.

1. 10 ml of venous blood from the subject is taken in a heparinised tube. This serves as the control sample.
2. 5 ml of a 5% solution of Evans blue in distilled water is then injected intravenously. 10 minutes after beginning of the injection another l0 ml sample is withdrawn from the vein of the opposite side into another heparinised tube.
3. The haematocrit of both samples are determined.
4. The optical density of the dye stained plasma is estimated. 0.0l ml of the dye is then diluted to 5 ml (dilution l : 500) with control plasma and its optical density is determined.

Radioactive methods

(a) *Radio-iodine plasma albumin method*

1. To a sample of plasma, iodine-containing radioactive isotope ^{131}I or ^{132}I are added, and allowed to incubate for some time. This plasma is slowly injected intravenously.
2. The degree of dilution of its radioactivity is determined, which is a measure of plasma volume.
3. The advantage of this method is that the albumin, cannot permeate through the capillary endothelium and is not affected by lipaemia and haemolysis.
4. The plasma and blood volume are measured in a similar way as the dye dilution method. The plasma volume determined by this method is about 2.5 litres in women and 3 liters in men.

(b) *Radioactive iron method*

1. Radioactive iron (^{55}Fe or ^{59}Fe) is incorporated into ferric ammonium citrate which is given by intravenous injection to a person (donor) belonging to group O (vide blood group). The radioactive iron is taken up by the newly formed blood cells which appear in the circulation within twenty-four hours.
2. 75 ml of blood having a radioactivity of about 3,000 counts per minute per ml (determined by Geiger) is injected intravenously to recipient, whose blood volume is to be determined.
3. After about 20 minutes of the injection, 15 ml of blood is withdrawn from the recipient.
4. After interval of 20 minutes, another two samples of blood of the same quantity are withdrawn.
5. The blood samples after proper dilution are centrifuged for half an hour.
6. The samples taken out both from the donor and the recipient are wet ashed and the iron is deposited electrolytically on copper.
7. The radioactive iron present in the two samples is determined with the help of *Geiger counter*.
8. The red cell volume is calculated from the number of millilitres of *donor's* cell injected, the radioactivity of the donor's cells and the radioactivity of the recipient's cells.
9. The blood volume is determined by measuring the plasma volume by the dye method and the result is added to the value of the red cell volume.

Similarly red cell volume can be determined either with labelled radioactive chromium (^{51}Cr) or radioactive phosphorus (^{32}P). So from all the above methods it is clear that the dye Evans blue or plasma labelled with radioactive iodine help in the measurement of plasma volume whereas radioactive iron, chromium or phosphorus is used to determine the red cell volume. No substance has yet been found which helps in measurement of the total blood volume directly. For an accurate measurement of blood volume, plasma and cell volumes must be determined simultaneously.

REGULATION OF BLOOD VOLUME

Although it is customary to talk about a constant blood volume yet it has been already shown that total blood volume does not remain constant and varies widely under different physiological conditions. This variation is mostly *due to alteration of the cells* and not due to that of plasma. *The plasma volume remains fairly constant* under normal conditions. The problem of regulation of blood volume is intimately linked up with that of water balance.

The maintenance of *blood volume depends upon a balance between water intake and water loss and also upon the adjustment of fluid interchange between plasma and tissue spaces through the capillary walls.*

A number of factors is involved:

1. *Physical factors:* Blood pressure, osmotic pressure, diffusion, the state of permeability of the capillaries, etc. are the important factors concerned in the regulation of the blood volume. The *tissue spaces*, due to their enormous capacity, act as a ready reservoir. Any increase in the blood volume will lead to passage of more fluid from the plasma to the tissue spaces. While any decrease will draw in more fluid from the tissue spaces and maintain the blood volume.
2. *Vitamins:* Some vitamins, specially C, by controlling the permeability of the capillaries—take part in the process.
3. *Endocrines:* A number of endocrine factors are also involved here. (a) The *antidiuretic factor* of the posterior pituitary controls excretion of water through the kidneys. When blood is diluted the secretion of the factor is inhibited and thus more water is lost. When blood becomes concentrated, reverse changes occur. (b) *Parathyroids*, by their effect on calcium metabolism, control the permeability of the blood vessels and thereby the rate of interchange between blood and tissues. (c) *Adrenal cortex* is believed to exert important influence upon salt balance, kidney function and excretion of water.
4. *Thirst:* Another important mechanism for replenishing the reduced blood volume is the phenomenon of thirst. When the water content of the body becomes low, 'thirst' is felt. The subject takes water and thus the blood volume is kept up. (Effects of saline injection, fluid loss and such others have been discussed elsewhere.)

Causes of decrease of blood volume

Blood volume is reduced in the following conditions

1. Loss of whole blood, e.g. haemorrhage.
2. Reduction in number of RBC, e.g. anaemia.
3. Loss of plasma alone.
4. Loss of blood water or anhydraemia.
5. Acute exposure to cold causes moderate loss.
6. Posture: Blood volume is low in the erect position than in the recumbent state.

Blood volume is increased due to

1. High temperature.
2. Muscular exercise.
3. Emotional excitement.
4. Pregnancy.
5. Congestive heart failure.
6. Administration of mineralocorticoids (deoxycorticosterone and aldosterone).

Intracellular Fluid Measurement

There is no direct method has yet been developed. It can be determined by subtracting the value of the extracellular compartment from the value of the total body water.

Ionic Concentration in Intracellular and Extracellular Fluids (Fig. 5.2)

The electrolytes are the major solutes in body fluids. The electrolytes have larger osmotic power because as they dissociate in water and contribute at least two particles to the solution. There is unequal distribution of ions (electrolytes) on the both sides of the cell membrane. The ions move from high to low concentration by diffusion and as a result of electrical gradient which favours movement of ions away from like charge toward opposite charge. Equilibrium is achieved by principles of Nernst potential and Goldman-Hodgkin-Katz equation as studied earlier. The ionic equilibrium generates the resting membrane potential. The concentration of ions in intracellular and extracellular fluids is as follows.

Terminology associated with body fluids

- ***Mole:*** A mole is the amount of a substance that contains the number of molecules equal to Avogadro's number.
- ***Avogadro's number:*** This is the number of molecules in one mole of a substance (i.e. 6.022×10^{23}).

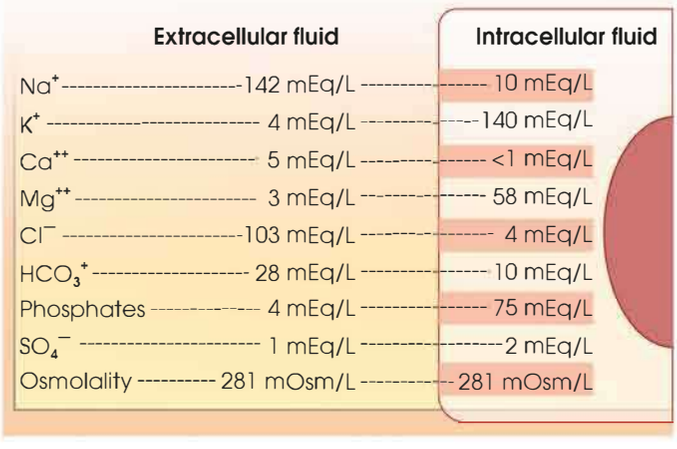


Fig. 5.2: Ion concentration in extracellular and intracellular fluids

- ***Osmole:*** This is the amount of a substance that yields, in ideal solution, that number of particles (Avogadro's number) that would depress the freezing point of the solvent by 1.86 K.
- ***Osmolality*** of a solution: It is the number of osmoles of solute per kilogram of solvent.
- ***Osmolarity*** of a solution: It is the number of osmoles of solute per litre of solution.
- ***Colloids*** are a term used to collectively refer to the large molecular weight (nominally MW >30,000) particles present in a solution. In normal plasma, the plasma proteins are the major colloids present. As the colloids are solutes they contribute to the total osmotic pressure and referred to as colloid osmotic pressure (or sometimes as the oncotic pressure).

Hydrogen Ion Concentration of the Body Fluids

The normal pH value for the body fluids ranges between 7.35 and 7.45. When the pH value of body fluids is above 7.45, it is referred to as alkalosis while pH below 7.35, is called acidosis. The bicarbonate, ammonium and protein buffers in the body fluids help restoring changes in the pH of body fluids, the respiratory system and the kidneys mainly regulate the pH of the body fluids.

EXAM-ORIENTED QUESTIONS

Essay

1. Discuss the composition of body fluids. Describe the fluid compartment.
2. Discuss the methods for measurement of fluids in various body compartments.

Short Notes

1. Body fluid composition
2. Extracellular fluid volume
3. Intracellular fluid volume
4. Plasma volume
5. Extracellular fluid measurement
6. Ionic composition of body fluids
7. pH of the body fluids

CLINICAL CASE SCENARIO

General Physiology

Q1. A patient of Kwashiorkor presented with oedema. What is the pathophysiological basis of development of oedema?

Ans. The increase capillary hydrostatic pressure and decreased oncotic pressure (decrease plasma protein concentration) leads to increase capillary permeability and fluids leak from capillary into interstitial spaces producing oedema. The obstruction in lymphatic drainage prevents circulation of lymph and may produce oedema.

Q2. What is the cause of Charcot-Marie-Tooth disease? What are the characteristic features observed in this disease?

Ans. The Charcot-Marie-Tooth disease results due to mutation that produces defects in neuronal protein which affects the axon and the myelin sheath. The characteristic features of the disease are the manifestation of hereditary motor and sensory neuropathy with progressive loss of touch sensations and muscle atrophy throughout the body. It may also produce stiffness of joint leading to contractures and scoliosis.

Q3. A child of six years was diagnosed as a case of adrenoleukodystrophy (ALD). Explain the cause for the diagnosed condition. Describe the characteristic features of clinical presentation of the disease.

Ans. ALD is caused due to accumulation of long chain fatty acids in the tissue. The lysosomes lack a second protein on the membrane due to which enzyme which break long chain fatty acids cannot enter into preoxisomes and this leads to accumulation of fatty acid in brain and spinal cord especially. The clinical symptoms observed in this condition are poor memory, low academic achievement or poor performance in school, weakness and paralysis of lower limb, stiffness of joint and ataxia.

Q4. A 32-year-old patient was known case of mitochondrial myopathy. Discuss regarding the cause for mitochondrial disease and signs and symptoms observed in this disease.

Ans. The mutation in the mitochondrial DNA affects its functions leading to mitochondrial disease which is group of disorders caused by dysfunctional mitochondrion. The signs and symptoms observed in mitochondrial diseases are fatigue, muscular weakness, growth retardation, loss of muscle coordination, dementia, learning disability, neurological disorders, autonomic dysfunction, and systemic pathological manifestations (kidney disease, GIT disorders, heart disease, respiratory disease, visual and hearing problem, etc.).

Q5. Discuss the cause for the following membrane transport disease: Hartnup disease, Gitelman syndrome and Pendred syndrome.

Ans. Hartnup disease is autosomal recessive metabolic disorder which affects the absorption of non-polar amino acids such as tryptophan.

Gitelman syndrome: It is an autosomal recessive type of disorders. The patient presents with hypokalaemic metabolic alkalosis, hypomagnesaemia and hypocalciuria. It is due to loss of function the mutation of sodium chloride symporter in thick ascending limb of loop of Henle.

Pendred syndrome: It is a genetic disease with characteristic presentation of sensorineural hearing loss with goitre. It is due to mutation in the PDS gene which is located on long arm of chromosome gene which codes for pendrin protein. The pendrin protein is found in kidney, thyroid and cochlea. It is involved in secretion of bicarbonates in kidney and functions as iodide/chloride transporter in thyroid.

Q6. As a medical student you are to interpret your opinion about channelopathies. Discuss regarding them in brief and cite a few examples.

Ans. The altered function of ion channels subunits or proteins that regulate them lead to channelopathies. The disease may be congenital or acquired (autoimmune attack on ion channel).

1. Catecholaminergic polymorphic ventricular tachycardia is due to mutation of genes encoding a calcium channel.
2. Familial hemiplegic migraine is an autosomal dominant disorder. This is classical migraine subtypes in which patient complain of weakness in one half of the body which may last for hours, days or week. It is caused by mutation in gene coding for P/Q type calcium channel.
3. Generalized epilepsy with febrile seizures plus: It is an autosomal dominant disorder in which patient exhibit various epilepsy phenotypes. It has been associated with mutations in three voltage gated sodium channels (SCN1B, SCN1A) or the gene encoding the γ_2 subunit of γ aminobutyric acid (GABA) A receptors (GABR G2).
4. Cystic fibrosis: It is caused due to mutation in cystic fibrosis transmembrane conductance regulator (CFTR) gene. The CFTR protein is chloride ion channel which plays important role in production of sweat, mucus and digestive enzymes.
5. Brigada syndrome: It is a genetic disorder. In these patients ECG is abnormal and there is higher risk of sudden calcium death. It is cause due to mutation in α subunit of sodium channel, α subunit of cardiac L type cardiac channel, β_1 subunit of sodium channel, β subunit of voltage-dependent L type calcium channel, etc.

Q7. A 42-year-old male was identified as a case of cardiac action potential anomaly. What can be the likely cause for the same? Name some antiarrhythmia drugs which act on cardiac action potential.

Ans. The cardiac action potential anomaly may be due to congenital mutation or injury to the cardiac muscle. The anti-arrhythmia drugs which act on cardiac action potential are lidocaine, quinidine, verapamil and propranolol.

Q8. There were the cases for study of water electrolyte imbalance, water intoxication and inward during early dehydration clinical exposure session. What are the causes for the conditions?

Ans. The profuse sweating due to summer heat or over exercise practice can lead to sodium and water loss through sweat producing electrolyte imbalance. The deficit in total body water leads to dehydration. It may occur due to sweating, osmotic diuresis, vomiting and diarrhoea.

Q9. The resident trainee instructed not to administer oral rehydration therapy but intravenous therapy in certain conditions of dehydration. Enlist such conditions in which oral rehydration therapy is contra- indicated.

Ans. Oral rehydration therapy is contraindicated in patients of haemodynamic shock, intussusceptions, paralytic ileus and in patients in comatose state.

Section

II

Blood

Chapter

6

Composition and Functions of Blood

INTRODUCTION

Blood may be described as a specialized connective tissue in which there is liquid intercellular substance known as plasma and formed elements, the red blood cells, the white blood cells and the platelets suspended in the plasma. The specific gravity of whole blood varies from 1.055 to 1.060. The freshly shed, blood is red, thick, opaque and slightly alkaline. In order to understand the full significance of blood, it is imperative to understand the composition and functions of blood.

FUNCTIONS OF BLOOD

1. **Transport of respiratory gases:** It carries oxygen from the lungs to the tissues and CO_2 from the tissues to the lungs.
2. **Transport of nutrition:** It carries digested food material absorbed from the intestine to the tissue cells for utilisation. It also carries nutritive material from one place of the body to the other. For instance, from the storage depots to the tissue cells.
3. **It acts as a vehicle** through which the hormones, the vitamins and other essential chemicals are brought to their places of activity.
4. **Drainage of waste products:** It carries the waste products of cellular activity and brings them to the organ of excretion, viz. kidney, lungs intestine, etc.
5. **Maintenance of waterbalance:** Vide 'Waterbalance'.
6. **Maintenance of acid–base equilibrium:** By its efficient buffering power (e.g. plasma proteins, reduced and oxidized haemoglobin, etc.) and with the help of kidney, skin and lungs. It helps to maintain a constant reaction of the body.
7. **Maintenance of ion balance** between the cells and the surrounding fluid.
8. **Regulation of body temperature:** The water content of blood possesses three qualities which make it very suitable for this purpose. (a) Due to its high specific heat* it can absorb a large amount of heat and thereby prevent sudden change of body temperature. (b) High conductivity—the thermal conductivity of water is higher than that of any other ordinary liquid. This helps quick distribution of heat. (c) High latent heat of evaporation—latent heat of evaporation of water is very high and since water is constantly evaporating from skin and lungs. A large amount of heat is lost in this way.
9. **Defensive action:** Blood acts as a great defensive mechanism in two ways: (a) The white cells due to their phagocytic properties engulf bacteria and foreign particles. (b) It develops antibodies which combat toxic agents.
10. **By the property of coagulation it guards against haemorrhage.**
11. **The plasma proteins of blood** have various functions (vide functions of plasma proteins).
12. **Regulation of blood pressure,** by changes in volume and viscosity (haematocrit value) of blood.

COMPOSITION OF BLOOD

Blood is a highly complex fluid which is composed of two parts—a liquid, called the plasma and different types of cells which remain suspended in the plasma. The cells are called the blood corpuscles. The plasma constitutes about 55%, and the cells about 45% of the total volume of human blood.

The general composition of the whole blood is as follows

Whole blood cells: (a) Red blood corpuscles or erythrocytes (RBC). (b) White blood corpuscles or leucocytes (WBC). (c) Platelets or thrombocytes.

Plasma: (a) Water, 91 to 92%, (b) Solids, 8 to 9%.

Inorganic constituents: 0.9% sodium, potassium, calcium, magnesium, phosphorus, iron, copper, etc.

Organic constituents

1. *Proteins:* 7.5% serum albumin, serum globulin, fibrinogen, prothrombin, etc.

* Specific heat of a substance is the number of calories required to raise the temperature of one gram of the substance to one degree centigrade.

2. *Non-protein nitrogenous (NPN) substances:* Urea, uric acid, xanthine, hypoxanthine, creatine, creatinine, ammonia, amino acids, etc.
3. *Carbohydrate:* Glucose, etc.
4. *Fats:* Neutral fat, phospholipid, cholesterol, cholesterides, etc.
5. *Other substances:* Internal secretions, antibodies and various enzymes (amylases, proteases, lipases, phosphatases, etc.).
6. *Colouring matter:* The yellow colour of plasma is due to small amounts of bilirubin, carotene and xanthophyllin.

Note that all values in Table 6.1 are expressed per 100 ml of blood. Table 6.2 denotes values of ion concentration in extracellular and intracellular fluid in millimoles/liter.

Relative Volume of Corpuscles and Plasma

In normal human blood (Table. 6.1), plasma volume is proportionally more than total corpuscular volume. The plasma volume varies between 52 and 55% and the packed corpuscular volume varies from 45 to 48%. The normal packed cell volume for males is about 45% while that in case of females is a bit lower and is about 40% of whole blood.

The ratio of red blood corpuscles to plasma is expressed as the haematocrit value. This can be estimated by an instrument called *haematocrit*. It consists of a specially prepared graduated capillary (Wintrobe's) tube of uniform bore in which a specimen of blood, treated with an anticoagulant, is taken. It is centrifuged at a speed of 3,000 revolutions per minute

Table 6.1: Normal value of blood constituents commonly of clinical importance

Blood constituents	*per 100 ml*	*Whole blood*	*Plasma*	*Serum*
Haemoglobin	gm/100 ml	12–17 (in male) 11–15 (in female)		
Proteins	gm/100 ml			6.0–6.8
Albumin	gm/100 ml			4.7–5.1
Globulin	gm/100 ml			1.3–2.8
Fibrinogen	gm/100 ml			
A/G ratio				1.2–1.8
True glucose	mg/100 ml	65–90		
Total lipids	mg/100 ml	360–820		
Fats	mg/100 ml			150–250
Total fatty acids	mg/100 ml		100–500	
Pyruvic acid	mg/100 ml	0.8–1.1		
Lactic acid	mg/100 ml	6–16		
Non-protein nitrogen	mg/100 ml	28–39		
Urea	mg/100 ml	19–33		
Urea N	mg/100 ml	9–15		
Creatinine	mg/100 ml	1.2–1.1		
Uric acid	mg/100 ml	1.0–3.0		
Total cholesterol	mg/100 ml			150–250
Free cholesterol	mg/100 ml			40–70
Esterified cholesterol	mg/100 ml			100–180
Calcium	mg/100 ml			9–11
Sodium	mg/100 ml			310–350
Potassium	mg/100 ml			14.1–24.2
Sulphur as sulphates	mg/100 ml			2.0–4.0
Chlorides as NaCl	mg/100 ml			576–612
Phosphorus as organic phosphate	mg/100 ml			3.2–4.1
Ascorbic acid	mg/100 ml		0.7–1.5	
Iodine	µg/100 ml		0.2–0.8	
CO_2 capacity at 40 mm CO_2	vol/100 ml	55–75		
O_2 capacity (exposed to air)	vol/100 ml	16–24		

Average figures in healthy adult Indians: Sugar, 85 mg per 100 ml of whole blood; urea, 25 mg%; NPN, 23.4 mg%; uric acid, 40 mg%; creatinine 1.5 mg%; cholesterol 154 mg%; chloride (as NaCl), 396 mg per 100 ml of whole blood; calcium, 10.2 mg per 100 ml of serum; inorganic phosphorus, 3.6 mg% and lipid phosphorus, 9.7 mg per 100 ml of whole blood.

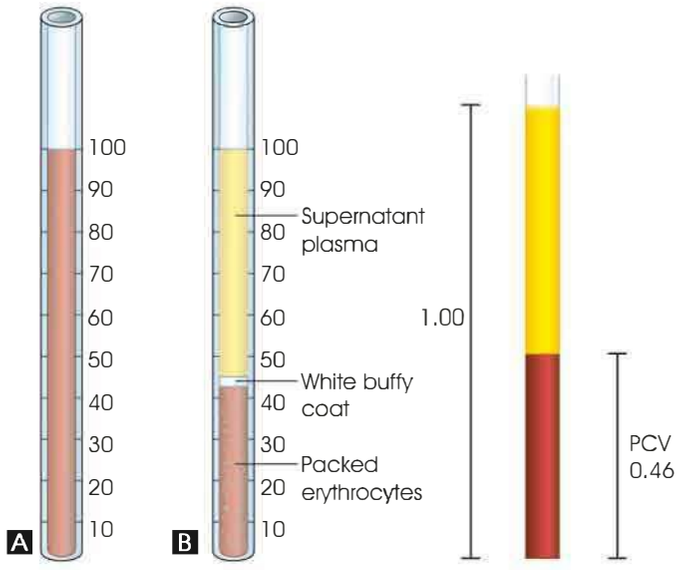


Fig. 6.1A and B: (A) Blood sample before centrifuging in the haematocrit; (B) The same after centrifuging in the haematocrit

Table 6.2: Intracellular and extracellular concentrations of ions of physiological importance

Component	*Intracellular concentration (mM/L)*	*Extracellular concentration (mM/L)*
Cations		
Na^+	5–15	145
K^+	140	5
Mg^{2+}	0.5	1–2
Ca^{2+}	10^{-4}	1–2
H^+	7×10^{-5} ($10^{-7.2}$ M or pH 7.2)	4×10^{-5} ($10^{-7.4}$ M or pH 7.4)
Anions		
Cl^-	5–15	110

for 30 minutes, until plasma and corpuscles are completely separated, and the sedimented corpuscles show no further shrinkage in volume. From the graduations on the tube the proportion of plasma and corpuscles can be known (Fig. 6.1A and B).

EXAM-ORIENTED QUESTIONS

Essay

1. Discuss the composition of blood and its functions.

Short Notes

1. Composition of blood
2. Functions of blood

Chapter

7

Specific Gravity of Blood

INTRODUCTION

Specific gravity is defined as the mass of any volume of substance by mass of equal volume of water at 4°C (the density of water is the maximum at 4°C).

Specific gravity of venous blood at 15°C (59°F) as determined by the psychometric method is in-between 1.048 and 1.066 with averages of 1.052 and 1.063. The clinical average is 1.057 in males and 1.053 in females. There is normal diurnal variation of specific gravity of blood and is about 0.003; and it is being generally lower in the afternoon and after meals and higher after exercise and during the night. The specific gravity of *blood serum* varies from 1.026 to 1.031 and that of *erythrocytes* is from 1.092 to 1.095. The specific gravity of the foetal blood in full term is highest and that of the mother at the same time is lowest (about 1.050). The specific gravity rapidly falls after birth due to the destruction of the red cells. The packed cell volume can be read after centrifuging blood sample in haematocrit.

Rise in Specific Gravity

Specific gravity rises in the following conditions:

1. When water is lost from the body, such as in excessive sweating, diarrhoea, cholera, etc.
2. By exudation of fluid into tissues or serous cavities due to inflammation or surgical operation.
3. When water intake is inadequate.

Fall in Specific Gravity

1. When large quantity of water is taken.
2. In severe haemorrhage after which fluid is drawn in from the tissue spaces and the blood is diluted.
3. Injection of saline into the veins causing dilution of blood.

Method of Determination

The specific gravity (SG) of the provided blood sample is determined by Philips and Vanslykes $CuSO_4$ method. The method of estimation is named after the biochemist Donald Dexter Van Slyke (1883–1971). The principle adopted, is to add drops of blood to a series of copper sulphate ($CuSO_4$) solutions of known specific gravity and to note in which particular solution the blood drop neither floats nor sinks. The specific gravity of that particular solution indicates the specific gravity of the blood sample. A series of mixtures of benzene and chloroform and mixtures of glycerine and distilled water of known specific gravity are also used.

Donald Van Slyke
1883–1971

Significance: Specific gravity measures the haemoglobin content and packed cell volume. The specific gravity indicates blood proteins status and degree of dehydration; hence the measurement of specific gravity of blood helps to confirm physiological homeostasis state of patient prior to any surgery.

EXAM-ORIENTED QUESTIONS

Short Notes

1. Significance of specific gravity
2. Method of determining specific gravity
3. Rise and fall in specific gravity

Chapter

8

Plasma Proteins

INTRODUCTION

Although it is customary to state that plasma contains several types of proteins yet it is highly probable that in the living animal, all these different varieties remain combined together forming a single protein complex. This complex is very loose and is easily broken down into different parts by addition of salts, alteration of pH, etc. The so-called serum albumin, serum globulin, fibrinogen, etc. are the parts of the same parent complex, isolated by different techniques of separation.

Serum is the fluid part of blood after clotting. It contains only serum albumin and serum globulin. The albumin/globulin (A/G) ratio is ordinarily 1.5:1.0. Different methods of separation indicate slightly different A/G ratio. Electrophoretic method of separation gives a ratio 1.2: 1.0. This ratio varies in different species but in the same species it remains almost constant in blood, lymph and serous transudations. In liver disease, however due to diminish formation of albumin, the ratio may be reversed. Chemical analysis of the total serum proteins reveals that arginine/lysine ratio is 10:18. This ratio remains more constant than the albumin/globulin ratio.

PLASMA PROTEINS VARIETIES

In normal individuals, total amount of plasma protein varies from 6.5 to 7.5% and average is about 7.0%. It is made up of following varieties: Serum albumin: 4.7–5.7%, serum globulin: 1.3–2.5%, fibrinogen: 0.2–0.4%, prothrombin (approximately 20 mg/100 ml), and seromucoid (are also present).

CHEMISTRY AND SEPARATION OF PLASMA PROTEINS

Serum Albumin

It constitutes the major part of the total plasma proteins.

Characteristics

1. It is albumin in nature and is having a molecular weight, about 69,000.
2. It is soluble in distilled water.
3. It is precipitated by full saturation with ammonium sulphate.
4. Its isoelectric pH is 4.7.
5. The albumin molecule is an ellipsoid, made up of a single polypeptide chain.
6. It is smaller and more compact than other plasma proteins and is heat coagulable.

Serum Globulin

It is globulin in nature.

Characteristics

1. It is having a molecular weight, varying from 90,000 to 1,300,000.
2. It is insoluble in distilled water, but soluble in salt solutions.
3. It is coagulated at about 70°C.
4. Globulin, like albumin, is also a mixture of several globulins.
5. By means of electrophoresis serum globulin has been separated into three fractions: Two fractions: (a) One fraction combines with bilirubin, (b) another fraction helps in the carriage of lipids, steroids and glycoproteins. α_2-globulin consists of α_2-macroglobulins, mucoproteins, ceruloplasmin, hepatoglobulins—the latter combine with free haemoglobin in the plasma.

β-globulin: Its molecular weight ranges from 90,000 to 1,300,000 and its isoelectric pH is 5.6. β-globulins are:

a. β-lipoprotein which helps in the carriage of lipid, steroid and carotene.
b. Globulin which helps in the transport of iron, e.g. transferring (siderophilin) which can also combine loosely with cupric copper.
c. Prothrombin is a β-globulin.

α-globulin: Its molecular weight ranges from 150,000 to 190,000 and its isoelectric pH is 6.0. They inhibit blood proteases and show significant inhibitor activity.

a. They are highly mobile in alkaline or electrically charged solution.
b. By careful electrophoresis, several varieties of functionally different proteins have been isolated more or less in pure form from the globulin fraction. They are immune globulins, hypertensinogen, isohaemoagglutinins (α, β, αβ, etc. *vide* blood groups), prothrombin, plasma thromboplastins, certain anterior pituitary hormones, etc.
c. They are of two types α_1 globulins: Examples: α_1-antitrypsin, α_1-antichymotrypsin, orosomucoid, α_1-lipoprotein, etc. α_2 globulins: Examples: Protein C, α_2-lipoprotein, angiotensinogen, haptoglobin, α_2-globulin, α_2-macroglobulin, ceruloplasmin, thyroxine-binding globulin, α_2-antiplasmin.

Gamma Globulin

The γ-globulins which are immunologically active are also called "immunoglobulins" or "antibodies".

Fibrinogen

It is globulin in nature. Its molecular weight ranges from 341,000 and its isoelectric pH is 5.8.

a. It is coagulated at about 56°C and precipitated by one-fifth saturation with ammonium sulphate and half saturation with NaCl.
b. It is insoluble in distilled water. It is distinguished from other plasma proteins by its property of clotting, during which fibrinogen is converted into fibrin.

SEPARATION OF PLASMA PROTEIN

The three proteins can be separated and isolated in the following ways.

Estimation of Plasma Proteins and Separation of Their Fraction: Method

1. Plasma is taken and its total protein is estimated by Kjeldal's process. This includes all the three proteins.
2. Then the total protein of serum from same sample is estimated.
3. Since, serum contains only albumin and globulin, the difference between the two total protein estimations will give the amount of fibrinogen.
4. Another sample of serum is half saturated with ammonium sulphate. This will cause precipitation of all globulins. It is filtered. The filtrate which contains albumin is fully saturated with ammonium sulphate. Albumin will be precipitated.

In this way the three fractions can be separated (other methods of separation of proteins are electrophoresis, isoelectric precipitation, ultracentrifugation, fractional separation by alcohol, etc.).

Michael Rubinstein was the first to use plasmapheresis for treating an immune-related disorder in an adolescent boy diagnosed as a case of thrombotic thrombocytopenic purpura (TTP) at the old Cedars of Lebanon Hospital in Los Angeles in 1959.

ORIGIN OF PLASMA PROTEINS

1. In the embryo, the primitive plasma and the plasma proteins are produced either by secretion or actual solution of the mesenchymal cells. The albumin fraction is the first to be formed. The other varieties appear later.
2. In the adults, all the four fractions are produced by the liver. This is supported by isotopic experiments. Fibrinogen, prothrombin and albumin are manufactured only in the liver.
3. Globulin is also suggested to be formed from other sources; such as: (a) From the disintegrated blood cells, (b) from the reticuloendothelial system (specially the γ-globulin), (c) from the tissue cells in general, (d) from lymphoid nodules.
4. The plasma proteins are not static entities. The isotopic experiments indicate that they are completely used up and replaced every fourteen days.
5. Albumin synthesis is stimulated by osmotic pressure changes and by hypoproteinemia; globulin, by a depressed blood protein pool and fibrinogen by systemic inflammation.

RATE OF REGENERATION OF PLASMA PROTEINS: WHIPPLE'S EXPERIMENT

George Hoyt Whipple an American physician and biomedical researcher shared Nobel Prize in medicine with George Richards Minot and William Parry Murphy for their discoveries concerning liver therapy in cases of anemia in 1934.

George Whipple
1878–1976

Whipple's Experiment

After depletion of plasma proteins, such as by severe haemorrhage or after blood donation, the plasma proteins come to the normal level in about fourteen days. Fibrinogen is regenerated first, then comes globulin and last of all serum albumin.

Relation of Diet to Plasma Proteins

Whipple performed the experiment of plasmapheresis upon dogs.

1. The dog was bled, and the cells were separated from plasma.
2. The plasma was rejected and the cells were re-injected being suspended in Ringer-Locke's solution. This is continued for several weeks after a protein concentration of 4% has been attained in order to exhaust the protein reserves.
3. Then it is found that on a standard diet, the rate of plasma protein regeneration is constant. It is seen that during fasting only 2 to 8 gm of plasma proteins are formed weekly by the body tissues.
4. Regarding the efficacy of food protein, the more a particular protein resembles the plasma proteins in the quality and quantity of its amino acid content the more effective it will be in this respect. Obviously, plasma itself will be the best. If whole plasma is given by mouth, for every 3 gm of plasma protein ingested, 1 gm of plasma protein is formed. The potency ratio is therefore 3:1. The potency ratio of the proteins in grains, potatoes, kidney or liver is 5:1. That of red cell, heart or spleen is 10:1.
5. From this, it is seen that excepting plasma proteins, a diet containing grains, potatoes, kidney or liver will regenerate the lost plasma proteins in the earliest time. Moreover, it is found that plant and grain proteins favour globulin formation, whereas animal proteins favour albumin formation. Role of essential amino acids is noteworthy.
6. Plasma proteins can be synthesized from amino acids if all the essential amino acids are freely supplied. Methionine is essential for long continued production. Cystine is less effective and can replace methionine for short periods only.
7. Vitamin K helps in the formation of prothrombin in the liver.
8. Inference from the experiment: There are three types of proteins in human body
   a. Dispensable reserve proteins: These proteins from the reserve store such as glands and skeletal muscle are the source of energy during fasting, starvation, etc.
   b. Labile reserve proteins: These proteins are released immediately into circulation from liver and other sources of protein synthesis to compensate for protein loss due to hemorrhages, burns, etc.
   c. Indispensable proteins: These proteins cannot be mobilized into circulation for compensation and are the fixed cell proteins utilized for various cellular functions.

FUNCTIONS OF PLASMA PROTEIN

1. ***Coagulation of blood:*** Fibrinogen and prothrombin are essential for coagulation of blood and takes part in the process (Fig. 8.1).
2. ***Maintain colloidal osmotic pressure*** of blood and aid in regulating the distribution of fluid between blood and tissue. All three proteins take part in the process.
3. ***Osmotic pressure:*** Albumin having the smallest and the most symmetrical molecule exerts maximum osmotic pressure. Osmotic pressure depends upon a number of molecules in the solution. Albumin has a considerably smaller molecular weight than globulin and comprises 52% of plasma protein. One gram of albumin in 100 ml will exert a pressure of 5.5 mm of Hg. Under the same conditions globulin exerts only 1.5 mm Hg. The total colloidal OP varies from 25 to 30 mm of Hg and albumin is responsible for 80% of it.
4. ***Maintain viscosity and blood pressure:*** The proteins of plasma, mainly globulins due to larger molecules and asymmetry of their structure are responsible to some extent for the viscosity of blood, and viscosity is an important factor in maintaining blood pressure which is essential for efficient heart action. Whole blood is isoviscous with 25% albumin, 15% α-globulin and 2% fibrinogen. The relative viscosity of blood is 4.7 for men, 4.4 for women, 4.2 for children; that of plasma as 1.8 and that of serum as 1.5 relative to distilled water at 37°C (98.6°F).
5. ***Concerned with erythrocyte sedimentation rate (ESR):*** The plasma proteins exert a great influence upon the suspension stability of blood. This is chiefly dependent on fibrinogen, less on globulin and least on albumin. An increase in fibrinogen raises the sedimentation rate of red blood corpuscles by increasing the speed of rouleaux formation. Since, the plasma proteins alter in various diseases, determination of sedimentation rate is of considerable clinical importance and constitutes a guide to the progress of disease. It is determined by the methods of Wintrobe, Westergren and Cutler.

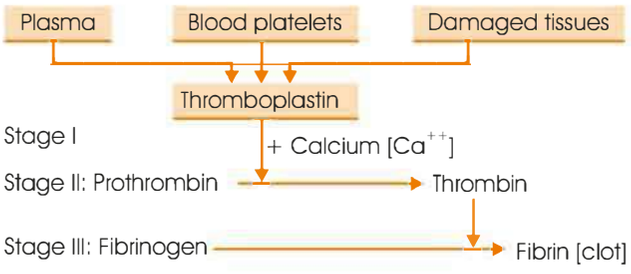


Fig. 8.1: Role of fibrinogen and prothrombin in blood coagulation

8

6. ***Act as buffers:*** They act as buffers in maintaining acid–base balance.
7. ***Act as a protein reserve:*** The plasma proteins serve as a storehouse of proteins from which the tissue can draw during starvation or inadequate protein diet.
8. Help ***CO_2 carriage*** by forming carbamino proteins (*vide* CO_2 carriage).
9. ***Form trephones:*** The leucocytes prepare substances from the plasma proteins, called trephones, which are necessary for the nourishment of the tissue cells grown in culture.
10. ***Antibodies:*** The antibodies being γ-globulin in nature make up a small fraction of the globulins of plasma for defence against infection.
11. ***Help transport of certain substances in blood:*** Plasma proteins combine with certain substances and help to carry them in the bloodstream: (a) Some hormones, enzymes, and clotting factors are part of globulin fraction of plasma proteins, (b) iron (transferrin) and copper (ceruloplasmin) are bound to globulin fractions.

RECENT ADVANCES: INTRINSIC SIGNALS PROTEINS

Günter Blobel is a German biologist and was awarded 1999 Nobel Prize in Physiology and Medicine for the discovery that proteins have the intrinsic signals and these intrinsic signals govern their transport and localization in the cell. The signal peptide forms an integral part of protein targeting, a mechanism for cells to direct newly synthesized protein molecules to their proper location by means of signal peptide within the molecule.

Günter Blobel
1936

EXAM-ORIENTED QUESTIONS

Essay

1. Enlist the various types of plasma proteins. Discuss the functions of plasma proteins.

Short Notes

1. Plasmapheresis
2. Origin of plasma protein
3. Whipple's experiment
4. Enlist the functions of plasma protein

Chapter

9

Viscosity and Erythrocyte Sedimentation Rate (ESR)

INTRODUCTION

Viscosity of blood is an important factor, since it determines the peripheral resistance of the blood flow through the blood vessels and thus helps to maintain blood pressure.

Viscosity: Characteristics

1. Human blood is five times more viscous than distilled water.
2. The viscosity of the whole blood is mainly due to cells and that of plasma is due to plasma proteins.
3. The viscosity of plasma is less than that of whole blood. The relative viscosities of water, plasma and whole blood are roughly 1, 1.8 and 4.7 respectively.
4. Viscosity is measured by noting the time of flow of a given volume of blood through a specially prepared tube (viscosimeter) and comparing the time taken by the same volume of distilled water to pass through the same tube.

Viscosity is Affected by Various Factors

1. It depends on the amount of plasma proteins, the number and volume of corpuscles and CO_2 tension.
2. Viscosity rises in acidosis, hyperglycaemia, hypercalcaemia, polycythemia, cyanosis, icterus, diabetes mellitus, etc. and is reduced in anaemia, fever, exercise, edema, lymphatic leukaemia and malaria. Rise of temperature reduces the viscosity of blood.

SUSPENSION STABILITY OF BLOOD (ERYTHROCYTE SEDIMENTATION RATE OR ESR)

Erythrocyte sedimentation rate (ESR): While in circulation, the red cells remain uniformly suspended in the plasma. If an anticoagulant is added to a specimen of blood and let stand it in a glass tube, the corpuscles (being heavier than plasma) are found to sediment gradually at the bottom of the tube, while the plasma remains as a clear supernatant fluid (Fig. 9.1).

This process is called sedimentation and the rate of settling down or sedimentation is known as the erythrocyte sedimentation rate (ESR). Thus, the erythrocyte sedimentation rate (ESR) is the rate at which red blood cells sediment in a period of one hour.

Sedimentation Continues in Three Phases

- A short stage of aggregation with a little fall.
- True sedimentation following maximum velocity of fall.
- Finally slowing until packing is complete leaving an upper layer of clear plasma.

It depends upon (a) on the differences in densities between red blood corpuscles and plasma, (b) on the degree of adherence of red blood corpuscles to one another (rouleaux formation) related to the plasma protein content and (c) on the resistance that plasma exerts on the red cell surface.

ESR INCREASED AND DECREASED

The ESR is increased in inflammatory conditions, anemia, pregnancy, autoimmune disorders (rheumatoid arthritis and lupus erythematosus), varied infections, lymphoma and multiple myeloma, etc. The ESR is decreased in polycythemia, sickle cell anemia, leukemia, hypoproteinemia (secondary to liver or kidney disease) and congestive heart failure.

SIGNIFICANCE OF ESR

ESR estimation is done to evaluate the response to therapy in certain inflammatory diseases such as polymyalgia rheumatica, temporal arteritis and rheumatoid arthritis. In many of the chronic diseases such as tuberculosis, rheumatoid arthritis, infective endocarditis, multiple myeloma, inflammatory bowel disease, etc. The ESR value may be markedly increased.

Procedure for estimation of ESR

ESR constitutes a guide to the progress of a disease. It is measured either in relation to a fixed time or distance by the methods of Wintrobe, Westergren and Cutler. The tube is put in a vertical position, the lower end of which is placed on a rubber cap and the upper end remains open. Height of supernatant plasma in mm separated out at the top of the vertical column of blood after an hour is noted. This is expressed as the erythrocyte sedimentation rate.

The erythrocyte sedimentation stages are

1. Stage 1: Rouleaux formation: It occurs in first 10 minutes
2. Stage 2: Sedimentation or settling stage occurs in nearly next 40 minutes.
3. Stage 3: Packing stage occurs in further 10 minutes after completion of stage 2 (cells start to pack at the bottom of the tube).

Normal ESR values by Wintrobe's method is 0–6.5 mm per hour for males, average being 3.7 mm per hour and 0–l5 mm per hour for females, average being 9.6 mm per hour.

PHYSIOLOGICAL VARIATIONS IN ESR

1. Lowest in newborn, 0–2 mm per hour.
2. In children, it is from 3 to l3 mm/hour, average 9 mm/hour.
3. In pregnancy there is a definite acceleration in beginning from the tenth to twelfth week. The rate gradually increases and reaches normal level only at third or fourth week postpartum.
4. In old age the ESR increases.
5. It is a non-specific reaction, and gives information of a general character, but is useful supplement when temperature, pulse, etc. show no clinical disorder.
6. ESR is increased in all acute general infections.

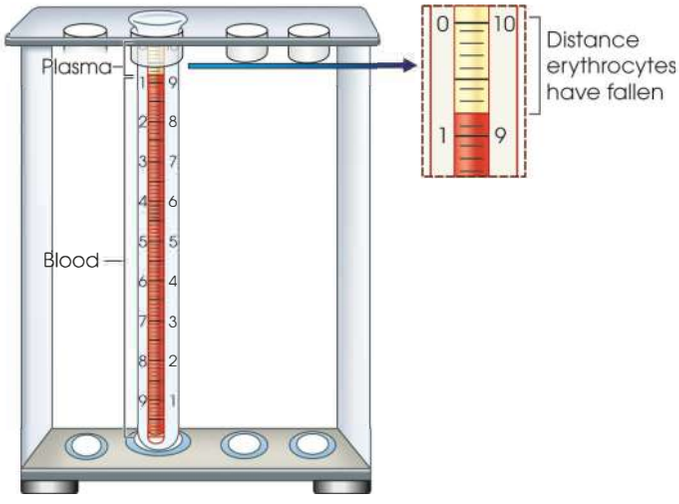


Fig. 9.1: Erythrocyte sedimentation rate reading observation: Note the observed value of ESR of 18 mm at end of one hour by Wintrobe's method

7. One of the most important uses of the test is in calling attention to the presence of more or less occult disease. It is also useful as an aid in differential diagnosis.
8. Other things being equal, an accelerated rate suggests organic disease rather than functional disorder.
9. It acts as a guide to the progress of a disease.

EXAM-ORIENTED QUESTIONS

Short Notes

1. Viscosity
2. Enlist conditions in which viscosity is altered
3. Define ESR and state its normal value in males and females
4. Physiological variation in ESR
5. pH mediated blood reaction
6. Methods of determination of blood reaction

Chapter

10

Coagulation of Blood

INTRODUCTION

When blood is shed, it loses its fluidity in a few minutes and sets into a semisolid jelly. This phenomenon is called *coagulation or clotting*. On further keeping, the clot retracts to a smaller volume and presses out a clear straw-coloured fluid, called the *serum*. Serum will not clot any more. When the process of coagulation is studied under the ultra microscope, it is seen that, minute granules appear at first, often near a clump of disintegrating platelets. These granules join together to form needles, which again unite with one another to form long threads across the whole bulk of blood. These threads cross one another and form a sort of network, into the meshes of which the red and white cells become entangled. The clot gradually retracts and serum separates out.

It is to be noted that coagulation is the property of plasma alone. The red and white cells do not take part in it. They only become caught up in the meshes of the clot and are thereby removed. It is due to this fact that the *clot has a red colour, and the serum is a clear non-cellular fluid*. Blood platelets take some part in the process.

IMPORTANCE OF COAGULATION OF BLOOD

The phenomenon of coagulation is of enormous physiological importance. *Its purpose is to stop further haemorrhage*. When bleeding occurs, the shed blood coagulates and the bleeding vessels become plugged off by the clot. The retraction of the clot compresses the ruptured vessels further and in this way bleeding is stopped.

The phenomenon of coagulation involves primary and secondary haemostasis.

Primary haemostasis: The platelet adheres to vascular wall forming a platelet plug and the process up to this stage is known as primary haemostasis.

Secondary haemostasis: The formation of fibrin clot by the interaction of clotting factor aided by enzymatic reactions is termed as definite haemostasis.

MECHANISM OF COAGULATION OR CLOTTING OF BLOOD

The theory that thrombin is generated by the presence of tissue factor was consolidated by Paul Morawitz in 1905.

Paul Oskar Morawitz
1879–1936

As early as 1904 Morawitz described the basic facts about the mechanism of blood clotting in the following manner.

1. When blood is shed, the *platelets* (by coming in contact with rough water-wettable surface), disintegrate and liberate *thromboplastin*. Certain amount of thromboplastin is also derived from the injured damaged tissues locally.
2. Thromboplastin converts *prothrombin* into *thrombin* with the help of calcium ions and
3. Thrombin interacts with *fibrinogen* forming *fibrin*. This is the *clot* (Fig. 10.1).

Haemostasis is process of stoppage of bleeding.

GENERAL CHARACTERISTICS OF COAGULATION

The three main mechanisms involved in blood coagulation are

1. **Vasoconstriction of blood vessel:** Whenever there is a vascular injury there is vasoconstriction of

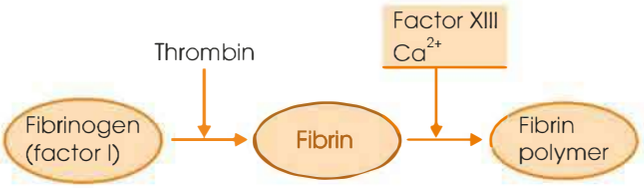


Fig. 10.1: Formation of fibrin clot

injured vessel as a result of contraction of the smooth muscle in the wall of the vessel. The collagen is exposed at the site of injury which promotes platelets adhesion to the injury site. Platelets release cytoplasmic granules which contain serotonin, ADP and thromboxane A_2. These contents of cytoplasmic granules increase the effect of vasoconstriction. Vascular spasm reduces the blood flow and blood loss is prevented but not stopped.

2. **Platelet plug formation:** As platelets are exposed to collagen; platelets release ADP (adenosine diphosphate) and thromboxane. Thromboxane A_2 and serotonin which are released from granules of platelets enhance vascular spasm. The formation of platelet plug is activated by a glycoprotein called von Willebrand factor (vWF). Adenosine diphosphate (ADP) attracts more platelets to the injured site. ADP (adenosine diphosphate) and thromboxane cause the surfaces of nearby platelets to become sticky and, as 'sticky' platelets accumulate, leading to plug formation. In the process more chemicals are released and further more platelets adhere to injured site and release their chemicals; creating a platelet plugs in a positive feedback loop. Platelets alone are responsible for cessation of the bleeding and this process is called primary haemostasis.

Note

After the platelet plug is formed then the clotting factor begins forming a clot. The clotting factors begin to form a collagen fibre called fibrin and further fibrin mesh is produced all around the platelet plug; moreover red and white blood cells become caught up in the fibrin mesh which causes the clot to become even stronger. This step of coagulation is referred to as secondary haemostasis. This is discussed below in the clotting cascade mechanism.

3. **Clotting:** Tissue factor produced by platelets activates intrinsic and extrinsic mechanism of blood clotting; which further activates prothrombin activator; which causes conversion of prothrombin into thrombin; thrombin converts fibrinogen into fibrin which polymerizes to form a blood clot.

Since 1940, research work has indicated that the clotting mechanism is a complex process. In 1954, an International Committee was established. The committee suggested an international system of nomenclature time to time with the appearance of new factors (Table 10.1).

Table 10.1: International nomenclature of blood coagulation factors

Factor	*Name*
I	Fibrinogen
II	Prothrombin
III	Tissue factor or thromboplastin
IV	Calcium
V	Proaccelerin (labile factor)
VII	Proconvertin (stable factor)
VIII	Antihaemophilic factor A, antihaemophilic globulin
IX	Antihaemophilic factor B, plasma thromboplastin component, Christmas factor
X	Stuart-Prower factor
XI	Plasma thromboplastin antecedent
XII	Hageman factor
XIII	Fibrin stabilising factor, Laki-Lorand factor

BLOOD COAGULATION FACTORS

Factor I or Fibrinogen

1. It is globulin in nature but has a much bigger molecule than serum globulin. The molecular weight is about 330,000.
2. It is coagulated at about 56°C and precipitated by one-fifth saturation with ammonium sulphate and saturation with NaCl.
3. It is distinguished from other plasma proteins by its property of clotting, during which fibrinogen is converted into fibrin.

Factor II or Prothrombins

1. It is protein in nature and present in normal plasma.
2. It has a molecular weight of about 62,700.
3. It is very labile in aqueous solution and is inactivated by acids at pH 4.8, by alkali at pH 10 and by heat at 60°C; but is stable indefinitely when dried from the frozen state. (The pH of any fluid is the measure of the hydrogen ion (H^-) concentration. The normal blood pH ranges between 7.35 and 7.45.)
4. In oxalated plasma, two forms of prothrombin are found 'A' and 'B'. The 'A' form is destroyed by oxygen and is heat labile. The 'B' form is removable by aluminium hydroxide. In normal plasma the two forms remain united as calcium compound.
5. When oxalate is added, calcium is removed and the two components become separate.
6. The prothrombin activity of blood is measured by the clotting time of recalcified oxalated plasma, to which *tissue emulsion* has been added.
7. *In human subject, the average 'prothrombin time' is 12 seconds. Prothrombin time* will be longer in deficiency of factor V or factor VII or Stuart factor.
8. *Prothrombin is manufactured in the liver. Vitamin K is essential for the formation of prothrombin. During clotting, prothrombin is converted into thrombin.*

Factor III or Thromboplastin

It is derived from two sources.

Intrinsic in the Plasma

1. *Intrinsic thromboplastin* is formed in the plasma due to interaction between different plasma factors, e.g. Hageman factor or factor XII, PTA or factor XI, Christmas factor or factor IX, anti-haemophilic globulin or factor VIII, calcium ions, factor V and factor X.
2. Prothrombin is converted into thrombin with the help of intrinsic thromboplastin in presence of calcium ions.
3. It should be noted that blood flowing normally through the circulatory system will not clot. But if the surface of the blood vessel becomes rough due to any reason, blood will clot even without the addition of tissue extract (extrinsic thromboplastin).

Extrinsic or Tissue Thromboplastin

1. It is formed from different tissues, e.g. extracts of brain, lungs, etc. as a result of injury. So long, it was known that prothrombin was converted into thrombin with the help of calcium ions and thromboplastin liberated from damaged tissues.
2. But recently it has been found that various plasma factors, e.g. factor VII or proconvertin, are required for such conversion and the process is called *extrinsic thromboplastin formation*.

Factor IV or Calcium

Ionic calcium is essential for blood clotting; by acting as a cofactor in the coagulation process. It is essential for the formation of both intrinsic and extrinsic thromboplastins and also in the conversion of prothrombin into thrombin.

Factor V or Labile Factor Accelerator Globulin or Proaccelerin

This factor is necessary for the complete conversion of prothrombin into thrombin by the extrinsic or intrinsic thromboplastin. It is a protein, heat-labile and is activated within half an hour at 56°C or by increasing the pH to 10.5. It is present in plasma but is used up during clotting.

Factor VI or Accelerin

This factor is a hypothetical activation product of proaccelerin (factor V).

Factor VII or Stable Factor or Proconvertin

1. This factor is present in plasma and is not used up during clotting. It is heat-stable and can withstand temperature up to 56°C.
2. It is a protein and remains associated with prothrombin. It accelerates extrinsic or tissue thromboplastin formation, being activated by extract released from damaged tissue.
3. Its formation is retarded after administration of *Dicoumarin* and in deficiency of vitamin K. *During blood clotting proconvertin is changed into convertin.*

Factor VIII or Antihaemophilic Factor (AHF) or Antihaemophilic Globulin (AHG) or Platelet Cofactor I

1. It is adsorbed on barium sulphate and has a molecular weight greater than 200,000.
2. This factor helps in the formation of intrinsic thromboplastin and intrinsic prothrombin conversion. It is present in the plasma and disappears when the blood clots.
3. It is protein in nature and remains in close association with fibrinogen. This factor is antihaemophilic.
4. In *haemophilia (bleeder's disease)* the defect is not in the platelets but it is due to the absence of this factor which helps in the breakdown of platelets and liberation of platelet cofactor I or thromboplastin factor.
5. The deficiency of AHG resulting in ***classical haemophilia*** in the male is transmitted as a sex-linked recessive trait. The body fails to synthesize this essential globulin due to the absence of the specific enzyme which is controlled by the mutant gene.

Factor IX or Christmas Factor or Plasma Thromboplastin Component (PTC) or Platelet Cofactor II

1. It is absorbed by aluminium hydroxide, is labile to heat but is relatively stable on storage.
2. It is precipitated by 59% ammonium sulphate.
3. This factor is necessary for intrinsic thromboplastin formation. Absence of this factor leads to a disease stimulating haemophilia known as ***haemophilia C*** and is transmitted as a sex-linked recessive in the male.
4. This type of disease was first found in a patient named *Christmas* and hence the name Christmas factor. This factor is not used up during clotting.

Factor X or Stuart Factor

1. In 1959 the international nomenclature has been given to this factor. Chemically it has many of the properties similar to that of factor VII.
2. Its synthesis is also retarded after administration of *dicoumarin*.
3. Absence of this factor leads to mild haemorrhagic diathesis.
4. It is stable in room temperature, but destroyed rapidly at 56°C in serum.

Factor XI or Plasma Thromboplastin Antecedent (PTA)

This is activated by *active Hageman factor*, and ultimately leads to formation of thrombin. Deficiency of this causes mild bleeding tendencies of *haemophilioid D type* and is transmitted as a *sex-linked dominant* to both sexes.

Factor XII or Hageman or Surface Factor

This is protein in nature. Inactive form is activated on surface contact. This in turn activates the protein-splitting enzyme *kallikrein* to produce plasma *kinins*. The resulting effects are increased vascular permeability and dilatation of blood vessels.

Factor XIII or Fibrin-stabilising or Laki-Lorand Factor (LLF)

The active form along with Ca^{++} converts soft fibrin clot to a solid, fibrous one. Its action also decreases the solubility of the clot in urea solution. Persons having *congenital malformation of LLF* suffer from *poor wound healing*.

Fitzgerald Factor also known as High Molecular Weight Kininogen

It is the contact factor for haemostasis. This non-enzymatic factor in circulation forms a complex with prekallikrein and factor XI. High molecular weight kininogen is synthesized in the liver and it forms a cleavage to bradykinin with aid of prekallikrein. Bradykinin has vasodilator effects.

Fletcher Factor also known as Prekallikrein

This factor is synthesized in the liver. In form of complex with factor XI and high molecular weight kininogen while in circulation. It is mainly responsible for process of contact activation for process of homeostasis.

Other Important Factors Participating in Coagulation Mechanism

Thrombomodulin

The molecular weight of thrombomodulin is 78,000. Thrombomodulin forms a cleavage with factor II, V and VII. The binding of thrombin with thrombomodulin activates protein C and this thrombin thrombomodulin complex serves as anticoagulant. Thrombin as a single entity is a procoagulant.

Protein C

1. The molecular weight of protein C is about 59,000.
2. The half life of protein C is about six hours.
3. It inactivates factor Va and factor VIIIa; it is a vitamin K-dependent protein which is activated by thrombo-modulin.

Protein S

The molecular weight of protein S is 75,000. It is synthesized by liver, platelets, osteoblast and Leydig cells. Protein S forms a complex with protein C and this complex inactivates factor Va and factor VIIIa.

Anti-thrombin III

Anti-thrombin III is a protease inhibitor and inhibits factor IXa and factor Xa.

The complex of tissue factor and factor VIIa is also inhibited by anti-thrombin III.

Tissue Factor Pathway Inhibitor

This factor is synthesized by endothelial cells of blood vessels and it inhibits factor VIIa and factor Xa.

EXTRINSIC AND INTRINSIC MECHANISMS OF COAGULATION OF BLOOD

Davie and Ratnoff (1965) have proposed what has been termed a 'waterfall sequence hypothesis' to explain the succession of events taking place in the coagulation of blood. Each protein coagulation factor exists in the plasma in an *inactive* (*proenzyme*) form and is activated sequentially until finally thrombin is formed which then converts fibrinogen into fibrin.

Key Points

1. The extrinsic system varies forms the intrinsic system, by the appearance of factors derived from outside the plasma. These extrinsic factors interact to perform active Stuart factor (factor X).
2. From this point the intrinsic and extrinsic systems pursue the same pathway to fibrin. Although either the extrinsic or the intrinsic system can initiate the formation of fibrin, yet the presence of both systems results in the formation of an extensional mount of it.
3. The needs for contribution of many clotting factors are involved in the coagulation process. But the *plasma protein clotting factors* usually *interact in pairs*. Due to this interaction each of the clotting factors is in turn converted from an inactive form to an active one. Since all the clotting factors are not supposed to possess enzyme actions, yet this conversion of enzymes from an inactive form to an active one is initiated in the sequence of action of clotting factors.
4. The process of intrinsic and extrinsic systems has been shown schematically in Fig. 10.2.

Intrinsic pathway

Step I: Factor XII activation: The contact of factor XII with exposed collagen of blood vessel or with negatively charge surface activates the intrinsic pathway. The factors which facilitates activates factor XII a are cofactors high molecular weight kininogen and kallikrein.

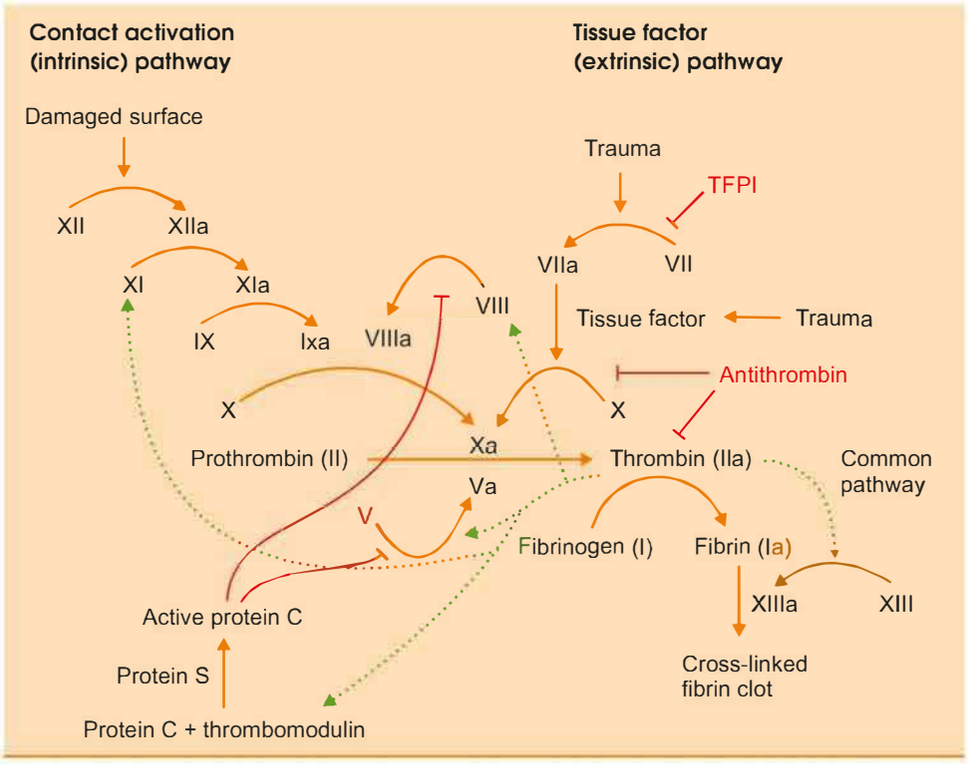


Fig. 10.2: Mechanism of extrinsic and intrinsic blood coagulation (*Courtesy:* Davie EW and Ratnoff OD)

Step II: Factor XI activation: The factor XIIa converts factor XI to activated form as XIa in presence of high molecular weight kininogen.

Step III: Factor IX activation: Factor XIa converts factor IX to activated form.

Step IV: Factor X activation: Factor IXa activates factor X to Xa in presence of cofactors activated factor VIII, calcium and membrane phospholipids. (The factor VIII forms a complex with vWF. The separation of factor VIII from vWF activates factor VIII and this is promoted by Xa and thrombin.)

Extrinsic mechanism

Step I: The tissue factor tissue thromboplastin is released from the injured tissue.

Step II: Factor VII activation: Tissue thromboplastin converts factor VII to factor VIIa which directly activates factor IX and factor X.

Step III: Factor X activation: The factor VIIa converts factor X to factor Xa in presence of calcium, tissue thromboplastin and platelet phospholipids.

5. **Conversion of prothrombin to thrombin:** The activated factor Xa converts prothrombin to thrombin in presence of activated factor Va, platelet phospholipid and calcium.

Role of thrombin, phospholipid and protein in blood coagulation

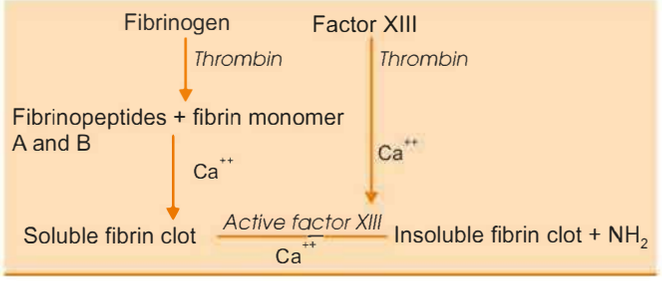
Role of thrombin (Flowchart 10.1)

1. Thrombin is a homogeneous glycoprotein of molecular weight 40,000 acting as a proteinase.
2. During the process of coagulation the complex of factor Va and Xa formed on phospholipid or platelet membrane converts prothrombin into thrombin in presence of calcium.
3. It also activates platelets and factor V, VIII and XI.
4. It activates protein C pathways and by releasing plasminogen activator promotes fibrinolysis.

Role of phospholipid: Phospholipid kephalin (cephalin) helps in the formation of *prothrombinase*. In the intrinsic system it is in the platelet factor 3 and in the extrinsic one in tissue thromboplastin.

Role of protein: Blood clotting factors, from V to XII, are plasma proteins mostly β-globulins. A few of them are however either α-globulin or β-globulin. Th excess thrombin binds to thrombomodulin. The resulting complex activates protein C and its cofactor protein S. They inhibit further thrombin formation directly—by inactivating factor 5 and indirectly by inactivating factor 8.

Flowchart 10.1: Role of thrombin



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6. **Transformation of fibrinogen to fibrin:** Thrombin as an enzyme helps in conversion of fibrinogen into fibrin by proteolysis of soluble fibrinogen and stabilization of fibrin polymer.
   a. The binding of thrombin to central domain of fibrinogen releases fibrinopeptides which forms fibrin monomer (soluble fibrinogen).
   b. The fibrin monomers unite to form protofibrils. These protofibrils branches and forms interconnected thick fibrin fibers and this process is termed as polymerization of fibrin monomers. Thrombin activates factor XIII in this stage.
   c. The covalent cross-linking of fibrin polymers stabilize the clot. The red blood cells and platelets are also trapped in fibrin network.

CLOT RETRACTION

Usually blood clot retracts to about half its initial volume within *20 to 24 hours*. When blood is shed, *fibrins* form a network like structure. The platelets adhere to these fibrin networks and form knots. The main function of platelets is clot retraction. The spicules which are known as filopodia are formed by the platelets. The fibrin stand gets embedded in the filopodia. The fibrin framework then becomes twisted and shortened, and clot retraction occurs. Calcium and thrombin hastens clot retraction. The glycoprotein IIIa and IIb receptors are involved in clot retraction. The clot retraction prevents thrombolysis, it blocks or seals the damaged injured blood vessel and also facilitates wound healing.

FIBRINOLYSIS

Clotted blood if kept sterile remains intact for several weeks. But if it is not kept sterile the clot breaks up. In human body the blood coagulation initiates the blood anti-clotting mechanism by activation of protein C and plasmin.

Thrombin and so also thrombomodulin activates protein C into its active form. The protein C inactivates the inhibitors of plasmin activators. This activates plasminogen activator which converts plasminogen into plasmin. It with help of cofactor protein S inactivates factor Va and VIIa and prevents coagulation.

Thus, fibrin breakdown in the clot which is known as *fibrinolysis* is brought about by a proteolytic enzyme in the plasma known as *plasmin or fibrinolysin*. The fibrin is degraded by plasmin into fibrin degradation products. Plasmin apart from its role in clot retraction regulates development of neurons, controls embryogenesis and ovulation, activates growth factors and aids in proliferative response of arteries in blood injury.

Normally fibrinolysis is prevented by the presence of another substance in the blood known as *antiplasmin* which remains attached to the plasma albumin (Fig. 10.3).

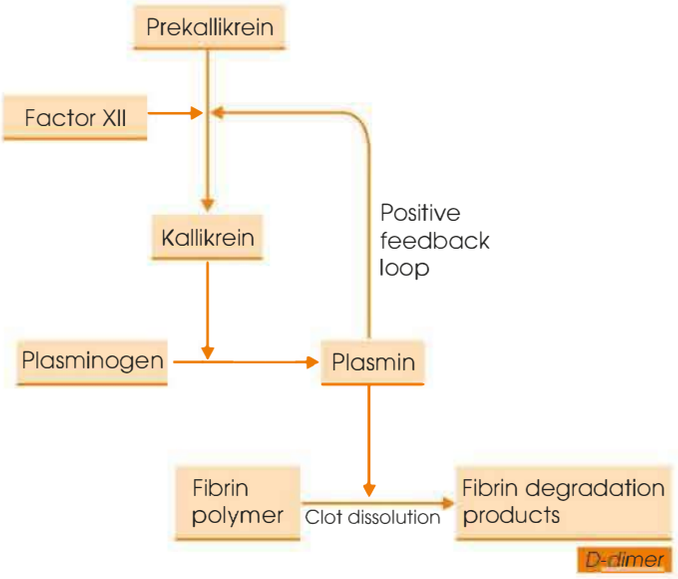

Fig. 10.3: Fibrinolysis

PLASMINOGEN

Plasminogen has molecular weight of 92,000 and made up of 791 amino acids. The cleavage by plasminogen activators at the bond between arginine and valine at 560 and 561 positions activates plasminogen.

There are two types of endogenous plasminogen activators:

1. **Tissue plasminogen activator (t-PA):** It is a polypeptide having molecular weight of 72,000 and is made up of 527 amino acids. The tissue plasminogen activator secretion is controlled by histamine, bradykinin, epinephrine, acetylcholine and gonadotrophins.
2. **Urokinase and streptokinase:** Urokinase, a plasminogen activator is a glycoprotein made up of 411 amino acids. Urokinase was originally identified from human urine sample, but is also found in bloodstream and the extracellular matrix. This urokinase plasminogen activator (u-PA) is a potent plasminogen activator. It is a thrombolytic agent and is used for therapeutic purpose in management of severe or massive deep venous thrombosis, myocardial infarction, etc. **Streptokinase** (**SK**) an enzyme secreted by several species of streptococci binds and activates plasminogen. The α (residues 1–150), β (residues 151–287) and γ (residues 288–414) are the three domains to streptokinase. Plasminogen binds to each domain but none can activate plasminogen independently. Streptokinase is administered intravenously immediately after the onset of a ST elevation myocardial infarction (STEMI) and has found to be immensely helpful in preventing any further pathological damages to the cardiac tissue.

3. The plasminogen activator inhibitors are secreted by adipocytes, monocytes, liver and endothelial cells. It has a rapid action for inhibiting tissue plasminogen activator and urokinase plasminogen activator.

NATURAL INHIBITORS OF COAGULATION

To maintain blood in a fluid state in the normal condition, retarding influences coexist with positive coagulation inducing factors in the circulating blood. Some of the ingrained safeguards against intravascular clotting are: (a) The relative slowness of thrombin production, (b) the unbroken continuity of the vascular endothelium and (c) removal of clotting intermediates by the RE cells. Besides these, other definite inhibitors of coagulation are present.

Antithrombin Activities Remove Thrombin from Blood

1. Antithrombin I is the thrombin-adsorbing effect of fibrin but whether it plays a role in normal coagulation is unknown.
2. Antithrombin II is a factor which acts jointly with heparin.
3. Antithrombin III is the so-called physiological antithrombin because it is present naturally and inactivates thrombin progressively. Heparin is described separately below.
4. Antithromboplastins are present in normal blood, and one or more circulating antithromboplastins are claimed to be present.

INTRAVASCULAR CLOTTING OR THROMBOSIS

Thrombus

It is a clot formed inside the blood vessels. Thrombus is formed due to slowing of circulation and damage of the vascular endothelium. Atheromatous patches occur in blood vessels and the vascular endothelium is damaged in some abnormal conditions. Masses of platelets are deposited in the damaged endothelium. Filaments of fibrin form also a network in this region. The platelets liberate thromboplastin. The fibrin, entangled in the lamellae of platelets, forms the thrombus or clot. Intravascular thrombosis sometimes occurs in coronary and cerebral vessels which are called coronary thrombosis and cerebral thrombosis respectively. After surgical operations, etc. thrombosis may occur in big veins.

HEPARIN AS AN ANTICOAGULANT

At first it was isolated from liver by McLean in Howell's laboratory, hence the name. Subsequently, it has been extracted from many tissues in the body.

Characteristics

1. It is anticoagulant, *in vivo* and *in vitro*.
2. One unit of heparin is defined as the quantity of material which will prevent the clotting of 1 ml of cat's blood for 24 hours when kept in cold.
3. Chemically it is mucoitin polysulphuric acid. Mucoitin is a polysaccharide, composed of glucosamine, glucuronic acid and esterified sulphuric acid forming an ester with molecular weight of about 17,000.
4. It has been shown that any substance with a high molecular weight, and being composed of polysaccharides and several SO_4 groups, can act as an anticoagulant. Hirudin, found in cervical glands of the common medicinal leech (Hirudo), is a compound of this nature.
5. Heparin is normally secreted by the mast cells. These cells are found in blood to about 1%. They remain scattered throughout the reticuloendothelial system and found abundantly along the course of many blood vessels, such as those of liver. Sometimes they replace the intima of the blood vessels. These cells are found to contain granules which are supposed to be the precursors of heparin.
6. It is doubtful whether heparin is present in normal blood in any appreciable amount and as such it probably takes no part in preventing intravascular clotting normally.
7. Heparin helps to maintain the normal fluidity of the blood within the vascular bed.

It inhibits the transformation of prothrombin to thrombin when accompanied by a plasma cofactor albumin X and neutralises the action of thrombin on fibrinogen.

FACTORS PREVENTING COAGULATION

1. By lowering temperature, coagulation can be prevented.
2. By avoiding contact with water-wettable surface and injured tissues. This prevents thrombokinase action. When blood is collected in a tube-coated with paraffin, the surface not being water-wettable, the platelets will not break down and coagulation will not take place.
3. Removal of calcium ions: (a) By precipitation: This is the commonest practice in clinical laboratories. This is done by adding citrates or oxalates of Na or K. Sodium fluoride (0.3% solution) is also used, (b) by formation of a complex compound. The substances used are di- and trisodium citrate and ethylenediaminetetra acetic acid (EDTA).
4. Precipitation of fibrinogen: By adding various salt solutions in adequate amounts. When blood is mixed with one quarter of its volume of magnesium sulphate or with an equal volume of half saturated sodium sulphate solution, clotting is prevented.

5. By the addition of substances of biological origin:
   a. *Protamines:* Simple proteins found in some fish.
   b. *Peptone:* When it is injected into the veins, the coagulability of blood is reduced (but peptone does not prevent the coagulation of a sample of blood *in vitro*). Extracts of cray fish and nussels act in somewhat similar manner. They act by increasing secretion of heparin by the mast cells.
   c. *Heparin:* Mucoitin-polysulphuric acid produced by mast cells.
   d. *Hirudin* (leech extract) and the venom of certain snake. Heparin, hirudin and venom inhibit coagulation of blood by inhibiting activation of prothrombin and thrombin fibrinogen reaction.
   e. *Cysteine:* Same as heparin and hirudin.
   f. *Dicoumarin or dicoumarol:* It is chemically related to the naphthoquinone derivative. It is antagonist to vitamin K. It inhibits the synthesis of prothrombin in the liver by preventing the action of vitamin K. Dicoumarol lowers the plasma prothrombin level and depresses the activity of factor VII.
   g. *Phenindione:* Action similar to that of dicoumarol. Its action is quick and depresses the activity of factor VII more than prothrombin.
   h. *By adding azo dyes and synthetic products:* Chicago blue, trypan red, trypan blue act as anticoagulants both *in vivo* and *in vitro*.

FACTORS HASTENING COAGULATION

1. Warmth.
2. Contact with water-wettable surface and contact with rough surface.
3. Additions of foreign bodies into a sample of blood (*vide* 'defibrinated blood'*).
4. Addition of thrombin.
5. Addition of thromboplastin.
6. Vitamin K injection or oral administration in high doses increases the prothrombin content of blood and increases the coagulability.
7. Addition of calcium chloride, both *in vivo* and *in vitro*.
8. Adrenaline injection produces constriction of blood vessels and helps in haemostatis mechanism.

Lipid and Coagulation

Recently a good number of evidences has been put forward to suggest that elevation of both neutral and phospholipid concentration of blood leads to aggregation of fibrin deposit, possibly due to retardation of the fibrin-dissolving system. This leads to augmentation in thrombus formation, and might result in increased incidence of thrombosis and atherosclerosis. Saturated fats are more agile in decreasing clotting time than unsaturated ones.

Normal Coagulation Time

Measured according to the method of Lee and White, it is 6 to 17 minutes in glass tube and 19 to 60 minutes in siliconised tube.

METHODS OF DETERMINATION OF COAGULATION OF BLOOD

1. **Capillary glass tube method:** This method is usually adopted as a bed side procedure. The finger is pricked and the blood is made to flow into a capillary glass tube about 15 cm (6 inches) long. A small bit of the glass tube is carefully broken off every fifteen seconds until a fine thread of clotted blood appears while the tube is being broken. The period between the appearance of blood in the finger and the formation of this thread is taken as the coagulation time. The average time, by this method, is 3–4 minutes.
2. **Wright's coagulometer:** The principle is same as above. Blood is allowed to flow into a dozen capillary tubes of equal calibre. The tubes are sealed on both sides and placed in water bath at 37°C. After 4 minutes, the first tube (the tube which was first filled with blood) is removed from the water bath, the ends are broken and the blood inside is expelled into water. The same procedure is repeated with all the other tubes at intervals of 30 seconds. When the blood expelled from a particular tube has the form of a worm-like clot, the end point is reached.
3. **Lee and White method:** 1 ml of blood is drawn from a vein by a dry syringe and placed in two clean test tubes, 8 mm in diameter. The tubes are closed by a rubber cork. At 5 minutes after withdrawal of the blood in the manner described, the first tube is gently tilted 45 degrees at one minute intervals until it can be inverted 180 degrees without blood flowing. This time is recorded and the same procedure is repeated with the second tube. As handling favors coagulation, the time for the second tube is taken as the true coagulation time since it was tilted less than first tube.

Bleeding Time

Normal average is 3.25 minutes, the range being 2–5 minutes. It is usually determined by Duke's method. The lobule of the ear is punctured and the time is noted. The blood oozing out is mopped up with a piece of filter paper every half a minute until bleeding stops. This indicates the end point.

Prothrombin Time (Quick)

An approximate prothrombin time is generally 11 to 16 seconds.

Key Points

1. When tissue extract (thromboplastin) and calcium chloride are mixed (added) in an optimum amount to blood of normal fibrinogen content, the only factor which has an inadequate concentration of prothrombin, can vary the coagulation time.
2. If the prothrombin is diminished; the coagulation time increases.
3. This test is a quantitative one for prothrombin in blood based on the coagulation time of oxalated blood plasma in the presence of tissue extract (thromboplastin) and calcium chloride.
4. In each laboratory a curve of prothrombin concentration in the blood to the prothrombin time is usually drawn for the evaluation of the prothrombin time.
5. The only precaution is that blood removed from the patient is immediately oxalated so that none of the prothrombin can be converted into thrombin.

Method

1. In a test-tube 0.2 ml of commercial thromboplastin containing calcium is kept at 37°C.
2. After 30 seconds, 0.1 ml plasma is quickly added from a pipette, and a stopwatch is started simultaneously.
3. The tube is kept in the water bath and is shaken constantly but gently for 10 seconds. Then in bright direct illumination the tube is tilted continually from vertical to almost horizontal position once per second until a gel appears. This is the end point.
4. A value of 11 to 16 seconds is satisfactory, but the test should always be performed in duplicate.

Applied Physiology

COAGULATION DISORDERS

1. **von Willebrand disease:** The deficiency in the formation of quality or quantity of von Willebrand factor (vWF) leads to this disease. The von Willebrand factor (vWF) is required for platelet adhesion. The three forms of von Willebrand factor (vWF)/disease are hereditary, acquired, and pseudo or platelet type. The types of hereditary von Willebrand factor (vWF)/disease are: vWD type 1, vWD type 2, and vWD type 3. The platelet type of von Willebrand factor (vWF)/disease is also an inherited condition.

   The characteristic features of this disease are varying degrees of bleeding tendency, such as easy bruising, bleeding gums and nosebleeds. The vWF gene is located on the short arm p of chromosome 12 (12p13.2). The types 1 and 2 are inherited as autosomal dominant, type 3 is inherited as autosomal recessive. Desmopressin is used in management of this disease as it stimulates the release of vWF from the Weibel-Palade bodies of endothelial cells, and increases levels of vWF nearly upto five-fold.
2. **Lack of fibrinogen or factor I:** Afibrinogenaemia or fibrinogenopenia is a rare congenital disease due to lack of fibrinogen. Sometimes it is found during abnormal pregnancy.
3. **Due to diminution of prothrombin or factor II:** Vitamin K helps in the formation of prothrombin in the liver. Vitamin K is a naphthoquinone derivative. It is absorbed from the small intestine in the presence of bile salts. In the liver it helps in the synthesis of prothrombin and factor VII or stable factor or proconvertin. In the liver disease, e.g. cirrhosis of liver, malignant disease of the liver, etc. there is diminution of synthesis of prothrombin in the liver. In obstructive jaundice due to absence of bile salts, vitamin K is not absorbed. Due to lack of vitamin K, synthesis of prothrombin and factor VII is decreased. Prothrombin time is prolonged and haemorrhages often occur.
4. **Due to lack of anti-haemophilic globulin (AHG) or factor VIII—haemophilia:** It is a disease which occurs in males but is transmitted through females. The coagulation time is abnormally prolonged. There is a tendency to bleed severely after trivial injuries. The knee or elbow joint may be distended with blood. The platelet count remains normal. There is lack of factor VIII or anti-haemophilic globulin (AHG). Blood transfusion temporarily supplies AHG and stops bleeding. Sometimes it has been observed that if blood taken from two subjects is mixed together, coagulation time is normal although the coagulation time of each individual subject has got prolonged coagulation time. From this it has been assumed that there are two types of hemophilic subjects, one lacking in AHG and another lacking factor IX or Christmas factor or PTC.
5. **Due to diminution of factors V, VII and IX—pseudohaemophilia:** In this disease there is congenital deficiency of factors V, VII and IX. The hemorrhagic condition stimulates haemophilia.

Note

Haemophilia A is due to factor 8 deficiency, haemophilia B is due to factor 9 deficiency and haemophilia C is due to factor 11 deficiency. The genes encoding for the factors 8 and 9 are on the X chromosome. Factors 8 and 9 can be extracted from donated blood. Recombinant factor 8 and recombinant factor 9 made by genetic engineering are immensely useful in management of haemophilia. The von Willebrand disease is most common type of bleeding disorder and a mutant version of the factor eliminates its protective effect on factor 8.

6. **Disseminated intravascular coagulation (DIC)** is a pathological condition in which there is widespread activation of the clotting cascade and this leads to formation of blood clots in the small blood vessels. The blood supply is compromised further leading

to multiple organ damage. The consumption of clotting factors and platelet in the process of coagulation the normal clotting is disturbed which may lead to severe bleeding from various locations.

The common causes of DIC are

1. Septicaemia
2. Massive tissue injury in cases of severe trauma, burns, rhabdomyolysis, extensive surgery
3. Snakebite
4. As a complication of mismatched blood transfusion
5. Giant haemangiomas (Kasabach-Merritt syndrome)
6. Large aortic aneurysms
7. Secondary to obstetric complications such as abruptio placentae, pre-eclampsia or eclampsia, septic abortion, postpartum haemorrhage, etc.
8. Fungemia

Treatment of DIC is focused towards treating the underlying condition. Infusion therapy of platelets or fresh frozen plasma in cases of significant bleeding. Cryoprecipitate is administered in patients having a low fibrinogen level.

7. **Thrombocytopenia:** It is a condition of lowered platelet count leading to mild to serious bleeding. The normal platelet count is 150,000 to 450,000 platelets per microliter of blood. The risk for serious bleeding occurs when platelet count becomes low as 10,000 or 20,000 platelets per microliter. Mild bleeding sometimes occurs when the platelet count is less than 50,000 platelets per microliter.

EXAM-ORIENTED QUESTIONS

Essay

1. Describe the intrinsic and extrinsic mechanisms of coagulation. Add note on fibrinolysis.
2. Define haemostasis. Enlist the clotting factors. Discuss the various stages of coagulation.

Short Notes

1. Clot resolution
2. Fibrinolysis
3. Heparin and its functions
4. Factors preventing coagulation
5. Factors hastening coagulation
6. Bleeding disorders
7. Hemophilia
8. Antithrombin III
9. Protein C
10. Methods of determination of coagulation time
11. Methods of determination of bleeding time

Chapter

11

Functions of Bone Marrow

BONE MARROW

The terms 'bone marrow' and 'myeloid tissue' are often used synonymously. *Myelos* meaning marrow is confined in the cavities of the bone in the postnatal life.

Bone marrow is the cellulovascular tissue occupying the medullary cavities and the cancellous spaces of the bone.

Key Points

1. Active marrow in the adult is estimated at from 3.5–6% of body weight. The volume of the marrow is 70 ml at birth and about 4000 ml in the adult.
2. In the adult only about half the marrow is in active state, known as red bone marrow, the remainder being inactive—yellow bone marrow.
3. Though the yellow bone marrow is inactive, yet it has the capacity of resuming its activity to produce blood cells during urgent need. Under such condition a certain portion of the yellow bone marrow is converted into the red bone marrow. Although only half the marrow is active in the adult, yet it has enormous functional capacity and considerable room for expansion.

Red Bone Marrow

Red cells are actively manufactured here, hence the colour. In foetal stage most of the bones contain red bone marrrow. But with the advancement of age and in postnatal life the red bone marrow is only located in the upper ends of humerus and femur, the bones of skull and thorax, the vertebrae and the innominate bones of the pelvis.

Yellow Bone Marrow

It is made up of fat and a little reticular tissue with blood vessels. Here, red cells are not manufactured. In the adult life they occupy the spaces where red marrow is absent.

Formation of Bone Marrow

1. *From birth up to the fourth year* all the bones contain red bone marrow.
2. *By seven years* the marrow becomes less active and is pale red in color.
3. *Between ten to fourteen years* a patch of yellow bone marrow appears in the distal ends of the shafts of the long bones and gradually extends on both sides.
4. At the age of twenty, entire red bone marrow of the long bones is replaced by yellow bone marrow except the upper ends of femur and humerus. Throughout adult life this distribution persists.
5. As age advances yellow bone marrow proportionally increases.
6. *By seventy years* more than half the ribs and half of sternum contains yellow bone marrow.

VASCULAR ARRANGEMENT IN THE BONE MARROW

The nutrient artery breaks up into smaller branches which widens out and becomes blood sinuses. These sinuses are lined by single layer of endothelium, the cells of which divide and give rise to red cells. These sinuses, where active erythrogenesis is going on, remain collapsed, thus creating the anoxic condition favourable for red cell formation. When the red cells are sufficiently mature, these collapsed sinuses open up, blood stream enters and the newly born cells are washed away into the circulation. There is some evidence that marrow activity depends to some extent upon the sympathetic system. Turnbull (1936) and later Gilmour (1942) demonstrated that erythropoiesis is also extravascular.

METHOD OF EXAMINING BONE MARROW

In animals, the bone is taken out and the marrow is collected after breaking the bone. A smear is prepared in the same way as drawing a blood film on a glass slide. In the human beings the sternum is punctured

with a special needle, the marrow is drawn out and the slides are prepared just like blood smears.

Staining

a. For staining the smear, Jenner's stain or Leishman's stain may be used in the same way as in staining blood film. For staining the reticulocytes cresyl blue should be used.
b. Vital staining of the living cells of the bone marrow can be carried out by injecting suitable preparation of Janus green and neutral red in the circulation of living animal.

It is to be noted that in the circulation the average ratio between white and red cells is 1:700. In other words, the red cells are much more in proportion to the white cells. But in the red bone marrow, the relation is reversed. Myeloid cells are more in number than the erythroid cells. The proportion between the cells of the myeloid series and the erythroid series varies from 8:1 to 2:1. This reversed relation is due to the fact that the life of the white cells is much shorter in the circulation than that of the red cells.

Consequently, the white cells should be more speedily manufactured than the red cells. Due to this reason, myeloid cell count is much higher than the erythroid cells in the bone marrow. The red bone marrow also contains giant cells known as megakaryocytes having a diameter of about 40 microns. Each cell contains a ring of lobed nuclei. From these megakaryocytes, platelets are formed.

FUNCTIONS OF BONE MARROW

1. **Haematopoietic (haemopoietic) function (production and release of blood cells):** Production of myeloid elements is the important function of bone marrow. It has been described that red bone marrow is active and has the capacity of forming red cells as well as other blood cells. In the embryo and even in the newborn, only red bone marrow is formed, but in the adult stage nearly 50% of the red bone marrow is converted into yellow bone marrow. This ratio is not constant and is changed with the advancement of age and also with the degree of the need of haemopoietic elements. All the blood cells like erythrocytes, granulocytes, platelets, monocytes and lymphocytes are formed in the red bone marrow. It has been studied that the marrow contains about 5.6 $\times 10^9$ erythroid precursors per kg body wt and 11.4 $\times 10^9$ neutrophilic precursors per kg body wt Mechanism by which the blood cells are released in the blood are not clear. Under certain urgent need and in case of anaemia, mature and even immature cells may be released in the circulation.
2. **Erythroplasia or destruction of RBC:** In the bone marrow not only the blood cells are formed but also the abnormal, imperfect, damaged and aged RBC is destroyed. These cells are sequestered or trapped and phagocytised in the macrophages of the bone marrow. Iron portion is stored as haemosiderin and ferritin in the liver, spleen, RE cells and bone marrow and the rest of heam is ultimately converted into the bile pigments.
3. **Storage functions:** Bone marrow is an important site for storage of iron in the form of ferritin and of haemosiderin coming from food sources as transferrin and also from destruction of RBC through phagocytosis. These stored irons are easily utilized for the synthesis of haemoglobin.
4. **Reticuloendothelial function:** Bone marrow plays an important role in the inactivation of toxins or other toxic substances of the body. The free macrophages of the bone marrow are increased during the invasion of toxins or during rapid haemolysis.
5. **Immunological function:** Regarding its immunological function, the marrow is not as competent as it is found in spleen and lymph nodules. Presence of lymph nodules in the bone marrow has been reported by many.
6. **Osteogenic function:** The cellular elements which take part is the formation of bone are formed in the marrow. The osteoclast, osteoblast, osteocyte, endosteum of blood vessels are formed within the marrow.
7. **Connective tissue functions:** Due to its different connective tissue contents, the bone marrow performs several functions associated with the connective tissues.

Formed Elements of Blood

There are three types of cellular elements in blood, i.e. (1) red blood corpuscles (RBC), (2) white blood corpuscles (WBC), and (3) platelets. These three are collectively known as 'formed elements of blood'.

Theories of formation: There are two theories regarding their origin are monophyletic theory and polyphyletic theory.

1. The monophyletic theory holds that all the blood cells are derived from a common primitive ancestor, which is called the 'stem cell' or haemocytoblast.
2. The polyphyletic school, in its complete form, holds that for every variety of blood cell, there is a distinct type of blast cell, viz. erythroblast, myeloblast, lymphoblast, monoblast, etc.

Other theories of formation of blood cells:

3. The dualistic school believes that there are two distinct types of primitive cells. One of them remains in the bone marrow and gives rise to the red cells, granulocytes and megakaryocytes, from which the platelets develop. The other cell remains in the lymphoid tissue from which the lymphocytes are derived.

4. Similarly, there is a trialistic school believing in three primitive cells. All school agree that in the early embryo, all the blood cells are derived from a single primitive reticuloendothelial cell.

It is generally conceded that, in postnatal life, the development of leucocytes is completely extravascular. The granulocytes are derived exclusively from the red marrow; while the lymphocytes and the monocytes

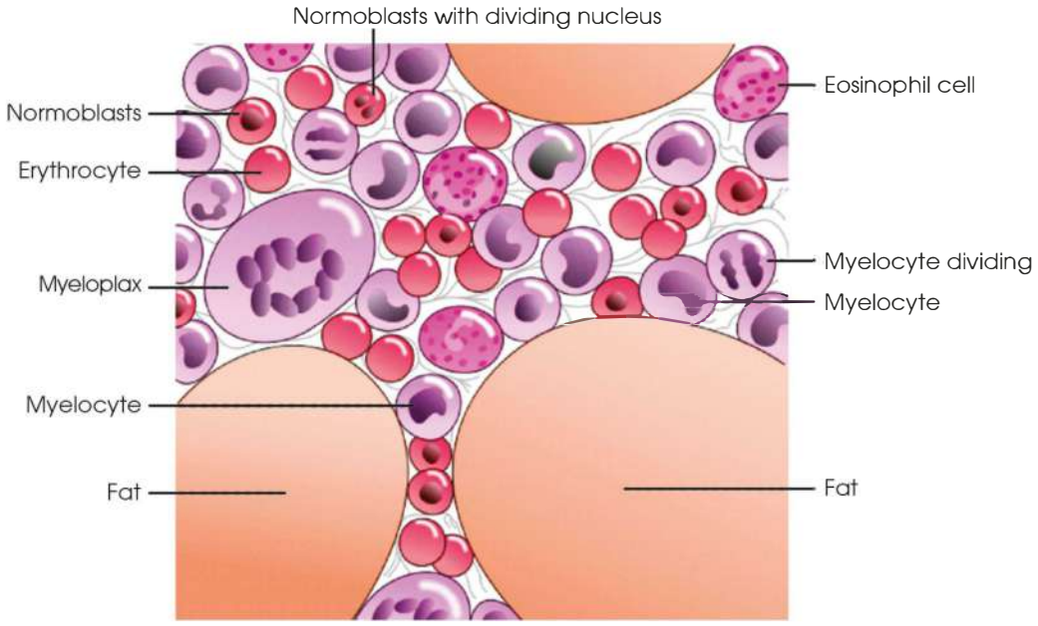

Fig. 11.1: Human bone marrow (Ganong, 1919)

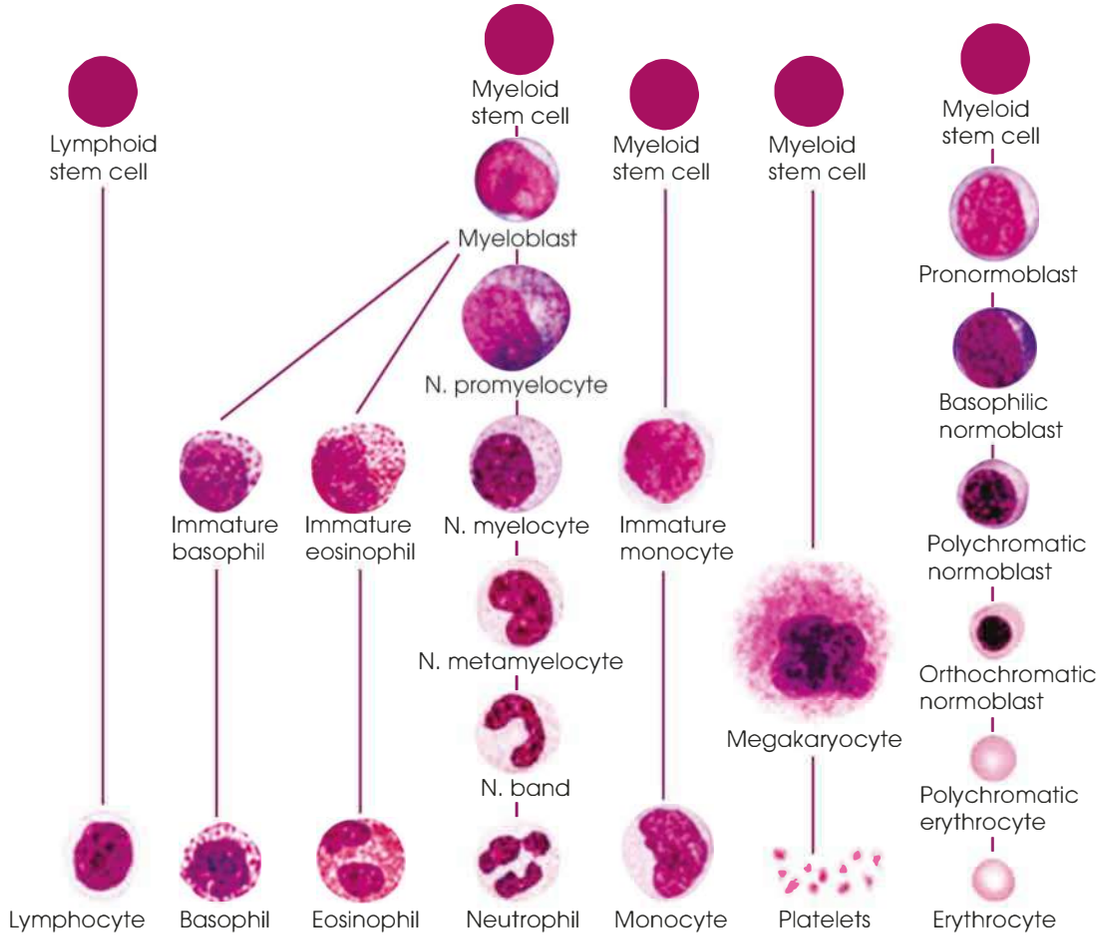

Fig. 11.2: Diagrammatic representation of genesis of erythrocyte, leucocytes and platelets

come mainly from the spleen and lymphatic glands and to some extent, from the bone marrow.

Precursors of Blood Cells

Stem Cells

The monophyletic theory which is accepted by haematologist holds that all the blood cells are derived from a common primitive ancestor, which is called the 'stem cell' or haemocytoblast. These stem cells self replicate and differentiate into progenitor cells.

Progenitor Cells

Myeloid progenitor cells differentiate into producing erythroid progenitor cell lines, granulocytes monocyte progenitor cell lines and megakaryocyte progenitor cell lines. The erythroid progenitor forms red blood cells, granulocytes monocyte progenitor forms white blood cells and megakaryocyte progenitor forms platelets.

Characteristic Features of Progenitor Cells

The progenitor cells form colony forming units: BFU-E: Burst forming unit-erythroid which forms colonies of erythroid series, CFU-E: Colony forming unit- erythroid which forms red blood cells; CFU-GEMM: Colony forming unit-granulocyte, erythroid, megakaryocyte and macrophages. These colony forming units are called multi-potent progenitor cells; Ba-CFU: Basophil-colony forming unit, Eo-CFU: Eosinophil-colony forming unit, M-CFU: Monocyte- colony forming unit and G-CFU: Granulocyte-colony forming unit.

Role of Cytokines in Haemopoiesis

The cytokines that are the colony stimulating factor stimulates formation of various types of blood cells: G-CSF stimulating granulocyte precursors, GM-CSF stimulating granulocytic and monocytic precursors, M-CSF stimulating monocyte precursors and interleukins I, II, III, V, etc. stimulating lymphocytic precursors.

The details of genesis of erythrocyte, leucocytes and platelets with the role of progenitor cells and cytokines; are detailed along with the chapters of RBC, WBC and platelets.

EXAM-ORIENTED QUESTIONS

Short Notes

1. Red bone marrow
2. Yellow bone marrow
3. Functions of bone marrow
4. Vascular arrangement of bone marrow
5. Staining and examining of bone marrow

Chapter

12

Red Blood Corpuscles (Erythrocytes)

INTRODUCTION

The mature human erythrocyte is a circular, biconcave, non-nucleated disc. The edges are rounded and thicker than the centre. Hence, the central portion appears to have a lighter shade. When viewed from the side it looks like a dumb-bell.

Features

1. In the early part of foetal life the mammalian red cells are all nucleated. But in the later part the nucleated cells disappear from the circulation.
2. The matured red cells are soft and flexible; and can readily squeeze through narrow capillaries.
3. Inside the corpuscles there is a frame work, chiefly composed of proteins and lipids. The meshes of this framework remain filled up with haemoglobin.
4. Under the microscope a single red cell seems to have a light brown or yellowish colour. But when seen in bulk the red cells appear to be red.
5. Histologically, no definite cell membrane has been demonstrated, but still, there seems to be a delicate outer envelope formed by the condensation of surface molecules.
6. The red blood cell membrane is composed of proteins, phosphatides and cholesterol. The inner and outer layers are made up of proteins and the middle layer of lipids.
7. The permeability of this membrane is highly selective. The bigger colloidal molecules as well as the cations (K^+, Na^+, etc.) are not allowed to pass. But certain crystalloids (urea, etc.) and the anions (Cl^-, HCO_3^-, etc.) are freely permeable.

COMPOSITION OF THE RED CELLS

Each cell is composed of a colourless envelope enclosing semiliquid material, 65% water and 35% solids of which 33% is haemoglobin bound to 2% stromal meshwork of protein, phospholipid, cholesterol, cholesterol esters and neutral fat. Other organic substances, such as urea, amino acids, creatinine, adenyl pyrophosphates diphosphoglycerates, etc. are also present, but in very small amounts. Of the total lipids, 60% is phospholipid (half of this is cephalin), 30% free cholesterol and 10% fats and cholesterol esters. Of the salts in the corpuscles, potassium phosphate is the chief (it should be noted that the chief salt of plasma is sodium chloride).

Normal Red Cell Count

The normal average count in adult male is taken as 5 million and in female 4.5 million per cubic millimetre. But most observers agree that the actual figures are a little higher, 5.4 million in males and 4.8 million in females. In infants, the count is 6 to 7 million, whereas in foetus 7.8 million. In the first ten days of the postnatal life large number of red cells; are destroyed (this is one cause of jaundice in the newborn).

VARIATIONS OF RED CELL COUNT UNDER VARIOUS PHYSIOLOGICAL CONDITIONS

1. **Diurnal variation:** Variations amounting to about 5% occur in twenty-four hours. The count is lowest during sleep, then gradually rises and becomes maximum in the evening.
2. **Muscular exercise:** Exercise raises the count temporarily.
3. **Altitude:** At higher altitude the count rises, whereas at lower altitude (i.e. high barometric pressure) the count falls.
4. **High external temperature:** Increases the red cell count.
5. Any condition which lowers the oxygen tension of arterial blood increases the red cell count.
6. **Injection of adrenaline and excitement** increase the count.

Size, Volume, Thickness, etc. of Red Blood Corpuscles

The diameter of the red cells when in the body varies from 5.5 to 8.8 µm and about one-third of this in thickness. But in the dried and fixed films (as done for clinical purposes) the cells shrinks to some extent; and has a mean diameter 7.2 µm (Fig. 12.1A and B). The average size of the red cells can be determined from a film preparation with the help of an instrument known as halometer.

It can also be measured directly under the microscope by an instrument known as micrometre. The average thickness of a red cell is about 2.2 µm (Fig. 12.1C), and the average volume is about 87 cubic microns.

Abnormal Forms of Erythrocytes

1. A variation in size is known as anisocytosis.
2. Red cells that are larger than normal are macrocytes, those smaller microcytes.
3. Deviation from normal shape is poikilocytosis.

Characteristic Features of RBC

1. The small size and the great number of the red cells are of considerable importance. This makes the available surface area very large and thereby facilitates rapid exchange of gases and other materials between the cells and the plasma. It is estimated that the total surface area of the red cells is about 1500 times greater than the surface area of the whole body. The total surface area of the red cells per litre of blood is about 600 sq metres.
2. The diameter and the volume of red cell increase when blood tends to become acid. Hence, increased CO_2 tension, anoxia, acidosis, etc. increase the volume and diameter of the red cells. Due to these reasons, the red cells in the venous blood are slightly larger than those in the arterial blood. Alkalosis produces opposite effects.
3. The absence of nucleus is of a great benefit. It gives the red cells, their biconcave shape and also makes room for more haemoglobin. The biconcave shape of the red cells is also of a great advantage for many reasons. For instance, (a) it allows considerable alteration of the cell volume without increasing the tension on the cell membrane. The concave part freely moves out and in as the volume increases or diminishes and in this way, can withstand considerable change of osmotic pressure and resist haemolysis.
4. In the venous blood about 7.5% increase of the cell volume occurs. This is due to the shift of the Cl ions (*vide* under chloride shift) into the cells, increasing the osmotic pressure and consequently drawing more water into the cells. (b) It allows easy 'folding' of the red cell when the latter passes through capillaries whose diameter is often narrower than its own. (c) Due to biconcave shape the haemoglobin remains distributed in a very thin layer. This facilitates quick saturation or desaturation with the gases.

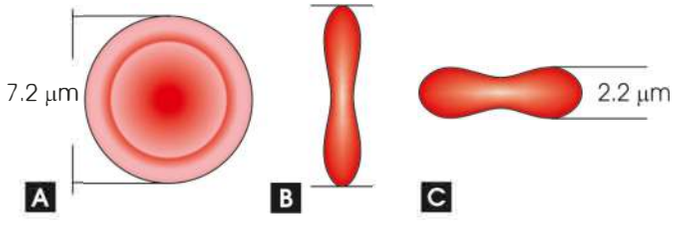

Fig. 12.1A to C: Red blood cell (erythrocyte) from a film preparation (schematic representation)

DEVELOPMENT OF RED BLOOD CORPUSCLES

Theories of Origin

There are two theories; intravascular and extravascular. Formerly, it was believed that the red cells were formed only intravascularly from the capillary endothelium. But Turnbull and Gilmour (1941) have shown that they are undoubtedly produced from the extravascular sources. They have shown that the parent cell is an extravascular cell, known as haemocytoblast which by active amoeboid movement burrows into the blood sinuses, multiply there and mature into normal erythrocytes.

The general trend of opinion seems to be in favour of the extravascular theory now.

STAGES OF BLOOD FORMATION IN THE EMBRYO AND FOETUS

Site of Development

In the embryo, the red cells develop from the area vasculosa of the yolk sac.

RBC development in foetal life

1. The mesodermal cells in this area remain as a mass of protoplasm with scattered nuclei.
2. Fluid droplets appear in it, and run together to form channels.
3. This fluid is the primitive plasma and those cells which line these channels become the vascular endothelium, from which the early red cells develop.
4. At first the cells are all nucleated. From the middle of foetal life the nucleated cells disappear from the peripheral circulation.

There are three successive stages of blood formation in the embryo and foetus:

1. ***Mesoblastic haemopoiesis*** is first demonstrable in the first two months of embryonic life. Throughout

this period, no blood forming organ is present and most other cells are formed outside the embryo. This stage is markedly diminished in a human embryo of nine weeks.

2. ***Hepatic haemopoiesis*** constitutes the second stage and includes the splenic and thymic blood formation. This stage occurs from the second to the fifth month.
3. The final or ***myeloid period of haemopoiesis*** begins approximately at the fifth month, with the establishment of the placental circulation. At first, the liver is chiefly occupied with erythropoiesis and the bone marrow leucopoiesis, but the bone marrow soon takes overall haemopoietic activity. The other sites however retain their haemopoietic potentialities throughout life.
4. After birth the bone marrow is the main site of erythrogenesis. During early years all bones are filled up with blood forming red marrow, but by twentieth year almost all the long bones are replaced with inactive yellow marrow and RBC formation in this location stops. Only the upper ends of femur and humerus contain red marrow and continue to form red cells throughout life. In addition to this, the vertebrae, the ribs and the flat bones produce red cells continuously.

Erythropoiesis = Arterial O_2 content, tissue O_2 tension

The most important factor controlling the rate of red cell production is the oxygen content of the arterial blood, a decrease in oxygen content stimulates erythropoiesis. The oxygen content of the blood may fall either due to diminution of the amount of haemoglobin content of blood or due to inadequate oxygenation of haemoglobin. Decrease in oxygen content in the arterial blood leads to decrease of oxygen tension in the tissue. During haemorrhage there is fall in circulating haemoglobin which leads to increased production of reticulocytes. In high altitude also there is increased red cell production.

The lowering of oxygen tension in the tissues has got no direct stimulating effect but acts through humoral mechanism. It is the erythrocyte-stimulating factor or erythropoietin or haemopoietin which stimulates erythropoiesis. Erythropoietin is a glycoprotein of low molecular weight. It is formed in the renal tissue probably due to the effect of adrenocorticotropical hormone (ACTH) or some other hormones of the anterior lobe of the pituitary.

Maturation and Multiplication

It should be noted that the phenomenon of development involves two distinct processes—one is multiplication and the other is maturation. By the latter process, the cell becomes specialised to perform that particular work for which it is meant.

Key Points

1. It should also be noted that, these two distinct multiplication and maturation are antagonistic attributes. They cannot go hand in hand in the same proportion, which mean maturation cannot take place when multiplication is actively proceeding and multiplication will cease in the same ratio as maturation is in progress.
2. In the case of red cells the process of maturation involves three different changes: First, a gradual reduction of cell size; secondly, the acquirement of haemoglobin; and thirdly, the disappearance of the nucleus. Of these three, again, haemoglobin formation seems to be the most important. For this reason it will be seen that as soon as haemoglobin begins to appear, cell division gradually ceases.

Stages of Development (Table 12.1)

The red blood cells are formed from the burst forming unit erythrocyte (BFU-E) and colony forming unit-erythrocyte (CFU-E) and these are derived from the progenitor cells. The cytokine erythropoietin stimulates the erythroid series of cells. The distinguished well defined erythroid series lineage form different stages of erythropoiesis (Fig. 12.2) which are as follows.

The stages are

Haemocytoblast: A big cell, 18–23 µm in diameter, with a large nucleus and a thin rim of deep basophilic cytoplasm (according to intravascular theory, this stage starts with endothelial cells. They are large, undifferentiated reticuloendothelial cells, lining the sinusoids of bone marrow. They proliferate and give rise to megaloblast).

Proerythroblast: 14–19 µm in diameter, basophilic cytoplasm, large nucleus with distinct nucleoli and a reticulum of fine chromatin threads. Haemoglobin absent. Actively multiplies into the next form, only in states of stress.

Table 12.1: Stages of development of erythrocytes

The stages of cells used by British school	*The stages of cells used by American school*
Proerythroblast	Megaloblast
↓	↓
Early normoblast	Early erythroblast
↓	↓
Intermediate normoblast	Late erythroblast
↓	↓
Late normoblast	Normoblast
↓	↓
Reticulocyte	Reticulocyte
↓	↓
Erythrocyte	Erythrocyte
A	B

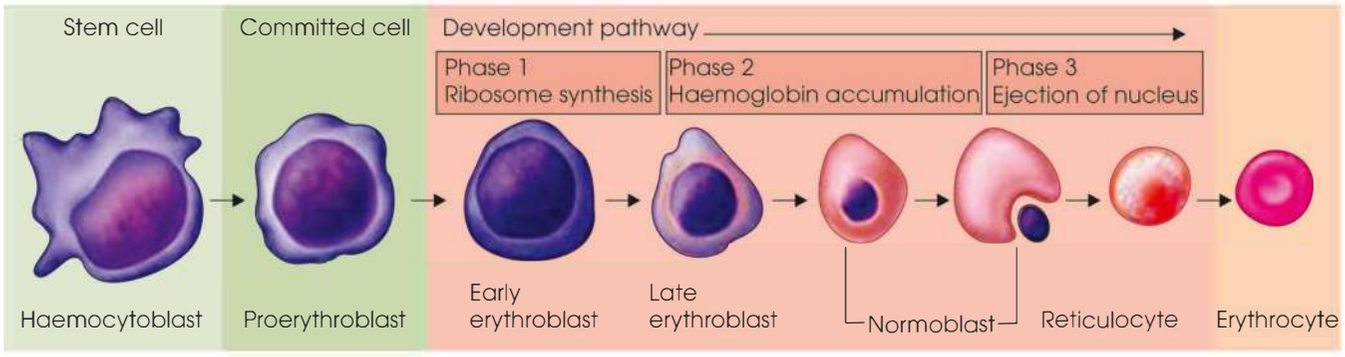

Fig. 12.2: Stages of erythropoiesis

Early normoblast: Smaller in size, 11–17 µm nucleus and chromatin are dense. Nucleoli are absent or rudimentary. Actively divides and passes into the next stage.

Intermediate normoblast: Size still smaller, l0–14 µm with fewer mitochondria. Nucleus more condensed, no nucleolus. Haemoglobin appears at this stage and consequently the cytoplasm becomes polychromatic. The later forms do not divide; they mature to form late normoblasts. The process of maturation involves the acquisition of more haemoglobin and a condensation of the nuclear chromatin.

Late normoblast: The size is more reduced; so that it is just a little larger (7–10 µm) than a mature red cell. The nucleus is very dense and takes a deep stain (pyknotic), looking like a drop of ink (ink-spot nucleus).

The amount of haemoglobin has increased. The further maturation of the normoblast involves the complete loss of the nucleus. There are two views; (a) nucleus undergoes fragmentation (karyolysis). The amount of haemoglobin increases at the expense of the nucleus, (b) nucleus is extruded from the cell as a whole. The factors that cause dissolution of the nucleus are not at all understood. The normoblast, after the loss of the nucleus, passes into the next stage.

Reticulocyte: When stained with vital stain (such as cresyl blue), these cells show a net-like structure (reticulum) in the cytoplasm. From the reticular appearance its name has been derived.

Key Points

1. In normal blood they are present to about 1%; in the newborn baby, about 30–50%. In the first week of life the number drops to 1%. It is from this stage that the red cells begin to appear in the peripheral circulation.
2. Their number increases when active regeneration of red cells takes place, for instance during recovery from anaemia. Under this condition even normoblasts may be found in the peripheral circulation.
3. Regarding the origin of the reticulum, it is held that they are the remnants of the original basophilic cytoplasm of the immature non-haemoglobinised red cells.
4. Instead of using vital stains, if ordinary Leishman's stain is used, the reticulocytes may appear in two other forms. They may either take a diffuse blue stain (polychromatophilia) or may display a number of discrete blue particles in the cytoplasm (punctate basophilia). The latter variety is especially prominent in cases of lead poisoning for no obvious reasons.
5. The reticulocyte matures into erythrocyte. It takes about 7 day's time to pass from the stage of proerythroblast (megaloblast) to that of reticulocyte and another two days from reticulocyte to mature erythrocyte.

FACTORS CONTROLLING ERYTHROPOIESIS

The red cells are constantly being destroyed and are regenerated. The rate of destruction and regeneration are same, otherwise, a constant red cell count would not be possible. These facts signify that some stimulus, exactly proportional to the number of red cells destroyed, is constantly acting upon bone marrow in order to replace the lost cells. The exact nature of the stimulus is not known.

Certain factors are necessary for the formation and maturation of red cells. They are as follows:

1. ***Diet:*** Food, rich in first class proteins (or proteins of high biological value), is important. First class proteins supply essential amino acids for the synthesis of globin of haemoglobin. It is also necessary for the formation of stromaproteins and the nucleoproteins of the red cells.
2. ***Anoxia and erythropoietin or erythrocyte-stimulating factor (ESP):*** The stimulus becomes more effective or is supplemented when there is low O_2 tension in the tissues. When air with low oxygen tension (as in high altitude) is breathed for some length of time, the red cell count rises due to liberation of erythropoietin or haemopoietin or erythrocyte-stimulating factor (ESF). It stimulates the bone

marrow and increases the rate and maturation of red cell formation.

3. ***Stimulus for maturation:*** It is now generally agreed that as the red cells mature, various factors influence the passage of the maturing red cell from stage to stage. Our knowledge in this respect though far from complete, may be briefly summarized as follows:
   a. *Haemocytoblast:* Nothing definite is known as to the factors that come into operation in this stage. In certain diseases this stage fails to occur. Red cell formation stops and the results are known as aplastic anaemia.
   b. *Proerythroblast:* Haematinic principle of Castle (haemopoietic principle or PA factor) vitamin B_{12} (extrinsic factors) and folic acid are required for the conversion of proerythroblast (megaloblast) into early normoblast (erythroblast). For the proper absorption of extrinsic factor, intrinsic factor present in gastric mucosa is essential.
   c. *Early normoblast:* A number of factors is influence this process.

Metals

a. **Iron:** Essential for haemoglobin formation especially for synthesis of haem. Dietary intake of iron is required for the formation of haemoglobin. Deficiency of iron in the diet leads to hypochromic or iron deficiency anaemia.
b. **Copper and manganese:** Help in the conversion of iron into haemoglobin by catalytic action.
c. **Cobalt:** As a component of vitamin B_{12}. It is of proved value in man and lower animals. The nature of action probably same as Mn and Cu.
d. **Calcium:** Helps indirectly by conserving more iron and its subsequent assimilation.
e. **Bile salts:** Presence of bile salts in the intestine is essential for the proper absorption of these metals.

Endocrine glands

a. Thyroxine is of undoubted value. Hypothyroidism is associated with hypochromic, macrocytic anaemia due to lowered metabolic activity in bone marrow.
b. Adrenal cortex: Adrenocortical insufficiency is often associated with anaemia; polycythemia might be present in Cushing's syndrome. The changes are possibly due to general metabolic alterations and not due to direct effect on bone marrow.

Vitamins: Vitamins C, B_6, and B_{12}, folic acid, riboflavin, pantothenic acid and nicotinic acid are all important.

Pigments

a. Bile pigments.
b. Chlorophyll and other porphyrins: Deficiency of these factors will give rise to less haemoglobin formation and therefore hypochromic anaemia. Their mode of action is unknown.
c. *Late normoblast:* The same factors that operate in the previous stage are also acting here. But the exact nature of the forces that lead to the disappearance of the nucleus is unknown.
d. *Erythrocyte:* The normal mature red cell.

ENERGY METABOLISM OF RBC

Mature red cells lack nucleus, DNA, RNA and mitochondria. These cells are not capable of synthesising haemoglobin. Krebs' cycle is absent. But nucleated RBC of bone marrow can be compared with other nucleated tissue cells of the body in respect of its metabolic processes.

Key Points

1. Mature red cells contain no glycogen and for metabolic processes, it has to depend upon plasma glucose that has constant access through erythrocyte membrane. The exact mechanism of transport of glucose through the membrane is not clearly known. But most of the investigators are of opinion that this is happened mostly through active transport mechanism.
2. As Krebs' cycle (TGA cycle) is absent in mature RBC (non-nucleated), the metabolic breakdown of glucose takes place through (a) Embden-Meyerhof glycolytic pathway (*vide* metabolism) and (b) pentose phosphate pathway or hexose monophosphate shunt (*vide* metabolism). Thus, the energy requirement of the RBC is obtained from the above two metabolic processes.
3. The longevity of red cells depends upon the maintenance of these energy-producing metabolic processes. The energy is required for the active transport mechanisms of the RBC. During active transport, Na is pumped out of the RBC and K is pumped in. Besides this, structural integrity and the transport of glucose depends upon the availability of ATP.

RBC Lifespan and Destruction

Normal lifespan of RBC is about 120 days. In young RBC the enzymes concerned with the metabolic pathway of glucose breakdown are present in a large amount. With the aging processes of the RBC, level of certain enzymes is decreased.

- Hexokinase, which acts in the first step of glucose metabolism, is decreased in amount during aging process.
- Glucose-6-phosphate dehydrogenase, which catalyses the first step of hexose monophosphate shunt is decreased in amount considerably during ageing.
- Other enzymes which take part in different metabolic processes of RBC are also decreased considerably.

With the alteration of glucose metabolism, the ATP source of the RBC is decreased. Due to reduction of available ATP, the structural integrity of the RBC is lost, transport mechanisms are disrupted and ultimately destruction of the cells occurs.

Span of Life

The average span of life of a mature red blood corpuscles were formerly believed to be about 3–4 weeks. But recent experiments (using radioactive Fe, or glycine labelled with isotopic 15N, which enters into the composition of haemoglobin) indicate that it is about 120 days in man.

Fate of the Red Blood Corpuscles

As the cells grow senile they change their shape and size and become more brittle. At first the cells throw out processes like pseudopodia and become flask-shaped. These are called poikilocytes. These processes are broken off and in this way the RBC disintegrates.

Degradation

1. This fragmentation takes place in the circulation and the fragments are swallowed up by the RE cells. The RE cells of the spleen, liver, etc. can also engulf the senile red cells as a whole and break them down intracellularly.
2. Haemoglobin is released and by degradation opening of the porphyrin ring system occurs. The degraded compound is known as verdohaemoglobin or choleglobin where the four pyrrole nuclei form a chain instead of a ring.
3. In the next stage it is broken down into protein and haem. Protein is broken down into amino acids. The iron present in the haem is stored in the body as ferritin and haemosiderin which help in the formation of new haemoglobin. The rest of the haem molecule is converted into a yellow pigment bilirubin which is oxidised into a green pigment biliverdin or according to some, biliverdin is first formed and which by reduction forms bilirubin.
4. Bilirubin and biliverdin probably combines with α_2-globulin and circulate in the bloodstream and enters the liver, and in the liver cells bilirubin and biliverdin are separated from globulin and conjugated with uridine diphosphate glucuronate to produce bilirubin monoglucuronide and bilirubin diglucuronide (cholebilirubin), the uridine diphosphate is set-free.
5. These compounds enter the duodenum through the bile duct and then into the intestine. In the large intestine by bacterial action they are changed into stercobilinogen (urobilinogen). Some urobilinogen is reabsorbed and excreted in the urine as urobilinogen. The rest is excreted in the faeces as stercobilinogen and stercobilin which are responsible for the brown colour of the stool (Fig. 12.3).

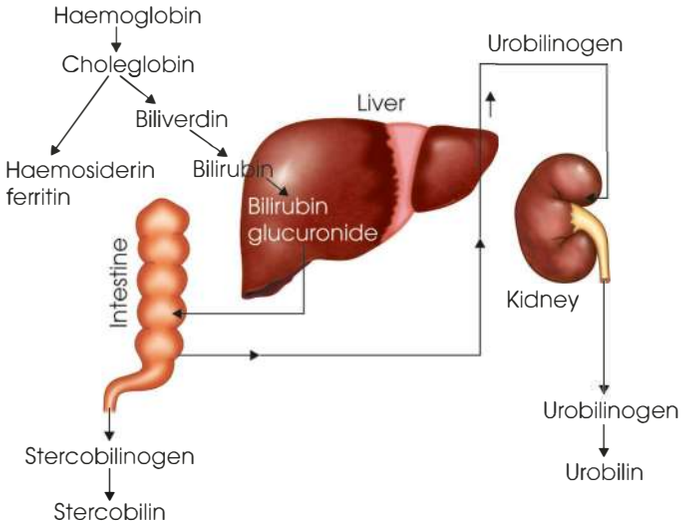

Fig. 12.3: Fate of red blood corpuscles and haemoglobin

Functions of Red Blood Corpuscles

1. ***Respiratory:*** Red cells carry oxygen and carbon dioxide.
2. ***Acid–base balance:*** They help to maintain acid–base balance. It is carried out by the buffering action of haemoglobin and other intracellular buffers.
3. ***Red cells maintain ion balance:*** By the special permeability of the cell membrane, the red cells help to maintain balance of positive and negative ions in the blood.
4. ***Viscosity of blood:*** Red cells help to maintain the viscosity of blood.
5. ***Pigments:*** Various pigments are derived from haemoglobin after the disintegration of the red cells, e.g. bilirubin, biliverdin, etc.

EXAM-ORIENTED QUESTIONS

Essay

1. Describe the stages of erythropoiesis with well labelled diagram.
2. Discuss the factors affecting erythropoiesis. Add note on functions of RBC.

Short Notes

1. Genesis of red blood cell
2. Enlist causes for physiological variation of red blood cell count
3. Energy metabolism of red blood cell
4. Fate of red blood cell

Chapter

13

Haemoglobin

INTRODUCTION

Chemistry

Haemoglobin is the red pigment of blood. It is a chromoprotein consisting of two parts: One part (96%) is a specific simple protein known as globin (histone) and the other (4%) is a non-specific prosthetic group—an iron-containing pigment called haem (Fig. 13.1A). Haem is a protoporphyrin compound and consists essentially of four pyrrole groups joined together. The porphyrin molecule can combine with metals forming metalloporphyrin compounds. Haem is a metalloporphyrin where the metal is iron. The iron content of haemoglobin is about 0.34% and about 3 g iron is present as haemoglobin in the total amount of blood of an adult. Iron remains in ferrous (Fe^{++}) form. Globin helps haem to keep the iron in ferrous state and to combine loosely and reversibly with molecular oxygen.

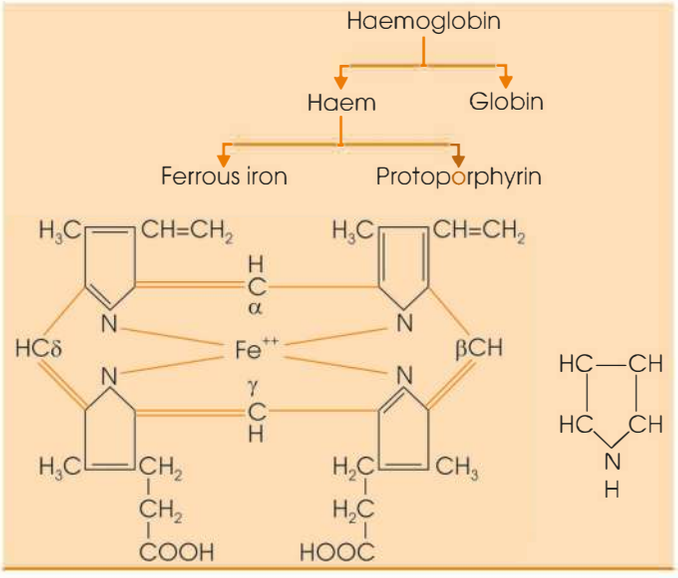

Fig. 13.1A: Composition of haemoglobin

Structure of Haemoglobin (Fig. 13.1B)

- The adult haemoglobin in normal individuals contains two α-chains and two β-chains. Each haemoglobin molecule has a haem prosthetic group which contains an atom of iron. Each molecule of globin consists of four polypeptide chains to each of which is attached a molecule of haem with Fe^{2+} capable of combining with one molecule of oxygen, thus each haemoglobin molecule has the capacity to carry four oxygen molecules.
- As soon as one of the molecules of haem combines with O_2, the other three molecules in the same polypeptide complex of the globin acquires a great affinity for O_2 and rapidly combines with it. This rapid oxygenation of haemoglobin in the initial phase of its exposure to O_2 is known as allosteric activation and explains the sharp rise in the dissociation curve in the lower range of O_2 tension and also the gradual levelling off of the curve at higher range as the haemoglobin reaches the near saturation point.

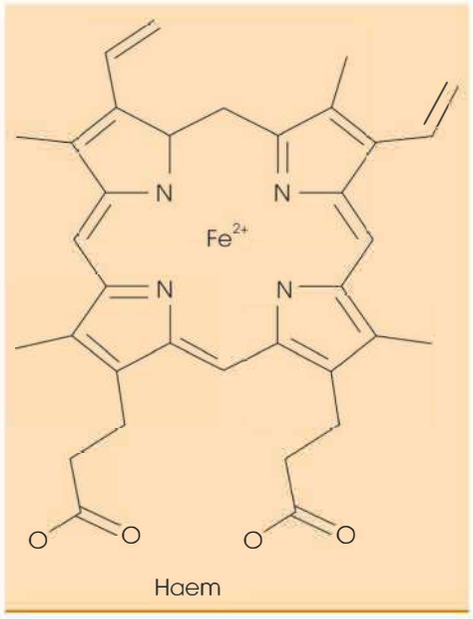

Fig. 13.1B: Structure of haemoglobin molecule

Max Ferdinand Perutz was an Austrian-born British molecular biologist, who shared the Nobel Prize for Chemistry with John Kendrew for their studies of the structures of haemoglobin and myoglobin in 1962.

Max Ferdinand Perutz
1914–2002

Properties

Key Points

1. **Oxygen association:** The most characteristic property of haemoglobin is the ease with which it combines with oxygen and dissociates from it. 100 ml of water will absorb one-third of oxygen at body temperature under atmospheric pressure. But 100 ml of blood, under the same condition, will take up 20 ml of oxygen (60 times), due to the presence of haemoglobin (about 1,200 ml of oxygen can be carried by the total amount of blood in an adult man). One gram of haemoglobin combines at normal (standard) temperature pressure (NTP) with 1.34 ml of oxygen. This corresponds to two atoms of oxygen for each atom of iron. The compound oxyhaemoglobin gives off its full oxygen content when placed in vacuum.
2. Oxyhaemoglobin holds its oxygen loosely which can be easily displaced by many other gases forming more stable compounds, e.g. CO, NO, H_2S will form carboxyhaemoglobin (carbon monoxyhaemoglobin), nitric oxide haemoglobin, sulphaemoglobin respectively.
3. The globin part of haemoglobin directly combines with CO_2 and forms carbaminocompounds.
4. **Crystallisation:** Haemoglobin can be easily crystallised. The form of the crystals, their solubility and the ease of crystallisation are characteristic of the species from which haemoglobin is obtained. Most bloods, including human blood, form rhombic prisms or needles. Haemoglobins of different species are said to be immunologically distinct. The distinction lies in the globin part of the molecule and not in the haem part. It is known that the amino acid composition of the various globins (derived from haemoglobin of different species) varies considerably specially in respect to their cystine content.
5. The haemoglobin of different species also shows different affinity for oxygen.
6. Isoelectric pH of haemoglobin (reduced Hb) is 6.8, that of oxyhaemoglobin is 6.6.

Spectroscopic appearance: Haemoglobin (reduced Hb) gives one broadband between the Fraunhofer's lines D and E (corresponding to the wavelength of γ559). That of oxyhaemoglobin consists of two bands between D and E. The band nearer D is called α-band (corresponding to the wavelength of γ579). The band nearer E is broader and is called the β-band (with a corresponding wavelength of γ542).

HAEMOGLOBIN VARIETIES

In man probably there are at least two varieties of haemoglobin, the foetal haemoglobin (HbF) and the adult haemoglobin (HbA). Foetal haemoglobin differs chemically and spectroscopically from the adult haemoglobin. It has a greater affinity for oxygen and releases CO_2 more readily. This is due to some difference in the globin fraction. This property helps to compensate the relative anoxia of foetal blood. At low O_2 pressure foetal haemoglobin can take up larger volumes of O_2 than adult haemoglobin.

Advantage of foetal haemoglobin: It is 70% saturated at 20 mm of O_2, pressure whereas adult haemoglobin is only 20% saturated at this pressure. A small quantity of foetal haemoglobin persists for some weeks or months after birth.

Thus, to summarize the well known varieties of normal human which include Hb are HbA1 (two α-chains and two β-chains), HbA_2 (two α-chains and two δ-chains), HbF (two α-chains and two γ-chains), embryonic Hb (two zeta [$\zeta_2 \in_2$] and two epsilon chains, HbA1C (glycosylated Hb), etc.

The Form in which Haemoglobin Exists in Cells

The actual state in which haemoglobin exists in the red cells is not yet fully understood. The quantity of haemoglobin in the red cells is too great for it to be in simple solution and it is also known that it is not present in crystalline form. From these observations it has been suggested that haemoglobin remains in some combined form. It is believed that haemoglobin remains absorbed to the lipid material of the stroma and the envelope of red cells. In certain lower invertebrates, haemoglobin remains free in the plasma. Its inclusion in the red cells has taken place gradually in the course of evolution.

CAUSES OF INCLUSION OF THE HAEMOGLOBIN IN RED CELLS

Though haemoglobin is protein having its molecular weight 68,000 yet it passes through the endothelial lining of the blood vascular system as well as through the normal glomerular membrane.

Haemoglobinuria is the condition when free haemoglobin is excreted through the urine. If Hb is free in the plasma then it is excreted through the urine. In plasma, Hb is present normally bound to protein at a concentration of about 5 mg/100 ml of whole blood.

- In plasma Hb remains normally as haemoglobin–heptoglobin complex which cannot pass through the

urine. Under normal condition about l00 to 150 mg of free Hb is released from red cells due to haemolysis. Hb can be bound as haemoglobin–heptoglobin complex by the heptoglobin present in the plasma. lf released Hb exceeds the capacity of the heptoglobin to bind the haemoglobin, then it is excreted through the urine.

- The free Hb passes readily through the glomerulus and of which certain amount is reabsorbed and rest is excreted through the urine. During reabsorption of Hb through the tubular epithelium certain amount of it, is converted into haemosiderin and excreted in the urine. So plasma Hb concentration is always associated with haemosiderinuria though the condition of haemoglobinuria may or may not be happened. Thus, if the Hb was not enclosed by the cell membrane of erythrocytes then this Hb would have been passed quickly through glomerular membrane and excreted through the urine. Because RBC cannot pass through the glomerular membrane in normal condition.

Haemoglobinuria may be caused under the following conditions

1. In strenuous exercise.
2. Due to mismatched blood transfusion.
3. *Black water fever* due to virulent type of malaria and *red water fever* due to another type of parasite which invades the erythrocyte causing release of Hb in the plasma.
4. Paroxysmal nocturnal haemoglobinuria
5. Hypotonicity of plasma
6. Thermal or chemical injuries
7. Paroxysmal cold haemoglobinuria

In transport of CO_2 from the tissues to the lungs the erythrocyte with its haemoglobin plays an important part, because the enzyme carbonic anhydrase is present within the erythrocyte. If haemoglobin instead of staying within the corpuscles, remains free in the plasma (as occurs in haemolysis); many injurious effects will be produced. The degree of the deleterious effects will depend upon the number of red cells haemolysed and the rapidity of haemolysis. Briefly the following ill effects will be produced:

1. Viscosity of blood will rise.
2. The colloidal osmotic pressure, normally about 30, will rise to 100 mm of Hg or more. This will seriously disturb interchange of various substances in the capillary area and will also disturb the formation of urine.
3. Loss of haemoglobin will reduce the amount of blood buffers and will cause acidosis. This is all the more enhanced by the disintegration of red cells, the loss of available surface area of the erythrocytes, which plays a considerable role in maintaining the acid–base balance and the ion balance in blood.
4. Loss of haemoglobin will reduce the oxygen-carrying capacity of blood, thus producing anoxia and acidosis.
5. Bile pigments will be produced in larger amounts by the RE cells from the released haemoglobin and in this way additional pressure will be put upon the liver to deal with them.
6. While being excreted through kidneys, haemoglobin will be precipitated in the acid urine and in this way the kidney tubules will be blocked. This will cause serious disturbance of the kidney function. As a delayed result, hypochromic anaemia will be produced.

SYNTHESIS OF HAEMOGLOBIN

Haemoglobin is synthesised inside the red cells in the bone marrow. *A number of factors is necessary* for the synthesis of haemoglobin. They are as follows:

1. ***First class proteins*** (or proteins of high biological value): It is necessary for the synthesis of the globin part of haemoglobin. Certain individual amino acids, such as histidine, phenyl alanine, leucine, etc. have been found to possess a special stimulating action on haemoglobin formation. A diet containing kidney, spleen, heart and certain fruits are very helpful. Four kinds of globin peptide chains—α, β, γ and δ have been isolated. Human haemoglobin contains two α and two β-chains. The haem portions are attached to the α- and β-chains.
2. ***Metals***
   - *Iron:* It is an essential constituent of haemoglobin. Daily intake of 12 mg is adequate.
   - *Copper, manganese and cobalt:* These metals, particularly copper, help in the incorporation of iron in the protoporphyrin molecules for the formation of metalloporphyrin. The ratio between Cu:Fe in the daily diet should be 1:100. Cobalt has a definite value as a constituent of vitamin B_{12}. They act as catalytic agents.
3. ***Endocrine:*** Of the endocrines, only thyroxine is of proved value.
4. ***Vitamins:*** Vitamins C and B_{12} are definitely helpful in this respect. It is also believed that folic acid, riboflavin, nicotinic acid, pantothenic acid and pyridoxine also play some parts in the formation of haemoglobin.
5. ***Porphyrins:*** Of the two types of porphyrins, I and III that are found in nature, the latter is utilised for haemoglobin formation. Studies with radioactive carbon show that protoporphyrin III, required for this purpose, is synthesised in the animal body from simpler substances like glycine, acetic acid, aceto-acetic acid, succinic acid or any amino acid that can give rise to the formation of succinic acid during metabolism or through tricarboxylic acid cycle.

- Glycine and succinate help in the synthesis of protoporphyrin.
- Aminolevulinic acid (ALA) is formed by the interaction of succinate and glycine. Two molecules of aminolevulinic acid after condensation forms porphobilinogen (PBG).
- From porphobilinogen ultimately uroporphyrinogen III formed. On decarboxylation, the uroporphyrinogen III is converted into coproporphyrinogen III and which on oxidation ultimately gives rise to protoporphyrin III.
- Protoporphyrin III in presence of globin and Fe^{++} is converted into haemoglobin. The steps involved in the synthesis of Hb (Hgb) can be presented schematically in Fig. 13.2.

AMOUNT OF HAEMOGLOBIN IN NORMAL BLOOD

The average adult figure, irrespective of sex is 14.5 g%. Different observers give different figures for haemoglobin. For instance: Sahli: 17.3%, Haldane-14.8%, Gower: 15%, and Hellige: 14% (the best method of expressing the amount of haemoglobin in blood is to mention it in absolute figure, viz. as so many grams in 100 ml of blood. If it is expressed in percentage, the scale used should be mentioned).

METHODS OF ESTIMATION OF HAEMOGLOBIN

1. ***Tallqvist's method:*** A drop of blood from the patient's finger is soaked in a piece of filter paper and compared against a standard colour scale, before the blood on the filter paper dries up. This method does not give accurate results.
2. ***Haldane's haemoglobinometer*** (Haldane's modification of Gower's method): The instrument consists of two tubes, one of which contains 20 cu mm of blood haemolysed with distilled water and saturated with CO gas. The colour of this tube is used as standard. In the other tube a little distilled water is taken and 20 cu mm of patient's blood, collected from the finger tip by a special pipette, is added. When blood becomes fully haemolysed, it is saturated with CO by passing coal gas through it. The colour developed is compared against that of the standard. If the colour of the unknown is stronger, it is diluted with distilled water until the tinge is same in both. The graduation up to which the blood has been diluted gives the percentage haemoglobin.
3. ***Gower's haemoglobinometer:*** Here, the standard used is a solution of picrocarmine gelatin. Otherwise the method is same as Haldane. The disadvantage of this method is that the colour of the standard gradually fades away.
4. ***Sahli's method:*** Here, instead of distilled water (N/10) HCl is used. This converts haemoglobin into acid haematin. The colour developed is matched against that of standard and the result is obtained as in Haldane's method.
5. ***Von Fleischl's haemometer:*** Here, the standard used consists of a set of coloured glasses.
6. ***Colorimetric method:*** Here, the comparison is done with the help of a colorimeter.
7. For very accurate work the method of Van Slyke and Stadie should be used. In this method iron of

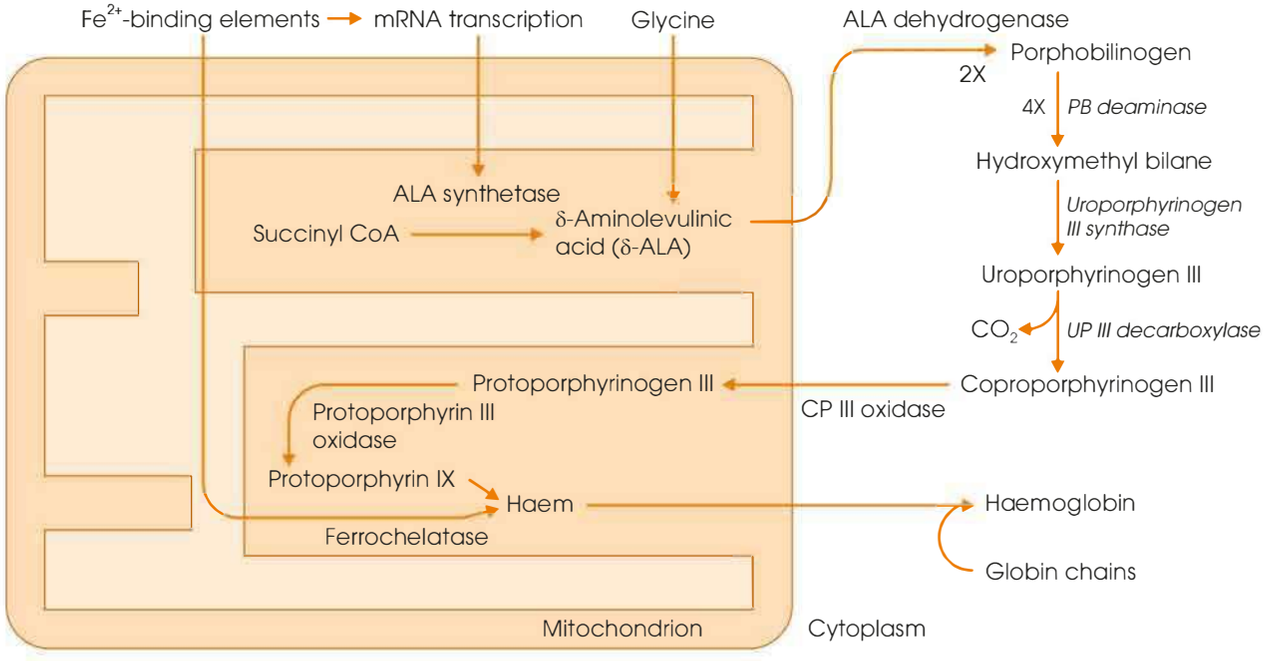

Fig. 13.2: Synthesis of haemoglobin

haemoglobin is estimated and from that the corresponding amount of haemoglobin is calculated. The photoelectric methods of Haliday, Kerridge and Smith (1935) may be adopted.

8. ***Spectrophotometric method:*** It is the modern method and is based as the measurement of absorption of light at certain wavelengths of cyanmethaemoglobin formed by treating the haemoglobin with ferricyanide and then with KCN.

VARIATIONS OF HB UNDER DIFFERENT PHYSIOLOGICAL CONDITIONS

1. ***Age:*** In the foetus, the concentration is highest. At birth, the average concentration is about 23 g per 100 ml. By the end of third month it falls below normal, probably, because of deficiency of iron in milk. After this gradual recovery takes place and at the end of the first year, the average amount is l2.5 g. Then it rises gradually up to normal figures.
2. ***Sex:*** In females, the amount of haemoglobin is slightly lower than in males. In adult females, the average is 13.7%, in adult males the average is 15.8%.
3. ***Diurnal variation:*** Variation of at least 10% occurs throughout the day. In the morning it is lowest; in the evening it is highest.
4. ***Altitude:*** At higher altitude haemoglobin percentage rises.
5. ***Exercise, excitement, adrenaline injection,*** etc. increase the amount of haemoglobin.
6. It should be noted from the above that normal variation of haemoglobin is mostly due to alteration of number of red cells and not due to any change in the absolute quantity of haemoglobin in each cell. Anything that alters the red cell count will alter the percentage of Hb proportionally.

Functions of haemoglobin

1. It is essential for oxygen carriage.
2. It plays an important part in CO_2 transport.
3. It constitutes one of the important buffers of blood and helps to maintain its acid–base balance.
4. Various pigments of bile, stool, urine, etc. are formed from it.

Derivatives of Haemoglobin

Compound

Oxyhaemoglobin: It is a compound of haemoglobin with oxygen. Iron remains in the ferrous (Fe^{++}) state in haemoglobin. It is not a stable compound. Oxygen may be removed when the blood is exposed to a vacuum. It has got two absorption bands between D and E.

Methaemoglobin: It is also a compound of haemoglobin with oxygen. It can be produced after treating the blood with potassium ferricyanide. It is chocolate-brown in colour. It is a stable compound. Oxygen cannot be removed by exposing the blood to a vacuum. Iron remains in the ferric (Fe^{+++}) state. It has got one absorption bands between C and D.

Carbohaemoglobin: It is a compound of haemoglobin with CO_2. The compound is formed by union of CO_2 with the globin portion.

Carboxyhaemoglobin or carbon monoxyhaemoglobin: Haemoglobin combined with CO instead of oxygen. It is present in blood in coal gas poisoning. It has got two absorption bands between D and E. The affinity of human haemoglobin at 38°C, for CO is 210 times greater than O, the extremely poisonous.

Sulphaemoglobin: It is formed by the combination of haemoglobin with H2S. The compound is very stable and is sometimes found in the blood after certain kinds of drug poisoning.

Nitric oxide haemoglobin: Haemoglobin combined with NO instead of oxygen, found in nitric oxide poisoning.

Derived Product

Iron Containing

Haematin: This derivative can exist in two forms—acid and alkaline and may be prepared from haemoglobin by the action of acid or alkali. This is sometimes found in the urine in old cases of haemorrhage. It is a ferric compound. Acid haematin has got an absorption band between C and D. The absorption band of alkaline haematin is near D line.

Haemin: Haemin is haematin hydrochloride. It is prepared by boiling oxyhaemoglobin with NaCl and glacial acetic acid. It is a ferric compound.

Haemochromogen: When alkaline haematin is reduced by ammonium sulphide, this derivative is obtained. It is a ferrous compound. Haem with ferrous iron is combined with denatured globin. Of all the haemoglobin derivatives, haemochromogen possesses the most characteristic spectrum. It has a very distinct band between D and E, as well as a fainter band between E and B lines. Due to this property this compound is often used to identify doubtful blood stains.

Cathaemoglobin: It is a compound of haem containing ferric iron with denatured globin.

Haem: It is a ferrous compound produced by the reduction of haematin in alkaline solution.

Iron Containing

Haematoporphyrin: This derivative can exist in two forms—acid and alkaline. It is prepared by mixing blood with sulphonic acid. When mixed with alkali the alkaline variety is formed. Normal urine may contain traces. It is found in the blood and urine in sulphonal poisoning and in certain cases of liver disease.

Haemopyrrole: When haematoporphyrin is reduced, this compound is formed. It is probably a mixture of several pyrrole compounds.

Haematodin: This compound is produced by the breakdown of haemoglobin in the body. It is found as yellowish-red crystals in the region of old blood extravasations. Some authors believe that it is identical with bilirubin.

Bilirubin: It is the chief pigment of bile and is produced from haemoglobin in the whole of the reticulo-endothelial system. From it are derived all the other bile pigments, the pigment of the stool, stercobilin; the pigments of the urine, such as urobilinogen, urobilin and urochrome.

EXAM-ORIENTED QUESTIONS

Essay

1. Discuss the steps involved in synthesis of haemoglobin. Describe the factors affecting haemoglobin synthesis.
2. What are the types of haemoglobin? What are the various methods of estimation of haemoglobin? What are the functions of haemoglobin?

Short Notes

1. Normal values of haemoglobin in males and females
2. Causes of variations of Hb count in physiological conditions
3. Types of haemoglobin and advantages of foetal haemoglobin
4. Haemoglobinuria
5. Derivatives of haemoglobin

Chapter

14

Iron Absorption, Transport, Storage and Excretion

INTRODUCTION

Iron: Sources: *All animal food*, e.g. meat, liver, egg, etc. excepting milk and butter, *vegetables*, e.g. peas, lentils, green leaves, fruits.

Daily Requirement

The daily requirement is 15–20 mg, per day and is generally enough in the normal diet. Pregnant and lactating females should have more. Milk being deficient in iron, the infant may develop anaemia. The foetal liver contains a large store of iron which is used up in the first three months. After third month, infants should have added amounts.

Distribution of Iron

Iron is distributed in the body (a) as iron porphyrins in haemoglobin, myoglobin, and also (b) as iron enzymes in catalase, cytochrome and peroxidase. Besides these, the iron is also present as non-iron porphyrins in transferrin, ferritin and haemosiderin.

ABSORPTION AND TRANSPORT

Iron is absorbed mostly from the whole of the gastro-intestinal tract but a large amount is absorbed from the upper part of the small intestine particularly the duodenum (Fig. 14.1).

Key Points

1. Dietary iron is absorbed through the mucosal cells as ferrous (Fe^{++}) form. Iron in diet is mostly present as ferric (Fe^{+++}) state which is reduced to ferrous form during absorption. Vitamin C, glutathione and amino acid—SH groups help in reduction of ferric to ferrous form.
2. After entering the mucosal cell as ferrous form, the iron molecules are rapidly reconverted into ferric state. The ferric iron as ferric hydroxide phosphate combines with a protein, *apoferritin* of the mucosal cells with the formation of iron–phosphorus protein complex, ferritin. This *ferritin* is one of the storage forms of iron in the tissue.
3. From the mucosal cell the ferritin iron passes into the blood. At first the ferritin iron is reduced into ferrous form and as such enters the blood stream. Here vitamin C also helps in transformation of ferric to ferrous form. However, the ferrous iron after entering the blood stream is re-oxidised into ferric form and combines with beta globulin apotransferrin to form transferrin.
4. It has been described by Osaki and others (1966) that ceruloplasmin (copper-binding protein) of the plasma also exerts a catalytic activity in plasma to convert Fe^{++} to Fe^{+++} form and thus incorporation of iron in the plasma transferrin is hastened.
5. Transferrin iron complex is the transport form of iron of the plasma and is carried to the myeloid tissue, liver, spleen, lymph node and other tissues of the body. Transferrin is thus the iron binding protein of the plasma and it shuttles iron atoms between tissues without itself being utilised appreciably. The transferrin can bind two atoms of Fe^{+++} per molecule of protein to form the red-coloured ferric protein complex.
6. Normal protein bound iron (PBI) in the plasma of males is approximately 120–140 µg%, and that of in females is 90–120 µg%. The total iron-binding capacity (TIBC) is about 300 to 360 µg per 100 ml in both sexes.
7. In the capillary blood vessels, the Fe^{+++} of transferrin passes through the peripheral capillary wall directly into the tissue spaces. But from the tissue spaces, the iron enters the tissues as ferrous (Fe^{++}) state and is stored as ferritin (Fe^{+++}) state (Fig. 14.1). If the parenteral administration of iron exceeds the capability of the tissue to store as ferritin, then the excess is stored as haemosiderin.

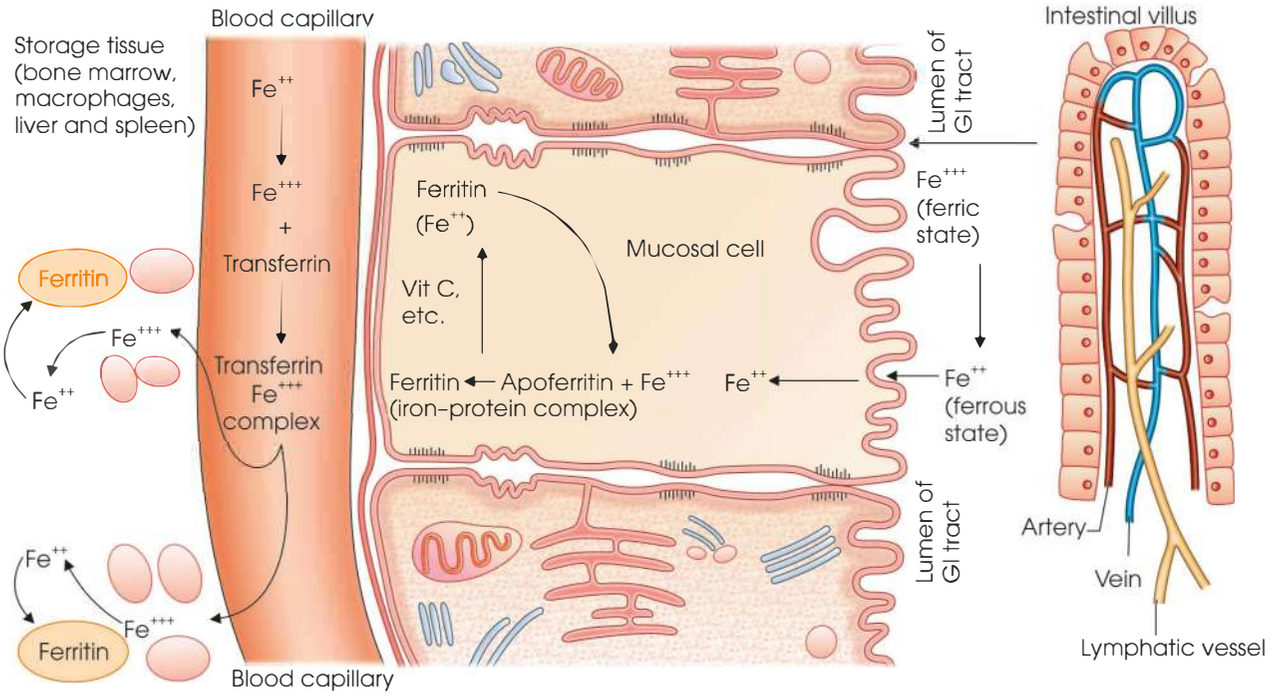

Fig. 14.1: Pathway of absorption and transport of iron (diagrammatic representation)

ABSORPTION OF IRON

Absorption of iron depends upon the following factors:

1. **Iron requirement of the subject:** In some unknown way, the degree of immediate need of the body for iron determines the rate and amount of absorption from the small intestine.
   - Miller (1954) suggests that iron absorption is under the control of a regulatory mechanism geared to the erythron need rather than to the reserve stores.
   - Other workers suggest that reserve iron stores control iron absorption via the mucosal cell through a low oxygen supply induced by low Hb in the blood stream.
   - Iron absorption is increased during growth, menstruation, pregnancy and in blood disorders. Absorption is increased markedly when the iron needs are acute as in haemorrhage or in anaemias.
2. **Form of the compound:** It is said that iron is best absorbed in ferrous (Fe^{++}) form. Most of the iron taken with food is in ferric (Fe^{+++}) form. They are at least partly converted into ferrous compounds before absorption. Organic iron of food is much less available for absorption than the inorganic. Insoluble forms are not absorbed.
3. **Reaction of the gastrointestinal contents:** The acidity of the gastric juice helps absorption. The gastric HCl helps the liberation of iron from the organic compounds in diet. Reduction from ferric form to ferrous one takes place in stomach with the help of gastric secretions. Partial gastrectomy often leads to iron deficiency anaemia.
4. **Pigments:** Absorption of iron is increased by chlorophyll and bile pigments. It is believed by certain workers that bile or gastric HCl is not needed for the absorption of inorganic iron salts.
5. **Calcium and vitamin C:** A small amount of Ca^{2+} decreases the formation of insoluble iron phosphates and thus helps absorption, but large amounts of Ca^{2+} inhibit iron assimilation. Vitamin C increases the absorption of iron from foods, possibly by reducing the ferric iron to the ferrous state.

Absorption is retarded by excessive mucus, administration of alkalis or low gastric acidity.

Time Taken for Absorption

The rate of absorption of iron is determined by the iron requirement for Hb synthesis. In anaemic cases, after a single dose of iron, a rise of serum iron takes place in 30 minutes, reaching its maximum in 3–5 hours (0.35 mg%, compared to normal value, 0.10 mg %) and returns to normal in about 12 hours. Maximum absorption is completed in 18 hours. The body observes a rigorous principle of economy in this respect. Iron derived from the disintegration of the red cells remains stored, and is utilised for the synthesis of further haemoglobin.

If a man be in iron balance any excess of iron administered per mouth will not be absorbed, but passes out in the faeces. If iron salts are given by injections, only traces appear in the urine, the rest

remains stored in the reticuloendothelial cells as haemosiderin. Haemoglobin in the blood falls when iron loss exceeds that of iron absorption and anaemia develops.

IRON IN BLOOD

Whole blood contains about 45–50 mg of iron per 100 ml. The total quantity present in all the red cells is about 3 gm. Another 1–3 gm is present in the rest of the body.

Iron is present in blood in two forms:

1. **Plasma iron:** Only traces of iron (average 0.10 mg per 100 ml) are present in plasma. It represents the form in which iron is transported in blood, from place to place, the compound known as transferrin or siderophilin. It is increased when the red cell formation is diminished, e.g. in aplastic anaemia, pernicious anaemia, etc. It is diminished when there is rapid red cell formation.
2. **As haemoglobin:** This accounts for about 92–98% of the total blood iron. It corresponds to about 50 mg of inorganic iron per 100 ml of blood.

STORAGE OF IRON

Iron is stored in two forms; ferritin and haemosiderin. The former is water-soluble while the latter is granular and insoluble in water. Reticuloendothelial system in general, particularly, liver, spleen and bone marrow store iron. Liver iron is readily increased by iron administration. In condition of excess haemolysis iron is deposited in these places in large quantities. Normally the iron liberated from the breakdown of red blood cells is also stored in these places and is utilised for Hb synthesis.

EXCRETION

Iron is excreted only in traces in urine, bile and faeces. In an adult the urinary loss is on the average 0.2 mg per day. It is believed that the iron content of the body is controlled by regulation of its absorption and not by excretion. Iron loss occurs during pregnancy, during labour, in the menstrual period, due to loss of blood, etc. (Fig. 14.2).

FUNCTIONS OF IRON

1. **Formation of Hb:** The primary function of iron is to form haemoglobin.
2. **Development of red cells:** Iron is not only necessary for Hb synthesis but also for the formation and maturation of red cells.
3. **Oxygen carriage in blood** in the form of Hb. One gm of Hb carries about 1.34 ml of oxygen (when fully saturated with oxygen).
4. **Related to tissue oxidation**
   - Cytochrome is an iron-containing compound. It is concerned with the oxidation of metabolites in the cells.
   - Indophenol oxidase is also an iron compound.

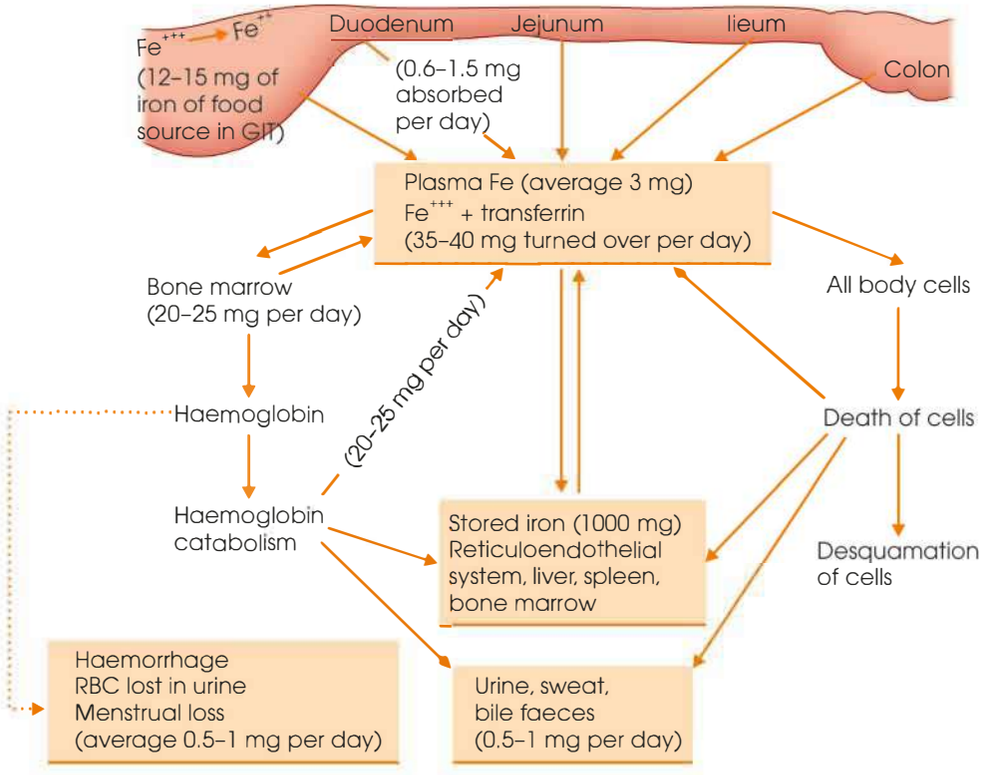

Fig.14.2: Iron metabolism and its pathway (schematic representation)

5. **Supplies O_2 to the muscle:** Myoglobin of muscle is an iron-containing chromoprotein like haemoglobin. It combines with O_2 and acts as an oxygen store for muscle.
6. **Relation with the cell nucleus:** The chromatin of the nucleus contains iron. It is possible that this iron takes an essential part (may be oxidative) in the functions of nuclei.
7. **Relation with oxidation in nerve cells:** Nissl granules, present in the cytoplasm of the nerve cells, contain organically combined iron. Here, iron serves some essential roles probably in oxidation. These granules disappear during activity of the nerve cells, and reappear during rest.

APPLIED: IRON DEFICIENCY ANAEMIA

The deficiency of iron in diet, increase physiological demand during pregnancy, menstruation, decrease absorption in gut due to malabsorption syndrome, secondary due to acute or chronic blood loss (GIT bleed, piles, etc.) or any chronic diseases produces iron deficiency anemia. Iron deficiency causes secondary anaemia (microcytic, hypochromic). The haemoglobin content of the red cells is diminished. The size and volume of the red cells are below average. There is normoblastic hyperplasia in the red bone marrow. Iron-deficiency anaemia occurs in children and adults due to severe blood loss.

EXAM-ORIENTED QUESTIONS

Essay

1. Discuss the absorption, transport, storage and excretion of iron.

Short Notes

1. Absorption of iron
2. Functions of Iron
3. Deficiency of iron

Chapter

15

Anaemia, Polycythemia, Osmotic Fragility and Blood Indices

INTRODUCTION

Anaemia: Deficiency of RBC in blood causes anaemia. Anaemia might be due to either excessive blood loss or increased destruction of RBC (haemolysis). It might also result from either defective formation in bone marrow (aplastic anaemia) or deficiency of maturating factor or nutritional defects.

Classification of Anaemia

Blood loss anaemia or haemolytic anaemia: After severe haemorrhage the lost plasma is restored within a short time but not the RBC. The blood is thus diluted and there is a fall in haematocrit. This anemia as per morphological classification of anemia is of normocytic normochromic type. After continued haemorrhage along with RBC, a considerable amount of iron is also lost which cannot be replaced by ordinary diet. Tissue anoxia stimulates bone marrow to form RBC which morphologically is of microcytic hypochromic type. Administration of iron is an effective cure in such a case.

Increased destruction of RBC: There are several types of anaemia. In all cases, RBC formation is abnormal and making it very fragile.

1. In familial haemolytic anaemia, the cells are small, spherical and can be easily broken down.
2. Sickle cell anaemia is due to cells having a sickle shape. The haemoglobin of such patients is of haemoglobin S type (instead of normal haemoglobin A).
3. Mediterranean anaemia (or thalassemia or Cooley's anaemia) is due to very small fragile RBC containing haemoglobin F.
4. Certain diseases like malaria and syphilis cause increased destruction of RBC as also the haemolytic agents described under fragility of RBC.

The increased RBC destruction, due to haemolysis, causes a rise in plasma, bile pigment (bilirubin) and there might be jaundice along with the anaemia.

Aplastic anaemia: *Failure of functions of bone marrow (aplasia):* The resulting anaemia is called aplastic anaemia.

This failure might be due to:

1. Primary failure of bone marrow itself
2. Enormous exposure to X-ray or γ-ray, the latter resulting from atomic explosion
3. Cancer in bone marrow
4. Poisoning from aromatic organic chemicals or some toxins
5. Kidney disease

There occurs substitution of normal marrow with fibrous tissue. The resulting anaemia is normocytic normochromic type as per morphological classification of red blood cells.

Pernicious anaemia and megaloblastic anaemia: *Defective formation of RBC:* For the conversion of proerythroblast also called megaloblast to mature RBC, several maturating factors are essential.

Characteristic features

1. This includes 'extrinsic factor' present in certain foods which are essentially vitamin B_{12} and folic acid and a gastric 'intrinsic factor' which helps in the proper absorption of the extrinsic factor. The haematinic principle absorbed is stored in the liver and transported to bone marrow. In absence of any of them, pernicious (Addison) anaemia and megaloblastic anaemia results.
2. In such a condition RBC count is greatly reduced, the diameter of red blood cell increases to 8 μm or more, mean corpuscular volume and mean corpuscular haemoglobin concentration increases and peripheral smear picture reveals nucleated red blood cells and marked poikilocytosis and anisocytosis. Pernicious or megaloblastic anaemia is of the macrocytic type and is usually normochromic.
3. The anoxia produced stimulates the bone marrow which is filled up with nucleated RBC. Along with blood changes, achlorhydria, lowered pepsin content and subacute combined degeneration of tracts of the

spinal cord (the demyelination of tracts of the spinal cord occupying the dorsal and lateral columns) also occur.

4. Vitamin B_{12} which is essentially the extrinsic factor, if injected at this stage (but not fed orally) cures not only the blood condition but also the neurological manifestations. Folic acid can cure the blood condition only. Possibly both the vitamins act as co-enzymes for the synthesis of DNA. Vitamin B_{12} alone is necessary for the formation of RNA the nucleic acid required for the integrity of the central nerves system.
5. The basic cause of pernicious anaemia is atrophy of the stomach mucosa. Gastrectomy or stomach cancer also leads to similar blood changes. In all such cases there is loss of intrinsic factor.
6. Absence of extrinsic factor, defect in liver storage or reduced response of bone marrow to maturating factor (as in myxoedema) might also be a contributory factor.
7. Atrophy of the intestinal mucous membrane, leading to the failure of absorption of vitamin B_{12} leads to non-tropical sprue. This anemia is morphologically classified as macrocytic hypochromic type.
8. The clinical sign and symptoms include decreased appetite, mental apathy, diarrhoea, soreness of tongue, motor deficit, tingling numbness in extremities, etc.
9. The patients are treated with vitamin B_{12} supplementation and folic acid.

NUTRITIONAL ANAEMIA

A diet deficient in essential amino acids or vitamins (vitamin C, pyridoxine, riboflavin, nicotinic acid, pantothenic acid), combined with or without diarrhoea, produces similar blood picture as that of tropical nutritional anaemia and is termed as nutritional anaemia. Inadequate intake or increased requirement of iron, as in pregnancy, causes hypochromic, microcytic, iron deficiency anaemia.

IRON DEFICIENCY ANAEMIA

Deficiency of iron in diet, increase physiological demand during pregnancy, menstruation, decrease absorption in gut due to malabsorption syndrome, secondary due to acute or chronic blood loss (GIT bleed, piles, etc.) or any chronic diseases produces iron deficiency anaemia. The red blood cell count is low and mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration values are decreased in iron deficiency anaemia. The clinical features observed in iron deficiency anaemia are fatigue, palpitation, breathlessness; recurrent infections and characteristics signs observed are dry spoon-shaped nails with longitudinal striations and bright red tongue.

SICKLE CELL ANAEMIA

Sickle cell disease is an inherited disorder. The patients of sickle cell disease have peculiar type of haemoglobin molecules called haemoglobin S. On exposure to hypoxia the red blood cells of sickle cell patient achieve a sickle shaped. The sickle red blood cells break down prematurely causing anaemia. The patients of sickle cell diseases present with history of fatigue, generalized weakness, shortness of breath, repeated infections, and periodic episodes of pain. Patient in crisis experience extreme painful episodes called vaso-occlusive crisis and when these cell blocks the capillaries in various tissue it may produce infarcts for example splenic infarct, stroke, avascular necrosis of bone, acute papillary necrosis in kidney, stroke, etc.

THALASSEMIA

Thalassemia is a disease of genetic origin in which there is defective synthesis of haemoglobin. There is a mutation or deletion of the genes which control globin synthesis. This decreases the formation of the corresponding globin chains and an abnormal hemoglobin ratio. This abnormal ratio decreases hemoglobin synthesis and the expression of thalassemia. The globin that is produced in normal amounts winds up in excess and forms red cell aggregates or inclusions. Thalassemia manifests as α-thalassemia disease or β-thalassemia disease. The excess of α-globins leads to the formation of α-globin tetramers in patients of β-thalassemia. The α-globin tetramers (α^4) accumulate in the erythroblast. These aggregates precipitate interfering with erythropoiesis and cell maturation leading to anaemia. Similarly, the α-thalassemia results in a β-globin tetramers (β^4) formation which is also called hemoglobin H. These tetramers under certain circumstances can lead to haemolysis, shortening lifespan of the red cell.

GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY

The G6PD enzyme deficiency manifests with failure of formation of NADPH. NADPH enzyme maintains glutathione in reduce state. The decreased concentration of glutathione in reduce state prone the red blood cell for haemolysis producing haemolytic anaemia.

CONGENITAL SPHEROCYTOSIS

It is an autosomal dominant disorder. The defective make up of spectrin protein as a result of genetic glycolysis defect lead to formation of microspherocytes (small size red blood cell). The characteristic biconcave shape is lost due to which red blood cells rupture easily as they are passing through the capillaries. This condition is also known as congenital spherocytosis.

HAEMOLYTIC ANAEMIA

By the term haemolysis is meant the disruption of red cells with the escape of haemoglobin from the corpuscles to the plasma.

- When red cells are suspended in isotonic solution, no change occurs. When suspended in hypertonic solutions, water is drawn out of the cells, the cell volume shrinks and they become crenated.
- When placed in hypotonic solutions, water enters the cells; they gradually swell up, become spherical and ultimately burst. Haemoglobin comes out into the plasma and the phenomenon is called haemolysis of blood. The fragility of the red cells depends upon the readiness with which the cells are haemolysed, when placed in hypotonic solution.

It can be determined quantitatively as follows

1. The blood sample to be examined is diluted two hundred times in a series of red cell-counting pipettes, using hypotonic salt solutions of gradually diminishing strength, as diluting fluids.
2. After half an hour the cells in each pipette are counted and the number is compared with the count done in the same way by using normal saline. From this the percentage of ruptured cells are found.
3. That particular concentration of the hypotonic salt solution, where 50% of the corpuscles are found to be haemolysed is determined and is used as the standard for comparing the fragility of other blood samples.

Normal haemolysis: It starts at 0.48% and is completed at 0.33% of NaCl solution. Fragility increases in acidosis.

- The red cells in the venous blood show increased fragility than in the arterial blood. This is due to the fact that in the venous blood the cell volume is already larger and consequently can withstand much less degree of further swelling. Hence, the cells become more easily haemolysed when placed in hypotonic solution.

Blood may be haemolysed in the following different ways

1. *By adding fat solvents:* Ether, chloroform, benzene, etc. causes leaking of blood by dissolving away the fatty red cell membrane.
2. *By causing osmotic disturbance:* Addition of distilled water or hypotonic salt solution increases the cell volume and causes haemolysis.
3. By disturbing the surface tension of the red cells. Addition of bile salts or saponin causes leaking of blood.
4. *Physical methods:* Alternate freezing and thawing of blood break down the RBC.
5. *Mechanical:* Vigorous stirring and shaking lead to haemolysis, but complete haemolysis of all the red cells in a sample is unusual by this process.
6. *Addition of incompatible blood:* This at first agglutinates the red cells and then causes haemolysis.
7. *Adding bacterial haemolysins.* Adding snake venom (viper).

Drugs: Quinine, phenacetin, nitrites, chlorates, etc. cause haemolysis. In addition to above there are various pathological conditions in which the fragility of the red cells is seriously disturbed.

POLYCYTHEMIA

Increase in number of RBC is called polycythemia. This might result in persons living at high altitude usually 15,000 feet above sea level. In some animals a large dose of cobalt might produce polycythemia possibly due to increased production of erythropoietins. Polycythemia vera (erythremia) is a pathological condition in which RBC count is well above normal.

BLOOD INDICES

Some important indices about red blood corpuscles and haemoglobin

1. *Colour index* (*CI*): It is calculated as follows:

$$= \frac{\text{Percentage of Hb}}{\text{Percentage of red cells}}$$

The haemoglobin percentage is determined as well as the red cell count. A count of 5 million red cells per cu mm is taken as 100%. If a subject is found to possess 60% haemoglobin and only 4 million red cells (i.e. 80% of the normal 5 million), then the colour index will be 60/80 = 0.75. The normal colour index is 1, but slightly lower index, i.e. 0.8, is more commonly found and is also not abnormal. Colour index indicates the proportion of haemoglobin present in each red cell with respect to normal. In hypochromic anaemia the index is low, in hyperchromic or macrocytic anaemia the index is increased.

2. *Mean corpuscular diameter (MCD):* In film preparations the average diameter is 7.2–7.3 µm.
3. *Mean corpuscular volume (MCV):* Anaemia is macrocytic when the MCV is over 96 cu µm, microcytic if below 85 cu µm and normocytic at normal level of it.

$$= \frac{\text{Volume of packed red cells in ml per litre}}{\text{Red cells in million per cu mm}}$$

= 87 cubic µm ±7

4. *Mean corpuscular thickness* (*MCT*): 2.1–2.2 µm. Mean corpuscular thickness can be determined from the formula:

$$\text{MCT} = \text{MCV} \times \frac{1}{\{\pi\,(\text{mean diameter}/2)^2\}}$$

Volume index

$$= \frac{\text{\% of corpuscular volume (45 ml – 100\%)}}{\text{\% of red cells (5 million per cu mm – 100\%)}}$$

= Normal average 1 (range 0.85–1.5)

Relative volume of packed red cells and plasma: This is determined by centrifuging oxalated blood in haematocrit. The cells occupy 45% of the total volume and the rest is made up by plasma. The ratio, cell; plasma = 45:55 is a good normal.

5. *Mean corpuscular haemoglobin (MCH):* It is calculated as follows:

$$= \frac{\text{Haemoglobulin in grams per litre of blood}}{\text{Red cells in million per cu mm}}$$

It refers to the average weight of the haemoglobin in the red blood sample. It is expressed in picograms. One picogram is 10^{-12} grams. The normal range of MCH in adult is 26–32 Pg.

6. *Mean corpuscular haemoglobin concentrations (MCHC):* It is calculated in the following ways:

$$= \frac{\text{Haemoglobin in grams per 100 ml}}{\text{Volume of packed red cells in 100 ml}} \times 100$$

The average figure is 35% ± 3.

This figure indicates how much of the average volume of the corpuscle is filled up by haemoglobin.

Normally 35% of the average volume of each red cell is filed up with Hb. If the MCHC is below 30% the anaemia resulting in hypochromic type. Value is never higher than above normal. Anaemia with normal MCHC is normochromic.

Saturation index: It indicates the concentration of haemoglobin in the red cell and is obtained by:

$$= \frac{\text{\% of weight of haemoglobin per 100 ml}}{\text{\% of corpuscular volume}}$$

= Average 1 (range 0.9–1.1)

EXAM-ORIENTED QUESTIONS

Essay

1. Define anaemia. Classify anaemia and discuss its physiological basis.
2. Discuss the significance and method of estimation of following blood indices: Colour index, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration.

Short Notes

1. Polycythemia
2. Osmotic fragility

Chapter

16

White Blood Cells and Platelets

WHITE BLOOD CELLS

White blood corpuscles (WBC) are an important variety of cells in the blood.

These cells differ from the red cells in many respects. For instance:

1. They do not contain any haemoglobin.
2. They are bigger in size.
3. They are nucleated, living cells.
4. They are actively amoeboid. They are much less in number. Their span of life is shorter.
5. Their origin is purely from extravascular tissue.
6. Their functions are absolutely different from those of red cells.
7. There are several varieties of leucocytes, whereas red cells are only of one variety.

Chemistry

Leucocytes are rich in nucleoprotein and also contain lipids, glycogen, cholesterol, ascorbic acid and a variety of enzymes, especially proteolytic.

Total Number

The average total number of white cells is 6,000 to 8,000 per cu mm, the normal range being 4,000–11,000 per cu mm. The average ratio of the total white cell count with the total red cell count is about 1:700, i.e. for; one WBC there is 700 RBC.

VARIATIONS IN NORMAL COUNT OF WHITE BLOOD CORPUSCLES

1. ***Diurnal variation:*** The total count varies from day to day and even from hour to hour. Sometimes, such variations are found to be random and without any obvious cause. In the morning or after rest, the count is lowest. The count rises after mid-day and usually becomes highest in the evening.
2. ***Muscular exercise*** and any condition leading to asphyxia (O_2 lack and CO_2 excess) increase the total count. Relation with meal: Formerly it was believed that there is leucocytosis after meals, which is now thought to be only a diurnal variation.
3. ***Injection of adrenaline*** increases the count.
4. ***Emotional stress:*** Fear, pain, etc. increases the count due to liberation of adrenaline.
5. ***Age:*** In the newborn, the count is very high, about 20,000 per cu mm. After second week, the count starts falling, but throughout infancy and childhood, the count remains proportionally higher. During infancy and childhood, the lymphocytes constitute about 40–50%.
6. ***Relation with pregnancy and labour:*** In pregnancy and at full term the count is higher, being highest (17,000) during labour. There is also an increase during menstruation.
7. ***Increased cellular destruction*** from infections, surgical operations, etc. produces derivatives of nucleic acid and causes a rise in neutrophil leucocytes.
8. ***Asthma, hay fever, skin disease,*** etc. and also after hostile invasion with parasites there is an increase in eosinophil (eosinophilia).
9. ***Adrenal cortical steroids and ACTH*** (adrenocorticotrophic hormone) cause an increase in neutrophil and decrease in lymphocytes and eosinophil. The fall in eosinophil is very specific for this hormone, and is used as an assay method.
10. ***Starvation and administration*** of certain chemicals like benzene, sulphonamide, etc. produce leucopenia (fall in leucocytes).
11. ***Infections leading to Leucopenia:*** Also occurs after bacterial, viral or protozoal infection and also in aplastic *bone marrow* disease or after exposure to ionising radiation. It is generally agreed that stores of white blood corpuscles remain in various parts of the body and under different conditions they may rapidly appear and disappear from the blood.

CLASSIFICATION AND DIFFERENTIAL COUNT OF WHITE BLOOD CORPUSCLES OR LEUCOCYTES

There are several varieties of white blood corpuscles, each type possessing characteristic morphology and staining property. Determination of the percentage of different varieties of leucocytes is known as the differential count of white blood corpuscles. The classification and the differential count, as generally accepted.

All white blood cells are produced and derived from multipotent cells in the bone marrow which are the haematopoietic stem cells.

GRANULAR LEUCOCYTES OR GRANULOCYTES

Cells with granular cytoplasm they are formed in the bone marrow from the time of birth onwards. The granules may take any of the three different stains neutral or acid or basic. Consequently this group includes the three varieties as listed in Table 16.1.

Neutrophil

Characteristic Features

1. It is about 10–14 µm in diameter. Most numerous in the adult blood is about 60–70%. The nucleus is many-lobed; the number of lobes varies from 2 to 7 or more.
2. The youngest cell has a single nucleus.
3. The number of lobes increases with the degree of maturity of the cells. Due to presence of lobes in the nuclei these cells are called polymorphs.
4. In the peripheral blood, the cells with three- to four-lobed nuclei are the most numerous.
5. The cytoplasm contains fine neutrophilic granules which appear pale-violet with Leishman's or Giemsa's stain. They are actively amoeboid and phagocytic.
6. The neutrophil leucocytes contain a variety of enzymes like phosphatase, nucleotidase, protease, amylase, lipase, etc. They also contain ascorbic acid, glutathione and glycogen. Metabolically these cells are much more active than erythrocytes.
7. Some physiologists have described certain sex differences in the nuclei of neutrophil (polymorphonuclear). The sex chromatin body is a small mass, usually adjacent to the nuclear membrane which stains deeply with haematoxylin, and is attached to the nucleus by a slender thread, giving a drumstick-like appearance. This sex difference is found in polymorphs of about 17% of normal females and is absent in the males.

Arneth Count or Arneth Index (Modified by Von Bonsdorff and Later by Cooke)

Following the idea that the age of the neutrophils is proportional to the number of lobes in their nuclei, Arneth (1904) divided them into five groups.

The **Arneth index** can be determined by counting the number of nuclear lobes in each of hundred neutrophils. Of 100 neutrophils counted the proportion of different groups are as follows; group I (one-lobed nucleus—round, oval, indented or horseshoe-shaped)—5–10%; group II (two-lobed nucleus)—25–30%; group III three-lobed nucleus)—45–47%; group IV (four-lobed nucleus)—16–18%; group V (nucleus with five or more lobes)—2%.

Cells with three-lobed nuclei are maximum in number, fully mature and functionally most efficient (Fig. 16.1). Cells with less nuclear lobes are immature, while cells with more lobes are increasingly senile. A shift to the left [(group I + group II) > 45%] means a relative or absolute increase of immature cells and consequently a less efficient leucocyte defensive system. A shift to the right means diminished leucopoiesis with the increase of hyper mature and senile cells.

Schilling index: It is simpler classification of neutrophils into four groups such as (a) myelocyte having only one lobe, (b) juvenile metamyelocyte in

Table 16.1: Classification of white blood corpuscles

Type	*Percentage*	*Absolute number*
I. Granular leukocytes or granulocytes	60–70%	3,000–6,000 per cu mm
a. *Neutrophil (polymorphonuclear)* The nucleus is many-lobed (2–7 lobes) and the granules in the cytoplasm taken neutral stain		
b. *Eosinophil* The nucleus is commonly two- or three-lobed The granules are coarse and stain with acid dyes (*eosin*)	1–4%	150–400 per cu mm
c. *Basophil* The nucleus is lobed the cytoplasm contains granules of various sizes which take deep basic stain	0–1%	0–100 per cu mm
II. Lymphocytes a. *Small lymphocyte* b. *Large lymphocyte*	25–30%	1,00–2700 per cu mm
III. Monocytes (including transitionals)	5–10%	Alsolute no. 350–800 per cu mm

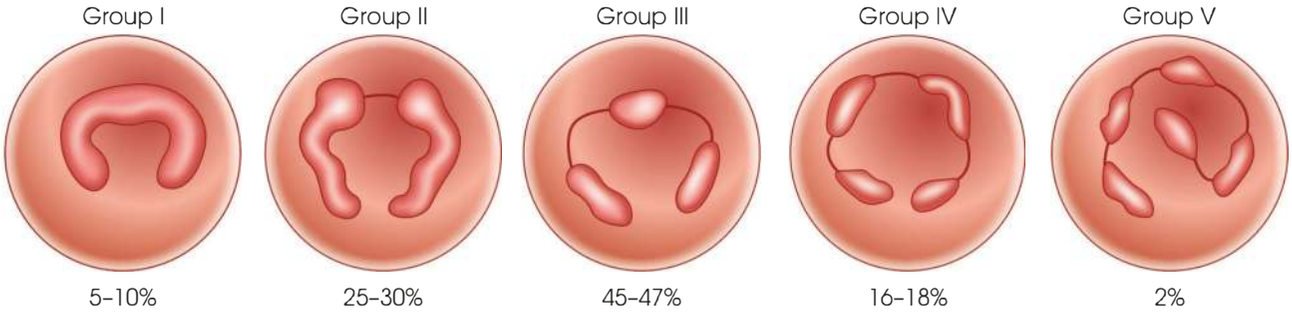

Fig. 16.1: Different numbers of lobes in polymorphs corresponding to different groups in Arneth count

which the nucleus is indented, (c) staff cell or band cell of Schilling having unsegmented but T- ,V- or U-shaped nuclei. It is an older metamyelocyte and corresponds to the first stage of Arneth, and (d) older or segmented neutrophils.

In response to acute need certain younger forms of neutrophils such as myelocyte and juvenile type are used to come out from the bone marrow. This has been indicated by Schilling as regenerative shift. His concept regarding the degenerative shift to the left is referred to the failure of the neutrophils to mature as the result of the depression of bone marrow function. In this type of shifting, an increased number of immature forms are found in the blood. It is assumed that these cells can never mature.

Eosinophil

Characteristic Features

1. It is about 10–14 µm in diameter.
2. The nucleus commonly possesses two lobes and stains less deeply than those in the polymorphonuclear cells.
3. The cytoplasm contains coarse granules, oval or round in shape, taking acid stain and showing red colour with eosin.
4. The cell membrane seems to be very delicate and is often found to be broken in a blood film.
5. The eosinophils are amoeboid, but not phagocytic.
6. They contain variable amount of histamine. They are involved in type I hypersensitivity reactions. Eosinophil numbers are increased in infection or allergic reaction by the release of IL-3, IL-5 and GM-CSF by Th2 and mast cells. This promotes release of eosinophils into circulation. Eosinophils get attracted to the required site of action by chemicals known as eotaxins which are released by mast cells. The histamine and its breakdown products also act as attractants for more eosinophil to the site.
7. They attack parasites which are large to be engulfed by phagocytosis. The specific granules releases major basic protein, eosinophil cationic protein (ECP), eosinophil peroxidise (EPO) and eosinophil derived neurotoxin (EDN). These proteins act as stimulants to mast cells causing them to release histamine and thus attracting more eosinophils and thus counteracting the parasites and killing them.

Basophil

Characteristic Features

1. Their size is somewhat smaller, with a diameter of about 10–14 µm.
2. The nucleus is usually kidney-shaped or only slightly lobulated.
3. The cytoplasm contains a large number of round granules which take deep basophilic stain.
4. The granules are less numerous than in the eosinophil and differ much in size. Unlike the eosinophil, the granules are often found to overlap the nucleus and obscure the details of its morphology.
5. They are actively amoeboid but are less so than the other varieties of granulocytes. They are believed to belong to cells of the reticuloendothelial system.
6. The bosophils are similar to connective tissue mast cells and like them exhibit metachromasia, the granules containing both histamine and heparin and also 5-hydroxytryptamine (5-HT). Heparin acts an anticoagulant.

The increase of granulocytes in the blood is called granulocytosis. Diminution of granulocytes in the blood is known as granulocytopenia. Complete disappearance of granulocytes is known as agranulocytosis

Agranulocytes are lymphocyte and monocyte.

LYMPHOCYTES

Small Lymphocyte

Characteristic Features

1. It is slightly larger than a red cell, the diameter being about 7 to 10 µm.
2. The nucleus is relatively large and occupies the major part of the cell.
3. The cytoplasm is basophilic, showing no distinct granules and makes a thin rim around the nucleus.
4. In early childhood they make up about 50% of the total white cell count. Their number diminishes with age. At the age of 10 years it makes about 35%.

Large Lymphocyte

Characteristic Features

1. It is about 10–14 µm in diameter in size, the nucleus may be round, oval or kidney-shaped.
2. The cytoplasm is proportionally more and forms a wider zone around the nucleus. It is basophilic and shows no distinct granules.
3. They are considered to be the younger forms of small lymphocytes.
4. In the adults, they are very few in numbers (4–8%) but are more frequent in children. In well-stained specimens a few reddish-purple granules (metachromatic granules) are found in the cytoplasm, generally collected at one pole of the cell.

MONOCYTES (LARGE MONONUCLEAR CELL, TRANSITIONAL CELL, etc.)

Characteristic Features

1. They are about 10 to 18 µm in diameter.
2. The nucleus is round or oval when the cells are young. But as they grow older, the nucleus becomes convoluted kidney-shaped or horseshoe-shaped.
3. The nucleus is eccentric with a large amount of clear non-granular cytoplasm, may be, with vacuoles in it. Fine reddish-blue metachromatic granules may be found throughout the cytoplasm in well-stained specimens. When the nucleus is round it is difficult to distinguish monocytes from the large lymphocytes. But the following points may be helpful. In the monocytes, the nucleus may be eccentric and the cytoplasm resembles 'frosted glass'; whereas in the large lymphocytes the nucleus is central and the cytoplasm has a 'clear glass' appearance. In the warm stage the monocytes are found to be actively motile whereas the large lymphocytes show very little movement.

DEVELOPMENT OF LEUCOCYTES

Leucopoiesis is defined as a process of development and maturation of the white blood cells. It takes a period of 12 days for development of leucocytes. They develop from the pluripotent haemopoietic stem cell which differentiates into colony forming units which are the progenitor cells (Fig. 16.2A).

DEVELOPMENT OF GRANULAR LEUCOCYTES OR GRANULOCYTES

The CFU-GM forms the granulocytes and monocytes cell in the bone marrow. The progenitor cells which form other granulocytes are Ba-CFU: Basophil-colony forming unit, Eo-CFU: Eosinophil-colony forming unit, M-CFU: Monocyte-colony forming unit and G-CFU: Granulocyte-colony forming unit. The development of granulocytes is termed as granulopoiesis myeloid series while that of monocyte is referred as monocyte macrophage series. The leucocytes are stored in bone marrow.

1. ***Myeloblast*:*** They form nearly 2% of total marrow cells. They are smaller in size (16 to 20 µm), non-granular cytoplasm and a round or bean-shaped nucleus with several nucleoli. There are numerous mitochondria. They are non-motile or less motile. They divide and form the next stage. It should be noted that up to this stage the cytoplasm has no granules when stained with the ordinary Romanowsky methods. By the peroxidase method granules may be found in the more mature of the myeloblast. The number of nuclei is an important differentiating point from lymphoblasts. The latter have definite and distinct nucleoli whereas in the former, the nucleoli look like irregular gaps.
2. ***Promyelocytes:*** The myeloblast matures into promyelocytes. The size of promyelocytes is 14–18 µm, there are azurophil granules present in the cytoplasm, and the nucleus is round or oval having condensed chromatin and few nucleoli. They undergo mitosis.
3. ***Myelocyte:*** This stage is characterised by certain remarkable peculiarities which are quite distinct from the previous stages.

   The peculiarities are as follows: (a) Multiplicity—although all the cells before this stage are multiplying, yet the multiplicity of the myelocytes is maximum. It is by the multiplication of the myelocytes that the normal supply of the white cells is kept up, (b) granules appear in the cytoplasm. The granules may take neutral, acid or basic stains and accordingly the myeloytes are of three varieties: (1) Neutrophilic myelocyte, (2) eosinophilic myelocyte, (3) basophilic myelocyte, (c) cytoplasm is less basophilic, and (d) nucleoli disappear and chromatin is coarser.
4. ***Metamyelocyte:*** At this stage, again certain characteristic changes appear: (a) The nucleus becomes deeply indented and is almost bilobed. This shows that maturation is advancing, (b) the cells no more multiply but only mature henceforward. (c) amoeboid movement appears and due to this, a few of these cells may burrow into the blood vessels, and (d) appear in the peripheral circulation.
5. ***Band or stab form:*** These are young juvenile granulocytes. There size is smaller than the metamyelocytes. There pinkish coloured cytoplasm contains granules. Nucleus is further condensed and transformed into band or indented V shape.
6. ***Leucocyte:*** The nucleus contains many lobes which are produced by maturation of the previous stage.

*Ferrate recognises another cell, the promyelocyte, being the transitional stage from the myeloblast to the myelocyte.

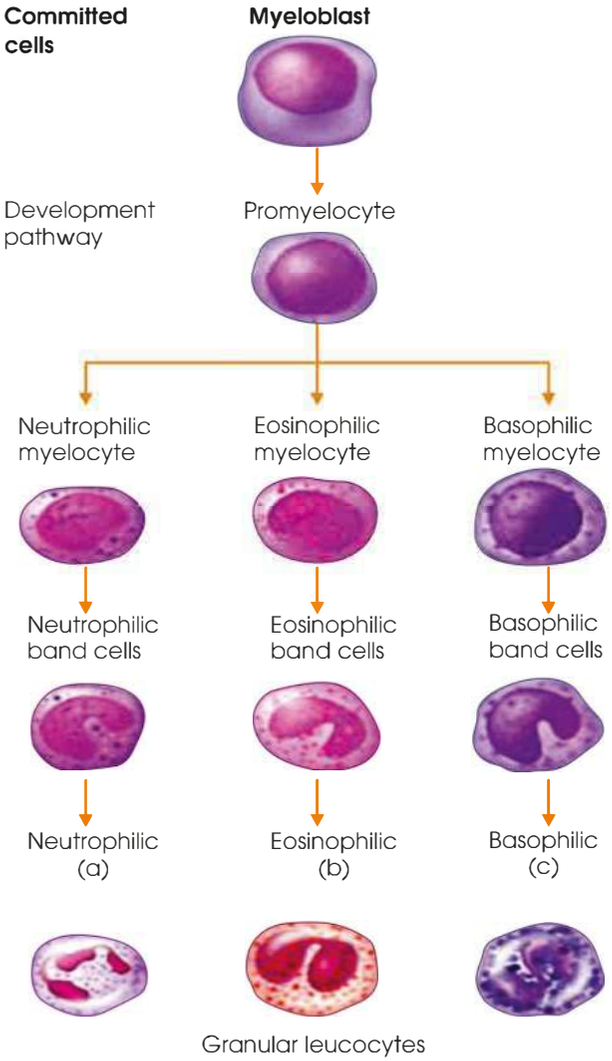

Fig. 16.2A: Formation of leucocytes

The maturation takes place in the bone marrow. Once the cells come in the peripheral circulation, no more maturation can take place. It is believed that the degree of maturation is proportional to the number of lobes of the nucleus.

Myeloblast progress into promyelocytes which under influence of various colony forming units and cytokines colony stimulating factor as described above matures into that specific series of cells that is neutrophils, basophils, eosinophils and agranulocytes; monocytes and lymphocyte are given in Fig. 16.2A and B.

Development of Lymphocytes

In the central part of lymph node, there is a small area about 1 mm in diameter and pierced by a small blood vessel. This is called the germinal centre or secondary lymphoid follicle. Some of the reticulum cells in this area proliferate and give rise to the lymphoblasts. In recent years, evidence has been accrued and shows that the thymus is an important and probably the main source of production of lymphocytes.

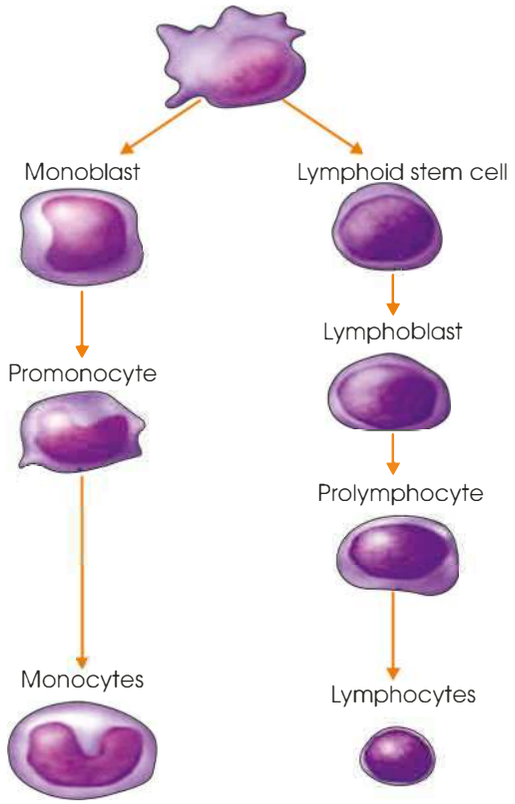

Fig. 16.2B: Formation of lymphocytes and monocytes

Lymphocytes are formed from lymphocytic stem cells that are the pluripotent haemopoietic stem cells in the bone marrow. The lymphocytic stem cells migrate to the peripheral lymphoid tissue and thymus. They develop and mature in peripheral lymphoid tissue and thymus.

The stages of proliferation are:

1. Lymphoblast is 15 to 20 µm in diameter, with a round or oval nucleus and a non-granular cytoplasm.
2. Prolymphocytes are 9–18 µm in diameter. They mature into lymphocytes. They have round indented nuclei and scanty non-granular cytoplasm.
3. Lymphocytes: Prolymphocytes divide and give rise to the small and large lymphocyte. The normal number of lymphocytes in the blood is maintained chiefly by the division of the lymphoblast cells.
   - The large lymphocytes are fairly mature cells and do not multiply any further. These cells are found in the circulation.
   - The small lymphocytes are derived by further maturation of the large lymphocytes. These cells leave the gland through the efferent lymphatics; thoracic duct and right lymphatic duct, and reach the circulation.

Role of Lymphocyte in Immunology

The lymphocytes include natural killer cells (NK cells), T cells and B cells.

NK cells are a part of the innate immune system involved in cytotoxic innate immunity and play a major role in defending the host from tumors and virally infected cell. T cells are involved in cell-mediated immunity. B cells are responsible for humoral immunity (mediated via antibodies).

As on antigen presentation T cells and B cells recognize specific non-self antigens. These cells generate specific responses to combat specific pathogens or pathogen-infected cells. B cells respond by producing antibodies which then neutralize foreign bacterial and viral antigens. T cells, called T helper cells produce cytokines on exposure to pathogens while cytotoxic T cells produce toxic granules containing enzymes which kill the infected cells. The activated, B cells and T cells forms sensitization memory against the antigens they have come across, in the form of memory cells.

Development of Monocytes

The monocytes are developed mainly from the monocyte macrophage series from progenitor cells CFU-M (colony forming unit-monocyte) under influence from colony stimulating factors from reticulum cells of spleen and lymph nodes and to a lesser extent of the bone marrow. The stages are—progenitor cells → monoblasts → promonocytes → monocytes.

1. **Monoblast:** It is a large size cell. These earliest precursors are called myelomonoblast.
2. **Promonocyte:** It is about 20 µm in diameter, having large, indented kidney-shaped nucleus, single nucleolus and basophilic cytoplasm.
3. **Monocyte:** It is about 12–20 µm in diameter. They have single, large horseshoe- or kidney-shaped nucleus. The nucleus has fine network of chromatin and have pale blue agranular cytoplasm.

Life and Fate of Leucocytes

The life of the different varieties of leucocytes differs. Sabin holds that granulocytes live from 1 to 2 days only. Osgood believes that neutrophils live from 2 to 4 days; eosinophils, 8–12 days; basophils, 12–15 days. The average life of lymphocyte seems to be more than a day, and may be 2 or 3 days.

Regarding the fate of leucocytes, it is known that all the varieties die, disintegrate and disappear. The neutrophils and other granular leucocytes undergo fragmentation in the blood stream and are also broken down by the reticuloendothelial cells. Regarding the fate of the lymphocytes, it is believed that most of the lymphocytes leave the body (and are destroyed) by passing out through the intestinal and other mucosa. But some are certainly destroyed by the phagocytic cells of reticulo-endothelial system like the other varieties.

FUNCTIONS OF WBC

1. **Phagocytosis:** The neutrophil polymorphonuclear leucocytes and the monocytes engulf foreign particles and bacteria, and generally digest them. This process is called phagocytosis. When the bacteria invade the body, the leucocytes pass out of the blood vessels and surround the threatened area and through their pseudopodial processes engulf the bacteria and destroy them. The eosinophils and lymphocytes have got slight phagocytic action. Eosinophils collect at site of allergic reaction and releases histaminases limiting effect of inflammatory mediator and inhibit mast cell degranulation. While monocytes follow the area of infections and form a second line of defence. Eosinophils enter the tissue and provide local mucosal immunity in urinary tract, gastrointestinal tract and respiratory tract. Monocytes kill tumour cells after sensitization by lymphocytes.
2. **Antibody formation:** Lymphocytes manufacture the γ-fractions of serum globulin. Immune bodies are associated with γ-globulin fraction. The significant γ-globulins are immunoglobulins. So the lymphocytes play an important role in the defensive mechanism of the body of an immunological nature. Adrenal cortical steroids are responsible for dissolution of lymphocytes and so increase in antibody concentration in blood.
3. **Synthesis:** Monocytes synthesize complement and other substances like prostaglandin E, and clot promoting factors.
4. **Manufacture of trephones:** Leucocytes manufacture certain substances from plasma proteins which exert great influence on the nutrition growth and repair of tissues. These substances are called trephones.
5. **Secretion of heparin:** The basophils are supposed to secrete 'heparin'; which prevents intravascular clotting.
6. **Antihistamine function:** The granulocytes, specially the eosinophil cells, are very rich in histamine. They are believed to defend against allergic conditions in which histamine like body are present in excess.

Control of Leucopoiesis

The factors which control the formation of leucocytes under physiological conditions are rather complex. From the fact that these cells are constantly dying away, and in spite of it, a fairly constant number is maintained in the circulation, it is evident that some stimulus must be constantly acting upon the leucopoietic tissues, so that the dead cells are replaced by new formation and a balance is kept up between production and loss.

- One thing is almost certain that for each variety of cells a specific stimulus is necessary and the stimulus is largely chemotactic.

- It has been shown that injection of nucleic acid or its derivatives, increases the neutrophil cells in the blood. From such observations it has been suggested that the nucleic acid, derived from the dead neutrophils, may act as the normal stimulus for the formation of fresh neutrophils. In this way the normal neutrophil count is maintained.
- Regarding the normal stimulus, for the other varieties of leucocytes, needs further exploration. Some suggest that proteins are responsible for lymphocytic proliferation and lipids for monocytic proliferation.
  - Experimental and clinical evidences suggest that some control regulating the release of cells into the circulation is exercised by the actual number of circulating leucocytes.
  - Menkin (1955) isolated two simple polypeptides, one thermolabile and the other thermostable as the specific leucocytosis—promoting factors in the pseudoglobulin fraction of exudates and two thermostable polypeptides as specific leucopenic factors.
  - Others in 1961 obtained evidence of the presence of a neutropoietin and still others in 1965 isolated from serum fraction which stimulates production and another which depresses production and maturation of cells.

ABNORMAL VARIATION IN WHITE BLOOD CORPUSCLES COUNT

Leucopenia: Leucopenia is a condition in which there is a decrease in the WBC count below 4,000 per cu mm. This is commonly due to a fall in the neutrophil cells.

Neutropenia occurs in children, typhoid, viral infection and bone marrow depression. Eosinopenia occurs after injection of ACTH or corticosteroid treatment.

Leucocytosis: A rise in the WBC count above 11,000 per cu mm is called leucocytosis. It might be due to an increase in neutrophil, eosinophil or basophil. There might also be lymphocytosis or monocytosis.

Physiologically neutrophil count increases after exercise, pregnancy, menstruation and lactation while the pathological causes are acute pyogenic infections, after burns, post-surgery, after haemorrhage, etc. Eosinophilia occurs in parasitic infections, and skin diseases.

Agranulocytosis: In such a condition there is a great fall in the number of circulating granulocytes, which usually result from the harmful effect of certain drugs.

Leukaemia: This is a malignant disease of one or other variety of the WBC, the number of which is greatly increased. Immature forms of the WBC make their appearance in the circulating blood in such condition.

PLATELETS

Platelets are non-nucleated round or oval, biconvex discs having various sizes and covered by unit membrane. The average size is 2.5 µm. But bigger forms (4–5 µm) are also seen. In ordinary blood film the platelets are generally not seen separately but in clumps. If antiagglutinant (EDTA) is applied, in the blood prior to drawing of film then the same can be seen separately in light microscope. In light microscope generally two components of platelets are seen in the stained slide. One is the clear ground substance—the *hyalomere* (*hyalos* = glass, *meros* = part) that is stained very faintly and the other is the deeply stained central portion—the *chromatomere* or *granulomere*.

Electron microscopic studies of platelets stained with glutaraldehyde followed by osmium tetroxide reveal additional components other than two components mentioned above. It is also claimed that the surrounding membrane of each platelet is covered by a thin film of carbohydrate.

In electron microscope the *hyalomere* is seen to consist of homogeneous fine granular materials. These hyalomeres in the periphery of platelets were also found to contain *microtubules* and *microfilaments*. The microtubules probably give the ovoid structure of platelets and the microfilaments are presumably associated with microtubules. The microfilaments contain *thrombosthenin* which can contract like actin and myosin in muscle. This contractile element—the thrombosthenin is responsible for change of the shape of platelets.

Similarly, under electron microscope, the *chromatomere* is seen to contain numerous components. These components (Fig. 16.3) are as follows:

α-granules: These granules are oval or sometimes round in shape and having diameter, 0.2 µm and length 0.3 to 0.4 µm. These granules are often seen enclosed in a membrane. A rounded dense osmophilic area is often present in these granular matrices of the organelle. The α-granules consist clotting mediators such as factor V, factor VIII, fibrinogen, fibronectin, platelet-derived growth factor, and chemotactic agents.

Mitochondria: These are 2–3 in number and are clearly seen in a thin section of platelets.

Sydersomes: These are iron-containing (ferritin) vesicles. These are not seen very frequently.

δ-granules, or dense bodies, contain ADP, calcium, serotonin which are platelet-activating mediators.

Glycogen granules: These granules are also distributed in certain parts of the platelets.

Ribosomes: These are generally seen in newly formed platelets.

Systems of tubules and vesicles: These are of two types. One is the surface-connecting system and the other is the dense-connecting system. The surface-connecting

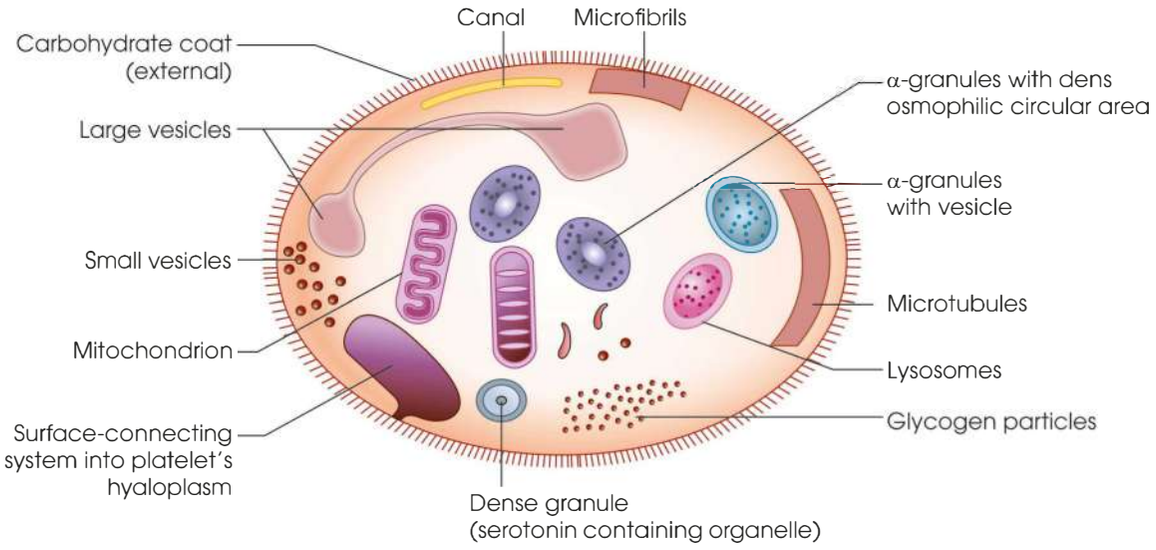

Fig. 16.3: Structure of platelet

system communicates; with the surface of platelet; and are concerned with phagocytic function. The membranes derived from megakaryocytic smooth endoplasmic reticulum are organised into a dense tubular system and it is responsible for thromboxane A_2 synthesis. As the dense tubular system is connected to the surface platelet membrane it aids in thromboxane A_2 release.

The average life of platelets is about 5 to 9 days. They are destroyed in the spleen and other reticulo-endothelial cells.

Properties

Characteristic properties are: (a) Sticking to water-wettable surface or otherwise rough surface (injured or diseased endothelium, etc.), (b) easy clumping, and (c) easy disintegration and thus liberation of thrombo-kinase.

Total Number and its Variations

The average number of platelets present per cubic millimetre of blood is about 250,000 to 450,000. Fairly rapid changes in number take place from day to day and even from one part of the day to another. 60–75% of the total platelets are circulating while rest remain in the spleen. Bone marrow depression, hypersplenism, viral infections, etc. platelet count decreases while splenectomy, after administration of epinephrine, etc. platelet count increases.

METHODS OF COUNTING OF PLATELETS

For avoidance of clumping, an anti-agglutinating agent, EDTA (ethylenediaminetetra-acetic acid) is generally used during counting of platelets. This can be done in ordinary light microscope by direct or indirect method.

In **ordinary method**, a drop of antiagglutinating substance is poured over the clear surface of the skin and a pin puncture is made through the drop. The blood that comes out is mixed up with the anti-agglutinating agent and an ordinary blood film is made through it. The platelet count is made along with the number of RBC present in each field. It is counted as the number of platelets present per 100 RBC in each field. If the erythrocyte count per cubic millimetre is made then the value of platelets number can be worked out from the above ratio.

In **direct method,** the platelet count is made by counting the same along with RBC in counting chamber. In this method, measured amount of blood and measured amount of anti-agglutinant along with a dye are taken in the pipette and are thoroughly mixed. The platelets and RBC present are counted in a counting chamber.

Development of Platelets

Thrombopoiesis is the process of formation and development of platelets. The pluripotent stem cell converts to form colony forming units Meg-CFU which passes through successive stages to form platelets. The giant cell (megakaryocytes) of the bone marrow introduces pseudopodia through the walls of the sinusoids. These processes are broken off in such a way that the individual fragment is surrounded by a unit membrane and are washed away by the blood stream. The haemocytoblast matures into megakaryoblast, promegakaryocyte, megakaryocyte and finally into platelets. These fragments with unit membrane are platelets (Fig. 16.4).

1. **Megakaryoblast:** It has size of 20–30 µm. The nucleus is oval or kidney-shaped with presence of numerous nuclei. The cytoplasm is blue and non-granular.
2. **Promegakaryocyte:** The megakaryoblast matures to promegakaryocyte stage. There is duplication of nuclear chromatin and the cytoplasm becomes granular.

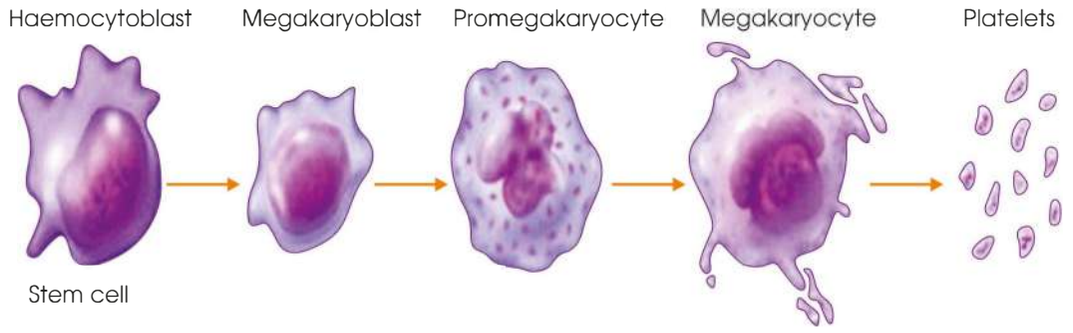

Fig. 16.4: Formation of platelets

3. **Megakaryocytes:** These are large cell having size of 30–90 µm. Cytoplasm is light blue in colour and has red-purple granules and nucleus is multilobed.
4. **Platelet:** They are 2–4 µm in diameter. They are colourless, ovoid or spherical structures. The detail structure has been discussed above.

FUNCTIONS OF PLATELETS

1. **Initiate blood clotting:** When blood is shed, the platelets disintegrate and liberate thromboplastin which activates prothrombin into thrombin.
2. **Repair capillary endothelium:** While in the circulation, the platelets adhere to the damaged endothelial lining of the capillaries and thus bring about a speedy repair. It is known that the capillary walls, being very delicate, are easily damaged and unless these weak spots are quickly mended, the vessels will break at these spots and capillary bleeding will take place. When the platelet count falls (below 50,000 per cu mm) such capillary bleeding occurs.
3. **Haemostatic mechanism:** This process seems to play by the dual functions of platelets such as agglutination and coagulation. The cessation of blood flow from ruptured blood vessels takes place through simultaneous coagulation and agglutination by platelets.
4. **Hasten clot retraction:** Speed of clot retraction (syneresis) is directly proportional to the number of platelets present and this retraction process is dependent upon the thrombosthenin (contractile protein of platelets) in presence of ATP and magnesium ions.
5. **When platelets disintegrate, 5-hydroxytryptamine is liberated.** 5-hydroxytryptamine (5-HT) has vasoconstrictor effect and helps in haemostatic mechanism.

Purpura

There are two types of purpura: Non-thrombocytopenic (normal platelet levels) and thrombocytopenic (lower platelet count). In this disease there is diminution of platelets in the blood. Haemorrhage occurs beneath the skin and mucous membrane. The appearance of lesions varies with the type of purpura, the duration of lesions, and the acuteness of the onset. The colour is first red, becoming gradually darker, then purple, fading to a brownish yellow. It may result in permanent pigmentation or it may disappear in course of 2 or 3 weeks but the damaged capillary endothelium is not repaired. The coagulation time remains normal but the bleeding time is prolonged. The clot does not retract.

6. **Contain some substances which are like ABO blood antigens:** Platelets may be agglutinated by specific antisera and in the presence of complement, lysis may occur.

EXAM-ORIENTED QUESTIONS

Essay

1. Classify white blood cell. Describe leucopoiesis. Describe the characteristics of all white blood cells.
2. Describe the structure of platelets and its functions.
3. Discuss the functions of platelet. Add note on purpura.

Short Notes

1. Physiological variations in normal count of white blood corpuscles
2. Arneth count
3. Schilling index
4. Life and fate of leucocytes
5. Functions of white blood cell
6. Functions of platelet
7. Methods of platelet count
8. Enlist causes for variation in platelet count

Chapter

17

Blood Groups and Blood Transfusion

BLOOD GROUPS

The blood groups are mainly of three types

a. A, B and O groups
b. Rh factor
c. M and N factors

Some other blood group characteristics (i.e. P, Lutheran, Lewis and others) excepting the above three are also present.

A, B and O Groups

The phenomenon of haemoagglutination is due to the interaction between two factors—agglutinogens*, present in the corpuscles and agglutinins, present in the plasma (or serum).

- There are two primary agglutinogens—A and B; and there are two corresponding agglutinins—α and β. The agglutinogens are inherited as mendelian dominants.
- The corpuscles of a particular subject may contain only A or only B or both A and B or no agglutinogens at all, i.e. O. Similarly, the serum may contain only α or only β or both α and β or no agglutinins.

Agglutinogens

Key Points

1. Agglutinogens start appearing in the sixth week of foetal life. Concentration gradually rises and at birth. It reaches one-fourth of the adult level. The adult level is reached at about puberty.
2. Agglutinogens A and B, are polysaccharides. They are not only found in red cells but in the cells of many other organs, such as salivary glands, pancreas, liver, lungs, testes, etc. They are soluble in water and as such diffuse out into the body fluids.
3. Thus, human beings may be out into four groups according to the nature of the agglutinogens possessed by their corpuscles. These groups are called O, A, B, and AB.
4. Group A is subdivided into A1 and A2.
5. Group AB is also divisible into A1B and A2B. Approximately 75–80% of group A belong to the subgroup A1 and 60% of group AB, to the subgroup A1B. The remainder belong to subgroups A2 and A2B.
6. Bernstein postulated that there are three allelic genes, A, B, O.

**Agglutinogens are also called isoagglutinogens, isohaemoagglutinogens or group specific substance. similarly, agglutinins are also known as isoagglutinins or isohaemoagglutinins. Agglutinogens and agglutinin in ABO blood group are listed in Table 17.1.*

From the Table 17.2 it is evident that group O can give blood to all but can take only from it own group. Hence, it is called universal donor. Group AB can take blood from all (universal recipient) but can give blood only to its own group. The terms universal donors and universal recipients are no longer applicable after the discovery of Rh factor. Group A and B can give blood to their own groups and also to group AB and can take blood from their own groups and from group O. Other combinations are not compatible (Fig. 17.1).

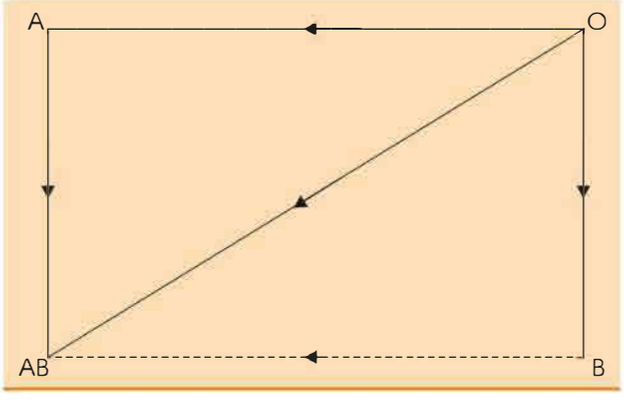

Fig. 17.1: The direction of the arrows indicates the type of donors who may give blood to the recipient of another group. It is self-evident that any patient is able to receive blood from donor of the same type as himself

Table 17.1: Agglutinogens and agglutinins in ABO blood group

Group	*Agglutinogens present in RBC*	*Agglutinins present in serum*
O	O	α and β
A	A	β
B	B	α
AB	A and B	Neither α nor β

Table 17.2: Reactions in red cells and plasma in ABO blood group

Donor's corpuscles	*Recipient's plasma*			
	0	*α*	*β*	*αβ*
O	–	–	–	–
A	–	+	–	+
B	–	–	+	+
AB	–	+	+	+

+ means agglutination (i.e. incompatible); – means no agglutination (i.e. compatible)

The four main ABO groups are listed in Table 17.1. While testing for compatibility the reaction between the donor's corpuscles and recipient's plasma, and the donor's plasma and recipient's corpuscles should be taken into account.

Taking into consideration all the above facts, the reactions between the red cells and plasma of the four blood groups are summarised in Table 17.2.

Rh Factor

Another important agglutinogens have been demonstrated (1940) in human red corpuscles also by Landsteiner and Wiener. It is the agglutinogens of the rhesus monkey and is present in 85% of White people. There is no corresponding agglutinin in the human plasma.

Karl Landsteiner
1868–1943

Recent studies indicate the Rh factor are not a single entity.

- There are six Rh agglutinogens—C, c; D, d; E, e. Of these, D and d are the commonest. These two will provide three subgroups—D, Dd and d. D is mendelian dominant, while d is recessive. Hence, groups D and Dd (collectively called D group) will be Rh positive (Rh +ve) and d will be Rh negative (Rh –ve).
- Practically all Rh positive people belong to D group and Rh negative people to group d.

Clinical importance: If Rh +ve blood be transfused to an Rh –ve patient, an anti-Rh factor will develop in the patient's blood in about 12 days. If a second transfusion of same blood be given to such a patient after this period, haemoagglutination of the donor's corpuscles will take place. In other words, blood which was compatible before has become incompatible now. So that before transfusion the test for Rh factor should be carefully done.

Erythroblastosis foetalis: During pregnancy the foetus may be Rh +ve whereas the mother Rh –ve. The Rh agglutinogens (slightly present also in the plasma) from the foetus pass into the maternal blood and stimulates the formation of anti-Rh factor. This antibody enters the foetal blood and destroys the red cells of the foetus. The foetus may die (causing miscarriage) or if born alive, suffers from severe anaemia (erythroblastosis foetalis). Such a mother becomes sensitised to Rh factor. In future if she gets a transfusion of otherwise compatible blood but containing Rh factor, agglutination will take place.

For the same reason, a Rh negative woman, before menopause should not be give transfusion of Rh positive blood. Because, in cases she becomes pregnant with Rh positive foetus, it can be a problem as described later.

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Rh Factor Details

- *Rh agglutinogens:* There are six or three pairs of Rh agglutinogens—C, c; D, d; and E, e. C, D and E are Mendelian dominants, while c, d and e are recessive.
- *Human red cells* will always carry three agglutinogens—one from each pair, but they will never carry both the members of any pair. Thus, CDE, CDe, cDE are possible but CcD and CDd are not.
- *Rh +ve and Rh –ve:* Groups containing the dominant agglutinogens, i.e. C, D, E will be Rh +ve. But since, C and E seldom remain without D practically all Rh +ve cases contain D, i.e. belong to group D.
- The Rh –ve cases will contains the recessive agglutinogens—c, d and e, and due to similar reasons stated above belong to group d. Every man carries some Rh agglutinogen. Majority have D and are Rh +ve. The rest carry d and are Rh –ve.
- All Rh incompatible reactions are due to interactions between group D (*donor*) and group d (*recipient*).
- *Rh antibody*
  - Each of the six agglutinogens has antigenic property, that is, they can stimulate antibody formation.
  - The corresponding antibodies are known as *anti-C*, *anti-D*, etc. D is strongly antigenic. Others are very feeble.
  - If D cells are repeatedly injected into a Rh –ve subject, anti-D will develop. This antibody may be of two types, early and late:
    - The early anti-D is formed first and is called complete antibody. It can agglutinate D cells *in vitro*, when they are suspended either in saline or albumin solution. Hence, it is also known as saline agglutinin.

- The late anti-D is formed later and is called incomplete antibody. It can agglutinate D cells *in vitro*, when they are suspended in albumin solutions only and not in saline solutions.

Specific Agglutinins

1. The specific agglutinins are not present in the foetal plasma. But maternal agglutinins, being filtered through the placenta, are found in the foetal plasma. Only 50% of newborn infants show an appreciable amount of this agglutinin.
2. Specific agglutinins start appearing from about the 10th day after birth and rises to the maximum at about the 10th year.
3. Agglutinins, like other antibodies, are found in the globulin fraction of the serum.
4. They are also present in low dilutions in body fluids which are rich in proteins, such as milk, lymph exudates and transudates. They are not found in urine and cerebrospinal fluid.
5. Haemoagglutinins increase temporarily during serum sickness and are reduced in leukaemia.
6. Like other antibodies, the concentration of specific agglutinin varies at all ages from man to man and even in the same individual under different conditions. They act best at a lower temperature.
7. The blood group of a particular subject is a fixed character and does not vary with age, or disease.
8. Non-specific agglutinins may sometimes appear in the blood which act in cold (at 0°–5°C.) and not at body temperature. These cold agglutinins may at times be sufficiently high to case auto-agglutination at body temperature. For this reason there may be intra-vascular haemolysis leading to haemoglobinuria (paroxysmal haemoglobinuria).

Haemolytic Disease of the Newborn

This disease is due to destruction of the Rh +ve RBC in the foetus by an anti-Rh agglutinin, present in the mother's serum, which has filtered through the placenta during pregnancy. The incompatibility between the blood of mother and child is caused by the inheritance of the Rh factor.

Incompatibility of the blood might arise only in case marked asterisk (*) in Table 17.3 (as in these two groups the mother is capable of producing an anti-Rh agglutinin to destroy the Rh +ve RBC), present in the foetus.

In this disease, destruction of the normal RBC leads to the presence of abnormal nucleated RBC in circulation. A few hours after birth there is anaemia, acute jaundice and related symptoms.

M and N Factors: Medicolegal Significance

Besides the A, B O system, other supplementary agglutinogens have been identified. They are known as M and N factors. This will provide three other independent groups M, N, and MN. These groups are of no importance for blood transfusion but have got medicolegal importance, e.g. paternity test.

Table 17.3: Genetic combination of rhesus factor

Father	*Mother*	*Children*
Rh +ve/Rh +ve	Rh +ve/Rh +ve	100% Rh +ve
Rh +ve/Rh +ve	Rh +ve/Rh –ve	50% Rh +ve/Rh +ve 50% Rh +ve/Rh –ve
Rh +ve/Rh –ve	Rh +ve/Rh –ve	50% Rh +ve/Rh –ve 25% Rh +ve/Rh +ve 25% Rh –ve/Rh –ve
Rh +ve/Rh –ve	Rh –ve/Rh –ve	50% Rh +ve/Rh –ve* 50% Rh –ve/Rh –ve
Rh +ve/Rh +ve	Rh –ve/Rh –ve	100% Rh +ve/Rh –ve*
Rh –ve/Rh –ve	Rh –ve/Rh –ve	100% Rh –ve/Rh –ve

Table 17.4: M and N factors

Child's group	*Parent's (Father) contributory group*	*Mother's group*	*Group absent in father*
M	M + M	Unrestricted	N
N	N + N	Unrestricted	M
MN	M + N	N	N
MN	M + N	M	M

There are two blood genes in each person, e.g. M + M, N + N or M + N. If a baby belongs to M group then the parents must have given M + M. If the baby has got N group then the parents must have given N + N. If the baby belongs to MN group then the parents must have given M + N. In the latter case if the mother's supplementary group is N then the father must belong to group M (Table 17.4). Other amino acids such as threonine, serine, protein, etc. The specificity of the blood group substances are dependent on the terminal components which may be non-reducing sugars discussed above.

This test can only show that the suspected person might or might not be the actual father.

In chemical composition, the blood group substances are nitrogenous, neutral, hetero-polysaccharide containing d-galactose, methylpentose fucose, d-glucosamine, d-galactosamine. Present as N-acetyl derivatives.

P System

Landsteiner and Levine in 1927 demonstrated still another system, P+ and P– by immunisation experiments in rabbits. Subsequently the antigen T1 was found and was shown to be part of the P system.

BLOOD TRANSFUSION: BLOOD GROUPS

Intravenous administration of blood to help replenish excess blood loss due to haemorrhage or otherwise, is known as blood transfusion. It is a most effective therapeutic tool when judiciously applied.

Total blood transfusion allows the transfer of those elements that are required for the defence against infection, the transport of oxygen, and the formation of platelet plug (a white thrombus). It permits the circulating blood to be re-established and corrects condition of shock and vascular collapse. It also supplies those clotting factors which are required for haemostasis. Modern transfusion methods not only use transfusion of stored blood from bottles in acid citrate dextrose (ACD) solution but it also uses cellular transfusions, plasma component transfusions, plasma transfusions, depending as the case may be, on the requirement of the patient. The blood may be stored for two or three weeks in a refrigerator at +4°C.

Indications for Blood Transfusion

1. Haemorrhage either acute or chronic (especially if the haemoglobin falls below 40%).
2. Shock in order to increase the blood volume.
3. Blood diseases, e.g. in all varieties of server anaemia where the haemoglobin is below 40% aplastic anaemia, haemorrhagic diathesis of the newborn, haemophilia, purpura haemorrhagica, etc. in haemorrhagic diseases blood transfusion increases coagulability.
4. In carbon monoxide poisoning, coal gas poisoning, etc. where haemoglobin has formed some other abnormal compounds, blood transfusion provides a fresh supply of oxyhaemoglobin.

Haemoagglutination: When blood of one species of animal is mixed with that of another; it is often seen that the corpuscles are agglutinated first and then haemolysed. Amongst human beings this phenomenon of haemoagglutination is also seen. The red corpuscles of one subject may be agglutinated by the serum or plasma of another.

These facts are of extreme importance in connection with blood transfusion. In various diseases, it is sometimes necessary to inject a large quantity (even 500 ml) of blood, taken from a healthy man (donor), directly into the vein of the patient (recipient). If the two bloods be not compatible haemolysis of the donor's corpuscles will take place, leading to disastrous effects to the recipient. For this reason, before undertaking blood transfusion, compatibility between the donor's blood and the recipient's blood must be carefully tested.

Autologous Transfusion

A procedure to withdraw the patients own blood in advance of elective surgery and then infuse this blood back if transfusion is needed during surgery has become popular.

Mismatched Blood Transfusion

The symptoms of a haemolytic transfusion reaction most often appear during or right after the transfusion. Symptoms include head, chest and flank pain, fever, chills, flushing, rigors, nausea and vomiting, urticaria, dyspnoea and hypotension. The further complications include haemoglobinuria, disseminated intra-vascular coagulation, transfusion related lung injury which manifest with signs of hypoxaemia, dyspnoea, cyanosis, fever, tachycardia and hypotension. The haemoglobinuria may further lead to anuria and still further may progress to renal failure. Mismatched blood transfusion leads to red blood cell haemolysis which releases haemoglobin which gets converted to bilirubin producing haemolytic jaundice. There is also risk of transmission of infections like malaria, syphilis, AIDS, etc.

17

BLOOD BANK

Maintenance of a constant volume of blood is essential for our life. If due to any reason, there is excessive loss of blood, it must be transfused from other sources. Due to this reason blood should always be kept handy for emergency and establishment of blood bank is essential in every hospital. So, blood bank is a specialised medical centre which collects and stores human blood for transfusion. The blood thus collected is separated into fractions, each of which has a particular use, serum, red cells, white cells, platelets, etc.

- Whole blood in presence of acid citrate as anti-coagulant can be preserved with dextrose solution at +4°C for several weeks.
- Red cells kept in such a condition are destroyed in a similar way as the normal RBC and so older samples contain lesser number of cells.
- Frozen separated plasma can be kept for years at –20°C.
- Lyophilised (frozen dried) plasma, from which the water has been removed, can even be stored at room temperature. Required amount of water is added to it during use.

EXAM-ORIENTED QUESTIONS

Short Notes

1. Classify blood group
2. ABO blood group system
3. Rh factor
4. Indication of blood transfusion
5. Mismatched blood transfusion
6. Storage of blood
7. Significance of MN blood group system

Chapter

18

Formation of Tissue Fluids

INTRODUCTION

Tissue fluid is formed from the plasma by process of diffusion and filtration. This fluid occupies the intracellular space and forms the connecting link in the transport of nutrition, gases and the metabolic end products between blood capillaries, tissue cells and the lymph. It constitutes the internal environment of the body, which surrounds tissue cells.

Tissue fluid is derived from two sources:

Blood capillaries: The amount of tissue fluid formed from blood depends upon:

a. Capillary permeability.
b. The difference of pressure between the capillary and the tissue fluid.
c. The difference of colloidal osmotic pressure of blood and tissue fluid.

It is obvious that anything that increases the capillary permeability will increase the amount of tissue fluid formed. Regarding blood pressure and osmotic pressure, it is known that at the arterial end of capillaries, the average blood pressure is about 32 mm of Hg and at the venous end, 10 mm of Hg. The colloidal osmotic pressure at both ends is same (25 mm of Hg on the average). At the arterial end, the net filtration pressure which is the difference between the two is 7 mm of Hg towards the tissue (interstitial) fluid. At the venous end due to fall in blood or hydrostatic pressure, the filtration pressure is 15 mm of Hg to the opposite side, i.e. from tissue fluid to the capillary (Fig. 18.1).

Tissue Activities

The amount of tissue fluid formed from the tissue cells depends upon the degree of metabolic activity of the cells. Tissue cells produce water as an end product of metabolism. This metabolic water is added to the already existing tissue fluid. More the degree of activity more will be the metabolic water formed and consequently the amount of tissue fluid will increase.

Two important exceptions to the capillary pressure are:

a. In capillaries of the lungs, hydrostatic pressure is about 6 mm of Hg
b. In capillaries of the kidneys, glomerular hydrostatic pressure is about 60 to 70 mm of Hg.

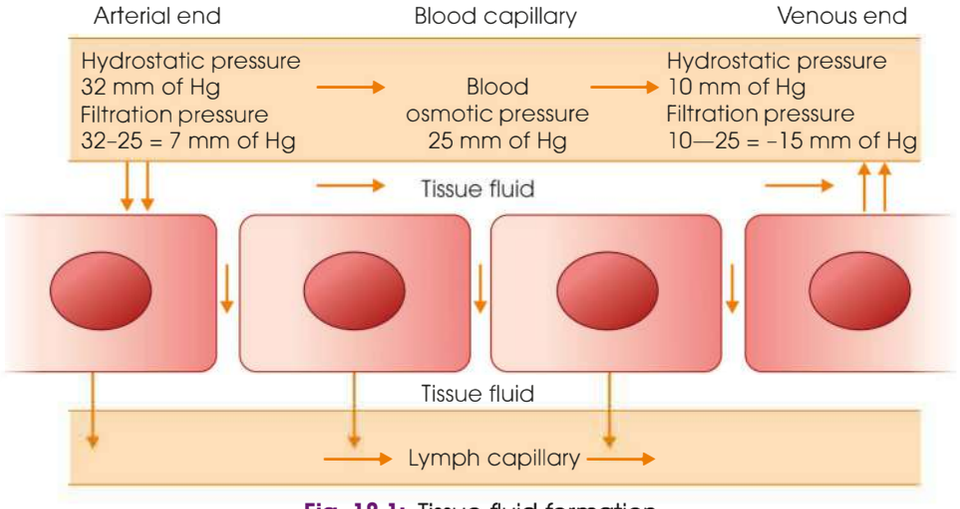

Fig. 18.1: Tissue fluid formation

If the hydrostatic pressure is increased within capillaries, then it will interfere the return of materials to the lymphatics or capillaries and will result in excess accumulation of tissue fluid (i.e. oedema).

Composition of Tissue Fluid

Its composition is same as that of lymph, except that its protein content is negligible; and as such, its colloidal osmotic pressure is very low. The specific gravity of the tissue fluid is about 1.015 to 1.023. It may contain a few erythrocytes. But regarding the white cells, the tissue fluid contains a good number of lymphocytes and a small number of granulocytes. Blood proteins and nutrient contents of it are very low. It does not contain platelets and may also clot, but with a very slow process. It contains higher concentration of waste products but glucose, salt and water contents are more or less same as those are present in blood.

Functions of Tissue Fluid

1. It constitutes the internal medium in which the tissue cells are bathed. The cells draw in oxygen and nutrition from the tissue fluid and excrete their metabolites into it. Hence, tissue fluid may be regarded as the medium which supplies all the immediate requirements of the cell.
2. It acts as a great reservoir of water, salts, nutrition, etc. This function is very important.
3. Under any condition, in which the blood volume is increased or diminished, physical forces are set up by which the blood volume is kept constant with the help of the tissue reserve. For example in haemorrhage, the capillary pressure becomes very low and goes below the colloidal osmotic pressure in the capillary which remains same.
4. **Restores blood volume:** Due to this higher OP in the capillaries, water is drawn in from the tissue spaces, so that blood volume is restored. When water is drawn away from blood, such as due to diuresis, excessive sweating or diarrhoea, blood volume and blood pressure will be lowered, but the plasma proteins will be more concentrated. This will increase the colloidal OP of blood. This increased osmotic pressure of plasma and reduced blood pressure will increase the rate of absorption from the tissue fluid, and thus blood volume will be kept constant.

   On the other hand, when blood volume increases, as for instance, by intravenous injection of large quantities of isotonic saline, fluid will pass out into the tissue spaces due to two causes:
   1. Saline will dilute the colloids and reduce the colloidal osmotic pressure.
   2. Increased volume of blood will raise the blood pressure and cause more filtration. Both these factors will cause more fluid to run out into the tissue spaces, until blood volume comes back to the original level.

OEDEMA AND ITS CAUSES

Aggregation of Tissue Fluid

1. Swelling or oedema observed sometimes in different parts of the body is due to the aggregation of the tissue fluid. This might result from several factors:
2. Increased capillary permeability resulting from dilated, damaged or inflammated capillary.
3. Increase in the capillary pressure which might be due to changes in posture (in lower extremities it is due to continued standing), obstruction to veins or rise in the venous pressure as observed in the cardiac failure.
4. Blockage of lymphatic nodes or vessels, as a result of inflammation of the node or blockage by very small worms like that of filaria.
5. Loss of the plasma proteins whether due to malnutrition or excessive loss resulting from the renal damage, causes decrease in plasma osmotic pressure and excessive aggregation of the tissue fluid.
6. Renal disease causes impairment of excretion of urine and the resulting water retention causes increase in the tissue fluid.
7. Unfamiliar exercise might cause swelling due to accumulation of metabolites.
8. Ingestion of a large amount of salts results in retention of water. Adrenal cortical extract also produces similar effects.

LYMPH AND LYMPHATICS

Characteristic Features

1. The lymphatic vessels at the periphery are microscopic blind (closed) end vessels, known as lymphatic capillaries. These tiny vessels are situated in the intercellular spaces and their walls formed by endothelial cells supported by the fibrous connective tissue (Fig. 18.2). These capillaries repeatedly join together to form bigger lymphatic vessels, which pass through the lymph nodes, receive more tributaries and gradually increase in size.
2. All the lymph from the body is finally collected into two big channels—the right lymphatic duct and the thoracic duct (or left lymphatic), which open respectively at the right and left subclavian veins. The right lymphatic duct, about 1.25 cm long, drains from the right forelimb and the right side of the neck and chest (Fig. 18.3).
3. The thoracic duct, being about 38–45 cm long and about 4–6 mm in diameter, emerges from the cisterna (receptaculum) chyli and also receives the left cervical duct, which collects lymph from the left forelimb, left side of the neck and chest. The cisterna

Fig. 18.2: Relationship of blood capillaries (containing RBC) to lymph capillaries

chyli, being situated on the front of the body of the second lumbar vertebra, receives all the lymph coming from two hind limbs and alimentary canal (Fig. 18.3).

4. The lymphatic vessels are provided with valves which help the lymph stream to flow in the direction of the chest.
5. The primary lymphatic vessels that remain in the centre of small intestinal villi are known as lacteals and during the course of digestion lacteals are filled with milk-white fluid, chyle.
6. The chemical composition of chyle, except for its high fat content, is similar to that of the lymph in other parts of the body. In the central nervous system there are no lymphatics. Here, cerebrospinal fluid takes the place of lymph.
7. Lymphatic capillaries are not also found in the cartilage, spleen, epidermis, internal ear and eyeball.
8. The function of lymphatics is to carry tissue fluid from tissues to veins and the return of water and protein from the interstitial fluid to blood from which they come. And the function of lacteals is to help in the absorption of digested food materials generally fats from the intestine.

PROPERTIES OF LYMPH

Lymph should be regarded as modified tissue fluid. Lymph is the clear watery-appearing fluid found in lymphatic vessels and is formed by the passage of substances from blood capillaries into tissue spaces. After a fatty food, the lymph of the thoracic duct appears milky due to the presence of minute droplets of emulsified fat absorbed from the alimentary canal.

COMPOSITION OF LYMPH

Microscopic examination of lymph depicts that it contains a large number of leucocytes (mostly lymphocytes) ranging from 500 to 75,000 per cu mm. No blood platelets present. The composition of the non-cellular part of lymph (fasting) is as follows: Water: 94% and solids: 6%.

Solids

Protein: Total protein content is roughly half that of plasma and varies from 2 to 4.5%.

Fats: In fasting condition fat content is low but after a fatty diet it may be 5–15%.

Carbohydrates: Sugar, 132.2 mg per 100 ml (dog's plasma contains 123 mg per 100 ml on the average).

Other constituents (expressed in mg per 100 ml): Urea, 23.5 mg (plasma, 21.7 mg); non-protein nitrogenous substance, 34.8 mg (plasma 32.6 mg); creatinine, 1.4 mg (plasma 1.37 mg); chlorides, 711 mg (plasma 678 mg); total phosphorus, 11.8 mg (plasma 22 mg); inorganic phosphorus 5.9 mg (plasma 5.6 mg) and calcium is 9.84 mg (plasma 11.7 mg). Enzymes and antibodies are also present.

Rate of Flow

Rate of flow of lymph along the human thoracic duct is from 1 to 1.5 ml per minute. In dogs, it is much higher. Lymphatogogue is the substance that increases the rate of lymph flow. Regulation of the rate of lymph flow depends upon (a) interstitial pressure, (b) arterial pulsation, (c) intra-thoracic pressure, and (d) muscular massage.

FACTORS RESPONSIBLE FOR FORMATION OF LYMPH

Since lymph is formed from tissue fluid, anything that increases the amount of tissue fluid will increase the rate of lymph formation. Lymph formation depends upon physical factors. There is no vital secretory process involved in it.

The following factors are responsible for lymph formation:

1. *Capillary pressure:* If the capillary pressure is raised, the rate of lymph formation increases. This is seen in venous obstruction. (But after some time, the rate slows down due to increased accumulation of fluid in the tissue spaces and the consequent rise of hydrostatic pressure of the tissue fluid.)

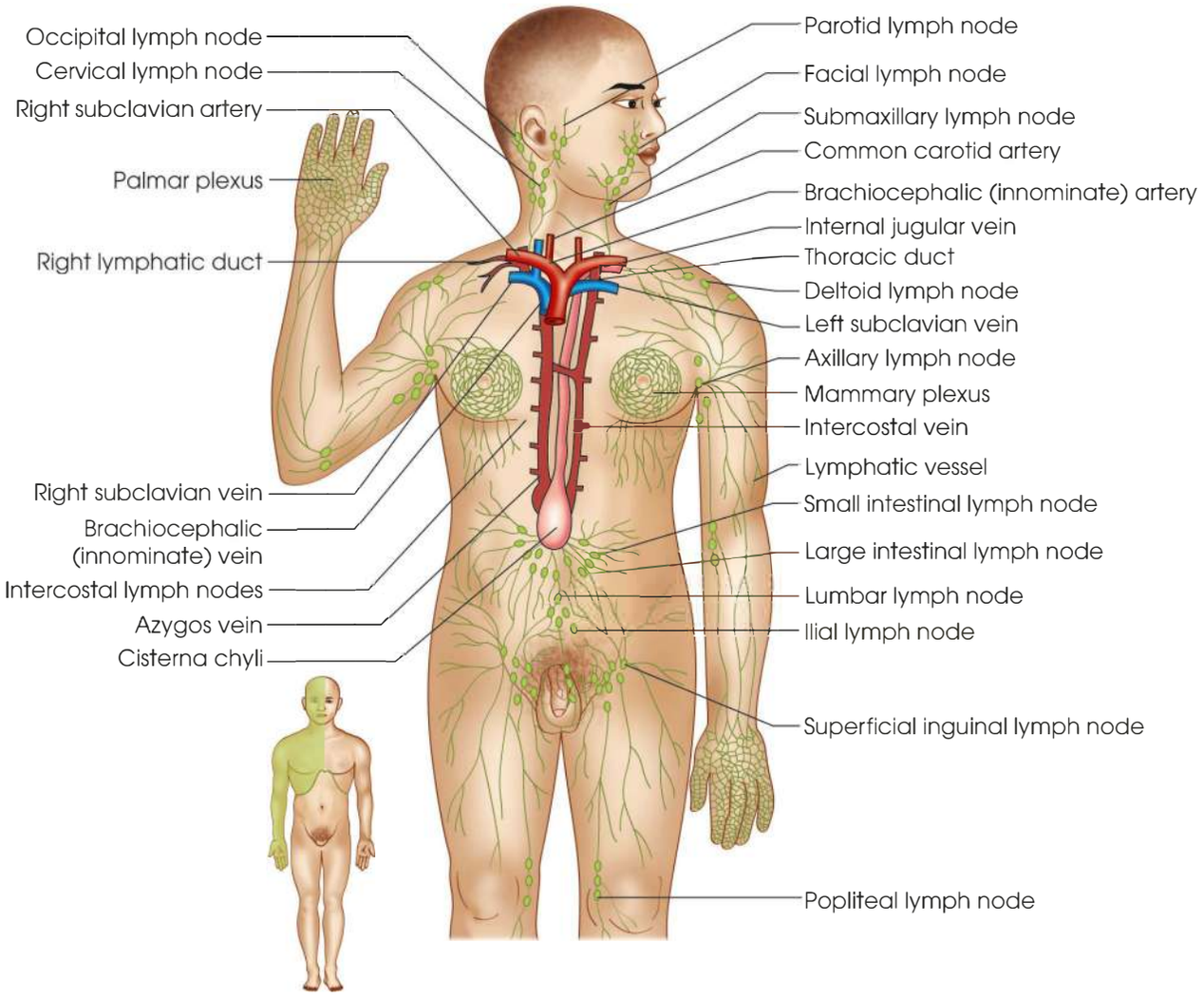

Fig. 18.3: Anatomical organisation of lymph circulation; Note: Shaded portion showing the regions of the body from which lymph flows into the right lymphatic duct and the other area representing lymph, from all the rest of the body, which enters the general circulation by way of the thoracic duct

2. *Permeability of the capillary wall:* Under any condition, where the permeability of the capillary wall is increased, more tissue fluid will be formed and consequently more lymph. The following factors increase capillary permeability:
   - *Rise in temperature:* Increased temperature of a particular locality increases capillary permeability.
   - *Substances acting directly on the capillary wall:* Peptone, foreign proteins, histamine and extracts of strawberries, crayfish, mussels, leech, etc. exert an injurious effect upon the capillaries and thereby increase their permeability.
   - *Reduced oxygen supply:* Under conditions of oxygen lack lymph flow increases due to higher permeability of the vessels. It acts probably by damaging the capillary endothelium. Anoxia, anaemia, stasis of blood due to vascular congestion, produces such results.
3. *Substances that alter the osmotic pressure:* Anything that reduces the colloidal osmotic pressure of blood will increase the formation of tissue fluid and lymph. Normal or hypotonic saline, when given intravenously, will dilute the plasma colloids and reduce the osmotic pressure. Moreover, blood pressure will be raised. Both these factors will favour formation of tissue fluid and lymph.
4. Hypertonic solutions will exert the same effect in a better way. Hypertonic solutions, introduced in the blood, will draw in more fluid from the tissue spaces at first and will increase the blood volume further. Blood pressure will be raised to a great extent, and plasma colloids will be further diluted. In this way hypertonic solutions will increase the formation of lymph much more than the normal or hypotonic solutions. Solutions of NaCl, glucose, Na_2SO_4, etc. may be used for this purpose.

5. *Increased metabolic activity of an organ:* Increased activity of a particular area increases the flow of lymph in the locality. It is due to:
   - Formation of more metabolites which increase the osmotic pressure of the tissue fluid.
   - Local vasodilatation and increased capillary pressure and permeability.
   - Relative anoxia.
   - Increased temperature of the locality.

   The last two also act by increasing the capillary permeability.
6. *Massage and passive movements:* These increase lymphatic flow to some extent just like active muscular contraction.

Functions of Lymph

1. **Nutritive:** It supplies nutrition and oxygen to those parts where blood cannot reach.
2. **Drainage:** It drains away excess tissue fluid and the metabolites and in this way tries to maintain the volume and composition of tissue fluid constant.
3. **Transmission of proteins:** Lymph returns proteins to the blood from the tissue spaces.
4. **Absorption of fats:** Fats from the intestine are also absorbed through the lymphatics.
5. **Defensive:** The lymphocytes and monocytes of lymph act as defensive cells of the body. The lymphatics also remove bacteria from tissues.

LYMPH NODE

Structure of Lymph Node

Lymph node is small, oval or bean-shaped body. It is a collection of lymphoid tissue enclosed in a connective tissue capsule and lying along the lymphatic stream. From the capsule, strands of tissue pass into the substance of the node and are known as the connective tissue trabeculae. Histologically, the node can be divided into an outer part—the cortex, and an inner part—the medulla (Fig. 18.4).

Cortex: The lymphoid tissues remain scattered throughout the node but in the cortex they are found in especially collected islands—the lymphoid nodules or follicles which vary from 0.35 to 1 mm in diameter. These nodules remain arranged parallel to the surface, sometimes two or three layers deep. Each lymph nodule is pierced by a small blood vessel and shows a less dense area at its centre, which takes a lighter stain. Surrounding this lighter zone there is a wider area packed with lymphocytes. The central zone (lighter) is called the germinal centre or secondary nodule, whereas the peripheral area is called the cortical nodule or primary nodule. These lymph nodules are separated from the trabeculae and capsule by blood sinuses.

Medulla: The medulla of the lymph node is much less dense than the cortex and is devoid of lymphatic nodules. It consists of scattered lymph cells, different

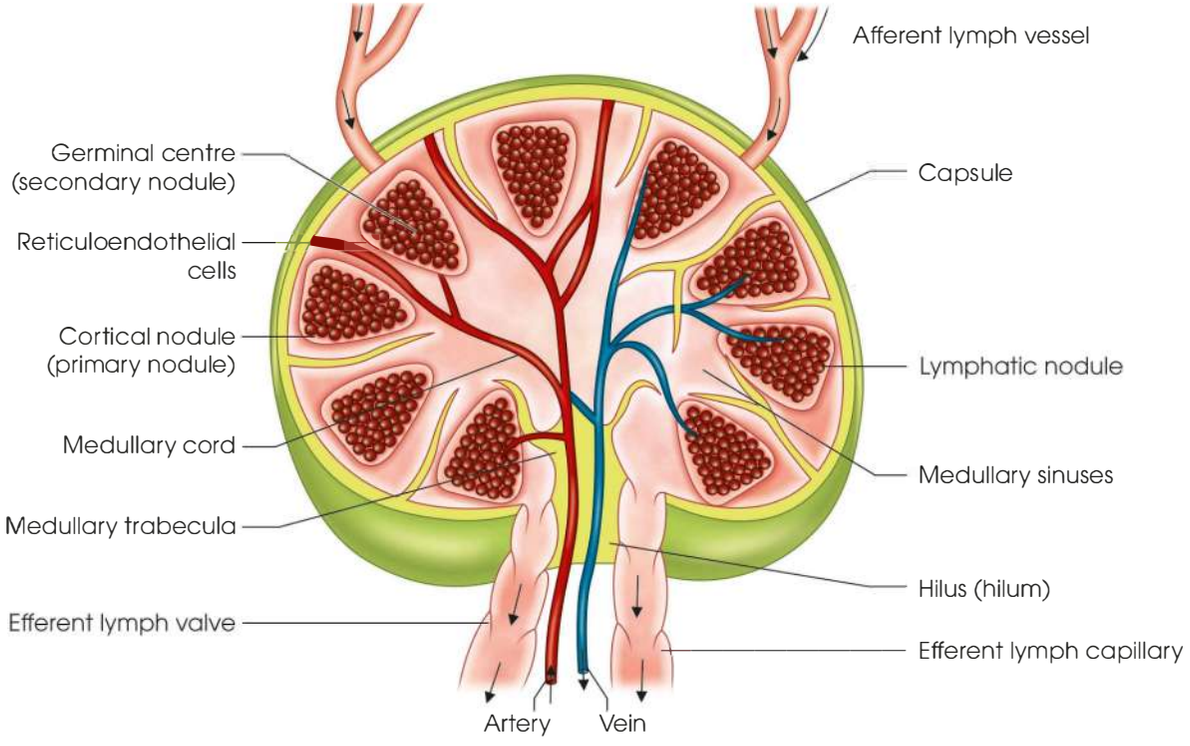

Fig. 18.4: Structure of a lymph node. Efferent lymph capillaries leave the lymph node at the hilus (hilum). The artery and the vein enter and leave at the hilus (diagrammatic representation)

varieties of reticuloendothelial cells and sometimes a few multi-nucleated giant cells (Fig. 18.5).

Hilus (hilum): At one side of the node there is a depression, known as the hilus were the capsule is thickened. Here the cortical part becomes very much thin and the medulla comes to the surface. At the hilus the node is pierced by three vessels—an artery, a vein, and the efferent lymph channel. The artery after entering the node breaks up into numerous arterioles which proceed at first along the trabeculae and then each arteriole breaks up into a bunch of capillaries which becomes surrounded by masses of lymphatic cells. The capillaries are then collected into venules, which join repeatedly to form the main vein that leaves the node through the hilus. The nerves supplying the lymph nodes are non-medullated autonomic fibres, which are distributed to the plain muscles in the blood vessels, the capsule and the trabeculae.

Functions of Lymph Node

1. They produce and supply lymphocytes to the blood and as a supportive function the trabeculae carry blood vessels which supply the node.
2. They make screening of the lymph by means of phagocytic activity.
3. They serve a great defensive role against bacterial infections (Fig. 18.5).
4. They temporarily stop the spread of cancer cells as those cells have to penetrate through the lymph vessels to the lymph nodes from where they spread in the body.
5. They act as mechanical filters to resist the entrance of poisonous substances into circulation.
6. They carry out immunological responses. They help in elaboration of antibodies and in the development of immunity.
7. Lymph nodes produce γ-globulin.

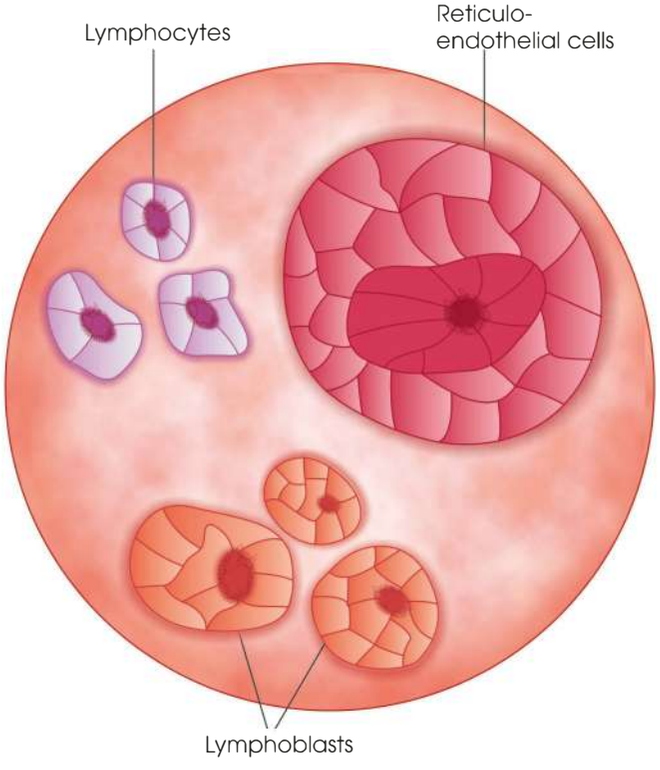

Fig. 18.5: Cells found in lymph node

Spleen

Spleen (*lien*) is the largest lymphoid tissue in the body and specialised, been-shaped organ for filtering blood. It is a highly vascular haemopoietic organ situated in the left hypochondrium directly beneath the diaphragm, above the left kidney and descending colon, behind the fundus of the stomach and weighing about 150 gm in adult. It also plays an important role in the metabolism and defense mechanism of the body. There are no afferent lymphatic vessels.

Structure of Spleen

Histologically it consists of (Fig. 18.6):

- Capsule with its outer covering the peritoneum
- Trabeculae with blood vessels or without blood vessels hilus (hilum)
- White pulp scattered throughout the red pulp.
- Red pulp
- Reticular meshwork
- Blood vessels.

Spleen is covered by a connective tissue capsule which is again enveloped by a serous membrane, the peritoneum. The peritoneum is closely adherent to the outside of the capsule. The capsule is deeply indented at the medial aspect of the organ and this indentation is known as hilus (hilum) of the spleen. Blood vessels, lymphatics and nerves pass through the hilus. From the inner surface of the capsule and from the hilus, many trabecuale radiate into the substance of the spleen and subdivide or delineate the organ into many communicating compartments or lobules. Each lobule is supplied with blood vessels that run along with the trabeculae. The lobules are not distinct because these are not completely surrounded by trabeculae.

Splenic Pulp

The parenchymal tissue which is enclosed within the capsule is the splenic pulp. The splenic pulp is of two distinct types: (1) White pulp and (2) red pulp. The white pulp is composed of typical lymphatic tissue whereas the red pulp is composed of an atypical lymphatic tissue.

1. **White pulp:** In a freshly sectioned spleen the white pulps are seen scattered all throughout the red pulp as grey patches. These grey patches at early periods were described as Malpighian bodies. But for

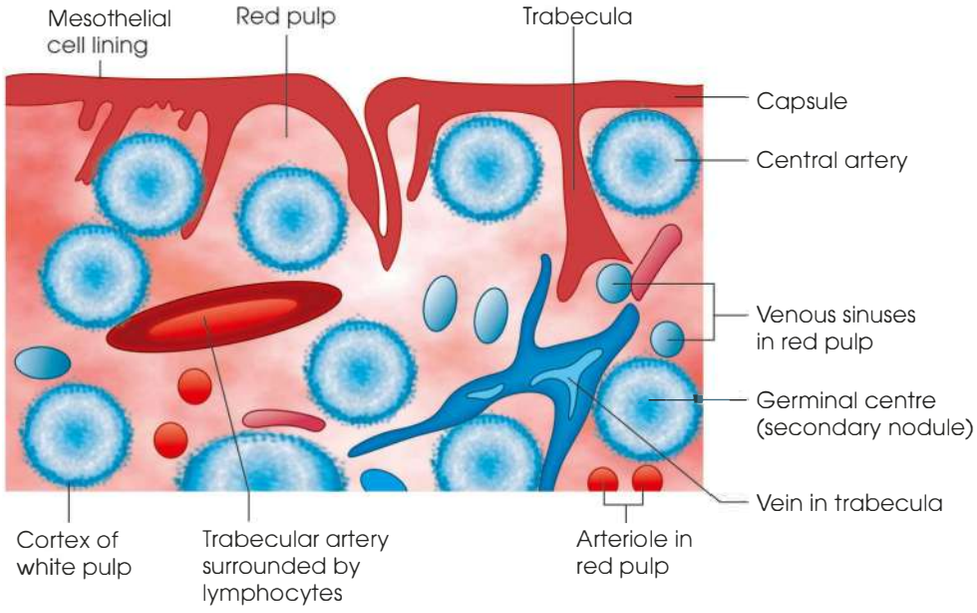

Fig. 18.6: Histological structure of spleen

confusion these terminology has been avoided in the literature in case of spleen and instead, white pulp has been used.

The white pulp is the accumulation of lymphatic tissue surrounding major arterial vessels of the spleen. This lymphatic tissue is comprised of lymphocytes, plasma cells, macrophages or other free cells lying in the meshwork of reticular fibres. In the white pulp, two distinct components are seen. As the arteries leave the trabeculae and enter the splenic parenchyma, the vessels lose its adventitia and are replaced by reticular tissue followed by invasion with lymphocyte. This constitutes the periarterial lymphatic sheath of the white pulp. At various points along the course of the vessels where the infiltration of lymphocytes is greater, it forms spherical or ovoid nodules, called splenic nodules of white pulp. The splenic nodules may have typical germinal centres.

2. **Red pulp:** It is a modified lymphatic tissue and is mostly infiltrated with cells of the circulating blood. It consists of two components:
   - *Splenic sinuses or sinusoids:* These are long vascular channels having 35 to 40 µm in diameter. They may have an irregular course and may vary in diameter. They extend throughout the red pulp.
   - *Splenic (or Billroth) cords:* They appear as continuous partitions in between the sinuses. These cellular cords ultimately form a spongy network of modified lymphatic tissue that gradually merges into the white pulp. In mammalian embryos the red pulp contains myelocytes, erythroblasts and also megakaryocytes. These types of cells are not present in adult spleen except in certain pathological conditions.

Marginal Zone

It is the junctional region in between the white pulp and the red pulp, and consists of a meshwork of branched reticular cells in association with extracellular reticulum, into which many arterial vessels open.

Blood Vessels and Nature of Blood Circulation

At the hilus of the spleen, arteries enter and divide into several trabecular branches. The trabecular (interlobular) branches pass along the trabeculae and after coursing for a certain distance the arteries may enter the splenic parenchyma. After entering the splenic parenchyma the artery loses its adventitia and takes the character of reticular tissue and afterwards become infiltrated with lymphocyte. In this way the vessels are ensheathed with lymphocyte constituting the periarterial lymphatic sheath. Along the blood vessels and at various points there are greater infiltrations of lymphocyte forming the so-called splenic nodule of the white pulp.

Thus, after leaving the trabeculae as central artery or arteriole passes through the white pulp where it gives off several branches. From here arterioles enter the red pulp. Here the arteriole is subdivided into several branches and as these vessels lie close like a brush, are called penicillar vessels. These penicillar vessels have three distinct successive components; the first long portion having thin smooth muscle is known as pulp arteriole; the middle one having thick sheath is known as sheathed arteriole or ellipsoid or Schweigger-Seidel sheath, the terminal one is arterial capillaries one to two in number. The arterial capillary ultimately ends in the splenic venous sinuses. The blood from the splenic venous sinuses empty into the pulp vein which combines to form the large veins and ultimately blood

return in the trabecular vein. In the hilus many trabecular veins join to form splenic veins and as such leave the spleen (Figs 18.7 and Fig. 18.8).

FUNCTIONS OF SPLEEN

1. **Formation of blood:** In the embryo, the spleen functions as a haemopoietic organ of some importance but in normal adult life it never functions in the formation of RBC. In some pathological conditions, the spleen may undergo myeloid metaplasia and a large number of erythroblasts, megakaryocytes and myelocytes appear in the tissue.
2. **Blood destruction:** The old red cells and white cells are destroyed by the RE cells of the spleen. Spleen has got some influence on the formation of platelets. In thrombocytopenic purpura, splenectomy causes increase in the number of platelets in the blood. Haemoglobin breaks down and formation of bilirubin also takes place in the spleen (biligenic function). Thus, in the post-natal life, spleen acts as a filter, which removes the old useless cells and allows only the young active cells to pass into the circulation.
3. **Reservoir of blood:** Spleen acts as a great reservoir of blood. It is observed that the spleen may release about 150 ml of blood (mainly erythrocytes) to circulation. In cat, as much as one-sixth of the total blood volume or one-third of the total red cells may remain in the spleen. In anoxic conditions (such as in haemorrhage, asphyxia, severe muscular exercise, high altitude, etc.), spleen contracts and sends out the stored blood into the general circulation. Adrenaline injections also have the same effect. All these conditions stimulate the sympathetic system, which causes contraction of spleen. In carbon monoxide (CO) poisoning, this is a great help. Due to the presence of slow circulation in the spleen, CO cannot saturate all the splenic red cells. When spleen contracts these healthy red cells are turned out into the circulation and are used for carrying oxygen.
4. **Relation to storage and metabolism**
   Pigment metabolism: Haemoglobin is broken down into haem and globin, the haem is further split into iron and pigment haematoidin, which becomes bilirubin of the plasma.
   Iron metabolism: The iron that is liberated from haemoglobin is at first stored in the splenic pulp cells.

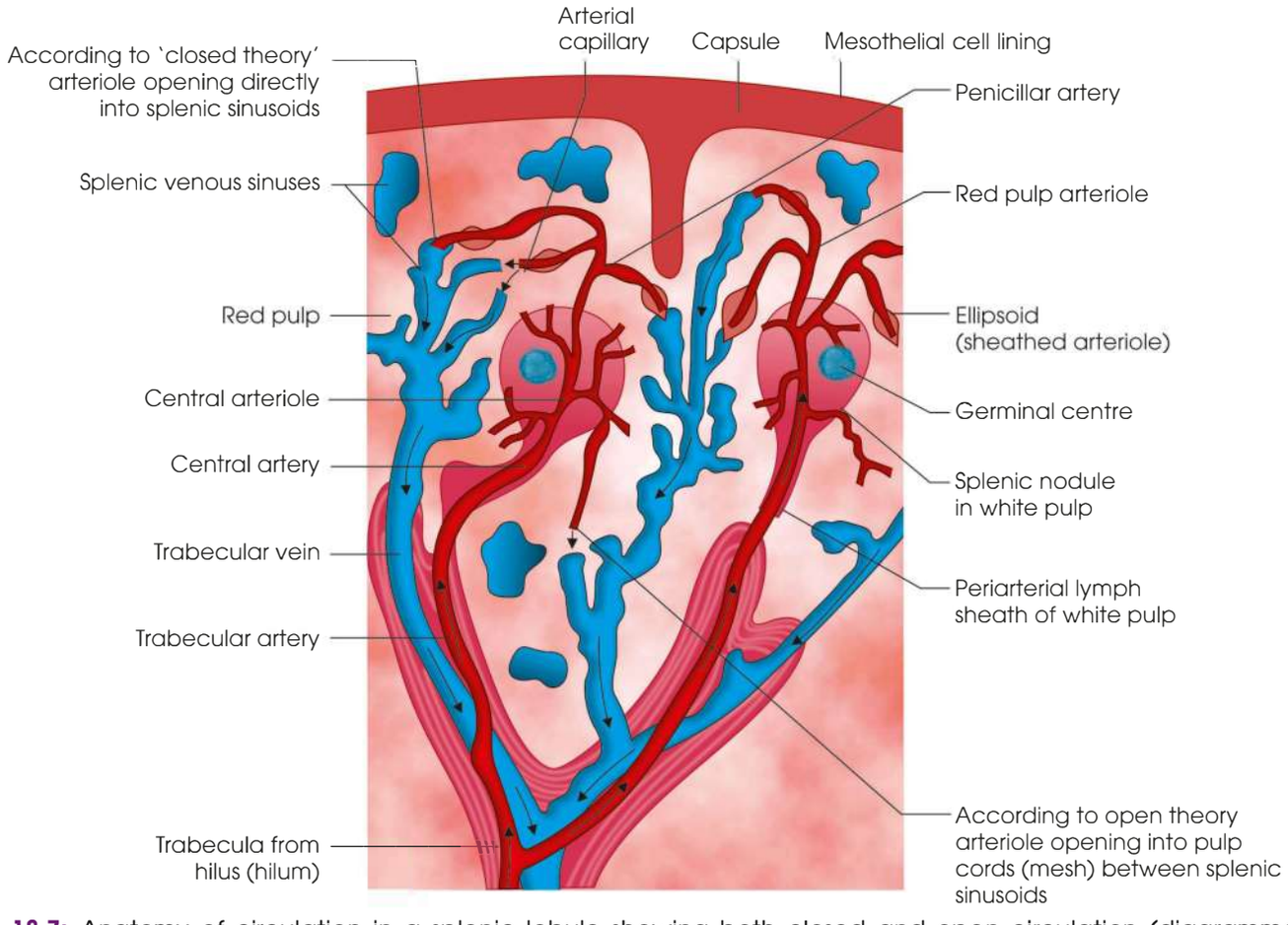

Fig. 18.7: Anatomy of circulation in a splenic lobule showing both closed and open circulation (diagrammatic representation)

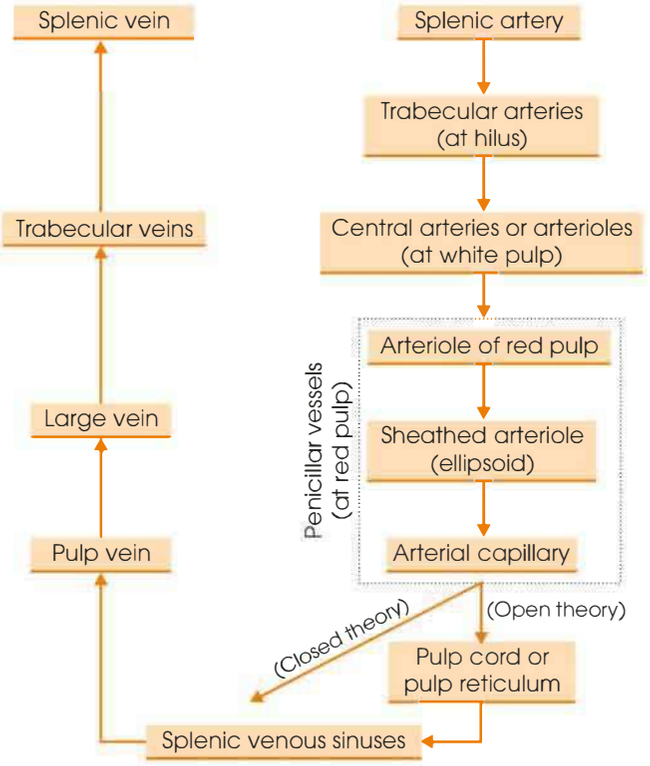

Fig. 18.8: Anatomy of circulation in spleen

Then it is gradually transferred to other places, being carried by the monocytes and the detached RE cells. It is specially taken to the liver for storage and to the bone marrow for further haemoglobin synthesis. After splenectomy this storage function suffers and more iron is lost and iron-containing pigment haemosiderin (storage form) are deposited in the spleen.

5. **Defensive action**
   - Many pyroninophil cells (probably plasma cells) are found in the splenic red pulp and hence the spleen is a chief site of immune body formation. Splenectomised animals cannot be immunized against tetanus toxin. Such animals easily succumb under intercurrent infection.
   - The RE cells engulf bacteria, parasites like those of Leishman-Donovan bodies in kala-azar and foreign particles.
   - The pulp cells unite with certain toxins, especially of diphtheria and remove them from general circulation.
   - The lymphoid cells of spleen also react against infections.
6. **Manufactures haemolysin:** When red cells of one species are repeatedly injected in another, a specific haemolysin is formed in the spleen.

Clinical cases have been reported, where spleen was found to elaborate a haemolysin-causing severe haemolytic anaemia in the patients. After splenectomy such cases were cured. From such evidence it is thought that spleen is either normally concerned with actual haemolysis of old red cells or prepares them for final haemolysis.

Classification, Varieties and Distribution of RE Cells (Fig. 18.9)

The cells of the RE system may be divided into two large groups.

Fixed Reticulo-endothelial Cells

They include the following varieties:

The tissue histiocytes: They are found in the connective tissues and loose areolar tissue of the serous membranes, viz., omentum,, pleura, etc. Although they are generally fixed, yet, under suitable stimulus (such as inflammation), these cells may become actively motile.

The reticulum cells of the spleen, lymphatic node and bone marrow: They remain in the reticular spaces of these organs. These are large cells and remain joined to one another by long branching processes. Under suitable stimulus they may be actively motile.

This method is generally adopted for staining the cells of this system. The vital dye is intravenously injected, the animal is then killed and the various tissues and organs examined under microscope.

- *The endothelial cells:* They are present in the lining membrane of the blood sinuses of spleen, bone marrow, adrenal cortex, pituitary, liver, etc. In the liver they appear as large, flat, stellate cells lining the blood capillaries and are known as the Kupffer cells.
- *Microglia:* These are small cells found in the central nervous system.

Wandering Reticulo-endothelial Cells

They comprise the following varieties:

- *The wandering histiocytes:* These are large wandering cells found in the omentum, splenic pulp, lymph nodes, bone marrow, etc. Due to their large size they are also called macrophages. Sometimes they may contain many nuclei, for instance, osteoclasts and megakaryocytes of the bone marrow. In addition to these the fixed tissue histiocytes, described above, may become wandering.
- *The wandering RE cells of the blood stream:* They are of two varieties:
  1. The normal monocytes of blood.
  2. Abnormal foreign cells which generally remain in the tissues but enter the blood stream under the influence of some pathological stimulus. Such cells are found in leukaemia, bacterial endocarditis, etc.

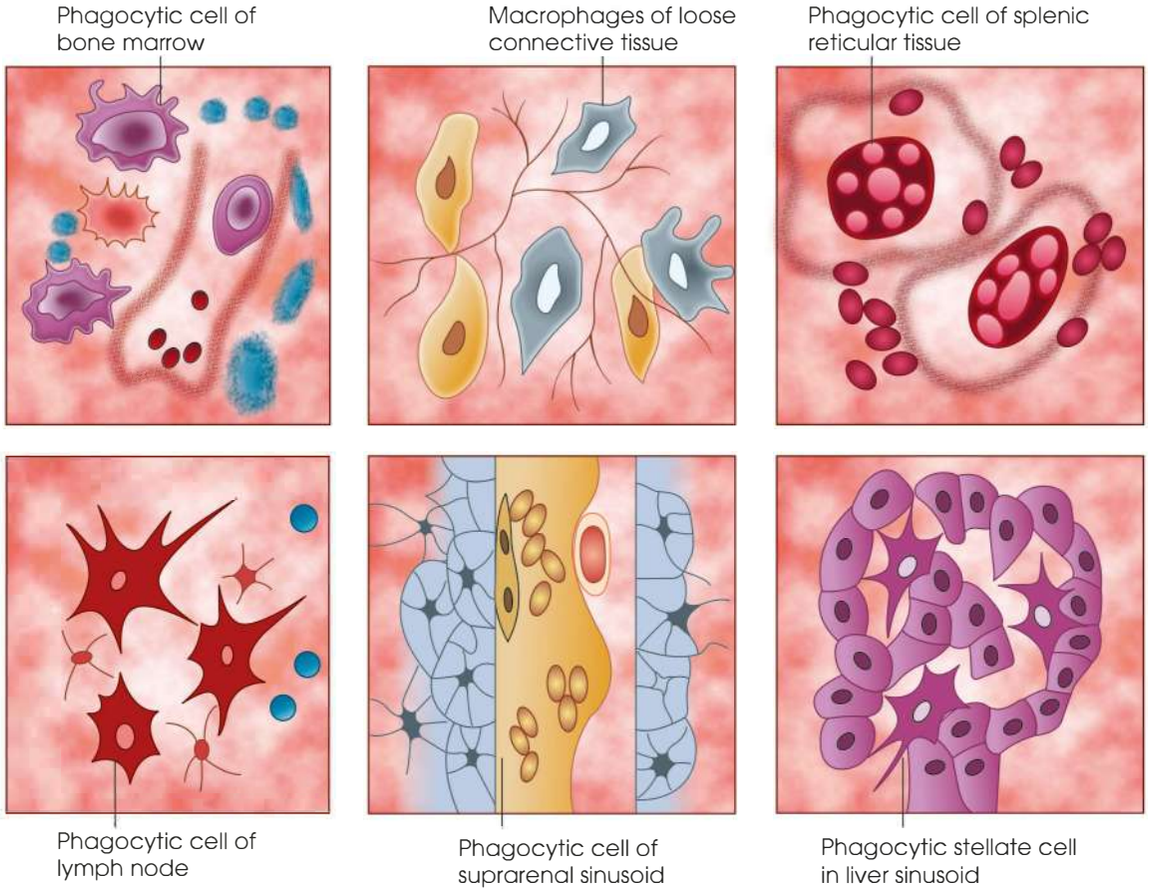

Fig.18.9: Different types of cells in reticulo-endothelial (macrophage) system (diagrammatic representation)

FUNCTIONS OF THE RETICULO-ENDOTHELIAL SYSTEM

1. **Phagocytosis:** This is the main characteristic property of this system of cells. They engulf foreign particles, bacteria and parasites and in this way, act as a great defensive mechanism.
2. **Formation of antibodies:** The antitoxic and antibacterial bodies are manufactured by this system. This is also a great protective mechanism.
3. **Formation of RBC:** Red blood cells develop from the RE cells. Turnbull holds that they develop from the extravascular RE cells (haemocytoblasts).
4. **Formation of leucocytes:** The granulocytes, lymphocytes and monocytes are all derived from the RE system.
5. **Destruction of red cells and white cells:** The senile red cells and white cells are engulfed and destroyed by the RE cells. In adult life, it takes place chiefly in the spleen and liver.
6. **Scavengers of tissue debris and bacteria in infections processes:** The RE cells help in the scavenging process.
7. **Formation of bile pigments:** The RE cells manufacture bilirubin from haemoglobin.
8. **Storage function:** A large amount of lipids, cholesterol and iron are stored in the RE cells.
9. **Manufacture of plasma proteins:** Serum globulin and certain other plasma proteins are manufactured, to some extent, by the RE cells.
10. **Formation of tissue cells:** Since the cells of this system are undifferentiated, they can be converted into ordinary connective tissue cells, such as fibroblasts, under suitable stimulus. This is seen during repair stage of inflammatory process.

EXAM-ORIENTED QUESTIONS

Essay

1. Explain in details the tissue fluid formation
2. Discuss the formation and circulation of lymph
3. Describe the structure of spleen and its functions
4. Describe the reticulo-endothelial system and its functions

Short Notes

1. Oedema
2. Functions of lymph
3. Functions of lymph node
4. Functions of spleen
5. Functions of reticulo-endothelial system

Chapter

19

Immunity

INTRODUCTION

The resistance exhibited by the host towards injury caused by micro-organisms and other products is termed as immunity. In the process of immunity the non-self molecules are to be destroyed and self molecules are to be preserved.

Body responds to antigenic response by innate defense and adaptive defense.

Innate immunity: The inherited immunity possesses by an individual in virtue of his genetic and constitutional make up. It is a non-specific system which responds quickly and consists of the following.

Innate Defenses

1. **First line of defense**
   a. Skin, mucous membranes, and their secretions make up the first line of defense. The keratin in the skin acts as a physical barrier to most micro-organisms. They are resistant to weak acids and bases, toxins and bacterial enzymes. The epithelial membranes produce protective chemicals that destroy micro-organisms: Skin acidity (pH of 3 to 5) inhibits bacterial growth while sebum contains insoluble fatty acids which possess broad antimicrobial activity.
   b. The stomach mucosae also provide identical mechanical barriers. The stomach mucosae secrete concentrated HCl and protein-digesting enzymes.
   c. The mucus aids in trapping micro-organisms which enter the digestive and respiratory systems. The upper respiratory tract mucosa is ciliated. The cilia sweep micro-particles (air pollutants) and bacteria laden mucus away from lower respiratory passages.
2. **Second line of defense:** This is nonspecific cellular and chemical components in human body to protect itself from infections such as:
   a. Phagocytes
   b. Natural killer (NK) cells
   c. Inflammatory response enlists macrophages, mast cells, WBCs, and chemicals
   d. Antimicrobial proteins in blood and tissue fluid

- *Phagocytes:* The macrophages are the main phagocytic cells in human body. The Kupffer cells (liver), alveolar macrophages (lung), osteoclast (bone), histiocytes (lymph nodes) and microglia (brain) are the fixed macrophages while others are mobile and circulate throughout a region in search of cellular debris. Process of phagocytosis: The microbes adhere to the phagocyte. The pseudopods of the invaded cell engulf the antigen into a phagosome. There is fusion of phagosomes with a lysosome to form a phagolysosome. The antigens in the phagolysosome are digested by proteolytic enzymes. The residual material is removed by exocytosis.
- *Natural killer cells:* These are a small, distinct group of large granular lymphocytes. They kill the target cells by releasing cytolytic chemicals and perforins. They react non-specifically and remove cancerous and virus-infected cells. They secrete vital chemicals which enhance the inflammatory response.
- *Inflammatory response:* Role of macrophages, mast cells, WBCs, and chemicals: The inflammatory response is initiated on injury of a body tissue to prevent the spread of infection to nearby tissues, and set the process of repair so as to dispose of cell debris and pathogens. The signs of acute inflammation are redness, heat, swelling, and pain. The inflammatory mediators are released by injured tissue, phagocytes, lymphocytes, and mast cells. These include kinins, prostaglandins (PGs), complement, and cytokines. They produce vasodilatation locally at site of injury resulting in hyperaemia. The mediators release exudates (fluid containing proteins, clotting factors, and antibodies) which enter into tissue spaces causing local edema (swelling), which produces

the sensation of pain. The protein-rich fluids which enter into tissue spaces (edema) help to dilute harmful substances, carry over large quantities of oxygen and nutrients needed for repair and also allow entry of clotting proteins, which prevent the spread of bacteria. There is infiltration of damaged area by neutrophils and monocytes macrophages.

The events occur in four main phases:

- *Leukocytosis:* Neutrophils are released from the bone marrow in response to leukocytosis-inducing factors released by injured cells.
- *Margination:* The neutrophils adhere to the walls of capillaries in the injured area.
- *Diapedesis:* The neutrophils squeeze through capillary walls and begin phagocytosis.
- *Chemotaxis:* The inflammatory chemicals attract neutrophils to the injury site.

As the inflammatory process continues the monocytes diapedesis into the inflammatory area and become macrophages. The macrophages engulfs the cellular debris and pathogens. Further the activation of the complement cascade and other adaptive immunity process take over for defense.

3. **Anti-microbial proteins:** These proteins defend infection by hindering the micro-organisms' reproductive process. The vitally important antimicrobial proteins are: Interferon and complement proteins.
   1. *Interferon:* Some of the cells produce and release interferons (IFNs) on being invaded by virus. The lymphocytes secrete gamma (γ) interferon, leucocytes secrete alpha (α) interferon while fibroblasts secrete beta (β) interferon. The released interferons stimulate neighbouring cells to produce proteins (PKR) that interfere with viral replication.
   2. *Complement:* These are group of about 20 proteins that are activated by the foreign antigens. They enhance both innate and adaptive defenses and thereby the inflammation. The pathogens are lysed by complement activation. The prime actors of complement system are 11 proteins and these are C1–C9, B and D.

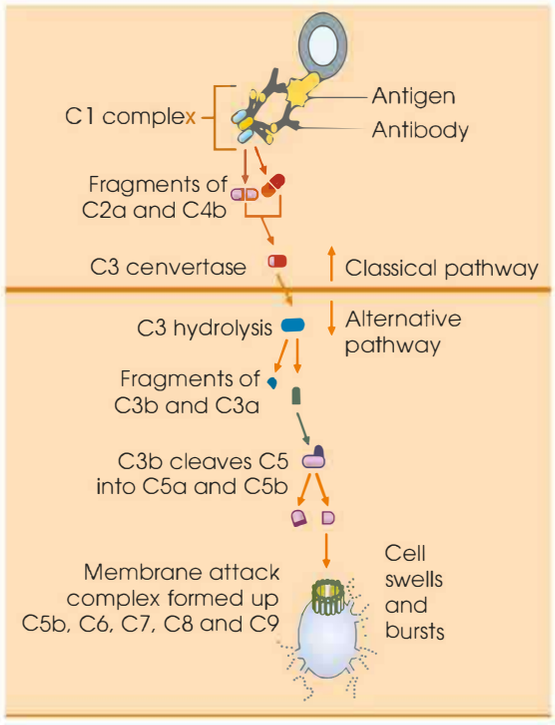

Fig. 19.1: Complementary system activation A: Action of complement factors C3a, C3b, C5a, C5b, C6, C7, C8 and C9

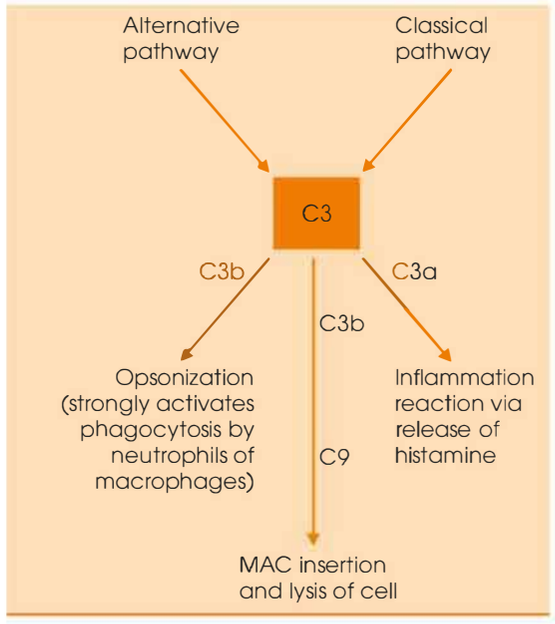

Fig. 19.2: Action mediated via complement factor C3

Complement

The activation of complement system occurs by two pathways: Classical and alternative (Figs 19.1 and 19.2).

Classical pathway: Its activation occurs when antibodies bind to invading organisms. Through this pathway the factor C1 binds to the antigen–antibody complexes (complement fixation) produce inflammatory response. The complement cascade product C3b activates phagocytosis by neutrophils and macrophages and there by these cells engulf bacteria to which antigen antibody complex are attached. The multiple complement factors C5b6789 brings over lysis by its direct effect of rupturing the cell membrane of invading bacteria or pathogens. The fragment C5a initiates chemotaxsis of neutrophils and macrophages to tissue area adjacent to antigenic agent. The fragments C3a, C4 and C5a activates mast cells and basophils releasing listamine, heparin and other substances which contribute to inflammation. The complement products by agglutination and neutrolization also defends infections.

Alternative pathway: It is triggered by interaction among factors B, D, and P, and polysaccharide molecules present on micro-organisms.

These pathways produce a cascade response in which complement proteins activation occurs in an orderly sequence and each of the steps catalyzes the next complement set. These pathways converge on C3, which cleaves into C3a and C3b. The factor C3b initiates formation of a membrane attack complex (MAC) which causes cell lysis by interfering with a cell's Ca^{2+} ejection ability. The factor C3a causes inflammation and C3b also causes opsonization.

C-reactive proteins (CRP): They are produced by the liver in response to inflammatory molecules. They activate the classical pathway by activating complementary factor C1 and binding to membrane. They participate in process of opsonization.

ADAPTIVE DEFENSES

The adaptive immune system is a functional system which recognizes specific foreign antigens and they act to immobilize, neutralize, or destroy foreign substances. The adaptive immune system is antigen-specific, systemic, and has memory.

They are mainly of two types:

1. *Humoral immunity:* It is the antibody mediated immunity.
2. *Cell-mediated (T cell) immunity:* The T cell mediated immunity amplifies inflammatory response and activate complement factors.

Terminology: The commonly involved terms in immunity process are:

1. *Antigens:* These are the entity which can mobilize the immune system and provoke an immune response.
2. *Immunogenicity:* The ability to stimulate specific lymphocytes and specific antibodies is termed as immunogenicity.
3. *Reactivity:* It is the ability to react with activated lymphocytes and antibodies.
4. *Hapten:* It is the incomplete antigen. It is a small molecule that is not immunogenic until attacked.
5. *Antigenic determinants:* These are the sites on an antigenic molecule that is immunogenic.
6. *Major histocompatibility complex (MHC):* It is the cell surface glycoproteins which is associated with self recognition. The body cells are dotted with protein molecules (self-antigens) that are not antigenic to self but are antigenic to others. The two classes of MHC proteins are: Class I MHC proteins—found on virtually all body cells and class II MHC proteins—found on certain cells in the immune response (Fig. 19.3).

Development of the Acquired Immune System

In the cell-mediated immunity process the lymphocytes directly attack invading antigen by lysis or indirectly by initiating inflammation and so also by activating other lymphocytes and macrophages.

The adaptive system cells are

1. ***Lymphocytes:*** T cells and B cells: The B lymphocytes via immunoglobulin's actions provide humoral immunity. T lymphocytes are non-antibody-producing cells that constitute the cell-mediated part of immunity.
2. ***Antigen presenting cells (APCs):*** They do not respond to specific antigens and has an auxiliary role in immunity.
   - *Lymphocytes:* The immature lymphocytes released from bone marrow remain identical. The lymphocyte matures into a B cell or a T cell depending on the site where it becomes immunocompetent. T cells mature in the thymus and B cells mature in the bone marrow. They are carried to secondary lymphoid tissue where they defend the antigens. They mature into fully functional antigen-activated cells upon binding with their recognized antigen.

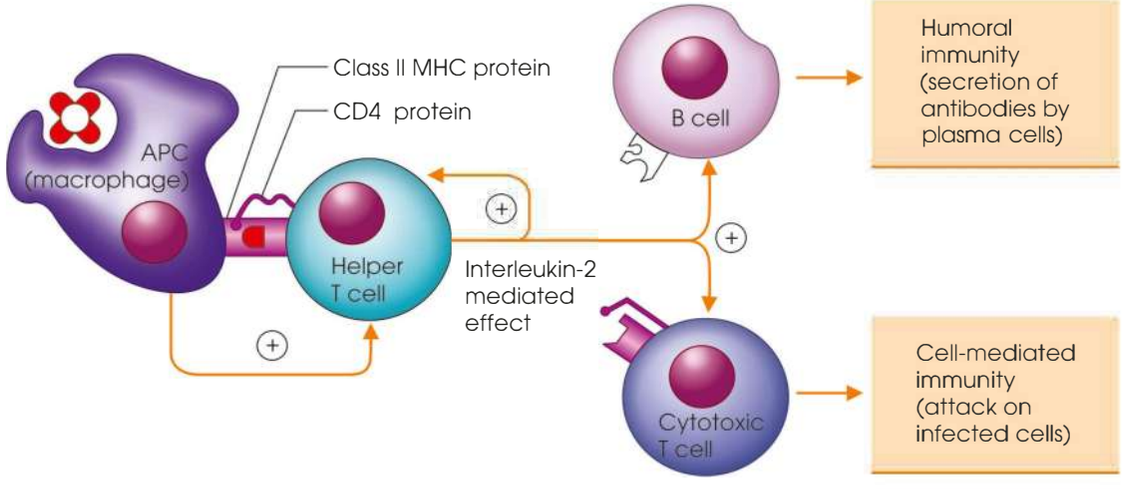

Fig. 19.3: Helper T cell-mediated humoral and cell-mediated immunity response

- *Antigen presenting cells (APCs):* The major APCs are dendritic cells, macrophages, and activated B cells. The major initiators of adaptive immunity are dendritic cells, which migrate to the lymph nodes and secondary lymphoid organs and present antigens to T and B cells.

Humoral Immunity

Humoral immunity response: The first encounter between an antigen and a naïve immunocompetent cell takes place in the spleen or other lymphoid organ. If the lymphocyte is a B cell; it provokes a humoral immune response and antibodies are produced against the antigens. B cell is activated when antigens bind to its surface receptors, these activating events along with T cell actions trigger clonal selection. These clone cells become antibody-secreting plasma cells. The plasma cells secrete specific antibody at the rate of 2000 molecules per second.

Acquired Humoral Response: Formation of Primary and Secondary Antibodies (Fig. 19.4)

Primary immune response: The cellular differentiation and proliferation, which occurs on the first exposure to a specific antigen. It takes period of three to six days after antigen challenge. The peak levels of antibodies are achieved around ten days. The antibody levels thereafter declines.

Secondary immune response: The re-exposure to the same antigen sensitized memory cells which respond within hours and the antibody levels peak in two to three days.

Types of Acquired Immunity

Active humoral immunity: B cells when presented with antigens produce antibodies against them. It may be naturally acquired in response to a bacterial or viral infection or artificially acquired in response to vaccination.

Passive humoral immunity: It is naturally acquired from the mother to her foetus via the placenta and may be artificially acquired from the injection of serum, such as γ-globulin.

Antibody Mediated Humoral Response

Antibodies: They are also called immunoglobulin. They contain four polypeptide chains linked by disulphide bond (Fig. 19.5). These polypeptide chains have two identical low molecular weight chains and two identical high molecular weight chains. These heavy and low molecular weight chains are divided into constant and variable region. Amino acid sequence in constant region remains similar among immunoglobulin's but it differs in variable region and is unique for individual

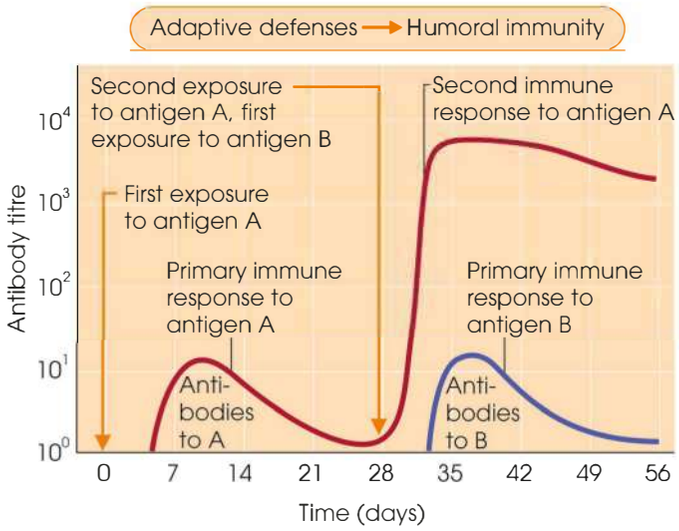

Fig. 19.4: Primary and secondary immune response

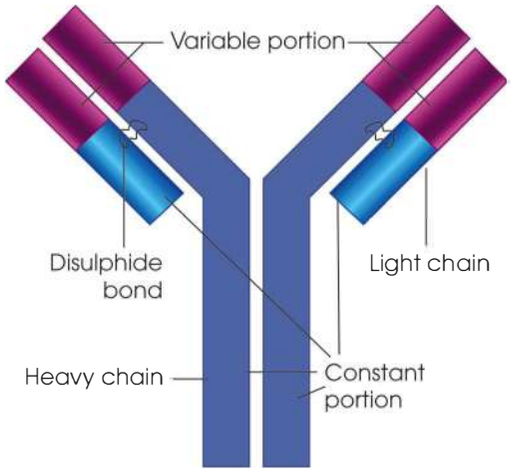

Fig. 19.5: Structure of immunoglobulin

immunoglobulin. They constitute the γ-globulin portion of blood proteins.

They are soluble proteins secreted by activated B cells and plasma cells in response to an antigen. There are five classes of antibodies: IgD, IgM, IgG, IgA, and IgE.

1. *IgG:* It play important role in primary and secondary response. They cross the placenta and confer passive immunity.
2. *IgE:* It binds to mast cells and basophils, and cause histamine release on activation.
3. *IgD:* It is attached to the surface of B cells which are responsible for activation of B cell.
4. *IgA:* It help to prevent attachment of pathogens to epithelial cell surfaces.
5. *IgM:* It is a pentamer released as primary immune response from plasma cell.

Role of Antibody

The antibodies do not destroy antigen by own-self; but they inactivate and tag it for destruction. The antibodies form an antigen–antibody (immune) complex and act

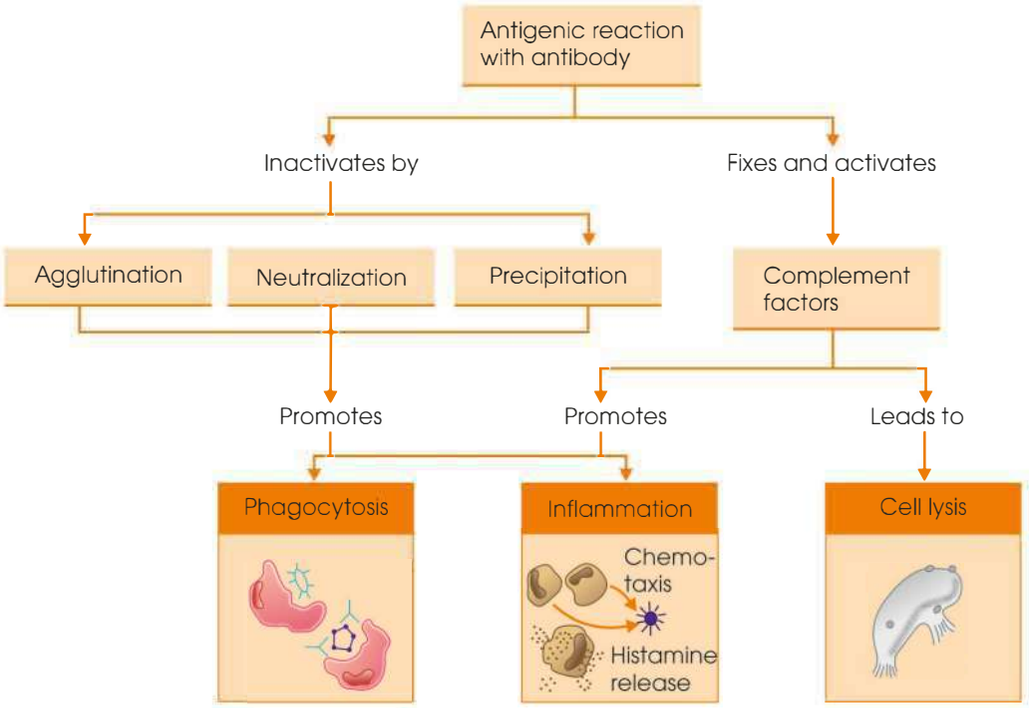

Fig. 19.6: Antigen–antibody complex action

via neutralization, agglutination, precipitation, and complement fixation (Fig. 19.6).

Monoclonal antibody: Monoclonal antibodies are pure antibody preparations are specific for a single antigenic determinant and are produced from descendents of a single cell. These are commercially prepared antibodies are used to provide passive immunity.

Cell-mediated Immune Response

Since antibodies are useless against intracellular antigens, cell-mediated immunity is needed. The T cells mediate cellular immunity through:

- CD4 cells (T4 cells) are primarily helper T cells (TH)
- CD8 cells (T8 cells) are cytotoxic T cells (TC) that destroy cells harbouring foreign antigens.

Other types of T cells are

- Suppressor T cells (TS)
- Memory T cells

T Cell Activation (Figs 19.7 and 19.8)

Key Points

1. T cell antigen receptors (TCRs) bind to an antigen-MHC protein complex which have variable and constant regions consisting of two chains (alpha and beta).
2. The T cell receptors: Bind to the MHC and are stimulated by the associated antigen. The co-stimulator (cytokines, interleukins, etc.) prompts the T cell to form a clone.
3. MHC occurs as two classes: MHC I on virtually all tissue cells and MHC II only on PM, some immune system cells. MHC I is present on virtually all tissue cells: The endogenous antigens produced by the cell (viral/cancer) stimulate the CD8+ cell population to form cytotoxic T cells (killer T, TC). MHC II is found only on PM of B cells, some T cells and APCs: The exogenous antigen from outside the cell when presented to PM surface stimulates the CD4+ cell population leading to formation of helper T cells (Th).

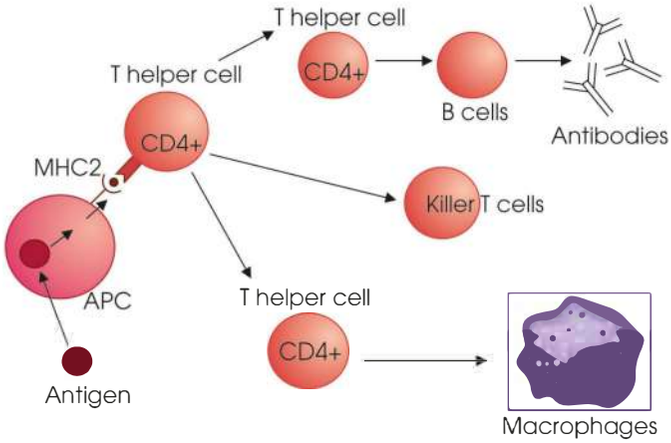

Fig. 19.7: Activation of T and B cells

T Cell Role

1. The helper T cells (Th) stimulate B cells and other T cells to proliferate.
2. The activated Th cells interact with B cells. They display antigen and produce cytokines which prompt the B cell to mature and form antibody. The TH cells also produce cytokines that promote TC

cells. TH cells recruit other WBCs and amplify innate defences (inflammatory).

3. The cytotoxic T cells (TC, killer T) directly attack and kill cells with specific antigen. The activated TC cells are co-stimulated by Th cells. TC binds to cell and releases perforins and granzymes. In the presence of Ca^{2+} perforins forms pores in target cell PM. The granzymes enter through pores and degrade cellular contents. TC then detaches and moves on. The macrophages remove the degraded products by exocytosis.
4. *Other T cells:* The regulatory T cells (TReg) release inhibitory cytokines that suppress B cell and T cell activity. This helps to prevent autoimmune events (Fig. 19.8).

Organ Transplant

Organ transplant needs to be safely carried out. There should be no rejection of the graft. The four major types of grafts are:

1. *Autograft:* It is the transplantation of graft from one site on the body to another in the same person.
2. *Allograft:* It is the transplantation of graft between individuals that are not identical twins, but belong to same species.
3. *Isograft:* It is the transplantation of grafts between identical twins.
4. *Xenograft:* It is the transplantation of grafts taken from another animal species.

The tissue rejection is prevented by supplementing immunosuppressive drugs in the patient of transplant. These drugs suppress patient's immunity.

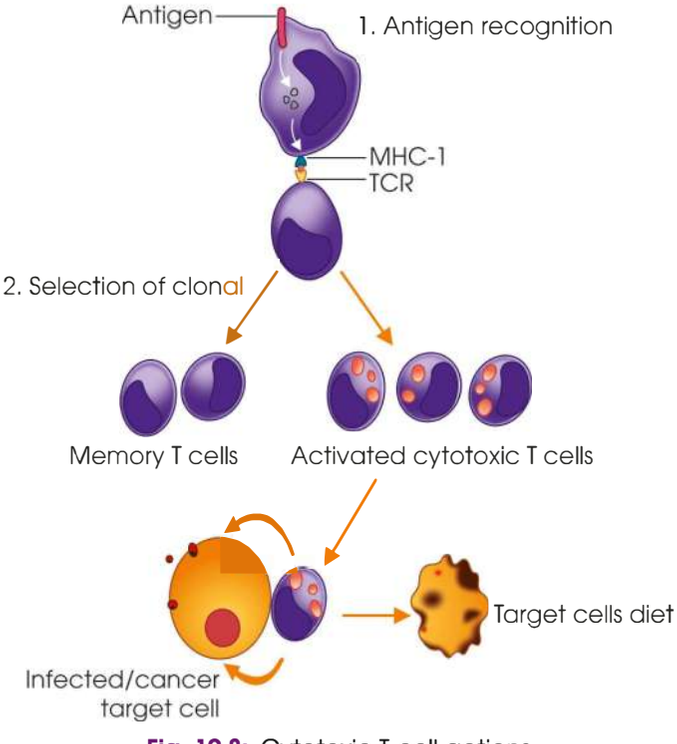

Fig. 19.8: Cytotoxic T cell actions

Severe Combined Immunodeficiency (SCID) Syndrome

It is the genetic defects that produce a marked deficit in B and T cells. The probable pathology may be due to abnormalities in interleukin receptors; and may also be due to defective adenosine deaminase (ADA) enzyme activity leading to accumulation of metabolites toxic to T cells. It is fatal if untreated; and responds to bone marrow transplants. Example: Hodgkin's disease leads to immunodeficiency by depressing lymph node cells.

Acquired Immune Deficiency Syndrome (AIDS)

It is caused by human immunodeficiency virus (HIV) transmitted via body fluids—blood, semen, and vaginal secretions. It affects the immune system by interfering with the activity of helper T (CD4) cells. It destroys the Th cells and depresses cell-mediated immunity.

Hypersensitivity

The immune responses cause tissue damage. The antibody mediated allergies are immediate and cell-mediated allergic condition is delayed hypersensitivity.

Types

Acute (type I) hypersensitivity: It commences immediately after contact with allergen. It produces anaphylaxis and is IgE mediated. The mechanism involves IL-4 secretion by T cells. This IL-4 stimulates B cells to produce IgE. The IgE binds to mast cells and basophils causing degranulation and histamine release and induce inflammatory response. The local effects include watering of eyes, itching, running nose, asthmatic attack, etc. Watery eyes, runny nose, itching and redness. Antihistamines counteract these effects. Anaphylactic shock is associated with allergens that have systemic distribution. There is widespread vasodilatation, constriction of bronchioles and restricted airflow.

Hypersensitivities: Types II and III

The sub-acute hypersensitivity is mediated via IgG and IgM immunoglobulin.

Cytotoxic reactions (type II): The antibodies bind to antigens on specific body cells, stimulating phagocytosis and complement-mediated lysis of the cellular antigens. Example, mismatched blood transfusion reaction.

Immune complex formation (type III): The widely distributed antigen reacts with antibody. The antigen–antibody complexes cannot be cleared; persistent inflammation/tissue damage. Examples: Farmer's lung; associated with autoimmune disorders.

Hypersensitivity: Type IV

It is delayed hypersensitivity and cell-mediated). It takes one to three days to react. It involves helper T cells and cytotoxic T cells and macrophages. Example: Allergic contact dermatitis (poison ivy, heavy metals, TB tine tests).

EXAM-ORIENTED QUESTIONS

Essay

1. Describe the first and second line of emmune defence mechanism of human body?
2. Describe the role of humoral and cell mediated response in immunity.

Short Notes

1. Adaptive defences
2. Innate immunity
3. Anti microbial protein
4. Complement factor
5. Hypersensitivity reaction
6. Organ transplant
7. AIDS

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CLINICAL CASE SCENARIO

Blood

Q1. An 18-year-old male subject complained of generalised weakness and fatigue. On clinical examination, yellowish discolouration was visible on skin and conjunctivae. His plasma bilirubin level was 2 mg%. van den Bergh reaction was indirect positive. What is the diagnosis? Enlist any three causes for the same.

Ans. The patient is suffering from haemolytic jaundice. It may be commonly observed in patient suffering from congenital spherocytosis, sickle cell anaemia or thalassemia.

Q2. A 32-year-old male patient suffering from liver disease was transfused four-pint of blood for severe anaemia prior to an emergency surgery for obstructive hernia. The patient blood profile after transfusion showed hyperkalaemia and hypocalcaemia. What are the reasons for the same?

Ans. The stored blood has high concentration of potassium. As it takes nearby a day for concentration of potassium to reach back to its physiological value as it is taken back to the red blood cell; and this is the cause of hyperkalemia after transfusion. The citrate is present in the transfused blood. Metabolism of citrate gets delayed in liver disease, therefore massive blood transfusion produces hypocalcaemia (due to prolong presence of citrate in circulation) in patient.

Q3. A 40-year-old female patient was diagnosed as a case of thrombocytopenic purpura. What are the characteristic signs observed in the disease?

Ans. The characteristic signs of purpura are spontaneous multiple haemorrhage under the skin and membranes' petechia haemorrhages (red spot on skin and mucous membrane) and evidence of spontaneous bleeding due to increases capillary fragility.

Q4. A newborn baby with physiology jaundice was advised phototherapy. What are the advantages of phototherapy?

Ans. The exposure of skin to white light produces conversion of lumirubin which acts by photo-isomerisation of bilirubin to soluble form which is excreted.

Q5. The blood peripheral smear report of 42-year-old female suffering from polymenorrhoea showed microcytic hypochromic anaemia. Diagnosed the condition. Describe the signs and symptoms of the disease.

Ans. The patient is suffering from iron deficiency anaemia. The various signs of the disease are dry, soft, spoon-shaped nails with longitudinal striations, angry red tongue and patient exhibits symptoms of breathlessness, palpitation, tiredness, generalized weakness, irritability, headache, difficulty in concentration, loss of memory, etc.

Q6. What is the cause for spur cell haemolytic anaemia and Rh deficiency syndrome?

Ans. Spur cell haemolytic anaemia: In the disorder, the cholesterol binds to red blood cell membrane producing thorny projection on RBC named acanthocytes. The disorder is due to lowered ability of liver to esterify cholesterol.

Rh deficiency syndrome: The membrane of red blood cell in the disease is deficient in Rh antigens.

Q7. A 40-year-old male reports of extreme tiredness, difficulty in concentration, feeling of fainting on sudden standing and decrease in appetite. Examination of oral cavity revealed signs of red beefy tongue, bleeding gums and cracking at the angles of mouth. Peripheral smear showed macrocytic hypochromic anaemia. What is the likely diagnosis? Enlist the signs and symptoms observed in the condition? What are the causes for the disease? What investigation may be prescribed?

Ans. The patient is suffering from megaloblastic anaemia due to vitamin B_{12} deficiency. The signs and symptoms seen in this condition are: Fatigue, shortness of breath, dizziness, pale or yellowish skin, irregular heartbeats, weight loss, numbness or tingling in hands and feet, muscle weakness, personality changes, unsteady movements and mental confusion or forgetfulness.

The common causes of vitamin B_{12} deficiency are

1. Inadequate dietary intake of vitamin B_{12} (diet devoid of egg and dairy products like milk, cheese, butter, etc.)
2. Impaired absorption of vitamin B_{12} due to intrinsic factor deficiency caused by the loss of gastric parietal cell as seen in chronic atrophic gastritis.
3. Malabsorption syndrome; which commonly occurs due to structural damage or wide surgical resection of the terminal ileum.
4. Surgical removal of the small bowel as in patients of crohns disease or celiac disease cause vitamin B_{12} deficiency.
5. Long-term use of ranitidine hydrochloride may contribute to deficiency of vitamin B_{12}.
6. Nitrous oxide abuse
7. Malnutrition and alcoholism

The investigations which can be carried out are in addition to the usual complete blood count (CBC) and peripheral smear to confirm the diagnosis are:

Serum B_{12} level, serum homocysteine and methylmalonic acid levels (the levels of serum homocysteine and methylmalonic acid are high in B_{12} deficiency and can be helpful in doubtful diagnosis) and Schilling test.

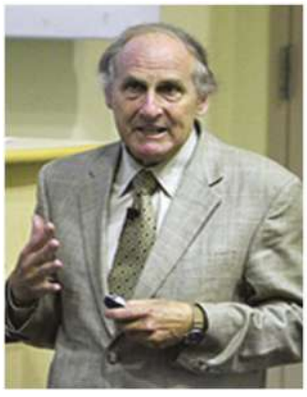
Recent Advances: Innate Immunity

Bruce Alan Beutler
1957

Bruce Alan Beutler an American immunologist and geneticist along with **Jules A Hoffmann** received one-half of the 2011 Nobel Prize in Physiology or Medicine, for "their discoveries pertaining to the activation of innate immunity" (the other half of the Nobel Prize went to Ralph M Steinman for "his discovery of the dendritic cell and its role in adaptive immunity").

Jules A Hoffmann
1941

Bruce Alan Beutler and Jules A. Hoffmann discovery revealed increased Drosomycin expression following activation of Toll pathway in microbial infection. The Toll-like receptor TLR-4 in mammals is the membrane-spanning component of the mammalian lipopolysaccharide (LPS) receptor complex. The TLRs helps in the identifying of microbes, as TLR detect signature molecules which produces infection. TLR-4 when artificially ligated using antibodies activate certain genes necessary for initiating an adaptive immune response. The TLR-4 function as an LPS sensing receptor was discovered by Bruce A Beutler and colleagues.



Ralph M Steinman
1943–2011

Ralph Marvin Steinman through his research protocol initiated antibody responses in tissue culture in the experimental laboratory. His finding revealed that the antigens, lymphocytes and "accessory cells" together are responsible for the observed immune responses. The accessory cells contain a new cell type with probing cell process or "dendrites". These accessory cells have been proven to be the missing link between innate and adaptive immunity.

Dendritic cells initiates T cell responses as a result the adaptive immunity develops in two stages:

1. The dendritic cell present antigens and initiate the afferent limb.
2. The other antigen presenting cell mediates the effectors to eliminate the antigen in tissue cultures. The immunity develops in clusters of lymphocytes and the dendritic cells.

Dendritic cells helped induce T cells response and also produce cytokines, interferons, chemokines, anti-microbial peptides thereby aid in combating infections. They also mobilize innate lymphocytes such as natural killer cells but they do not phagocytised or kill microbes.

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3. Canadian Jewish Review: Mark Charles Steinman: Multiculturalcanada.ca. December 17, 1965. Retrieved December 18, 2011.

2008 Nobel Prize for Physiology and Medicine: HIV VIRUS

Françoise Barré-Sinoussi
1947

Luc Antoine Montagnier
1932

Françoise Barré-Sinoussi a French virologist and **Luc Antoine Montagnier** were awarded the 2008 Nobel Prize for Physiology and Medicine for identification of the human immunodeficiency virus (HIV) as the cause of AIDS.

REFERENCE

Gallo RC, Montagnier L. "Historical essay. Prospects for the Future". Science 2002;298 (5599):1730–1.

Notable Contributions: Diseases, Drugs, Immunity and Recovery

1. Robert Koch was a German physician who is widely credited as one of the founders of bacteriology and microbiology. He investigated the anthrax disease cycle in 1876, and studied the bacteria leading to tuberculosis

Robert Koch
1843–1910

in 1882, and cholera in 1883. He also formulated Koch's postulates. Koch won the 1905 Nobel Prize in Physiology or Medicine.

REFERENCE

Metchnikoff, Elie. The Founders of Modern Medicine: Pasteur, Koch, Lister. Classics of Medicine Library: Delanco, 2006.

Alexander Fleming
1881–1955

Ernst Chain
1906–1979

Howard Florey
1898–1968

2. Sir Alexander Fleming (1881–1955), Ernst Boris Chain (1906–1979) and Sir Howard Walter Florey (1898–1968) received the Nobel Prize in Physiology or Medicine for their work on penicillin. Fleming while studying bacteria noticed that some had been killed and had dissolved away around a spot of blue-green mould which by-chance had contaminated one of his dishes. He transferred the mould to broth, and this had a strong effect on bacteria that even when diluted hundreds of times, the penicillin completely prevented bacterial growth. Chain and Florey went onto purify and extract penicillin, enabling it to be produced in large amounts to treat many different bacterial diseases.

REFERENCE

Hugh TB. "Howard Florey, Alexander Fleming and the fairy tale of penicillin". The Medical Journal of Australia 2002; 177 (1): 52–53.

3. Edward Jenner was born in Berkeley, Gloucestershire in 1749 philipps. He carried out his experiments on eight-year-old patient James Phipps in 1796. Jenner inserted pus taken from a cowpox pustule into an incision on the arm of James Philips his patient. Jenner subsequently proved that having been inoculated with cowpox James was immune to smallpox. He experimented on several other children, including his own son. He published his results of his experimentation in 1798 and Jenner coined the word vaccine from the Latin 'vacca' for cow and further carried out his contribution towards development of vaccine.

Edward Jenner
1749–1823

REFERENCE

Budai J. '200th anniversary of the Jenner smallpox vaccine'. Orvosi Hetilap (in Hungarian) 1996;137(34):1875–7.

4. The French chemist and biologist Louis Pasteur is famous for his germ theory and for the development of vaccines. He made major contributions to chemistry, medicine, and industry. His discovery that diseases are spread by microbes which are living organisms like bacteria and virus saved countless lives.

Louis Pasteur
1822–1895

Pasteur showed that airborne microbes were the cause of disease. Pasteur built on the work of Edward Jenner helped to develop more vaccines. Pasteur's career showed how conservative the medical establishment was at the time.

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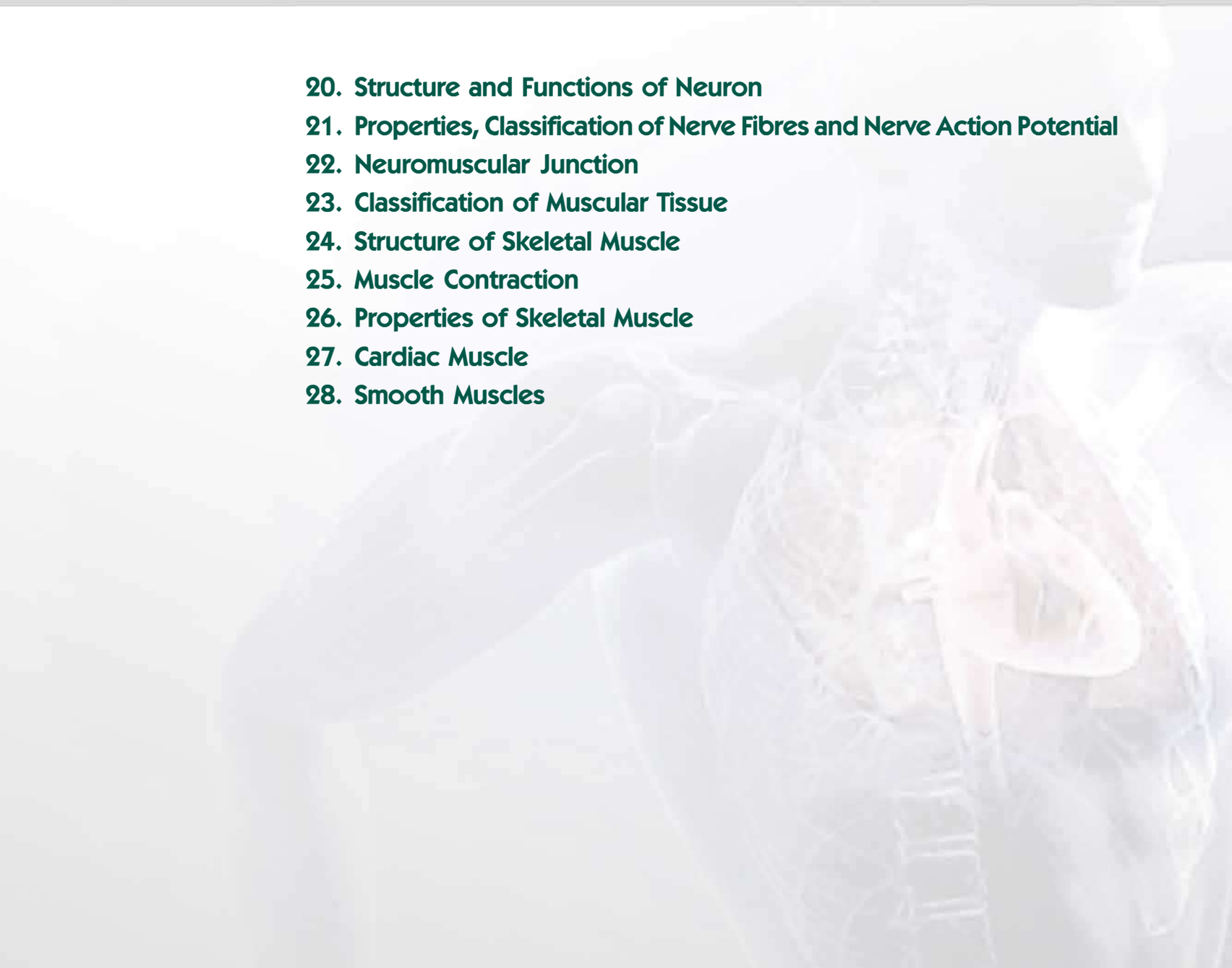
Section

III

Nerve and Muscle



Chapter

20

Structure and Functions of Neuron

INTRODUCTION

The nervous system is composed of nervous tissue. The nervous tissue consists of two elements: Nerve cell or neuron and neuroglia. The neurons are the structural and functional units. They communicate with each other directly or indirectly through synapses. They are the electrically excitable cells and they transmit impulses via chemical and electrical signals. The detailed histological structure will help in understanding the functions of the neurons.

HISTOLOGICAL STRUCTURE

Neuron

A nerve cell with all its processes is called a neuron (Fig. 20.1). It is the structural and functional unit of the nervous system. Neuron may consist of a nerve cell body or soma and two types of processes—axon and dendrite (dendron).

Structure of Nerve Fibres

Santiago Ramóny Cajal was a Neuroscientist and a Nobel laureate provided detailed descriptions of cell types associated with neural structures and strongly advocated neuron theory.

Santiago Ramóny Cajal
1852–1934

Axon

Axon is the process of a nerve cell body that carries impulse away from the soma. It is generally long with few branches (collaterals). The neurons have a single axon and its branches help it in communicating with its target cells. Axon hillock is part of the axon where it emerges from the soma. The axon hillock has highest density of voltage-dependent sodium channels and electro-physiologically it is the most easily excited part

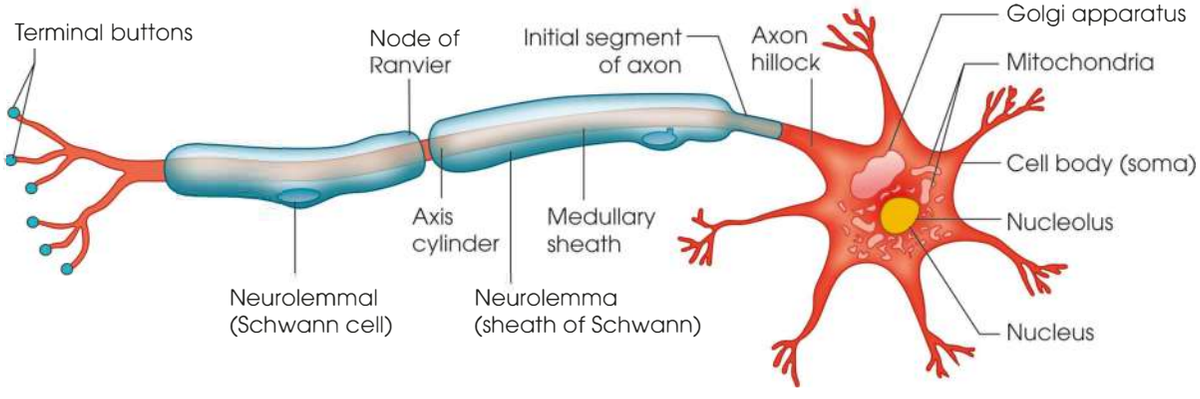


Fig. 20.1: A neuron with all its processes and the formation of a medullated nerve fibre

of the neuron. While the axon and axon hillock are generally involved in information outflow, this region can also receive input from other neurons. Axis cylinder in an axon contains axoplasm: A semi-fluid substance, essential for nutrition and growth of the nerve fibre. It also contains neurofibrils and mitochondria. Axon ends in numerous terminal buttons (axon telodendria). Neurotransmitters are released along the axon terminal into the synaptic cleft between the terminals and the dendrites of the next neuron. The neurotransmitter aids in synaptic transmission.

The term nerve fibres usually refer to the axons. Nerve fibres which carry impulses to the central nervous system are termed afferent and those carrying impulses from the central nervous system to the periphery are known as efferent. Thus, the peripheral mixed nerves containing both sensory and motor fibres are axons (Fig. 20.2).

Dendrite or Dendron

Dendrite or dendron is the process that carries impulse towards the cell body. It collects impulses from other neurons and carries them towards the cell body. It is generally short with many branches, and contains Nissl granules. Number varies from nil to numerous. Dendrites are, as a rule, multiple, relatively short and follow specific branching pattern. It is presumed that dendrites do not conduct impulse like axons in the central nervous system and instead they are the part of receptor membrane of the neuron.

Cell Body (Fig. 20.3)

The cell body is called the neurocyton (soma). Neurons are commonly classified according to the number of their processes:

1. **Apolar neurons** have no process.
2. **Unipoar neuron:** All developing neuroblasts pass through a stage when they have only one process the axon. In the adult human such true unipolar neurons are not commonly seen, but they are found in the mesencephalic nucleus of the 5th cranial nerve.
3. **Biopolar neurons:** Typically these neurons are spindle-shaped possessing the axon at one pole and a dendrite at the other. Numerous, developing neuroblasts pass through this stage. In the adult, they are usually found in the retina, in the vestibular ganglion, in the spiral ganglion of the cochlea, and in the olfactory neuro-epithelium.
4. **Pseudounipolar neurons:** These types of atypical bipolar neurons are found in all spinal ganglia and in the ganglia of the cranial nerves other than those of 8th cranial nerve. These types of neurons are at first typically bipolar and spindle-shaped, but as development proceeds the cell processes converge until these meet at one side of the cell body. Then this elongates so that a fine process is formed with a T-shaped division at the end, one branch of the T being the dendrite from the periphery and the other being an axon extends centrally.
5. **Multipolar neurons:** These neurons have varied forms. A few of the common types are Purkinje cell of the cerebellar cortex, pyramidal cell of the motor cortex, small neuron from the spinal nucleus of the trigeminal nerve, motor neuron from the ventral horn of spinal cord, etc. Usually the shape depends mainly on the number and position of the dendrites.

An alternative classification separates the neurons in two groups

1. **Golgi type I neuron** has a very long axon which has an extensive course outside that part of the grey matter of the CNS where the cell body lies, and passes in the white matter. These cells form the bulk of the neurons and they constitute the peripheral nerves and the main fibre tracts of the brain and spinal cord.
2. **Golgi type II neuron** is stellate and has a short axon which does not leave that part of the grey matter in

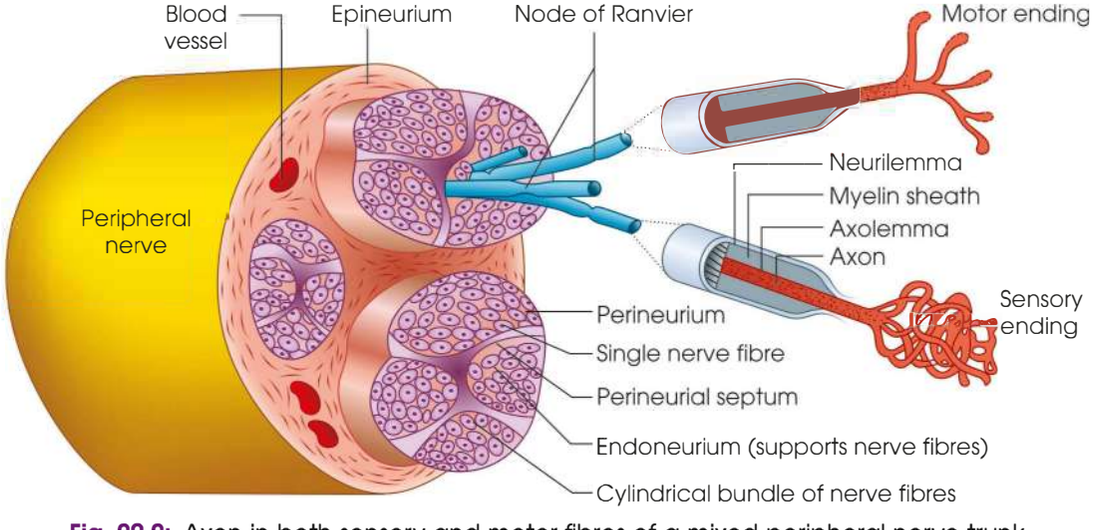


Fig. 20.2: Axon in both sensory and motor fibres of a mixed peripheral nerve trunk

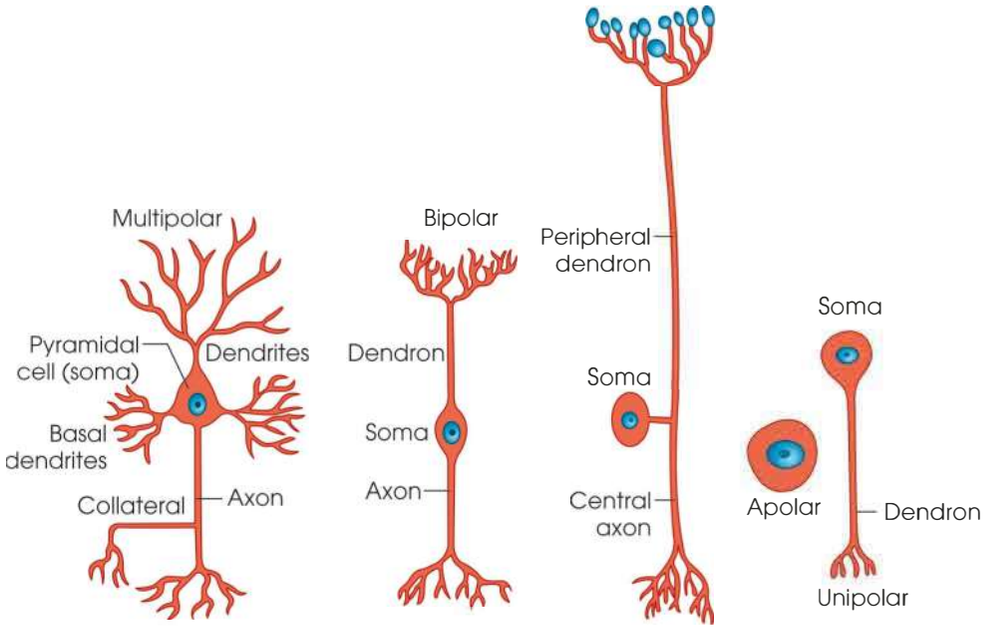


Fig. 20.3: Diagram to show morphological features of the types of neurons

which the cell body lies. These cells are to be found in the retina, the cerebellar and the cerebral cortices. These neurons vary greatly in size and shape. Spherical, oval, piriform, polyhedral, and fusiform types are described, but all have numerous radiating processes which give them a stellate or star-shaped appearance.

Anaxonic neuron: It is a type of neuron where the differentiation of axon from the dendrites cannot be done. Such anaxonic neurons are present in brain and retina.

Structure of Cell Body (Soma)

The soma (also known as cyton or perikaryon) is the bulbous end of neurons. It contains nucleus and other organelles.

The constituents of soma are (Fig. 20.4A to C)

a. ***Nucleus:*** The nucleus is commonly large, spherical or slightly ovoid and centrally placed within the perikaryon. It varies in size with the size of the cell and with its state of activity. The chromatin particles in the nucleus are finely dispersed and hence nuclei of the neurons appear pale and empty-looking when stained with basic dyes, e.g. thionine. One or two nucleoli are characteristically present. The sex chromatin of the female, which looks like a small dark staining body lies close to the nucleolus or nuclear membrane in the nerve cell nucleus. Although present in the male, it is too small to be seen under normal conditions. This helps in cell identity.

b. ***Neuroplasm:*** The neuroplasm or cytoplasmic matrix of the nerve cell contains filamentous, membranous and granular organelles and are arranged more or less concentrically around the nucleus. With a light microscope these organelles are neurofibrils. Nissl bodies or chromophilic substance, Golgi apparatus (Fig. 20.4B), mitochondria, a centrosome. A considerable number of inclusions are also found.

c. ***Neurofibrils:*** Fine filaments, passing through the neuroplasm, from the dendrite to the axon (Fig. 20.4C).

Franz Nissl
1860–1919

d. ***Nissl bodies (granules):*** It was named after Franz Nissl, a noted neuropathologist, who invented the Nissl staining method.

Characteristic features

1. Angular granules, staining with basic dyes (methylene blue, thionine or cresyl violet) are present in scattered form through the cytoplasmic portion of the cell and dendrites except at the axon hillock from which the axis cylinder arises. There is no Nissl granule in the axon (Fig. 20.4A).
2. Electron microscopic studies reveal that these basophilic granules are composed of thin, parallel arranged membrane bound cavities which are closely akin to endoplasmic reticulum containing cisternae, vesicles and tubules.
3. In dendrites, Nissl granules are rod-shaped. In motor neurons, they are coarse and flocculent while in sensory neurons they are almost dust-like.

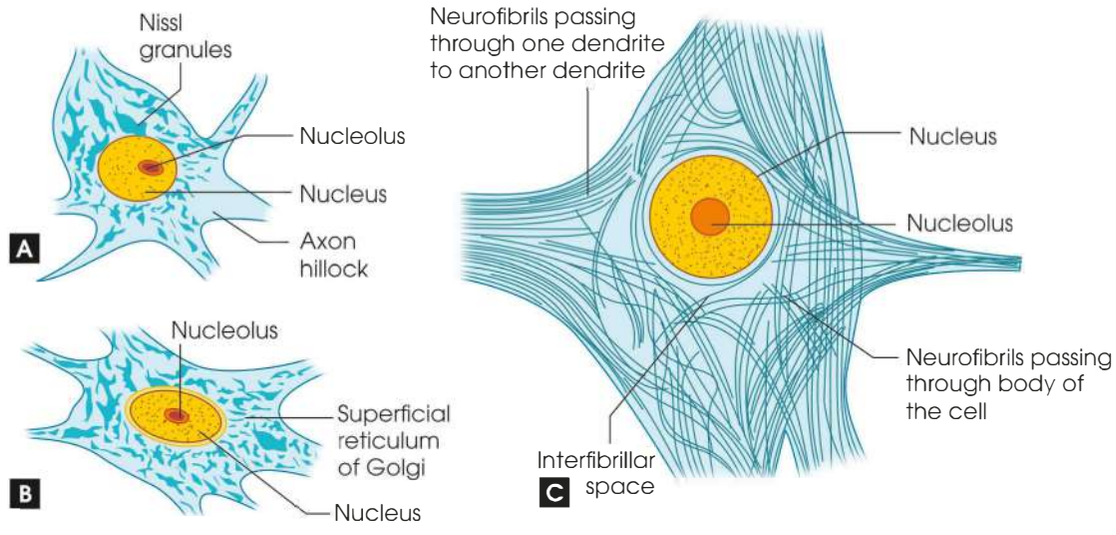


Fig. 20.4A to C: Nerve cells (diagrammatic). (A) Showing Nissl granules; (B) Showing superficial reticulum of Golgi; (C) Showing neurofibrils in a cell from the anterior grey column of the spinal cord

4. They disappear following injury to the nerve cell, damage to the processes, loss of function of the neuron, circulatory disturbance, hyperpyrexia, poisons, fatigue, etc. This process is known as chromatolysis. When the nerve cell regenerates, the granules reappear.
5. The main function of Nissl bodies is synthesis of proteins for intracellular use.

e. ***Mitochondria:*** Numerous and distinct. They are rod-shaped, spherical and widely distributed. There are phospholipid-protein inclusions which often appear in granules or filaments.

f. ***Golgi apparatus:*** Highly developed and complex-intracellular reticular network.

g. ***Superficial reticulum of Golgi:*** A fine network of the nerve cell (Fig. 20.4B).

h. ***Ribosome***

i. ***Endoplasmic reticulum***

j. ***Centrosome:*** The spherical centrosome contains a pair of centrioles and is characteristic of the immature, multiplying neuroblasts during early stages of embryonic development. When seen under EM a centrosome is often found in the cytoplasm, even though the neuron will never divide. Its role in the neuron is therefore not clear.

k. ***Inclusions:*** The inclusion bodies in soma include:

Melanin: It is present as dark-brown or black granules in some cell groups, particularly in the substantia nigra of the midbrain, locus coeruleus in the floor of the IVth ventricle, dorsal motor nucleus of the vagus, and in the spinal and sympathetic ganglia. Its significance is not clear.

Lipochrome granules: These are yellow or orange colour and are seen in certain cell groups, especially in those of the autonomic ganglia and in the region of axon hillock. The amounts of this yellow pigment (lipofuscin) increase with age. It is suggested that it represents a by-product of normal metabolic activity.

Lipid: It is encountered in the form of droplets in the cytoplasm of nerve cells. *Glycogen:* It is found not only in embryonic neurons but also in embryonic neuroglial cells, in embryonic cells of the ependyma and choroid plexus. But it cannot be seen in adult nervous tissue with a histochemical demonstration.

Iron-containing granular deposits: These are observed in the nerve cells of the substantia nigra, globus pallidus, etc.

l. *Neurosecretory material:* The hypothalamic neurons of the supra-optic and paraventricular nuclei contain droplets with characteristic staining properties. These are present in the cell body as well as in their axon.

Cell membrane: The surface of plasma membrane of a neuron appears to be basically similar to that of cells in general. Within the CNS, the nerve cells are closely invested by a network of neuroglial cells and fibres, whereas in the peripheral ganglia the nerve cells are surrounded by a capsule of satellite cells of similar origin.

NEUROGLIA

It is a special type of interstitial tissue and is present both in the grey and white matter. According to shape, size and number of processes, three main types have been described (Fig. 20.5A to D):

1. Astrocytes
   - Protoplasmic astrocyte
   - Fibrous astrocyte
2. Oligodendroglia or oligodendrocyte (few processes)
3. Microglia (small in size) (Fig. 20.5D).

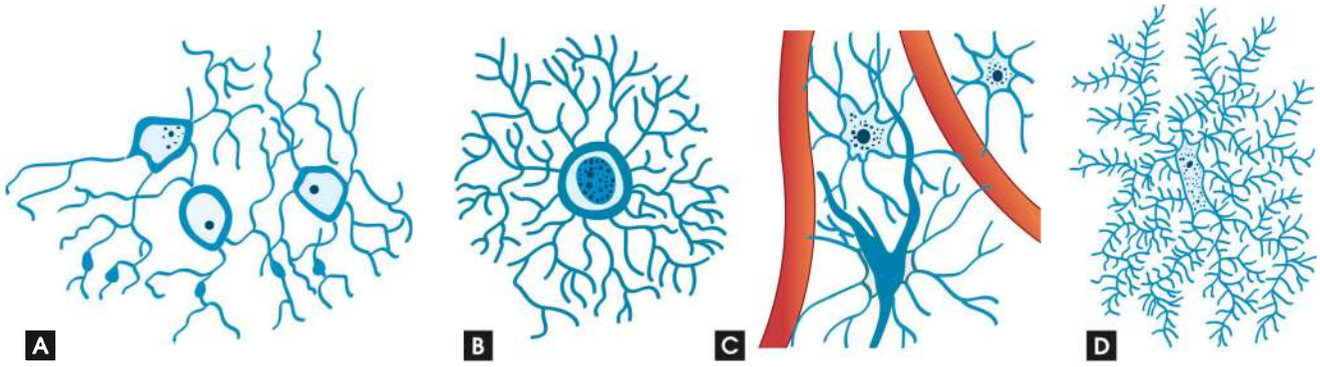


Fig. 20.5A to D: Diagrammatic representation of the neuroglial cells showing (A) Oligodendroglia; (B) Protoplasmic astrocyte; (C) Fibrous astrocyte; (D) Microglia

The first two are ectodermal in origin. But microglia is mesodermal and belongs to the reticulo-endothelial system.

Functions of Neuroglia

1. Support
2. Insulation
3. Phagocytosis

Under pathological conditions, the microglia becomes amoeboid and phagocytic and wanders in the meninges and blood vessels in the central nervous system. Their number is increased during inflammation.

Astrocytes are found around the blood vessel wall and appear to serve as regulator of the ionic environment of the neurons and are thought to make up the blood–brain barrier (Fig. 20.5C). Like the Schwann cells (oligodendroglia) (oligodendrocyte) take part in the formation of myelin sheath in the central nervous system.

Axonal Transport and Nourishment of Neurons

Axoplasmic transport is also known as the axonal transport. It is a cellular process regulating movement of mitochondria, synaptic vesicles, proteins, lipids and other cell organelles along the axoplasm. It also brings over the movement of molecules to be degraded (by lysosomes) from the axon back to the cell body. The movement toward the cell body is retrograde transport and movement toward the synapse is the anterograde transport. Axonal transport is essential to its growth and survival. The microtubules (tubulin) which traverse through the length of the axon provide the main cytoskeletal tracks for transportation. The movement of cargoes in the anterograde and retrograde directions respectively is brought over by motor proteins kinesin and dynein. Axonal transport can be fast or slow, and anterograde or retrograde.

Role of Neurotrophins in Neuronal Development

Neurotrophins are a family of proteins which induce the survival development, and function of neurons. They are of a class of growth factors capable of signaling particular cells to survive, differentiate, or grow. Neurotrophins promote the survival of neurons and are known as neurotrophic factors. Neurotrophic factors prevent the neuron from initiating programmed cell death, thereby promoting their survival. Neurotrophins are of different types:

1. Nerve growth factor which is important for the survival and maintenance of sympathetic and sensory neurons.
2. Brain-derived neurotrophic factor (BDNF) is a neurotrophic factor found in the brain, and the peripheral nervous system. It controls the growth and differentiation of new neurons and synapses by axonal and dendritic sprouting.
3. Neurotrophin-3, is a neurotrophic factor, and a protein growth factor that influences activity on certain neurons of the peripheral and central nervous system.
4. Neurotrophin-4 (NT-4) is a neurotrophic factor acting via TrkB receptor tyrosine kinase. It is also identified or called NT4, NT5, NTF4, and NT-4/5.

EXAM-ORIENTED QUESTIONS

Essay

1. Draw a well-labeled diagram of Neuron. Describe its structural component.

Short Notes

1. Axon
2. Classification of neurons
3. Myelin sheath
4. Myelinogenesis
5. Neuroglia
6. Functions of neuroglia
7. Nissl bodies

Chapter

21

Properties, Classification of Nerve Fibres and Nerve Action Potential

INTRODUCTION: PROPERTIES OF NERVE FIBRES

Nerve fibres show the following properties:

1. Excitability
2. Conductivity
3. All-or-none law
4. Refractory period
5. Summation
6. Adaptation
7. Accommodation
8. Indefatigability

1. EXCITABILITY

The nerve fibre can be stimulated by a suitable stimulus, which may be mechanical, thermal, chemical or electrical. In experiments, electrical stimulation is usually employed, because its strength and frequency can be accurately controlled.

The following changes will show that a nerve has been excited:

On stimulation of a nerve by a threshold stimulus will generate an action potential and this wave of negative potential passes along the nerve and can be detected by galvanometer or by CRO.

Generation of Action Potential and Excitability of the Nerve

Key Points

Phases of Action Potential

1. **Resting potential:** An electrical disturbance always accompanies the travelling nerve impulse. In resting cell, the surface is positively charged and the interior is negatively charged. When the surface is stimulated and the permeability is increased, as a result, there is reversal of polarization. The surface at the stimulated point becomes negative (cathode) causing catelectrotonic change.
2. **Depolarization:** When this change rises to threshold level, impulse will pass like self-propagated disturbance by drawing positively charged particles from the neighbouring points which in turn becomes cathode. The depolarization of the membrane is the first step of the manifestation of an impulse. After an initial slow rise, depolarization wave overshoots rapidly and reaches up to the potential line (zero line) to approximately +35 mV.
3. **Repolarization:** After that it reverse and begins to fall very rapidly towards the resting level (–70 mV). At approximately two-thirds of repolarization, the rate of fall is being abruptly slowed.

   This slower fall is known as negative after potential (after-depolarization). The rapid rise of depolarization wave and the rapid fall of repolarization wave are known as spike potential.
4. **Hyperpolarization:** After reaching the basal level the wave overshoots slightly but slowly in the hyperpolarizing direction. This is known as positive after-potential (after-hyperpolarization). The whole sequence of potential changes in the nerve following excitation is known as action potential or membrane potential (Fig. 21.1).

Ionic Basis of Excitability of Nerve

Key Points

1. **Resting state:** In resting state, the nerve fibre remains in polarized state and the membrane potential lies within –70 mV. The inside of the nerve is negative and the outside of the nerve is positive (Fig. 21.2). Na^+ concentration outside the membrane is higher than that of inside the membrane. K^+ concentration inside the membrane is also higher than that of outside the membrane. K^+ can permeate through the membrane at resting state but the Na^+ cannot permeate.

   The influx of sodium leads to depolarization while efflux of potassium leads to repolarization. The voltage-gated sodium channel and potassium channel are main contributors towards generation of action potential. The action potential occurs in

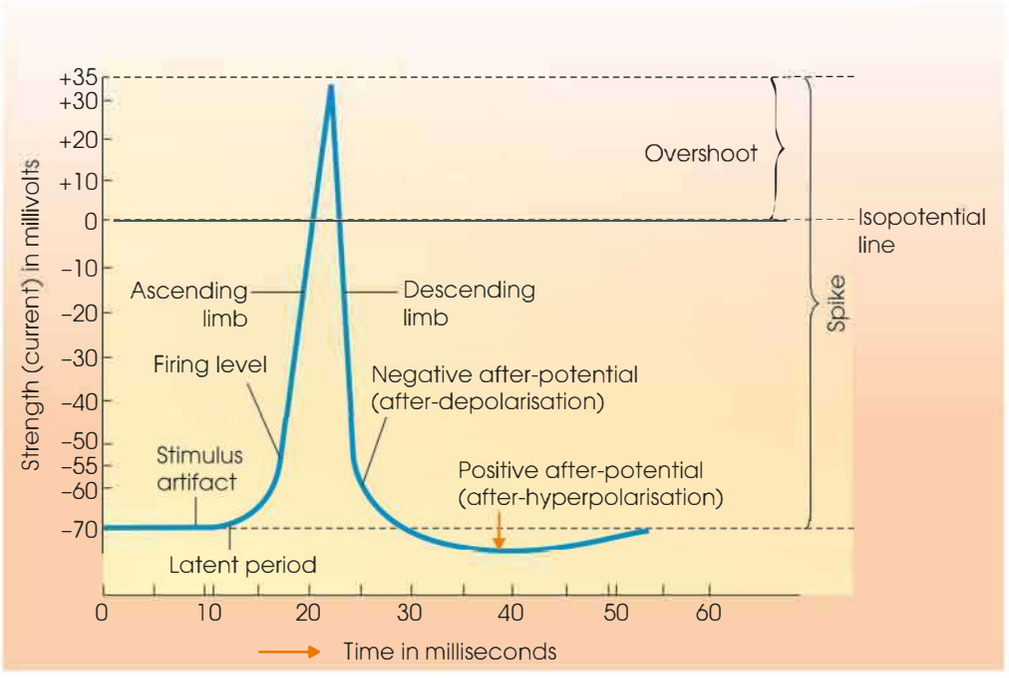


Fig. 21.1: Diagrammatic representation of spike potential (action potential) recorded with the help of microelectrode in the nerve cell

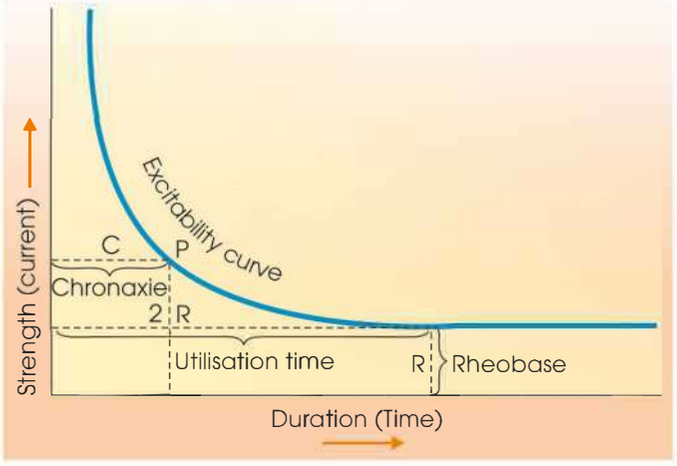


Fig. 21.2: Diagrammatic representation of excitability curve (strength–duration curve) applied to an excitable tissue producing response relating the strength of stimulus

successive stages of depolarization, repolarization, negative after-potential and positive after-potential.

2. **Depolarization (excitability):** Permeability of Na^+ to membrane is increased only after excitation and it is the first event of the action potential. The threshold stimulus leads to influx of sodium through leaky channels and via the opening of the voltage-gated sodium channel. The membrane potential decreases from –70 to –55 mV.

   As the depolarization proceeds further, a large number of voltage-gated channel opens. So the depolarization starts with the onset of Na^+ entry and thus an increase in Na^+ conductance is taken place. The tremendous increase is Na^+ conductance during this period is known as activation of membrane produce large and sweep depolarization and the membrane potential reaches to +35 mV. Thus, the *reversal* of potential is caused with the development of positivity inside the membrane and negativity outside. The Na^+ sodium influx stops due to inactivation of gates of sodium channel. The sodium channel remains open for very brief period of time. Thus, this speedy closure produces auto-deactivation of the sodium channel. The voltage-gated K^+ channels fully open at +35 mV causing efflux of K^+ ions.

3. **Repolarization:** But as soon as the action potential attains the voltage approximately +35 mV, K^+ efflux out from inside the membrane. The inside of the membrane becomes negative and outside becomes positive again. This stage is the repolarization phase and K^+ conductance is increased to the maximum. But at the later period of this phase (at the termination of spike potential) K^+ conductance is slowed down. As the membrane potential reaches to iso-potential level and as it is reaching towards the resting membrane potential the inside of the membrane achieves negativity; this limits efflux of potassium ions. Thus, a few milliseconds are delayed in restoring the membrane potential. This state is known after depolarization phase-potential and is attributed to slow efflux of potassium ions. In the later phase of repolarization the sodium channel is closed and then its inactivation gate opens slowly while the K^+ channel begin to close and gradually are completely closed. Thus, as membrane reaches

resting state the activation gates of sodium and potassium channel are closed while inactivation gate of sodium channel opens.

4. **After hyperpolarization:** This increased negativity inside hinder further efflux of K^+. Most of the voltage-gated K^+ channels are closed but as some of the voltage-gated K^+ channels the efflux continues and membrane potential becomes more negative producing the phase of after hyperpolarization. The resting membrane potential is yet to be achieved.
5. **Resting membrane potential:** It is achieved by the complete closure of voltage-gated K^+ channel. The resting ionic composition is restored by the active Na^+-K^+ pump mechanism (increased activity of Na^+-K^+-ATPase).

In this way resting normal ionic status is established.

Excitability Depends upon Following Factors

1. **Strength of stimulus** (Fig. 21.2): A minimum strength is essential to excite a tissue.

   Strength-intensity of stimuli: Current intensity of stimulus which is just adequate to cause an impulse is called the threshold. Intensity below the threshold is known as subliminal. Magnitude of current just sufficient to excite a nerve or muscle is called rheobase.
2. **Duration of stimulus** (Fig. 21.2)**:** The stimulus must continue for a certain minimum period, which varies inversely as the strength. The minimum time required to have a response is known as utilization time. The shortest duration of current flow which will excite the nerve or muscle under current strength equal to twice the rheobase is called the chronaxie. Chronaxie value is a useful index of the relative excitability of the tissues.

   Excitability of a nerve fibre can be determined by studying its strength–duration relationship (threshold stimulus intensity and duration) of the stimulus. To obtain an excitability curve (strength–duration curve) a minimum current strength for exciting a nerve or muscle is first determined and chronaxie is then obtained by determining the shortest duration of stimulus with double the rheobase voltage (Fig. 21.2).
3. **Direction of the current:** If the current is passed transversely across the nerve no effect will be produced. When it passes along the length of the nerve there is the maximum chance of stimulation.
4. **Frequency of stimulus:** A single stimulus will generate a nerve impulse, but if the stimulus be strong, more than one impulse may follow. Ca^{++} lack increases this tendency of multiple responses.

Injury: Excitability is increased near the site of injury. But later on it becomes depressed, and this depression slowly travels down the nerve, so that excitability disappears last from that part of the motor nerve which is nearest to the muscle.

Compound Action Potential

Action potential recorded from a group of nerve fibres (e.g. sciatic nerves) or a nerve trunk is called compound action potential as it is the summated action potentials of different types of nerve fibres having different conduction velocities (Fig. 21.3). The multi-peaked shape of the compound action potential is due to the activity of the different nerve fibres of varying conduction velocity. Most nerves are composed of myelinated nerve fibres of various diameter and also unmyelinated fibres of quite large number. The results obtained by stimulating one end of a frog nerve and recording from a point as far away as possible were described by Erlanger and Gasser.

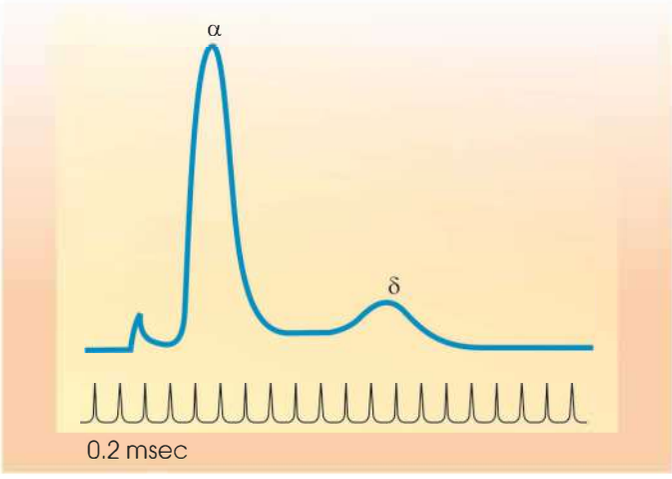


Fig. 21.3: Compound action potential

Joseph Erlanger
1874–1965

Herbert Spencer Gasser
1888–1963

Joseph Erlanger is an American physiologist along with **Herbert Spencer Gasser** identified and classified several varieties of nerve fibre and established the relationship between action potential velocity and fibre diameter. They received the Nobel Prize in Physiology or Medicine in 1944 for their achievements.

With a large shock, the action potential appears as in Fig. 21.4. It is conventionally split into three waves called A, B, C. The A wave itself is divided into α, β, γ and δ sections (Fig. 21.5).

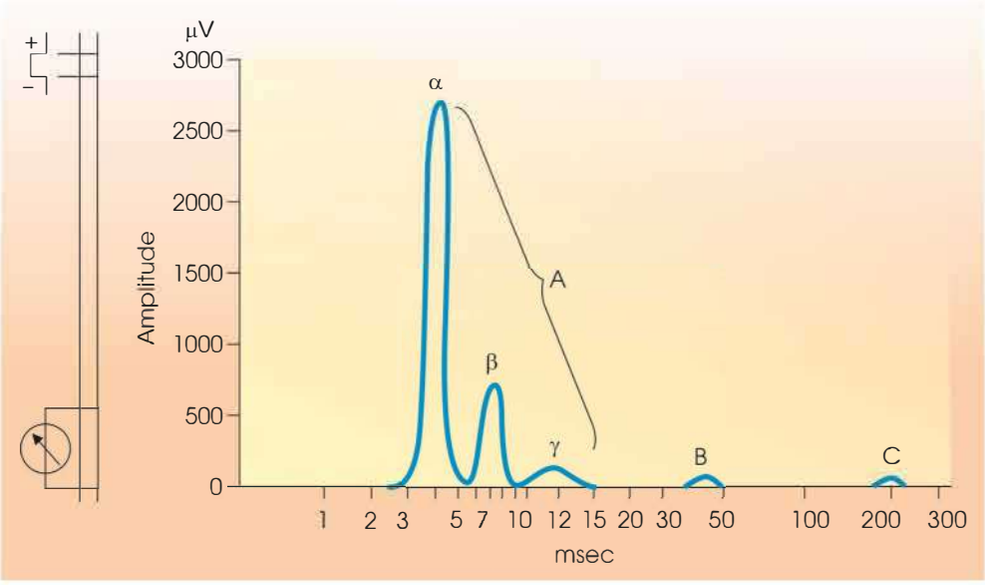


Fig. 21.4: A compound action potential as might be recorded from any nerve containing both myelinated and unmyelinated fibres. The A wave represent myelinated fibres and the C wave unmyelinated ones. The B wave may be mixed

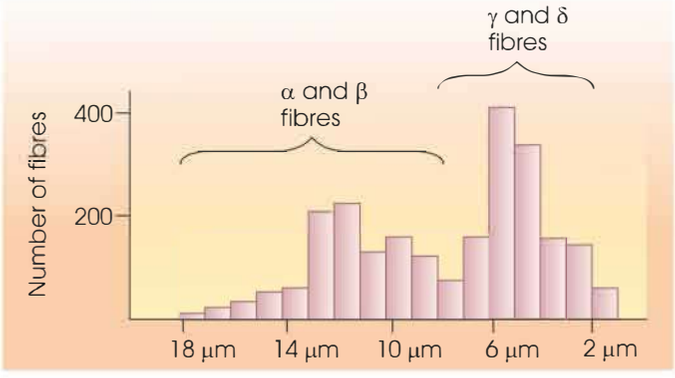


Fig. 21.5: The graph showing the numbers of myelinated nerve fibres of different diameters in nerve

The A wave represents activity in myelinated axons and the C wave that in unmyelinated axons. The B wave may represent both types. The reason for the spreading out of the waves lies in the differing conduction velocities of the fibres. If the recording electrode is close to the stimulating electrode, only a single wave can be detected. It is possible to activate selectively the axons of different sizes. With small shocks, only the wave is apparent. As the shock strength is increased, so other components of the A wave appear, followed by the B and C waves. The largest, fastest-conducting fibres thus have the lowest threshold to stimulations. If a maximum shock (one which fires all the fibres) is used, and pressure is carefully applied between the stimulating and recording electrodes, another phenomenon becomes apparent. The pressure blocks, conduction in the largest fibres first. The α wave is the first to go followed by β, γ, δ and B until only the C wave is left.

By adjusting both the stimulus strength and the pressure it is thus possible to record any component of the action potential. For example, if it is desired to fire γ fibres along, the stimulus strength will be increased until α, β and γ wave appeared and then knock out the β components by application of a pressure block.

2. CONDUCTIVITY

The nerve impulse is conducted along the nerve fibre. Conductivity shows the following characteristics:

1. Impulse is propagated along a nerve in both directions. [But under normal conditions the nerve impulse travels in one direction only—in the motor nerve towards the responding organ; in the sensory nerve towards the centre. This is due to the action of 'synapse'.]
2. ***Velocity of nerve impulse:*** The nerve impulse is propagated with a definite speed (other conditions remaining same). The conduction velocity depends upon the diameter of the nerve fibres, the thicker fibres showing higher velocity. The conduction velocity also depends upon the presence or absence of myelination and also on temperature.
   - *Myelination:* The conduction velocity of myelinated fibres is proportional to the diameter of nerve fibres. The conduction rate in msec is approximately 6 times the fibre diameter in microns in fibres larger than 3 μm. The unmyelinated fibres have a conduction velocity proportional to the square root of the diameter of nerve fibres. With a diameter of 1 μm the conduction velocities are approximately the same. Below 1 μm unmyelinated fibres have a faster conduction rate than myelinated fibres.
   - *Temperature* has got an immense role in conduction of nerve impulse. In the cold-blooded animal conduction velocity is lower than those of the warm-blooded animal.

Conduction velocity calculation: The conduction velocity of a bundle of nerve fibres can be studies by stimulating the nerve bundle at one end and recording compound action potential at other end. If the length of nerve in mm from the placements of the stimulating electrodes to the recording electrodes is noted then the conduction velocity V (msec) can be calculated easily by diving this length of nerve fibres (L) with the latency (in msec) of action potential: V (velocity in msec) = L (length of the fibre in mm)/latency in msec (Fig. 21.6). In general, greater the diameter of the nerve fibre, higher is the velocity of conduction. The conduction velocity of peripheral nerve fibres in man has been studied intensively and it lies in between 60 and 120 msec.

Velocity/diameter ratio of a mammalian nerve fibre has been worked out to be 6 **(Hursh factor)**. If the diameter of the fibre is known then the conduction velocity can be worked out by simply multiplying the diameter (in *u*) with the ratio 6. Conversely if the conduction velocity (msec) is divided by the ratio 6 then the diameter (in *u*) can be found out. The ratio 6 has been considered for peripheral nerve fibres.

Factors Affecting Conductivity and Excitability

1. Temperature—cooling diminishes and warming increases these properties.
2. Mechanical pressure—depresses conductivity and excitability.
3. Blood supply—if blood supply be cut off both these properties are lost.

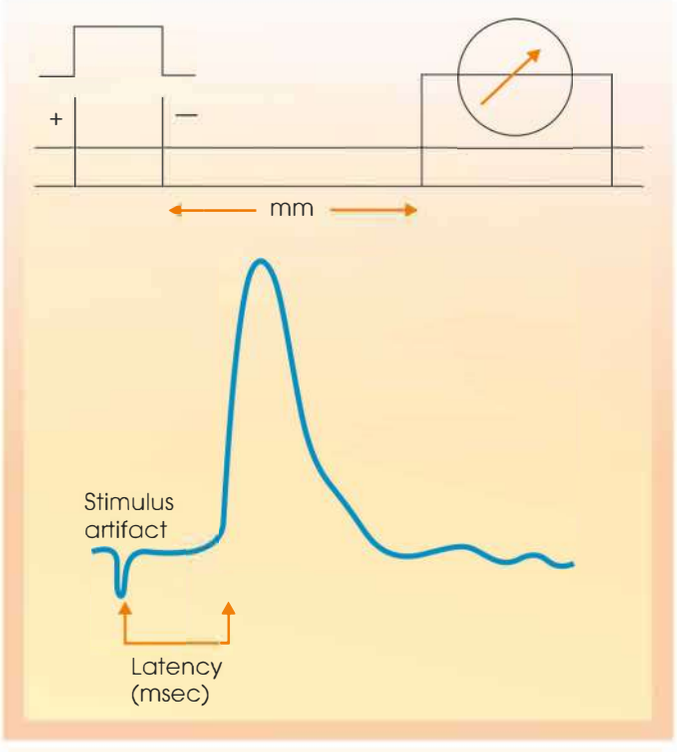


Fig. 21.6: Calculation of conduction velocity

4. Chemicals—CO_2 and narcotics, viz. ether, chloroform, alcohol, Novocain, etc. diminish and finally abolish excitability and conductively.
5. H-ion concentration—increased pH (alkali) increases and decreased pH (acid) diminishes conductivity and excitability. At pH 8.0 the nerve becomes hyper excitable and spontaneous discharge may occur. Even a single stimulus may cause multiple responses (repetitive response).
6. Increased Ca^{++} diminishes and decreased Ca^{++} increases conductivity and excitability.
7. Plasma K^+: Hyperkalemia causes membrane potential to become less negative which decreases excitability by inactivating fast sodium channels. Hypokalemia increases membrane potential hence reduces excitability.
8. O_2 lack—depresses conductivity and excitability and if continued abolishes these properties. If O_2 is readmitted, they return.

3. ALL-OR-NONE LAW

If the stimulus be adequate a single nerve will always give a maximum response. If the strength or duration of the stimulus be further increased no alteration in the response will take place. This property is present in single fibre preparation. In the whole nerve this property is different.

4. REFRACTORY PERIOD

When the nerve fibre is once excited, it will not respond to a second stimulus for a brief period. This period is called absolute refractory period. The absolute refractory period means that the nerve is completely refractory to stimulation—in other words in incapable of eliciting an action potential at any intensity of stimulation. During the absolute refractory period there is total inactivation of the sodium carrier mechanism and as the Na^+ ions cannot enter the fibre, there is no development of the action potential. Immediately following this, there is a brief relative refractory period, during which the excitability is subnormal but gradually rising. This is succeeded by a third brief period of increased excitability, known as supernormal phase. Lastly, there is a period of subnormal excitability—subnormal phase. Figure 21.7 demonstrates the different refractory periods.

When two stimuli are applied to one end of a nerve at different time intervals, compound action potentials are recorded and two different action potentials are recorded (I, II). But as the time intervals are gradually decreased, a time will come when the second shock will fail to produce compound action potential of equal length and the height will be decreased (III, IV). This is happened in absolute refractory period. But if the time interval is further

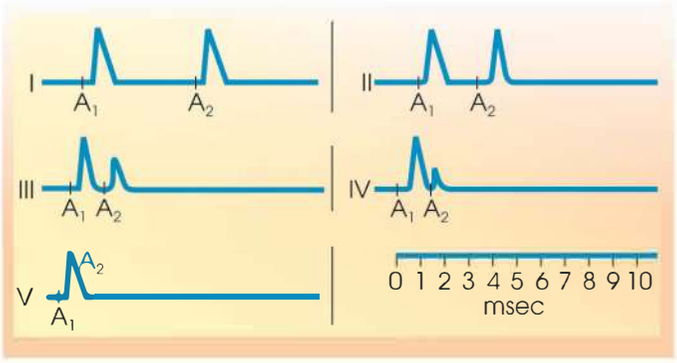


Fig. 21.7: Diagram shows the nature of action potential recorded by successive stimuli at different levels

decreased, then the second stimulation will fail to elicit any response. This period is the absolute refractory period (V).

In the large mammalian nerve fibres the durations are as follows: Absolute refractory period: 2 to 3 milliseconds in frog but in mammal it is 0.5 millisecond. Relative refractory period is 10 to 30 milliseconds in frog but in mammal it is 3 milliseconds. Supernormal phase is 12 milliseconds and subnormal phase may be up to 70 milliseconds.

5. SUMMATION (LATENT ADDITION)

In a nerve fibre summation of two submaximal stimuli is possible.

6. ADAPTATION

The nerve fibre quickly adapts itself. Due to this adaptation there is no excitation during the passage of a constant current. Only when the strength of the current is suddenly altered or the current is made or broken excitation takes place. A gradual change will fail to excite.

7. ACCOMMODATION

If a stimulus even with stronger strength is applied very slowly to a nerve, then there may have no response only due to lack of attaining the threshold strength. This phenomenon is called accommodation, i.e. slowly applied stimulus is accommodated by the nerve no matter how strong the stimulus is applied.

8. INDEFATIGABILITY

In the nerve muscle preparation, if the nerve is stimulated repeatedly, then after a certain period the muscle fails to give any response. Now if that nerve is isolated from the muscle and placed on a fresh muscle, then application of stimulus will excite the muscle. This shows that nerve is not fatigued.

Metabolism in Nerve Fibres

Metabolic changes are constantly going on in a nerve fibre at a very low level. During activity the metabolic rate increases. It is known by the following facts:

Oxygen consumption and CO_2 production: Nerve fibres continuously use O_2 and evolve CO_2. The resting sciatic nerve fibre consumes about 0.7 cu mm of O_2 and evolves about 0.6 cu mm of CO_2 per gram of nerve fibre per minute, the RQ being 0.8. During activity the extra metabolism consumes about 0.25 cu mm of O_2 and produces slightly smaller amount of extra CO_2 per gram of nerve fibre per minute. The RQ of this extra metabolism is about 0.99. The extra O_2 consumption during activity occurs during the period of evolution of delayed heat.

- When the frequency of stimulation is raised the total O_2 consumption increases but that per impulse, proportionally decreases.
- Increasing the strength of the stimulus above the threshold value does not increase the O_2 consumption.

Chemical Changes and their Relation with the Properties of Nerve Fibres

Key Points

1. Unlike muscles, the excitability, conductivity and the recovery process can go on in a nerve for a considerable period even in absence of oxygen.
2. The chemical changes in the nerve are roughly of the same nature as seen in the muscles. Pyruvic acid is formed and if O_2 supply be insufficient, lactic acid accumulates (same as in muscles). Thiamine which is essential for complete oxidation of these acids is found in good amount in the nerve fibres. Although carbohydrates burn, yet they are not the only source of energy (contrast with nerve cells, which possibly use galactose). The breakdown of phospholipids also takes an essential part here.
3. It is said that, the energy requirement of the resting nerve is supplied by combustion of sugar and phospholipids mainly.
4. During activity, ATP and creatine phosphate break down and supply energy for the propagation of the nerve impulse. Both ATP and creatine phosphate are then resynthesised but the source of energy of this recovery process is not known (may be in the same way as in muscles). During activity acetylcholine is liberated by the cholinergic fibres, while norepinephrine by the adrenergic fibres.
5. The nerve fibres are rich in K^+ and thiamine. During activity K^+ (and possibly thiamine) diffuses out and Na^+ enters the fibres. This migration of K^+ and Na^+ seems to be intimately related to the properties, viz. excitability, conductivity, etc. of the nerve fibres.

HEAT PRODUCTION IN NERVE FIBRE

As already mentioned the metabolism in the nerve fibre is very low. During rest a minute quantity of heat is produced which increases during activity. Heat is evolved in three phases: The first phase is called initial heat and the other two phases are seen during recovery and therefore known as the recovery heat or the delayed heat.

Initial heat: It is about 10% of the total heat (5–10 microcalories per second per gram of nerve fibre) but the rate of evolution is very brisk being 5000 times greater than that of delayed heat. It is anaerobic and coincides with the spike potential. Its cause may be the breakdown of ATP, creatine phosphate or, as Hill suggests, due to the discharge of an electric double layer located at the surface of the nerve fibre.

Delayed heat: It is aerobic and is 8.5 times more than the initial heat. This energy is possibly used for the resynthesis of ATP and creatine phosphate and as such, for restoring the normal excitability of the nerve fibre.

It comes in **two phases**

The *first phase* lasts for few seconds and the quantity of heat is small and is about the same as the initial heat.

The *second phase* may last for 10–30 minutes and contributes the greatest proportion of both total and delayed heat.

- Increase in the strength of the stimulus does not raise heat production. But increased frequency increases about 25%.
- It is to be noted that heat production in the grey matter (nerve cell) is enormously greater than that in the nerve fibre.

Classification of Nerve Fibres

Nerve fibres have been classified in different ways:

1. **Histologically:** Medullated and non-medullated
2. **Functionally:** Motor (efferent) and sensory (afferent).
3. **Chemically:** Adrenergic (producing norepinephrine) and cholinergic (producing acetylcholine).

According to diameter and conduction velocity (Erlanger and Gasser): The physiological properties of nerve fibres vary with their diameter and conduction velocity. Thicker the fibre, higher will be the impulse velocity and spike potential but lower will be the refractory period and stimulus threshold (chronaxie). Erlanger and Gasser have divided the nerve fibres into A, B and C.

On systematic examination of the compound action potential of various nerves, it reveals that:

1. A fibres are myelinated, somatic, afferent and efferent axons.
2. B fibres are pre-ganglionic, myelinated, efferent, and sympathetic axons.
3. C fibres are sympathetic and somatic, unmyelinated axons. The C fibres are differentiated into two groups—the sC and drC on the basis of differences in their after-potential. The drC group has got no negative after-potential. C groups of fibres are efferent, post-ganglionic sympathetic axons and the drC groups of fibres are the small afferent axons found in peripheral nerves and dorsal roots.

In peripheral somatic fibres, both A and C fibres are present. If such fibres are stimulated at one end and recorded through oscilloscope at other end, then the compound action potential formed in A fibres is of four different deflections—α, β, γ and δ. These different deflections are due to corresponding stimulation of different fibres of different conduction velocities.

- α deflection is due to stimulation of nerve fibres having comparative larger diameter with higher conduction velocity.
- δ deflection is due to stimulation of fibres having lowest diameter and slowest conduction velocity.

Tables 21.1 and 21.2 and Flowchart 21.1 classification according to conduction velocity and diameter of the nerves.

MECHANISM OF CONDUCTION OF THE NERVE IMPULSE

According to the membrane theory, the nerve impulse is a propagated wave of depolarization. It has been discussed more earlier that a resting nerve fibre remains in polarized state, with positive charges lined up along the outside of the membrane and negative charges along the inside. As soon as the fibre is excited at a point the polarity is changed and for a brief period, it is actually reversed. This reversed polarity is due to increased permeability of Na^+ to the membrane and this depolarization of wave is developed. A local circuit current flows between the depolarized membrane and the resting membrane areas. Positive current flows inward through the depolarized membrane and outward through the resting membrane and in this way circuit is completed. This local depolarization current then excites the adjacent portions of the membrane producing progressively more and more depolarization. The depolarization wave travels in all directions along the entire length of the nerve fibre. This type of conduction is observed in the non-medullated nerve fibre (Fig. 21.8).

Repolarization wave occurs from the point of stimulus, a few ten thousandths of a second later than the depolarization wave and spreads progressively along the membrane following same directions as the depolarization has spread previously.

Table 21.1: Numerical classification of nerve fibres

Types of fibre	*Diameter of fibre in μm*	*Velocity of conduction in msec*	*Duration of spike potential in msec*	*Absolute refractory period in msec*	*Function*
A-α	12–20	70–120	0.4–0.5	0.4–1.0	Proprioception; somatic motor
A-β	5–12	30–70	—	—	Touch, pressure
A-γ	3–6	15–30	—	—	Motor to muscle spindle
A-δ	2–5	12–30	—	—	Pain, temperature
B	Less than 3	3–15	1.2	1.2	Preganglionic sympathetics
C dorsal root (drC)	0.4–1.2	0.5–2.0	2.0	2.0	Pain reflex response
C sympathetic (sC)	0.3–1.3	0.7–2.3	2.0	2.0	Postganglionic sympathetics

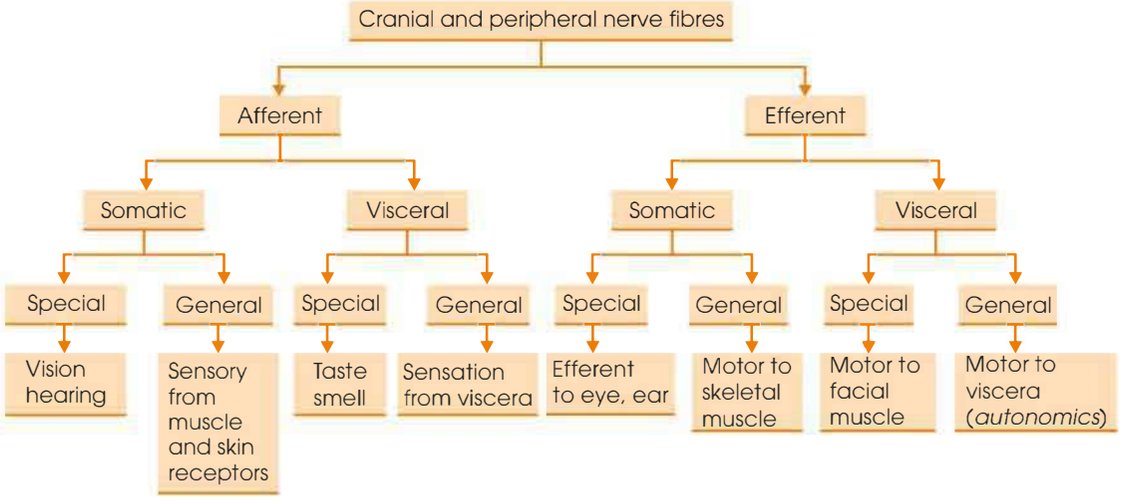
%Numerical classification of sensory nerve fibres: Sometimes sensory nerve fibres are numerically classified and have been presented along with Erlanger and Gasser latter system in the following Table.

Table 21.2: Classification of nerve fibres on physio-chemical basis

Group	*Letter system*	*Origin*	*Reflex response*	*Central reflex connection*	*Destination*
Ia	A-α	Muscle spindle: Annulo-spiral spindle ending	Myotatic reflex of muscle (tendon jerk)	Monosynaptic with motor neuron of muscle of origin	Spindle of extensor of flex or muscle
—	—	—	Relaxation of antagonist muscle during myotatic contration of agonist muscle	Disynaptic with antagonist motor neuron	Antagonist muscle
Ib	—	Golgi tendon organ	Reaction lengthened	Disynaptic with motor neuron of muscle of origin	Muscle of origin
II	A-β and A-γ	Muscle spindle Flower-spray ending, skin, touch-pressure receptors	Relaxation of extensors and excitation of flexors (flexor withdrawal)	Polysynaptic	Extensor and flexor motor neurons
III	A-δ	Muscle and skin, pain-temperature receptors	Flexion withdrawal of same limb, crossed extensor of opposite limb	—	—
IV	Dorsal root C fibres	Muscle and skin, pain receptors	—	—	—

% On physio-anatomic basis—the peripheral nerves can be classified into afferent and efferent and each of which is again subdivided as presented in the following Table.

Flowchart 21.1: Classification of nerve fibres on anatomical basis



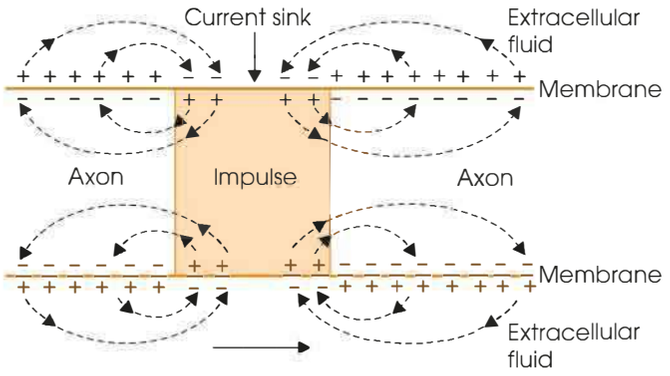


Fig. 21.8: Diagrammatic representation of current flow around an impulse of axon in unmyelinated nerve fibre representing movements of positive charges. A straight arrow indicates direction of propagation

Saltatory Conduction in the Myelinated Nerve Fibre

In the myelinated nerve fibre, condition depends upon a similar pattern of circular current flow. Myelin sheath is an effective insulator. Ions cannot pass through the myelin sheath, and nodes of Ranvier permeate ions to pass through it more easily. That is why the membrane at nodes of Ranvier is 500 times as permeable as it is in unmyelinated fibres. For this reason the impulse is transmitted from node to node rather than continuously along the entire length of the fibre (Fig. 21.9). The depolarization in myelinated axon jumps from one node of Ranvier to the next. This jumping or leaping of depolarization from node to node is known as saltatory (saltare = to dance) conduction (Figs 21.10 and 21.11).

The current which remains confined to the nodes depolarizes the internodal part by local circuit action. The myelin sheath increases the velocity of conduction. Huxely and Stampfli in 1949 demonstrated the following experiments.

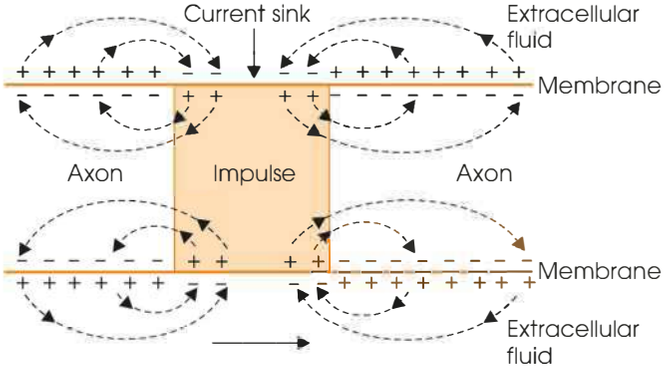


Fig. 21.9: Diagrammatic representation of local current flow around an impulse, an axon in the myelinated nerve fibre (saltatory conduction) representing movements of positive charges. A straight arrow reveals direction of propagation

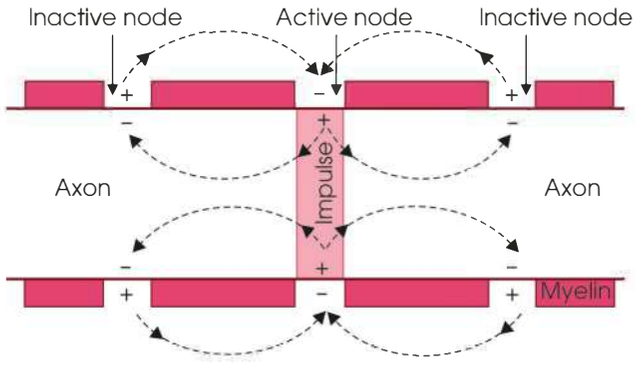


Fig. 21.10: Diagrammatic representation of the saltatory conduction along a myelinated axon. The straight arrow represents direction of propagation

PHYSIOLOGICAL PROPERTIES OF THE NERVE FIBRES

Physiological properties of the nerve fibre can be studies through the cathode ray oscilloscope (CRO). Action potential or membrane potential or a single nerve fibre can be studied by microelectrode placed within the nerve fibre and a differential electrode placed outside the nerve fibre. Each microelectrode is a minute capillary glass tube with a tip of 0.25 to 2 microns filled with a very concentrated potassium chloride solution acting as an electrical conductor. The other ends of both the electrode are connected to the cathode ray oscilloscope (CRO). The development of action potential in the nerve fibre is viewed on the oscilloscopic screen of the CRO.

Nerve Injury

By noting the electrical response of a muscle it is possible to know whether it is degenerating or not. The spot where the muscle receives its motor nerve is known as the motor point. These points have been mapped out for all skeletal muscles and during actual procedure reference is made to these maps. The active electrode (stigmatic electrode) is placed upon the motor point of the muscle and the other passive electrode (generally large and flat) is placed on a distant indifferent spot, such as the back of the neck. By reversing the current

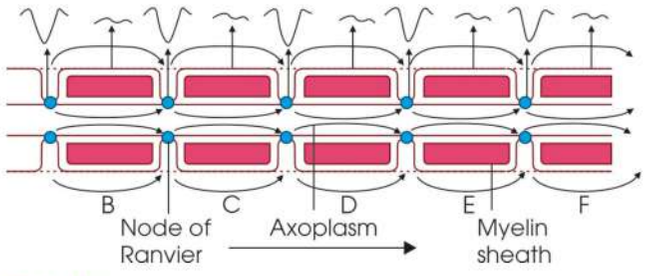


Fig. 21.11: Huxley and Stampfli's experiment to demonstrate the conduction of nerve impulse depending upon action current flowing outside the myelin sheath

with a commutator, the stigmatic electrode may be either made anode or cathode. With such arrangements, four sets of reactions are seen. When the stimulating electrode is cathode the response at make is called cathode-closing contraction (CCC). The response at 'break' is known as cathode-opening contraction (COC). When the stimulating electrode is anode similar two contractions are obtained and are known respectively as anode-opening contraction (AOC) and anode-closing contraction (ACC). The quantitative relation is as follows: CCC > ACC > AOC > COC. When the muscle degenerates:

ACC becomes greater than CCC

No response is obtained to Faradic stimulation; muscle starts responding to galvanic stimulation because the chronaxie is increased and excitability of the degenerating muscle is decreased. A slow worm-like contraction to galvanic stimulation. This is called reaction of degeneration (RD).

Degeneration and Regeneration of Nerve

Nerves injury may occur due to compression, ischaemia, laceration, traction or burning. The damage to nerve may vary in severity. The injury might be transient and quick recovery of lost functions or it may lead to degenerative changes.

Sunderland Classification of Nerve Injury

First degree injury: It constitutes transient ischaemia and neurapraxia, the effects of which are reversible.

Second degree injury: Axonal degeneration takes place, but as the endoneurium is preserved, regeneration can lead to complete, or near complete recovery.

Third degree injury: The endoneurium is disrupted but the perineurial sheaths remain intact and there is limited internal damage. The chances axonal regeneration exist but fibrosis and crossed connections will limit recovery.

Wallerian degeneration: It occurs when a nerve axon is cut, crushed, or frozen.

It is called anterograde degeneration. It is named after Augustus Waller, a neurophysiologist (1816–1870), who first described the process degeneration of injured nerve fibres. Post injury as the axon is disrupted from the neuron's cell body it degenerates distal leading to Wallerian degeneration. Degeneration usually occurs within a day or two after a nerve injury. The axon's neurolemma is the outermost layer of the neuron made of Schwann cells. It does not degenerate and remains as a hollow tube.

The changes due to nerve injury may progress as follows:

Early changes

1. Synaptic transmission disruption.
2. The cut ends pull apart and seal up, and swell, due to axonal transport in both directions.

After few hours later

3. Synaptic terminal degenerates and there is accumulation of neurofibrils, vesicles, etc.
4. Astroglia surrounds terminal normally; after axotomy. It interposes between terminal and target due to which terminal get pulled away from post-synaptic cell.

After days–weeks

5. Myelin breaks up and leaves debris (myelin hard to break down).
6. Axon undergoes Wallerian degeneration
7. Chromatolysis: Cell body swells; nucleus of the nerve cell becomes eccentric and Nissl bodies are sparse.

Regeneration

Regeneration takes place only outside the central nervous system where neurolemma is present. Presence of neurolemma is, therefore, essential for the process. Hence, in the central nervous system, neurolemma being absent, nerve fibres do not regenerate at all.

The following steps are seen during regeneration:

1. The axis cylinder grows out from the central cut end as a rounded sprout and proceeds towards the solid neurolemmal cord.
2. The proliferated Schwann tissue in the peripheral cut end and its prolongation towards the central cut end provide an influence (neurotropism) which guides the approaching axis cylinder.
3. Each growing fibre splits up into numerous neurofibrils (even up to 100), the Schwann cells disappear and the fibrils enter the newly-made neurolemmal tubes (2–3 weeks after the section, the inner walls of the tube may contain a number of fibrils. All the fibrils degenerate, excepting a single one, which gradually enlarges and occupies the central part of the whole length of the tube proceeding peripherally.
4. The daily rate of growth is about 0.25 mm in the scar tissue between the two cut ends and 3–4 mm in the peripheral neurolemmal tubes.
5. Myelin sheath begins to appear in about 15 days and proceeds peripherally along the fibre at a slower rate than the growing axis cylinder. Increase in the diameter of the fibre takes place very slowly. The diameter of the fibre is limited by the size of the neurolemmal tube and that of the parent nerve cell.
6. With a clean sharp wound and the cut ends being in apposition, some degree of recovery usually takes place in 6–24 months. For a motor nerve, recovery may be complete. But for a mixed nerve, it is rarely so.
7. In the regenerated fibres the axis cylinder and myelin sheath are reduced in thickness, the internodal distance is also diminished. But the rate of conduction of nerve impulses in the regenerated fibres remains the same.

DEGENERATION AND REGENERATION OF NERVE

The framework of both sensory and motor endings can resist degeneration for months. If the nerve fibres fail to regenerate, the endings also atrophy. But if the fibres regenerate, the living frameworks of the nerve endings quickly establish connection with the growing fibres and start functioning. Some of the newly growing fibres may establish connection with new types of endings in new situations. It is also possible that some growing nerve fibres may reach a place where there was no nerve ending at all and absolutely fresh nerve endings may develop around them. Complete functional regeneration occurs after histological regeneration—3 weeks in case of motor nerve fibres and 5 weeks in case of sensory nerve fibres.

Transneuronal Degeneration

When a neuron or its motor fibre degenerates the neuron next in the chain is often found to degenerate also. This takes place in spite of the fact that there is no anatomical conditions continuity through the synapses. It is probably an example of disuse atrophy. In many conditions, this type of degeneration occurs, e.g. after section of the optic nerve, the cells in the lateral geniculate body degenerate. After section of the posterior spinal root, the posterior horn cells degenerate. In lesions of the motor cortex or pyramidal tracts, the anterior horn cells may degenerate. This type of degeneration may be the underlying cause of the so-called system diseases, viz. amyotrophic lateral sclerosis, etc. where degeneration of anterior horn cells follows that of the pyramidal tracts.

Thus to summarize: The process of nerve injury and repair includes the following steps

Example: Injury to target organ (striated muscle).

1. Following the nerve injury the distal part of the axon will undergo disintegration and the myelin sheath breaks up.
2. The nucleus of the nerve cell becomes eccentric and Nissl bodies are sparse.
3. During stage of recovery the new axonal tendrils grow into the mass of proliferating Schwann cells.
4. The tendrils find its way into the old endoneurial tube and the axon thereafter slowly regenerate.

CATHODE RAY OSCILLOSCOPE (CRO)

Karl Ferdinand Braun
1850–1918

The cathode ray oscilloscope is used to estimate or measure the electrical changes of the living tissues. A cathode emits electrons when a high voltage is applied to it with a suitable anode in a vacuum. These emitted electrons are directed into a focused beam which strikes the face (screen) of the glass tube of the CRO. The screen of the CRO is coated with a number of fluorescent substances (phosphors) which emits light when struck by electrons.

Karl Ferdinand Braun invented the Cathode Ray Tube Oscilloscope in 1897 when he was experimenting on physics principles of applying an oscillating signal to electrically charged deflector plates in a phosphor-coated CRT.

In a CRO to record the action potentials, two electrical circuits must be employed which are as follows:

1. An electronic sweep circuit
2. An electronic amplifier.

The electronic sweep circuit is connected with two vertical metal plates (X-plates) on either side of the beam in the cathode ray (CR) tube. By altering the electrical potentials at a very high speed it moves the beam of electrons horizontally across the screen of the tube from left to right and when the beam reached the right extremity, it jumps back to the left side and in this way the beam is always moving from to right. There are two metal plates, in the cathode ray tube, which

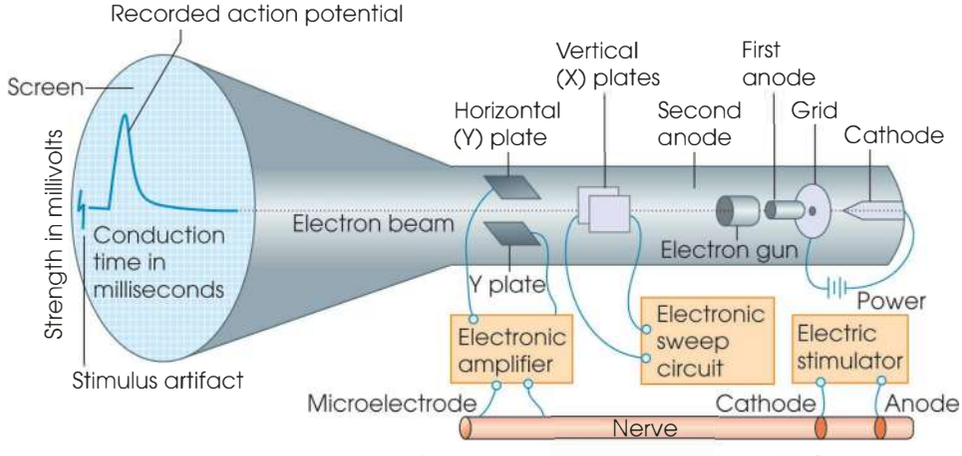


Fig. 21.12: Simplified, diagrammatic representation of the main connections of the CRO to action potential changes

arranged horizontally, one above and the other below the electron beam. The Y plates (horizontal) are connected, with the electronic amplifier. This set of deflecting plates move the electron beam up and down with the change of action potential in them. For experiments, the electrodes are place on the nerve fibre or the tissue of which the electrical potential change is to be measured. These electrodes are connected with the amplifier circuit thereby to the horizontal plate (γ-plates). The potential change in the tissues after proper amplification is transmitted to the Y plates and recorded as vertical deflections of this electronic.

Beam on the screen which may be photographed for permanent record. Within the CR tube itself, cathode serves as a source of electrons; grid controls the intensity of electron beam and brightness of the spot. The first anode compresses the flow of electrons into narrow beam whereas the second anode (electron gun) is highly positive and accelerates the beam of electrons. The electric stimulator, of which one is connected with cathode and the other with anode, applies a short (or as chosen) voltage (pip) to the nerve stimulating microelectrodes (Fig. 21.12).

EXAM-ORIENTED QUESTIONS

Essay

1. Discuss the properties of nerve fibre.
2. Classify nerve fibres. How is the impulse conducted in various nerves?
3. Describe the mechanism of Wallerian degeneration and regeneration of nerves.

Short Notes

1. Excitability of nerve
2. All-or-none law in nerve
3. Conductivity in nerve
4. Metabolism in nerve fibres
5. Electrotonic current
6. Electrotonus
7. Cathode ray oscilloscope

Recent Advances: Nerve Growth Factor

Stanley Cohen
1922

Rita Levi-Montalcini
1909–2012

Stanley Cohen and Rita Levi-Montalcini jointly received the Nobel Prize for Physiology and Medicine in 1986 for their discovery of Nerve Growth Factor. Cohen later discovered the epidermal growth factor too. His research on cellular growth factors has helped in understanding the development of cancer and designing anti-cancer drugs.

Chapter

22

Neuromuscular Junction

INTRODUCTION

Anatomical Considerations (Fig. 22.1)

The motor nerve before ending into the muscle fibre loses its myelin sheath. The nerve fibre, at its termination, branches into several expanded structures which are known as axon terminals or sole feet.

Under light microscope (Fig. 22.2) the myoneural junction shows naked motor nerve endings with the Schwann cells. The expanded nerve endings or sole feet lie within the corrugated sarcolemma—the junctional folds of the muscle fibre. The corrugated sarcolemma is formed by the numerous invaginations of the sarcoplasm. These sarcolemma invaginations are, collectively called synaptic gutter. The sole feet do not end within the sarcoplasm but a gap is present in between the axon membrane and the sarcolemma.

This gap is known as synaptic cleft. Synaptic cleft is filled with extracellular fluid (gap substance). The synaptic cleft is having a diameter of 20–30 nm (nanometres). It is the space between the axon terminal and the muscle cell membrane. It contains the enzyme cholinesterase which can destroy ACh. Numerous muscle nuclei are also seen in the sarcoplasm. Under electron microscope (Fig. 22.3), the sole feet show the presence of mitochondria, numerous vesicles, smaller granular elements and also endoplasmic reticulum. The whole sole foot is covered by the cytoplasm of the Schwann cell. This substance is stored in the vesicle and released when the propagated impulse reaches the post-junctional membrane (PJM) or post synaptic membrane. The axon terminal contains around 300,000 vesicles which contain the neurotransmitter acetylcholine (ACh). Cholinesterase is present in the rim of the synaptic gutter.

Due to presence of multiple folds in the sarcolemma, the total surface area at which the transmitter substance acts is increased. Mitochondria are also present in the sarcoplasm.

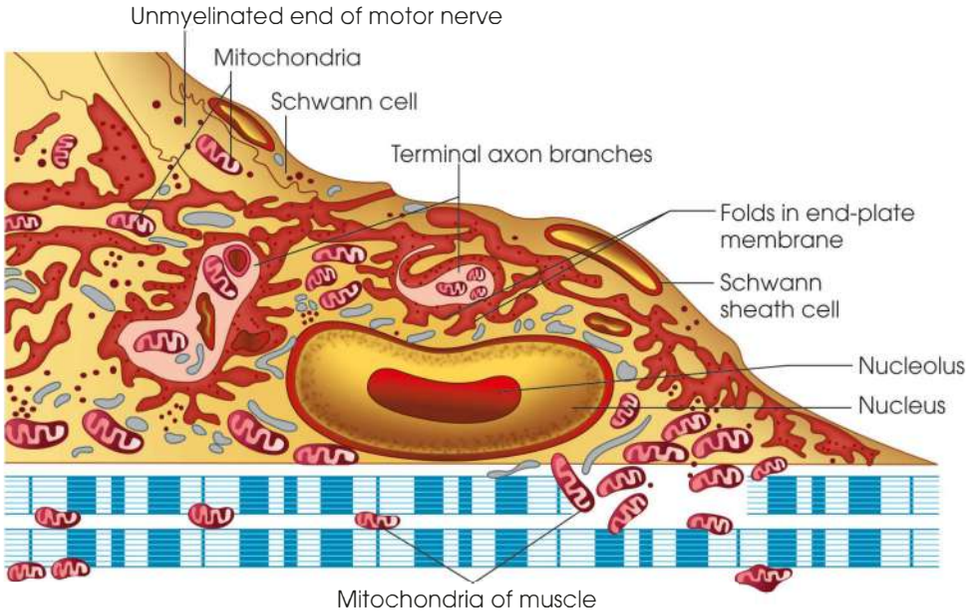


Fig. 22.1: Diagrammatic representation of an electron microscopic structure of the neuromuscular junction

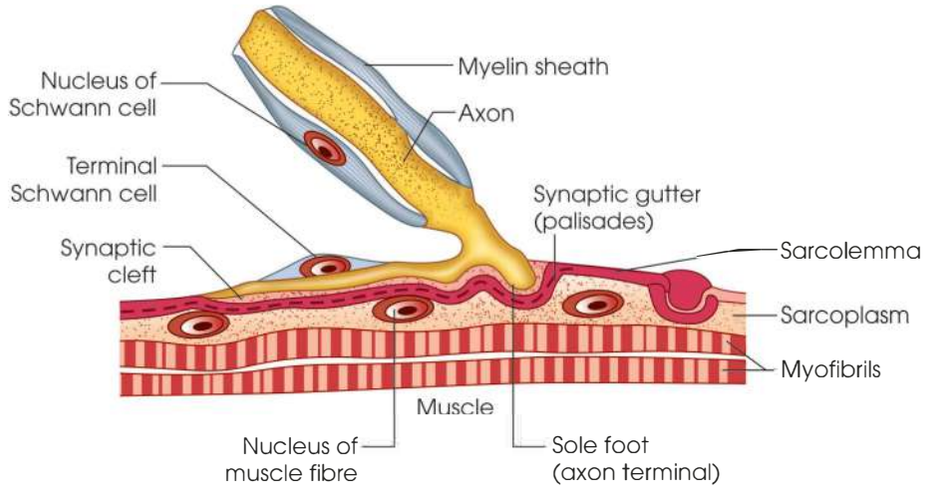


Fig. 22.2: Diagrammatic representation of the relationship of nerve ending to the muscle fibres (*neuromuscular junction*)

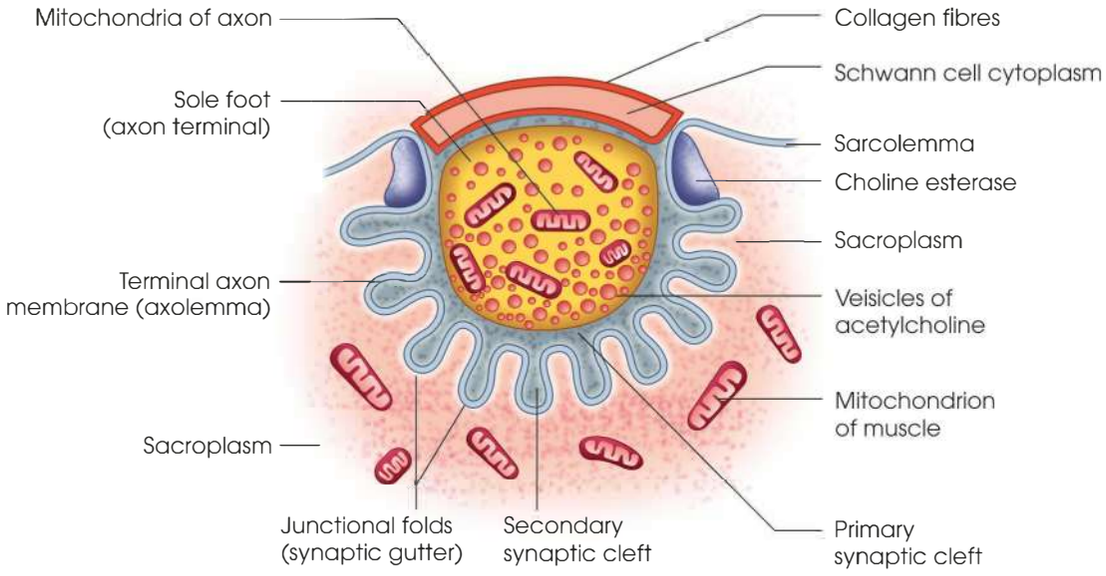


Fig. 22.3: Electron microscopic appearance of myoneural junction at the region of an axon terminal (sole foot) ending in the motor endplate

Synthesis of Acetylcholine in Motor Neuron

The ACh is synthesized locally in the cytoplasm of the nerve terminal, from active acetate (acetyl coenzyme A) and choline. Then it is rapidly absorbed into the synaptic vesicles and stored there. The synaptic vesicles themselves are made by the Golgi apparatus in the nerve soma (cell-body). Then they are carried by axoplasmic transport to the nerve terminal which contains around 300,000 vesicles. Each vesicle is then filled with around 10,000 ACh molecules. As action potential reaches the synaptic knob the Ca channels open increasing calcium permeability. The vesicle fuses with presynaptic membrane to release the neurotransmitter (NT) from synaptic knob to synaptic cleft. The neurotransmitter combines with specific receptors on the other membrane post-synaptic potential to generate end-plate potential. EPP then spread by local current to adjacent muscle fibres which are depolarized to threshold and fire action potential.

Sequence of Events in Neuromuscular Transmission (Fig. 22.4 and Flowchart 22.1)

Pre-synaptic Events

1. The action potential is initiated in the pre-synaptic motor neuron and invades the endplate region.
2. The depolarization of motor neuron up to terminal buttons result in the opening of voltage-dependent calcium channels. There is influx of Ca^{2+}, down its concentration gradient.
3. The increased cytoplasmic concentration of calcium enhances the movement of microfilament and microtubules which moves the vesicle to the pre-synaptic membrane. The fusion of vesicles containing

acetylcholine (ACh) to the membrane of the terminal buttons, resulting in exocytosis of ACh. Acetylcholine diffuses across synaptic cleft to the muscle cell.

Synaptic cleft events: The acetylcholine is then degraded by acetyl cholinesterase present in the synaptic cleft and some acetylcholine diffuse out of the cleft. About 50% of choline is returned to the pre-synaptic terminal by Na^+ choline transport to be reused for ACh synthesis.

Post-synaptic Events

1. Acetylcholine binds to nicotinic ACh-receptors at endplate. The receptor binding of acetylcholine causes opening of cation channels, leading to influx of Na^+.
2. The resulting depolarization of muscle cell membrane at the endplate is referred to as the endplate potential (EPP). The small quanta (packets) of ACh are released randomly from nerve cell at rest, each producing smallest possible change in membrane potential of motor endplate, the miniature EPP. When nerve impulse reaches the ending, the number of quanta release increases by several folds and result in large EPP.
3. EPP then spread by local current to adjacent muscle fibres which are depolarized to threshold and fire action potential.
4. The local depolarization causes adjacent regions to be depolarized, causing an AP in the muscle cell membrane.
5. AP spreads out in all directions from the endplate, propagates along muscle cell, initiating contraction.

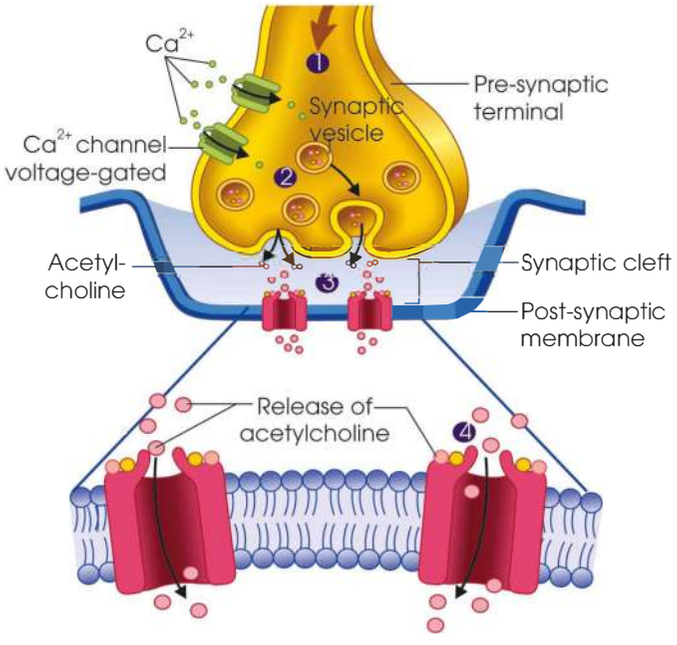


Fig. 22.4: Events in neuromuscular transmission

Flowchart 22.1: Neuromuscular transmission

Propagated action potential

↓

Depolarizes the neuron terminals—ACh released from synaptic vesicles with the Ca^{++} influx

↓

ACh diffuses across the synaptic cleft—ACh forms a transmitter-receptor-complex with receptor substance of the PJM

↓

Production of end-plate potential (EPP). Initiation of propagated muscle action potential in the sarcolemma —transmission of action potential from sarcolemma to the contractile units of muscle through excitation—contraction coupling and development of muscle tension: Muscular contraction takes place

To ensure purposeful movement muscle cell electrical response is turned off by acetylcholinestrase (AChE), which degrade ACh to choline and acetate. Now muscle fibre can relax, if sustained contraction is needed for the further desired movement another motor neuron AP leads to release of more ACh.

Neuromuscular Blockers

These are non-depolarizing or competitive and depolarizing neuromuscular blockers.

1. **Non-depolarizing neuromuscular blocker:** The prototype of non-depolarizing neuromuscular blocker is tubocurarine. The new generation non-depolarizing neuromuscular blockers are pancuronium and gallamine. In small clinical doses they act the predominantly at the nicotinic receptor site to block ACh. At higher doses they can block pre-junctional Na channels thereby decreasing ACh release. Because of the competitive nature of the postsynaptic blockade, transient relief of the block can be achieved by increasing ACh levels at the synaptic cleft (i.e. use cholinesterase inhibitors).
2. **Depolarizing neuromuscular blocker:** The prototype of depolarizing agent is succinylcholine. The actions are similar to ACh but are longer acting. The membrane is depolarized by opening acetylcholine receptor channels causing brief period of muscle fasciculation. The endplate eventually repolarizes but because succinyl choline is not metabolized like ACh it continues to occupy the acetylcholine receptor to desensitize the endplate. Because of the mechanism of action of depolarizing drugs is similar to ACh, their blocking effects are augmented by acetylcholine esterase inhibitors.

Reversible acetylcholine esterase (AChE) inhibitors: They compete with acetylcholine to bind to acetylcholine esterase inhibitors and prevent hydrolysis of

acetylcholine. The example of AChE inhibitors are physostigmine and neostigmine.

1. **Myasthenia gravis:** It is a disease involving neuromuscular junction and is characterized by the extreme muscular weakness. It is an autoimmune condition in which the body produces antibodies against its own motor endplate ACh receptors. All of the ACh molecules do not find functioning receptors site to bind. As a result AChE destroys much of ACh before it ever has a chance to interact with receptor site and contribute to EPP. It is characterized by muscle weakness and it gets typically aggravated by repeated contraction. The typical clinical presentation attributed to myasthenia is weakness of ocular muscles causing drooping of the eyelid. The disease may progress and affect facial muscles, limb girdle muscles, and respiratory muscles. The patient may suffer from respiratory paralysis leading to death. It is treated with long acting anticholinesterase inhibitor pyridostigmine or neostigmine.
2. **Lambert-Eaton myasthenic syndrome:** It is an autoimmune disorder affecting the presynaptic portion of the neuromuscular junction. This unique triad of symptoms; presented by patient are proximal muscle weakness, autonomic dysfunction and areflexia. Proximal muscle weakness is a product of pathogenic autoantibodies directed against voltage-gated calcium channels, and this leads to decreased release of acetylcholine from motor nerve terminals on the presynaptic cell.

SYNOPSIS OF THE NEUROPHYSIOLOGICAL MECHANISM

This mechanism involves reception of stimulus by the receptor organ, transmission of the impulse from one neuron to another and transfers of the impulse through the motor endplate to the muscle.

Let the pacinian corpuscle be the receptor which is lamellated like onion with naked axis cylinder of the sensory nerve arborising in the central core responds to changes in pressure.

When a stimulus is applied to corpuscle it gets deformed and the mechanical stimulus is transformed into electrochemical signal which is conducted along the sensory nerve fibre. The membrane of the outer side the axis cylinder within the receptor contains abundance of sodium and chloride ions, whereas there is predominance of potassium ion inside the axoplasm. Under resting condition the outer side of the membrane is positive and the inner side is negative. When the lamella is deformed it causes ionic movement across the membrane of the axis cylinder which has got specific permeability. There is at first inward flow of sodium and chloride ions which is followed by the outward flow of potassium ion. Due to these ionic movements slight depolarization of the membrane occurs. This is known as generator potential. This again depolarizes the adjacent axon membrane. When the stimulus is adequate, the depolarization reaches the threshold level and the depolarization spreads at the first node of Ranvier. This is action potential. It is generated at the first node of Ranvier and then there is saltatory conduction, i.e. it is conducted in 'jumps' or 'leaps' at each successive node along the sensory nerve. At the node of Ranvier there is major inward and outward flow of ions along with electrical currents. The current which remains confined to the nodes depolarizes the inter-nodal part by the local circuit action. This action potential lasts for a very short time, i.e. 1 millisecond. The action potential follows each other very quickly along the sensory nerve. The refractory period of the nerve is also 1 millisecond. So there is the saltatory conduction of the impulse into the grey matter of the spinal cord through the sensory neuron and is transmitted to the motor neuron of the anterior horn. At the synaptic junction in the spinal cord the axon terminals make an intimate contact with the membrane of the motor neuron. The pre-synaptic and post-synaptic membranes form the synapse. The synapse acts as a functional unit of the transmission of impulses. At the membranes there is predominance of sodium and chloride ions on the outer side, whereas potassium ion on the inner side. Due to this ionic distribution there is difference of potentials across the synaptic membranes. The small synaptic vesicles are also activated and there is liberation of acetylcholine. The permeability of the postsynaptic membrane is increased and there is ionic movement along with the small electrical current. Due to these changes excitatory postsynaptic potential (EPSP) develops. Then the impulse is propagated to the anterior horn cell. Under normal condition the impulses may be excitatory or inhibitory. When there is preponderance of excitatory stimulation over the inhibitory stimulation there will be depolarization of the motor neuron membrane and the discharge of the action potential. When there is predominance of inhibitory stimulation over the excitatory stimulation there will be hyperpolarization of the motor neuron membrane. The inhibitory postsynaptic potential (IPSP) will develop and will inhibit the discharge of any impulses. When the motor neuron is excited, the impulse is transmitted to the motor endplate. The postjunctional membrane and the axon are quite separate and remain at a slight distance. When the motor endplate is excited, it gets depolarised. There is inflow and outflow of ions. Acetylcholine plays an important part in this process. Endplate potential (EPP) develops. The membrane of the muscle fibre is subsequently depolarised to the threshold level and the action potential in the muscle is produced. Subsequently the contraction of the myofibrils occurs.

EXAM-ORIENTED QUESTIONS

Essay

1. Describe the neuro-muscular junction? Discuss the sequence of events in neuromuscular transmission.

Short Notes

1. Neuromuscular blockers
2. Myasthenia gravis
3. Lambert-Eaton syndrome
4. Duchenne muscular dystrophy

Recent Advances: Humoral Transmitters in the Nerve Terminals and the Mechanism for their Storage, Release and Inactivation

Julius Axelrod is an American biochemist shared the 1970 Nobel Prize in Physiology or Medicine with **Bernard Katz** and **Ulf von Euler**. They explained through their discovery the mechanism of release and reuptake of catecholamine neurotransmitters epinephrine, norepinephrine and dopamine.

Julius Axelrod
1912–2004

Bernard Katz
1911–2003

Ulf von Euler
1905–1983

They proved by the experimentation that catecholamine neurotransmitters after being released into the synapse are recaptured ("reuptake") by the pre-synaptic nerve ending, and recycled for later transmissions. This research laid the groundwork for discovery of selective serotonin reuptake inhibitors (SSRIs) which block the reuptake of neurotransmitter, serotonin.

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2. Raju T.N. "The Nobel chronicles. 1970: Bernard Katz (b 1911), Ulf Svante von Euler (1905–1983), and Julius Axelrod (b 1912)". Lancet 1999;354(9181): 873.

Chapter

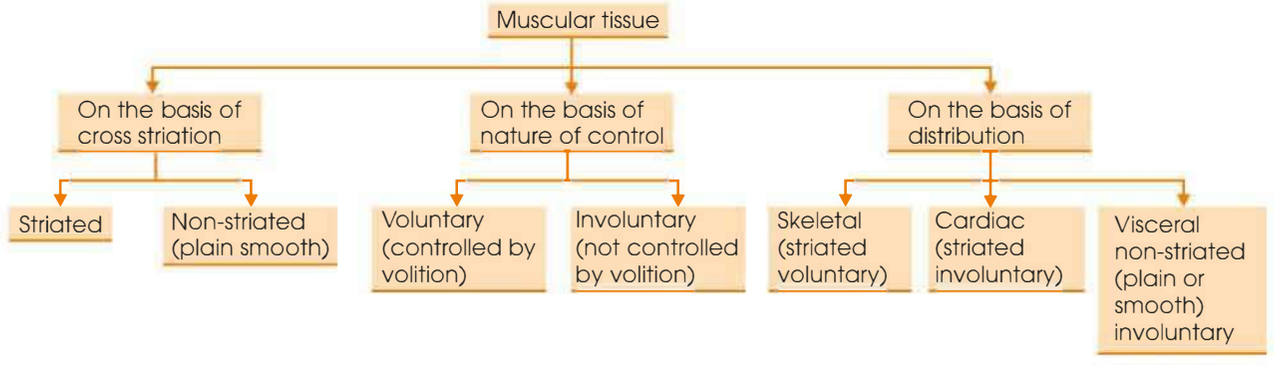
23

Classification of Muscular Tissue

INTRODUCTION

The characteristic property of muscular tissue is its ability to contract when excited. The property of conductivity is also well developed in muscular tissue though not to the degree as that of nervous tissue (Flowchart 23.1). These special functional characteristics are present due to the special modification of the general properties of the protoplasm. The energy required for the performance of the muscular work is obtained through the metabolism of the food substances. So for their prolonged performance, an efficient blood circulation is essential for constant supply of food materials and oxygen and removal of metabolic waste products.

Flowchart 23.1: Classification of muscular tissue



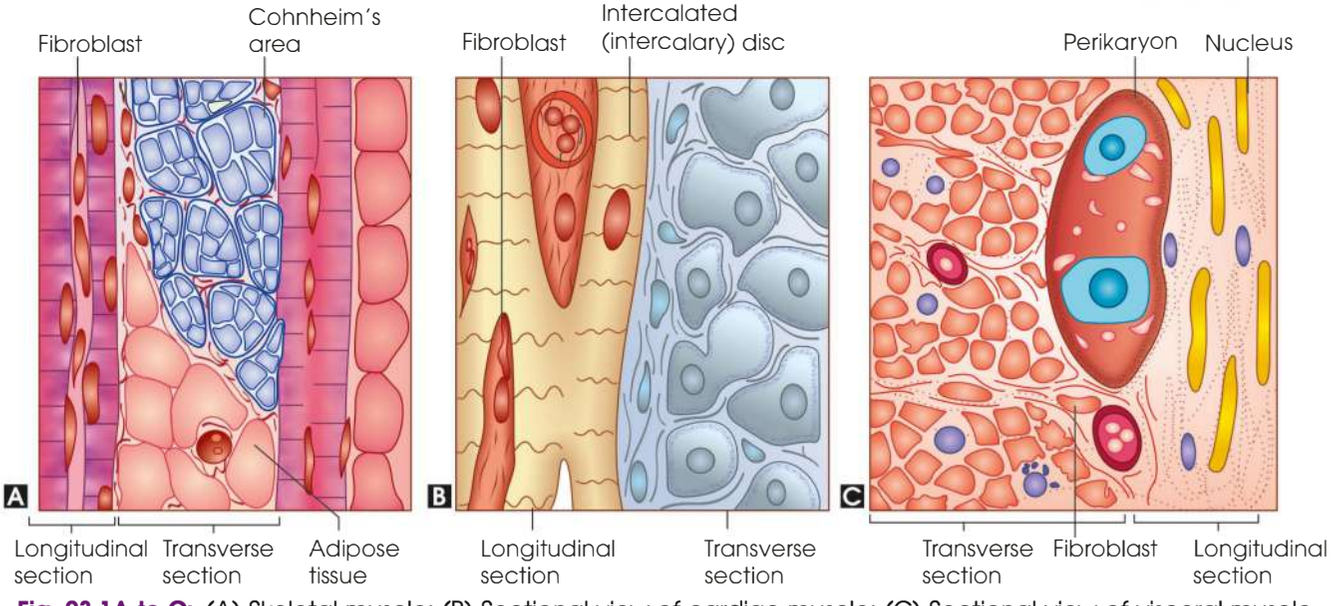


Fig. 23.1A to C: (A) Skeletal muscle; (B) Sectional view of cardiac muscle; (C) Sectional view of visceral muscle

MUSCLE CLASSIFICATION

Muscles are classified into three types (Fig. 23.1A to C):

1. *Morphological basis:* As striated and non-striated (plain smooth)
2. *Functional basis:* Basis of their control: Voluntary and involuntary.
3. *Basis of distribution:* Skeletal (striated voluntary), cardiac (striated involuntary) and visceral (plain or smooth and non-voluntary)

The term muscle fibre is attributed to a muscle cell due to its elongated shape which is specially adopted for the contractile function. These muscle fibres have no similarity with the various fibres present in the connective tissue except their elongated thread-like structure. They are fundamentally of different natures.

EXAM-ORIENTED QUESTION

Short Note

1. Discuss the classification of muscle.

Chapter

24

Structure of Skeletal Muscle

INTRODUCTION

The skeletal (voluntary and striated) muscle fibres are multinucleated cylindrical structures having a clear display of longitudinal and cross-striations. This muscular tissue is responsible for voluntary movement of the living system. It can perform works of rapid, powerful contraction as well as that of prolonged slow sustained tonic contraction.

Distribution

These muscles mostly in all instances are attached to osseous tissues (bones), innervated with somatic nerves through which volitional control is performed. In the fresh state the human skeletal muscle is pink in colour due to the presence of muscle pigments and high vascularity. Due to variation in colour there are red and white (or pale) muscles.

Origin and Development

The skeletal muscle is developed from the solid mass of the mesoderms (myotomes) except the muscle of the head which is developed from the loose mesenchyme. The cells, which give rise to the muscular tissue, are named as the myoblasts. In the myotome they are regular cylindrical but gradually they become elongated, spindle-shaped, parallel bundles and ultimately the multinucleated myofibrils with characteristic cross-striation appearance.

There are three theories for the appearance of the multinucleated skeletal muscle cell:

1. The myoblasts fuse together during the process of development of the muscle fibre which was supported by many with electron micrography (observed in developing myotome of amphibian larvae).
2. In the course of the development of the skeletal muscle fibre (cell), the nucleus is multiplied by mitotic division, but the cytoplasm does not divide accordingly.
3. Both the processes as described under (1) and (2) may take place. During the early phase of development of the skeletal muscles of mammals the nucleus of the muscle fibre migrates towards the periphery from the centre of the cell to accommodate myofibrils at the central core.

General Features

Characteristic Features

1. The skeletal muscles are attached to a bone by means of the tendons nearly in all cases.
2. A tendon is composed of densely packed white fibrous (non-elastic) connective tissue.
3. The fibres of the tendons are affixed to the sarcolemma of the muscle fibres at the junctional point. This is surrounded by areolar tissue; to strengthen the junction.
4. Like other muscles, the skeletal muscle is also supported by various connective tissues.
   - *Epimysium:* It is the connective tissue coat, and the outermost covering for each whole skeletal muscle bulk.
   - *Perimysium:* The whole muscle bulk is divided into smaller bundles, the fasciculi, bounded by the perimysium (the connective tissue septa).
   - *Endomysium:* Now again each fasciculus consists of muscle fibre which is enclosed in a delicate areolar connective tissue jacket, the endomysium.
5. The connective tissue content of different muscle varies widely. The proportion of connective tissue is highest in the muscles responsible for the fine and precise movements.

Histological Structure of Muscle Fibres

Key Points

1. The skeletal muscle fibres are cylindrical, elongated cells with multiple nuclei.
2. The length and breadth of the muscle fibre vary from 1.0 to 4.0 mm and 0.01 (10 µm) to 0.1 mm (100 µm) respectively.

3. The transparent cell wall of the muscle fibre is named as *sarcolemma*. It is visible under light microscope when fresh muscle fibres are teased.
4. Electron micrograph shows that it is made up not only of the plasmalemma but also of an extrinsic coat of amorphous material, similar to the basement membrane. This amorphous layer is pierced and encircled by reticular fibres.
5. Inside the plasmalemma elongated multiple nuclei and transversely striated myofibrils (bundles of myofilaments) are embedded in the sarcoplasm.
6. The sarcoplasm contains other constituents as that of any other cell, e.g. numerous mitochondria), a small Golgi apparatus near each nucleus, myoglobin, lipid, glycogen, sarcoplasmic reticulum (endoplasmic reticulum in case of other tissue), etc. Fibres which are richer in sarcoplasm are darker in colour and *vice versa*.

Myofibrils (Fig. 24.1): Characteristic Features

The characteristic features of skeletal muscle, the alternate light and dark shades (transverse striations) and thick longitudinal strands can be studied with light microscope.

1. Electron microscopy reveals that the longitudinal striation is due to the presence of myofibrils of different thickness whereas the transverse striation is due to the presence of alternate light and dark segments of longitudinally arranged elements.
2. In cross-section, myofibrils appear as fine dots either distributed uniformly or in a group of polygonal areas. They are separated from adjacent bundles by clear sarcoplasm. The separated myofibrils by the sarcoplasmic areas are known as fields of Cohnheim (*see* Fig. 23.1).
3. The dark band is doubly refractive (anisotropic) when studied under polarized light, hence the name A band (sometimes called Q-band).
4. The light band is monorefractive (isotropic) under polarized microscope, from which the name is derived I-band (sometimes known as J-band).
5. This I-band is bisected at the midpoint by a thin darkly stained line—the Z-line (sometimes called Dobie's line) which is also known as Krause's membrane.
6. The Z-line is made of a membrane extending across the myofibrils. The myofibrils are tortuous at these Z-lines. The portion enclosed by two adjacent Z-lines of a myofibril is considered as the contractile unit and is named as sarcomere. It extends 2 to 3 µm in length.
7. In certain exceptional preparations, central portion of the A-band is paler in colour and called the H-band (Hensen's line).
8. At the midpoint of the H-band, i.e. also of the A-band, there is a narrow dark line, M-line or M-band, where the myosin filaments are thickened.
9. The myosin and actin filaments are overlapped at the peripheral dark portion of the A-band, which is named as the O-band.
10. On both sides of the Z-line, somewhere in the mid-region of the I-band there is a comparatively darker thin transverse line—the N-line.

Fine structure: Electron micrography reveals that fine thread-like protein filaments together forms the myofilaments, of which the thicker component is myosin filament (100 Å in diameter) and thinner one is actin filament (50 Å in diameter).

The primary structure of myosin and tropomyosin is characterized by a large amount of acidic and basic amino acid content, that confer a very high charge on the molecules. Actin, troponin and α-actin in contrast have got low charge and are further distinguished by their high proline content.

Myosin Filaments

The myosin filaments (Fig. 24.1) are present as parallel strands throughout the whole length of the A-band.

1. The length of myosin filament is about 1.5 µm (15,000 Å) and is slightly thicker at the midline.
2. On further dissociation of these filaments, the myosin molecules (1500 Å in length) appear as rod-shaped with a globular projection at one end (Fig. 24.2E).
3. The peripheral bead-like structures of myosin filaments are due to the presence of globular heads of the myosin molecule (Fig. 24.2D). Rod-like portion is named as light (L) meromyosin and the head portion, i.e. the globular portion is as heavy (H) meromyosin (Fig. 24.2D).
4. The heavy meromyosin has got two components. The head portion is known as heavy meromyosin sub fragment 1 and the neck is heavy meromyosin sub-fragment II (Fig. 24.2C). The heavy meromyosin sub-fragment I possesses all the enzymatic and actin-combining properties of the parent (myosin) molecule.
5. In each 400 Å segment of the myosin filament, there are six heavy meromyosin heads (cross-bridges). These heavy meromyosin heads are arranged in a helical manner with a radial pattern of 60° and each set of six bridges complete one revolution around the myosin filament.
6. Each heavy meromyosin head is pointed towards a separate actin filament and thus the cross-bridges with actin occur at approximately 400 Å intervals along the myosin filament (Fig. 24.2E).
7. The heavy meromyosin is responsible for the ability in formation of the cross-bridge with actin and also for the ATPase activity essential for muscular contraction. This heavy meromyosin is sometimes named as the active point of myosin molecules.

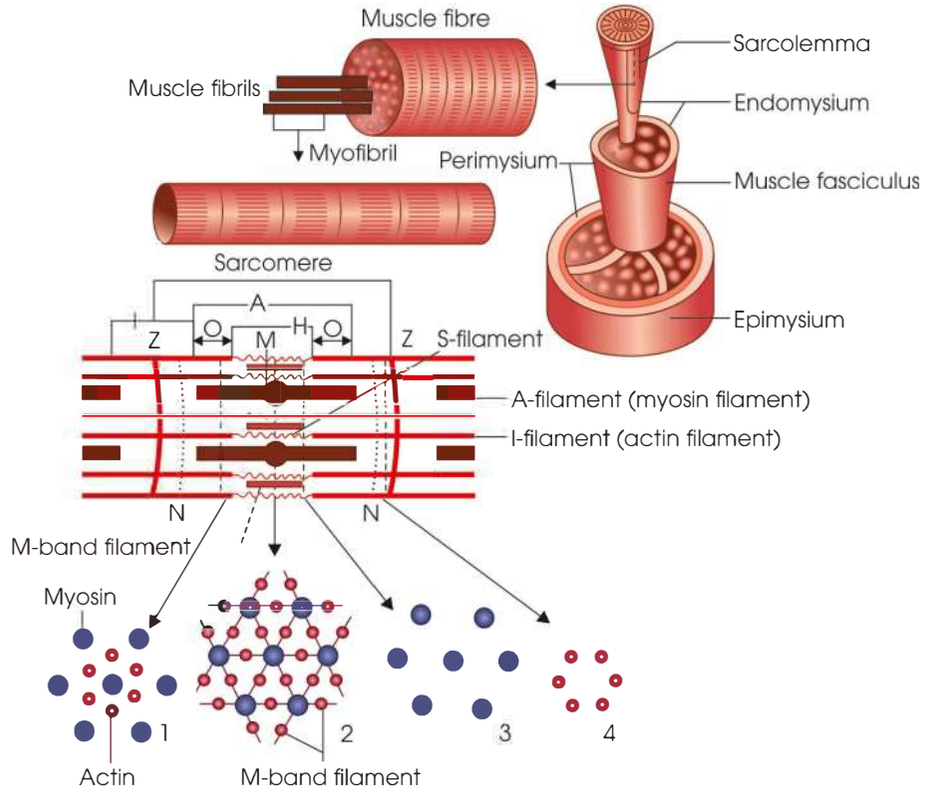


Fig. 24.1: Anatomical organization of skeletal muscle from gross to molecular level

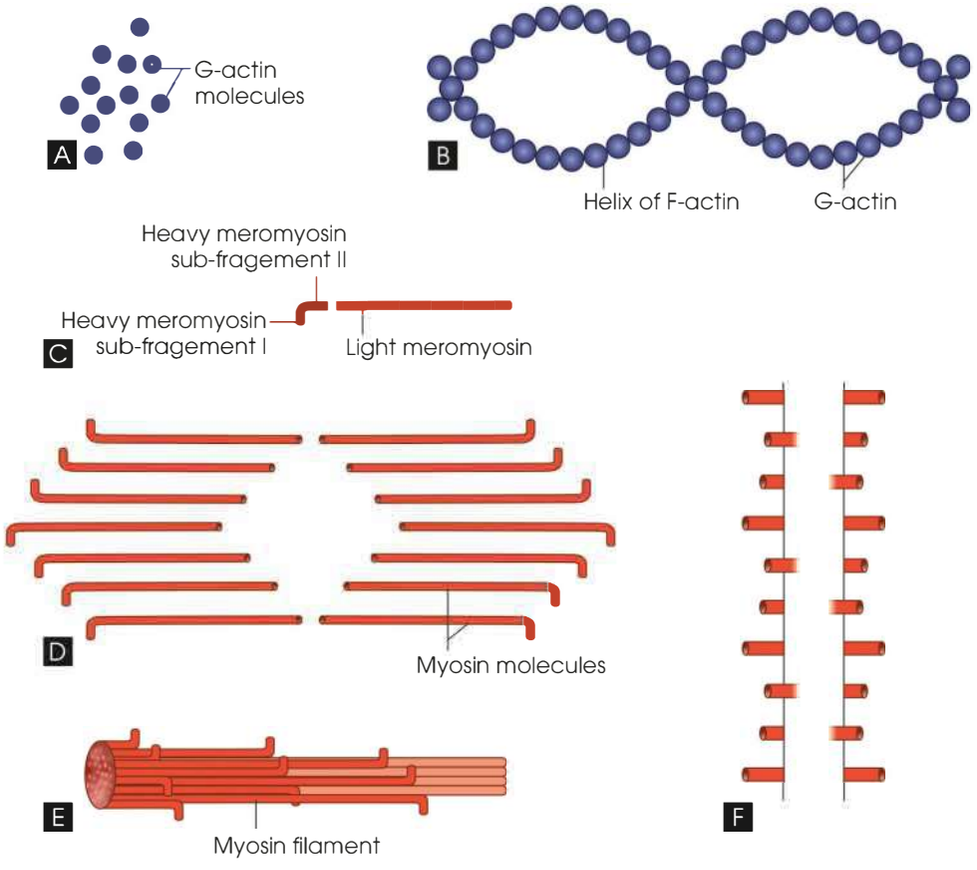


Fig. 24.2A to F: Minute structures of actin and myosin filaments: It is to note that the model of myosin molecule in E (according to EM evidence is different from F (according to X-ray analysis by Huxley), where these are pairs of heads in opposite direction at 140 AU interval in the myosin filament (diagrammatic representation)

8. At the M-band region (midpoint of myosin filament) the union of myosin molecules occurs tail-to-tail, but at this region the myosin filaments appear thicker indicating that there is possibility of presence of some proteins of unknown nature (attached to central portion of myosin filament, Fig. 24.3A) which may help in strengthening the tail-to-tail union of the myosin molecules.

Functions of M-bands are

i. To maintain the parallel alignment of the myosin filaments

ii. To guide the actin filaments during contraction.

Actin Filament

Actin filaments are extended from each Z-line within a sarcomere towards the H-band and connected with the other actin filaments by the S-filament (Fig. 24.1) within the H-band. It extends 1 μm in both sides of the Z-line.

Characteristic Features

1. According to Knappeis and Carlsen, the length of the actin filament is about 1 μm.
2. With very high magnification, the F-actins–fibrous actins appear as beaded and seem to consist of globular sub-units (55Å)—G-actins (Fig. 24.2A) forming two strands entwined in a helix. These strands are therefore a polymer of G-actin.

   Containing 13 G-actins; at each turn of the helix (Fig. 24.2B).
3. Low-angle X-ray studies and also chemical investigations suggest that the tropomyosin and newly known troponin are oriented in the grooves of the actin helices.
4. The actin filaments, approaching the Z-lines, appear to be continuous with four fine diverging filaments—Z-filaments (Fig. 24.3B). It is believed that these Z-filaments contain the muscle protein—tropomyosin.
5. In cross-section, the arrangement of the actin filaments appears in hexagonal shape with one myosin at the centre, but again when that of the thick filaments is considered then the myosin filaments are forming triangles with one central actin filament (Figs 24.1 and 24.3).
6. The thin filaments actin is connected to each other longitudinally by means of the still far more thinner S-filaments (Fig. 24.1).

Sarcotubular System

Under electron microscope, the myofibrils are seen to have surrounded by a canalicular network of membrane-limited tubules—sarcoplasmic reticulum.

Characteristic Features

1. The sarcoplasmic reticulum is identical with the endoplasmic reticulum of other cell type but with the difference that its membrane does not possess ribosome.
2. The sarcoplasmic reticulum is extended longitudinally along the A-band with frequent anastomosis in the region of the H-band and also in the I-band.
3. The sarcoplasmic reticulum is connected at its both longitudinal and terminal ends by another set of the transverse cisterns—terminal cisternae (Fig. 24.4).
4. The terminal cisternae have got larger caliber and are thus continuous and confluent with the longitudinal reticulum (sarcotubule).
5. Pairs of parallel terminal cisternae (adjacent terminal cisternae) are separated from each other by a slender transverse tubule which is known as T-tubule.
6. This T-tubule is not confluent with the terminal cisternae and is a tubular invagination of the sarcolemma but not a part of sarcoplasmic reticulum. It is continuous with the extracellular space. These tubules are generally called T-system. The pairs of terminal (transverse) cisternae and the central T-tubules are collectively called triads (Fig. 24.4).

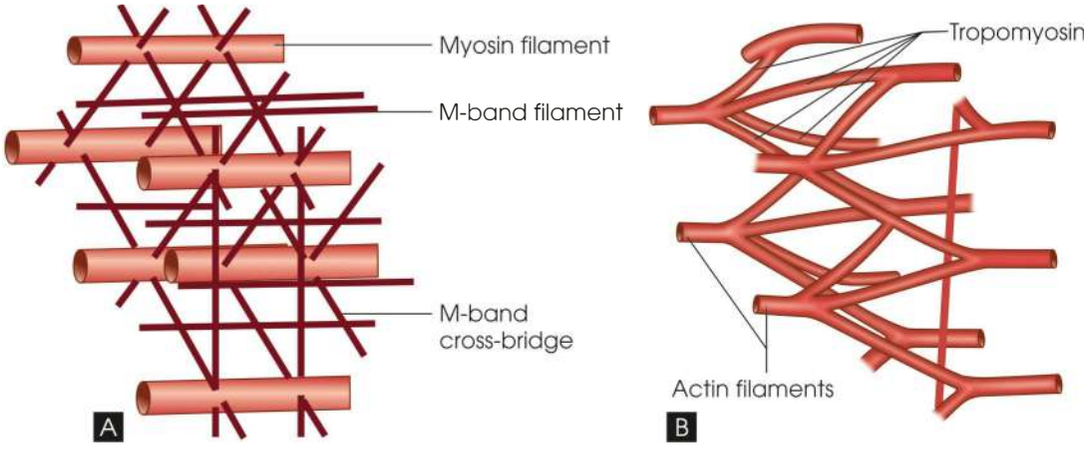


Fig. 24.3A and B: Arrangements of myosin M-band filaments at M-band. (A) actin-tropomyosin at Z-line (B)

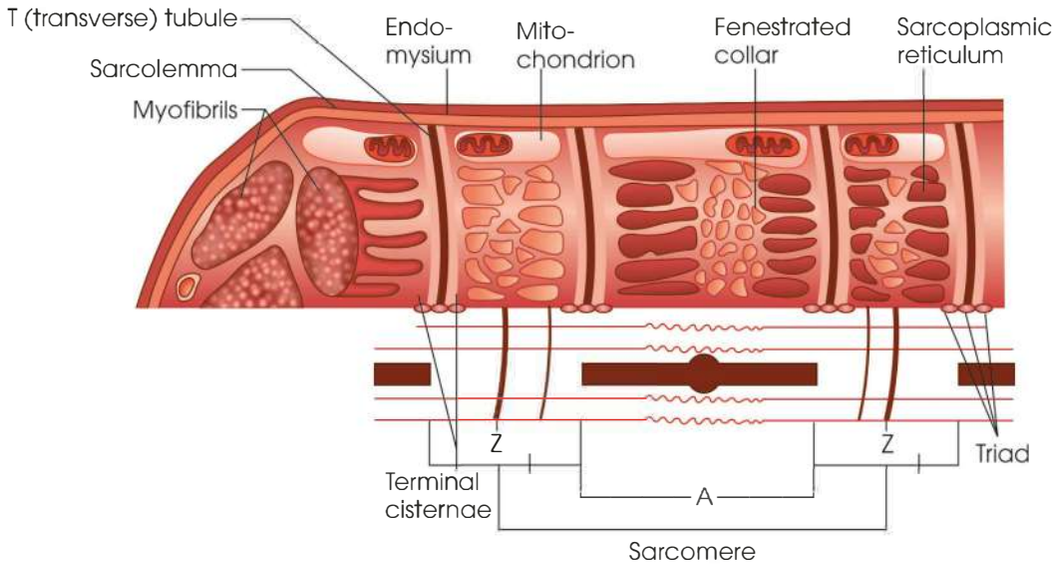


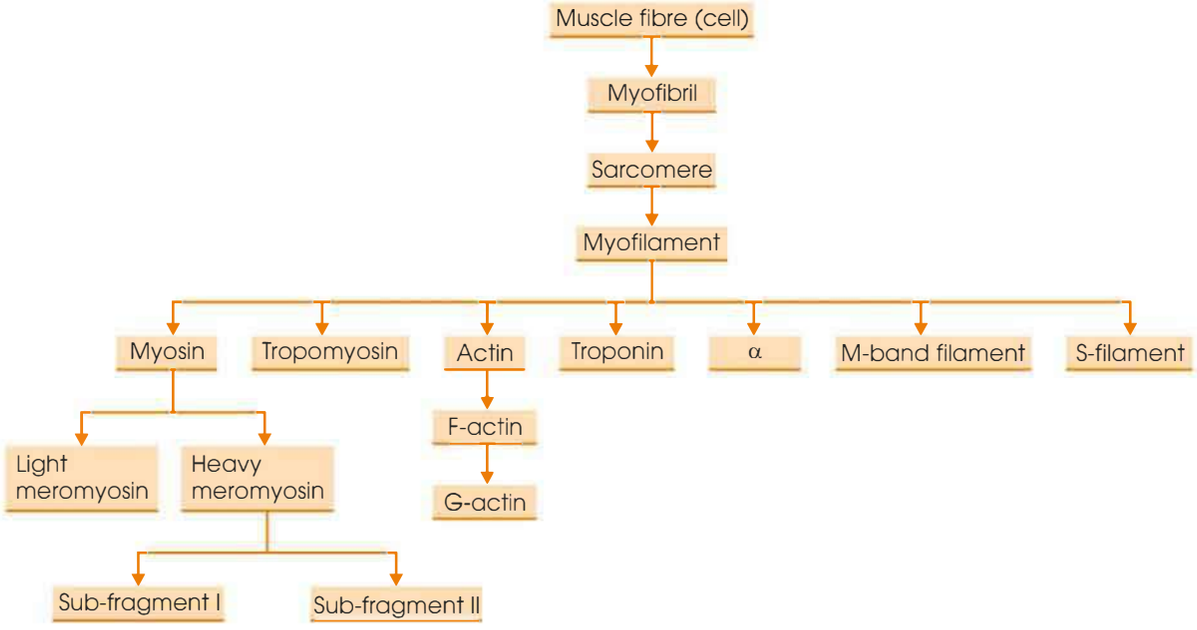
Fig. 24.4: Sarcotubular system of the mammalian skeletal muscles showing the triad to pass at the A-I junction (diagrammatic representation)

7. In amphibian muscle the triads encircle the I-band at the region of the Z-line but in mammalian muscle, the same is present at the junction of each A-band with the adjacent I-band. So in mammals there are two sets of triads in each sarcomere (Fig. 24.4). The T-system plays an important role in quick transmission of impulse from the cell surface to each myofibril.

Blood Vessels, Lymphatics and Nerves of Skeletal Muscle

- Skeletal muscles are richly supplied with networks of anastomosing capillaries which run longitudinally with intercommunicating transverse branches. No capillaries penetrate sarcoplasm. The great arteries and veins are seen in the perimysium. The largest and smallest veins possess valves.
- The lymphatic supply communicates with blood vessels of epimysium and perimysium. But lymphatics are not found between these muscle fibres (Flowchart 24.1).
- Myelinated nerve fibres supply striated muscle. The motor nerve endings terminate at end plates. The sensory nerves end in groups of modified muscle fibres known a muscle spindles. Functions of sympathetic nerve here are not known.

Flowchart 24.1: Micro-anatomical organization of the muscle fibre has been presented schematically



Ending of Muscle in Tendon

At the musculotendinous junctions the endomysium, perimysium and epimysium of the muscle become continuous with the fibrous tissue of the tendon.

Red and White (or Pale) Muscles

A muscle fibre, being composed of a number of delicate fibrils surrounded by a more fluid sarcoplasm and having mitochondria and sarcoplasmic reticulum, possess respiratory pigment, myoglobin (muscle haemoglobin) within the sarcoplasm.

1. Red colour of the muscle fibre is due to the presence of myoglobin. This myoglobin acts in the transport of oxygen from blood vessels (capillaries) in the extracellular space to the sites of oxidation (mitochondria). In most mammals, all the muscle fibres contain cytochrome and more myoglobin and look red. These red muscle fibres possess more nuclei frequently central in position, much granular sarcoplasm, well-marked longitudinal striation and irregular transverse striation. Red muscle fibres have a high capacity for oxidative metabolism with a strong activity of Krebs cycle and electron transport enzymes and slower in their contractile action.

   Red muscle fibres undergo fatigue less rapidly than white (or pale) muscle fibres and are well adapted for static or postural contractions.
2. White (or pale) muscle fibres are deficient in myoglobin and generally present in frogs. These white fibres are small, regular and have poor sarcoplasm, peripheral nuclei. These white fibres possess a high rate of anaerobic glycolysis with intense activity of glycolytic enzymes and phosphatase. White fibres being predominant in flexor muscles help phasic contractions by which changes in the position of the body or a limb are done. In some mammals (e.g. rabbits) including the human and birds have both red and white (or pale) muscle fibres.

EXAM-ORIENTED QUESTIONS

Essay

1. Describe the structure of skeletal muscle.

Short Notes

1. Origin of muscle
2. Distinguish between red and white fibres
3. Characteristic of sarcoplasmic reticulum in skeletal muscle

Chapter

25

Muscle Contraction

INTRODUCTION

Activation of tension-generating sites within muscle fibres activates the contractile forces in a muscle leading to muscular contraction. The muscle tension can be produced without any change in muscle length. The muscle fibres returns back to their low tension-generating state with termination of muscle contraction and occurrence of relaxation.

Mechanism of Contraction

1. **Theory of folding of myofilaments (contractile proteins)** stated that certain muscle proteins are shortened or folded during the muscular contraction by forming actin–myosin complex. But morphological studies do not show any such evidence that during muscular contraction the myosin filaments are shortened. Now if the actin filaments attain any shortening or folding at all, still it is not possible during normal life.
   - Interdigitation or sliding of myofilaments: The sliding filament hypothesis (Fig. 25.1) states the alteration of relative position of the myofilaments during the muscular contraction but neither the actin nor the myosin filaments are shortened themselves. The cross-bridge interaction between actin and myosin leads to muscle contraction by means of the sliding filament mechanism.

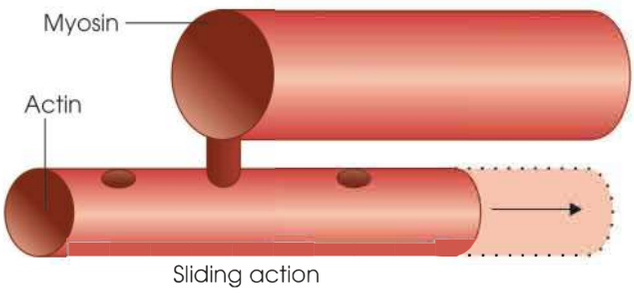


Fig. 25.1: Sliding action of actin and myosin

   - During contraction, the actin filaments slide past the myosin filaments and thereby the actin filaments are further extended into the A-band causing shortening of the length of the H-zone and narrowing the sarcomere (Fig. 25.2).
   - In this process the myosin filament gradually approaches the Z-line; and the actin filament the M-line; thereby altering the site of attachment of the cross-bridges at one point ahead in the manner of an animated cog-wheel (Fig. 25.2B and C).
   - At certain stages of contraction, the ends of the two adjacent actin filaments may touch each other and the I-band is of minimum length.

There are two theories regarding the position of two opposite actin filaments of the same sarcomere, during maximum contraction (Fig. 25.2D and E).

   - At the free end of the actin filaments in the M-band there is a slide over each other (Fig. 25.2D)
   - The zigzag Z-line is straightened causing an increment in distance between the adjacent actin filaments.

Due to this there will be a stretching of the actin filaments towards the Z-line causes shortening of the sarcomere (Fig. 25.2E).

Thus, contraction is accomplished by thin filaments from opposite sides of each sarcomere sliding closer together between thick filaments. Energy required for this sliding process is maintained from the breakdown of the ATP by the myosin ATPase present at the local heavy meromyosin molecule.

Molecular Mechanism of Muscle Contraction

The skeletal muscle contraction involves the following steps of action (Fig 25.3A to D and Flowchart 25.1):

1. The action potential which reaches the axon of the motor neuron activates voltage-gated calcium ion channels on the axon, and calcium enters in.
2. The calcium causes acetylcholine vesicles in the axon to fuse with the membrane, and releases acetylcholine

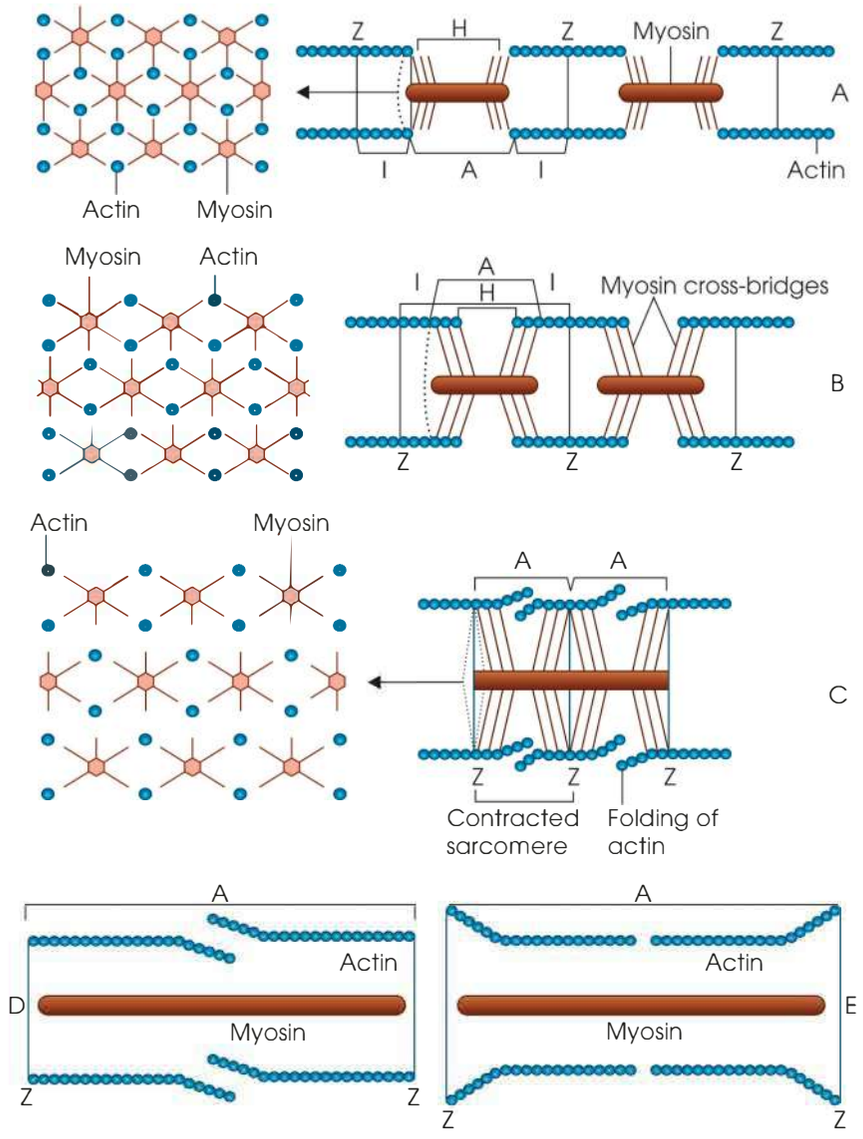
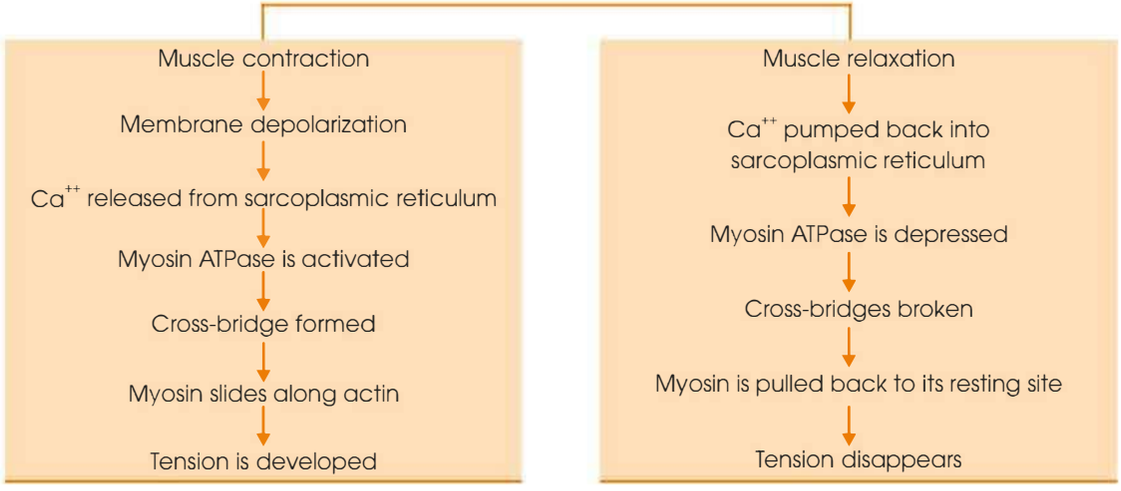


Fig. 25.2: Diagrammatic (hypothetical) representation of the sliding filament mechanism of muscular contraction showing also the cross-bridges in-between actin and myosin filaments. (A) Shows the sarcomere in resting condition; (B) Represents the sarcomere in partially contracted state; (C to E) Depict the sarcomere in maximally contracted states

Flowchart 25.1: Mechanism of muscle contraction and relaxation



into the cleft between the axon and the motor end plate of the muscle fibre.

3. The acetylcholine then diffuses across the cleft and binds to nicotinic receptors on the motor end plate, in the membrane and sodium enters in, and potassium rushes out. However, because sodium is more permeable, the muscle fibre membrane becomes more positively charged, and triggers an action potential.
4. The function of the T-system, as already mentioned, is to propagate the impulse from the sarcolemma to the myofilaments within a short time. Following stimulation the impulse is transmitted to the myofibrils by the T-system and the depolarization causes the release of calcium from the sarcoplasmic reticulum which in turn activates the myosin ATPase. This activated ATPase breaks the ATP to ADP and the ADP to AMP with the release of certain amount of energy which is required for the process of contraction.
5. The released calcium binds to the troponin present on the thin filaments of the myofibrils. The troponin then allosterically modulates the tropomyosin. At rest the tropomyosin physically obstructs binding sites for cross-bridge; and as calcium binds to the troponin, the troponin forces the tropomyosin to move, unblocking the binding sites.
6. Myosin heads form a cross-bridge with actin binding sites.
7. ATP binds to the myosin heads and breaks the cross-bridge.
8. The hydrolysis of ATP causes the myosin heads to change shape and swivel—this moves it to the next actin binding site.
9. The movement of the myosin heads causes the actin filaments to slide over the myosin filaments, shortening the length of the sarcomere and by the repeated hydrolysis of ATP, the skeletal muscle contracts.

Mechanism of relaxation: A relaxing factor has been isolated from muscle homogenates. From electron microscopic studies of the muscle homogenates, it has been claimed that membrane-limited vesicles are present which are derived from the fragmentation of sarcoplasmic reticulum. These vesicles have the capacity of binding Ca^{++} in presence of ATP. The calcium ATPase pumps calcium from sarcoplasm into sarcoplasmic reticulum, the activity of calcium ATPase decreases as no more action potential arrives at the neuromuscular junction. As adequate calcium ions are not available to bind with troponin the interaction between myosin head and active site on actin filament is stopped and muscle undergoes relaxation (Flowchart 25.1).

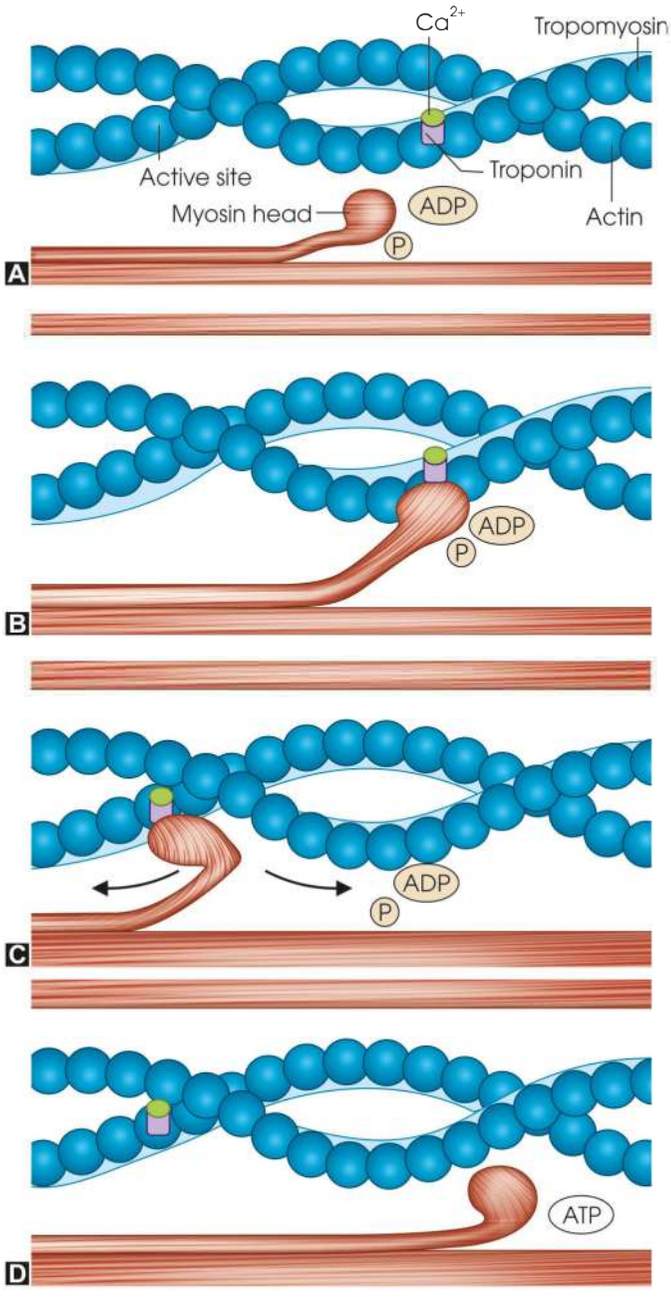


Fig. 25.3A to D: Mechanism of muscular contraction. (A) Calcium binds to troponin exposing the active site for myosin head; (B) Cross-bridge formation between myosin head and actin; (C) Power stroke mechanism with liberation of energy due to ATP breakdown; (D) Detachment of cross-bridge as a new ATP molecule attaches to the myosin head

Changes during Muscular Contraction

Mechanical Changes

During contraction, the muscle fibre shortens in length, increases in thickness, but the total volume remains same (or slightly increases). The muscular contraction may be isometric when the length of the muscle fibres remains constant but the tension increases, or isotonic when the muscle becomes shorter and thicker.

Chemical Changes

Following chemical changes take place during contraction in the anaerobic and aerobic phases.

Glycolysis and Oxidative Breakdown (Fig. 25.4)

This process involves many separate enzymes and different intermediates. The end products are pyruvic or lactic acid.

1. The first step in the liberation of energy for contraction is the breakdown of glycogen in the muscle fibre. The glycogen reacts with inorganic phosphate and splits up into glucose-1-phosphate (Cori ester) with the help of phosphorylase. Muscle phosphorylase contains pyridoxal phosphate as a cofactor. The process of uptake of the phosphate and its cleavage into glucose phosphate is called phosphorolysis.
2. Glucose-1-phosphate is converted into glucose-6-phosphate.
3. Glucose-6-phosphate is changed into fructose-6-phosphate, then into fructose-1,6-diphosphate and into two triose phosphates. The triose phosphate is finally converted into pyruvic acid.
4. Pyruvic acid is reduced to lactic acid by reduced nicotinamide adenine dinucleotide (NAD.2H) [previously called reduced coenzyme-1 or $DPNH_2$] and lactic dehydrogenase in the absence of oxygen.
5. Out of the total quantity of the lactic acid formed under anaerobic conditions one-fifth of it is oxidized to CO_2 and H_2O and four-fifths are re-synthesised into glycogen in the liver.

In the presence of oxygen the pyruvic acid is oxidized through a series of steps known as the Krebs tricarboxylic acid (TCA) cycle.

- In this cycle one molecule of pyruvic acid loses one molecule of CO_2 and is converted into active acetate (acetyl coenzyme A). The active acetate is metabolized through a series of reactions known as citric acid cycle.
- Each molecule of pyruvic acid at each turn of the cycle liberates three molecules of CO_2 and two molecules of water. There is net production of 39 ATP per one hexose unit of glycogen metabolized. But as there is peroxidation of one molecule of glucose into CO_2 and water, net 38 ATP are produced.
- In anaerobic condition only 2 ATP are produced per molecule of glucose metabolized and 3 ATP are produced per hexose unit of glycogen metabolized under such condition.

Energy for muscular contraction is thus provided as ATP from (a) anaerobic glycolysis leading to breakdown of glycogen and glucose to pyruvic and lactic acids, (b) oxidation of lactic acid to pyruvic acid and further oxidation of pyruvic acid in TCA cycle via acetyl CoA into H_2O and CO_2, (c) fatty acid oxidation through β-oxidation and through TCA cycle also provides some amount of ATP as muscle energy, (d) during muscular activity creatine phosphate also maintains the ATP level of the muscle.

Role of Creatine Phosphate or Phosphagen and Adenosine Triphosphate (ATP)

Creatine phosphate ($CrPO_4$) plays an essential role in the muscular contraction. The role of carbohydrate metabolism is to provide energy for the re-synthesis of creatine phosphate. The breakdown process of creatine phosphate involves the re-synthesis of ATP. Creatine phosphate reacts with ADP and as a result of which creatine phosphate loses its phosphate radical and ATP is formed.

Key Points

- ATP is again broken down by adenosine triphosphatase (ATPase) to form ADP and inorganic phosphate. The breakdown of ATP precedes that of creatine phosphate.
- ATP is composed of adenine, d-ribose and 3-molecules of orthophosphate. The energy-rich* phosphate bonds are contained in creatine phosphate

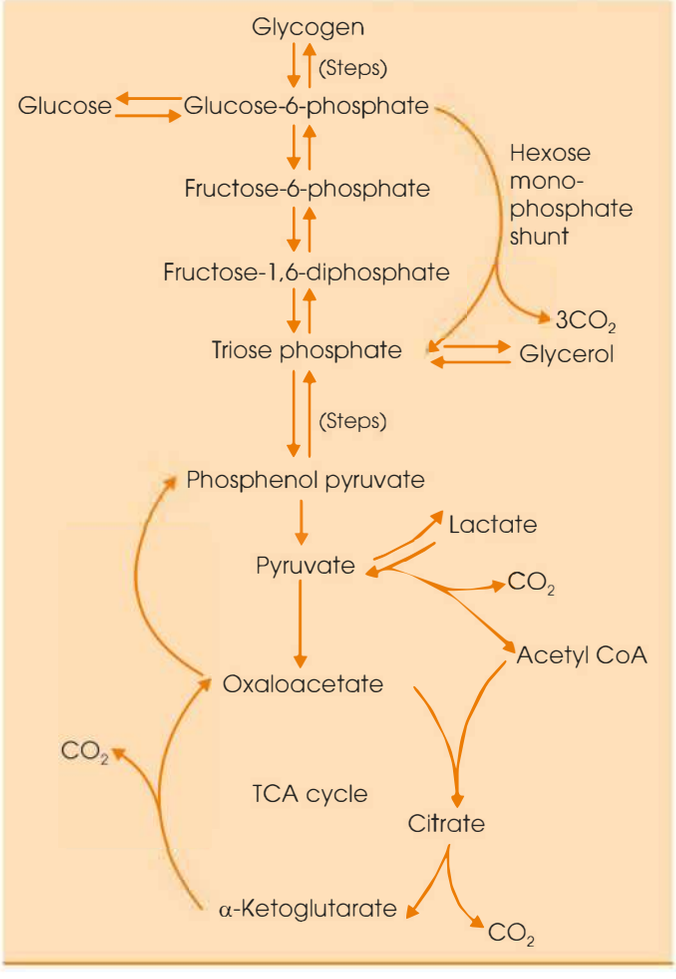


Fig. 25.4: Diagrammatic representation of glycolysis and oxidative breakdown

and ATP. The terminal phosphate group which split from ATP; or the phosphate bond energy may be transferred to other compound, e.g. in the conversion of fructose-6-phosphate into fructose-1,6-diphosphate and ADP is formed.

- The resynthesis of creatine phosphate takes place with the help of phosphate released from ATP in a reversible reaction. As a result of transference of phosphate, ATP changes into ADP.
- Creatine phosphate also serves as a reserve source of phosphate bond energy for rapid re-synthesis of ATP in case of muscle poisoned with the iodo-acetic acid. In the iodo-acetate poisoned muscle the carbohydrate breakdown is inhibited but resynthesis of ATP can take place for a considerable period with the help of creatine phosphate.

The sequence of events may be summarized in the following order

1. ATP breaks down into ADP and there is release of phosphate and energy. Actin and myosin are the contractile substances. ATP helps in the shortening of actomyosin threads.
2. Creatine phosphate breaks down and the phosphate with its energy is transferred to ADP and forms ATP. The creatine phosphate store is thus a constant supplier of ATP.
3. Glycogen of the muscle breaks down in a series of stages and releases energy-rich phosphate bond in the intermediate stages which in turn help in the re-synthesis of ATP and also of resynthesis of creatine phosphate.

Under anaerobic conditions, glycogen breakdown occurs as far as pyruvic acid, which again takes up hydrogen from NAD. 2H and changes into lactic acid. Dehydrogenase catalyzes this reversible reaction. Muscle glycogen is formed from this lactate by a process known as Cori cycle. Under aerobic conditions lactic acid is also produced. It diffuses out into the circulation and other body fluids. It is re-oxidized into the pyruvic acid mainly in the liver and enters the citric acid cycle or may be transformed into glycogen.

Muscular Contraction and its Relationship with the Breakdown of ATP

The ***mechanism of muscular contraction*** begins into the breakdown of ATP. The contraction takes place due to release of phosphate bond energy from ATP. When the muscle is stimulated the impulse travelling over the fibre is associated with an increase in sodium and calcium permeability of the membrane. As a result, inflow of sodium ions to the inside of the muscle fibre is accompanied by a slight inflow of calcium ions at the same time. The calcium ions then stimulate adenosine triphosphatase (ATPase), which helps in the release of energy from ATP surrounding the muscle filaments. The energy produces momentarily an electrostatic charge between the actin and myosin filaments which in turn pulls the actin filaments into the spaces between the myosin filaments. ATPase remains in an activated state so long calcium ions are present inside the muscle fibre.

Surrounding the filaments and sarcoplasmic reticulum there is another substance known as relaxing substance which binds calcium ions within a fraction of a second after they enter the interior of the fibre so that the calcium is changed into unionized form. As a result of inactivation of calcium ions in the interior of the muscle fibre no more energy is released from adenosine triphosphate. The electrostatic charges between the actin and myosin filaments disappear, thus allowing the muscle to relax.

There are two types of phosphate bonds—high-energy bond (indicated by the sign ~) and low-energy bond.

1. A high-energy bond when split up releases about 10,000–12,000 cal/gm/mol, whereas a low-energy bond liberates only 2,000 cal/gm/mol.
2. ATP contains two high-energy and one low-energy phosphate bonds (ATP = A-P~P~P) and phosphagen contains one high-energy phosphate bond (Cr~P). It is believed that energy released from the high-energy bonds is not wasted as heat, etc. but is utilized for work and other cellular processes.

Utilization of other Fuels during Muscular Contraction

It is possible that skeletal muscle also utilizes ketone bodies and free fatty acids for the re-synthesis of ATP. This is particularly applicable in the case of flight muscles of migratory birds, where glycogen store is not sufficient to supply energy for a long period of muscular contraction.

Cardiac muscle is similar to the above flight muscle, biochemically, and consumes ketone bodies and free fatty acids in harmony with them for continued activity.

Changes in H-ion Concentration

The normal reaction of muscle is slightly on the alkaline side (pH 7.3). During muscular activity both acid and alkaline products are formed. These compounds try to neutralize each other and thereby prevent any serious change of reaction. Besides this, the muscles possess an efficient buffer system in the form of muscle proteins, their Na and K salts, the inorganic phosphate, the bicarbonates, etc. all of which help to prevent marked change of muscle pH. But in spite of this, in the initial stages the reactions become more alkaline due to the liberation of free creatine (which is strongly alkaline). In prolonged activity, especially under inadequate oxygen supply, the reaction becomes distinctly acid (pH about 6.0) due to accumulation of lactic acid.

Other Chemical Changes

In the resting muscle the RQ varies from 0.85 to 0.90, showing that the resting metabolism involves the oxidation of certain non-carbohydrate foodstuff. Hill, by determining the osmotic pressure of the muscle under various conditions, has proved that, unknown reactions of considerable magnitude other than carbohydrate oxidation, take place. It has also been shown that glycerol, ketone, etc. may join the path of glycogen metabolism and undergo a process of oxidation. Moreover, little is known about the changes seen in the nitrogenous constituents of muscles, such as carnosine, inositol, free creatine, etc. All these indicate that muscles utilize not only carbohydrates but other substances as well (Fig. 25.5).

Oxygen Utilization and CO_2 Production

The broad facts about this are as follows: (a) The resting muscle uses O_2 and liberates CO_2, the RQ being 0.85–0.90. (b) O_2 is not required for contraction and lactic acid formation. The muscle maintains its irritability and contractility for a good length of time even under complete anaerobic conditions. (c) Oxygen is essential during recovery process when lactic acid burns and glycogen is re-synthesized. The RQ of this period is unity.

Cori Cycle

In the living body under normal conditions, the oxygen supply to the muscles is so efficient that slight or moderate exercise does not produce lactic acid accumulation. Because due to ready oxygen supply, lactic acid quickly disappears. But in heavy exercise, the oxygen supply becomes inadequate; excess of lactic acid accumulates in the muscle fibres and diffuses out into the blood stream. A considerable amount of this blood lactate is utilized by the heart and small amounts by other tissues. A little portion of it may re-enter the muscle to form glycogen. But the major part of the blood lactate is taken up by the liver and is converted into glycogen. Liver can prepare glycogen from lactic acid more quickly than it is done by the muscle. This liver glycogen is converted into glucose, which enters blood stream, and then into the depleted muscles and is converted into glycogen. The muscle can form glycogen more readily from glucose than it is done by the liver. In this way the muscle gains back its depleted glycogen store. Liver and muscle help each other to organize a bigger cycle through which lactic acid moves. This cycle is as follows:

Muscle glycogen → Muscle latic acid → Blood lactate → Liver glycogen $\rightleftharpoons$ Blood glucose → Muscle glycogen.

This cycle is called Cori cycle (Fig. 25.6).

Thermal Changes

During muscular contraction (Fig. 25.5), heat is produced in the muscle, which has been measured with the help of an instrument called *thermopile*. Heat is produced in two stages; (a) initial heat, which occurs at the onset of contraction, (b) recovery heat or delayed heat, which occurs, following the contraction. The initial heat is the rapid outburst of heat, whereas the recovery heat is slow and prolonged production of heat. The recovery heat is equal to the total energy set free during muscular contraction. In the recovery process the chemical changes, which are responsible for the initial liberation of energy, are reversed. AV Hill had shown that the heat produced in a single twitch of a frog's muscle may be divided into three stages:

a. ***The heat of activation:*** The heat of activation occurs immediately after stimulation and diminishes while the contraction proceeds. The heat production occurs about ten milliseconds after stimulation.
b. ***The heat of shortening:*** This is the second stage of heat liberation and depends upon the amount of shortening—ax, where x denotes the amount of shortening and a is the constant (multiplication of constant and variable).

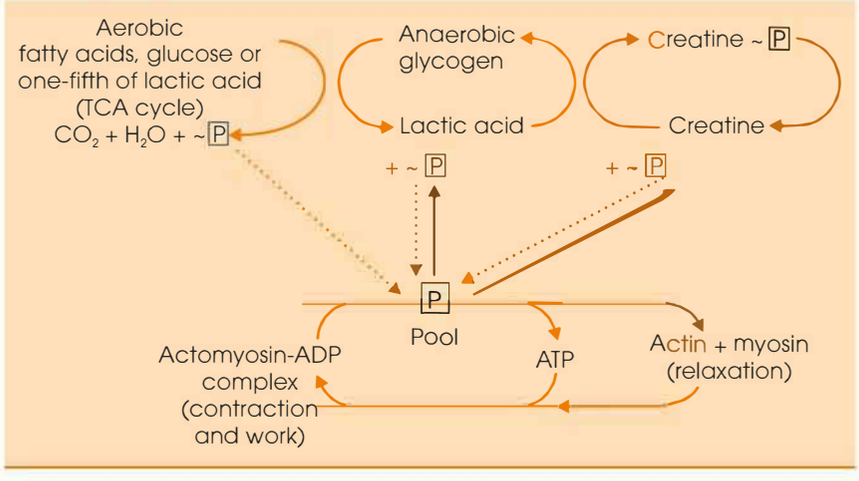


Fig. 25.5: Chemical reactions during muscular contraction

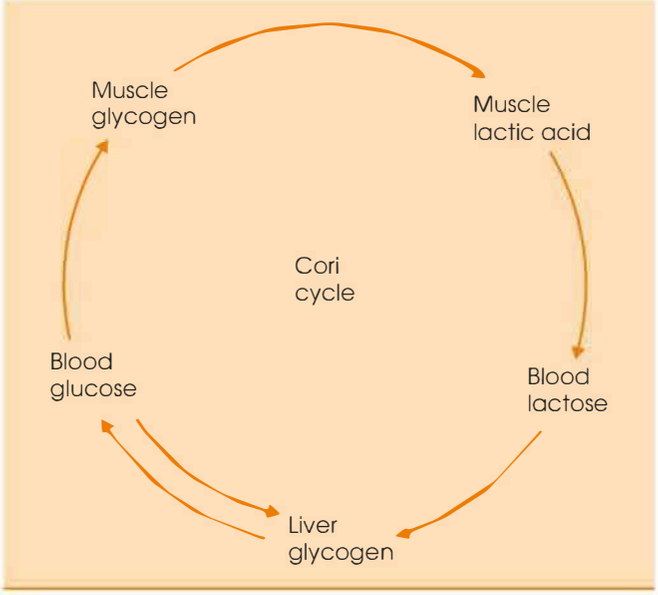
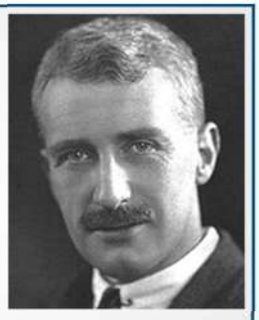


Fig. 25.6: Cyclical relationship of lactic acid and glycogen

c. ***Recovery heat:*** There is slow production of heat during recovery process in the presence of oxygen. The heat of shortening begins later. There is no heat of relaxation.

Archibald Vivian Hill shared the 1922 Nobel Prize in Physiology or Medicine for his elucidation of the production of heat and mechanical work in muscles.



Archibald Vivian Hill
1886–1977

Electrical Changes

Instruments used for the purpose are capillary electrometer, Einthoven's string galvanometer or cathode ray oscillograph. The electrical changes are of the same nature as seen in the nerve. The cut surface or the stimulated spot is negative to the rest of the muscle fibre. When the injured and the uninjured areas are connected, current of injury will be found. By repeating the same experiments as in nerves current of action with diphasic variation and current of injury with monophasic variation can be found. Mechanical contraction starts when the electrical change attains its maximum, which takes place very early during the latent period. The action potential as well as the wave of mechanical contraction is propagated at the same rate (3–4 meters per second). Under no condition the electrical wave (excitation process) can be propagated without the accompanying wave of contraction. Hence, the electrical wave can be regarded as the index of the contraction wave. Cold slows and warmth hastens both these waves.

Chronaxie and Rheobase

The excitability of different tissues varies widely. Some tissues are highly excitable whereas others are dull. While considering the excitability of a tissue, two factors are to be considered; (a) the strength of the current used, (b) the duration of the current flowing through the tissue. These two factors bear an inverse relation. The stronger the current the lesser will be the duration required. But there is a limit. Two standards have been established to measure the excitability of various tissues. (Instruments used: Lapicque's liquid potentiometer or Keith Lucas's spring rheotome.) The strength standard (intensity threshold) is called rheobase. The duration standard (time characteristic) is called by Lapicque as chronaxie.

- ***Rheobase*** is defined as the minimal galvanic current which when allowed to flow indefinitely will excite a tissue.
- ***Chronaxie*** is defined as the shortest duration of stimulus required to excite a tissue by a current strength equal to twice of rheobase voltage. Chronaxie of a tissue is a definite measure of its excitability. A less excitable tissue has a longer chronaxie, whereas a more excitable tissue has a shorter one.

The speed of activity of tissue and its chronaxie bear a direct relation. A tissue, having shorter chronaxie, has shorter period of action, shorter refractory period and a quicker rate of propagation. Sluggish tissues, such as some form of plain muscle, have longer chronaxies. Owing to this, they fail to be excited by Faradic stimulation (induction shock), because the duration of the current here is very short. Chronaxies of the various tissues are as follows:

1. The tissues of the newborn child possess chronaxies which are ten times longer than those of adults. This explains the slower movements of infants.
2. Tissue of cold-blooded animals generally possesses longer chronaxies (i.e. they are more sluggish) than those of warm-blooded types.
3. *Muscles:* The chronaxies of the skeletal muscles are shorter than those of plain and cardiac. Flexor muscles have shorter chronaxies than extensors. The pale muscle fibres (more active) have shorter chronaxies than the red muscles (sluggish). Fatigue lengthens and adrenaline shortens the chronaxie of skeletal muscles. Similarly, cold lengthens and warm shortens it. A degenerating muscle has longer chronaxie; that is why it fails to be excited by Faradic stimulation in the early stage of reaction of degeneration.

Chronaxie of the plain muscle is longer than that of the skeletal muscle. Vagal stimulation shortens and sympathetic stimulation lengthens the chronaxie of the plain muscles of the stomach. Stretching diminishes their chronaxies and increases excitability.

Cardiac muscle: The chronaxies of ventricular and atrial muscles are same but that of the junctional tissues is three times longer. Stimulation of vagus, stretching, cold, and inhibitory durgs like acetylcholine shortens the chronaxie. Warmth, sympathetic stimulation and accelerator drugs like atropine, adrenaline, etc. lengthen chronaxie.

4. *Nerves:* The chronaxie of the autonomic nerves is more than that of the somatic nerves. Nerve fibres of larger diameter (which conduct more rapidly), have shorter chronaxies than thinner ones (having less conductivity). The chronaxies of the corresponding sensory and motor nerves are about the same. In a degenerating nerve the chronaxie becomes longer. (The chronaxies of certain frog's tissues are as follows: Skeletal muscles: 0.3–0.9 millisecond, ventricles: 3.5 milli seconds, stomach: 30–100 milliseconds, pigments cells of the skin: 11,000–15,000 milliseconds.)

Applied Physiology

Electromyography: Electromyography is a study of the action potential in human skeletal muscle. It is the technique for recording the activation signal of muscles. EMG is performed by an electromyograph which records an electromyogram. A part of the electrical currents generated; is transmitted to the outer surface of the body. The changes in action potential can be studied by either putting electrodes at the surface of active muscle area or inserting them directly into the muscle concerned. The potential can be recorded by cathode ray oscillograph. The measured EMG potentials range between <50 µV and 20–30 mV, depending on the muscle under observation. The typical repetition rate of muscle unit firing is about 7–20 Hz. Damage to motor units can be expected at ranges between 450 and 780 mV. The study has a great clinical value in the diagnosis of different types of neuromuscular functional impairment in which either the rate or rhythm of nerve impulse is influenced.

Muscular Disorders

The few common muscular disorders are

The muscular dystrophies are hereditary disorders characterized by signs and symptom of progressive muscular atrophy and weakness.

1. **Duchenne muscular dystrophy (DMD):** This disease is due to the absence of a gene located on the short arm of the X chromosome at the Xp21 site. This results in the absence of the gene product dystropin in skeletal muscle. Dystropin is a membrane-associated structural protein that maintains muscle fibre integrity during contraction. There is disruption of the membrane covering the muscle fibre; leading to excess amounts of entry of calcium ions into the cell and also cause cell degeneration.
2. **Becker muscular dystrophy:** The disease is identical to the Duchenne type as it is due to mutation in the dystrophin gene; the same gene on the X chromosome that causes Duchenne muscular dystrophy but this dystrophy appears later in life and progresses more slowly.
3. **Vitamin D deficiency:** The muscular wasting and weakness is associated with lack of vitamin D. In this disease marked atrophy of type 2 fibres may occur. The influence of vitamin D over muscles is not fully understood, but it has been observed that at least one of its metabolites, 25-hydroxycholecalciferol, influence the protein turnover and the resting energy state of the muscle.
4. **Viral myositis:** Pleurodynia which is also known as Bornholm disease or epidemic myalgia is caused by the coxsackievirus. The patient recovers completely after a brief period of intense muscular pain and fever.

EXAM-ORIENTED QUESTIONS

Essay

1. Discuss the molecular mechanism of muscular contraction.
2. Describe the mechanical, thermal and chemical changes during muscular contraction.

Short Notes

1. Electromyography
2. Muscular dystrophy

Chapter

26

Properties of Skeletal Muscle

INTRODUCTION

Skeletal muscle shows the following properties: (1) excitability and contractility, (2) refractory period, (3) tonicity, (4) conductivity, (5) extensibility and elasticity.

1. Excitability and Contractility

With an adequate stimulus, muscles are excitable. The stimulus may be mechanical, thermal, chemical or electrical. For facilities of accurate adjustment, electrical stimulus is used in laboratory experiments. When excited, the muscle contracts. This is immediately followed by relaxation. A single induction shock will produce a single contraction (twitch). The record of this on a moving drum (Fig. 26.1) will produce a curve, called the simple muscle curve (Fig. 26.2). But if the stimulus be strong, it may cause stronger contraction.

Simple Muscle Curve

The simple muscle curve (Fig. 26.2), as obtained with a frog's gastrocnemius, and has a total duration of about 0.1 second. It consists of three parts:

1. *Latent period (0.01 second):* It is the interval between the application of stimulus and the beginning of contraction. Latent period is due to time taken: For propagation of impulse from the point of stimulation to the neuromuscular junction and hence to the sarcolemma and for initiation of contraction.
2. *Period of contraction* (*0.04 second*): It is a period from beginning of the contraction up to the maximum contraction.
3. *Period of relaxation* (*0.05 second*), from the summit up to the original level.

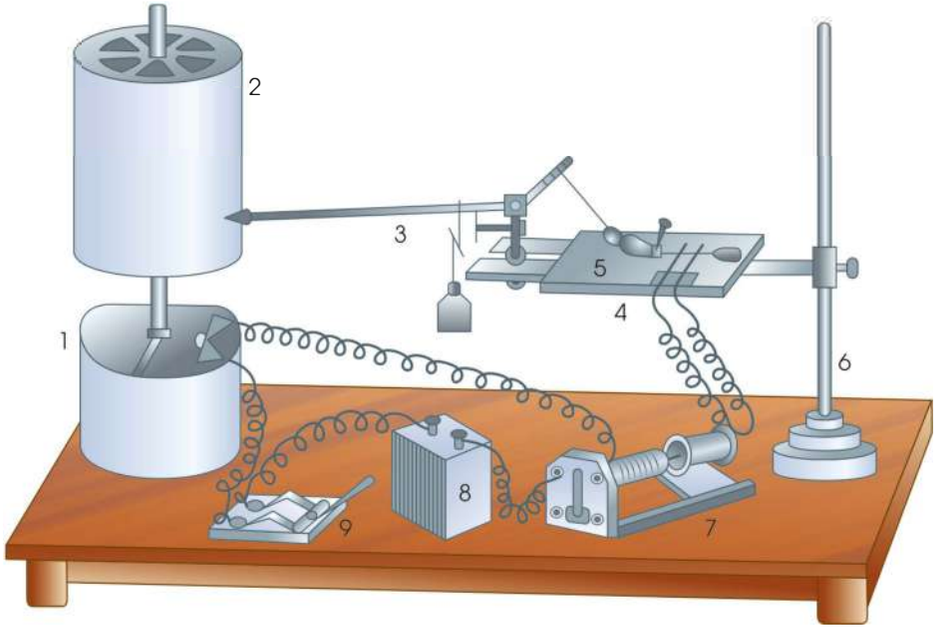


Fig. 26.1: Diagram shows the experimental set-up for the demonstration of simple muscle curve. (1) Motor, (2) Drum showing the curve, (3) Writing lever, (4) Myograph board. (5) Muscle and nerve with stimulating electrode, (6) Myograph stand, (7) Induction coil, (8) Battery, (9) Mercury key

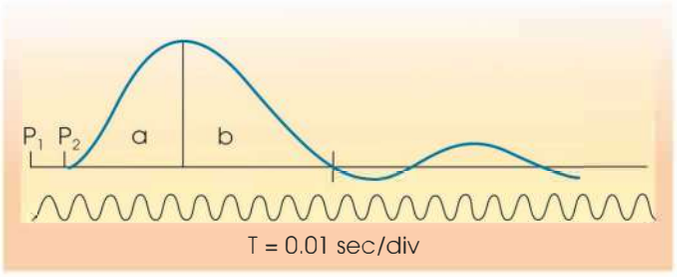


Fig. 26.2: Simple muscle curve: P—Point of stimulation, P—beginning of contraction, P_1 P_2—latent period, a—contraction phase, b—relaxation phase

Factors Affecting Excitability and Contractility

The following factors affect excitability and contractility and therefore alter in various ways, the nature of the simple muscle curve.

Strength and duration of stimuli

For stimulation to occur, two factors are necessary—a minimum strength and an adequate duration of the stimulus. Chronaxie, which includes both these factors are the measure of excitability of a tissue.

Effects of two successive stimuli *(Fig. 26.3)*: If the second stimulus is applied after sufficient intervals both first and second stimuli will cause contraction and two simple muscle curves will be recorded (Fig. 26.3, stage a). The second curve will be slightly higher than the first one due to the beneficial effect of contraction. If the second stimulus is applied in the relaxation period of the first, more or less two separate curves will be produced and the second curve will be higher (Fig. 26.3, stage b). If the second stimulus is applied within the contraction period of the first one the second curve will be superimposed on the first one with a higher height of contraction (Fig. 26.3, stage c). These are known as summation of effects (contraction). If the second stimulus is applied within the latent period but after refractory period of the first, then their effects are added together giving a simple muscle curve of larger height than either of them produced separately (Fig. 26.3, stage d). This effect is known as summation of stimuli. By using maximal or submaximal stimuli, these summated effects can be obtained. This is true for either of the single muscle fibre; or a whole muscle bulk. In case of submaximal stimuli, the summated, effects are due to activation of more nerves and muscle fibres, but in case of maximal stimuli, the greater response cannot be due to an increase in numbers of responding nerves and muscle fibres, but due to the difference in contractile mechanisms. It is claimed that these effects are due to beginning of second twitch before the active state for the first twitch is complete.

That is the duration of the active state of the first response makes possible the augmentation of the second response. Thus, this effect goes against the law of all-or-none.

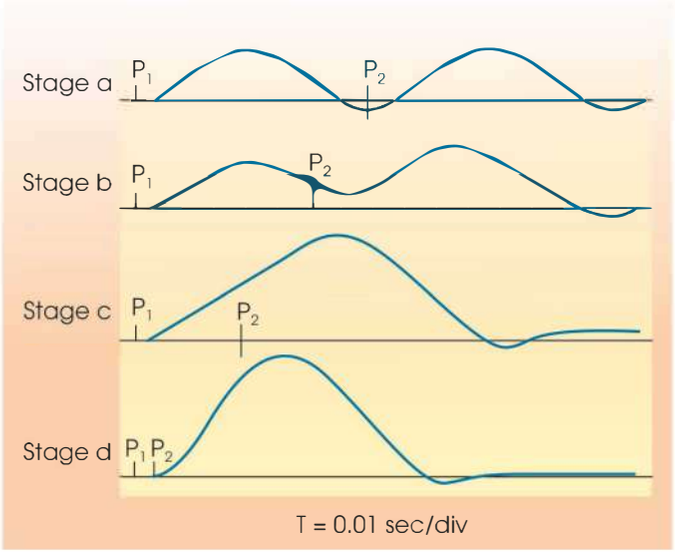


Fig. 26.3: Effect of two successive stimuli. Stage a: The second stimulus is applied after sufficient interval; Stage b: The second stimulus is applied in the relaxation period of the first; Stage c: The second stimulus is applied before the contraction of the first one is over; Stage d: The second stimulus is applied within the latent period of first one

Effects of repetition of stimuli

Following phenomena are observed:

Staircase phenomena: When a freshly excised muscle is stimulated with a single induction shock of sufficient strength then a contraction of certain amplitude is recorded. If second stimulus of same strength is applied at an interval of about 1 second to the muscle after completion of the effect of first stimulus, then an increased amplitude of contraction is recorded. With a series of such stimuli (5 to 6 stimuli) but under the condition that each contraction is allowed to be completed before the next stimulus is applied, then a gradual increase in amplitude of contraction is obtained. This stair-like rise is called the staircase (treppe) phenomenon. Increased H^+; Ca^{2+} ion concentration and an increase of temperature within the muscle create a favourable condition for more work (beneficial effect). Hence, the contraction becomes stronger.

Clonus: When repeated stimuli are applied, the type of response will vary according to frequency. When the frequency is such that each successive stimulus falls within the period of relaxation of the previous curve the record will show a series of wavy oscillations. This is called clonus or incomplete tetanus (Fig. 26.4b to e).

Tetanus: When the frequency is more, so that the stimuli fall within the latent period of the previous curve, the record traces a clear steady line, which rises at first abruptly and then gradually, till maximum contraction takes place. This is called tetanus (Fig. 26.4f). Here the fusion is complete and the muscle, instead of vibrating, exerts a steady pull. Due to summation, the height of

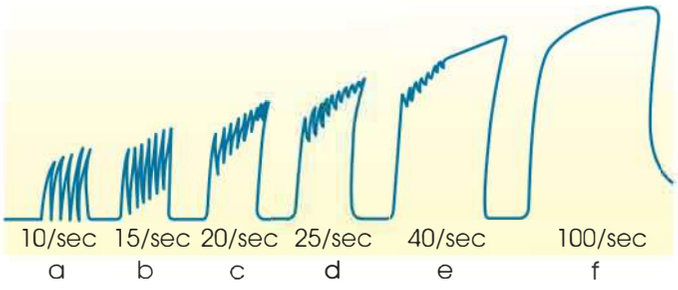


Fig. 26.4: Genesis of tetanus

tetanic contraction is usually higher than that of a single twitch. Frequency of stimulation, required for the induction of tetanus, varies with the nature of the muscle. In external eye muscle it is about 350/sec, in gastrocnemius muscle it is about 100/sec.

Clonus may be described as summation of successive contraction, whereas tetanus is summation of successive stimuli. The mechanical movement in response to voluntary stimuli is neither a twitch nor a tetanus (Lippold, 1957). During voluntary movement, the skeletal muscles are stimulated at low frequency, which is less than fusion frequency, and also asynchronously, so that the infrequent contraction of a large number of fibres gives the appearance of a smooth response.

Fatigue: When repeatedly stimulated, the muscle has lost its irritability, becomes gradually less excitable and ultimately ceases to respond. This phenomenon is called fatigue (Figs 26.5, 26.6 and 26.8). Muscular fatigue can be defined as the inability of the muscle to do further work. In the fatigue curve, all the periods are lengthened. The relaxation period is so much prolonged that the curve fails to reach the baseline before the next stimulus arrives, thus leaving a contraction remainder (Fig. 26.5). The causes of fatigue might be due to (a) exhaustion of sources of energy of the muscle, (b) accumulation of the end products of chemical reactions, such as lactic acid, carbon dioxide, ketone bodies and (c) decrease of local synthesis of acetylcholine-like substances during prolonged exercise (Torda and Wolff, 1945). Oxygen is required for the removal of these substances and so for recovery. Fatigued muscle left in nitrogen does not recover. In studying fatigue in muscle with circulation and without circulation, the muscle gets fatigue more earlier in case of the latter and does not recover on rest (Fig. 26.6A). But in case of muscle with circulation, fatigue comes later and the muscle recovers on rest (Fig. 26.6B). This shows that oxygen, which is supplied through blood circulation, is required for recovery of fatigue. The seat of fatigue lies in the muscle when it is directly stimulated. But when it is stimulated through the motor nerve, the seat of fatigue is in the neuromuscular junction. In physiological exercise, the seat of fatigue is neither in the muscle nor in the neuromuscular junction but at the synapses in the central

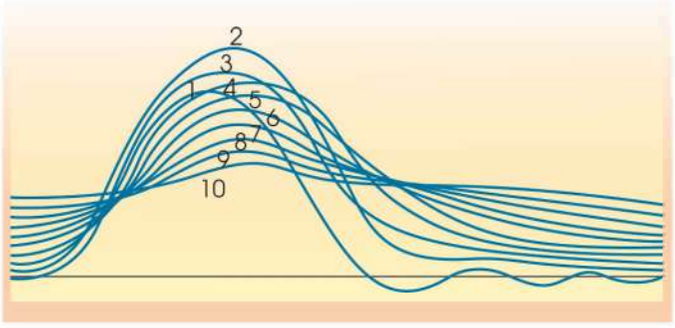


Fig. 26.5: Fatigue curve of a muscle. The diagram indicates successive stage of the curve (beginning from 1). 2 and 3 are higher than 1 due to beneficial effect of contraction. 10 shows the maximum development of the contracture

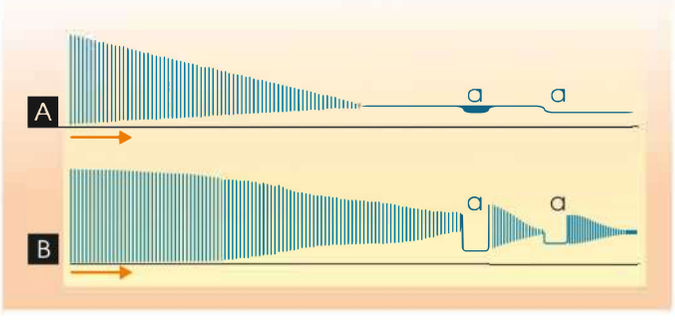


Fig. 26.6A and B: Muscle fatigue in slow moving drum (diagrammatic). Diagram shows the effect of circulation on muscular fatigue. (A) Without circulation; (B) With circulation; 'a' shows the effect of rest on recovery from fatigue, B—a shows recovery from fatigue, whereas A—a does not

nervous system (central fatigue). On comparison, it is seen that fatigue after voluntary work first appears in the synapses, then in the neuromuscular junctions and lastly in the muscle itself. In human subjects fatigue can be studied with the help of an instrument called *ergograph* (Fig. 26.7).

Fatigue can be experimented by noxious stimulation at the foot of a spinal frog. If a reflex withdrawal of foot is obtained and by continuous stimulation the reflex contraction of the muscle is lost, the foot does not withdraw. At this stage if the flexor nerve is stimulated, then contraction of the muscle again occurs. This cessation of contraction is due to changes in the spinal cord. This can also be demonstrated in man by protective mechanism. When the arm or leg muscles of a subject are used to contract repeatedly with a weight attached to the part and he is unable to lift weight voluntarily, then electrical stimulation of the motor nerve through the skin produces a powerful contraction.

All-or-none law

It means that if a single muscle fibre contracts at all, it will contract to its maximum, provided the conditions remain constant. If internal and external conditions are changed, the amount of contraction will vary. This law

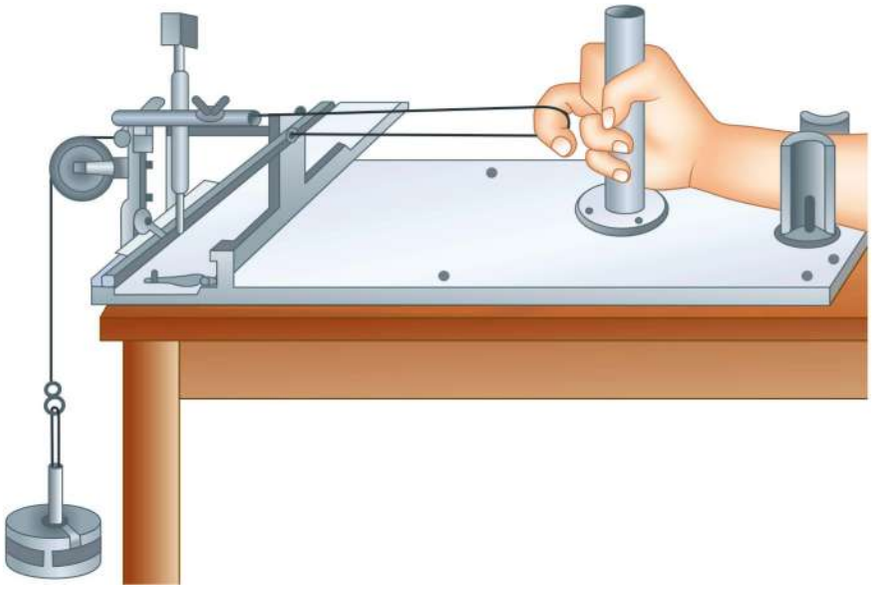


Fig. 26.7: Ergograph

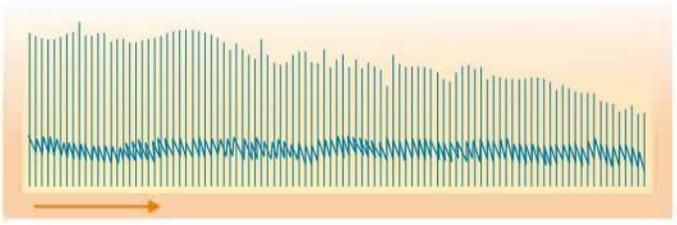


Fig.26.8: Fatigue curve of the middle finger in the ergograph

holds good for a single muscle fibre and does not apply for the whole muscle, which is composed of innumerable muscle fibres. Because, in the latter case, as the strength of the stimulus is increased, more and more muscle fibres will be affected and the degree of contraction will be raised (staircase phenomena) and a stage will be achieved when there will be no further rise (all-or-none law for whole muscle). But modern theory claims that the all-or-none law is applicable in case of development of the action potential but not for the activation of the contractile materials.

Effects of temperature

Moderate warmth (25°C.) increases and cold (5°C.) depresses both excitability and contractility. The former shortens and the latter lengthens all the periods of the muscle curve (Fig. 26.9). Temperature above 42°C produces heat rigor due to coagulation of proteins present in the muscle.

Effects of load

Load lengthens the latent period but reduces the periods of contraction and relaxation. It also reduces the degree of contraction, i.e. the height of the curve (Fig. 26.10). The effect of load on the work done by the muscle depends on the way in which the load is applied. If the weight is allowed to stretch the muscle prior to

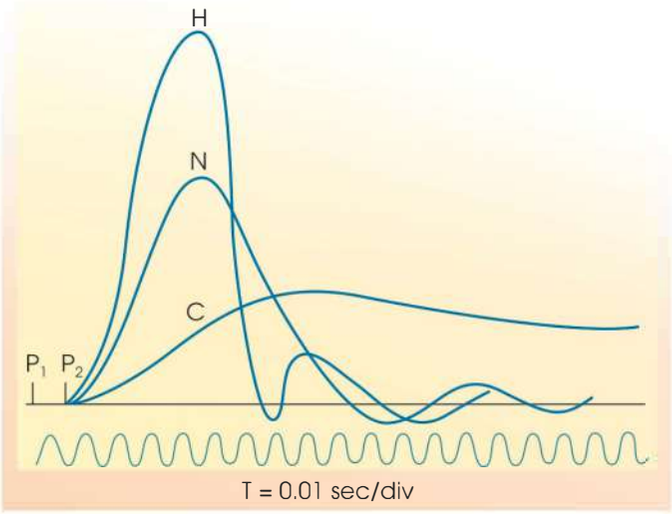


Fig. 26.9: Effect of temperature. P1: Point of stimulation, P2: Onset of contraction, H: Effect of heat, N: Normal curve, C: Effect of cold

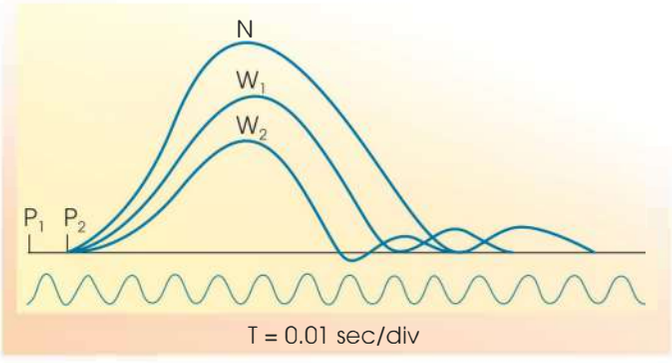


Fig. 26.10: Effect of load. P1: Point of stimulation: P2: Beginning of contraction. Curve N: Unloaded, W_1 and W_2 curves are due to application of different graded loads

its contraction, the muscle is said to be free-loaded but if the lever is supported then the muscle is only stretched when the contraction begins, the muscle is said to be after-loaded. The mechanical efficiency in free-loaded muscle is higher than that in after-loaded muscle. This is mostly related with the increase in initial length of muscle fibres.

Work done by the muscle: The work done may be calculated by multiplying the weight with the actual height of the curve.

Actual height of contraction h in cm can be determined by dividing the observed height of contraction H in cm by the magnification of the lever (Fig. 26.11). The magnification of the lever can be determined by dividing the length L in cm of the lever from the fulcrum to the tip of the lever, with the length l in cm from the fulcrum to the point of the lever from which the weight is lifted. So the magnification will be (L/l). Thus, the calculation for actual height will be $h = H \div (L/l)$ or $(H \times l)/L$. if W is the weight in grams lifted then

$$\text{Work done} = W \times \frac{H \times l}{L} \text{ gm cm}$$

$$= W \times \frac{H \times l}{L} \times 981 \text{ ergs}$$

Work done by the muscle in free-loaded muscle within the physiological limits increases gradually with the increase of load but the same in after-loaded muscle does not follows linearly with the gradual increase of weight. The working efficiencies in such after-loaded muscle increase with a certain weight and then decreases gradually.

The chemical energy liberated during contraction is partly converted into mechanical energy which manifests itself as work. The term mechanical efficiency indicates what fraction of the total energy liberated is converted into work. In untrained men, the mechanical efficiency is 20% in athletes, 25–30% in isolated muscles, under optimum conditions, it may be as high as 40%.

It is seen that, the slower the speed of shortening, the greater is the amount of work done. But evidently, there is an optimum limit beyond which slowing the rate of contraction will reduce the work done.

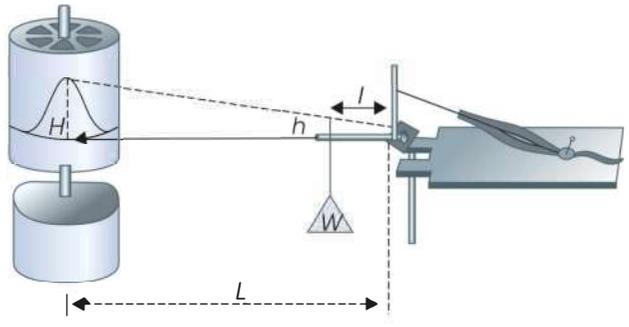


Fig. 26.11: Experiment set up to calculate the work done by a single twitch

Effects of salts and ions

(a) Sodium salts exert an excitatory effect. (b) Calcium salts have got role in initiation of contraction. Development of tension of the muscle is prevented if the Ca^{++} is not present in the medium. It is claimed that the Ca^{++} stimulates ATPase activity so that the association of actin and myosin in presence of ATP takes place promptly. Calcium ions and to a less extent Mg ions help the enzyme action of myosin. (c) Potassium salts reduce excitability and hasten fatigue. On stimulation, potassium ions escape from the interior of the muscle. It is believed that the excitability, contractility and electrical phenomenon of the muscle depend largely upon this migration of the potassium ions. The resting muscle membrane is permeable to potassium ion but not to sodium ion. (d) Magnesium ion is essential for the action of the enzyme phosphorylase which is very important for the phosphate transfer during muscular contraction. (e) Increased H-ion changes exert the same effect as excess potassium.

2. Refractory Period

After stimulation there is a brief period during which the muscle is not excitable to a second stimulus. This period is called refractory period. In the case of smooth muscles, refractory period is very short and falls within the latent period. In the skeletal muscles of frog, it is about 0.005 second, in the mammalian muscles it is about 0.002 second. Cold lengthens and heat shortens this period. During the first part of the refractory period, the muscle remains inexcitable to any strength of stimulus and is known as absolute refractory period. But in latter part of the refractory period the muscle may be excitable only with a stronger stimulus, and is known as relative refractory period. The absolute refractory period in skeletal muscle is shorter than in cardiac muscle and for this reason the skeletal muscle can be tetanised or fatigued.

The earliest chemical change during muscular contraction is the breakdown of adenosine triphosphate (ATP). So long as the broken ATP is not re-synthesised in adequate amounts, the muscle cannot be excited.

Refractory period is that period during which this resynthesis of broken ATP takes place.

3. Tonicity

In the body the skeletal muscles always remain in a state of light tension. This is called tone. Tone of the skeletal muscle may be defined as the reflex sustained and partial contraction. In isolated muscle, tone is absent. If the motor nerve of the muscle is excised, tone is also lost. These experiments show that muscle tone is a reflex process, the centres being situated in the spinal cord. A muscle under tone does not show fatigue. This is due to the fact that in tone production, the whole

muscle is not contracting simultaneously. Only a few muscle fibres are contracting at a time. The muscle fibres contract in batches. When one batch is contracting, the other is relaxing. Hence, the whole muscle does not show any fatigue.

4. Conductivity

After simulation the wave of contraction starts at the point of stimulus and is propagated both ways along the muscle. In frog's muscles the rate of propagation is 3–4 metres per second. In warm-blooded animals 6–12 metres per second.

5. Extensibility and Elasticity

Muscle extends when stretched. When the tension is released, it goes back to its original length. But this elastic return is a little slower. A rubber band is found to extend uniformly by increasing loads of equal weights upon it and returns quickly to the original position as soon as the weights are removed. But under the same conditions, muscle does not immediately come back to its original position. It takes a little longer time. This is called extension remainder.

Contraction of Skeletal Muscle

Characteristics of Isotonic and Isometric Contraction

Contractions are of two types—isotonic and isometric. In isotonic contraction physical shortening of the muscle is allowed when one end of it is attached to a light weight which is lifted. This type of contraction has already been described and gives simple muscle curve. In isometric contraction physical shortening of the muscle is reduced to minimum by making it contract against a strong spring. The slight change in the spring is magnified by suitable instruments and is recorded. The curve of isometric contraction shows the following peculiarities: (a) The latent period is longer. (b) The period of contraction is longer and the tracing at first shows an upward concavity followed by an upward convexity up to the summit. (c) Relaxation period is more gradual and the curve shows a gentle slope with a slight upward concavity. The rise of tension is abrupt and commences very early. The amount of tension is also much higher than in isotonic contraction. Other things remaining constant the tension developed in the muscle fibres is directly proportional to the initial length of the fibre. The evolution of heat is also much more than in isotonic contraction.

Nature of Voluntary Contraction

Voluntary contraction differs from simple muscle twitch in two respects. First it lasts much longer and secondly, the degree of contraction can be finely adjusted at will. The voluntary contraction is neither a simple muscle twitch nor a tetanus. The rate of discharge is slow and less than fusion frequency and the number of cells discharging may vary in number and consequently the muscle fibres affected will, vary. The cells do not all discharge at the same time. But they work in 'batches'. While one group is discharging the other group is resting. For this reason, the muscle fibres of all the motor units are not in the same state at the same time. They are at different phases of contraction and relaxation. Their algebraic sum gives a smooth, steady pull. The accurate gradation in the strength of contraction is due to the involvement of varying number of anterior horn cells.

Contracture: Under certain conditions skeletal muscles show a peculiar type of persistent contraction. This is called contracture. It differs from tetanus or any other forms of physiological contraction in two ways. First, in contracture only a part of the muscle may be involved, while the rest of the muscle remains relaxed. Secondly, the contractile process is not propagated along the muscle fibre and also no action potentials are seen with it. Contracture may be found under various conditions, such as (a) by strong, prolonged or multiple stimuli, (b) in fatigue, (c) in certain pathological states of the body.

EXAM-ORIENTED QUESTIONS

Essay

1. Discuss the properties of skeletal muscle.

Short Notes

1. Characteristics of isotonic and isometric contraction
2. Contracture

Chapter

27

Cardiac Muscle

INTRODUCTION

The cardiac muscle (involuntary striated) contracts rhythmically and automatically, which is particularly maintaining the life process of the living system by assisting the supply of nutrients, O_2 and removal of metabolic waste products. Morphologically, it can be distinguished from smooth and skeletal muscles, though it carries some common characters to each other.

The main differences between cardiac and skeletal muscles are

1. The spontaneous nature of cardiac contraction and rhythmicity is not subjected to voluntary control.
2. The cardiac fibres are not simple cylindrical but they bifurcate and come in contact with that of the neighbouring fibres and ultimately form a three-dimensional network which causes the false syncytial appearance under light microscope.
3. The nucleus is single and placed deep in the sarcoplasm more or less at the centre.

Distribution: The cardiac muscles are actually forming the muscular body of the heart (muscle layer of the heart). These muscles are also present in small amounts in the great vessels ending in or opening from the heart.

Origin and development: Cardiac muscles in the embryo are originated from the mesenchymal tissue. They are formed of the splanchnopleure adjoining the endothelium of the cardiac primordium. The processes of the star-shaped cells have got desmosome-like attachments to each other which are ultimately developed into the intercalated discs. The cells (myoblasts) divide mitotically and gradually the fine bundles of myofibrils begin to appear. Electron micrographically, the Z-lines are the first appeared cross-striations. The Purkinje fibres also develop from same primary star-shaped cell reticulum as that of the myocardium.

Histology: The cardiac muscle fibres are separated from each other by the connective tissue endomysium along with blood vessels and lymphatics. The cardiac muscle fibres are not made up of one straight simple cylinder but they have got short cylindrical branches in all directions (in any dimension). These branches are coming in contact with that of the adjacent fibres, ultimately forming a three-dimensional network. Under light microscope these networks appear as syncytium (cytoplasmic continuation in-between neighbouring cells) which was also supported as the property of the cardiac muscle that if it contracts, it will contract as a whole. But electron micrograph reveals that these branches are not acting as cytoplasmic bridge but they are separated from each other by special surface specialization, the intercalated disc. These discs appear as dark lines under light microscope and pass in irregular step-like configuration across the fibres at fairly regular intervals and at the level of the I-band.

The sarcolemma of the cardiac muscle is more or less similar to skeletal muscle. The mitochondria are more numerous and the cytoplasm is more abundant. The mitochondria are arranged longitudinally and are present in-between the myofibrils in rows. The nucleus is elongated centrally placed in the diverging myofibrils. A small Golgi apparatus is present at one pole with a few lipid droplets. As age increased the lipofuchsin (lipofuscin) pigments are deposited near the nucleus and may be much extensive to give the brownish appearance of the heart—the brown atrophy of the heart. The sarcoplasm contains more glycogen than that of the skeletal muscle. The patterns of the A-, I-, Z-, H-band, etc. are identical with that of the skeletal muscle.

Fine structure as visualised under electron microscope which are

1. The myofibrils made up of myofilaments, are more or less similar to that of the skeletal muscle.
2. The myofilaments are not continuous in the adjacent fibres of the longitudinal order, i.e. they are limited to the individual fibres, in other words, similar to the skeletal muscle. Yet groupings of the myofilaments

(made up of actin and myosin) are not complete to form the myofibrils as that of the skeletal muscle. These myofibrils are delineated by the sarcoplasmic reticulum and sarcoplasm.

3. Sarcoplasm contains a large amount of mitochondria (average 2.5 µm in length, but often they appear as 7–8 µm in length) along the long axis. Mitochondria often remain completely surrounded by myofilaments. So the myofilaments of the cardiac muscle fibres form a continuum which looks like a large cylindrical mass made up of parallel myofilaments subdivided by fusiform clefts of the sarcoplasm occupied by mitochondria.

Sarcotubular System

T-system: This T-system is the tubular imagination of the sarcolemma of the cardiac muscle and is larger in diameter than that of the skeletal muscle. The T-tubules are present at the Z-line in the cardiac muscle fibres, but the same are at the A-I junction in case of the mammalian skeletal muscle. The functional significance of the locational difference is not yet understood. These tubules also increase the surface for metabolic exchange in-between the interior of the cardiac muscle fibre and the intercellular space, over and above they function for quick propagation of impulse from the cell surface to the interior.

Sarcoplasmic reticulum: This reticulum of the cardiac muscle fibre is ill developed. They are consisted of longitudinal interconnected tubules which are expanded into small terminal sacs at the Z-line. T here is no well-developed transverse cisternae in the cardiac muscle (Fig. 27.1). So the transverse section of T-tubules at different regions may show triad diad (dyad) or only T-tubule depending upon the presence of sarcotubules running in association with the T-tubules.

Transmission of Impulse and Mechanism of Contraction

The mechanism of contraction in the cardiac muscle is essentially same as that of the skeletal muscle. Impulse originated in the pacemaker area is transmitted through different conducting tissues and ultimately reaches the cardiac muscle fibre, and from which the impulse is transmitted rapidly from cell to cell through different junction surfaces of the intercalated discs. From the cell surface, the impulse is transmitted to the contractile elements of the myofibrils through the sarcotubular system.

Intercalated (Intercalary) Discs

These are the areas of extensive cell contact and run transversely across the fibre. These also appear as dark line under the light microscope (Fig. 27.2). Electron micrograph reveals that they are made up of the unit membranes of two adjacent cells. A complex pattern of ridges and papillary projection of the unit membrane on each cell fit into corresponding grooves and pits in the other cell membrane which forms an elaborately interdigitated junction, specialized for cell-to-cell

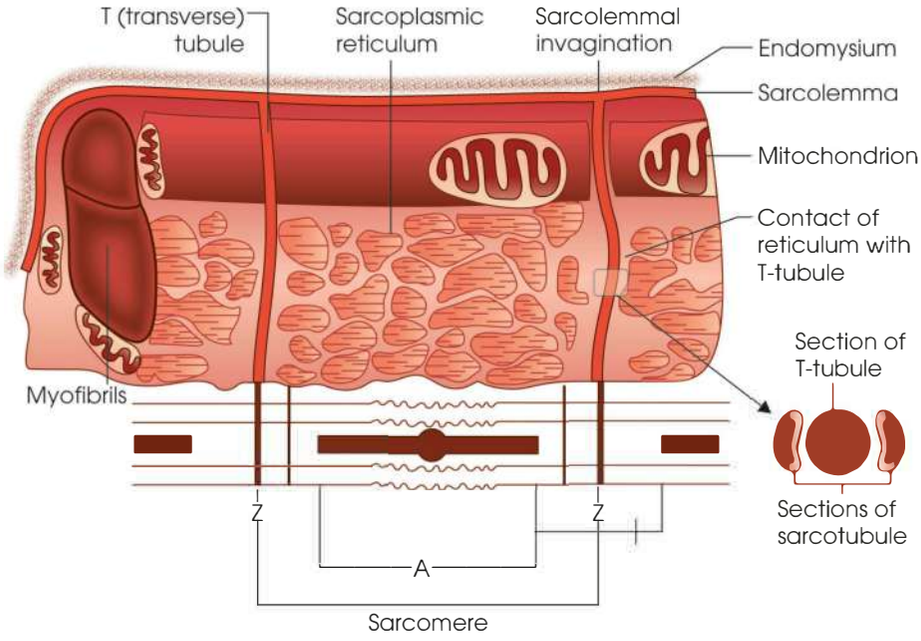


Fig. 27.1: Sarcotubular system of the mammalian cardiac muscle (diagrammatic representation) showing the contact of the sarcoplasmic reticulum with tubules at the Z-line. It also shows the absence of terminal cisternae but transverse sections at certain points of T-tubule may have triads due to presence of sections of T-tubules in the middle and reticular tubules (sarcotubules) on both sides.

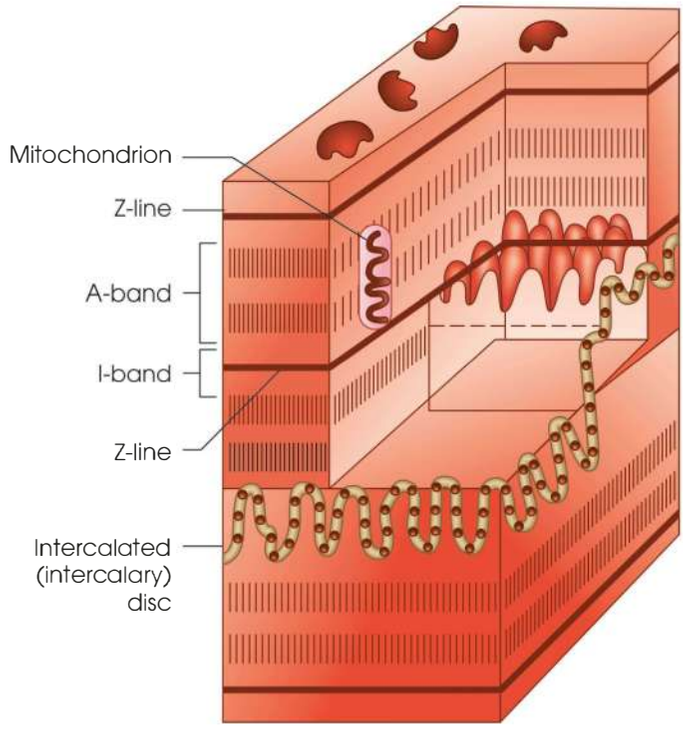


Fig. 27.2: Cardiac muscle (diagrammatic representation) showing their relation with adjacent fibres (cells) at the intercalated discs

cohesion. This interdigitated junction is mostly similar in structure with the epithelial cell junction. In the transverse portion of the intercalated disc, there is desmosome-like cell-to-cell junction with 200Å intercellular cleft (Fig. 27.3). At desmosomes (maculae adhaerentes), the inner layer of each of the opposing unit membranes is added up by a thin layer in which the myofilaments of the ajacent I-band terminate. At irregular intervals of the transverse portion of the intercalated disc there are also small tight junctions which are known as maculae occludentes. At the maculae occludentes the layer of the opposing membranes are fused together by obliterating the intercellular gap. The longitudinal portion of the step-like cell-to-cell junction contains the more extensive areas of close membrane contact—fasciae occludentes (intercellular space is obliterated). These areas of the intercellular space obliteration are of low electrical resistance which helps in the rapid propagation of excitation from cell-to-cell throughout the whole mass of the heart and thus assisting the myocardium to behave as syncytium. The desmosomes of the transverse portion are concerned with the cell-to-cell cohesion and transmission of the pull of contractile unit of one cell to the other in the longitudinal direction. The mechanism acting behind the binding of the cells together is not yet much clear still it is evident that Ca^{++} has got some important role. The individual cells of cardiac muscle perfused with Ca^{++} free-Ringer are seen to have separated at the intercalated discs. Electron micrography depicts that the separation takes place at the maculae adhaerentes (desmosomes) where the individual unit membrane remains intact but the intercellular space is opened up. At the close junction (maculae occludentes), the membrane cannot be separated due to their fusion but one of the cells may be denuded of its membrane (Fig. 27.4: b-b, c-c, d-d).

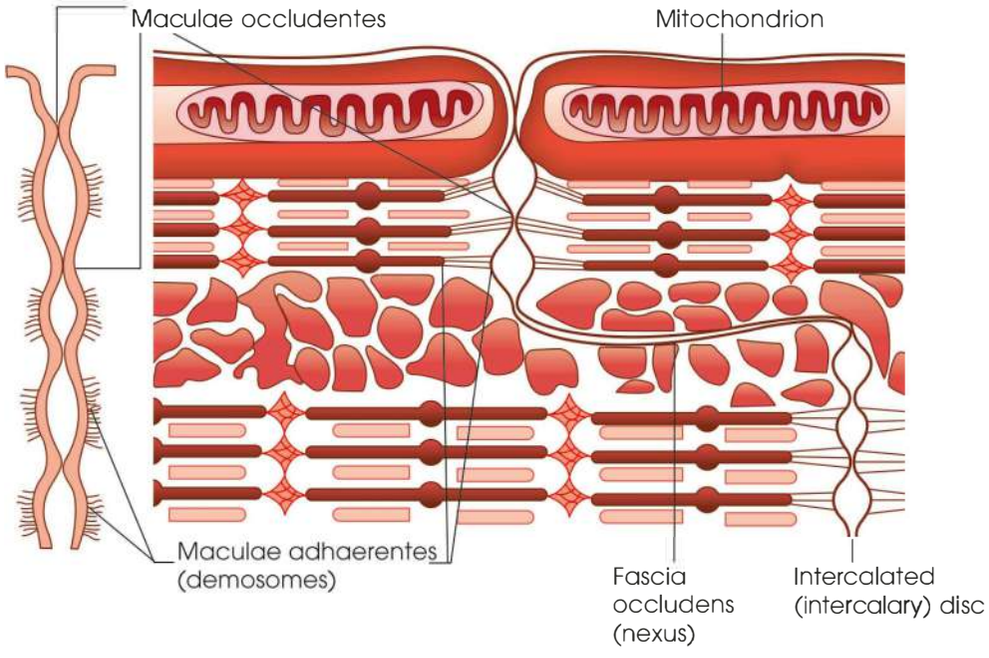


Fig. 27.3: Junctional peculiarities at the intercalated (intercalary) disc of the cardiac muscle (diagrammatic representation). Inset shows structural peculiarities at the transverse portion of the intercalated disc

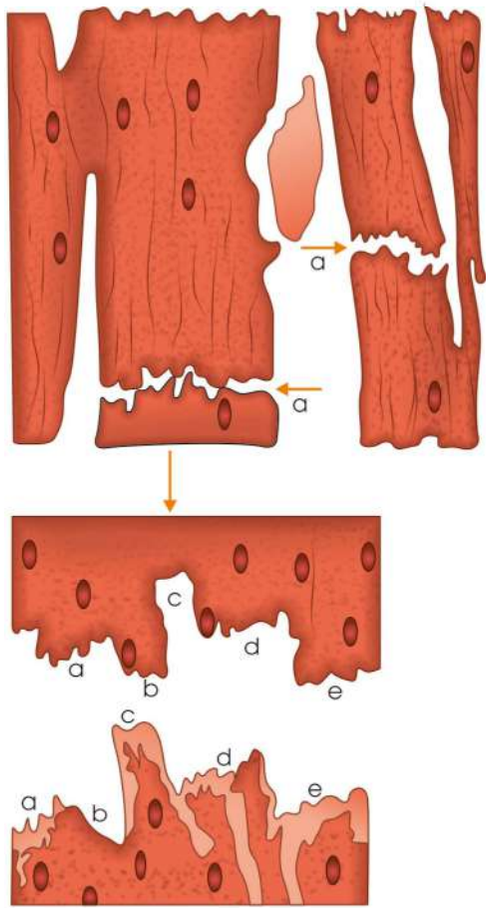


Fig. 27.4: Separation of the cells at the intercalated disc (at 'a' in upper inset) after perfusion of cardiac muscle (diagrammatic) with Ca-free Ringer fluid (after Bloom and Fawcett)

Blood Vessels, Lymphatics and Nerves of Cardiac Muscle

Cardiac muscle fibres receive blood vessels from coronary arteries and these vessels form a basket-like capillary network around the muscle fibres. Much anastomoses between arterioles of cardiac muscles are seen. But these anastomoses are to a certain extent quite capable of supplying blood through backflow in the case of a sudden occlusion of one of vessels. Lymphatic vessels give a rich supply to the interstitial (between cells) connective tissue. Myelinated and non-myelinated nerve fibres supply richly cardiac muscle fibres. Efferent nerve fibres form a fine network with varicose swelling and end on the surface of cardiac muscle fibres.

Note

Properties of cardiac muscle will be discussed with the cardiovascular system.

EXAM-ORIENTED QUESTIONS

Essay

1. Discuss the structural morphology of cardiac muscle. Discuss the histological features.

Short Notes

1. Describe the intercalated disc
2. Describe the transmission of impulse and mechanism of contraction

Chapter

28

Smooth Muscles

INTRODUCTION

The visceral muscle is also known as the plain, non-striated, smooth involuntary muscle. It is called non-striated because it has got no cross-striations. The contraction of this muscle is not controlled by volition or will.

Distribution: These muscles are present in all hollow viscera, e.g. gastrointestinal (GI) tract, ducts of the glands, blood vessels, respiratory, urogenital and lymphatic systems of the body. These are also present in the dermis, ciliary body and iris of the eye. The automatic contraction of the smooth muscle fibres (GI tract, ureter, uterus, etc.) facilitates the movements of the contents that are passing through the above-mentioned viscera. Again in the dermis they are responsible for the erection of hairs.

Origin and development: The smooth muscles are mesenchymal in origin. The mesenchymal cells first start to stretch out. The nucleus becomes elongated, and myofilaments appear in the cytoplasm. In the case of blood vessels, the mesenchymal cells are arranged along the wall of the tube at regular intervals and developed in the same fashion as already mentioned and ultimately forming the continuous circular and longitudinal muscular layers of blood vessels. It is claimed by some workers that the new smooth muscle fibres that appear in the uterus during pregnancy are originated from the undifferentiated connective tissue. The smooth muscle fibres can increase in size and also in bulk during physiological requirement (e.g. uterus in pregnancy) and in pathological stimuli (e.g. arterioles in hypertension). There is also evidence that smooth muscle cells themselves can divide by mitosis.

Histology

The smooth muscle fibres are elongated, fusiform (spindle-shaped) with a wider central portion where the single nucleus is situated (Fig. 28.1). Both the ends of the fibres are tapering towards the periphery. The average length is about 0.2 mm which varies much (20 µm at the wall of the blood vessels to 500 µm, in the pregnant women's uterus) and the width is about 6 µm at the central widest portion. The cells of the smooth muscles are so arranged that the thick-middle portion of one is juxtaposed by the thin-end portion of the other. So in the transverse section, there will have rounded or irregularly polygonal profiles of various sizes (1.0 µm to several µm in diameter), only the largest profiles will demonstrate the centrally placed nucleus. The nucleus is elongated, oval. Delicate uniform chromatin network is present in the nucleoplasm. There are two or more nucleoli. In ordinary preparation, the sarcoplasm is quite homogeneous. But in special preparations (e.g. macerated with acid), fine longitudinal striations may be demonstrated running full length of the fibre. These are the myofibrils and are interpreted as the contractile

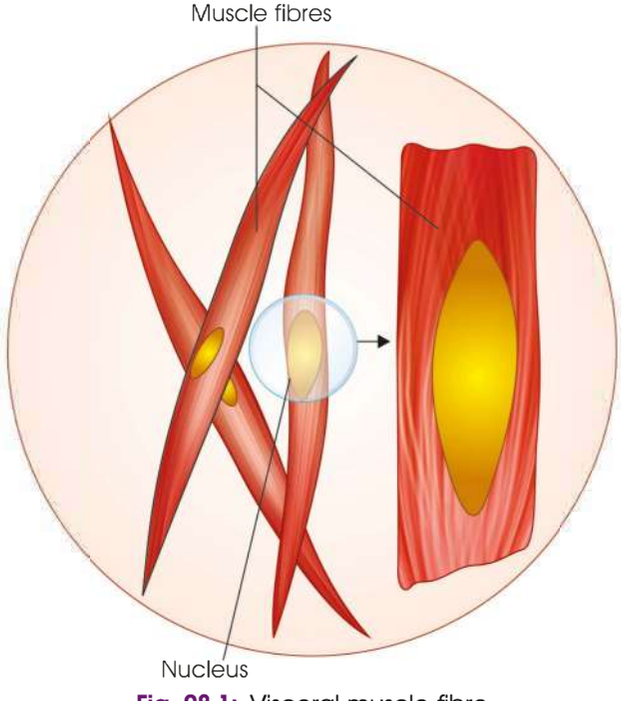


Fig. 28.1: Visceral muscle fibre

material of the smooth muscle. They are doubly refracted but have got no isotropic (I-band) and anisotropic (A-band) regions, i.e. no cross-striation which is the characteristic feature of the other two varieties of muscles (skeletal and cardiac muscle).

A small Golgi apparatus is also present near the nucleus. Sarcoplasm contains a considerable amount of glycogen. The surface of the smooth muscle has a thick basal lamina (similar to the basement membrane of the epithelium). The sarcolemma (the plasma membrane) is surrounded by an extraneous glycoprotein coat similar to the basement membrane of epithelial cells.

Fine structure: In electron micrograph, the nucleus appears as elongated, smooth-surfaced and rounded at the ends. The mitochondria are present at the poles of the nucleus. The sarcoplasm also contains the sarcoplasmic reticulum, free ribosomes and a small Golgi apparatus. The remaining sarcoplasm is occupied by the thin myofilaments along the long axis of the fibre. Myofilaments are interpressed by the mitochondria which are also arranged along the long axis. There is a dense area along the inner aspect of the sarcolemma which corresponds to the Z-line of the skeletal muscle where the myofilaments are attached. The final contractile elements, myosin and actin, are present chemically. The actin filaments are seen readily but the myosin filaments are not yet demonstrated as a whole, though small processes of actin filaments are assumed as myosin. At high magnification it is seen that the actin and the myosin filaments are 30 Å and 80 Å in thickness respectively and they are not arranged in any definite order as that of the skeletal muscle.

Cell-to-cell relation: The adjacent smooth muscle cells are separated by thick basilar lamina (total distance 400–800 Å). Typical desmosome is not present. The specialized dense areas of adjacent cells are present often opposite to one another but are distributed entirely in random. Within these specialized dense areas, the myofilaments terminate. These are the suggested site for cell-to-cell cohesion. At some areas the basal laminae are absent where the unit membranes are coming in close contact to each other, having a similar fusion pattern as that of the tight junctions of the cardiac intercalated disc (zonula occludens of the epithelia)—the fasciae occludentes or nexuses. The nexus is probably a low resistant area through which rapid propagation of excitation impulse is possible from one cell to other in case of the smooth muscle (Fig. 28.2).

Contractile Mechanism

It is assumed that the mechanism of contraction of the smooth muscle is same as that of the skeletal muscle because it is known that the actin and the myosin filaments of smooth muscles also do not change in length during contraction. The visceral smooth muscles (e.g. GI tract, ureter, uterus) are capable of contracting automatically like the cardiac muscle. The contraction of these muscles is slower. Sustained forceful contractions are also possible without loss of relatively greater expenditure of energy. Stretching of the muscle, as in case of GI tract due to bolus of food passing through it, is presumably one of the causes of initiating an impulse and thereby producing contraction. These particular groups of muscles have got some functional similarities with that of cardiac muscles particularly in their automatic rhythmicity. The vascular smooth muscles have got some similarities with the skeletal muscles, because the activities of both groups of muscles are modified by the stimulation of the motor nerve.

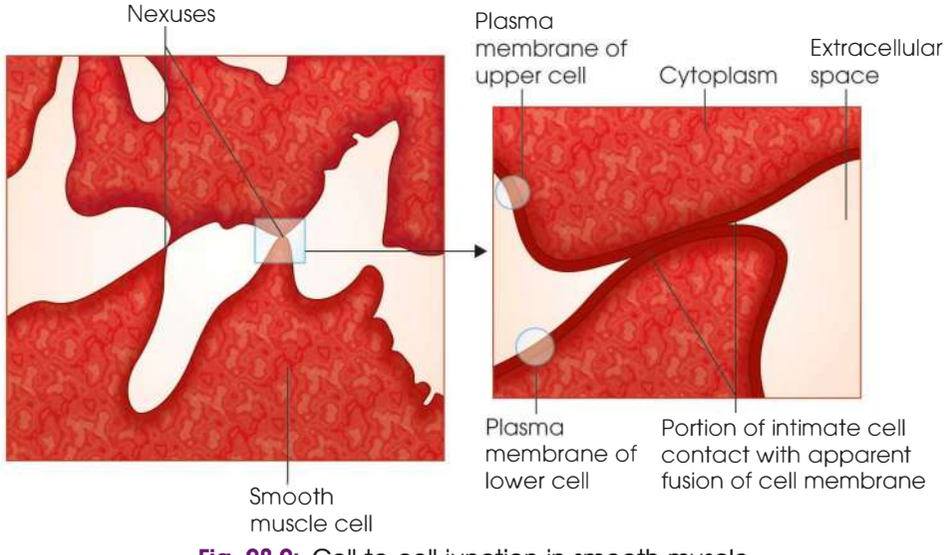


Fig. 28.2: Cell-to-cell junction in smooth muscle

Molecular Mechanism of Contraction and Relaxation in Smooth Muscle

Contraction: The contractile activity in smooth muscle is initiated by a Ca^{2+}-calmodulin which activates myosin light-chain kinase which causes phosphorylation of the light-chain of myosin. The process of phosphorylation activates myosin ATPase activity. The myosin–actin interaction and the myosin ATPase activity brings over the cross-bridge cycle and contraction by sliding of the filament. The attachment of ATP molecule to myosin head energizes it so that it attaches to the active site on actin filament producing a power stroke and shortening of muscle releasing energy due to ATP breakdown. When another molecule attaches to the myosin it leads to detachment of cross-bridge from actin. The cross-bridge cycle detaches when there is fall in concentration of calcium level below critical level.

Note

A Ca^{2+} sensitization of the contractile proteins is signaled by the RhoA/Rho kinase pathway to inhibit the dephosphorylation of the light-chain by myosin phosphatase, maintaining contractile force and is responsible for the latch bridge mechanism of contraction of smooth muscle. The removal of Ca^{2+} from the cytosol and stimulation of myosin phosphatase initiate smooth muscle relaxation.

Relaxation of smooth muscle: Smooth muscle contract as long as there is calcium bound to the calmodulin and the myosin light-chains are phosphorylated. Once the stimulus for the release of calcium has been withdrawn, a series of Ca-ATPases move the Ca^{++} back into the SR or out of the cell. As a result of decrease in intracellular calcium levels, the calcium leaves the calmodulin and the myosin light-chain kinase gets inactivated. As the myosin light-chain is dephosphorylated, the myosin head no longer has significant affinity for the actin active site and relaxation follows.

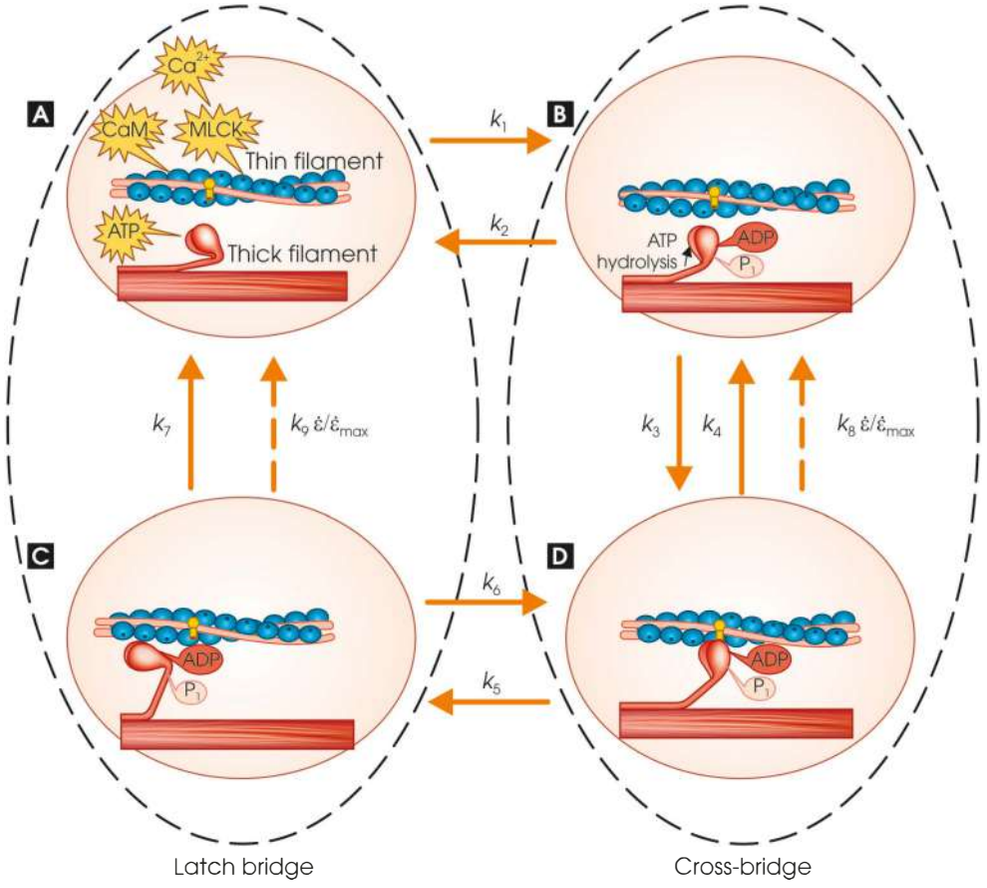


Fig. 28.3A to D: Diagrammatic representation of four-state biochemical model for the contraction of smooth muscle in response to calcium stimuli: Depicts free unphosphorylated myosin and formulation of actin filament (A), detached phosphorylated myosin (B), attached phosphorylated myosin (C), attached unphosphorylated myosin (D). (Reference: Tao Liu. A constitutive model for cytoskeleton contractility of smooth muscle cell. Proceedings of the Royal Society. 08 April 2014; volume 470, issue 2164)

PROPERTIES OF PLAIN (SMOOTH) MUSCLES

Broadly speaking, properties of the plain (smooth) muscle are same as those of the skeletal muscle, with the following differences.

Excitability and Contractility

- Plain muscle is less excitable. Contraction is slow, and worm-like. All the periods are longer. The latent period varies from 0.2 to 2 second, period of contraction from 0.5 to several seconds; the period of relaxation is proportionately longer than that of contraction.
- The refractory period is also much longer. One more peculiarity is that, a particular stimulus (electrical or mechanical) may cause either contraction or relaxation. If the muscle is relaxed, the stimulus will cause contraction; but if it is already contracted, the stimulus will relax the muscle.
- The effect of temperature is also different. Moderate warming cause relaxation and cold temperature produces contraction.
- *Phasic and tonic contraction:* Type of contraction in smooth muscle is phasic and tonic type. The rate of phosphorylation, calcium concentration in cytoplasm and rate of cross-bridge cycle returns to basal resting level in phasic contraction, while they do not in tonic contraction. The attach cross-bridges are known as latch bridges. The rate of cross-bridge cycle when decreased produces sustained state of contraction with increased muscular tension and lower level of myosin light-chain kinase activity and less expenditure of energy. This sustained state of smooth muscle contraction is the latch bridge mechanism.

Rhythmicity

It has automatic inherent rhythmicity. When slightly stretched most varieties of plain muscles will exhibit rhythmic contractions. These contractions are independent of all nerves and are purely myogenic. The 'stretch' acts as a stimulus causing the relaxed muscle to contract and the contracted muscle to relax. In this way as long as the 'stretch' is maintained, the alternate rhythmic contraction and relaxation will continue.

Conductivity

The wave of contraction is propagated along the muscle fibre but the rate of propagation is much slower. One peculiarity is that in the plain muscle the stimulus travels from fibre-to-fibre through the nexus and the wave of contraction is propagated through the whole muscle sheet. The electric changes during contraction are also peculiar because, two negative potential changes are found to accompany each contraction.

Tonicity

Plain muscles remain in a state of light tension.

Factors affecting the activity of plain muscle

1. *Temperature:* Heat diminishes both excitability and tonus. Cold, on the other hand, increases tonus.
2. *Salt balance:* The activity of the smooth muscle is modified by K; Ca ratio. Ca^{++} influences the Na^{+} permeability mechanism and increases the rate of rise of spike; K^{+} also increases the tone of the smooth muscle.
3. *pH:* Increase in pH lifts tonus and rhythmic activity, decrease in it produces quiescence and relaxation.
4. *Previous state of activity:* A stimulus which generally causes contraction, will induce relaxation in a smooth muscle if it is already in a state of high tonus.
5. *Hormones:* The secretion in smooth glands has profound influence over the activity of plain muscle. Contraction of uterus on stimulation is dependent on the state of oestrus of the organ. Acetylcholine stimulates and adrenaline depresses the tone of muscle. Posterior pituitary extract also increases tonic contraction of the uterine and intestinal muscles.

Functions of the Muscular Tissue

1. The specialized function of the muscular tissue is to contract and thereby produce motion. Although the skeletal muscle fibres contribute to the functioning of the body as a whole, yet the extent is much greater than the production of movement *per se*.
   - Movement of the osseous tissue at the joints encompasses:
     - Locomotion of the body from here to there (ambulation)
     - Changes in position of the body (i.e. standing, lying down, stooping over, moving of eyeballs, tongue and extremities, etc.).
2. Development of muscular skills involved in our activities and in our speech.
3. Much production of body heat and fluid balance is a concomitant contribution of muscles to whole body.
4. Muscles help in maintaining the posture of the body, the formation of walls of body cavities and the support of the organs within cavities.
5. As muscles are dependent upon the circulatory system, they reciprocate by helping to protect blood vessels and assist in maintaining the circulation of the body by forceful contraction of the heart.
6. As muscles help in respiration, they contribute to the provision for oxygen and eliminate carbon dioxide, maintaining the vital acid–base balance of the body.
7. Muscles are agents of the brain by means of which we maintain our independence and give overt expression to our inner thoughts and feelings.
8. They also assist in reacting the threats of danger to our well-being and that of others dear to us.

Rigor Mortis

Rigor mortis is a state of rigidity that develops in the muscles after death. Isolated muscles also develop rigor mortis. While in this condition the muscle (a) loses its excitability, (b) shortens in length, (c) increases in thickness, (d) becomes viscous and loses translucency, (e) becomes distinctly acid (pH 5.8), (f) gradually becomes stiff, (g) glycogen disappears, and (h) the muscles give off carbonic acid. Severe muscular exercise immediately before death hastens the onset of rigor mortis. This is found in soldiers killed in action and also in hunted animals. The time of onset is not the same in all muscles. On the average, rigor mortis starts in the second hour and is completed in three hours after death. The muscles of the body are involved in the following order—lower jaw, face, neck, thorax, abdomen, upper extremities and lastly the lower extremities. Rigor mortis disappears 24 to 36 hours after death due to autolysis. The disappearance also follows the same order. Caffeine, arsenate, chloroform vapour, increased temperature and acidity hasten rigor mortis. Rigor mortis is a state of permanent irreversible contraction and is associated with deficiency of ATP causing establishment of permanent link between actin and myosin. This deficiency of ATP fails to bring about dissociation of actomyosin to actin myosin. It is postulated that during activity creatine phosphate level is associated with the ATP level of the muscle. The creatine phosphate is the high-energy resource of the muscle and its level is dependent upon the ATP. When ATP falls to 85% and creatine phosphate to 30% of its initial value, the symptoms of rigor mortis begin to appear.

Characteristics of skeletal, smooth and cardiac muscles are given in Table 28.1.

Table 28.1: Characteristics of skeletal, smooth and cardiac muscles

Characteristics	*Skeletal*	*Smooth*	*Cardiac*
Histology			
1. Size and shape	Cylindrical; 1–40 mm long, 10 to 100 μm in diameter	Elongated, fusiform, length 0.2 mm (average), width—6 μm (central portion)	Short cylindrical, rectangular in longitudinal section, polyhedral in transverse section
2. Striations	Transverse, longitudinal	Longitudinal (special preparation)	Both longitudinal and transverse
3. Sarcolemma	Present and complete	Present and complete	Present and complete
4. Nucleus	Multiple, just under the sarcolemma	Single at the centre with distinct nucleoli	Single and central
5. Sarcotubular system	Present, T-systems are at junctions of A-I band (in mammals). Terminal cisternae are prominent	Present but not so characterised	Present, but without terminal cisternae. T-system is more prominent and present at Z-line (in mammals)
6. Branch	Nil	Nil	Multiple branches. In all directions—three-dimensional network without cytoplasmic continuity (false syncytial appearance)
7. Cell-to-cell conduction	Nil	Through nexus	Through specialised areas of intercalated use
Properties			
1. Rhythmicity	Nil	Present	Present and characteristic
2. Conductivity	Very fast	Slower	Slower, different in different parts
All-or-none law	True for single fibre	True for single fibre	True for the whole heart, because of functional syncytium
3. Contractility	Simple muscle curve with characteristic features	Slow and worm-like. All periods of the curve longer	Characteristic muscle curve Contraction, longer than relaxation
4. Refractory period	Short—within latent period	Longer than smooth	Longest, whole contraction period is absolute refractory
(a) Tetanus	Possible	Not so	Impossible
(b) Fatigue	Possible	Possible. Difficult to demonstrate	None, long refractory period ensures recovery
5. Tonicity	Tone depends on nerves	Independent of nerve	Independent of nerve

(*Contd.*)

Table 28.1: Characteristics of skeletal, smooth and cardiac muscles (*Contd.*)

Characteristics	*Skeletal*	*Smooth*	*Cardiac*
Composition			
1. Protein	Maximum	Less	Less
2. Glycogen	Less	More	More
3. ATP and phosphagen	Present	Present	Present
4. Carnosine	Maximum	Less	Less
5. Fats	Mostly neutral fats	Mostly neutral fats	More phosphatides and cholesterol than in others
6. Inorganic	Na/K—1/5 Ca^{++}	About same	Na/K—1/2, i.e. more Na, Ca^{++}
Action of ions			
1. Sodium	Excitation	Probably same	Initiates and maintains heartbeat
2. Calcium	Present mostly in sarcoplasmic reticulum and stimulates ATPase activity during muscular contraction	Same as skeletal	Increases strength of contraction and duration of systole
3. Potassium	Reduces excitability and hastens fatigue	Probably same	Inhibits contration and produces relaxation
Metabolism			
1. Carbohydrates			
(a) Lactic acid	Oxidised less easily than glucose and other incomplete	Oxidised less easily than glucose and often incomplete	Completely and more readily than glucose
(b) Glycogen	Reduced in starvation and diabetes mellitus	Reduced in starvation and diabetes mellitus	Increased
2. Blood supply and O_2 consumption	Moderate	Less	High
Distribution	Skeletal	Hollow viscera, capsules, skin, etc.	Only in heart
Control	Under the will, so voluntary	Not so, involuntary	Not so, involuntary
Nerve supply	Somatic with special nerve endings	Autonomic with ganglia and free nerve terminals	Same as in involuntary

EXAM-ORIENTED QUESTIONS

Essay

1. Describe the histological structure of smooth muscle.
2. Discuss the property of smooth muscle.
3. Describe the mechanism of contraction of smooth muscle.

CLINICAL CASE SCENARIO

Nerve Muscle

Q1. A 17-year-old female patient complained of generalized weakness, fatigue, inability to sit in squatting position and drooping of eyelids. Her anti-acetylcholine antibody titer is increased. What is the diagnosis? What are the commonly affected muscles in this disease? Which is the most common risk in such patients?

Ans. The patient is suffering from myasthenia gravis. The muscles most commonly involved are facial, ocular and muscles of swallowing and mastication. The most life-threatening complications in such patients is paralysis of respiratory muscle leading to cardio-respiratory arrest.

Q2. Identify the condition in which there are circulatory antibodies in circular against calcium. Name the muscle which is commonly affected in this disease.

Ans. Lambert-Eaton syndrome. The muscle which is most commonly affected is proximal muscle of lower limb.

Q3. A 25-years-old female suffering from generalized muscular weakness and disability died of heart failure. Her case profile revealed mutation of dystrophin gene. What can be the likely diagnosis? What effects will mutation of dystrophin gene lead to?

Ans. The patient is suffering from X-linked disease Duchenne muscular dystrophy. The mutation of dystrophin gene leads to altered structure of dystrophic glycoprotein complex in muscle. The dystrophin glycoprotein complex aids in strengthening the myofibrils by connecting thin filaments to B dystroglycan in the sarcolemma.

Important Historical Background of Nerve Discovery

1. **Jan Swammerdam (1637–1680)**

He discovered that stroking the innervating nerve of the frog's gastrocnemius muscle generates a contraction.

2. **Alessandro Volta (1745–1827)**

He developed a device that produced electricity and this could be used to stimulate muscles. He invented the electric battery. The term "volt" comes from his name and is in use till date.

3. **Luigi Galvani (1737–1798)**

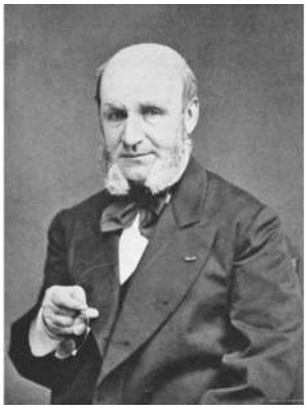
He was an Italian physicist who discovered animal electricity; and is recognized as the pioneer of bioelectromagnetics. He showed that electrical stimulation of muscular tissue produces contraction and force.

4. **Carlo Matteucci (1811–1868)**

He was an Italian physicist and neurophysiologist who pioneered work in bioelectricity showing that

bioelectricity is connected with muscular contraction. He demonstrated the existence of the action potential accompanying a frog's muscle in 1842.

5. **Guillaume Duchenne (1806–1875)**



He was a French neurologist, who was first to describe neuromuscular disorders and also development treatment for them and also created electrodiagnosis and electrotherapy.

6. **Herbert Jasper (1906–1999)**

He constructed the first electromyograph from 1942–1944 at McGill University (Montreal Neurological Institute). He used his instruments to perform groundbreaking work with epilepsy and neurology.

7. **John Basmagian (1931–2008)**

He was a Canadian scientist. He was known for his well known for his work in electromyography and biofeedback. He performed some of the earliest studies using fine-wire EMG (electromyographic) to demonstrate that subjects could voluntarily control even at the single motor unit level and this is controlled by a single neuron in the spinal cord. He authored a book on biofeedback which is well defined text on EMG and biofeedback.

Recent Update: A Newly Discovered Muscle: The Tensor of the Vastus Intermedius

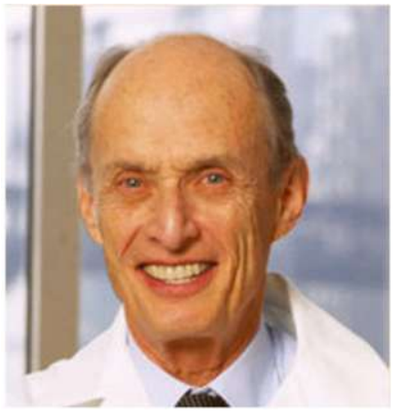
The quadriceps femoris is recognized as a muscle group composing of the rectus femoris and the three vasti. But the clinical experience and investigations of anatomical cadaver descriptions are not consistent with the textbook description. A new muscle referred as second tensor-like muscle between the vastus lateralis (VL) and the vastus intermedius (VI), has been identified and named as the tensor VI (TVI). This study demonstrated that there is an additional muscle belly between the VI and VL, which cannot be clearly grouped or assigned to the former or the latter. Distal exposure shows that this muscle belly becomes its own aponeurosis, which continues distally as part of the quadriceps tendon.

Reference: Gorb K, Acland T, Kuster MS, Manestar M, Filguiera L. A newly discovered muscle: The tensor of the vastus intermedius. Clin Anat 2016; 29(2):256–63.

Recent Advances in Signal Transduction

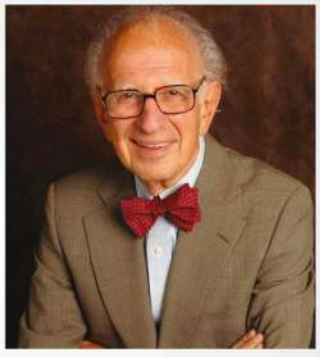
Paul Greengard is a neuroscientist from United States of America and well known for his work on the cellular and molecular function of neurons. He along with Arvid Carlsson and Eric Kandel were awarded the Nobel Prize for Physiology or Medicine in the year 2000 for their discoveries concerning signal transduction in the nervous system.

Greengard and Eric Richard Kandel demonstrated how dopamine interacts with a receptor on the cell membrane of a neuron to cause increase in cyclic AMP, which in turn activates protein kinase A which further



Paul Greengard
1925

Arvid Carlsson
1923



Eric Richard Kandel
1929

activates other protein by phosphorylation. The activated protein carries functions bringing various changes in the cell: Transcribing DNA to make new proteins by moving ion channels to the cell surface increasing the cell's excitability or moving more receptors to the synapse thereby increasing the neuron's sensitivity.

It was Carlsson who developed a method for measuring the amount of dopamine in brain tissues. His research finding revealed that dopamine levels in the basal ganglia were especially high. He administered reserpine drug in animals and demonstrated that there is decrease in dopamine levels and a loss of movement control after the same. These symptoms were similar to those of Parkinson's disease. He could alleviate the symptoms by administering L-dopa to these animals. These findings led to use of L-dopa for treating Parkinson's disease.

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1. Les Prix Nobel. The Nobel Prizes 2000, Editor Tore Frängsmyr, Nobel Foundation: Stockholm: 2001.
2. Barondes, Samuel H. Better than Prozac. New York: Oxford University Press; 2003: 21–22, 39–40.
3. Kandel, Eric R. The Age of Insight: The Quest to Understand the Unconscious in Art, Mind, and Brain, from Vienna 1900 to the Present, New York: Random House; 2012.

Section

IV

Cardiovascular System

Chapter

29

Introduction to Cardiovascular System

INTRODUCTION

The primary function of the cardiovascular system is to provide an adequate supply of oxygen and nutrients to all cells of the body and carry away the waste products of their metabolism. It is a well-organised transport system of the body by which the blood being circulated within a closed system under different pressure gradients, created by the pumping mechanism where heart acts as the central pump (Fig. 29.1).

The cardiovascular system includes (a) heart, (b) arteries, (c) capillaries and (d) veins. They all differ in structures as well as in functions. Blood is in circulation and is carried out to various tissue delivering oxygen and nutrients to them. Blood gets deoxygenated in the tissues and oxygenated in the lungs. Consequently, it has to pass alternately through lungs and tissues, doing opposite functions at these two places. Hence, circulatory system has been divided into two functionally opposite parts:

1. *Systemic circulation* (greater circulation with high resistance circuit)—passing through the tissues.
2. *Pulmonary circulation* (lesser circulation with low resistance circuit)—passing through the lungs. The two systems again meet in the heart (Fig. 29.2).

The main functions of circulation are to make available to the tissues its different metabolic needs and on the other hand to carry away from the tissues the CO_2 and other metabolic waste products for elimination from the body. These are done in two ways: (1) By

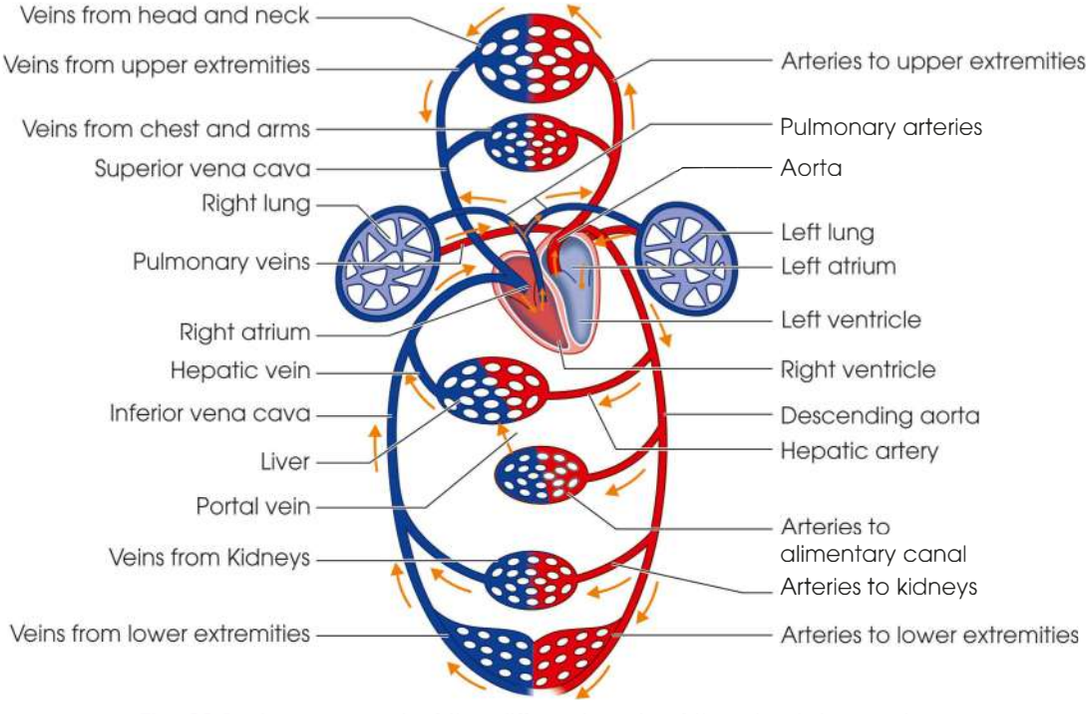


Fig. 29.1: Arrangement of the different parts of the circulatory system

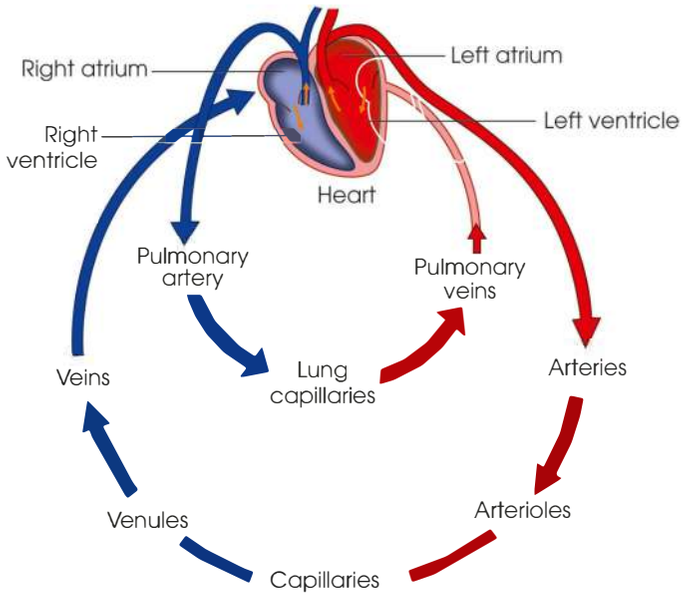


Fig. 29.2: Relationship between systemic and pulmonary circulation

maintaining patent circulation, so that blood is supplied adequately to every part of the body (in rest and activity). (2) By maintaining an optimum blood pressure which is essential for capillary exchange.

ANATOMICAL CONSIDERATIONS OF THE HEART

The heart rests obliquely in the thoracic cavity (Fig. 29.3). The anterior surface of the heart faces the sternum, the posterior surface—the base of the cone faces the vertebral column and the inferior or diaphragmatic surface rests on the diaphragm.

Key Points

1. The heart has got four chambers (Fig. 29.4); two ventricles and two atria: both right and left. The two left chambers are separated from the two right ones, by a continuous partition, the atrial portion of which is called the interatrial septum (fibrous).
2. Atrium is sometimes called auricle. Strictly speaking, the auricles mean the ear-like flaps protruding from the atria, although atria and auricles are often used synonymously.
3. The ventricular part is known as the interventricular septum (upper one-fourth fibrous, lower three-fourths muscular).
   - From the left ventricle arises the aorta, carrying oxygenated blood to the tissues.
   - From the right ventricle, which is less muscular than the left, arises the pulmonary trunk, carrying reduced blood to the lungs.
   - The right atrium receives all the venous blood from the body through three veins; the inferior and the superior venae cavae, and the coronary sinus.
   - The left atrium receives all the oxygenated blood from the lungs through pulmonary veins.
4. Thus, the four chambers of heart perform four different functions. The course of circulation is as follows (Figs 29.1 and 29.2).
   a. The left ventricle propels oxygenated blood to the tissues. Here, it gives up oxygen and becomes reduced. The reduced blood comes back to the heart through the veins and is received by the right atrium.
   b. From the right atrium it passes into the right ventricle, which then propels it into the lungs.

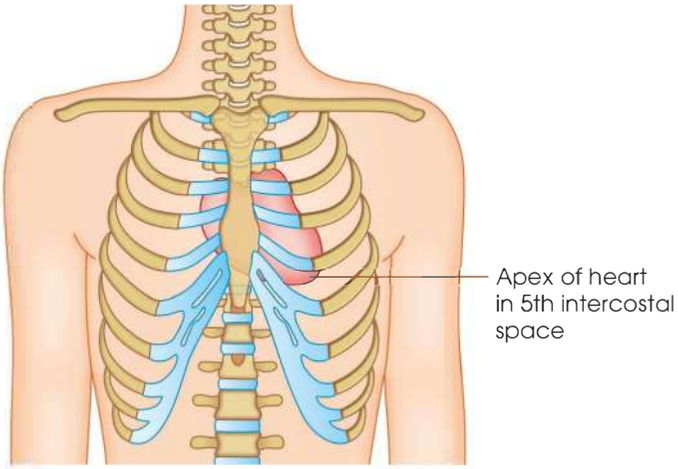


Fig. 29.3: Anatomical position of the heart in the thoracic cavity

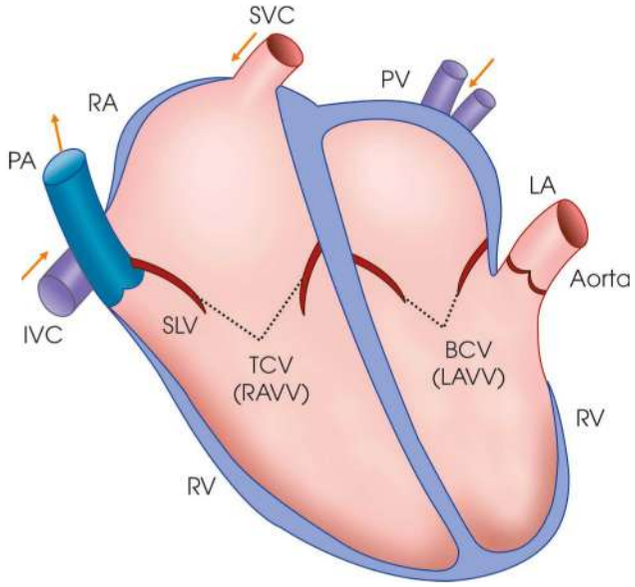


Fig. 29.4: Showing the chambers, valves and vessels of heart TCV (RAVV): Tricuspid valve (right atrioventricular Valve); BCV (LAVV): Bicuspid valve (left atrioventricular valve); RV: Right ventricle; LV: Left ventricle; RA: Right atrium; LA: Left atrium; SVC: Superior vena cava; IVC: Inferior vena cava; PA: Pulmonary artery; PV: Pulmonary vein; SLV: Semilunar valve

Here, it becomes re-oxygenated, and is returned to the left atrium through the pulmonary veins.

c. From here it enters the left ventricle and is pumped out into the greater circulation again. In this way circulation goes on.

5. The systemic circulation, therefore, begins in the left ventricle and ends in the right atrium.
6. The pulmonary circulation starts in the right ventricle and ends in the left atrium (Fig. 29.2). The right half of the heart is concerned with reduced blood, while the left half with oxygenated blood.
7. Two technical terms are used in connection with heart, e.g. systole and diastole. The term systole means contraction and diastole means relaxation.

Valves of the Heart (Figs 29.4 and 29.5)

There should not be any admixture between arterial and venous blood. In other words, circulation must be strictly one way. This is done by the action of valves. There are four sets of valves in the heart.

- The right atrioventricular opening is guarded by tricuspid valve [anterior (infundibular), posterior (marginal) and medial (septal) cusps].
- The left opening is guarded by the mitral (due to its resemblance to a Bishop's mitre) or bicuspid valve [anterior and posterior cusps].
- The openings of the aorta and pulmonary artery are guarded by semilunar valves (three cusps).

Characteristic Features

1. The cusps of tricuspid valve and mitral valve are triangular in shape and are attached at their bases to margins of fibrous connective tissue encircling the atrioventricular orifices. These valves open when the blood passes from the atria to the ventricles. The apices of the valves are projected within the ventricles during flowing of blood into ventricles. But the apices are restricted to bulge within the atrium during ventricular contraction by the presence of chordae tendineae which attach the apical end of the valve and the papillary muscle in the ventricular wall at the other (Fig. 29.6). During ventricular contraction the pressure of blood forces the cusps into apposition and closes the orifice and the chordae tendineae prevent the cusp to go inside the atria or allowing blood to be ejected back into the atria.
2. The term artery means a vessel which carries blood away from the heart. The term vein indicates a vessel carrying blood towards the heart. In the systemic circulation arteries carry oxygenated blood (arterial blood) and the veins carry reduced blood (venous blood). But in the pulmonary circulation their functions are opposite.
3. The larger and stronger aortic semilunar valve guards the orifice between the left ventricle and the systemic aorta, whereas the pulmonary (pulmonic) semilunar valve guards the opening between the right ventricle and the pulmonary trunk.
4. Thicker nodules or corpora arantii are attached at the centre of free margin of each semilunar pocket.
5. Sinuses of Valsalva or aortic sinuses are present between aortic wall and cusps of the valve (Fig. 29.5). During ventricular contraction the intraventricular pressure rises and the push opens the valves. During ventricular relaxation the blood begins to flow back and the cusps are thus kept in apposition and the orifice is closed (Fig. 29.7).

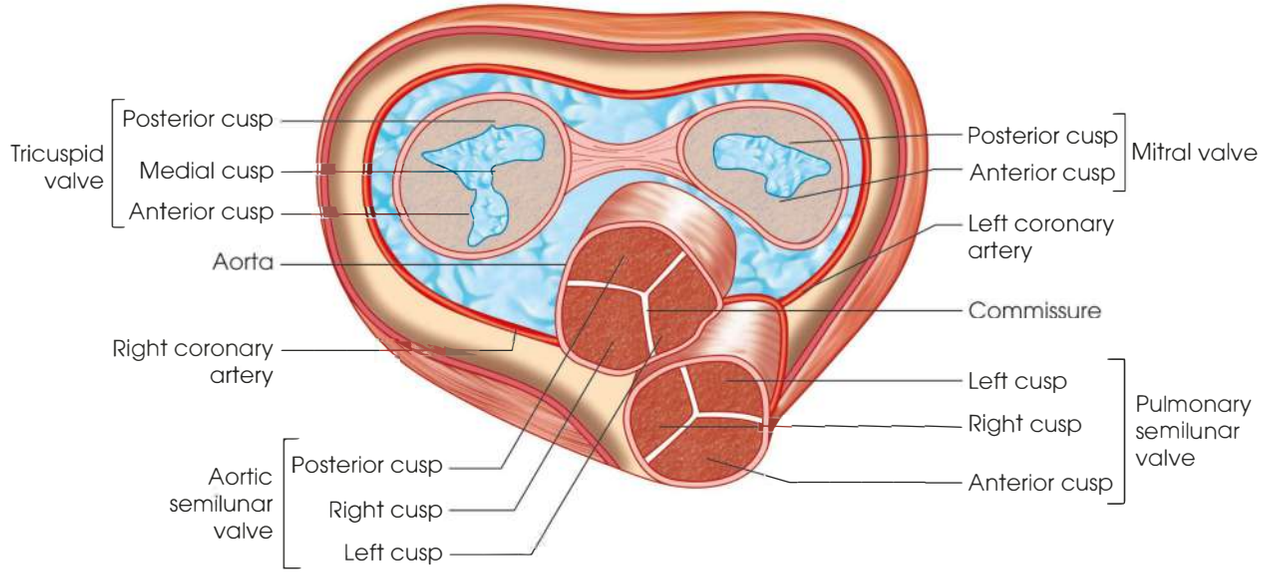


Fig. 29.5: Opened AV valves of the heart in diastole (atria removed) permitting blood flow into ventricles

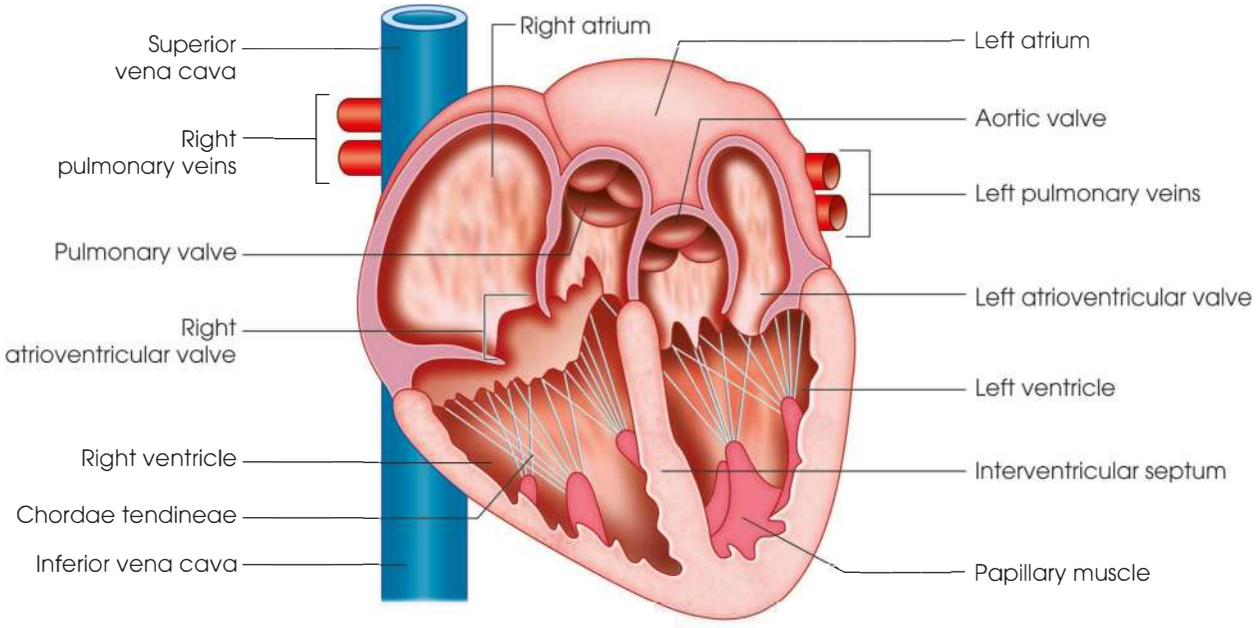


Fig. 29.6: Diagram shows the arrangement of AV valves and their attachment with chordae tendineae and papillary muscles

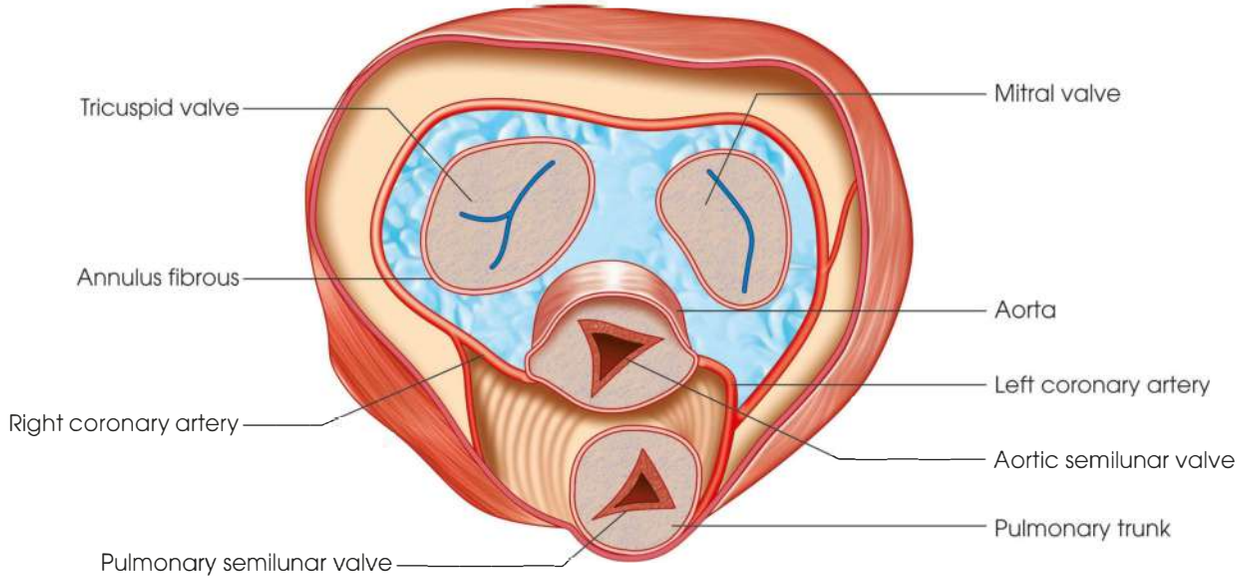


Fig. 29.7: Diagram showing the heart after removal of atria in systole with open semilunar valves

Action of the Valves

Key Points

1. The atrioventricular (AV) valves open towards the ventricles and close towards the atria.
2. The semilunar (SL) valves open away from the ventricles and close towards the ventricles. So that when atria contract, atrioventricular valves open and blood passes into the ventricles (Fig. 29.8). When ventricles contract, atrioventricular valves close, but semilunar valves open. This prevents regurgitation of blood into the atria but allows it to flow out of the ventricles (Fig. 29.9). In this way circulation becomes one way.
3. Heart is a miracle of constants. The two ventricles contract simultaneously, as also the two atria. The same amount of blood passes out of the ventricles at the same time during systole. The same amount of blood enters the heart at the same time during diastole. Any discrepancy in the time or in the quantitative relations may ultimately cause heart failure.

Histology of the Cardiac Muscle

The aorta divides successively into branches of gradually diminishing calibre. It, at first, breaks up into a number of big arteries, each one of which divides into

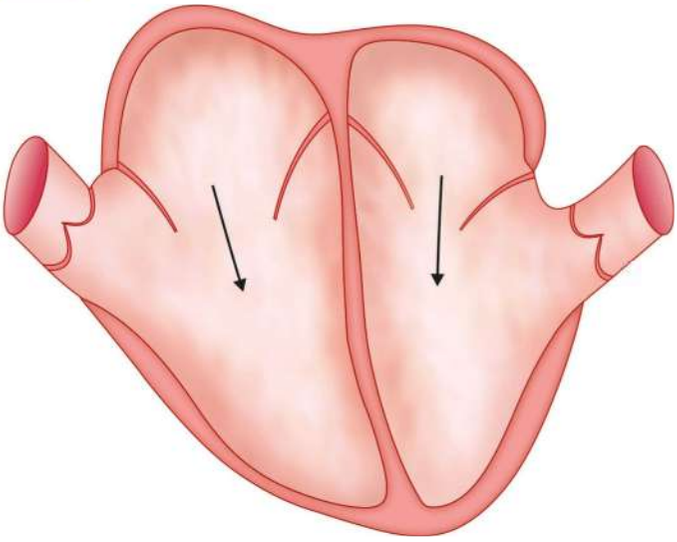


Fig. 29.8: Condition of heart when atria contract. AV valves open and SL valves shuts

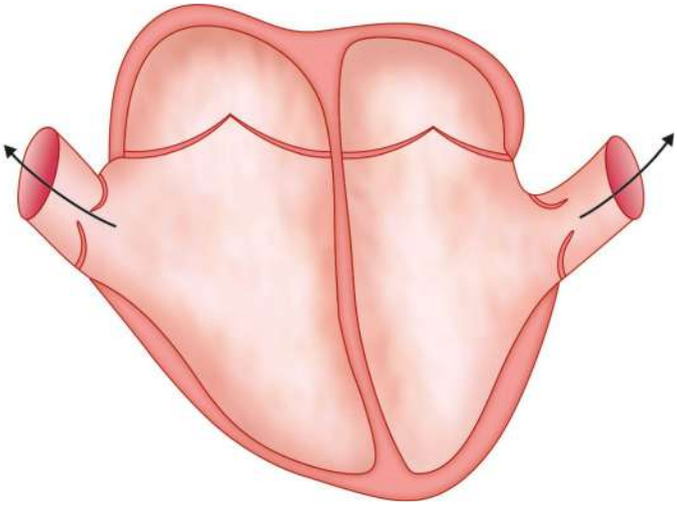


Fig. 29.9: Heart during ventricular contraction: AV valves closed and SL valves open

a number of smaller arteries. Each small artery gives rise to a bunch of arterioles (0.2 mm outside diameter). Blood flows from the arteriole into a metarteriole and then into the capillaries (7–9 µm in diameter). Metarterioles lead into thoroughfare (preferential) channels which lead into venules. The true capillaries which are shorter in length arise from the metarterioles, arterioles and thoroughfare channels (Fig. 29.10). In each capillary there is a precapillary sphincter which is under the control of the sympathetic nervous system. By means of this sphincter blood flow through the capillary may be adjusted.

At each division, the vascular bed enlarges and blood pressure falls—because the total cross-section of the branches is greater than the parent vessel. The capillaries collect into venules, venules into veins and ultimately come back to the heart through the two venae cavae.

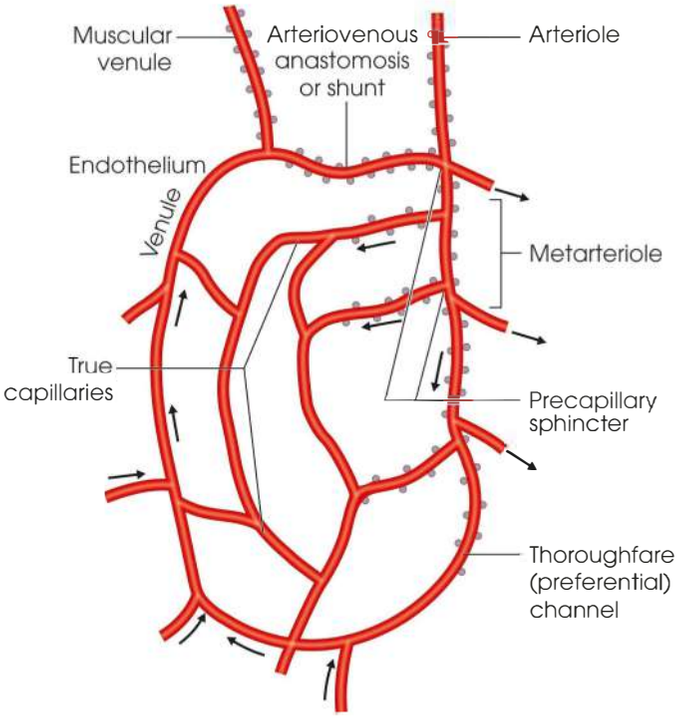


Fig. 29.10: Structural pattern of the capillary bed

The whole circulatory system is built up on the same histological plan. There are three layers; the outer layer is made-up of fibrous tissue (collagen) and elastic tissue (tunica adventitia or externa), the middle layer of plain muscles and a network of elastic fibres (tunica media) and the inner layer of endothelium surrounded by an elastic layer (tunica intima or interna) (Figs 29.11 and 29.12). But all the layers are not found equal proportions in all the vessels (Fig. 29.13). In the heart, tunica adventitia is represented by the fibrous pericardium; the media by cardiac muscle and the intima, by the endocardium.

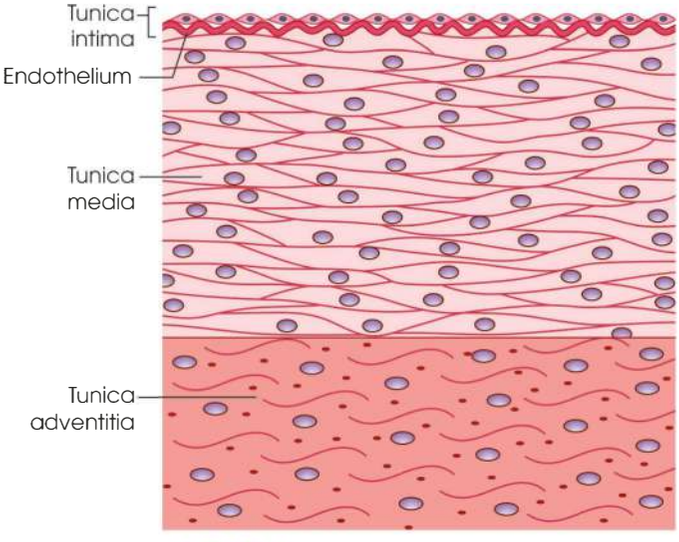


Fig. 29.11: Layers of artery

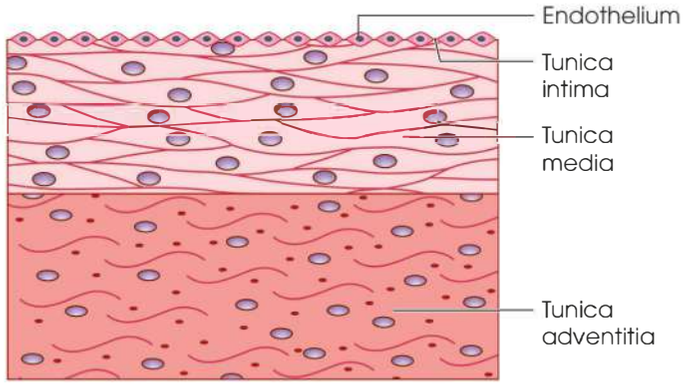


Fig. 29.12: Layers of vein

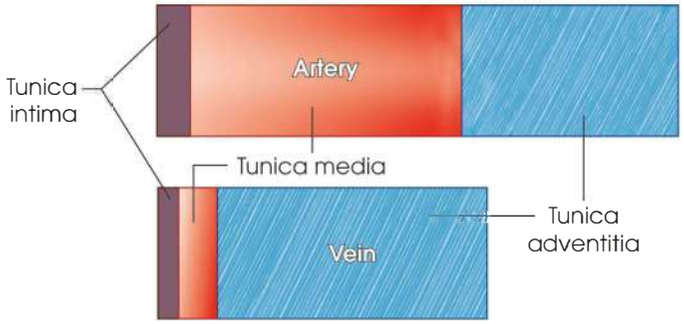


Fig. 29.13: Diagram shows the comparative thickness of different layers in artery and vein

Arteries

1. In the arteries, all the layers (Fig. 29.14) are present. The two outer layers are very thick, because it has to withstand considerable blood pressure.

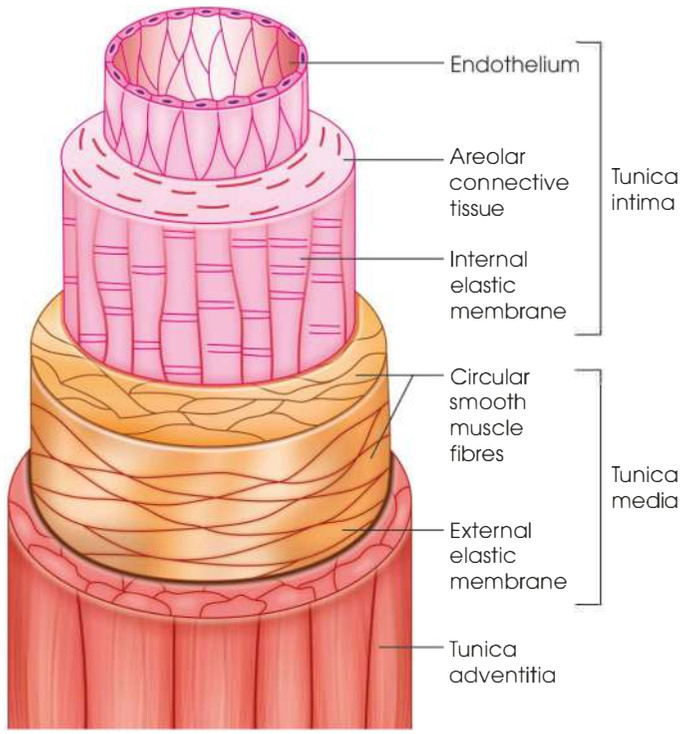


Fig. 29.14: Diagrammatic structure of arterial wall

2. The tunica media consists chiefly of circularly arranged smooth muscle cells.
3. The tunica adventitia is composed chiefly of white fibrous connective tissue that runs parallel to the long axis of the blood vessels and a definite external elastic membrane is present very close to the media. This outer coat is comparatively non-elastic and limits the stretching of the artery and thus prevents undue distension and rupture.
4. The tunica intima consists of a single layer of endothelium, set upon a basement membrane of elastic tissue known as the internal elastic membrane (elastic lamina). Besides these, there is also an inner endothelial lining which is subendothelial layer of delicate fibro-elastic (areolar) connective tissue.
5. A special system of vessels—the vasa vasorum—passes into the arterial wall to supply blood to these layers.

Arterioles

1. The arterioles have got relatively thick walls and narrow lamina. These vessels are rich in vasomotor innervations and are capable of distributing blood to the different portions of the body by vasodilatation and vasoconstriction. Pressure and flow of the vascular systems are principally maintained through modifying the lumen of the arterioles.
2. The arterioles also possess three layers (Fig. 29.15). The tunica intima consists of endothelial lining and internal elastic membrane. But it is devoid of sub-endothelial fibro-elastic connective tissue.
3. The tunica media is composed of one to five layers of muscle cells and contains scattered elastic fibrils.
4. The tunica adventitia is also present and is very thin. It is composed of loose connective tissue. There is no definite external elastic membrane.

Capillaries

These make a connective link in between the arterioles and venules. They are linked by a single layer of flat endothelial cells which are the major component of the wall (Fig. 29.16). The capillary endothelium does not directly touch the elements of other tissues and is always separated from a supporting bed of the connective tissue by an intervening layer—the basal lamina. The average diameter of the capillaries is 7–9 µm and just allowing blood corpuscles to pass through. In resting state the most of the capillaries remain closed and during functional state all of the capillaries mostly open up.

Sinusoids: Sinusoids and sinusoidal capillaries are not true capillaries and they have got relatively large calibre (30 µm) with irregular and tortuous walls. The continuous endothelial lining is absent. There is also some incomplete lining of phagocytic cells. Due to absence of basal lamina, the blood gets direct contact with the tissue cells.

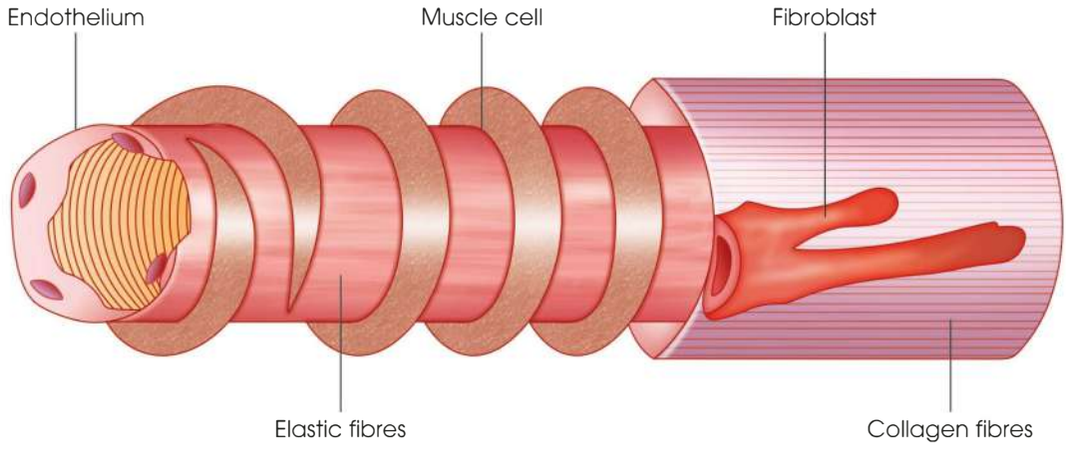


Fig. 29.15: Structure of an arterial wall showing endothelium, muscle cells, elastic fibres, etc.

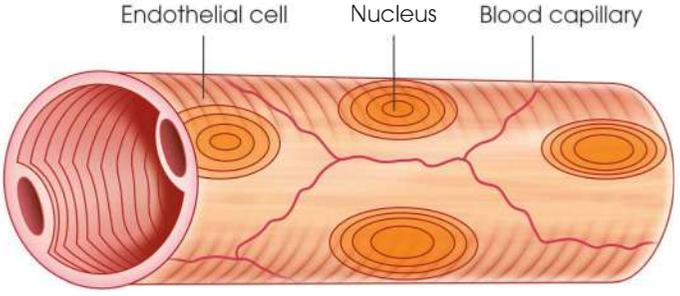


Fig. 29. 16: Arrangement of endothelial cells along the wall of a blood capillary

Veins: In veins all the three layers are present but the intima and media are comparatively thinner than those of arteries and this is only due to reduction of muscular and elastic components. Though the walls are very thin yet the vessels are very strong due to presence of connective tissue components.

In tunica intima, the endothelial cells are less elongated than those of the arteries. A little connective tissue along with a few fine elastic fibres is present. In tunica media, there is a little elastic tissue and muscle. There are also considerable collagen fibres. The tunica adventitia is much developed and much thicker than the tunica media due to presence of muscle, collagen and elastic fibres. Structures of the veins all throughout are not same and differ from one place to another. Some veins do not possess smooth muscle. These are the cerebral veins, meningeal veins, retinal veins, etc.

Valves of the Veins

Valves are present in most of the veins particularly of those of the lower limb. These valves prevent backflow from the heart. These are semilunar pocket like flaps (Fig. 29.17) formed by the local folding of the intima.

Blood Vessels (Vasa Vasorum)

Large arteries and veins of diameter above 0.1 mm are generally supplied with nutrient blood vessels which arise from the adjacent small arteries. In large arteries

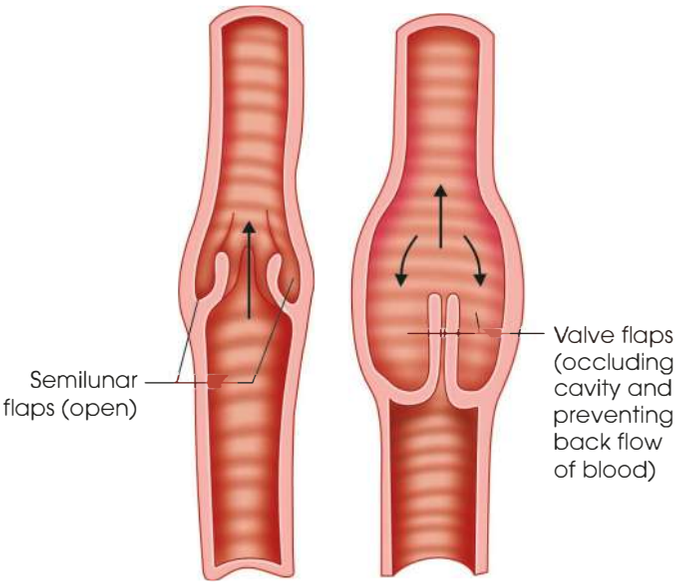


Fig. 29.17: Diagram depicts the action of valves

the vasa vasorum penetrates or may penetrate all the layers of the blood vessels and ends ultimately with the lumen of the artery. In true sense, the vasa vasorum actually supplies the tunica adventitia and the external part of the tunica media.

Lymphatics of Blood Vessels

Lymphatic vessels are also present abundantly in the adventitial and peri-adventitial tissue.

Factors that Maintain Circulation

1. ***Pumping action of the heart:*** This is the main motive force of circulation.
2. ***Elastic recoil of the arteries:*** Ventricles contract during systole and relax during diastole. So during systole certain amount of blood is being led directly into the aorta and thus the vessel is stretched (potential energy is gained). But due to elasticity of blood vessel wall, the aorta recoils during diastole of the ventricle and thus blood moves forward (due

to change of potential energy into kinetic energy for flow) towards the periphery as the backward movement is restricted due to presence of semilunar valves.

3. ***Pressure gradient:*** Blood pressure gradually falls from the left to the right side of the heart. In the big arteries the average pressure is 120 mm of Hg; in the arterioles the pressure sharply falls to about 50–60 mm of Hg; in the capillaries the pressure is about 15 mm of Hg; in the veins the pressure falls further, while near the heart the pressure is 0 mm of Hg or even negative. Due to this pressure gradient blood passes from the higher to the lower pressure, i.e. from the left to the right side of the heart.
4. ***Respiration:*** During inspiration intrathoracic pressure falls and intra-abdominal pressure rises. Hence, with each inspiration, venous blood is sucked up by the thorax and is pumped out by the abdomen.

   In this way respiration helps venous return and acts as a great force in maintaining circulation.
5. ***Muscular exercise:*** When muscles contract, they squeeze the capillaries and veins, and thus help venous return. This is aided by the valves of veins, which prevent the passage of blood back towards the capillary bed (Fig. 29.18).
6. ***Effect of gravity:*** Above the level of heart, it helps venous return. But below the level of heart, it works against it.

Special Junctional Tissues

Cardiac muscle consists essentially of certain specialised structures which are responsible for initiation and transmission of cardiac impulses at a higher rate than the rest of the muscle. Those specialised cardiac tissues operate such mechanism are collectively known as the junctional tissues of heart (Fig. 29.19). They comprise the following structures; (a) Sino-atrial (SA) node, (b) Atrioventricular (AV) node, (c) bundle of His (atrioventricular bundle), (d) the right and left branches of the bundle ending, (e) Purkinje fibres.

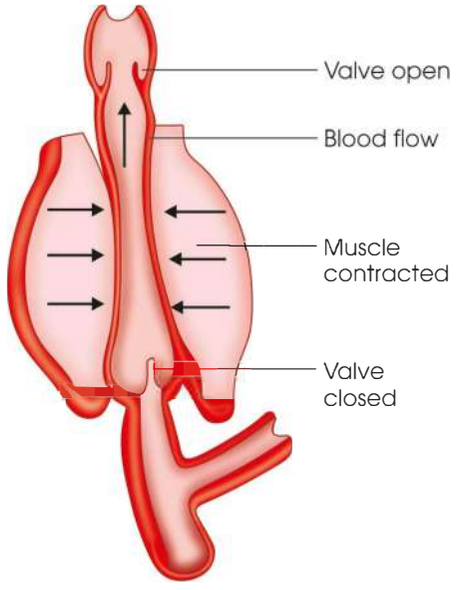


Fig. 29.18: Showing the action of valves of a vein during muscular exercise

Histology of the cardiac muscle has been already described earlier. The sino-atrial and atrioventricular nodes and bundle of His are composed of specialised cardiac tissue (Fig. 29.20) and contain high amount of glycogen. These have got more sarcoplasm than the rest of the cardiac muscle fibres. Purkinje fibres also contain high amount of glycogen in their sarcoplasm. The atrial muscle fibre is connected with the ventricular muscle fibre only through the bundle of His because a fibrous tissue ring keeps the atrial muscle separated from the ventricular muscle. Damage of bundle of His causes dissociation of atrial and ventricular rhythm.

Sino-atrial Node (Keith and Flack, 1907)

Sino-atrial node is situated in the right atrium at the junction of superior vena cava and the right auricular appendage. It extends downwards along the sulcus terminalis for about 2 cm (three-fourths of an inch). It is broader at the top and tapering below, and measures about 5 × 20 mm.

Goldman (1970) has described that there are three internodal atrial pathways originating from the SA node go to the AV nodal region (Fig. 29.21).

These internodal tracts contain Purkinje type of fibres.

1. The anterior internodal tract after coming out from the SA node curves round the superior vena cava and anterior wall of the right atrium. Here it bifurcates into two branches, one of which goes to the left atrium and other goes to the anterior superior region of the AV node.
2. The middle internodal tract and posterior internodal tract after coming out from the SA node curve behind the superior vena cava and end in the superior margin and posterior margin of the AV node respectively.

Functions

It generates the normal cardiac impulse at the rate of 70 to 80 per minute in the adult and acts as the pacemaker of heart and the rhythm originated from this region is generally designated as sinus rhythm.

Atrioventricular Node (Tawara, 1906)

Atrioventricular node is situated in the right atrium at the posterior part of the interatrial septum close to the opening of the coronary sinus. It measures about 2 × 5 mm. The presence of the atrioventricular node was first identified by Kent in 1892 and afterwards His in 1893 described a band of modified muscle fibres to course from the atrium to the ventricle.

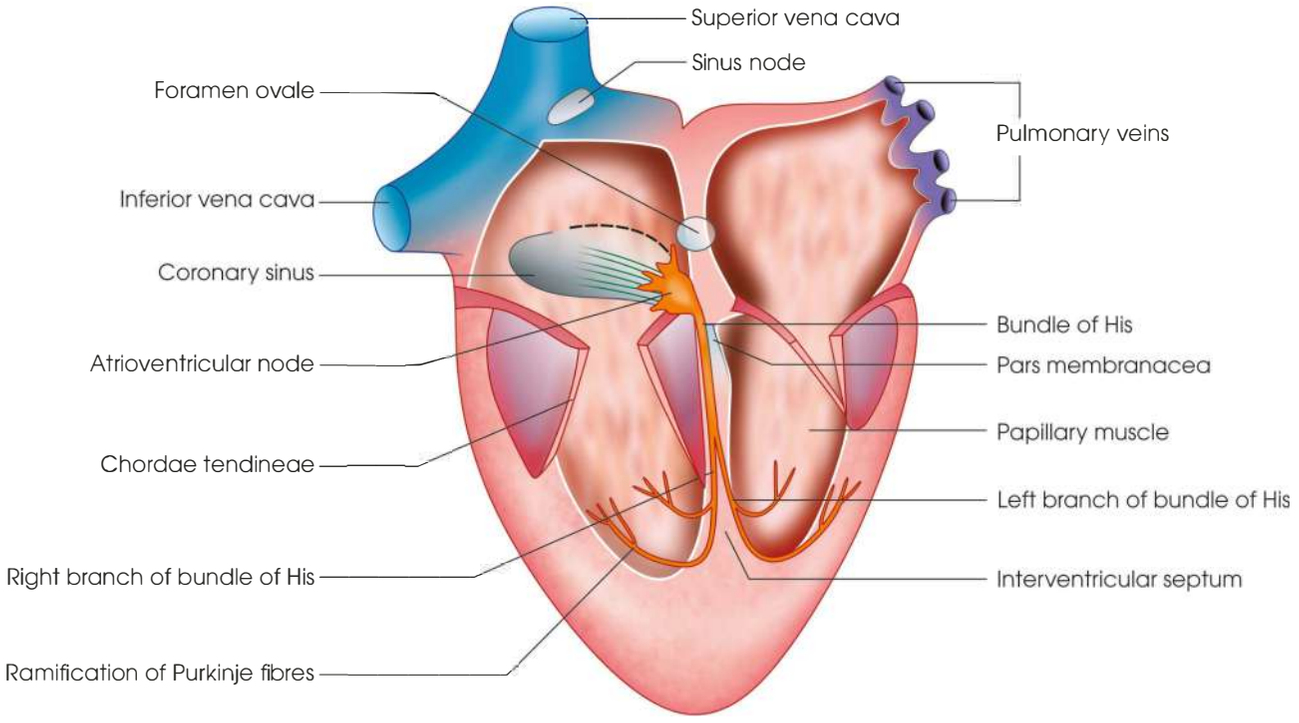


Fig. 29.19: Specialised nodal tissues and conducting tissues in human heart

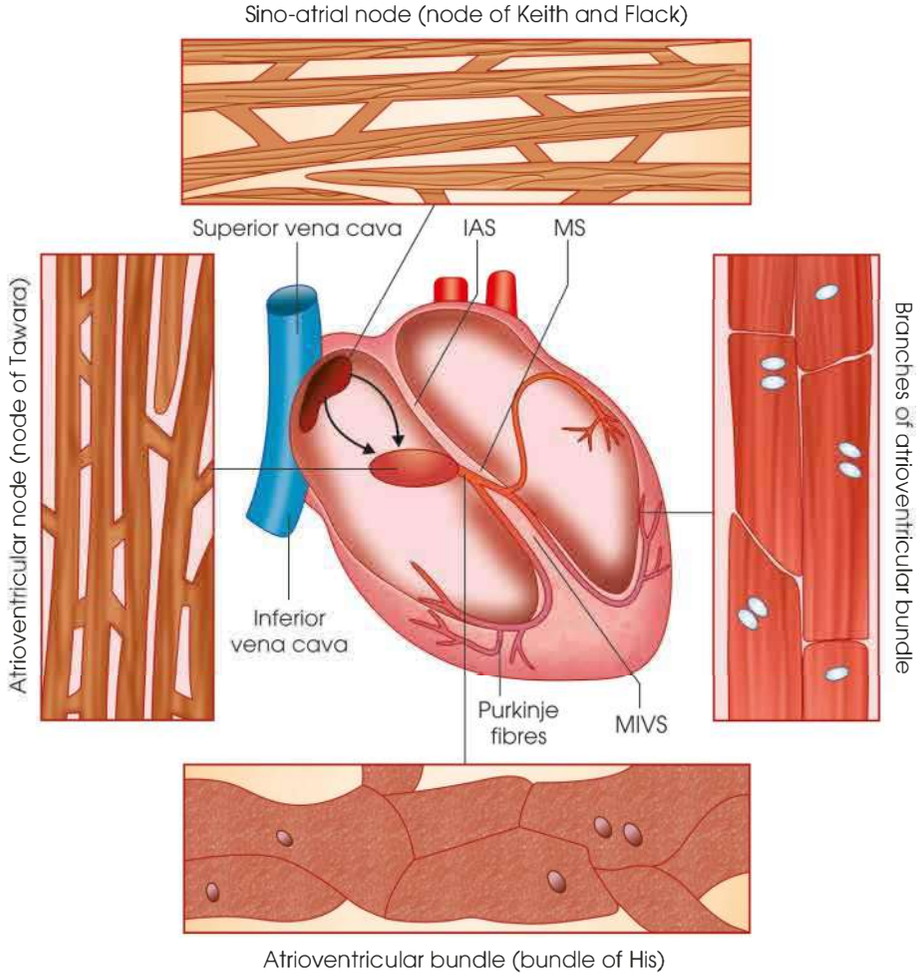


Fig. 29.20: Specialised cardiac tissues. IAS: Inter-atrial septum, MS: Membranous septum, MIVS: Muscular interventricular septum

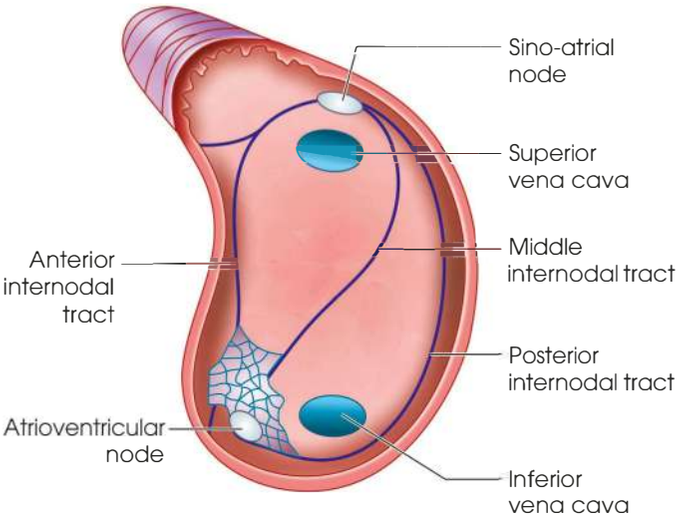


Fig. 29.21: Diagram shows the internodal pathways of impulse—conducting systems from SA node to AV node

Functions

a. It receives the impulse originating from the SA node and transmits it to the ventricles through the bundle of His.
b. It acts as reserve pacemaker. The rhythm that is originated in the AV node is known as nodal rhythm.
c. It also initiates the cardiac impulse, but at a slower rate (40 to 60 per minute). In abnormal conditions, when the SA node fails, the AV node generates the impulse (nodal rhythm).

Bundle of His

The main trunk of this bundle is continuous with the AV node and passes upwards until it reaches the posterior margin of the membranous part of the interventricular septum and then forwards below it. It measures about 20 mm long.

Bundle Branch

Just above the muscular part of the septum, the bundle divides into right and left branches. The right bundle branch is longer than the left one. The left bundle branch bifurcates into superior and inferior divisions. It pierces the membranous septum, enters the left ventricle and passes along the muscular septum towards the apex. The left branch ends in the Purkinje systems of the ventricular subendocardial tissue (Fig. 29.22). The right branch passes down the right side of the septum. These branches remain just under the endocardium. They are finally distributed through the terminal arborisations of a special type of cardiac muscle fibres, known as the Purkinje fibres. The main bundle (bundle of His), its right and left branches and the finer ramifications of the latter remain ensheathed in a special connective tissue covering and thus separated from the surrounding cardiac muscles.

Functions

a. *Conduction:* The normal function of bundle branch is to conduct the atrial impulse into the ventricles.

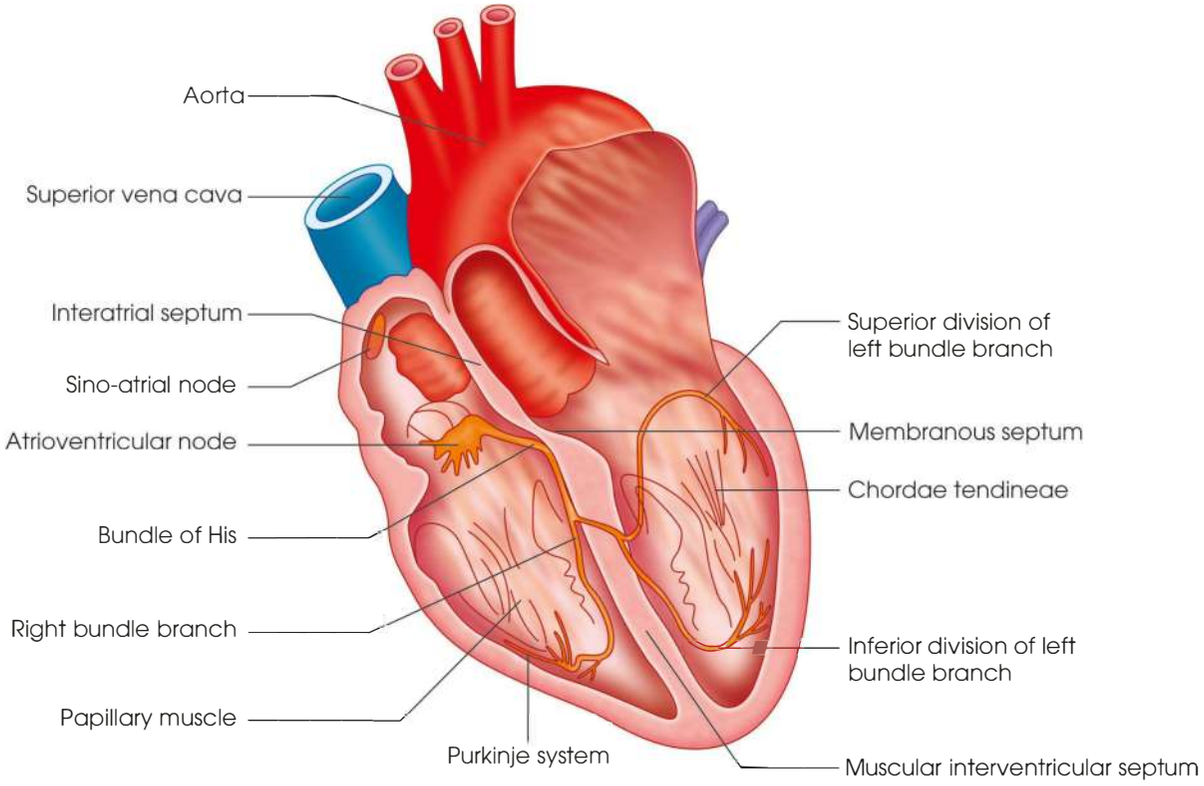


Fig. 29.22: Conducting system of heart muscle

b. *Rhythmicity:* When the SA and AV nodes fail, the bundle can originate cardiac impulse. But the rate is very slow, about 36 per minute.

Purkinje Fibres

The purkinje fibres which arise from the branches of the bundle of His, spread from the interventricular septum directly to the papillary muscle and then to the lateral walls of the ventricle ending ultimately within the subendocardial network. Purkinje (1845) first observed the presence of these fibres in the subendocardial tissue of the ungulate heart. Purkinje fibres have got a larger diameter (50 to 70 µm) than the ordinary cardiac muscle fibre (15 µm). It also contains relatively more sarcoplasm with large amount of glycogen. Myofibrils in the fibre are present mostly in the periphery of cells and the central space is occupied by glycogen.

Functions

Main function of these fibres is to conduct impulse quickly to every part of the ventricular muscle fibre. These fibres also can initiate impulse (30–35 per min) in case of atrioventricular dissociation.

"I am obliged to conclude that in animals the blood is driven round a circuit with an unceasing, circular sort of movement, that this is an activity or function of the heart which it carries out by virtue of its pulsation, and that in sum it constitutes the sole reason for the heart's pulsatile movement".

William Harvey
1578–1657

EXAM-ORIENTED QUESTIONS

Essay

1. Describe the conducting system of the heart.

Short Notes

1. Functions of Sino-atrial Node
2. Functions of Atrioventricular Node
3. Functions of Bundle Branch
4. Functions of Purkinje Fibres
5. Valves of the heart

Chapter

30

Initiation and Spread of Cardiac Impulse

ORIGIN OF THE HEARTBEAT

Initiation of Impulse and Localisation of Pacemaker

By separating artria from the ventricles of excised hearts, William Harvey in 1628 had shown that the artial rhythm was higher than the ventricular rhythm. Keith and Flack (1907) have described that from the SA node the heart beat first starts. If the impulse conduction from this area is cut off by giving a Stannius ligature between the sinus and artrium of the frog heart (first Stannius ligature), the heart beat does not cease but a new slower rhythm dovelops within a few seconds from the new pacemaker area. This indicates that the SA node (sinus) is not the only pacemaker; there is other pacemaker area-the AV node which can maintain the beat in its absence. If second ligature is applied in the same heart between the atrium and ventricle then ventricle begins to beat at a more, slower rate indicating that ventricle can function as pacemaker in the absence of SA node or AV node. Now it shows that the three separate components of the heart beat at descending order of frequency are represented by the sequence; → sinus → atrium → ventricle. In normal heart the SA node controls the rest of heart muscle by its higher rhythmical activities.

This classical experiment, described above cannot be possible in mammalian heart because, SA node and AV node are both present in the atrium. So by simple ligature the SA node cannot be separated from the AV node by first stannous ligature. Ventricle however can be separated from the atrium by the second Stannius ligature (Fig. 30.1).

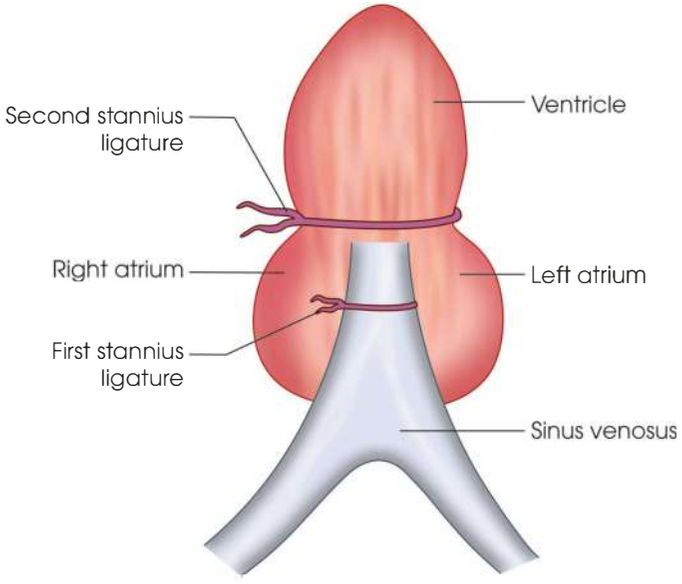


Fig. 30.1: Diagram showing the location of first and second stannius ligatures in the frog heart

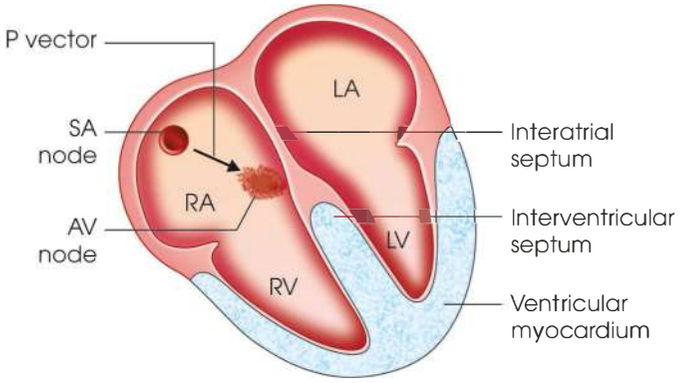


Fig. 30.2: Diagrammatic representation of atrial activation showing the transmission of impulse

Conduction Over Atrial Muscle

- Cardiac impulse originated at the SA node is transmitted over both the atria like concentric waves and thus the P wave is produced in ECG (electrocardiogram).
- The spread of electrical impulse through the SA node is very slow (0.05 m per sec) but the same through the junctional tissues that connect the node to the atrial musculature or to the AV node is higher (1 m per sec).
- The impulses from the SA node are transmitted directly to the AV nodes through the internodal atrial bundle (Fig. 30.2).

Conduction Over AV Node

- There is also a considerable delay of 0.07 to 0.1 sec in transmission of impulse in the AV node before excitation spreads over the ventricle. This AV nodal delay allows the atrial systole to complete before the ventricle is excited.
- This delay is observed maximally at the junctional region between the atrium and atrioventricular node. The conduction velocity of impulse at this region is approximately 0.05 m per sec. The delay is however minimised by sympathetic activity and the same is increased by vagal stimulation.
- Besides nodal delay in the AV node, the impulse is transmitted through this region in one direction only because if the ventricle is stimulated, the impulse fails to reach the AV nodal region in retrograde fashion.

Conduction Over Bundle of His and the Right and Left Bundle Branches

Beyond the atrioventricular region, the impulse is transmitted along the bundle branch at a higher velocity (4–5 m per sec). The impulse from the bundle of His passes quickly through the right and left bundle branches and ultimately reaches the Purkinje fibres and ventricular muscle fibres as well.

Conduction through Purkinje Systems

The impulse, after passing through the right and left bundle branches, passes into the Purkinje fibres and also its multiple ramifications within the subendocardial surfaces of both ventricles. The impulse then travels from the endocardium to the epicardium of ventricular muscle perpendicularly.

Conduction through Ventricular Muscle

In human beings, the mid-portion of the interventricular septum is activated normally in a left to right direction. So, the depolarisation of the ventricular muscle begins at the left side of the interventricular septum because the Purkinje fibres arise more proximally from the left bundle branch than from the right bundle branch and activates the left side of the septum initially. After mid-septal activation from the left to the right direction (Fig. 30.3), the impulse comes down the septum to the apex of the heart and next portions of myocardium that is activated is the anteroseptal region of the ventricular myocardium (Fig. 30.4). The impulse then proceeds to basal portion of the heart.

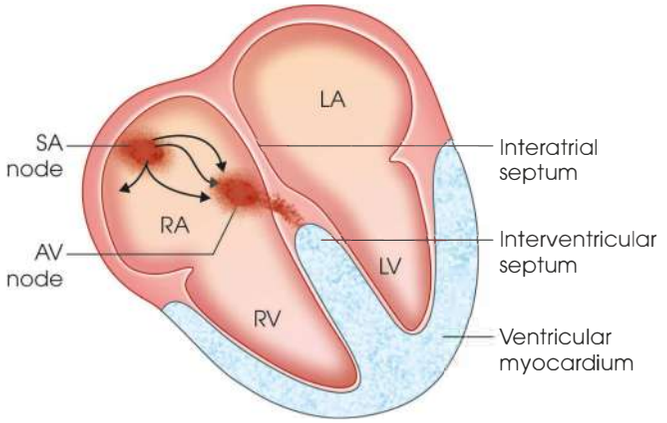


Fig. 30.3: Showing activation of maximal portion of left and right ventricular myocardium

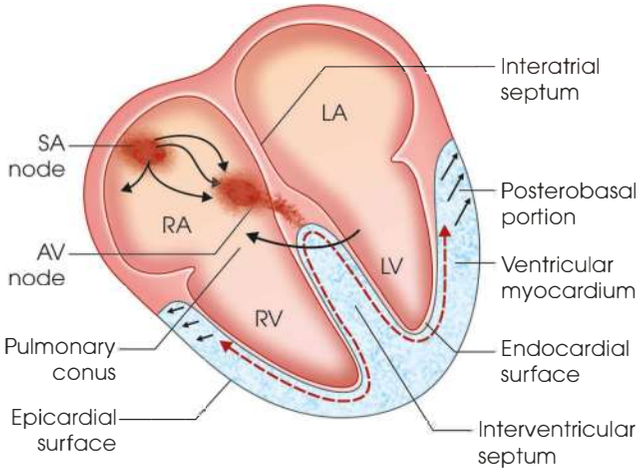


Fig. 30.4: Late activation of posterobasal region of left ventricle, uppermost region of interventricular septum and pulmonic conus

HEART BLOCK

Defective production of the sino-atrial impulse or its conduction in the heart is called heart block. There may be four main types according to site of damage.

1. **Sinoatrial nodal block:** The SA node may fail to generate the impulse occasionally, so that the whole heart misses one beat. This is called sinoatrial nodal block.
2. **Atrioventricular nodal block:** The impulse may be normally generated but its transmission into the ventricles is faulty. The defect lies in the AV node or in the main bundle before division or in both. This is called atrioventricular nodal block.

   This may be of three grades: (a) The impulse is transmitted but is merely delayed. The P-R interval in ECG is prolonged. (b) The impulse occasionally fails to reach the ventricles, so that the atria contract but the ventricles do not. This type of block is often found to have some regular rhythm, viz. every 2nd or 3rd or 4th impulse is conducted. So that for every two or three or four atrial beats there will be one ventricular beat. In this way 2:1, 3:1, 4:1, etc. block is produced. (c) The sino-atrial impulse does not reach the ventricles at all, so that atria and ventricles dissociate and beat at their own rhythms—atria at the rate of 60, ventricles about 36 per minute. The pulse rate at ventricular rhythm will be 36 per minute. This is called idioventricular rhythm.

The first two grades are often called incomplete heart block; the third grade complete heart block.

3. **Bundle branch block:** One branch of the bundle may be defective producing either right or left bundle branch block. The ventricle on the normal side will contract a little earlier than the other one producing reduplication of the first sound. The QRS duration is prolonged.
4. **Fascicular block:** The block in the anterior or posterior fascicles of left bundle branch produces hemi block which is called fascicular block.

EXAM-ORIENTED QUESTIONS

Essay

1. Discuss the physiological functioning of initiation and spread of cardiac impulse.

Short Notes

1. Heart block
2. Atrioventricular nodal block
3. Complete heart block
4. Fascicular block

Chapter

31

Properties of Cardiac Muscle

INTRODUCTION

The properties present in other muscle are also shown by the cardiac muscle.

Cardiac muscle tissue **consists** interlocking cardiac muscle cells, or fibres, which impart cardiac muscle and its properties. The cardiac muscle fibres are striated and consist of a single nucleus. The light and dark bands are observed under the microscope. The dark bands are the thick protein filaments made of myosin proteins and thin filaments are made of actin protein. The myosin of cardiac muscle is a hexamer consisting of two myosin heavy chains (MHC), each associated with two myosin light chains (MLC). In cardiac muscles, there are multiple isoforms in each subunit, and is a determinant of force-generating ability of cardiac myosin by modulating cross-bridge kinetics producing contraction.

Cardiac muscle exhibits certain special features. The properties of cardiac muscle are rhythmicity, excitability, conductivity, contractility, all-or-none response, staircase phenomenon, refractory period, tonicity and functional syncytium. They are briefly summarised below.

1. Rhythmicity

The cardiac muscle has its own rhythmicity. The cardiac muscles are self excitable.

One of the main characteristic features of the cardiac muscle is that it can initiate its own impulse rhythmically. They undergo contraction on excitability. This inherent rhythmical property is present throughout the cardiac muscle and is evident from the SA node (Fig. 31.1), AV node, atrial muscle, Purkinje fibre and from damaged the ventricular muscle fibre.

The rate of rhythmicity in the SA node is 70 to 80 per minute, in AV node 40 to 60 per minute, in atrium 60 per minute, in ventricle 20 to 40 per minute. Due to higher rhythmical property of the SA node, it controls the rest of cardiac muscle and thus heartbeats at the rhythm of the SA node. When the SA node fails, the AV node takes the charge and if it fails, the atrium and afterwards ventricle take the charge of maintaining heartbeat.

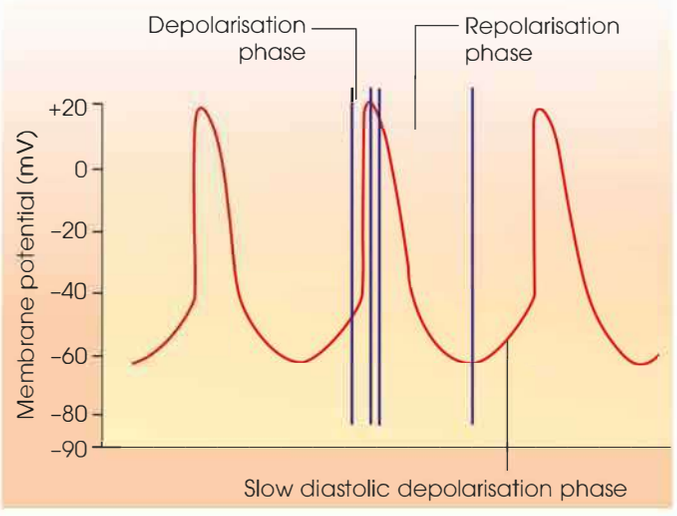


Fig. 31.1: Transmembrane action potential recorded from the single fibre of the SA node

2. Excitability

On excitability the cardiac muscle generates action potential.

Cardiac Action Potentials

The cardiac muscles exhibit two types of action potentials, these are fast action potentials (Fig. 31.2) and occur in atrial and ventricular muscles and Purkinje fibres while slow response action potential occurs in sino-atrial node and atrioventricular (AV) node (Fig. 31.3). Cardiac action potential of atrial and ventricular muscles and Purkinje fibres has a true resting potential, a fast depolarization phase, and a prolonged plateau phase.

The ionic conductance (Fig. 31.2) which are responsible for various phases are as below:

Phase 0: Rapid depolarization and overshoot: The hundred-fold opening of voltage-gated sodium

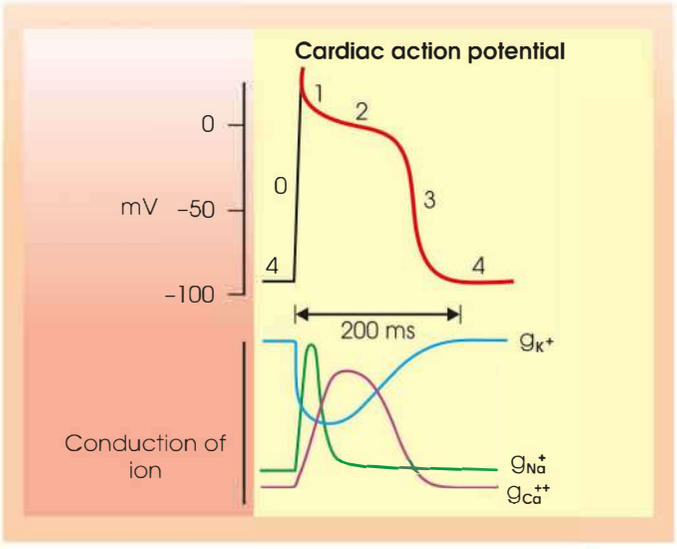


Fig. 31.2: Cardiac action potential

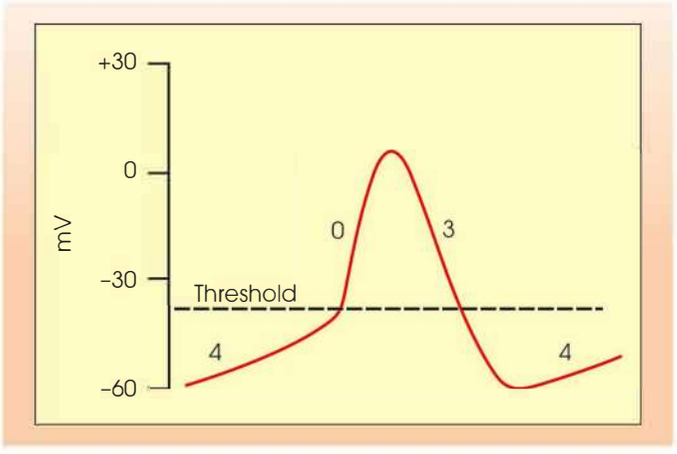


Fig. 31.3: Pacemaker action potential (SA node)

channels increases the influx of Na^+ ions. And this occurs when membrane potential is –60 mV. The opened sodium channel further activates the opening of the other voltage-gated sodium channels by process of auto-activation. This is short-lived and accompanied with marked increase in Ca^{++} permeability at –30 to –40 mV. The membrane potential reaches to peak at +20 to + 30 mV with positivity inside the cell.

Phase 1: Initial rapid repolarization: Decreased Na^+ and increased K^+ conductance. There is inactivation of the fast Na^+ channels. The sodium influx ceases. The outward transient rectifying K^+ channels opens leading to efflux of potassium ions.

Phase 2: Plateau phase: The increased Ca^{++} conductance due to slow and prolonged opening of calcium channel leads to plateau phase. There is efflux of potassium ions through the slow delayed rectifier K^+ channel. The influx of calcium into the cell is balanced by the efflux of potassium to the exterior of the cell, resulting in the plateau in action potential graph recording.

Phase 3: Repolarization: This phase is produced due to increased K^+ and decreased Ca^{++} conductance. The closure of L-type Ca^{2+} channels prevents calcium influx. The outward rectifying K^+ channels open increasing potassium permeability and more potassium moves outside.

Phase 4: Resting potential: It denotes the membrane potential when the cell is not being stimulated. This phase is observed as a horizontal line in non-nodal tissue action potential. This phase is produced due to increased K^+ and decreased Na^+ and Ca^{++} conductance. The opening of the inward rectifying K^+ channels restores membrane permeability to potassium ions and thereby reinstating resting membrane potential.

Pacemaker Action Potential

Phase 0: It occurs due to opening of T-type voltage-gated calcium channel (transient low voltage activated calcium channel) which further activates opening of L-type voltage-gated calcium channels and the depolarization produce is due to influx of calcium. This phase is less steep.

Phase 1 and **phase 2** are absent in nodal action potential.

Phase 3: Repolarization: The voltage-gated potassium channel opens leading to increased potassium efflux while calcium channel closes down.

Phase 4: Diastolic depolarization: The early part of this phase is due to decreased potassium conductance as the potassium channels close. The opening of the transient low voltage calcium channel contributes towards development of diastolic depolarization.

The spontaneous diastolic depolarization after every action potential phase is responsible for automaticity of SA node. The diastolic membrane potential (resting potential) which depolarizes forms the prepotential. This prepotential which triggers action potential is the pacemaker potential.

Effect of sympathetic and parasympathetic stimulation and role of drugs on nodal action potential

1. *Sympathetic influence on pacemaker potential:* Any activity which will produce activation of sympathetic system releases norepinephrine and also increases calcium ion permeability in cardiac muscle fibres due to opening of voltage-gated L-type calcium channel. This increases the cardiac muscle excitability by the increase rate of conduction of impulse eventually leading to increased contractibility of cardiac muscles.
2. *Parasympathetic activity* releases acetylcholine. This acetylcholine binds to M2 muscarinic receptors, and via the G protein, produces opening of potassium channels. The increases potassium efflux, which hyperpolarizes the cell. This hyperpolarization decreases the firing rate of SA node and there by decreases the heart rate and rate of transmission of cardiac impulse.

3. *Drugs*
   a. *Calcium-channel blockers* reduce the slope of phase 4, decreases the rate of spontaneous depolarization, thus decreasing the rate of pacemaker firing. These drugs also decrease the slope of phase 0, thus slowing the conduction velocity within the AV node.
   b. *Potassium-channel blockers:* They mainly delay phase 3 of the repolarization thus lengthening the duration of action potential.

3. Conduction

The impulse originated at the SA node spreads over the atria and reaches the AV node through the internodal fibres. The AV node transmits the impulse through the bundle of His and its branches to the ventricles. From the apex of the heart through the Purkinje fibres the impulse is conducted to the base. Conduction in the bundle of His and the Purkinje fibres is 1 metre per second, still less in the ventricular muscles 0.4 metre per second and least in the SA node 0.05 metre per second and AV node 0.1 metre per second.

4. Contraction

Like other muscles, the cardiac muscle is excitable by adequate stimuli and responds by contraction. The fundamental contractile unit of the cardiac muscle is myofibril which contains the protein units, actin and myosin. During contraction these two units are associated in presence of ATP and thus the fibre is shortened, but during rest these are dissociated again with the re-synthesis of ATP. Myosin itself is an enzyme 'ATPase' capable of dephosphorylation of ATP. Ca^{++} ion activates the ATPase activity-favouring prompt association of actin–myosin and ADP complex. Excess calcium always keeps the muscle unit in contracting state (calcium rigor) due to association of more contractile units. K^+ ions do not favour association of actin and myosin. So if excess K^+ is added in the extracellular fluid then the heart muscle gradually stops in diastole.

5. All-or-none Response

If a quiescent heart muscle is stimulated at widely spaced electrical shocks of increasing strength then muscle contracts as a whole only when the threshold strength is reached. But there was no such increasing amplitude of contraction with increasing intensities of stimulation. This was observed by Bowditch (1871). Single skeletal muscle fibre behaves like this but if the entire muscle is stimulated with graded intensities of stimuli then graded responses are encountered.

Thus, to conclude the muscle does not respond to sub-threshold stimuli and when strength of stimuli is up to threshold, it contracts maximally.

6. Staircase Phenomenon

In a stannius preparation if the ventricular muscle is stimulated with inducted current, the first few contractions gradually increase in size and then it becomes steady. This is known as treppe or staircase phenomenon.

Key Points

a. This staircase phenomenon is only observed in quiescent heart but not in active normal heart. Bowditch (1871) has described that contractions of ventricular muscle following a stoppage are weaker, but the heart regains its full strength gradually in the subsequent contractions, which on the record form a kind of staircase. In other words, every contraction leaves a condition more favourable than it found, but this favourable condition deteriorates in time.
b. This favourable condition following a contraction is known as beneficial effect and is due to increase calcium ion availability and increased temperature; while visco-elastic properties or the accumulation of metabolic products have got a little role on the staircase phenomena.

7. Refractory Period

This is another characteristic property of the heart muscle. The refractory period of the heart is long and can be divided into three parts:

a. *Absolute refractory period:* This period extends throughout the whole period of contraction. Any stimulus, however strong, will fail to elicit a response if it falls within this period. For this reason, heart muscle cannot be tetanised. This long refractory period ensures enough time for recovery of the cardiac muscle. This is the reason why cardiac muscle

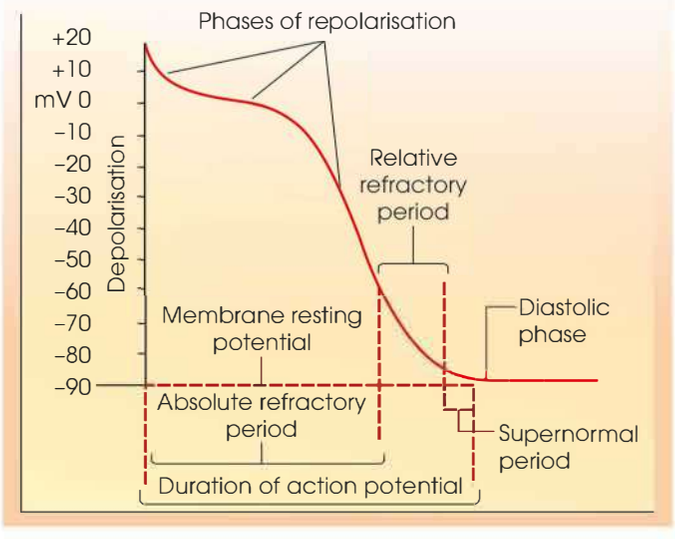


Fig. 31.4: Diagrammatic tracing showing the different phases of transmembrane potential, refractory periods of a single ventricular muscle fibre following excitation

Table 31.1: Different properties of cardiac muscle fibres

Fibres	*Size of fibre*	*Glycogen content*	*Rate of conduction (metre)*	*Length of systole (refractory period)*	*Rhythmicity*
SA nodal	Fine	Present	0.05 per sec	Highest	Highest
AV nodal	More or less similar to SA node	Present	0.1 per sec	Lower than SA node	Lower than SA node
Atrial	Broader than ventricular	Higher than ventricular	1.0 per sec	Lower than ventricular	Lower than ventricular
Ventricular	Broader	Higher than nodal	0.4 per sec	Lower than nodal	Lower than nodal
Purkinje	Broadest	Same as that of atrial	1.0 per sec	Same as that of atrial	Same as that of atrial

cannot be fatigued. This period coincides the period from the onset of depolarisation phase to the repolarisation up to the threshold potential (Fig. 31.4).

b. *Relative refractory period:* This starts immediately after the absolute refractory period and involves the first part of relaxation. Only a very strong stimulus will be effective. This period begins when the transmembrane potential during repolarisation phase has just reached the threshold potential (–60 mV) and ends just before the repolarisation phase is ceased.

c. *Supernormal period:* There is another type of refractory period observed after the relative refractory period which is known as supernormal period. This period is limited from the point of termination of repolarisation to the beginning of slow diastolic repolarisation phase.

The refractory period is longest in the AV node, intermediate in the ventricles and least in the atria. Drugs like digitalis and quinidine prolong the absolute refractory period. Stimulation of the vagus reduces the systole and as such, diminishes the refractory period. The length of the refractory period is directly proportional to the duration of systole and inversely to that of the diastole of the heart. Hence, it will depend upon the heart rate. For rates up to 100 per minute, the absolute refractory period is about 0.2 second.

8. Tone

Heart muscle possesses tone. This tone is independent of nerves and can be adjusted. In this way, it can maintain a fairly constant tension upon its varying contents.

It has been observed that the different properties of cardiac muscles (Table 31.1) are not developed in all the tissues of heart in the same order. Certain properties have developed specially in certain tissues while others are not. It is also seen that these functions are related to the size and chemical composition of the muscle cells.

Hence, cardiac muscle can be divided into four groups:

1. The smallest fibres with least glycogen at the nodes.
2. The broader fibres with more glycogen in the ventricles.
3. The still broader fibres with more glycogen in the atria.
4. The broadest fibres with abundant glycogen in the Purkinje fibres, bundle of His and its branches.

As the size of the fibres increases, the rate of conduction and the glycogen content also increase. But the duration of systole, the refractory period and the rhythmicity increase in the reverse order.

9. Functional Syncytium

The individual muscle fibres of heart are interconnected via gap junction and electrical potential can spread through the cardiac muscle easily without any resistance. Thereby cardiac muscle acts as a functional syncytium unit.

EXAM-ORIENTED QUESTIONS

Essay

1. Discuss the properties of cardiac muscle.
2. Describe the effect of sympathetic and parasympathetic stimulation and role of drugs on nodal action potential.

Short Notes

1. Rhythmicity
2. Conductivity
3. Refractory period
4. All-or-none law
5. Excitability of cardiac muscle
6. Tone of cardiac muscle

Chapter

32

Cardiac Cycle

INTRODUCTION

Changes that occur in the heart during one beat are repeated in the same order in the next beat. This cyclical repetition of the various changes in heart, from beat to beat, is called cardiac cycle.

Cardiac Cycle Time

This is the time required for one complete cardiac cycle. With the normal heart rate of 75 per minute, this time will be 60/75 = 0.8 second. It means that every event in the cycle will be repeated at the interval of 0.8 second. It is obvious that the cardiac cycle time will be inversely proportional to the heart rate.

Interrelations of the Various Events in the Cardiac Cycle

In the cardiac cycle there are four main events:

1. Atrial systole
2. Atrial diastole
3. Ventricular systole
4. Ventricular diastole.

All the other changes are subsidiary to them.

Atrial systole: Atrial systole initiates the cycle, because the pacemaker SA node is situated in it. It lasts for 0.1 seccond, and is followed by atrial diastole, lasting for 0.7 second. At the end of diastole, the atrial systole returns, and in this way, the atrial cycle goes on (total duration 0.8 second).

Atrial diastole: Atrial systole is followed by atrial diastole and its duration is 0.7 sec.

Ventricular systole: At the end of atrial systole, ventricular systole starts and its duration is 0.3 second.

Ventricular diastole: This is immediately followed by ventricular diastole and its duration is 0.5 second. At the end of diastole, ventricular systole repeats and thus the ventricular cycle goes on (total duration 0.8 second).

In order to follow the march of events during the cardiac cycle and their interrelations, Fig. 32.1 should be carefully studied. In it, there are two concentric rings, divided into eight equal parts. The whole circle represents one complete cardiac cycle, so that each of its eight divisions represents 0.1 second.

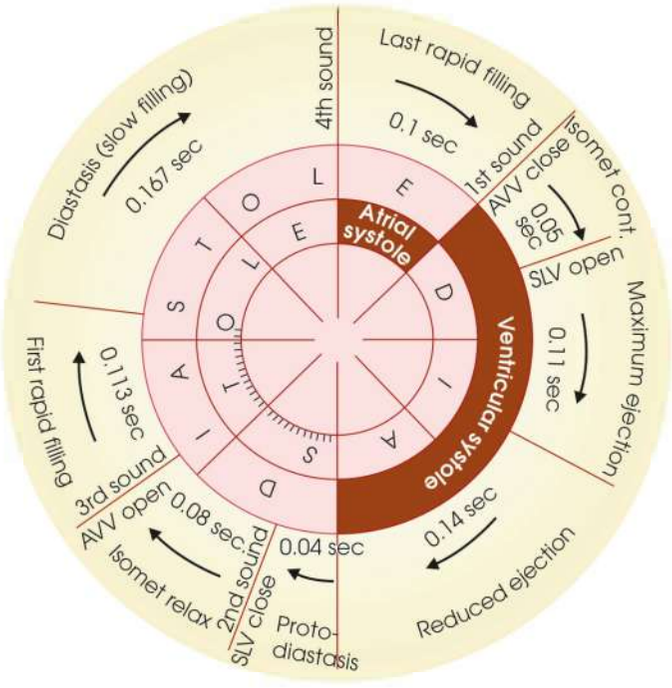


Fig. 32.1: Sequence of events during cardiac cycle

The Inner Ring Represents the Atrial Events and the Outer Ring Represents Ventricular Events

Let us follow inner ring first

- **Atrial systole:** The one shaded division in it denotes atrial systole (0.1 second). During this period the atria contract and expel their contents into the respective ventricles. The left atrium, being further away from the SA node, contracts a little after the right atrium. But practically their contractions are simultaneous. The force of contraction is stronger in the first half than in the second. Because during first half or at

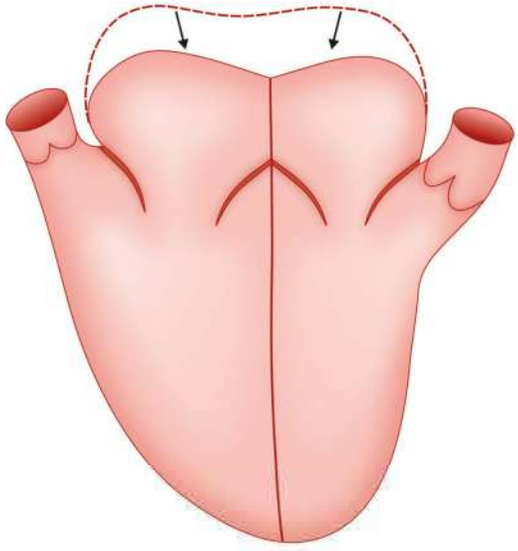


Fig. 32.2: Heart, during atrial systole. Atria shorten, AVV—open, SLV—closed

initial stage the intra-atrial pressure remains high and during last half the same is decreased due to expulsion of blood to the ventricle (Fig. 32.2).

- **Atrial diastole:** After atrial systole, comes its atrial diastole (0.7 second) being represented by seven unshaded divisions in the ring. During this period the atria relax and receive blood from the great veins—the right atrium from the venae cavae, the left atrium from the pulmonary veins. At the end of this period, the atrial systole comes again and in this way, the atrial events go on.

Let us now follow the Ventricular Events in the Outer Ring

There are three shaded divisions on it, representing ventricular systole (0.3 second). It is followed by five unshaded divisions, indicating ventricular diastole (0.5 second).

- **Ventricular systole:** On comparing the two rings, it would be found that ventricular systole commences at the end of atrial systole. The reason for this is very clear. The impulse originating at the SA node will certainly overtake the atrium first, and then it will travel down the junctional tissues, enter the ventricles and stimulate their contraction. Naturally then, ventricular systole will always come after atrial systole.*

From these interrelations we can deduce one fundamental rule of cardiac action that the systoles of atrium and ventricle will never overlap. In other words, when one chamber is contracting, the other must be relaxing.

1. At the onset of ventricular systole, the first sound occurs. It is caused by the sudden closure of the AV valves due to sharp rise of intraventricular pressure.
2. The semilunar valves open a little later, because, until the intraventricular pressure goes above that in the aorta and pulmonary artery, the semilunar valves will not open. Thus, at the beginning of ventricular systole, there is a brief period during which both the valves are closed and the ventricles are contracting as closed cavities (Fig. 32.3). No blood passes out and therefore, no shortening of the cardiac muscle will occur. Hence, this period is called isometric contraction period (0.05 second). It is marked at the onset by the closure of the AV valves (e.g. the first sound) and at the termination by the opening of the semilunar valves.
3. At the end of this period, the semilunar valves open and the ejection period starts (0.25 second). During this period, blood is expelled from the ventricles—from the left ventricle into the systemic aorta, from the right into the pulmonary trunk. In the first part of this period (0.11 second) the outflow is very rapid. Hence, it is known as the maximum ejection period (Fig. 32.4). In the last part (0.14 second) the rate of outflow slows down. Hence, it is called the reduced ejection period (Fig. 32.5). Here, the ventricular systole ends and diastole begins.

Let us follow the Outer Ring Further

It will be seen that after the three shaded divisions, come the five clear divisions—representing the duration of ventricular diastole (0.5 second).

- **Ventricular diastole:** As soon as ventricles relax, the intraventricular pressure starts falling. The blood columns in the aorta and pulmonary trunk try to roll back towards ventricles but are stopped by the sharp closure of the semilunar valves. This produces the second sound of heart. Thus, the onset of ventricular systole is marked by the first sound and its termination by the second sound (approximately).

Key Points

On comparing the two rings, it will be seen that the last one division (0.1 second) of ventricular diastole is overlapped by atrial systole. In other words, when atria are contracting, the ventricles are still in diastole and are having the last part of their filling. It will be seen further that the first four divisions of ventricular diastole coincide with the corresponding four divisions of the atrial diastole. From this we can come to another

*The time relations are so adjusted that the atrioventricular conduction time of the impulse (P-R interval of electrocardiogram) is a little longer (0.16 second) than the duration of atrial systole (0.1 second). This explains why ventricular systole must always come after atrial systole normally.

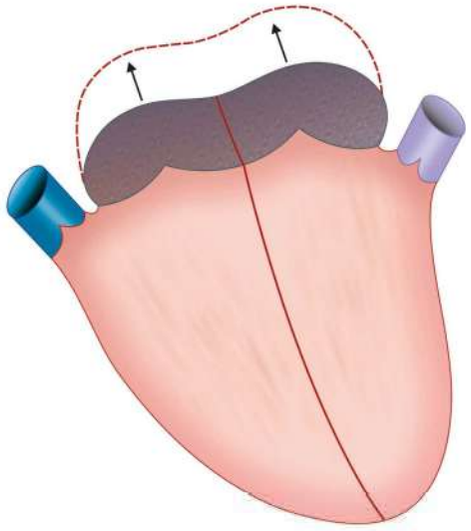


Fig. 32.3: Heart, during isometric contraction phase. Atria relaxing. Both valves closed. No shortening of ventricles

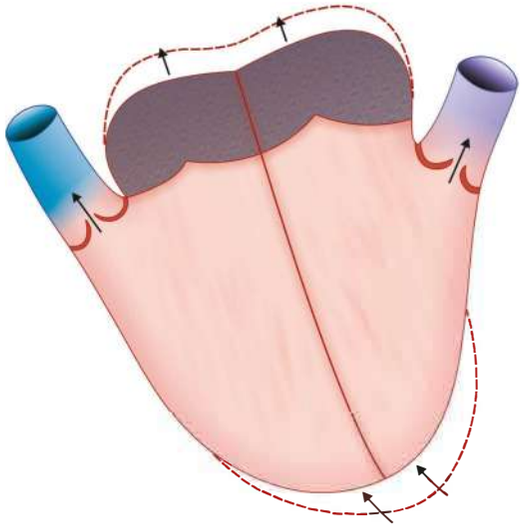


Fig. 32.4: Heart, during maximum ejection period. AVV—closed. SLV—open. Atria relaxing. Ventricles shortening

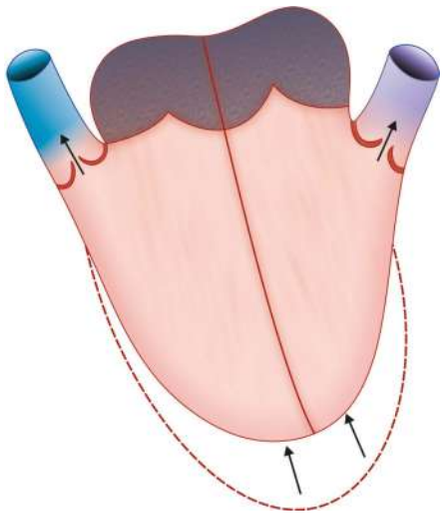


Fig. 32.5: Heart, during reduced ejection period. AVV—closed, i.e. SLV—open. Atria—full. Ventricles much shortening

fundamental rule of cardiac action that—the diastole of the two chambers will always partly overlap. In the left half of the unshaded division will be found (Fig. 32.1). In other words, both the chambers are in diastole here. This is called the diastole of the whole heart (0.4 second).

Let us Again follow the Ventricular Diastole on the Outer Ring

1. As mentioned above, the second sound occurs at the end of ventricular systole. But this statement is not exact, because, till the falling of intraventricular pressure goes below the intra-aortic pressure, the semilunar valves will not close. Consequently, there will be a short interval between the onset of diastole and the closure of the semilunar valves (i.e. the second sound). This period is called the protodiastolic period (0.04 second). From this it is clear that the second sound does not occur just at the end of ventricular systole but a little afterwards (i.e. after the protodiastolic period).
2. Although the semilunar valves have closed, yet the AV valves are still not open. Because, the falling intraventricular pressure takes a little time to go below that of the atria, so that the AV valves may open. Consequently, there will be a brief interval during which both the valves remain closed and ventricles are relaxing as closed cavities. Since no blood enters the ventricles there will be no lengthening of cardiac muscle fibres. Owing to this, it is called the isometric relaxation period (0.08 second Fig. 32.6).
3. At the end of isometric relaxation period, the AV valves open. Blood rushes into the ventricles and ventricular filling begins. The first part of this period is known as the first rapid filling phase (0.113 second). Because, as soon as the AV valves open, blood

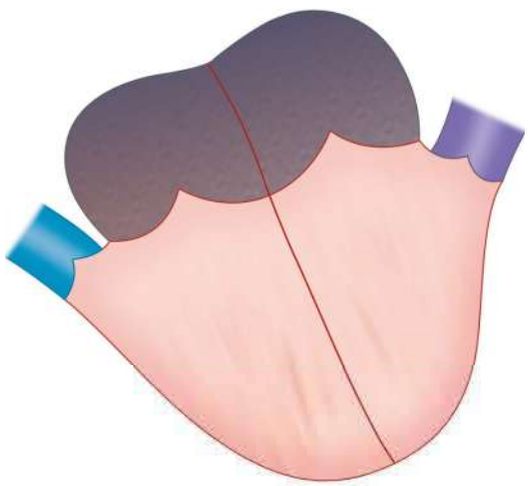


Fig. 32.6: Heart, during isometric relaxation period. Both valves closed. Ventricles relaxing. No blood entering, hence no lengthening of ventricles

accumulating so long in the atria, rushes into the ventricles. The steep fall of the intraventricular pressure during the isometric relaxation period, make the inflow all the more intense. Although the duration is brief yet the largest part of ventricular filling takes place during it. Due to rapid rush of blood a sound is produced, known as the third sound of heart (Fig. 32.7).

4. In the next phase of ventricular diastole, the rate of filling slows down. The ventricles are already full to a large extent and ventricular pressure slowly rises. Consequently, the rate of inflow from the atria will be gradually slower. This period is called diastasis or slow inflow phase (0.167 second). Although this is the longest phase of ventricular diastole, yet the amount of filling during this period is minimum. If one looks into the heart (Fig. 32.8) during this time, one will find that, the whole atrioventricular canal contains a continuous column of blood, more or less stagnant, in which the cusps of the AV valves are passively floating.
5. After this period comes the last part of ventricular diastole represented by the last unshaded division on the outer ring. It is obvious that this phase corresponds with atrial systole. Due to atiral contraction, blood rushes into the ventricles and ventricular filling again becomes rapid. This phase—the last rapid filling phase (0.1 second) is responsible for the last part of ventricular filling. Due to rapid rush of blood, again a sound is produced—known as the fourth sound of heart. Thus, the onset of filling period is marked by the third sound and its termination by the fourth sound. Here the ventricular diastole ends. They are completely filled up, the impulse from the SA node arrives in the mean time and the ventricles plunge into systole again. Thus, the cycle goes on.

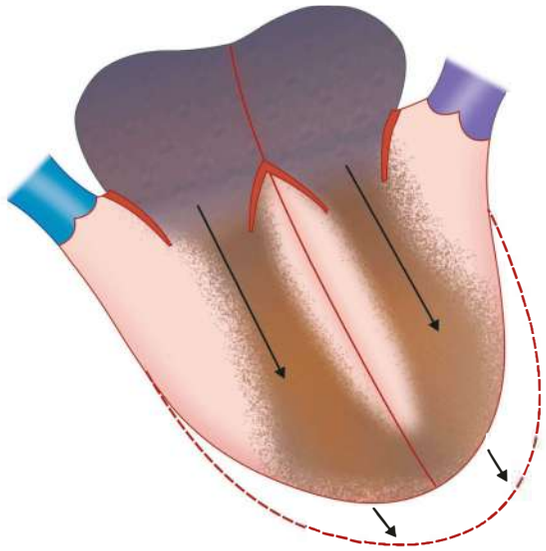


Fig. 32.7: Heart, during first rapid filling phase. AVV—open. Blood rushing into the ventricles relaxing and lengthening. SLV—closed

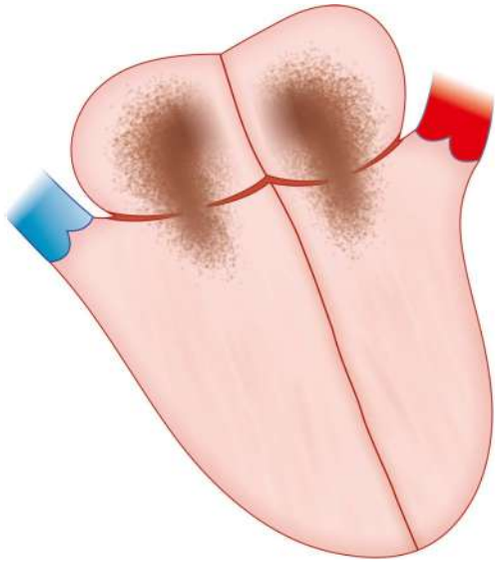


Fig. 32.8: Heart, during diastasis. AVV—floating in neutral position. SLV—closed. Both atira and ventricles filled up

Summary of the Sequence of Events in Cardiac Cycle

1. The atrial systole is the first event (0.1 second). It initiates the cardiac cycle, because the pacemaker SA node is situated here. Due to higher atrial pressure, the first half of atrial systole is stronger than that of the last half.
2. After systole comes the atrial diastole (0.7 second). These two alternately follow each other and constitute the atrial cycle (0.8 second).
3. Just after the atrial systole, the ventricular systole (0.3 second) begins and is immediately followed by its diastole (0.5 second). These two events repeat alternately and make up the ventricular cycle (0.8 second).
4. At the onset of ventricular systole, the AV valves close producing the first sound. The semilunar valves open a little later. The interval between the closing of the AV valves and opening of the semilunar valves is called the isometric contraction period (0.05 second). During this period ventricles contract as closed cavities and intraventricular pressure steeply rises.
5. At the beginning of ventricular diastole, the semilunar valves close producing the second sound. There is a brief interval between the beginning of diastole and the closure of the semilunar valves—known as protodiastolic period (0.04 second). So that, second sound occurs actually after this period.
6. The AV valves open a little after the closing of the semilunar valves. The interval between these two is called the isometric relaxation period (0.08 second). During this period ventricle relax as closed cavities and intraventricular pressure steeply falls.

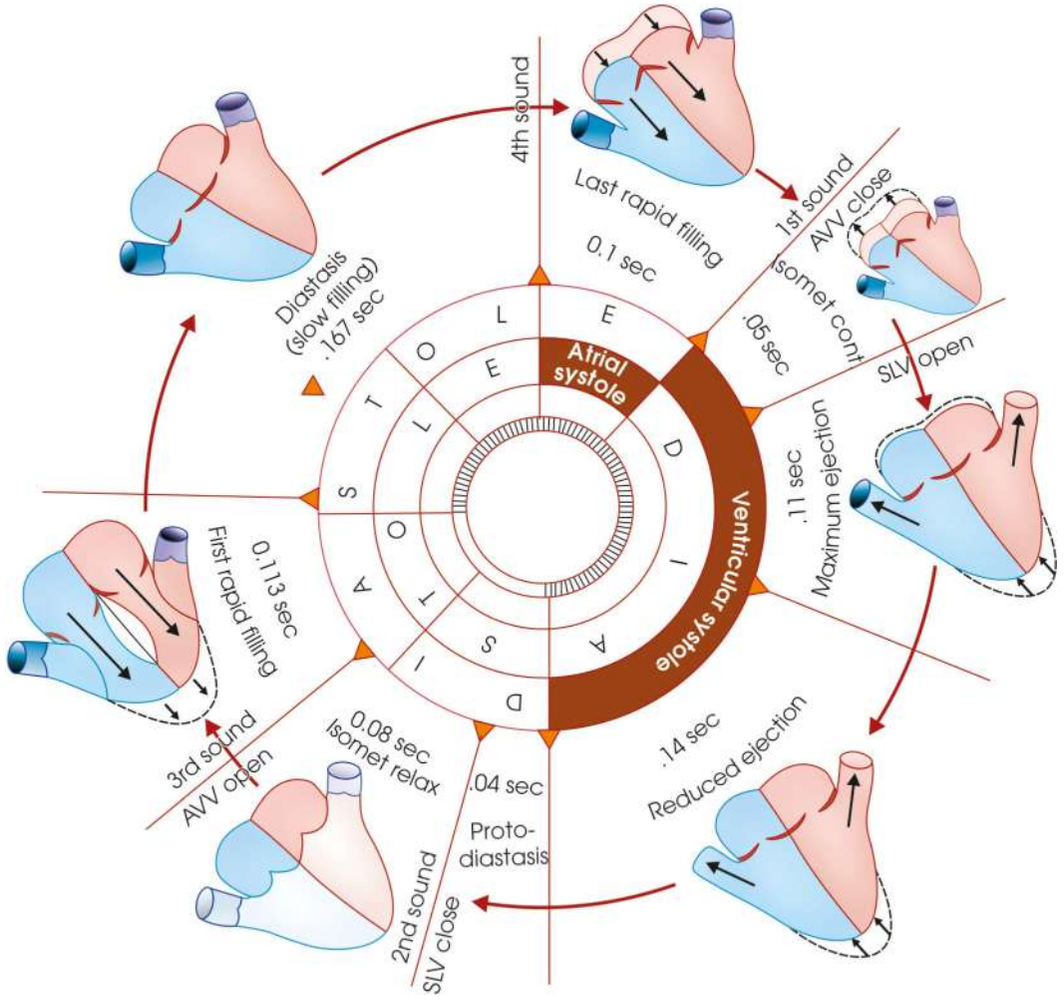


Fig. 32.9: The interpretations of important events of the cardiac cycle. Condition of heart corresponding to each important phase is shown

7. At the end of this period, the intraventricular pressure goes below that of the atria and the AV valves open. Atrial blood rushes into the ventricles—producing the third sound. Here, ventricular filling begins.
   - The first part of filling is very rapid, being known as the first rapid filling phase (0.113 second). The maximum filling takes place during this brief period.
   - The intermediate part of filling is very slow and is known as diastasis or slow inflow phase. Although this is the longest phase (0.167 second), yet the amount of filling is minimum.
   - The last part of diastole corresponds with atrial systole. Due to active contraction of the atria, filling becomes very rapid. This last rapid filling phase (0.1 second) is responsible for the last part of ventricular filling.

Due to rapid rush of blood, another sound is produced—the so-called fourth sound of heart. Here, ventricular diastole ends and systole commences again. In this way the cycle continues (Fig. 32.9).

Table 32.1: Cardiac event with their time relation

Cardiac event	*Duration*	
• **Atrial events**		
– *Atrial systole*		0.1 second
○ First phase (at rise of pressure)	0.05 second	
○ Second phase (at fall of pressure)	0.05 second	
– *Atrial diastole*		0.7 second
	Total	**0.8 second**
• **Ventricular events**		
– *Ventircular systole*		0.3 second
○ Isometric contraction period	0.05 second	
○ Maximum ejection period	0.11 second	
○ Reduced ejection period	0.14 second	
Total ejection period	(0.25 second)	

(*Contd.*)

Table 32.1: Cardiac event with their time relation *(Contd.)*

Cardiac event	*Duration*	
– *Ventricular diastole*		0.5 second
○ Protodiastolic period	0.04 second	
○ Isometric relaxation period	0.08 second	
○ First rapid inflow	0.113 second	
○ Diastasis or slow inflow phase	0.167 second	
○ Last rapid inflow (atrial systole)	0.100 second	
Total ventricular filling time	(0.38 second)	
	Total	**0.8 second**

Time Relations of the Various Events

It has been noted that the cardiac cycle time is inversely proportional to heart rate. But all the phases of cardiac cycle do not proportionally vary. The duration of diastole varies much more than that of systole. For instance, with a rate of 120 per minute the cardiac cycle time will be 0.5 second. The systolic period will be reduced to 0.23 second, and diastolic period to 0.27 second with a rate of 60 per minute the cycle time is 1 second. Here, the systole will be 0.33 second and diastole 0.67 second. Thus, when the rate rises from 60 to 120 per minute the systole diminishes only by 0.1 second whereas diastole diminishes by 0.4 second. In other words, heart rate varies more at the expense of diastole than that of systole.

Summary of the Time Relations

With 0.8 second, as the cardiac cycle time (heart rate 75 per minute), the time relations of the various events are given in Table 32.1.

EXAM-ORIENTED QUESTIONS

Essay

1. Discuss the various events of cardiac cycle.
2. Describe the cardiac event with their time relation.

Short Notes

1. Atrial systole
2. Atrial diastole
3. Ventricular systole
4. Ventricular diastole
5. Isovolumetric contraction
6. Protodiastolic phase

Chapter

33

Pressure and Volume Changes during Cardiac Cycle

INTRODUCTION

After being oriented to the events in cardiac cycle, the study of pressure and volume changes is important to understand the physiological functioning of the heart. The methods by which pressure and volume changes are studied during cardiac cycle are explained below.

METHODS OF STUDY

Pressure Changes

In animals—***optical method of Wiggers:*** A vertical glass tube filled with an anticoagulant solution is used as a cannula. One end of it is introduced into the chamber whose pressure is to be recorded. The other end of the cannula is covered with a tense elastic membrane, upon which a small, mirror is set. A beam of light is so arranged that it is reflected by the mirror and falls on moving photographic plate. The pressure changes in the chamber are transmitted through the solution in the cannula and moves the mirror. The oscillations of the reflected beam are recorded on the moving photographic plate.

In man—(a) Pressure changes in the right atrium and ventricle have been directly measured in man by introducing a thin rubber tube into the antecubital vein and gradually pushing it up through the corresponding bigger veins into the right atrium and then into the right ventricle. The pressure changes are transmitted through this tube and are recorded by suitable apparatus. (b) From the jugular pulse tracing indirect information about the pressure changes in the right atrium can also be obtained.

Volume Changes

In animals, it can be studied by cardiometer (Fig. 33.1). This is a rounded funnel in which the heart is placed in such a way that the ventricles remain inside and atria outside the funnel—the fitting being made airtight around the atrioventricular groove. When ventricles

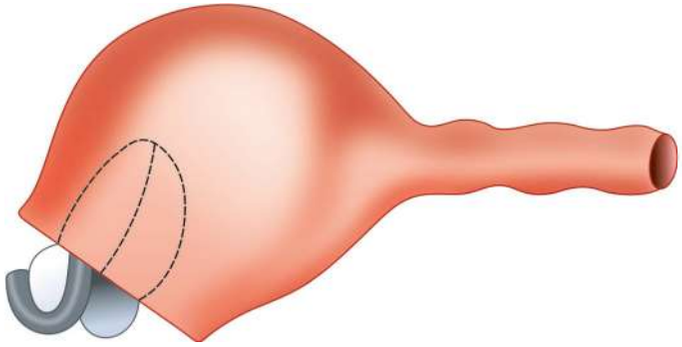


Fig. 33.1: Henderson's cardiometer

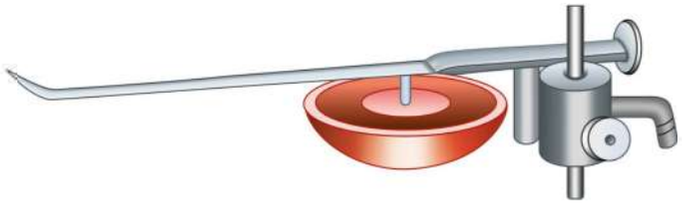


Fig. 33.2: Marey's tambour

contract, pressure in the funnel falls; when it relaxes, pressure rises. These pressure changes are transmitted into a tambour (Fig. 33.2) or a piston recorded through rubber tubings; and are recorded on a moving drum.

With such studies the following facts about the pressure and volume changes are known.

Intraventricular Pressure Changes

The ventricular curve (Fig. 33.3) is to be carefully followed. The following pressure changes are found on it.

Ventricular Systole

1. In the isometric contraction period both valves remain closed, blood cannot run out and ventricles forcibly contract upon the locked-up blood. Hence, intraventricular pressure sharply rises.
2. In the next phase, maximum ejection period comes. Since blood is running out, pressure should fall in

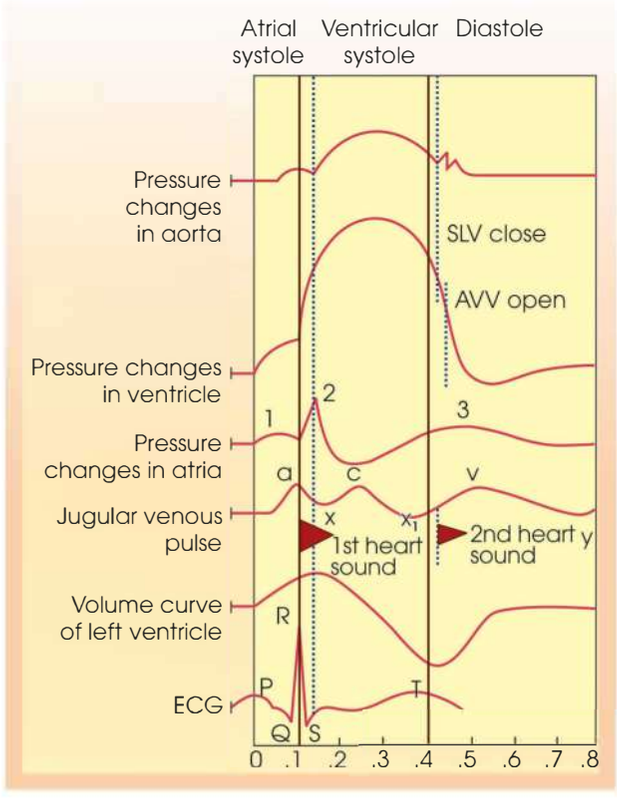


Fig. 33.3: Relation between pressure, volume and electrical changes in heart, aorta and jugular vein during the cardiac cycle of 0.8 second duration corresponding to heart rate of 72 per minute

the ventricles. But the force of contraction is stronger than the rate of outflow; hence intraventricular pressure continues to rise for a brief while, but at a much slower rate than before. Gradually, the force of contraction and the rate of outflow equalise. Hence, a horizontal plateau is produced at the summit.

3. In the next phase—the reduced ejection phase—the force of contraction is much reduced and is proportionally less than the rate of outflow. Hence, intraventricular pressure gradually falls.

Ventricular Diastole

1. In the protodiastolic period the pressure continues to fall as in the previous stage.
2. In the isometric relaxation period the ventricles are actively relaxing as closed cavities and the intra-ventricular pressure sharply drops. This continues until the AV valves open.
3. As soon as the AV valves open atrial blood rushes into the ventricles. But the rate of relaxation being more than filling, ventricular pressure continues to fall slowly to some extent.
4. Then comes the period of diastasis or slow inflow phase. Ventricles are no more relaxing, blood accumulates in it and pressure slowly rises.
5. In the last phase—corresponding to atrial systole—blood is pumped into the ventricles and ventricular pressure shows a small but sudden rise. Then ventricular systole comes again and the changes repeat.

Intra-atrial Pressure Changes

In the atrial curve (Fig. 33.3) there are three positive waves 1, 2 and 3, and three negative waves.

1. During atrial systole, intra-atrial pressure rises causing the first positive wave. In the later part of systole, the pressure falls because most of the atrial fibres have started relaxing.
2. During atrial diastole, the atria relax and pressure should continue to fall. But instead of that, there occurs a sharp rise causing the second positive wave. This corresponds to the isometric contraction period of the ventricles. As soon as ventricles contract, the AV valves become shut and bulge into the atrial cavity in a dome-shaped manner. Hence, intra-atrial pressure suddenly rises.
3. Then the pressure very sharply falls and corresponds to the maximum ejection period of the ventricles.

This sudden fall is due to the following three reasons

1. Atrial relaxation continues.
2. As the ventricular muscles shorten, the AV ring is pulled down, so that atrial cavity enlarges.
3. Due to reduction of ventricular volume, mediastinum pressure falls. Owing to this negative pressure—the thin-walled atria dilate and atrial pressure falls.
   a. In the later part of ventricular systole, intra-atrial pressure slowly rises causing the third positive wave. This is due to accumulation of blood in the atria. AV valves remaining closed. This rise slowly continues until the AV valves open (i.e. up to the end of isometric relaxation period).
   b. During isometric relaxation period, the AV ring rises up and is an additional cause for pressure rising. As soon as the AV valves open, atrial blood rushes into the ventricles, so that atrial pressure falls. The fall continues till about the middle of ventricular diastole. Then as ventricles fill up (diastasis), atrial pressure slowly rises. After this, atrial systole comes again.

Intra-aortic Pressure Changes (Aortic Curve, Fig. 33.3)

During isometric contraction of the ventricles, a slight rise of the intra-aortic pressure takes place due to the bulging-out of the semilunar valves.

With the opening of the SL valve, blood enters the aorta and aortic pressure smoothly rises and falls—running parallel to the intraventricular pressure.

The fall of intra-aortic pressure in the reduced ejection phase is due to three causes:

1. Ventricle is contracting less forcibly than before, so that a comparatively less amount of blood is entering the aorta now.

2. More blood is running out into the periphery than is entering the aorta from the ventricles.
3. Reflex vasodilatation through sino-aortic nerves, thus reducing the peripheral resistance and facilitating better ventricular emptying.
   a. With the onset of diastole, ventricular pressure sharply falls causing a backward flow of the aortic blood towards ventricles. Owing to this, aortic pressure drops causing the incisura. It corresponds to the dicrotic notch of the radial pulse.
   b. The blood column is reflected back by the sudden closure of the semilunar valves, thus causing a sharp rise in the aortic pressure. This is the cause of the dicrotic wave on the radial pulse. After this, aortic pressure shows a few elastic oscillations (after vibrations) caused by the recoil of the aortic wall.
   c. The aortic pressure then slowly falls due to the continuous passage of blood to periphery (propelled by elastic recoil of the vessels). The fall continues till ventricles contract again.

Jugular Pressure Tracing (Venous Pulse, Fig. 33.3)

The pressure changes in the right jugular vein will give a good idea as to those in the right atrium, because the right jugular vein is the direct continuation of the superior vena cava. It may be studied in a number of ways:

1. A small metal cup is placed on the vein. The cup communicates through a rubber tube with Marey's tambour (Fig. 33.2) as the vein pulsates pressure changes take place in the cup and are transmitted to Marey's tambour. The recorder of the tambour moves and the oscillations are recorded on a moving drum.
2. Mackenzie's polygraph. This is an instrument by which tracing of jugular pulse and radial pulse can be obtained simultaneously. This is a very useful instrument by which the pressure changes as well as their time-relations can be known.

Since jugular pressure follows the atrial pressure, it is obvious that jugular pulse will have three positive waves and three negative waves following those in the atria. But due to its distance, the waves will come a little later than the corresponding atrial waves. The three positive waves are called, a, c, v and three negatives ones as x, $x1$, y.

The waves are as follows:

1. The first positive wave-a is due to atrial systole. As atrial muscles contract, pressure in it rises, blood in the jugular vein cannot enter the atria, hence, jugular pressure rises. (It is not due to the regurgitation of atrial blood into the jugular vein. As a sleeve of atrial muscles surrounds the openings of great veins; so when atria contract, these sleeves of muscles also contract thus closing the openings of great veins like a purse string.)
2. This is followed by the first negative wave-x. This is due to the fall of atrial pressure during the adynamic phase of atrial systole.
3. After this, the second positive wave-c comes during isometric contraction phase. In this phase there is bulging of the AV valves into the atrium causing rise of intra-atrial pressure. The a-c interval indicates the conduction time of the bundle of His and corresponds to the P-R interval of the electrocardiogram. (The c wave begins 0.1 sec before the primary wave of the radial pulse.)
4. x1 is the second negative wave after c. It is due to the corresponding fall of pressure in the atrium.
5. v is the third positive wave, caused by the gradual filling of the atria and the return of the AV ring to its original position. The summit of the v wave indicates the end of ventricular systole.
6. The third negative wave y sets in after the opening of the AV valves and is caused by the corresponding fall in the atrial pressure.

Many informations regarding the conditions of heart in health and disease may be obtained from the jugular tracing.

Ventricular Volume Changes

The volume changes of the ventricles are to some extent the reverse of its pressure changes.

The following phases are seen:

1. During atrial systole, ventricular volume increases due to rapid filling. This rise is maintained during the isometric contraction phase of the ventricles, because no blood is going out.
2. As soon as ejection starts, ventricular volume smoothly and continuously falls up to the end of systole. In the isometric relaxation period, volume remains same, because no blood is entering.
3. In the next phase ventricular filling starts and its volume rises rapidly corresponding to the first rapid filling period.
4. After this, during diastasis or slow inflow phase, ventricular volume very slowly increases (Fig. 33.3).

Heart Sounds

There are two classical sounds of heart, known as the first and the second sounds. They can be easily detected with a stethoscope. Two other sounds have also been described—the third and the fourth, which, though difficult to detect clinically, are constantly found in graphic records. The first and the second sounds are

Note: Robert Emerald, Richard L et al., recorded the JVP using a modern method of photoplethysmographic system in 2017.

close to each other. After the second sound there is a longer pause. The sequence is like this; first sound → second sound → pause; first sound → second sound → pause. Thus, the sounds go on.

Biophysical Principle Underlying the Occurrence of Sound in the Heart or in the Circulation

In general blood flows, under most circumstances, through blood vessels in a streamline or in a lamina. Accordingly streamline flow is silence and must not be noisy. But under certain condition when this laminar flow or streamline flow is changed into turbulent flow then a noise may be produced.

According to Sir Osborne Reynolds (1890), turbulent flow is noisy and this turbulence depends upon the critical velocity. It is the velocity at which the turbulence occurs. Reynolds has shown that the critical velocity depends upon the viscosity of blood, density of blood and also on the radius of the blood vessels, by the relation $V_C = (K_{pr})$, where V_C = critical velocity, K = Reynolds number which is constant about 1,000, η = viscosity of blood, ρ = density of blood and γ = radius of the tube (Fig. 33.4).

Cause of Heart Sound

The cause of heart sound is due to (a) vibration of the leaves of the valves during closure and (b) occurrence of turbulence during rapid rush of blood from the atrium to the ventricle. During gradual closure of valves, the vibrations that occur are transmitted from the valvular area towards the apices of the ventricles in case of first heart sound and along the arteries (aortic and pulmonary) in case of second heart sound.

1. So, the first and second heart sounds are not necessarily due to cause of turbulence but for the cause of closure of the tricuspid and mitral valves in case of first heart sound; and aortic and pulmonary valves for the second heart sound.
2. The third heart sound occurs during opening of atrioventricular valve and where the rushing of blood through the narrow opening, produces turbulence.
3. The fourth heart sound is due to rapid rushing of blood from the atria to the ventricles due to forceful contraction of the atria.

Murmurs: However in the heart and in blood vessels abnormal sounds which are generally heard due to abnormally high velocity of blood flow through the valves and blood vessels. These abnormal sounds (murmurs) are mostly associated with the occurrence of turbulence. In mitral stenosis or in coarctation of aorta, murmurs are always associated with the occurrence of turbulence, because during such occurrence, the blood flow reaches its critical velocity during flowing of blood through the narrowed lumen of the valves and blood vessels. Besides this, during heavy exercise innocent systolic murmurs are heard in normal individual due to rapid rush of blood from the ventricles to the arteries at midsystole.

Methods of Study

Clinical stethoscope is the commonest instrument, used for detecting and demonstrating heart sounds. For more accurate work, a microphone is applied to the precordium and is suitably connected to an oscillograph. It is also connected with a mirror arrangement which reflects a beam of light on a moving photographic plate. Thus, the sounds can by graphically recorded (Fig. 33.5).

1. The first heart sound is manifested by a prominent set of vibrations. It occurs during the ventricular systole and is noted just before the onset of the c wave of the jugular pulse.
2. The second heart sound is manifested by a set of vibrations not as prominent as the previous one and occurs during the ventricular diastole and coincides with the notch on the ascending limb of the v wave of the jugular pulse.

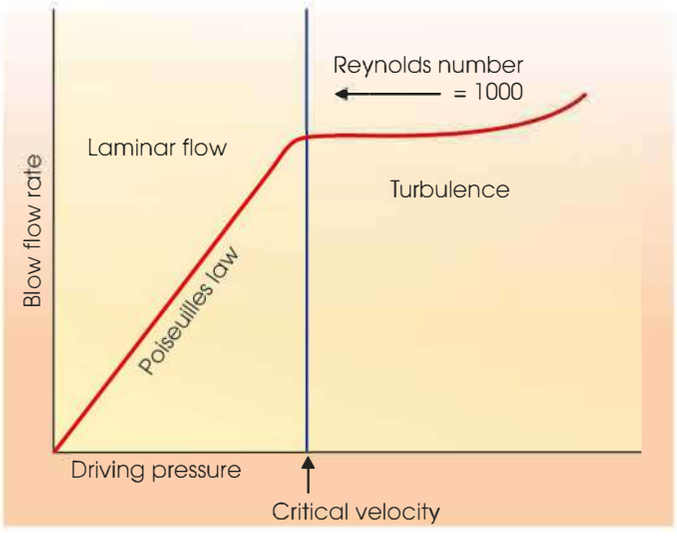


Fig. 33.4: Graphical tracing of laminar flow and turbulent flow in the tube

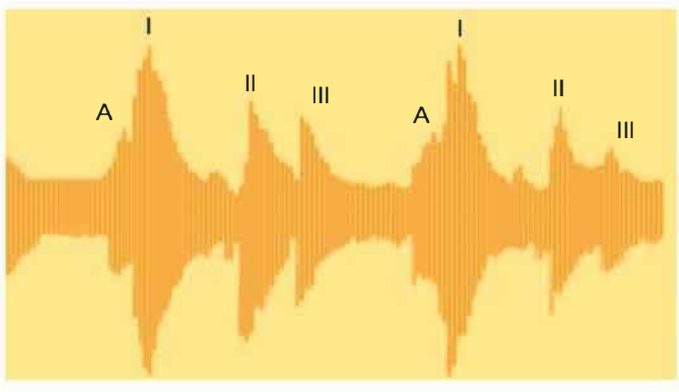


Fig. 33.5: Graphical records of heart sound

3. The third heart sound is manifested by a small set of vibrations and coincides with the end of the descending limb of the v wave of the jugular pulse.
4. The fourth heart sound is manifested by another set of vibrations which occurs during the atrial systole and coincides with the a wave of the jugular pulse.

The sounds are briefly described below.

First Heart Sound

It occurs at the onset of ventricular systole.

Nature: Dull and prolonged, like the word L-U-B-B (Fig. 33.6).

Duration: 0.1–0.17 sec (average, half the ejection period).

Clinical identification: It can be identified by the following features: Its nature, it is best heard over the left fifth intercostal space about 1.27 cm (half an inch) inside the mid-clavicular line. It coincides with the apex beat and with the commencement of the carotid pulse. It comes just after the pause and just before the radial pulse. It coincides with the spike of the R wave of the electrocardiogram. It precedes the onset of the c wave of the jugular pulse.

Cause: First sound is caused due to:

- Sudden closure of the AV valves and the vibrations set up in the valve leaflets due to increase in the intraventricular pressure.
- Contraction of the thick ventricular muscles (muscular element). It is doubtful whether the second factor takes any appreciable part. In graphic records often two distinct groups of vibrations are seen. The first group corresponds to the isometric contraction period and the second group to the maximum ejection period. The latter suggests that aortic vibrations, due to entry of blood, take part in causing the prolonged character of the first sound. The frequency of vibration varies from 25 to 45 per second

Significance: First sound indicates the onset of clinical systole of the ventricles.

The duration and the intensity of the first sound indicate the condition of the myocardium. In a strong healthy heart these features are prominent and become more so in a hypertrophied heart. If the myocardium is weak, the first sound will be short and low pitched. A clear first sound indicates that the AV valves is properly closing, i.e. there is no incompetence.

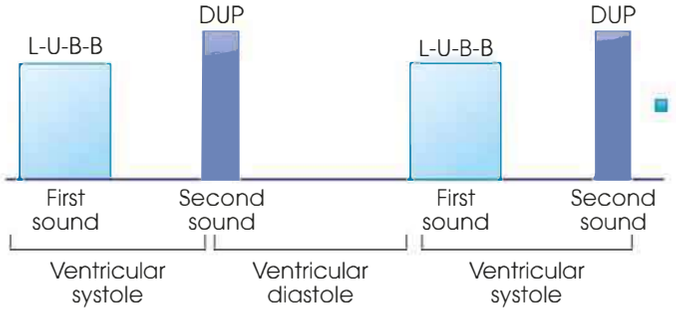


Fig. 33.6: Heart sounds (schematic representation)

Second Heart Sound

Nature: Short and sharp like the word DUP, the pitch being higher and duration shorter than the first sound (Fig. 33.6).

Duration: 0.1–0.14 second.

Causes: It occurs at the onset of diastole and is caused by the sudden enclosure of the semilunar valves in the aorta and pulmonary artery and just after the T wave of the ECG. At the end of systole, ventricular pressure falls below the pressure of the aorta and the pulmonary arteries causing a much pressure difference in between the aortic or pulmonary arteries and the ventricles. This increased pressure in aorta and pulmonary arteries artery tends to close up the valves through a backflow.

The two valves—aortic and pulmonary, do not close simultaneously. During inspiration but not in expiration, the sound is thus split into an aortic component and later a pulmonary component. Its intensity depends on the blood pressure. It consists of three or four chief vibrations with a frequency of about 50 per second.

Significance: It indicates the end of systole and beginning of diastole. Its pitch is directly proportional to the blood pressure. A clear second sound indicates that the semilunar valves are closing properly, i.e. there is no regurgitation. The interval between first and second sounds is taken as the clinical systole and that between the second and first as the diastolic period of heart. The diastolic period, generally known as the pause, is a little longer than the systolic period. When the heart rate rises, the long pause shortens and the sounds appear to be equidistant.

Third Heart Sound

Nature: It takes place just after the second sound and coincides with the opening of the AV valves, i.e. with the commencement of the ventricular filling.

Duration: About 0.04 second.

Causes: It is caused by the sudden rush of atrial blood into the ventricles when the AV valves open. Although it is stated that it can be detected in 60% of normal subjects yet, in actual practice, it is difficult to detect clinically.

Significance: It indicates the beginning of the ventricular filling.

Fourth Heart Sound

It is also called the atrial sound.

Causes: It is caused by the contraction of the atria and the consequent rush of blood into the ventricles. It is difficult to detect clinically but is found in the graphic records. It coincides with the rise of a wave of the venous pulse.

Significance: It occurs just before the first sound and indicates the end of ventricular filling.

It should be noted that each of the above sounds is really produced at two places—for instance, the first sound is produced at both the AV valves, the second sound at both the semilunar valves (aortic and pulmonary) and so on. Yet only one first sound, one second sound, etc. is heard. This is due to the fact that the two sets of valves move exactly at the same time. If any discrepancy occurs in the pressure and time relations between the different chambers of heart, instead of one sound, two sounds may be heard at each time. Thus, reduplication of the sounds occurs in cardiac diseases.

COMPOSITE REPRESENTATION OF THE SEQUENTIAL CHANGES IN THE PRESSURE AND VOLUME EVENTS IN THE HEART AND BLOOD VESSELS DURING THE CARDIAC CYCLE CORRELATING WITH PHONOCARDIOGRAM AND ELECTROCARDIOGRAM (Fig. 33.7)

1. I-II is the ventricular isometric contraction phase. This phase starts with the closure of atrioventricular (AV) valve and with the occurrence of the first sound. In this phase, the ventricle is a closed chamber and with the contraction of the ventricular muscle, the intraventricular pressure rises first slowly but at later

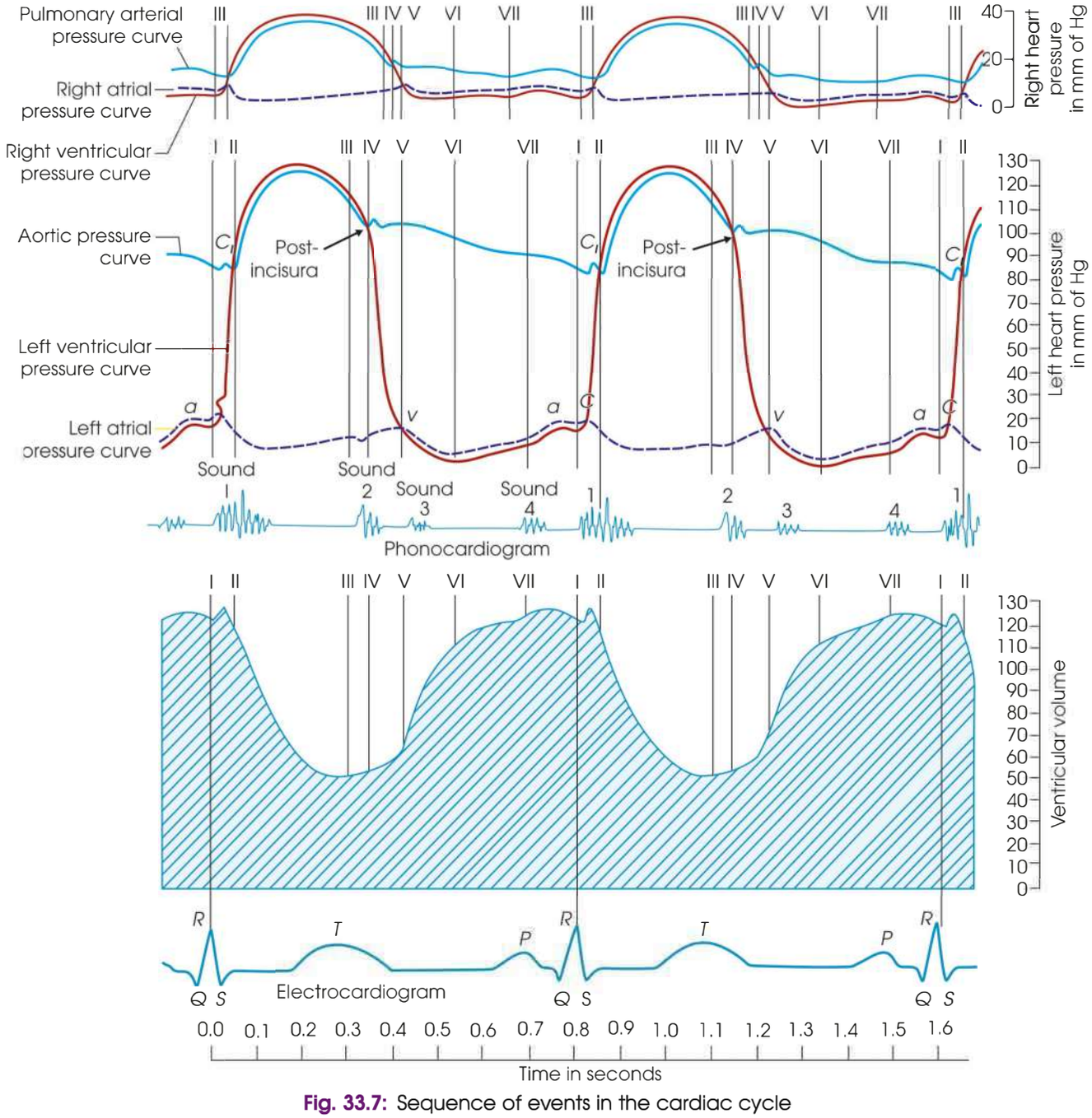


Fig. 33.7: Sequence of events in the cardiac cycle

stage, rapidly. Notch c1 in the aortic pressure curve and notch c in the atrial pressure curve—both are due to the transmission of the intraventricular pressure through the bulging of semilunar (aortic) valve and atrioventricular valve respectively.

2. II-III is the ejection phase of the ventricle. At II, the ventricular pressure overcomes the aortic pressure and the semilunar valve opens. The blood begins to flow through the aorta at a higher pressure head. The ventricular pressure remains at a higher level throughout this phase. The ventricular volume begins to decrease with the onset of ventricular systole. The atrial pressure though remains below the ventricular pressure head but begins to rise due to the venous filling.
3. III-IV is the protodiastolic phase. The ventricular systole ceases and the ventricle begins to relax. At IV, the ventricular pressure falls below the aortic pressure and the semilunar valve is closed with the occurrence of the second sound. The ventricle is again a closed chamber and relaxes isometrically. Due to the closure of the semilunar valve, a depression in the aortic pressure curve is generally observed. There are multiple oscillations in the aortic pressure curves which are known as postincisural vibrations.
4. At IV-V, the ventricular pressure curve drops abruptly and at V it falls below the atrial pressure and then the blood from the atrium begins to rush (first rapid filling phase) in the ventricle rapidly. Here the third sound is heard. Throughout the diastolic phase of the ventricle, the atrial pressure remains in higher pressure head (V-I). The ventricular volume curve begins to rise and maintains this stage until the systole begins. The third sound occurs with the rapid rush of blood in the ventricle.
5. At VII-I, the atrial systole coincide with the ventricular diastole and the blood rushes in the ventricle from the atrium very rapidly and the fourth sound occurs. *a* in the atrial pressure curve represents the cessation of the ventricular diastasis (slow filling) and the onset of the atrial contraction.
6. At I, the atrial systole ceases and the ventricular systole starts and the cycle is repeated. The ventricular volume begins to decrease with the onset of the ventricular systole.

EXAM-ORIENTED QUESTIONS

Essay

1. Describe the pressure and volume changes during cardiac cycle in atria, ventricle, aorta and jugular veins.
2. Describe the characteristics, causes and significance of heart sounds.

Short Notes

1. First heart sound
2. Second heart sound
3. Third and fourth heart sounds
4. Jugular venous pressure

Chapter

34

Electrocardiogram

INTRODUCTION

It has been discussed earlier that prior to each contraction, an electrical impulse is generated in the SA node and thus transmitted to the AV node, bundle of His, Purkinje fibre, ventricular muscle fibre and lastly to the surrounding tissue in which the heart is bathed in. Body is a volume conductor and the heart muscle, being the electrical generator with two opposite poles (dipole), is bathed in it (Fig. 34.1). Under this condition, electrical impulse that is initiated in the cardiac muscle will be transmitted throughout the body. If suitable electrodes (leads) are placed on the body opposite to the heart and connected to a very sensitive galvanometer with a recording device, then the electrical potential can be recorded. The record is known as *electrocardiogram* (ECG). The machine by which the electrocardiogram is recorded is spoken of as *electrocardiograph*.

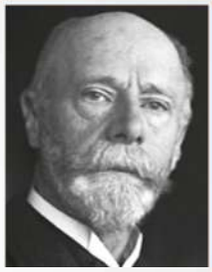
The currents generated from the different chambers of heart are not equally transmitted in all directions. Hence, the record will vary according to parts of the body from which the action current is led off. Consequently, to obtain accurate information, several records from different parts of the body should be taken.

Methods of Recording Electrocardiogram

Though Waller (1887) first recorded the electrocardiogram yet Einthoven (1903) was the real father of the electrocardiography. His method of recording ECG through string galvanometer was followed until 1930 when it was largely replaced by the amplifier—driven oscillograph provided with direct ink recording.

Principles upon which the string galvanometer was based are as follows: If an electrical current is allowed to pass through a string suspended in a magnetic field, then the string will move. In this string galvanometer there is a thin platinum wire suspended within the magnetic field created by the south and north poles of the magnets. The string is suspended in such a way that it can move freely. There is a thorough passage longitudinally through the two magnetic bars and the rays of light may be collimated through this passage in such a way so that it may fall directly over the photographic screen (Fig. 34.2). The light source is obstructed by the movement of the galvanometric string and the nature of impression is also changed according to the movement. The two free ends of the string are attached to the body. Electrical impulse that is initiated from the heart is transmitted all throughout the body because the body is a volume conductor. So this impulse will be transmitted through the electrodes to the string galvanometer which will vibrate in the magnetic field.

Figure 34.3 depicts the moving coil galvanometer in which a strong magnetic field is developed between south and north poles of an electromagnet or strong permanent magnet. There is a coil of wire whose one end is suspended from above and the other end is joined with the wire below the coil. At the middle of the coil there is an attached mirror which rotates when the coil rotates. When electrical current makes to pass through the coil of wire, one side of it moves backward in the



William Einthoven
1860–1927

William Einthoven, observed Waller demonstrating the use of the capillary electrometer to record an "electrograph" of the heart. In 1895, he was able to detect recognizable waves, which he labelled "P, Q, R, S, and T." He formulated the concept of "Einthoven's triangle" by mathematically relating the 3 leads (Lead III = Lead II–Lead I). He described bigeminy, complete heart block, "P mitrale," right and left and ventricular hypertrophy, atrial fibrillation and flutter, the U wave, and examples of various heart diseases. The "father of electrocardiography" was honoured with the Nobel Prize in medicine in 1924.

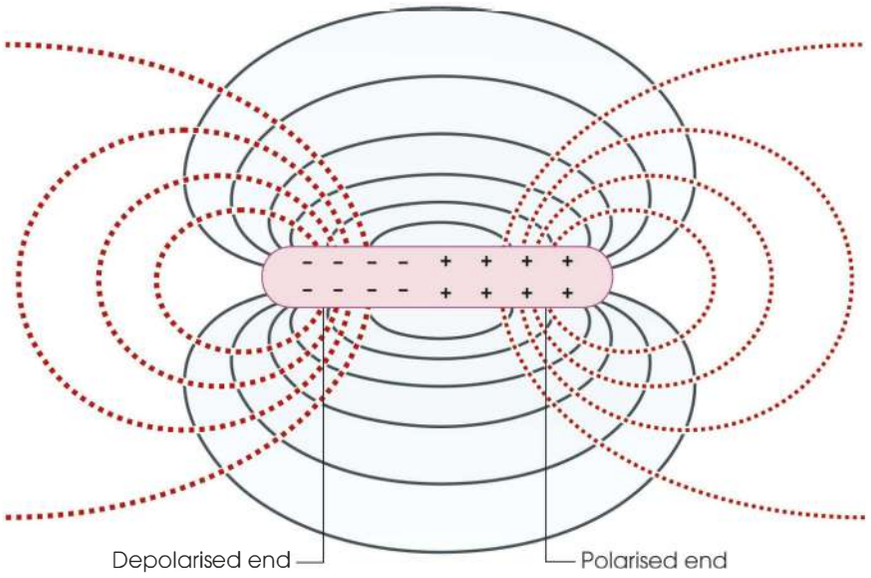


Fig. 34.1: Electrical generator of a dipole in a volume recorder showing current flow (solid lines) and iso-potential lines (dotted lines)

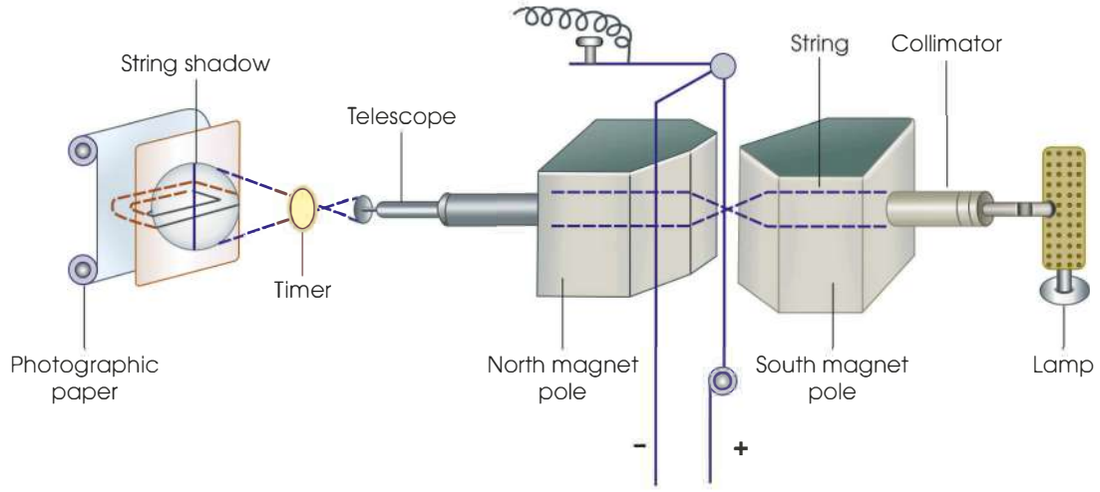


Fig. 34.2: String galvanometer of the electrocardiography

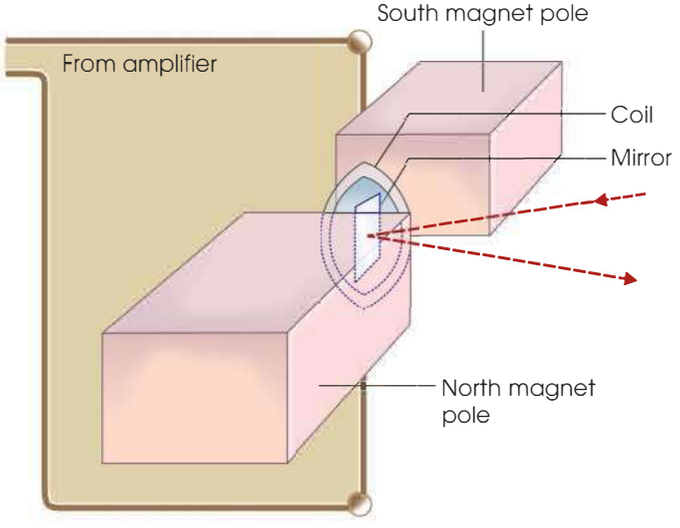


Fig. 34.3: Moving coil galvanometer of the electrocardiograph (diagrammatic)

magnetic field and the other side moves forward due to opposite direction of the current. If a ray of light is allowed to fall on the mirror as the coil rotates to left or right, the reflected beam of light can be recorded on a moving photographic paper. This type of galvanometer requires an amplifier to make potential recorded from the surface of body. This type of galvanometer was used in past for electrocardiographic studies due to rapidity of recording changes (several hundred cycles per second) in electrical potentials.

Present ECG recorders: Newer generation digital and computer based ECG recorder machines area available today and are in routine use. The direct pen recorder device is very suitable both clinically and experimentally because the nature of wave is recorded graphically and the wave is visualised on the spot.

Electrocardiographic Leads Used both Clinically and Experimentally

When electrocardiographic connections are made between two parts of the body, then this specific arrangement of each pair of connections is designated as lead. Figure 34.4 represents the arrangement of different standard limb leads. The different leads that are conventionally used:

1. Standard limb leads
2. Chest leads
3. Augmented unipolar limb leads.

Standard Limb Leads (Fig. 34.4)

The action current of the heart is recorded by placing two electrodes in two different positions in the body. Such arrangement of lead is called bipolar leads. The bipolar leads (standard limb leads) which are in common use are listed in Table 34.1.

According to convention, the combination of the lead I will be made by connecting the left arm to the positive pole of the galvanometer and the right arm to the negative pole of the galvanometer. The combination of the lead II will be made by connecting the right arm to the negative pole and the left leg to the positive pole of the galvanometer. The combination of the lead III will be made by connecting the left arm to the negative pole and the left leg to the positive pole of the galvanometer.

Einthoven's triangle: It is an equilateral triangle drawn arbitrarily around the area of the heart (Figs 34.4 and 34.5).

Einthoven's law: Einthoven's law states that if ECG is recorded through standard limb leads then the sum total voltage of the QRS (ventricular complex) in leads

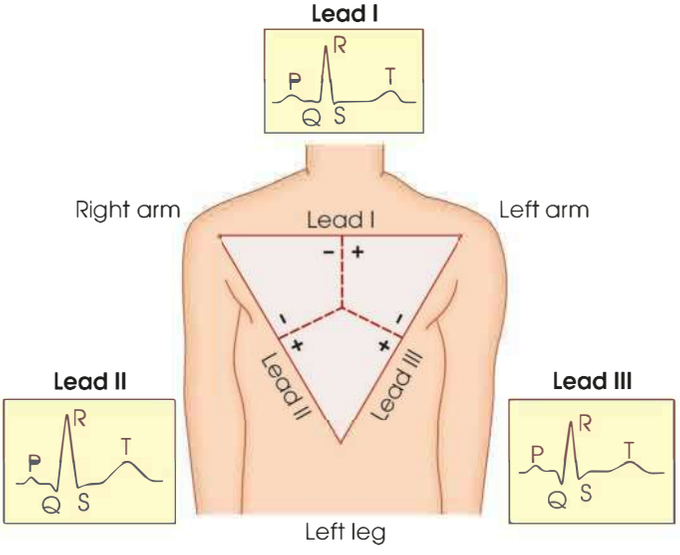


Fig. 34.4: Bipolar leads—standard limb leads of the electrocardiogram and general normal recordings from these leads (schematic representation)

Table 34.1 Bipolar leads (standard limb leads)

Lead	*Negative terminal*	*Positive terminal*
I	Right arm	Left arm
II	Right arm	Left leg
III	Left arm	Left leg

I and III is equivalent to lead II. If the voltage of QRS complex in lead III is + 2 and in lead I is +3, then the voltage of the same in lead II will be +5. On the other hand, if the voltage of QRS in any two leads is known then the other one will be obtained after deducting the latter from other two (Fig. 34.5).

Normal ECG Recorded in Standard Limb Leads

ECG recorded simultaneously in three leads shows mostly similar in shape, contour and other feature. P, R, T waves are positive waves in all the leads. The voltage of different complexes is different from lead to lead. For clinical purposes in diagnosis of the arrhythmia, it does not matter with which leads, the ECG has been recorded. But for determining the extent of cardiac damage either in the atria or in the ventricles, it does matter with which leads the ECG is taken.

Interpretation of Human Electrocardiogram (Fig. 34.6)

It shows the following five consecutive waves; P, Q, R, S, T. There are two isoelectric periods—the shorter one, between P and Q; the longer one, between S and T. P, R and T are upward deflections, while Q and S are downward waves. The waves are therefore alternately up and down. P is of atrial origin, hence called the atrial

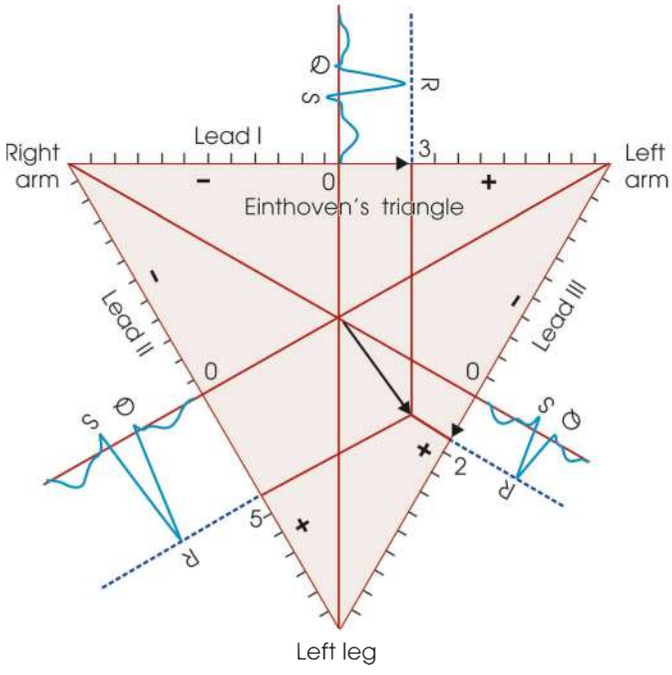


Fig. 34.5: Electrical axis of QRS complex shown by Einthoven triangle

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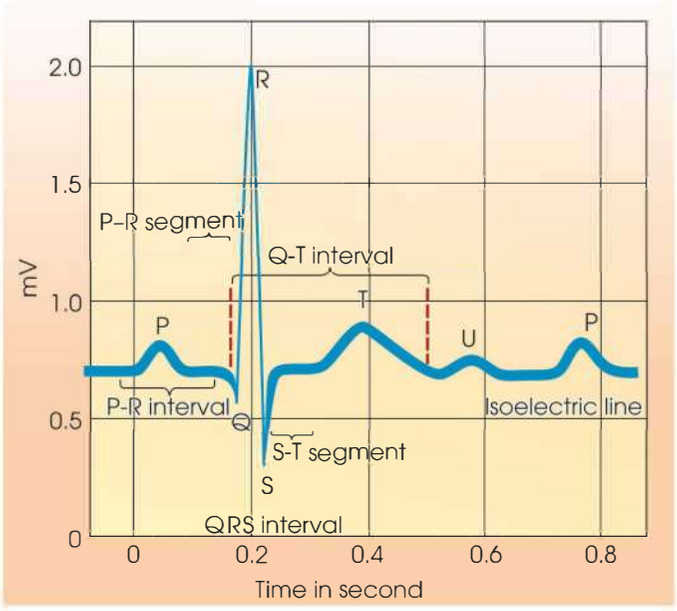

Fig. 34.6: Conventional terms for electrocardiographic deflections

complex, while QRS-T being of ventricular origin, and are collectively known as the ventricular complex. They are briefly described below.

P wave: This is the first upward deflection. It is a small but constant wave having a rounded or pointed top. It is depolarisation wave of the atria and, takes place when the impulse spreads over the atria and therefore, caused by the passage of the action over the atria. Its average duration is about 0.1 sec (same as atrial systole).

Repolarisation wave of the atria is submerged within the ventricular complex.

The impulse arrives at the AV node, at about the summit of P.

Normal P indicates that the impulse is originating at the SA node. It spreads over the atria in the usual direction. There is no defect of conduction. The strength of contraction, the mass of atrial musculature and its nutrition, are normal. If the P is inverted, it indicates that SA node fails to initiate impulse and the atrial muscle depolarises by the impulse originating in AV node.

Any abnormality of atrial activity will be reflected by corresponding changes in the P wave. For instance, in atrial fibrillation it will be absent; in atrial hypertrophy, it will be large and may be notched; in nodal rhythm, the direction will be reversed.

QRS-T waves: These four waves are caused by ventricular activity and are collectively known as the ventricular complex. QRS are depolarisation waves of the ventricle; and are the initial group of three waves—one following immediately upon the other. The average duration of QRS-T is 0.43 sec; that of QRS is usually less than 0.08 sec and should not exceed 0.1 sec.

Q wave: As soon as the impulse arrives at the muscular part of the septum, producing the first wave Q. Hence, Q is caused by the activity of the septum. It is a small, negative wave and often inconspicuous deflection.

Q wave is not found in those animals which do not possess an interventricular septum (reptiles and amphibian). It is also absent in infants suffering from congenital patency of the septum. Prominent Q wave indicates old infarction.

R & S waves: R is the most constant and conspicuous wave having the tallest amplitude. It is the first positive deflection during ventricular depolarisation. It follows immediately upon Q. S is the next downward deflection, constant but often inconspicuous. In lead I, R is mainly caused by right ventricle and S due to left ventricle. In lead III, it is just the reverse. In abnormal conditions of ventricles, the shape, size and duration of RS alter. For instance, in bundle branch heart block their duration is prolonged beyond 0.1 sec and their relative amplitude varies.

T wave: R is followed by a long isoelectric period, after which comes the last upward deflection T. It is a broad, smooth rounded deflection with an average duration of 0.27 sec. It is repolarisation wave of the ventricle. It is normally positive because the apex of the heart repolarises much earlier than the base of the heart. In young adults, T is very prominent. In old age it is flattened. Exercise increases the amplitude in a healthy heart. It is sometimes inverted in lead III without apparent reason. It is also altered by stimulation of the vagus and sympathetic nerves, by digitalis and other poisons, by anoxia caused by coronary constriction and by the damage of myocardium from any cause.

Abnormalities of T wave in shape, size, direction, duration and reaction to exercise in leads I and II are of great prognostic significance. It indicates serious myocardial damage and is often associated with cardiac hypoxia.

U wave: This wave is often seen just after the T wave. It is possibly due to slow repolarisation of the intraventricular conducting system.

R-R interval: It is the interval between the two successive R waves. If the R-R interval in next successive stages are same then they indicate that the ventricle is depolarising rhythmically.

P-P interval: It is the interval between the two successive P waves. Equal intervals in next successive stages indicate rhythmical depolarisation of the atrium.

P-R interval: This is the interval from the onset of P to that of QRS. It measures the conduction time of the impulse from the SA node to the ventricles. Normally, it varies from 0.13 to 0.16 sec and should not exceed 0.2 sec. A longer interval shows impaired conduction

through the bundle. Variable P-R interval in successive stages indicates AV dissociation. This indicates ventricle to beat without the influence of SA node. [True conduction time, i.e. from the AV node to the ventricles, should be measured from the summit of P to the beginning of Q. But since Q is often absent, P-R interval is taken as the conduction time for the sake of convenience.]

QRS interval: It measures the total ventricular depolarisation time. It is measured from the onset of Q wave to the cessation of S wave. It varies from 0.08 to 0.1 sec.

Q-T interval: It measures the ventricular total electrical activity time. It is generally measured from the onset of Q wave to the end of T wave. It is about 0.36 sec.

RS-T segment: Elevation or sagging of this segment indicates myocardial damage or hypoxia.

T-P interval: Alteration of this interval indicates the alteration of the heart rate. It is measured from the end of T wave to the beginning of P wave. T-P interval actually measures the diastolic period of the heart. If the T-P interval in successive stages is variable then it indicates atrioventricular dissociation.

Unipolar Limb Leads (Table 34.2)

Electrodes are placed respectively on the left arm, left leg and right arm. They are connected together to form a central terminal which passes through a suitable resistance (5,000 ohm) and is kept almost to zero potential. This is the indifferent electrode. Other electrode is put on different parts of the body surface. This is the exploring electrode. By this arrangement the indifferent electrode is kept at zero degree potential so that the exploring electrode records the local unmodified action current.

The following unipolar leads are in use:

- Lead VR: Right arm.
- Lead VL: Left arm.
- Lead VF: Left leg (foot).

VR lead: Q wave is bigger which is followed by small R wave, small S wave and an inverted T wave (Fig. 34.7).

Table 34.2 Unipolar limb leads

Lead	*Negative terminal*	*Positive terminal*
VR	Right arm Left arm Left leg	Right arm
VL	Right arm Left leg Left arm	Left arm
VF	Right arm Left arm Left leg	Left leg

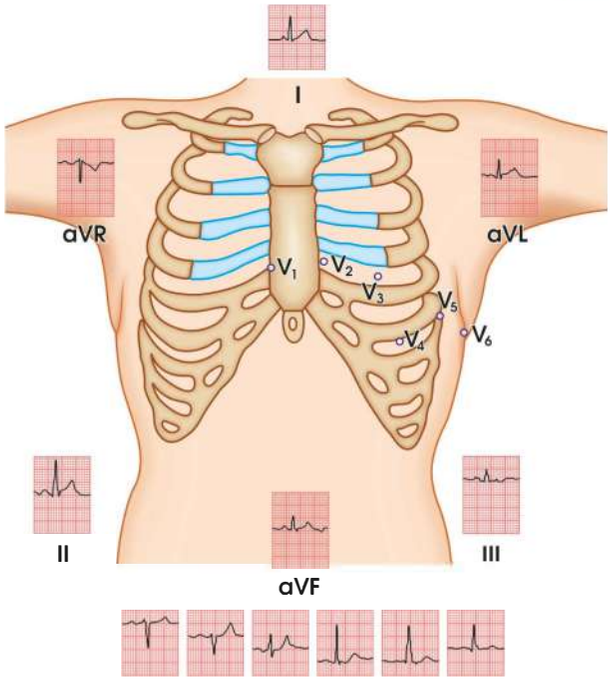

Fig. 34.7: ECG of bipolar leads and unipolar chest leads (*Courtesy:* Prof RN Chatterjee, MD (Calcutta)

VL lead: The waves in VL changes with the alteration of the position of the heart. When the heart is vertical, VL resembles with that of VR. R wave is small followed by big S wave. T wave is inverted.

VF lead: There is a large R wave followed by S wave. T wave is erect. Through the unipolar limb leads VR, VL, VF, electrical activities of the ventricular cavity, upper part of the left side of the heart and the inferior surface of the heart respectively are known. QRS complex in VF resembles with that of chest lead V_1 if the records are taken during expiration and with that of chest lead V6 if the records are taken during deep inspiration.

Augmented Unipolar Limb Leads

In these types of leads there are three leads widely used. These are aVR, aVL and aVF. In the unipolar limb leads (VR, VL, VF), the amplitudes are small but in augmented unipolar limb leads (aVR, aVL, aVF), the amplitudes are increased by 50%.

Key Points

In these types of recordings, one limb is connected to the positive terminal and the other two limbs are connected together to the negative terminal.

1. When the right arm is connected to the positive terminal and the left arm and left leg together to the negative terminals, then the combination will be a VR (denoting block R as right arm).
2. Similarly, when the left arm is connected to the positive terminal and the right arm and left leg

together to the negative terminals, the combination will be a VL (denoting L as left arm).

3. When the left leg is connected to the positive terminal, and the right arm and left arm to the negative terminals, then the combination will be aVF (denoting F as foot).

Normal ECG recorded in these leads (Fig. 34.8) shows more or less similar except that the recording in aVR is inverted. This is because the side of the heart nearest to the right arm is negative in respect to the rest of the heart.

Chest Leads (Unipolar)

There are six chest leads, viz. V_1, V_2, V_3, V_4, V_5, and V_6. In the chest leads one electrode is placed on the anterior surface of the chest and connected to the positive pole of the galvanometer and the other electrode—the indifferent electrode placed anywhere in the body is connected to the negative pole of the galvanometer.

One such combination actually makes one lead. The conventional precordial positions used are as follows:

V_1: Fourth intercostal space at 2.54 cm (1 inch) away from the right sternal border.

V_2: Fourth intercostal space at 2.54 cm (1 inch) away from the left sternal border.

V_3: At the midpoint between V_2 and V_4.

V_4: Fifth intercostal space at left mid-clavicular line.

V_5: At a point where the anterior axillary line intersects perpendicularly the horizontal line extended from V_4.

V_6: At a point where mid-axillary line intersects perpendicularly the same horizontal line extended from V_4.

Normal ECG Recorded in Chest Leads (Fig. 34.8)

Key Points

1. In leads V_1 and V_2, the QRS complex is normally negative, because the chest electrodes in these leads are nearer to the base of the heart which is the direction of electro-negativity during most of the ventricular depolarisation phase.
2. On the other hand, in V_4, V_5 and V_6 the QRS complex is positive because these chest leads are nearer to the apex of the heart and are electropositive during depolarisation. Localisation and the extent of damage can be determined by using the chest leads.
3. Normally V_1–V_3 indicates the activity of the right ventricle and is characterised by increasing height of R wave from V_1 and a progressively decreasing S wave. The height of R wave increases from V_1 to V_4 or V_5.
4. V_5, V_6 denotes the activity of the left ventricle and is characterised by a very small Q wave and a tall R wave. T wave is generally upright in all positions except sometimes in V_1.

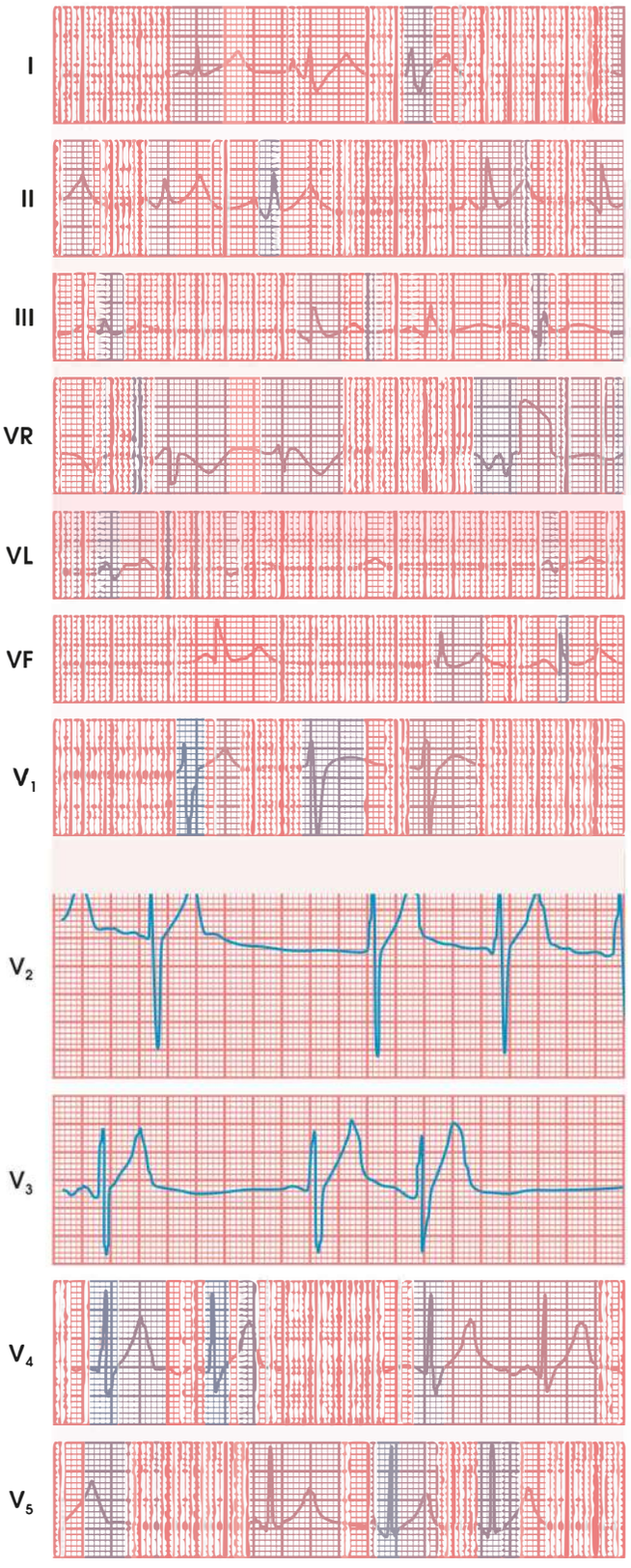

Fig. 34.8: Sino-atrial heart block

Significance of Various Leads and their Limitations

In general, the standard limb leads are most valuable for diagnosis of arrhythmia and also for preliminary studies of the functional abnormalities of the heart.

The ***precordial chest leads*** are very important for diagnosis or for (a) localisation of the recent or old ventricular damage, (b) bundle branch block, and (c) detection of ventricular hypertrophy.

The ***augmented unipolar limb leads*** are most valuable for (a) determining the position of the heart, (b) confirming the significance of Q and T waves in the standard leads, and (c) confirming the evidence of ventricular damage or hypertrophy.

Abnormalities in ECG may not always indicate heart disease, because these abnormalities may be functional, congenital or induced by drugs or by disease.

The clinical importance of electrocardiogram is obviously very great. It gives fairly accurate information as to the condition of atria and ventricles—almost in all of its functional aspects. In cardiac abnormalities, characteristic variations occur in the electrocardiogram along with side-by-side clinical findings may act as a dependable guide to the diagnosis, prognosis and treatment.

Electrocardiographic Appearances under Certain Cardiac Disorders

Heart Block

Heart block is the condition when conduction of impulse from pacemaker area through the conducting tissue is interrupted at any degree.

Sino-atrial block: Sometimes impulse from SA node fails to reach the atrial muscle due to block (sino-atrial block). In ECG (Fig. 34.8), P is absent due to atrial standstill, but the ventricle beats at the rhythm of the AV node and QRS-T complex remains unaltered.

Incomplete heart block or partial heart block is the condition when the bundle of His is partially blocked.

Thus to conclude: In first degree heart block (Fig. 34.10), The P-R interval is abnormally increased from 0.16 to 0.38 sec. In the second degree heart block (Fig. 34.11), QRS-T complex follows each second or third P wave (2:1 or 3:1 heart block). In certain partial heart block, P-R interval increases with each successive beat until one P wave is not followed by a QRS-T complex. So, in this form of heart block, the P-R interval is gradually increased in successive beats until a ventricular beat fails to occur (Fig. 34.12). This type of block is known as Wenckebach phenomenon.

Sometimes, block occurs in either branch—left or right bundle branch by injury or by some pathological processes.

- In ***left bundle branch block***, there is delay in activation of the left ventricle. Ventricular rate is normal, but duration of QRS complex is greater. R waves in V_6 are slurred or notched.
- In ***right bundle branch block***, there is delay in impulse conduction through the right ventricle. QRS duration is prolonged and the R waves in V_1 and aVR are slurred. In V_5, V_6, and aVL there is late slurred S wave.

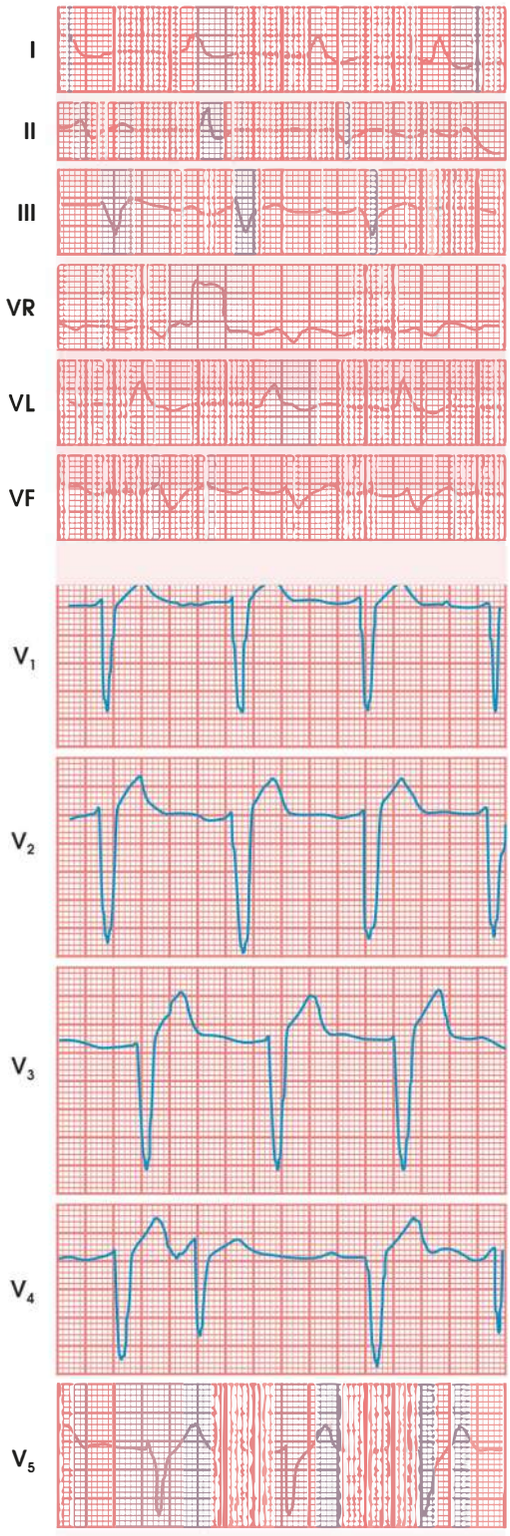

Fig. 34.9: Prolonged P-R interval
(*Courtesy:* Dr Sunil Sen, MB, MRCP (Edin))

In complete heart block, where the impulse conduction is completely interrupted due to damage of bundle of His, there are complete atrioventricular dissociations. Atrium beats at the rate of sinus rhythm

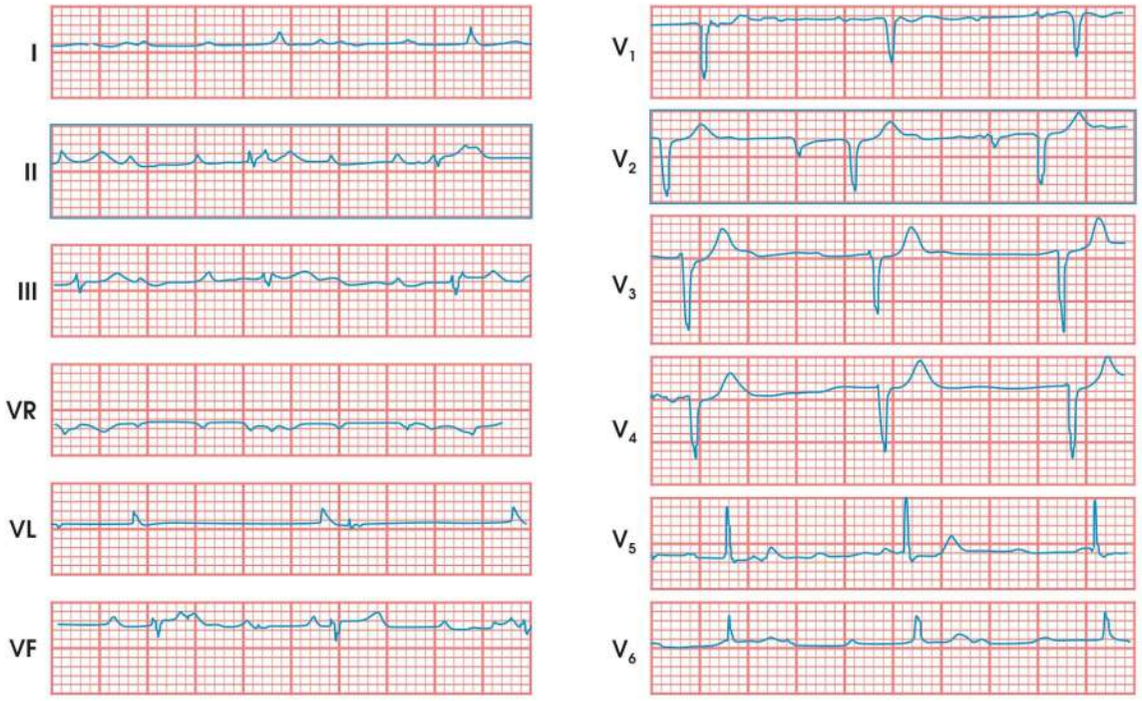

Fig. 34.10: Complete heart block

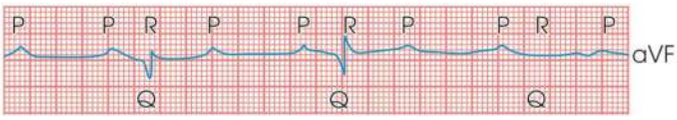

Fig. 34.11: Second degree heart block

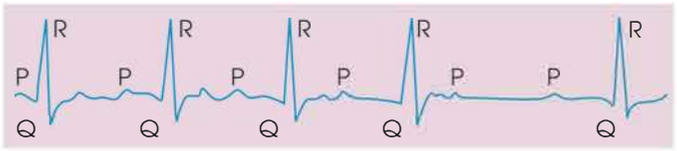

Fig. 34.12: Second degree heart block of Wenckebach type

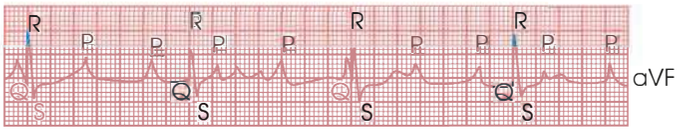

Fig. 34.13: Complete heart block showing atrioventricular dissociation

and ventricle at its own. This type of block is known as complete or third degree heart block. In the ECG, P-P interval and R-R interval will be same all throughout but P-R interval will be variable because P will not be followed by the QRS-T complex (Figs 34.11 and 34.13).

Resultant cerebral ischaemia may cause dizziness and fainting (Stokes-Adams syndrome).

Ventricular Premature Beat or Extra Systole

Figure 34.14 illustrates the series of ventricular premature beat or extra systole. Sometimes, a portion of the myocardium becomes irritable and ectopic beat

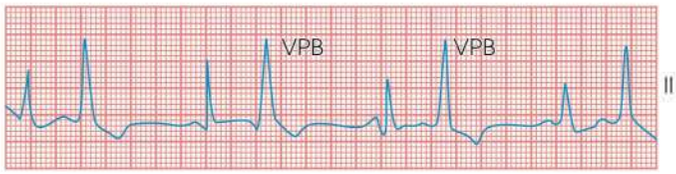

Fig. 34.14: Ventricular premature beats

occurs before the expected next normal beat. This beat causes transient interruptions of the cardiac rhythm. This type of ectopic beat is known as ventricular extra systole or premature beat.

Ventricular Paroxysmal Tachycardia

This is the condition when the ventricular muscle is damaged considerably. The ECG shows the series of ventricular premature beats or extra systole occurring successively for several beats without any normal beats interspersed (Fig. 34.15). This is the early stage of ventricular fibrillation.

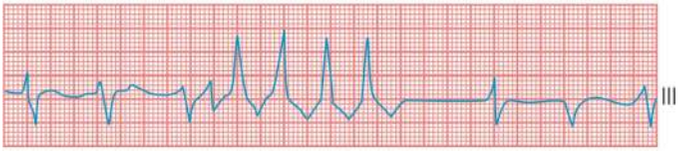

Fig. 34.15: Ventricular paroxysmal tachycardia

Ventricular Fibrillation

This is the condition of massive damage at multiple areas of the ventricles. In the ECG, the complexes are bizarre and the individual component cannot be deciphered (Fig. 34.16).

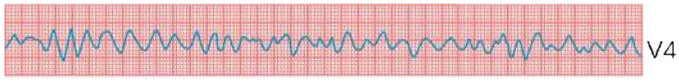

Fig. 34.16: Ventricular fibrillation

Wolff-Parkinson-White Syndrome

The impulses pass directly from atrium to the ventricular muscle via an abnormal route, called the bundle of Kent, which bypasses the AV junctions. This leads to only partial activation of ventricular muscle before normal activation reaches it via the conduction system (as due to delay in the AV junction). ECG changes seen are shorter PR interval and broader QRS complex that exceed over 0.12 s.

Ischemic Heart Disease

This is the condition when the circulation of the heart muscle is interfered with curtailment of blood due to ischaemic changes. In the ECG, there is inversion of T waves. The S-T segments is sagged.

Myocardial Infarction

It is the condition when an area of the muscle is necrosed due to permanent arrest of blood supply to that area. The cause of arrest of blood supply is due to formation of thrombus in a region narrowed by atherosclerotic plaques (coronary thrombosis). The ECG shows deep Q wave, S-T segment elevation and T wave inversion. In early stage, the prominent Q wave may not be observed. Only elevation of RS-T segment in lead I, aVL and V_5–V_6 may be observed. Depression in RS-T segment may be observed in leads II, III, and aVF. In old infarction, there is prominent Q wave which is observed in lead I, aVL, and V_5–V_6.

Mean Electrical Axis of the Heart

It is the *resultant* electromotive force passing through the heart at a definite direction. The electromotive force having its definite magnitude and direction is known as vector. So each vector is the measure of the *instantaneous* electromotive force of the heart and its directions may be regarded as the instantaneous electrical axis.

Determination of Electrical Axis

Electrical axis of the heart is determined on the hypothesis of Einthoven equilateral triangle. According to the hypothesis of Einthoven triangle, the heart assumed to be a centre point of electrical generator in the equilateral triangle which is formed by joining the three parts of the limbs and from which the different leads in standard limb leads have been led off. According to the Einthoven principle, the sum total voltage of QRS in lead II is equivalent to the sum total voltage in leads I and III. According to this method, the centre point of the triangle is drawn by drawing perpendicular line from middle point of each of the sides of the equilateral triangle. From the centre, a circle is drawn outside the triangle. Conventionally the upper half of the circle is taken as negative and the lower half of the circle as positive. The upper half is divided equally so as to represent 0° to – 180° and the lower equally to represent 0° to + 180° (Fig. 34.17). A scale is drawn from the middle point of each side of the triangle. The scale represents the amplitude in mV. Negative and positive representation in the scale is generally made according to the nature of connection in each lead (Fig. 34.5). For determining the axis, simultaneous ECG at least in two standard limb leads are required. The sum total value of the QRS complex of two leads is plotted on the sides of the triangle in the positive or negative direction depicting the specific lead and a new point is obtained for each lead (A, B). If the value is positive then it is plotted in the positive direction and if it is negative then it is plotted in the negative direction depicting the specific lead. From each new point a perpendicular is drawn which intersects the other line inside the triangle at point C. The centre O is joined with the point C and is extended outward so as to reach the circle at a point which is the actual axis of the heart (Fig. 34.18). The normal range of axis deviation lies within –30° to + 110°. If the axis is beyond –30° then it is called abnormal left axis deviation and if it is beyond + 110° then it is called abnormal right axis deviation (Fig. 34.17).

The abnormal right axis deviation occurs in right bundle branch block, posterior myocardial infarction and right ventricular hypertrophy. The abnormal left axis deviation occurs in left bundle branch block, left right ventricular hypertrophy and antero-lateral myocardial infarction.

Ventricular Conditions that may Cause Axis Deviation

Change of position of the heart: Change of position of the heart has got a little effect on the axis. The change is only 20° to 30° from the original value. If the heart is shifted to the left then the axis is deviated to the left. During expiration left axis deviation and during inspiration right axis deviations are often observed.

Hypertrophy of the ventricle: Hypertrophy of the ventricle alters the axis greatly. It is generally due to increase of muscle mass. Axis is generally deviated towards the hypertrophied side. This is due to increase of muscle mass in the hypertrophied side; the depolarisation wave takes much longer time to cover the hypertrophied side than the normal one. Accordingly the normal ventricle becomes depolarised earlier than the hypertrophied one and strong vector is shifted towards the hypertrophied side. For this reason, the right axis deviation is observed in hypertrophy of the right ventricle and left axis deviation in case of the hypertrophy of the left ventricle.

Bundle branch block: In bundle branch block the axis is deviated towards the side of the block. Because in

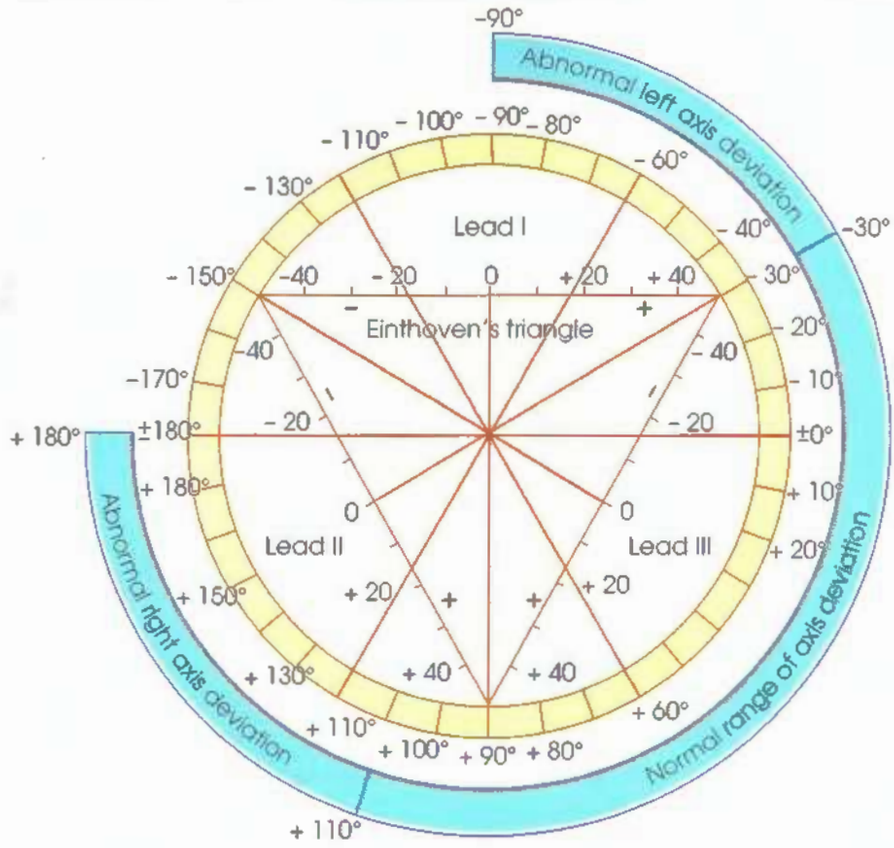

Fig. 34.17: Diagram showing the normal and abnormal ranges of axis deviation

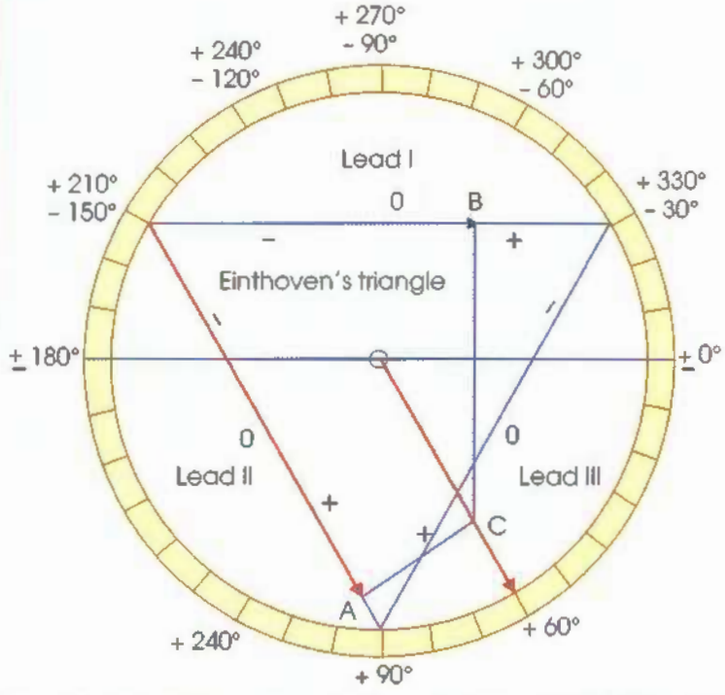

Fig. 34.18: Calculation of the electrical axis. The net deflection of QRS complex plotted on equilateral triangle

normal condition the impulse is transmitted to the lateral walls of two ventricles through the two branches almost at the same instant and two ventricles depolarises almost at the same time. If one branch is blocked then the impulse will be transmitted earlier by the normal one. The affected side will be depolarised earlier and the affected side will remain still in polarised state. So, the axis will be deviated towards the side of injury or a block. In left bundle branch block the deviation will be towards the left and in the right bundle branch block the axis will be deviated towards the right.

ECG changes due to altered ionic composition

1. Increased potassium levels in extra-cellular fluid (hyperkalaemia) produces tall T waves and abnormal and prolonged QRS complex.
2. Decreased potassium levels in extra-cellular fluid (hypokalaemia) produces prolonged PR interval, T wave inversion, depression of ST segment and prominence of U wave.
3. Decreased concentration of sodium in extra-cellular fluid (hyponaetraemia) produces low voltage waves in ECG.
4. Decreased concentration of calcium in extra-cellular fluid (hypocalcaemia) produces prolonged QT interval and ST segment.
5. Increased concentration of calcium in extra-cellular fluid (hypercalaemia) produces calcium rigor.

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EXAM-ORIENTED QUESTION

Short Notes

1. Explain the various methods of recording of an ECG.
2. Describe the significance features of ECG recording in standard limb leads, chest leads and augmented leads.
3. Describe the significance of vector analysis in ECG.
4. Discuss the ECG changes in various pathological disorders.

Chapter

35

Innervations of Heart and Heart Rate

INTRODUCTION

Nerves of the Heart and their Action

The regulation of the heart is effected through the afferent (centripetal) and efferent (centrifugal) nerves of the heart (Fig. 35.1).

Afferent Nerves

1. Impulses are carried from the heart through the vagus nerve and from the aortic arch via the aortic nerve to the cardiac centre in the medulla.
2. Sympathetic nerve supply is mediated via the inferior cervical and first four thoracic ganglia and first four thoracic nerve roots into the spinal cord.
3. The impulses are carried from the carotid sinus through the sinus nerve, a branch of glossopharyngeal nerve to the cardio respiratory centres in the medulla.

The *efferent nerves* are: Vagus and sympathetic (Fig. 35.1).

Vagus Nerves

The pre-ganglionic fibres of the vagus nerves arise from the dorsal nuclei of the vagus situated in the floor of the fourth ventricle in the medulla (Fig. 35.1). The parasympathetic output is from nucleus ambiguus and to a lesser extent from the dorsal motor nucleus. The solitary nucleus, being an integrating hub for the baro-reflex, receives sensory input about the state of the cardiovascular system. After their origin, they descend downwards. The cardiac fibres separate from the main nerve trunk in the neck and proceed towards the heart and form deep and superficial cardiac plexuses with the fibres of the sympathetic. The fibres reach the atrial muscle and make synaptic connections with intra-ganglionic cells situated near the sino-atrial and atrio-ventricular nodes. From here the post-ganglionic fibres arise and supply the specialised tissue of the sino-atrial and atrio-ventricular nodes and also extend between the muscle fibres. The post-ganglionic fibres do not extend beyond the upper part of the bundle of His and the base of the ventricles. Ventricular myocardium of the apex is said not to receive any vagal fibres. The vagus nerves also supply fibres to the coronary vessels.

Tonic Action of the Vagus Nerves

The vagus nerve exerts a tonic inhibitory control over all parts of heart. Acetylcholine is released by the post-ganglionic fibres on stimulation. Atropine prevents action of acetylcholine on the cardiac muscle and the heart rate is increased even up to 150 per minute in human beings. This proves that a constant inhibitory influence is exerted by the vagus on heart. The vagal tone is of reflex in origin, being produced by the sino-aortic nerves. Section of these nerves reduces the vagal tone and increases the heart rate.

Stimulation of the Vagus Nerves

Stimulation causes the following effects:

1. *The heart rate is slowed down:* Even it may stop. This is caused by reducing the rhythmicity of the SA node. This is known as negative chronotropic effect of the vagus nerve.
2. *The conductivity of the bundle is reduced:* Thus, producing various degrees of heart block. This effect is known as negative dromotropic effect. This may be an additional cause for slowing.
3. *The force of contraction is diminished:* In the frog, the vagus nerve directly depresses ventricular muscle. This is known as the negative inotropic effect of the vagus nerve.
4. The duration of systole is diminished, but the duration of diastole is increased.
5. The length of refractory period (which depends upon the systolic length) is diminished.
6. Excitability of the heart is also reduced and this condition is known as negative bathmotropic effect.

On the whole, the vagus acts as the inhibitor of heart.

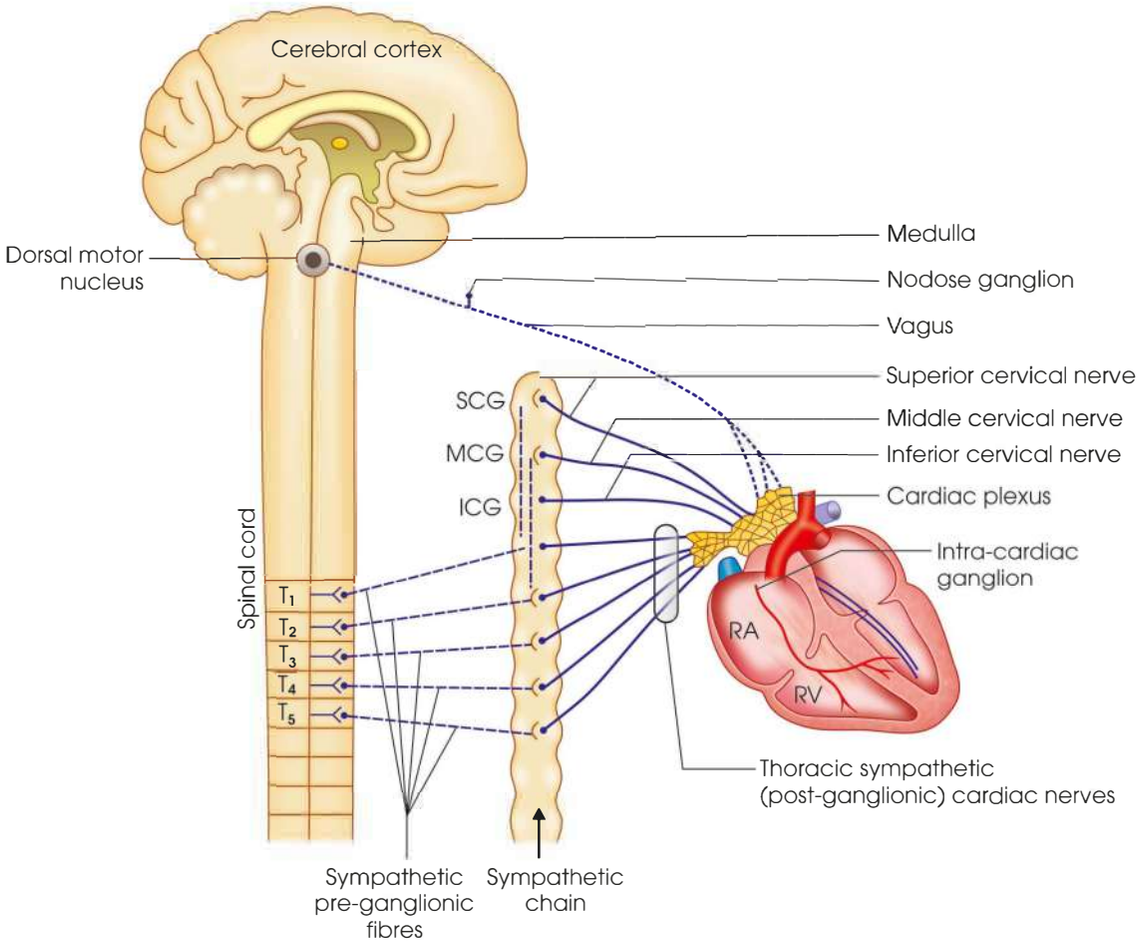

Fig. 35.1: Autonomic innervations of cardiac nerves

Sympathetic Nerves (Fig. 35.1)

The sympathetic fibres arise from the intermediolateral cell of the spinal cord (thoracic cords T_1 to T_5) before merging into the sympathetic trunk. The preganglionic fibres synapse in the sympathetic ganglia of the cervical and thoracic regions, which in turn, pass on post-ganglionic fibres to the cardiac plexus. The superior cardiac nerve emerges from superior cervical ganglion; middle cardiac nerve emerges from middle cervical ganglion and inferior cardiac nerve emerges from inferior or stellate cervical ganglion.

The cardiac nerve fibres supply the specialised tissues of the sino-atrial and atrio-ventricular nodes, atrial and ventricular muscles. The sympathetic fibres carry fibres to the coronary vessels and α-adrenergic receptors mediate vasoconstriction and β-adrenergic receptors mediate vasodilation. The sympathetic exerts a light tonic accelerating action on the human heart. Noradrenaline is released by the post-ganglionic fibres of the sympathetic on stimulation.

Effects of Stimulation of the Sympathetic Nerves

1. *Increases the frequency of heart rate* (*accelerator*): It is due to its effect on sino-atrial node. This is known as (positive) chronotropic effect of the sympathetic nerves.
2. *Increases the force of contraction (augmentor):* It is due to its effect on atrial and ventricular muscles. This is known as (positive) inotropic effect of the sympathetic nerves.
3. Increases the excitability (positive bathmotropic) and irritability of heart and thereby may cause extra-systoles (ectopic beats).
4. Increases the conductivity (positive dromotropic) of the myocardium and the bundle of His. On the whole the sympathetic nerve is taken as the accelerator and augmentor of heart tone. (Of the two nerves—vagus and sympathetic—the former exerts a much stronger influence on the heart than the latter).

Cardiac Centres

1. The dorsal motor nucleus of vagus in the medulla (nucleus tractus solitarius and nucleus ambiguus) is the cardiac inhibitory centre and it transmits continuous tonic inhibitory vagal impulse to the heart. This centre has got direct connection with the afferent nerves coming from the baroreceptors or chemoreceptors. Reflex bradycardia during the rise of systemic blood pressure is due to stimulation of the cardio-inhibitory centre. On the other hand,

tachycardia is observed during the fall of blood pressure, which is due to inhibition or depression of the cardio-inhibitory centre along with the withdrawal of vagal tone from the heart. Under such state sympathetic cardiac centres get the upper hand.

2. The higher cardio-accelerator centre or vasomotor centre is located in the reticular formation of medulla oblongata. With the increase in the activity of the pressor centre along with the rise of blood pressure, heart rate and stroke volume are also increased. This pressor centre possibly functions with the superior control of the same in the hypothalamus and cerebral cortex.
3. The sympathetic supply is by sympathetic nerve cells which lie in intermedio lateral horn cells of the upper thoracic segments of the spinal cord (T.1–5). The pre-ganglionic fibres enter the sympathetic ganglia to connect with the cells of thoracic ganglia and inferior, middle and superior cervical ganglia. In animals and even in human beings, the first thoracic ganglion and inferior cervical ganglion are fused to form the stellate ganglion from which post-ganglionic fibres (accelerator fibres) run directly to the heart. The function of the cardio-accelerator centre of the spinal cord is modified by the higher centres.
4. *Hypothalamus:* The caudal hypothalamus controls sympathetic activity while rostral hypothalamus is responsible for para-sympathetic response. Sympathetic activities on the heart are increased following stimulation of these centres. These centres have got connections with motor and premotor areas (centres) of the cerebral cortex. If these cortical areas are stimulated, the increase of heart rate is encountered cerebral cortex exerts influence via limbic system and hypothalamus.

Heart Rate

The normal heart rate in males is 72 beats per minute (the range is from 70 to 80 per minute) and it is slightly higher in females than in males. The higher heart rate in females may be due to (a) lower blood pressure, (b) more sympathetic tone.

Apart from sex, heart rate depends on the following factors:

1. *Age:* Roughly the heart rate is inversely proportional to age. Heart rate during various stages of life is listed in Table 35.1. At younger ages, the cause may lie in the higher basal metabolic rate (BMR). In old age, the rate is slightly higher—probably due to compensatory circulatory adjustment against gradual circulatory failure with the aging process.
2. *Metabolic rate:* Heart rate is directly proportional to the metabolic rate. They always run parallel. Anything that increases the basal metabolic rate, viz. exercise, excitement, etc. will also increase the heart rate. Similarly, factors which reduce basal metabolic rate diminish the heart rate.
3. *Respiration:* During inspiration heart rate increases; during expiration it falls. This phenomenon is known as sinus arrhythmia. It is very often found in healthy children. In adults, it becomes prominent after deep voluntary respiration.
4. *Size of the animals:* It is seen that under normal conditions, heart rate has an inverse relation with the size of the animal. Smaller the size, greater the heart rate. For instance, heart rate of canary is about 1,000 per minute, that of an elephant, only 25 per minute.

Table 35.1: Heart rate during various stages of life

Age/Stage	*Heart rate*	*Age/Stage*	*Heart rate*
Foetus	140–150 per min	3rd year	95–100 per min
Newborn	130–140 per min	7–14 years	80–90 per min
1st year	115–130 per min	>15 years	70–80 per min
2nd year	100–115 per min	Old age	75–80 per min

Regulation of Heart Rate

Heart rate can be adjusted according to the metabolic needs. In exercise the rate increases; during sleep it falls. The purpose of this regulation is to maintain an optimum blood pressure and an optimum rate of blood supply to the tissues.

Mechanism of Regulation

The mechanism involves two factors:

1. *The local mechanism:* This includes the SA node and the junctional tissues. Any factor, which by its local action on the SA node can alter its rhythmicity, will also alter the heart rate.
2. *The nervous mechanism:* It includes:
   Cardio-inhibitory centre—connected with the vagus.
   Cardio-accelerator centre—connected with the sympathetic. Stimulation of the vagus depresses and that of the sympathetic increases the heart rate.

Inhibitory effect: Of the two nerves, the vagus exerts a stronger action. Atropine prevents action of acetylcholine to the cardiac muscle and increases the heart rate even up to 150 per minute. This proves that the vagus exerts a tonic inhibitory control over heart rate. This vagal tone is reflexly produced through the sino-aortic nerves. The sympathetic also exerts a tonic accelerating effect but to a lesser extent.

The variations of heart rate, under normal physiological conditions, are mainly brought about by alteration of vagal tone. It is only in extreme cases that sympathetic stimulation comes into play.

Thus, alterations of heart rate may be brought about in two ways; (a) by affecting the local mechanism, i.e. the node and the junctional tissues or (b) by acting through the nervous mechanism. The cardiac centres, again, may be influenced in two ways directly and reflexly. There is evidence to show that the two cardiac centres are in reciprocal relation. Stimulation of one will depress the other and *vice versa*.

Factors Affecting Heart Rate (Fig. 35.2)

1. *Impulses from the higher centres:* Excitement generally quickens the heart rate but sudden shock may slow or even stop the heart rate.
2. *Respiration:* During inspiration heart rate increases; during expiration it falls. This phenomenon is known as sinus arrhythmia. The normal sinus arrhythmia also known as respiratory sinus arrhythmia (RSA) is variability in heart rate in synchrony with respiration. The heart rate is under control of regulatory centres in the medulla oblongata. The nucleus ambiguus (one of the cardiac regulatory centres) via the vagus nerve increases parasympathetic influence over the heart. The vagus nerve inhibits the heart and decreases the rate of SA node firing thus decreasing the heart rate. Inspiration triggers inhibitory signals to the nucleus ambiguus and the vagus nerve remains unstimulated while on expiration the cells in the nucleus ambiguus are activated and heart rate is slowed down via vagal effect.
3. *Reflexes:* Factors influencing the cardiac centre have been presented in Fig. 35.3. Heart rate can be altered by various reflexes. For instance, (a) cardio-inhibitory reflexes, (b) cardio-accelerator reflexes, (c) reflexes from other parts of the body.
   Cardio-inhibitory reflexes (sino-aortic or Marey's reflex): There are stretch receptors in the carotid sinus and aortic arch. When blood pressure rises, these nerve endings become stimulated due to stretching; sensory impulses pass up through the sino-aortic nerves and increase the vagal tone, so that heart rate falls. When blood pressure falls, no inhibitory impulse passes up and heart rate rises. Thus, heart rate and blood pressure have an inverse relation. This is known as Marey's law. This sino-aortic effect is best seen when the initial blood pressure remains near about the normal range. Since the sino-aortic nerves help to maintain blood pressure variations through a limited range by regulating the heart rate, these nerves are called the buffer nerves of heart. (The inverse relation between heart rate and blood pressure does not hold well in all conditions. For instance, exercise, emotion, anoxia, etc. increase both heart rate and blood pressure.)
4. *Cardio-accelerator reflexes:* Venous engorgement of the right atrium and the great veins reflexly increases the heart rate. The afferent fibres, arising from the roots of the great veins and right atria, pass along the trunk of the vagus to the cardiac centre. Engorgement of these parts stimulates the nerve endings, reflexly inhibits the vagal tone and also stimulates the sympathetic to some extent. Thus, heart rate rises. This reflex is called Bainbridge reflex (more appropriately venous reflex). This happens in muscular exercise and in deep inspiration when venous return increases. This is also a cause of increased heart rate during congestive cardiac failure.
5. *Reflexes from other parts of the body:* Sensory stimuli from other parts of the body may alter the heart rate in either direction. Moderately painful stimuli generally quicken the heart rate. Stimulation of the central end of the fifth nerve or of the splanchnic nerve slows the heart rate. A sudden blow on the abdomen may stop the heart rate.
6. *Anoxia:* It increases the heart rate which is directly proportional to the degree of anoxia. The effect is due partly to its action on the cardiac centre and partly to reflex stimulation through the chemoreceptors in the carotid and aortic bodies. This is one cause of rapid pulse in heart failure, anaemia, haemorrhage, high altitude, CO poisoning, etc.
7. *CO_2 excess:* In moderate amounts it increases the heart rate, partly by its direct action and partly reflex. But in larger amounts it produces heart block and reduces heart rate. (It should be noted that circulation is more sensitive to O_2 lack but respiration is more sensitive to CO_2 excess.)
8. *Body temperature:* Rise of body temperature (muscular exercise, fever, hyperthyroidism, etc.) increases the heart rate (a) by acting on the SA node, and (b) by stimulating the cardio-accelerator centre.
9. *Increased intra-cranial pressure:* It slows the heart rate by directly stimulating the vagus.
10. *Adrenaline:* Since it acts like the sympathetic, it should accelerate the heart rate, but in intact animals the rate is often reflexly reduced. Because, by causing vasoconstriction, adrenaline raises blood pressure and thus mobilises the sino-aortic reflex, so that heart rate may be reduced. But the force of contraction invariably rises.
11. *Thyroxine:* It quickens the heart rate (a) by directly stimulating the metabolic rate of the sino-atrial node, (b) by increasing the basal metabolic rate of the body, and (c) by a probable stimulating effect on the sympathetic.
12. *Muscular exercise:* It increases the heart rate. Because most of the factors concerned with acceleration, are operating during exercise. For

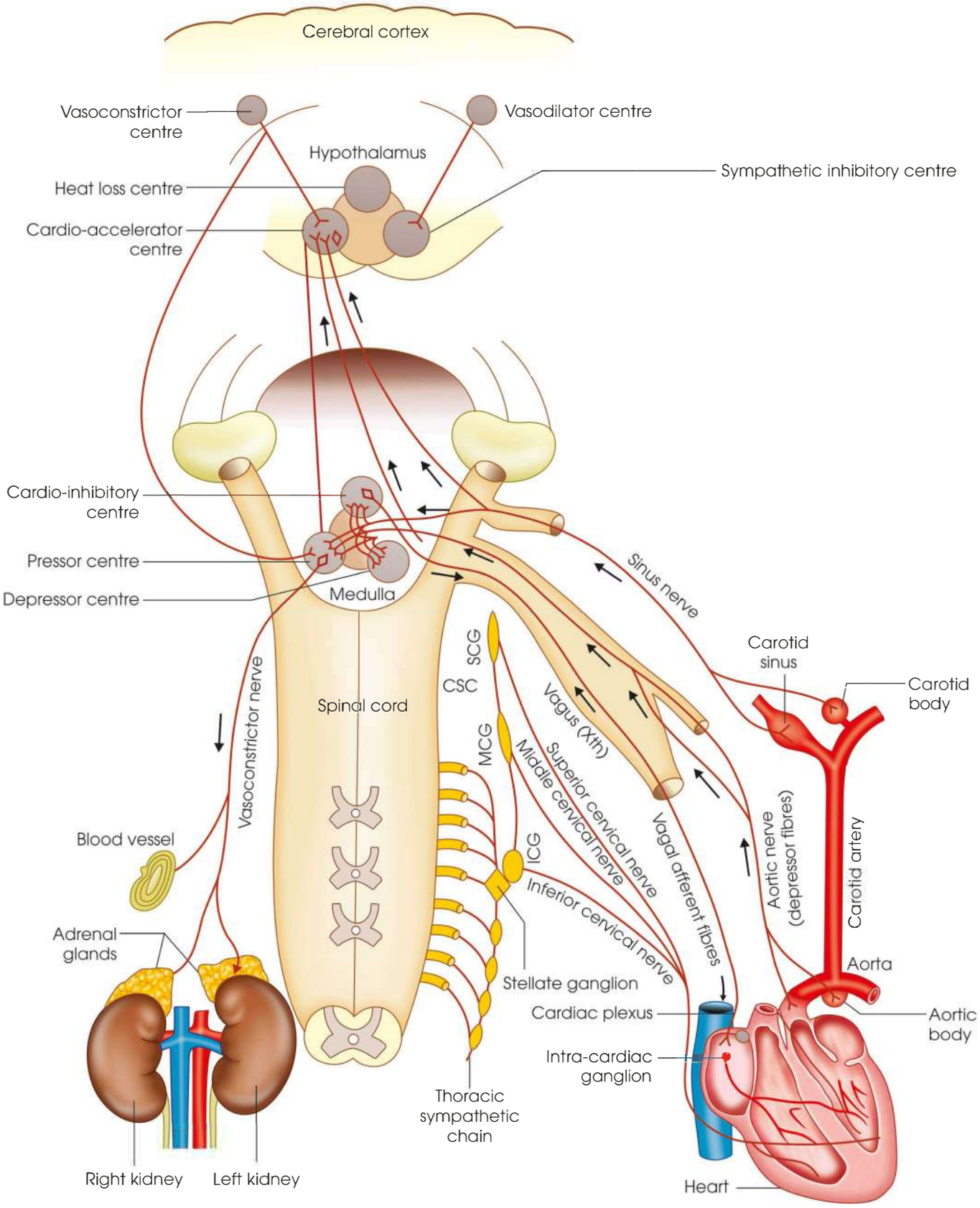

Fig. 35.2: Factors influencing the cardiac centre

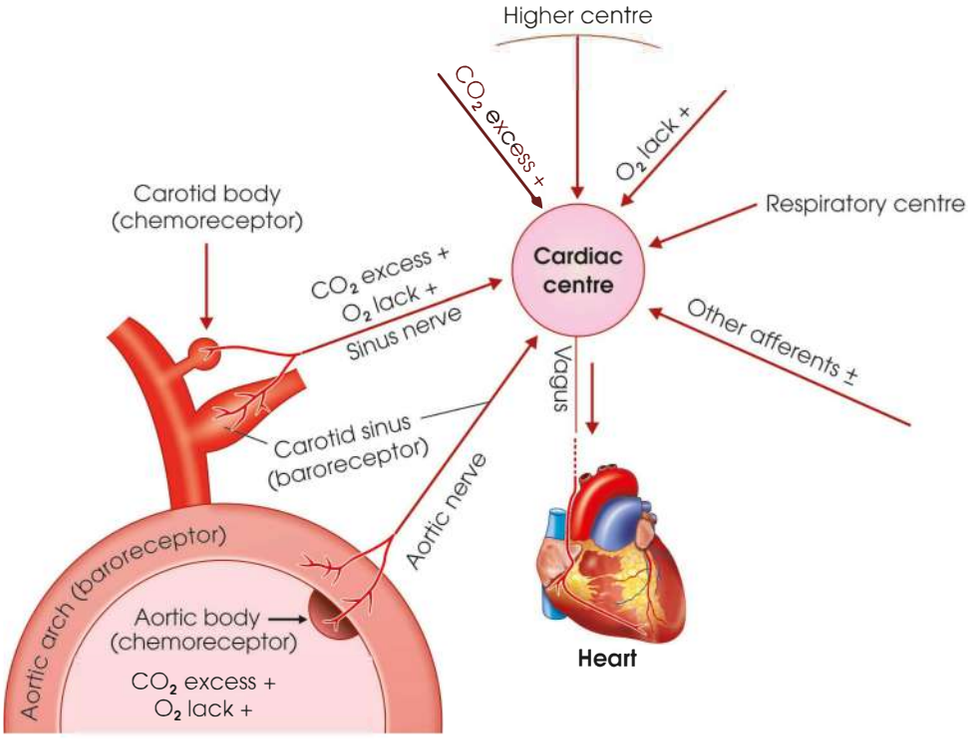

Fig. 35.3: Factors affecting heart rate

instance, increased venous return initially mobilises Bainbridge reflex. Increased respiration, excitement, anoxia, CO_2 excess, adrenaline secretion, etc. all come in and increase the heart rate.

EXAM-ORIENTED QUESTIONS

Essay

1. Describe the nerves of heart and their actions.
2. Define heart rate and normal physiological values. Discuss the factors affecting heart rate.
3. Explain the mechanism involved in regulation of heart rate.

Short Notes

1. Sino-aortic reflex
2. Bainbridge reflex
3. Cardiac centres

Chapter

36

Cardiac Output

INTRODUCTION

At each beat certain amount of blood is pumped out by each ventricle into the circulation. This is called cardiac output.

Two terms are used

1. Stroke volume or systolic discharge (output)
2. Minute volume (minute output).

Stroke volume means the output per ventricle per beat. Minute volume means the output per ventricle per minute. Hence, minute volume = stroke volume × heart rate. As the volume of blood put out by both sides of the heart is same, cardiac output is to be multiplied by 2 so as to calculate the quantity of blood pumped by the heart as a whole.

Normal Values

In adults, the average stroke volume is 70 ml and the minute volume about 5–6 litres. In other words, the amount of blood expelled per ventricle per minute, is approximately the same as the total blood volume of the body.

The output is directly proportional to the metabolic rate, and as such, to the surface area and body weight. The cardiac output per minute per square metre of body surface is known as cardiac index. The average value is 3.3 litres. The surface area of an average-sized adult is about 1.7 m^2. Accordingly, the average cardiac index is about 3.3 litres/min/m^2 (5.6/1.7). The stroke volume per square metre of body surface is known as stroke volume index. The average value is 47 ml. Any factor that increases or diminishes basal metabolic rate, body weight or surface area, also alters the minute volume proportionally.

DISTRIBUTION OF CARDIAC OUTPUT

Since venous return per minute should be the same as the minute output, it follows that blood flow through the tissue per minute must also be the same. In other words, 5 litres of blood passes out per ventricle per minute, 5 litres of blood flows through the tissues per minute and the same 5 litres come back to heart per minute. It is really astonishing, how accurately these constant relations of time and quantity are maintained.

Although full data are not known, yet the minute volume of heart is mainly distributed as follows:

1. Kidneys: 1,300 ml per minute
2. Brain: 700–800 ml per minute
3. Coronary: 200 ml per minute
4. Muscle: 600–900 ml per minute
5. Liver: About 1,500 ml per minute

Total quantity of blood distributed in these organs does not exceed 4,500 ml per minute. So the remaining amount is distributed to the skin, bones and gastrointestinal tract.

CARDIAC RESERVE

It is the capacity of heart to generate sufficient energy for expelling a large quantity of blood and for raising blood pressure above the basal level during emergency. Generally, the normal heart expels about 5 to 6 litres of blood per minute per ventricle and during exercise this amount may be about 30 to 40 litres per minute. According to Starling's law, it is nothing but physiological capacity of the heart.

CONTROL OF CARDIAC OUTPUT

Cardiac output depends upon the following four factors:

1. Venous return
2. Force of heartbeat
3. Frequency of heartbeat
4. Peripheral resistance

Venous Return

Anything that increases or diminishes the venous return will alter the cardiac output accordingly. Venous return depends upon the following:

1. *Muscular exercise:* When muscles contract, they squeeze the capillaries and venules and increases venous return.

This is aided by the valves of veins, which prevent the passage of blood back towards the capillary bed.

2. *Respiration:* During inspiration intrathoracic pressure falls and intra-abdominal pressure rises. Hence, with each inspiration venous blood is sucked up by the thorax and is pumped out by the abdomen.
3. *Pressure difference between capillaries and venules:* Normally, there is a slight positive pressure (32–12 mm of Hg) in the capillary area (capillary tone), while in the great veins it may be even negative. Vascular dilatation without fall of general blood pressure will increase the capillary pressure and thereby raise the venous return (viz. muscular exercise). But if it causes a general fall of blood pressure—as in shock, venous return will fall.
4. The vasomotor system adjusts the lumen of the arterioles and venules and thereby alters the venous return.

Force of Heartbeat

The strength of contraction depends mainly on three factors:

1. *The initial length of the cardiac muscle:* Within physiological limits, greater the initial length, stronger will be the force of contraction [Starling's law (Fig. 36.1) which is an inherent, self-regulating mechanism that permits heart to adjust to changing end-diastolic volumes]. Heterometric regulation of cardiac output: It is obvious that the initial length is proportional to the degree of filling, which again, depends on the venous return.
2. *The length of diastolic pause:* Filling, rest and recovery takes place during diastole. Hence, with a shorter diastolic period which is inadequate for these, the force of contraction will diminish unless the rate of venous return is raised.
3. *Contractility:* Sympathetic stimuli make myocardial fibres contract with greater strength at any given length (homeometric regulation of cardiac output). Contractility determines the change in peak biometric force at a given initial fibre length (end-diastolic volume). Contractility can be augmented by certain drugs such as epinephrine or digitalis or by tachycardia. The positive inotropic effect produced is reflected in incremental increase in developed force and velocity of contraction.
4. *Nutrition and oxygen supply:* An adequate supply of nutrition and oxygen is essential for efficient cardiac activity. In addition to this an optimum H-ion concentration, a proper balance of inorganic ions and an appropriate temperature and pressure, are also required for strong heartbeat.

Frequency of Heartbeat

1. Heart rate affects both stroke volume and minute volume by altering the length of diastole and thereby the degree of filling and force of contraction.
2. It should be noted that blood pressure depends upon the minute volume and not on the stroke volume. The following consideration will clarify. Venous return remaining constant, the rise of heart rate will reduce the diastolic pause and therefore the stroke volume. But the product–stroke volume × heart rate may not fall; even it may rise above the resting value. Thus, minute volume and therefore blood pressure (BP) may rise even if the stroke volume falls. This happens with a moderate rise of the heart rate.
3. But if the heart rate be too high, the stroke volume becomes so low that the minute output falls far below the normal (Fig. 36.2). Blood pressure drops and the subject may be unconscious. This happens in paroxysmal tachycardia when the frequency suddenly becomes 150–200 per minute. (Muscular

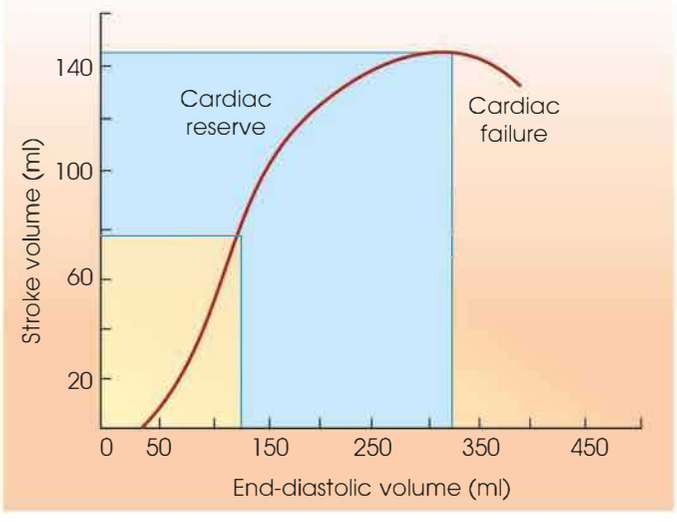

Fig. 36.1: Graphical representation of Starling's law of heart. Showing when the end-diastolic volume increases, ventricles contract more vigorously and thereby stroke volume increases

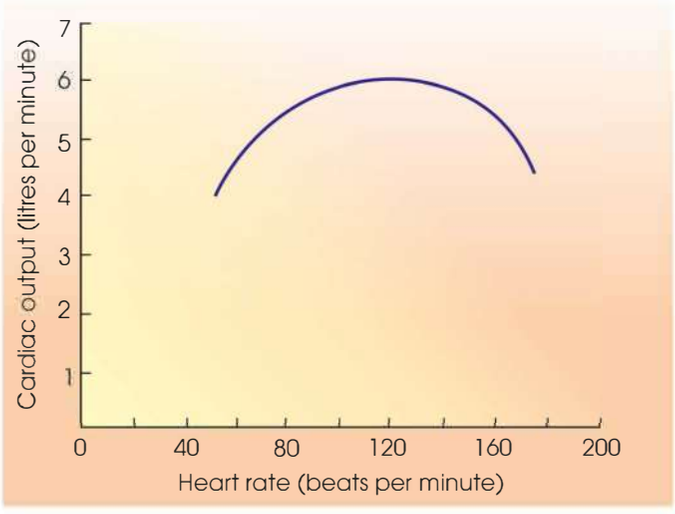

Fig. 36.2: Graphical representation of the relationship of cardiac output and heart rate assuming constant venous pressure and ventricular residue (diagrammatic)

exercise is an exception. Here, both the frequency of heartbeat and the rate of venous return increase. Cardiac filling becomes more than normal even during the short diastolic period. Hence, both stroke volume and minute output increase.)

4. On the other hand, when the heart rate becomes very slow (as in heart block)—though the stroke volume is much bigger than normal, yet the minute volume may fall, because the product may be less than normal. But with a moderate slowing the minute volume may not fall at all. In some instances it may rise (recovery from heart failure). Thus, alteration of heart rate on either side will generally raise the minute volume up to a certain extent. Beyond that, the minute output will fall.

But when normal functioning (shaded portion) is beyond the range, the stroke volume decreases and the relationship is reversed.

Relation with Peripheral Resistance

Heart maintains a constant cardiac output and blood flow even against increased peripheral resistance (after-load). An optimum blood pressure is essential for adequate cardiac activity. General vasoconstriction of the arterioles will cause an increase in blood pressure. Heart at first fails to expel all its blood but in the next heartbeat the filling becomes more, because the normal venous return is added upon the residual blood. Consequently, the initial length becomes bigger, the heart contracts with greater force and the normal output is restored.

Factors Influencing Cardiac Output

1. *Muscular exercise:* In heavy exercise the output may be 30–40 litres, i.e. 6–10 times the normal minute volume (stroke volume 170–200 ml; heart rate 150–180 per minute).
2. *Posture:* The minute volume is greater in the recumbent posture than in standing, because gravity retards venous return in the latter.
3. Stroke volume is also determined in part by neural input, with sympathetic stimuli making the myocardial muscle fibres contract with greater strength at any given length and parasympathetic stimuli having the opposite effect. The action of catecholamines liberated by sympathetic stimulation on strength of contraction is called their inotropic action. When the strength of contraction increases without any increase in fibre length more of the blood that normally remains in the ventricle is expelled that is the ejection fraction increases.

Other Conditions

1. *Fever, hyperthyroidism, excitement* (10–25%): Adrenaline, ingestion and digestion of food (10–20%), anoxia, CO_2 excess, intravenous (IV) saline, pregnancy (45–85% of full term), etc. increase cardiac output.
2. Hypothyroidism, haemorrhage, shock, heart failure, etc. reduce cardiac output.
3. *Sleep:* May reduce slightly but usually not much.

METHODS OF MEASURING CARDIAC OUTPUT

In animals, the output can be measured with the help of:

1. Dye method
2. Fick principle using O_2 or CO_2
3. Physical method (ballistocardiography)
4. Echocardiography

1. Dye Method: Stewart and Hamilton's Dye Dilution Method

The dye method is often the method of choice. A known quantity of Evans blue (non-diffusible dye, known as T-1824) is injected intravenously. The dye will circulate through the heart, lungs and appear in the carotid artery. The concentration of the dye when it first appears in the artery is determined with the help of colorimeter from the samples of arterial blood taken every few second interval. The concentration of each sample of blood is determined by photocolorimeter and plotted on a semilogarithmic paper. The dye concentration rapidly rises to a peak, then falls and rises again due to recirculation of dye. The mean concentration of the dye is mined with the help of the following formula; $F = D/ct$, where F = volume flow in litres per second, D is the quantity of dye (Evans blue) injected, c is the mean concentration of the dye and t is the duration in seconds of the first passage of dye through the artery.

2. Fick Principle Using O_2 and CO_2

Cardiac output (CO) can be evaluated indirectly from whole body oxygen consumption (VO_2) and the mixed venous (CvO_2) and arterial oxygen concentrations (CaO_2) employing Fick's principle. This technique is seldom used nowadays.

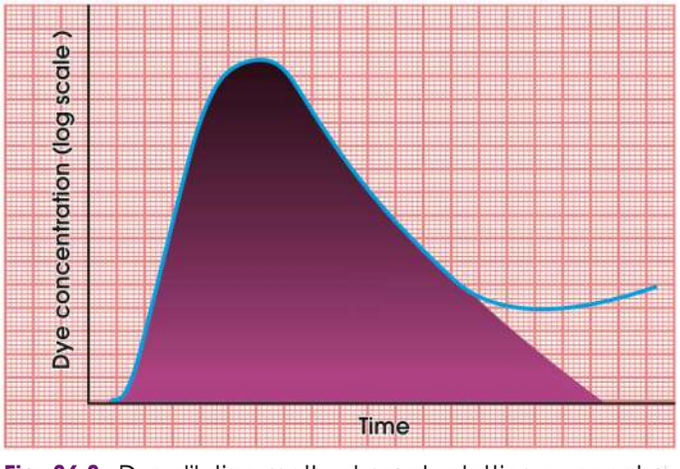

Fig. 36.3: Dye dilution method graph plotting concentration against time

Fick principle using O_2: (a) O_2 uptake in lung is measured over 5–10 minutes by spirometry or expired air collection in a Douglas bag. In present days, O_2 uptake is measured with spirometry. (b) O_2 content of arterial blood is calculated from Hb% assuming 95% saturation. (c) A sample of mixed venous blood is collected from the right atrium by introducing a fine rubber catheter into the cubital vein and pushing it gradually into the right atrium (Cournand's method) and its O_2% is determined. From these data minute volume can be calculated as shown below.

Fick principle: It was shown by Fick that cardiac output can be calculated by noting certain data about O_2 or CO_2 exchange.

When applied to oxygen it will be as follows: O_2% of mixed venous blood is determined, say 15 ml. O_2% of arterial blood is determined, and say 19 ml. **Arteriovenous O_2 difference per 100 ml** of blood is therefore, 4 ml.

Total O_2 consumption per minute (Douglas bag) say is 200 ml.

Hence, each 100 ml of venous blood while passing through lungs, takes away 4 ml of O_2.

Therefore, 200 ml of O_2 will be carried away by 100/4 × 200 ml = 5 litres of blood. Obviously, this is the minute output of right ventricle, which is same as that of left ventricle. Similar figures will be obtained by applying the principle to CO_2.

So, the cardiac output can be determined with the help of the following formula: Cardiac output (ml/min)

$$= \frac{\text{Consumption of } O_2 \text{ (ml per minute)}}{\text{Difference of } O_2 \text{ content in arterial and venous blood (ml/min)}}$$

Fick principle using CO_2: This method can be readily adopted at the bedside. The steps are as follows: (a) CO_2 output per minute is determined with Douglas bag. (b) Alveolar air is collected and its CO_2 tension, which is identical with that of arterial blood, is determined. (c) Alveolar air is again collected after holding the breath for 5 seconds. The CO_2 tension of this air is same as that of venous blood. CO_2% of arterial and venous blood is then determined from the CO_2 dissociation curves. From these data minute volume can be calculated.

Note

1. In man, there is difficulty in applying direct Fick's method during heavy exercise. So, the other method to dye method is preferred.
2. Fick's methods are infrequently in used for measurement of cardiac output as there is difficulty in collecting and analyzing exhaled gas concentration to level of accuracy.

Adolf Eugen Fick was the first to measure cardiac output, using what is now called the Fick principle. He introduced a law of diffusion called Fick law of diffusion in 1855. He was the first one to develop a technique for measuring cardiac output in 1870.

Adolf Eugen Fick
1829–1901

3. Physical Method: Ballistocardiography

a. *Ballistocardiography:* Another method of determining cardiac output in human beings is the ballisto-cardiography which was originally devised by Henderson and latter on modified by Starr and his associates. The method is based on the principle of Newton's third law of motion "every reaction has an equal and opposite reaction."
b. Ballistocardiogram is a record of the recoil of the body caused by the movement of heart and blood within it in opposite direction. It can be recorded by allowing subject to lie on a suitably suspended table.
c. The more convenient method is the recording of the movement of a steel rod kept on the stretched legs while lying supine on a fixed table. The movement of the rod can be recorded by suitable sensitive electronic instrument.

The procedure of ballistocardiography is not in practice today as method for evaluation of cardiac output.

4. Thermo-dilution Method (Cold Saline Method)

This method is based on the indicator-dilution principle applicable to injectates that cause changes in blood temperature detected downstream. An injectate of known volume and temperature is injected into the right atrium and the cooled blood traverses a thermistor in a major vessel branch downstream over duration of time. On application of indicator-dilution principle to estimate cardiac output; the cardiac output is found to be inversely proportional to the mean blood temperature depression and the duration of transit of cooled blood (i.e. area under the curve as in indicator-dilution method).

Limitations

- The result is affected by the phase of respiration and as a caution it should be measured at the same point of respiratory cycle.
- The variations in the speed of cold water injection can result in altered measurement.

History of Echocardiography

Drs. Inge Edler and Carl Helmuth Hertz at the History of Ultrasound Symposium in Washington D.C. in October 1988

Cardiologist Inge Edler and Hellmuth Hertz, Physicists were the pioneers of echocardiography. They first reported the continuous recording of the heart walls movement in 1954 and described the use of the ultrasonic cardiogram for mitral valve diseases in 1956.

In 1977, Edler and Hertz were joint recipients of the Lasker Prize, which is the American equivalent of the Nobel Prize in medicine.

5. Doppler Echocardiography

The cardiac output is determined by the Doppler echo by measuring cross-sectional area and velocity of blood flow along any one of the structures such as the aorta, the pulmonic artery, or across any of the valves. The pulsed ultrasound waves are emitted and directed parallel to flow of blood, e.g. down suprasternal notch into ascending aorta. The wavelength of sound is altered as it is reflects off moving red blood cells. The change in pitch indicates velocity of red blood cells. The aortic cross-section on Doppler is noted down.

Calculation

The length of blood flow—velocity in the ascending aorta in unit time is multiplied by the cross-sectional area of the aorta to give stroke volume. The stroke volume is multiplied to heart rate to give cardiac output.

EXAM-ORIENTED QUESTIONS

Essay

1. Define cardiac output and its normal value. Describe the factors affecting cardiac output.
2. Discuss the methods of estimating cardiac output. Describe the factors influencing cardiac output.

Short Notes

1. Factors affecting cardiac output
2. Methods of estimating cardiac output

REFERENCE

Acierno LJ, Worrell LT, Inge Edler: Father of echocardiography. Clin Cardiol 2002;25:197–9.

Chapter

37

Blood Pressure

INTRODUCTION

Blood pressure is the lateral pressure exerted by blood on the vessel walls while flowing through it.*

Four terms are in common use

1. *Systolic pressure (SP):* The maximum pressure during systole.
2. *Diastolic pressure (DP):* The minimum pressure during diastole.
3. *Pulse pressure (PP):* The difference between systolic and accepted diastolic pressure.
4. *Mean pressure (MP):* It is roughly the arithmetic mean of the diastolic pressure and the systolic pressure. But a close approximation to the mean pressure may be obtained by adding the diastolic pressure with one-third of the pulse pressure.** In true sense it is the level of the line having area between the pulse wave contour and the diastolic pressure level. In adults, the relation between the three-pressure is as follows; SP/DP/PP = 3/2/1, viz. if systolic pressure be 120, diastolic pressure should be 80 and pulse pressure 40 mm of Hg.

BASAL BLOOD PRESSURE

In adult males, the average systolic pressure is 125–130 mm of Hg + 15 (viz. from 110 to 145 mm of Hg); and average diastolic pressure is 70–90 mm of Hg. Although it is constant in a given individual, yet basal pressure varies in different ones with the following factors.

Physiological Variations

1. ***Age:*** Blood pressure rises with age. During infancy, the systolic pressure is from 70 to 90 mm of Hg; childhood, 90–110 mm of Hg; puberty, 110–120 mm of Hg; old age, 140–150 mm of Hg.*** At any age, a systolic pressure persistently above 150 mm of Hg and a diastolic pressure above 100 mm of Hg should be accepted as high. On the other hand, systolic pressure below 100 mm of Hg and diastolic pressure below 50 mm of Hg should be taken as low in the adults.

   Average blood pressure and standard deviations in apparently healthy persons (assuming diastolic end point is disappearance of sound) is listed in Table 37.1.
2. ***Sex:*** In females, both systolic and diastolic pressures are slightly lower than in males up to the age of 45–50 years.
3. ***Build:*** The systolic pressure is usually high in obese person. In most of the overweight persons the blood pressure is found to be high.
4. ***Exercise:*** In strenuous exercise the systolic pressure rises and may reach even up to 180 mm of Hg. In moderate exercise there is slight rise of systolic blood pressure.
5. ***Posture:*** The diastolic pressure is slightly higher in the standing position. In the recumbent position the diastolic pressure is lower than in the standing or in the sitting position.
6. ***Sleep:*** The systolic pressure falls by about 15 to 20 mm of Hg during sleep.
7. ***After ingestion of meals:*** There is a slight rise of systolic pressure.
8. ***Emotion of excitement:*** It causes increase of systolic pressure.

SIGNIFICANCE OF BLOOD PRESSURE

Systolic Pressure

It undergoes considerable fluctuations. Excitements, exercise, males, etc. increase it, while sleep, rest, etc.

*Lateral pressure is that pressure when force is exerted at right angles to the direction of flow at any point within a tube filled with a circulating fluid. Resistance is opposition to force.

**Sometimes, the MP is stated as the DP plus one-half of the PP.

***To obtain a rough estimate of the systolic pressure of a particular subject, several formulae are often used by clinicians. They are as follows: (1) 100 + age; (2) 100 + 2/3rds of the age; (3) 90 + age, etc.

Table 37.1: Average blood pressure and standard deviations in apparently healthy persons

	Males		Females	
Age group	*Systolic*	*Diastolic*	*Systolic*	*Diastolic*
20–29	124.0 ± 13.2	77.0 ± 9.5	116.5 ± 11.6	73.0 ± 9.4
30–39	126.5 ± 13.9	79.5 ± 10.0	122.0 ± 14.0	76.5 ± 10.4
40–49	129.5 ± 16.0	81.5 ± 10.2	129.0 ± 18.3	81.0 ± 11.1
50–59	136.5 ± 19.0	83.5 ± 11.4	138.0 ± 21.4	84.0 ± 12.0
60–69	142.5 ± 23.5	84.0 ± 11.2	149.0 ± 25.7	85.0 ± 13.4
70–79	145.5 ± 24.0	81.5 ± 14.1	158.5 ± 26.0	84.5 ± 14.2
80–89	145.0 ± 25.0	80.5 ± 12.4	155.5 ± 28.0	82.5 ± 15.2

diminish it. The height of systolic pressure indicates: (a) The extent of work done by heart, (b) the force with which the heart is working, (c) the degree of pressure which the arterial walls have to withstand.

Diastolic Pressure

It undergoes much less fluctuations in health and remains within a limited range. Consequently, variations of diastolic pressure are of greater prognostic importance than those of systolic. Diastolic pressure is the measure of peripheral resistance. It indicates the constant load against which heart has to work.

Pulse Pressure

It generally varies directly as the stroke volume. But this quantitative relation may not be true in all cases.

Physiological Significance of Blood Pressure

1. Blood pressure maintain a sufficient pressure head to keep the blood flowing.
2. Blood pressure provides motive force of filtration at the capillary bed, thus assuring nutrition to the tissue cells, formation of urine, lymph and so on.

From the above considerations, it is seen that, the range of blood pressure gives correct information's about the state of the circulatory system as a whole and also about the functional condition of the tissue cells and organs.

MEASUREMENT AND RECORDING OF BLOOD PRESSURE

Arterial Blood Pressure

This can be measured by two methods; (1) direct and (2) indirect.

Direct Method (Animal Studies)

The artery is exposed and an arterial cannula of which one tapering end is inserted directly into the lumen of the exposed vessel and the other end is connected to the U-shaped mercury manometer that shows the actual blood pressure in mm of Hg. As the mercury column in one limb (that has direct contact with the blood vessel) descends and the other limb of the U-tube ascends, the value in the scale will be doubled so as to get the actual blood pressure. For convenience it is generally considered to be 1 mm in the scale equivalent to 2 mm of Hg of pressure. Before recording the blood pressure, the mercury levels in both limbs of the U-tube must be adjusted to the 0 mark of the scale. For recording pressure a floating stylus with a writing pointer that marks on the smoked paper may be used (Fig. 37.1). This method is only suitable in animals and gives the idea about mean pressure. Due to high inertia of the mercury, the blood pressure changes associated with cardiac cycle are damped. Respiratory undulation of mean pressure waves are clearly seen in this direct method.

Direct methods in human: The blood pressure can be measured directly by an invasive method with the aid of pressure transducer. The cather/transducer is inserted into the antecubital vein via vena cava and reaches to right atrium, right ventricle up to pulmonary artery or cather/transducer can be inserted via brachial/femoral artery ⇒ aorta, left atrium up to left ventricle and thus this aids in evaluation of blood pressure and pressure in respective heart chamber. Though it is accurate, but is invasive.

Indirect Method

In indirect method, the pressure may be measured without any surgical procedure and thus it is very convenient clinically in human being. Riva-Roci (1896) first introduced this indirect method and afterwards Korotkoff (1905) introduced a convenient method by which the systolic pressure and diastolic pressure could be ascertained only through listening to a sound. This is the standard method of recording blood pressure all throughout the world. In this method commonly the pressure of the brachial artery is measured. The instrument used is known as ***sphygmomanometer***.

Three methods: (a) Oscillatory, (b) palpatory, (c) auscultatory.

Oscillatory method: Inspection of oscillation in spring gauge or mercury manometer is the basis of this

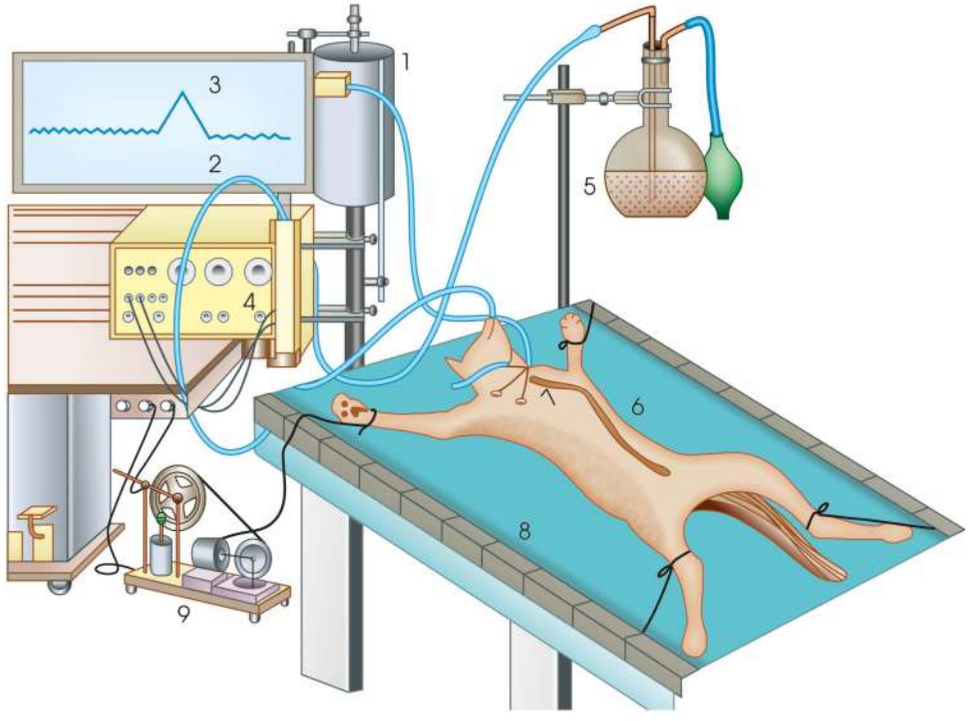

Fig. 37.1: Direct method for measurement of arterial blood pressure. 1: Kymograph, 2: Floating stylus for blood pressure recording, 3: Stylus for respiration recording, 4: Mercury manometer, 5: Reservoir, 6: Animal (cat), 7: Artery cannula, 8: Operation table, 9: Respiratory pump

method. In this method, a pressure cuff is wrapped over the brachial artery and the oscillations that are produced by the pulsations are observed. The instrument is always kept at the heart level. When the cuff pressure is increased and raised above the systolic pressure, the oscillations disappear, but on releasing the pressure gradually, the oscillations become larger and prominent. The pressure head at which the larger oscillations are seen is considered as systolic pressure. But on further release of pressure, the oscillations become smaller and disappeared. The pressure, at which the oscillation just becomes smaller or disappears, is known as diastolic pressure.

Palpatory method: The instrument is kept at the level of the heart and the cuff is tied round the upper arm. Pressure is raised to 200 mm of Hg and then gradually released. When the pulse just appears at the wrist, the pressure is noted. This is the systolic pressure. This method is not accurate. By this method, the diastolic pressure cannot be determined.

Auscultatory method: The instrument is kept at the level of the heart and the cuff is tied round the upper arm. Pressure is raised to 200 mm of Hg and then gradually released. Variations of sounds are heard with a stethoscope placing its chest piece on the brachial artery, a little below the cuff.

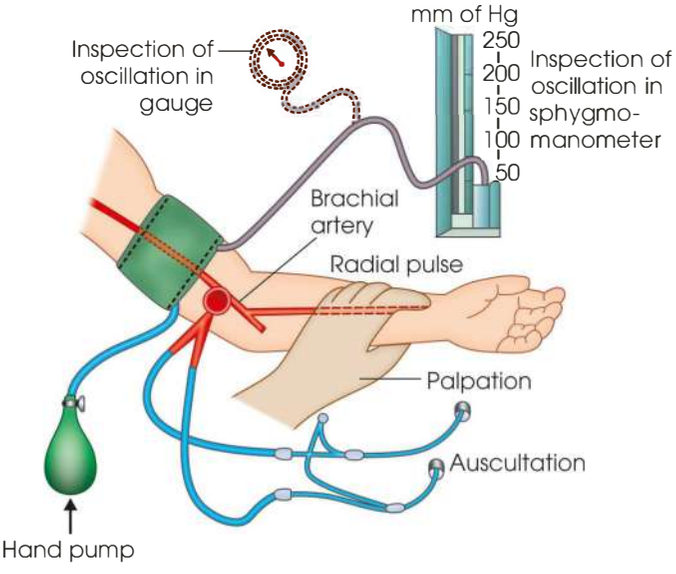

Fig. 37.2: Measurement of arterial blood pressure in human beings

He was the first to measure blood pressure in animals (Mare). He invented several devices, including a ventilator, a pneumatic trough and a surgical forceps for the removal of bladder stones.

Stephen Hales
1677–1761

The sounds are heard due to occurrence of turbulence in the flow of blood through the narrowed blood vessels when the manometric pressure just coincides with the systolic blood pressure. Due to giving air pressure in the cuff, the vessel is pressed and blood flow is obliterated. But while releasing the air pressure gradually, blood just begins to flow through the narrowed blood vessels and the pattern of flow is changed from streamline flow (silent) to turbulent flow (noisy). When the pressure is further released, normal streamline flow sets in and the sound is no longer heard. At this point manometric pressure coincides with the diastolic blood pressure. So, as the pressure is released the following variations of sounds are heard:

1. *First phase:* Sudden appearance of a clear tapping sound. This indicates systolic pressure. It persists while the pressure falls through 15 mm of Hg.
2. *Second phase:* The tap sound is replaced by a murmur persisting for another 15 mm of Hg.
3. *Third phase:* The murmur is replaced by a clear loud gong sound lasting for the next 20 mm of Hg.
4. *Fourth phase:* The loud sound suddenly becomes muffled and rapidly begins to fade. This point indicates diastolic pressure.
5. *Fifth phase:* Absence of all sounds.

Scipione Riva Rocci invented an easy-to-use cuff-based version of the mercury.

Riva Rocci
1863–1937

Venous Pressure

It is the pressure which is exerted by the blood within the veins. Average venous pressure of human being in recumbent position is about 60–120 cm of H_2O. The venous pressure can be measured by inserting a needle directly into the antecubital vein and by connecting the needle to a water manometer (Fig. 37.3). Venous pressure is a valuable index in determining the efficiency of the heart.

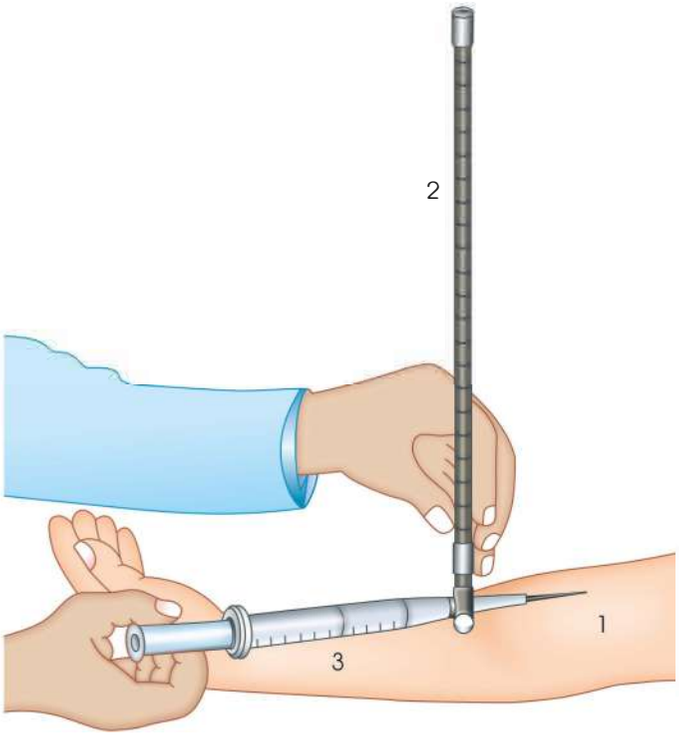

Fig. 37.3: Use of water manometer for measurement of venous pressure. 1: Anti-cubital vein, 2: Manometer, 3: Syringe

Invented Stethoscope in 1819.

René-Théophile-Hyacinthe Laënnec
1781–1836

FACTORS CONTROLLING ARTERIAL BLOOD PRESSURE

1. **Pumping action of the heart:** Effectual contraction of the heart is the main factor for controlling the cardiac output, blood pressure and flow within the blood vessel. Because in each effectual contraction of the ventricle certain amount of blood is ejected out into the aorta. The driving force of blood is mainly created by the pumping action of the heart. The efficiency of the heart is considered upon how much amount of blood is driven out by the heart into the aorta in each beat.
2. **Cardiac output:** Alterations of cardiac output will alter blood pressure. Cardiac output depends upon venous return, force and frequency of heartbeat. Blood volume affects blood pressure directly, by mainly modifying the cardiac output.
3. **Peripheral resistance:** It is the resistance which blood has to overcome while passing through the periphery. The chief seat of peripheral resistance is the arterioles and to a smaller extent the capillaries (vide below). Peripheral resistance depends on the following: (a) Velocity of blood, (b) viscosity of blood, (c) elasticity of arterial walls, (d) lumen of

blood vessels. Resistance is directly proportional to the first two and inversely to the last two factors.

- *Velocity:* A rapidly flowing stream will have more frictional effect than a slower one. Hence, pressure is high in the aorta but low in the capillaries.
- *Viscosity:* Other factors remaining constant, a more viscid blood will have a higher friction than a lesser one. For this reason, plasma transfusion is sometimes more effective to maintain blood pressure than ordinary saline.
- *Elasticity:* Due to elastic properties, the arteries can dilate and accommodate considerable amount of blood with relatively less rise of blood pressure. In old age, the arterial walls become stiff. Hence, blood pressure rises.
- *Lumen of the vessel:* Peripheral resistance is inversely proportional to the lumen of the vessel. In other words, smaller the vessel, higher will be the resistance. One should expect therefore that the capillaries, having the smallest lumen, should have the highest pressure.

$$\text{Peripheral resistance} = \frac{\text{Mean arterial pressure}}{\text{Cardiac output}}$$

Mean arterial pressure can be expressed in dynes per square centimetre by multiplying the pressure in mm of Hg by 1,332.

4. **Elasticity of the arterial walls:** In normal diastolic pressure arterial walls are stretched but due to the presence of elastic tissues in their walls, they tend to recoil. Due to elasticity of the arterial walls, the blood flow is pulsatile in the arteries. In the capillaries and venules, the flow is continuous. In old age, the expansion of the arterial walls becomes limited due to sclerotic changes and the blood pressure rises.
5. **Blood volume:** Increase in blood volume will raise both the systolic and diastolic blood pressures due to the increased quantity of blood in the arterial system and greater stretching of the arterial walls.
6. **Viscosity of the blood:** Alteration in blood viscosity will affect the diastolic pressure by its effect on the peripheral resistance. The intra-molecular friction is greater when the viscosity is high. Increase in viscosity increases diastolic blood pressure.

ADJUSTMENT OF BLOOD PRESSURE

In normal individual the constancy of the internal environment is being adjusted by the well-organised controlling system, which is called *Milieu interieur* after Claude Bernard and *homoeostasis* after Cannon. Adjustment of blood pressure, according to the needs of the body, may be carried out by the several complex reflexes whose centres are lying in *the cerebral cortex formatio reticularis, hypothalamus, medullary and spinal vasomotor centres*.

The (a) efferent and (b) afferent pathways constituting the above reflexes are lying within the sympathetic and parasympathetic nervous systems whose activities are modified by the hypothalamus and other centres.

EFFERENT PATHWAYS OF THIS SELF-ADJUSTMENT OR HOMOEOSTASIS OF BLOOD PRESSURE

These are the vagi and the sympathetic nerves which control the blood pressure by (a) modifying the cardiac activity, (b) altering the cardiac output, and (c) altering the lumen of the blood vessels. The relative activities of the vagi and the sympathetic of the efferent pathways are under the control of vasomotor systems, which are described below.

Vasomotor System

This system consists of: *Vasomotor centre, vasoconstrictor nerves and vasodilator nerves*. They supply vasomotor nerves, mainly to the arterioles but to some extent to the capillaries and venules. This vasomotor centre is highly developed in higher animals and human beings. In infants and children, it is imperfect. By regulating the radius of the blood vessels this system takes part in adjusting blood pressure and blood supply to a particular part. It also plays an immense role in heart regulation.

1. Vasomotor Centre (VMC)

Vasomotor centre is situated on the floor of the fourth ventricle in the reticular formation at the level of the calamus scriptorius. It is the diffuse area of the reticular formation in the lateral medulla containing neurons which control vascular tone; and encompass cardio-inhibitory centre and cardio-accelerator centre.

The dorsal motor nucleus of vagus in the medulla (nucleus tractus solitarius and nucleus ambiguus) is the cardio-inhibitory centre and it transmits continuous tonic inhibitory vagal impulse to the heart. This centre has got direct connection with the afferent nerves coming from the baroreceptors or chemoreceptors. Reflex bradycardia during the rise of systemic blood pressure is due to stimulation of the cardio-inhibitory centre. On the other hand, tachycardia is observed during the fall of blood pressure, which is due to inhibition or depression of the cardio-inhibitory centre along with the withdrawal of vagal tone from the heart. Under such state sympathetic cardiac centres get the upper hand.

The higher cardio-accelerator centre or vasomotor centre is located in the reticular formation of medulla oblongata. With the increase in the activity of the pressor centre along with the rise of blood pressure, heart rate and stroke volume are also increased. This pressor centre possibly functions with the superior control of the same in the hypothalamus and cerebral cortex.

The sympathetic cardiac centres or cardio-accelerator centres are situated in the lateral horn cells of the upper

thoracic segments of the spinal cord (T.1-5). The preganglionic fibres enter the sympathetic ganglia to connect with the cells of thoracic ganglia and inferior, middle and superior cervical ganglia. In animals and even in human beings the first thoracic ganglion and inferior cervical ganglion are fused to form the stellate ganglion from which post-ganglionic fibres (accelerator fibres) run directly to the heart. The function of the cardio-accelerator centre of the spinal cord is modified by the higher centres.

Vasomotor Reflexes

1. *Depressor reflex:* Blood pressure falls due to diffuse dilatation of the arterioles. Rise of blood pressure stimulates the baroreceptors of the carotid sinuses and aortic arch, and causes slowing of the heart and arteriolar dilatation. The vasodilatation is due to inhibition of vasoconstrictor effect of the sympathetic.
2. *Pressor reflex:* Blood pressure rises due to diffuse constriction of the arterioles. Diminution of blood pressure fails to stimulate the baroreceptors of the carotid sinuses and aortic arch, and the para-sympathetic inhibitory tone over the heart and blood vessels is withdrawn. Blood pressure is raised reflexly through over activity of the sympathetic. Vasoconstriction of the arterioles is due to activity of the vasoconstrictor centre. Reflex vasoconstriction also occurs due to stimulation of chemoreceptors during the fall of blood pressure.

Control of VMC

Vasomotor centre is under the superior control of cerebral cortex and hypothalamus (Fig. 37.4).

Factors influencing VMC have been described as follows:

1. *Higher centre (including hypothalamus):* Emotion generally stimulates, causing vasoconstriction. But shock may depress the centre—leading to a sudden fall of blood pressure and fainting (vasovagal attacks).
2. *Respiration:* During inspiration systemic blood pressure is generally decreased but increased during expiration. This is due to the decrease of left ventricular cardiac output during inspiration. Reverse is the effect during expiration. There is no evidence of direct respiratory centre—effect on vasomotor centre.
3. *CO_2 excess:* Excess stimulates. The action is mainly on the centre but partly reflexly through the sino-aortic nerves.
4. *O_2 lack:* Generally stimulates VMC. The effect is mainly reflex through the sino-aortic nerves and slightly direct on the centre.
5. *Sino-aortic nerves:* Variations of blood pressure, CO_2 tension, O_2 tension, etc., reflexly regulate the activity of the vasomotor centre through the sino-aortic nerves. Normally, a stream of inhibitory impulses is carried up by these nerves depressing the vasomotor centre. When blood pressure rises, VMC is depressed, vasodilatation occurs and further rise of blood pressure is checked. When blood pressure falls, the centre is released causing vasoconstriction and raising blood pressure. (Sino-aortic nerves also control cardiac centre, respiratory centre and adrenaline secretion.)

Other afferents: Local vasomotor tone is altered by afferent nerves originating from different baroreceptor and chemoreceptor areas, distributed all throughout the body. The baroreceptors are located in the right atrium, in the left atrium and left ventricle, in the pulmonary arch of aorta, in the junction of the superior thyroid artery and common carotid artery, junction of the subclavian artery and common carotid artery, and all throughout common carotid artery in between the superior thyroid artery and subclavian artery, mesenteric blood vessels (pacinian corpuscles), thoracic arch of the aorta and in the central vein (venous receptor). The chemoreceptors are located in the ventricular cavity and all throughout the blood vessels wall. Reactive hyperaemia is the consequence of local chemoreceptor activity on the blood vessels wall by the accumulated metabolites. Heat dilates and cold constricts the skin vessels reflexly.

2. Vasoconstrictor Nerves

The fibres pass along the sympathetic outflow from the first thoracic to the second lumbar segments. Brief details are as follows:

1. *To the skin and muscles:* Pass out through the grey rami communicantes—to the mixed spinal nerves and finally distributed through ordinary motor and sensory nerves. The distribution is strictly unilateral, stopping sharply at the midline.
2. *To the head and neck:* Come from the first to the fourth thoracic segments—enter the superior cervical ganglion from which postganglionic fibres arise and pass along the carotid artery and its branches.
3. *To the forelimbs:* Arise from the fourth to tenth thoracic segments—enter the stellate ganglion from which the postganglionic fibres arise and pass along the spinal nerves and supply the blood vessels.
4. *To the hindlimbs:* Arise from the eleventh thoracic to the second lumbar segments—relay in the lower lumbar and upper sacral ganglia, the postganglionic fibres accompany the nerves of the sacral plexus.
5. *To the abdominal viscera:* From the lower thoracic and upper two lumbar segments—pass through the splanchnic nerves to coeliac ganglion—the postganglionic fibres pass along the blood vessels.
6. *To the thoracic viscera:* Heart receives constrictor fibres through the vagus; lungs form the sympathetic.

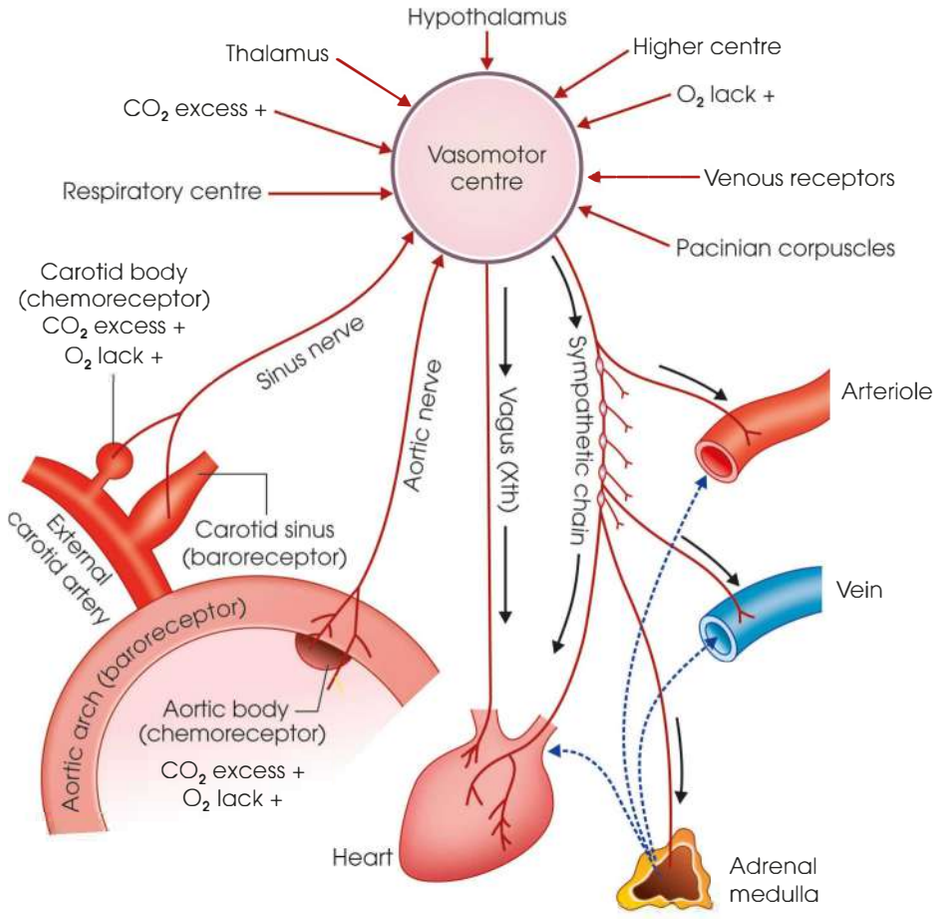

Fig. 37.4: Different factors that influence the vasomotor centre

3. Vasodilator Nerves

There are three types of vasodilator nerves:

1. **Para-sympathetic (cranio-sacral) vasodilators**
   Cranial
   - Chorda tympani—to the sub-maxillary or sub-mandibular gland.
   - Lesser superficial petrosal—to the parotid gland.
   - Lingual—to the vessels of tongue.

   Sacral: Nervi erigentes—to the vessels of genitalia.
2. **Sympathetic vasodilators:** Sympathetic fibres are mostly vasoconstrictor in nature. But some vasodilators are also present.
   For instance:
   - The dilator fibres of the coronary vessels come through the sympathetic.
   - Sympathetic dilator fibres have been demonstrated in the peripheral nerves in human beings.
   - Stimulation of the last anterior thoracic root produces dilatation of the kidney vessels.
   - Stimulation of the right splanchnic nerve sometimes causes vasodilatation and fall of blood pressure.
3. **Antidromic vasodilators** in the posterior spinal root (Fig. 37.5). When posterior spinal root is cut, distal to the ganglion and the peripheral end is stimulated—although the nerve is afferent, yet the vessels in the periphery—both skin and muscles, dilate (axon reflex). In the skin, it is due to liberation of histamine and as such produces the typical triple response; dilatation, flare and wheal. In the muscle it liberates acetylcholine and thereby causes vasodilatation.

Afferent Pathways

They are lying in two sets of receptors that carry information of the instantaneous circulatory status to the centre. These sensory receptors are: (1) Chemoreceptors and (2) baroreceptors distributed all throughout the cardiovascular system. The relative roles of the different afferent pathways have been described under separate headings, viz. (a) sino-aortic mechanisms controlling systemic blood pressure and flow, and (b) vascular receptors other than sino-aortic controlling mostly the local blood pressure and flow.

ROLE OF SINO-AORTIC MECHANISM IN THE REGULATION OF NORMAL BLOOD PRESSURE

From the above, it is evident that blood pressure can be adjusted according to the needs of the body in various ways. Of all the factors, the sino-aortic mechanism plays the chief role. The sino-aortic mechanism is carried on by baroreceptors and

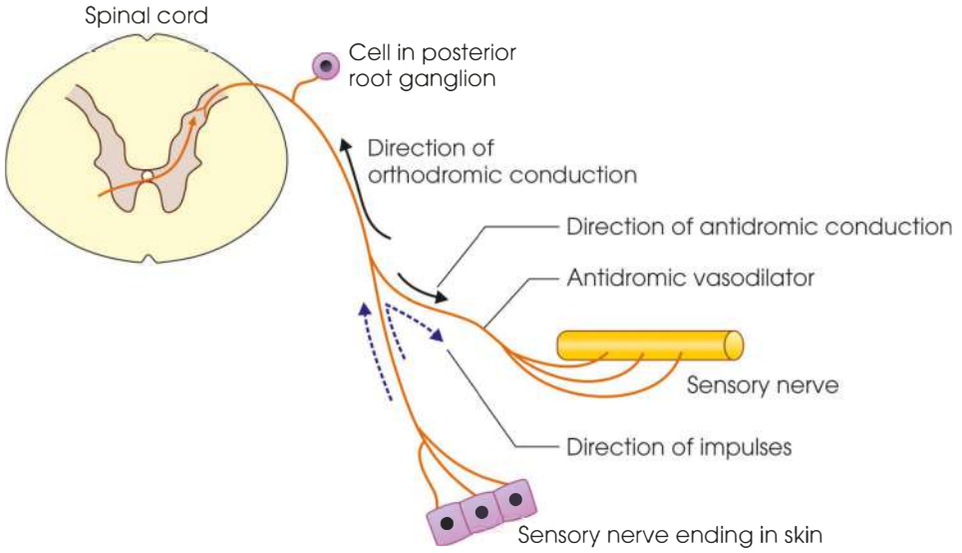

Fig. 37.5: Antidromic nerve fibres in posterior nerve root

chemoreceptors. This mechanism regulates blood pressure by adjusting the heart rate, vasomotor centre, and secretion of adrenaline and noradrenaline. It also adjusts respiratory centre in such a way that the functions of heart and respiration may run parallel.

Sino-aortic Mechanism

Baroreceptors Location

This includes carotid sinus and aortic arch (Fig. 37.6).

Carotid Sinus

It is a dilatation at the root of internal carotid artery, often involving the common carotid. The sinus nerve (afferent) arises from the carotid sinus and carotid body, passes along the glossopharyngeal nerve and ends in the medulla in close relation with respiratory, cardiac and vasomotor centres.

Aortic Arch

Afferent nerves and stretch receptors, similar to those in the carotid sinus, are also present in the adventitia of aortic arch, the roots of great vessels and even the adjoining parts of left ventricle. They serve the same function as the carotid sinus.

Aortic Nerve

This nerve arises from the arotic body, the aortic arch and the basal part of left ventricle. It is a purely afferent nerve. Its course varies in different species but in human beings it mostly passes in the vagus. Like the sinus nerve it ends in medulla being closely related to cardiac, vasomotor and respiratory centres.

Chemoreceptors Location

This includes carotid (G. *Karas* means sleep) body and aortic bodies (Fig. 37.7).

Carotid bodies: It is the small nodule situated on the occipital artery, a branch of the external carotid artery very close to the carotid sinus. It consists of lumps of polyhedral cells (glomus cells) richly supplied with blood vessel and nerve (Fig. 37.8). These vessels arise from the carotid artery. Numerous afferent nerve fibres surround the cell clumps and even individual cell and terminate in chemoreceptors. They are sensitive to chemical changes in body.

Aortic Bodies

These are small nodular structures supplied by special blood vessels and situated (a) on the thorax between pulmonary trunk and ascending aorta, (b) on the ventral surface of the root of the right subclavian artery, (c) on the ventral surface of the root of the left subclavian artery, and (d) on the ventral surface of aortic arch. Afferent pathways from these chemoreceptor areas are lying in the aortic nerves and vagi. Their structure nerve endings and functions are similar to those of carotid bodies.

Note

Haemorrhage or in enfeebled circulation a rhythmic blood pressure wave (vasomotor wave) is often encountered. These vasomotor waves are due to chemoreceptor activities under such state. These waves were observed by Mayer (1876) and known as Mayer's wave. Following inactivation of chemo-receptors, these waves disappear completely.

The functions of sino-aortic nerves may be described as follows:

1. Reflexly maintain the vagal tone and thus exert tonic inhibitory control on the heart.
2. Reflexly maintain the inverse relation between blood pressure and heart rate and thus keep the

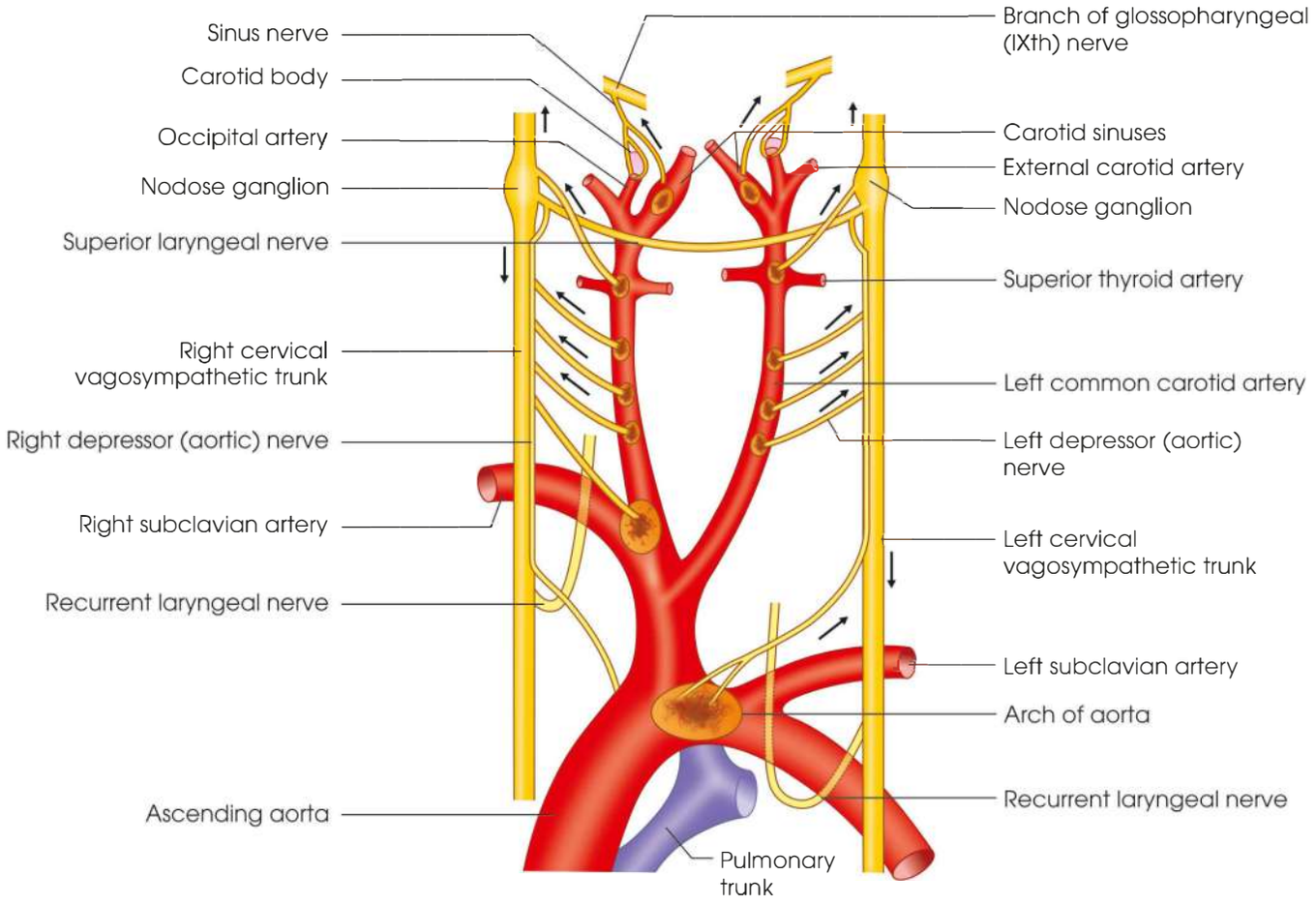

Fig. 37.6: Diagram represents the distribution of different baroreceptors on the walls of the blood vessels. (Carotid sinus, Baroreceptors at the junction of the superior thyroid artery and common carotid artery, Baroreceptor areas on the wall of the common carotid arteries, Baroreceptors at the junction of the subclavian artery and common carotid artery, and Aortic arch baroreceptors)

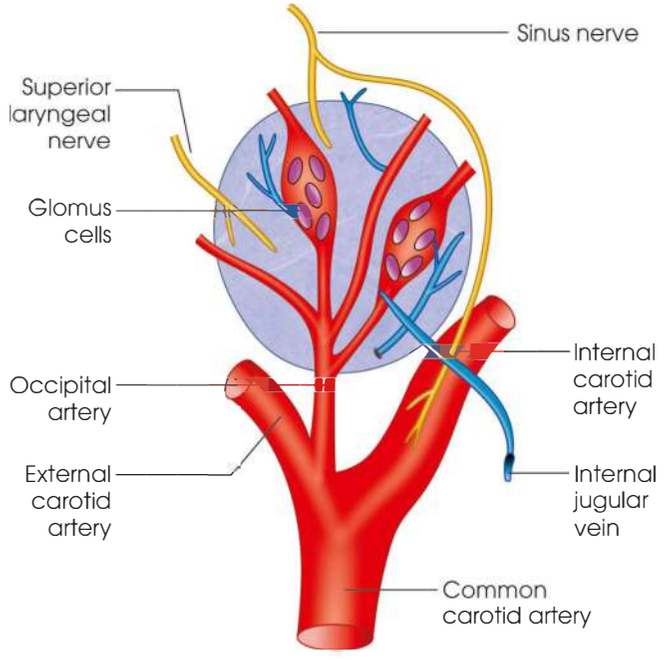

Fig. 37.7: Carotid body showing blood vessels and nerve supply

variations of blood pressure within an optimum range (hence called Buffer nerves).

3. Exert tonic inhibitory action on respiratory centre and vasomotor centre (vasoconstrictor).
4. Reflexly regulate the activity of the respiratory, cardiac, vasomotor centres and adrenaline secretion and thus bring about a perfect coordination among them.
5. Changes in viscera, viz. variations of movement, tone, etc. may be reflexly produced through autonomic nerves.

VASCULAR RECEPTORS OTHER THAN SINO-AORTIC FOR THE CONTROL OF BLOOD PRESSURE AND FLOW

The sino-aortic mechanisms are meant for the maintenance of systemic blood pressure and flow. But the vascular receptors other than sino-aortic are responsible mostly for control of local blood pressure and blood flow.

These are as follows:

1. *Several baroreceptor areas in the wall of right and left common carotid arteries are located* between the

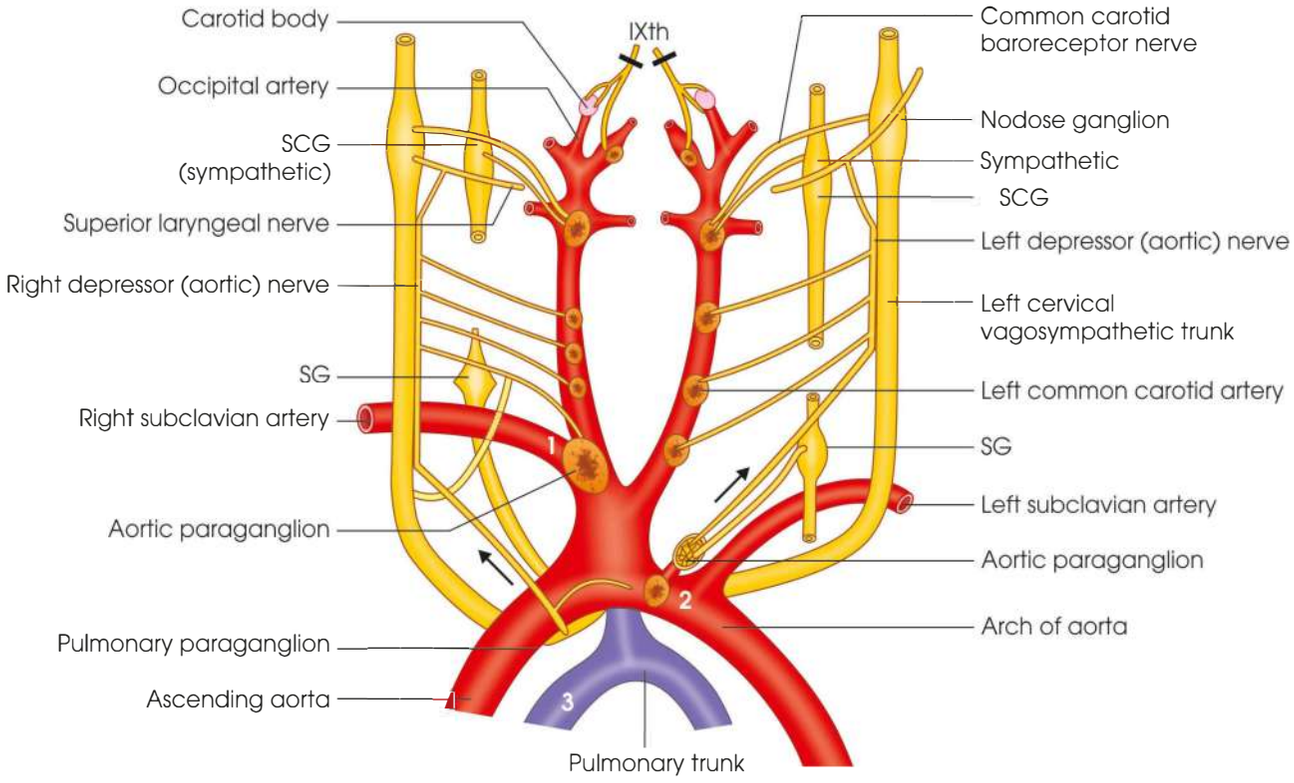

Fig. 37.8: Distribution of chemoreceptors at areas of the blood vessels. 1, 2, 3 are the chemoreceptor areas

level of the superior thyroid artery and subclavian bifurcation. Afferent impulses from these areas are carried through the branches of the aortic nerves.

2. *Baroreceptors of the pulmonary arch of aorta:* These baroreceptors are present in the pulmonary conus of bifurcation. Reflex bradycardia and hypotension are produced if these receptors are stimulated due to rise of pressure.
3. *Receptors in thoracic aorta:* Rise of blood pressure may produce reflex vasodilatation in the innervated limb through the stimulation of these baroreceptors.
4. *Mesenteric baroreceptors:* These receptors do not play any important role in the regulation of systemic blood pressure but they may play in the regulation of blood flow in the abdominal viscera.
5. *Peripheral vascular receptors:* Presence of other peripheral vascular receptors has been observed by many. It is claimed that this reflex decrease of blood flow is through venous—arteriolar reflex causing constriction of the arterioles due to (a) distension and (b) increase of transmural pressure of the veins. In congestive heart failure there is general occurrence of peripheral vasoconstriction (in the fingers and nose) is mostly due to reflex effect of increased central venous pressure.
6. *Löven reflex:* This is axon reflex. If any portion of the vessel is dilated then the neighbouring vessel is constricted. This was first observed by Lovett.
7. *Bainbridge reflex:* Bainbridge (1915) showed that intravenous administration of saline or blood produced reflex acceleration of the heart. He claimed this to be reflex arising from the stretch receptors present in the venous side of the heart (great vein and also right atrium) and bilateral sectioning of the vagi abolished the response. He claimed that if the heart rate is initially high (above 130 per min) then the reflex effect will be bradycardia instead of tachycardia.
8. *Right atrial receptors (A and B):* Increasing the perfusion pressure in the right atrium produces bradycardia. This reflex effect is abolished by atropine or vagotomy. Bainbridge effect is the cause of stimulation of chemoreceptors supplied by the vago-depressor trunk which might well be activated by the changes in gas content, acidity, tonic balance and viscosity of the blood associated with the massive intravenous infusion.
9. *Pulmonary deflation receptors:* Paintal (1955a) stated that these receptors are stimulated by congestion of lungs during rapid venous return and produce reflex bradycardia. These receptors are also a part of pulmonary depressor chemo-reflex.

10. *Reflexes from the inflation of the lungs:* The inflation and deflation reflexes aid in regulating the ventilation of the lungs, thus it prevents over distension and maintain sustainable deflation. The receptor sites are located in the respiratory tract chiefly in the bronchi and bronchioles. As the lung tissue is stretched by inflation, the stretch receptors then send impulses to the respiratory centre, slowing down inspiration. This is called the Hering-Breuer inflation reflex. When the expiratory phase starts, the receptors are no longer stretched and no impulses are send to respiratory centre thus promoting deflation. This is called the Hering-Breuer deflation reflex.
11. *Left atrial and left ventricular receptors:* Paintal (1955b) has shown that these receptors are excited by the increased pressure in the left side of the heart and produce bradycardia.

AS CHEMORECEPTORS

Bezold and Jarisch reflex: Bezold and Hirt (1868) and Jarisch (1938) observed profound bradycardia, hypotension and apnoea following intravenous injection of veratrine alkaloid. They considered being the direct effect of the drug on these cardiac receptors in left ventricle (mostly) that causes reflex cardiac and respiratory effects. Jarisch concluded that these are proprioceptive receptors and are normally responsive to stretch of the ventricular wall. Paintal (1955b) has shown that veratrine and related substances may stimulate the ventricular receptors and also some of atrial receptors ('A' and 'B'). He also observed that these drugs do not act directly on these receptors but act through changing the ionic status of the receptor areas.

MECHANISM OF REGULATION OF BLOOD PRESSURE

I. **Short-term regulating mechanisms:** Baroreceptor reflex, chemoreceptor reflex and CNS ischaemic response
II. **Intermediate mechanism of regulation:** Stress relaxation of vasculature and capillary fluid shift mechanism
III. **Long-term regulating mechanism:** Renin-angiotensin-aldosterone mechanism
IV. **Role of other hormone in regulation of blood pressure:** Anti-diuretic hormone and atrial natriuretic peptides
V. **Role of humoral vasoconstrictors and vasodilators**
VI. **Chemical control of blood pressure influenced by vasomotor mechanism**

I. Short-term Regulating Mechanisms

These are:

1. Baroreceptor reflex
2. Chemoreceptor reflex
3. CNS ischaemic response

1. **Baroreceptor reflex:** Baroreceptors are characteristic mechanoreceptor type of sensory neurons which are activated by a stretch of the blood vessel. An increase in pressure causes the receptors located at aortic arch and carotid sinuses to stretch, increasing frequency of action potentials. They send action potentials to vasomotor centres to the solitary nucleus in the medulla, which via autonomic reflex influence the cardiac output and total peripheral resistance. Baroreceptors exhibit baroreflex activity whenever there is change in the mean arterial blood pressure, bringing the blood pressure toward a normal level. Baroreceptors are more sensitive to decrease in pressure and sudden changes in pressure (Fig. 37.9).
2. **Chemoreceptor reflex:** Chemoreceptors are receptors located in carotid and aortic bodies. They are stimulated by chemical changes in blood mainly hypoxia ($\downarrow O_2$), hypercapnia ($\uparrow CO_2$), and pH changes. Chemoreceptors excite the vasomotor centre, which elevates the arterial pressure. When pCO_2 increases above 40 mm Hg, or pH decreases below 7.4 chemoreceptors are stimulated. They send the signal to the respiratory centre and respiratory activity increases in response to the chemoreceptor reflex. The increased sympathetic activity stimulates both the heart and vasculature to increase arterial pressure also. Cerebral ischaemia activates central chemoreceptors which also produce simultaneous activation of sympathetic and vagal nerves to the cardiovascular system.
3. **CNS ischaemic response:** If blood flow is decreased to the vasomotor centre in the lower brainstem and CO_2 accumulates, the CNS ischaemic response is initiated. The very strong sympathetic stimulator causing major vasoconstriction and cardiac acceleration. Sometimes called the "last ditch stand".

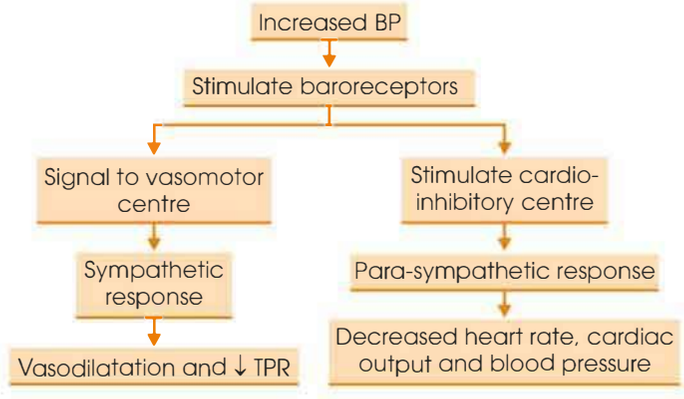

Fig. 37.9: Baroreceptor reflex

II. Intermediate Mechanism of Regulation of Blood Pressure

1. **Stress relaxation of vasculature:** When arterial pressure increases it stretches the arterial vessels and the vessel responses by relaxation as a normal vascular response and blood pressure is decreased. *Vice versa* in reverse stretch relaxation there is tightening of the vessel in response to lowered blood pressure thereby vascular adjustment it restores the normal blood pressure.
2. **Capillary fluid shift mechanism:** Fluid shift through the capillary wall: Increased blood pressure increases the capillary pressure which leads to shifting of fluid from capillary to interstitial region, thereby decreasing blood volume and restores the blood pressure. Similarly, decreased blood pressure draws fluid into capillary from interstitial region thereby restoring the normal blood pressure.

III. Long-term Regulation of Blood Pressure

Renin angiotensin aldosterone mechanism: This system is generally known as a long-term regulator of arterial pressure. Kidney compensates for loss in blood volume or decreased blood pressure by activating an endogenous vasoconstrictor angiotensin II. The kidneys control the level of H_2O and NaCl in the body, thus controlling the volume of the extracellular fluid and blood. By controlling blood volume, the kidneys control arterial pressure. Any drop of renal blood flow and/or ↓ Na^+, will stimulate volume receptors found in juxtaglomerular apparatus of the kidneys to secrete renin which will act on the angiotensin system leading to production of angiotensin I, angiotensin II and III. Angiotensin II is a vasoconstrictor which will increase blood flow to the heart and subsequently the preload, ultimately increasing the cardiac output (Fig. 37.10). Angiotensin II also causes an increase in the release of aldosterone from the adrenal glands and further increases the Na^+ and H_2O reabsorption in the distal convoluted tubules in the kidney.

IV. Role of other Hormone in Regulation of Blood Pressure

- **Anti-diuretic Hormone (ADH):** Hypovolemia and dehydration will stimulate the osmoreceptors in the hypothalamus, which will lead to release of ADH from posterior pituitary gland. ADH will cause water reabsorption at kidney tubules and thereby aid in regulating blood pressure.
- **Atrial Natriuretic Peptide (ANP):** This hormone is secreted from the wall of right atrium to regulate Na^+ excretion in order to maintain blood volume thereby aid in regulating blood pressure. It is released in response to stimulation of atrial receptors. It increases salt excretion via kidneys: By reducing water reabsorption in the collecting ducts, relaxes renal arterioles and it inhibits sodium reabsorption in the distal tubule.

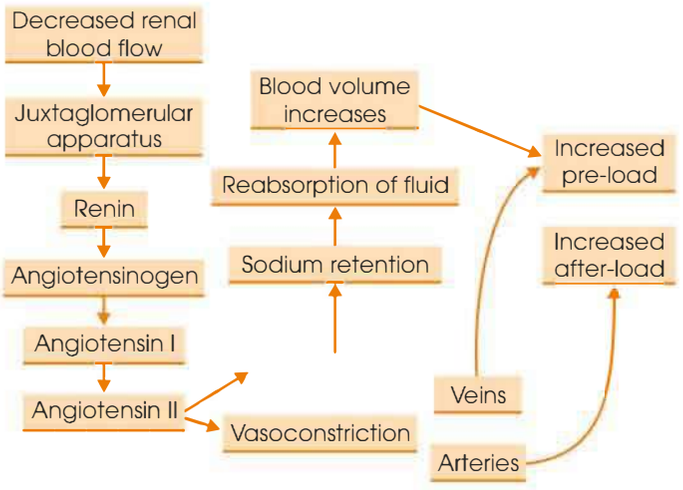

Fig. 37.10: Renin-angiotensin control mechanism

V. Role of Humoral Vasoconstrictors and Vasodilators

Local release of vasoconstrictor and vasodilators: This redistribution of blood is made by withdrawing or heightening the vasomotor tone. In condition of increased blood pressure, there is depression of sympathetic tone along with activation of parasympathetic tone—causing peripheral vasodilatation so as to shift the blood to the splanchnic bed (peripheral bed); but in condition of decreased blood pressure, there is increase of sympathetic tone along with decrease of parasympathetic tone—causing profound vasoconstriction in the splanchnic bed (other peripheral bed) so as to shift the blood to the vital organs

Humoral Vasoconstrictors

Apart from angiotensin and vasopressin, other vasoconstrictors are:

- *Norepinephrine and epinephrine:* Hormones of adrenal medulla: The release of norepinephrine and epinephrine from adrenal medulla; causes vasoconstriction and increases the blood pressure.
- *Endothelin:* It is a 21-amino acid peptide that is produced by the vascular endothelium. Endothelins are peptides that constrict blood vessels and raise blood pressure. There are three isoforms (identified as ET-1, -2, -3). It is the most potent known vasoconstrictors know and increased production of endothelin may lead to cerebral vasospasm, hypertension and heart disease.

Humoral Vasodilators

These are:

1. *Bradykinin:* They produce powerful arteriolar dilation and increased permeability of the capillaries.

2. *Histamine:* They are released from damaged or inflamed tissue; also during an allergic reaction. Also cases arteriolar dilation and increased permeability of the capillaries.
3. K^+ ions, Mg^{2+} ions, H^+ ions, anions—acetate and citrate, and CO_2—produces vasodilatation.
4. *Nitric oxide:* It is a potent vasodilator. It relaxes vascular smooth muscle by activating guanylate cyclase and increases the intracellular levels of cyclic—guanosine 3′,5′-monophosphate, producing vasodilatation.

VI. Chemical Control of Blood Pressure Influenced by Vasomotor Mechanism

Many substances produced in the body are known to increase or decrease blood pressure by influencing the vasomotor mechanism.

Some of these are:

1. *CO_2:* Tonic activity of the vasoconstrictor area may be due to stimulating action of CO_2 in blood. During early stage of asphyxiation this may bring about a great increase in blood pressure. It is observed that over ventilation of lungs, as by voluntary deep inspiration and expiration for 3 or 4 minutes, causes a feeling of giddiness. As a large amount of CO_2; is expelled from blood by over ventilation; the vasoconstrictor area is deprived from proper stimulation by CO_2. As a result, a fall in blood pressure and vasodilatation in splanchnic area occur.
2. *Epinephrine:* If it is injected into blood, epinephrine constricts the cutaneous and abdominal arterioles, and this result in a very sharp rise of blood pressure, but the elevated pressure does not stand for a long time. In contrast, there is a dilatation of the coronary and skeletal muscle arterioles.
3. *Histamine:* It causes a marked dilatation of capillaries and arterioles in lowering of blood pressure.
4. *Alcohol:* It causes a marked dilatation of blood vessels as a depressant on the vasomotor centre.
5. *Tobacco:* Smoking increases both systolic and diastolic blood pressure. There is an increase of pulse rate materially and a decrease of temperature of extremities. So the use of tobacco may be injurious in arteriosclerosis and also in cardiac diseases associated with arteriosclerosis or even high blood pressure. Vasopressin: Though it is an internal secretion of the posterior pituitary, it causes an increase of blood pressure. But this rise in pressure is not as great as that of epinephrine, yet this pressure continues for a long time.
6. *Acetylcholine:* Direct action of acetylcholine on coronary blood vessels is dilatation.

EXAM-ORIENTED QUESTIONS

Essay

1. Define the blood pressure, its component, normal values and causes of physiological variation in blood pressure.
2. Describe the various short-term, intermediate-and long-term mechanism in regulation of blood pressure.

Short Notes

1. Methods of recording of blood pressure
2. Factors affecting blood pressure
3. Physiological variation in blood pressure
4. Sino-aortic mechanism
5. Baroreceptors
6. Chemoreceptors
7. Renin-angiotensin system
8. Humoral vasoconstrictors
9. Humoral vasodilators

Chapter

38

Velocity of Blood Flow and Radial Pulse

INTRODUCTION

Velocity of Blood

Definition: It is the rate of blood flow through a given vessel. Blood flow is the volume of blood flowing through a particular vessel in given interval of time. Difference between the velocity and blood flow is that, the flow remaining constant, the velocity is inversely proportional to the cross-sectional area of the blood vessel. But blood flow is directly proportional to the cross-sectional area of the vessel. But velocity in local blood vessel, if constricted, is increased and sometimes it reaches the critical velocity producing sound (turbulent flow).

Velocity of blood depends upon the following factors

1. *Lateral pressure and kinetic energy for flow:* In a tube of varying diameter, the lateral pressure varies directly and velocity inversely with the cross-sectional area of the tube. That is where there is any increase of velocity; the lateral pressure will be decreased at that unit area due to conversion of certain amount of potential energy into kinetic energy for flow.
2. *Total cross-sectional area of the vascular bed:* It is inversely proportional to the total cross-sectional area of the vascular bed. As one proceeds to the periphery the total vascular bed enlarges and velocity falls. Hence, in the aorta and larger arteries, it is about 0.5–1 metre per sec, whereas in the capillaries, about 0.5–1 mm per sec. As the capillaries join up to form bigger and bigger veins, the vascular bed shortens and the velocity increases. Near the heart, the total cross-sectional area of the great veins is nearly double that of the aorta. Hence, velocity is about half.
3. *Pumping action of the heart:* It is directly proportional to the force with which blood is propelled. Other factors remaining constant the velocity will, therefore, depend upon the minute output of the heart. Velocity thus increases during systole and diminishes during diastole.
4. *Peripheral resistance:* Blood flow is inversely proportional to the peripheral resistance. Vascular dilatation will reduce the resistance and increase blood flow. While vasoconstriction will increase the resistance and reduce the blood flow. But the velocity of blood flow is just the reverse. If blood vessel is locally dilated or constricted then the velocity of blood flow is decreased or increased, respectively. This principle is very helpful in maintenance of blood flow in the locally constricted blood vessels due to atherosclerotic invasion (Bernouilli's principle). Because due to constriction at that area the kinetic energy for blood flow is increased but the lateral pressure is decreased. If the peripheral resistance is increased in general then there is possibility of decreasing the both (velocity and blood flow).

Methods of Measurement of Velocity of Blood

In the capillaries: The velocity can be measured by noting the rate of progress of red blood corpuscles under the microscope.

In the larger vessels (in animals):

1. *Ludwig's stromuhr* (Fig. 38.1): With this instrument the amount of blood passing through a vessel per unit time is measured. The velocity is then calculated by dividing the total volume flow (V) with the cross-sectional area of the vessel (μr^2 = area of circle).
2. *Differential stromuhr* (*Fleischl*): This is a more accurate instrument by which the change of velocity during each heartbeat can be recorded.
3. *Thermostromuhr* (*Rein*): The principle is to heat a particular spot on the vessel by high frequency current at a known rate and then the rise of temperature of blood is noted a little down the stream by a thermocouple. The rise of temperature is inversely proportional to the velocity.
4. *Electromagnetic method*: A more advanced method based on electromagnetic principle has also been devised.

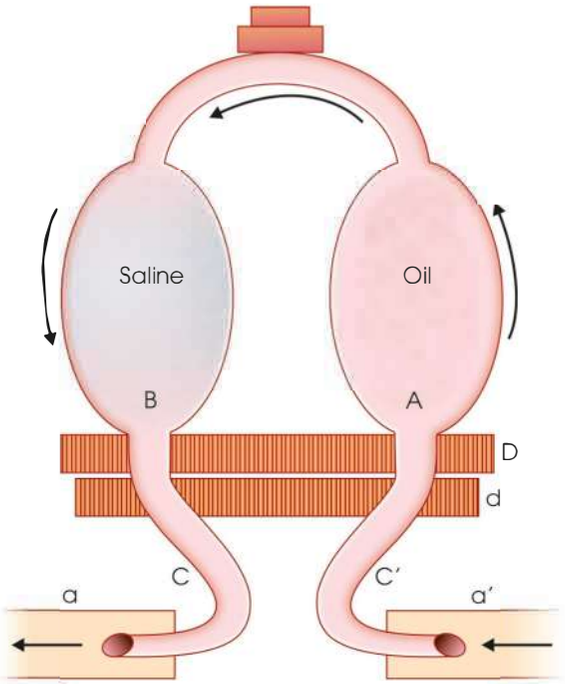

Fig. 38.1: Ludwig's stromuhr. Two intercommunicating glass bulbs A and B fitted to a disc D which can be rotated over another disc d. To d is fixed two cannula C and C'. C' is inserted into the proximal cut end of the artery and C to the distal end. A contains oil and B contains saline. Blood enters through C', fills up A, pushing out the oil into B. The saline of B in the mean time enters circulation. When B is just filled up with oil (or A is just filled up with blood), the bulbs with the disc D is quickly rotated through 180°, so that B now comes over C' and A over C. Thus, the process is repeated and calculation is made from the number of times the bulb, whose capacity is known, was filled with blood in a given period

Mean Volume Flow

Instrument used—plethysmograph (Fig. 38.2). The total volume of blood passing through an organ or any other part does not depend upon the velocity of blood flow through the corresponding artery. It depends upon three factors:

1. The total cross-sectional area of the vascular bed in the organ.
2. The rate of metabolism in the organ.
3. The degree of vasodilatation or vasoconstriction in the locality.

The following figures give the mean volume flow per minute for each 100 gm:

Thyroid: 560 ml.
Kidney: 150 ml.
Brain: 130 ml.
Liver: 150 ml (1/3rd arterial, 2/3rds portal).
Intestine: 70 ml.
Heart (coronary): 100 ml.

Alteration of volume of a limb is recorded by instrument called plethysmograph (Fig. 38.2). The cuff of the

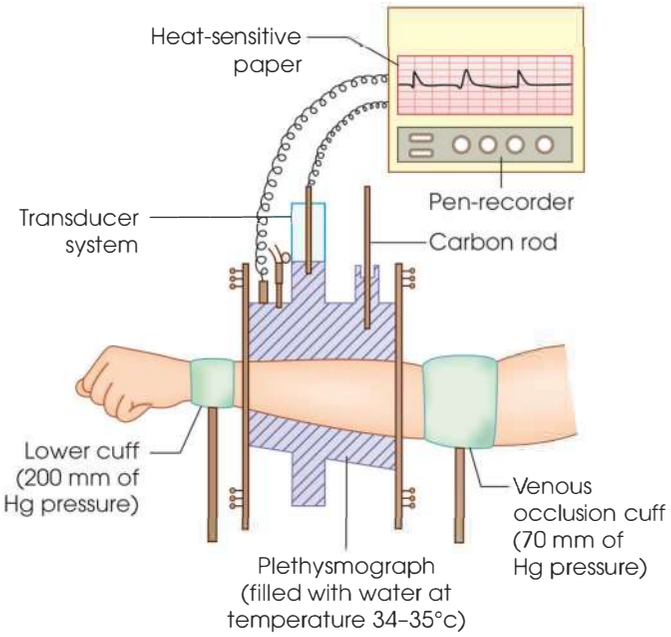

Fig. 38.2: Use of plethysmograph for recording of blood flow in the forearm

sphygmomanometer is applied to the arm and inflated up to a certain pressure which is less than the arterial pressure. The veins are occluded but the arterial blood flow in the limb remains unchanged. The volume of the limb gradually increases as no blood can leave due to occlusion of the veins. The limb is kept in a water-tight glass vessel which is connected with the volume recorder. Changes in the volume of a limb will displace water from the glass vessel and this displacement of water will be recorded in a sensitive electronic apparatus through transducer system.

Radial Pulse

By the term pulse is meant the expansion and elongation of the arterial walls passively produced by the pressure changes during systole and diastole of ventricles.

Pressure Pulse

It is the pressure wave created by the ventricle while ejection of blood into the fully distended aorta and then propagated wave through the blood column towards the periphery. The sudden dilatation of the aorta, to accommodate the output, is transmitted in the form of a wave throughout the arterial system and this wave constitutes the so-called pulse. (In the capillary area no pulsation is normally found. Due to enlargement of the vascular bed pulsatile circulation in the arteries becomes a smooth continuous flow in the capillary bed.)

Velocity of Pulse Wave

The velocity of pulse wave is much more rapid (about 6 times) than the velocity of blood flow; because, the former is conducted through the solid vessel walls,

whereas the latter by propelling a liquid. Pulse velocity depends on the degree of elasticity of vessels. By the term elasticity of a vessel is meant by the percentage increase in the volume of the artery with each mm of Hg rise in blood pressure. Its relation with pulse velocity is as follows:

The elasticity at different ages as found from this formula is as follows:

- Between the ages of five and six—0.47; between eighteen and twenty—0.33;
- Between forty and forty-five—0.24; between seventy and seventy-five—0.18. Thus, elasticity decreases with age, so that in older people the same cardiac output will cause a higher systolic pressure.

With age, the arteries become less elastic, hence the velocity increases. Blood velocity is inversely proportional to the total cross-sectional area of the vascular bed at any part. In the aorta it is about 1 m per second, whereas in the capillaries it is about 0.5 to 1 mm per second. The average velocity in metres per second at different ages are as follows; between the ages of five and six—5.2 m; between eighteen and twenty—6.2 m; between forty and forty-five—7.2 m; between seventy and seventy-five—8.3 m.

Recording of Radial Pulse

For clinical purposes, the commonest instrument used, is Dudgeon's sphygmograph. For more accurate work, elaborate apparatus for optical recording is used. The record of radial pulse is shown in Fig. 38.3. The upstroke is abrupt and without any secondary wave on it. Near the middle of the downstroke there is a sharp depression called the dicrotic notch. This is immediately followed by a small wave, the dicrotic wave. These two features are constantly present in a normal pulse tracing. The wave from the beginning of the tracing up to the **dicrotic** notch is called the primary wave or percussion wave. This whole wave corresponds to ventricular systole. The rise and fall of pressure in this wave follow the similar pressure changes in the aorta and ventricles. The dicrotic notch is due to the sharp fall of pressure caused by the rolling back of aortic blood towards the left ventricle at the beginning of diastole. The dicrotic wave is caused by the return of the same blood column being reflected back by the closed semilunar valves. Sometimes secondary oscillations are found on the downstroke both above and below the dicrotic wave, being known as the pre-dicrotic wave and and post-dicrotic wave, respectively. These are due to elastic oscillations of the aorta. The normal pulse is called catacrotic pulse. When a secondary wave is found on the upstroke, the wave is called anacrotic wave and the pulse is called anacrotic pulse. When the dicrotic wave becomes so prominent that it can be easily felt with the fingers the pulse is known as dicrotic pulse.

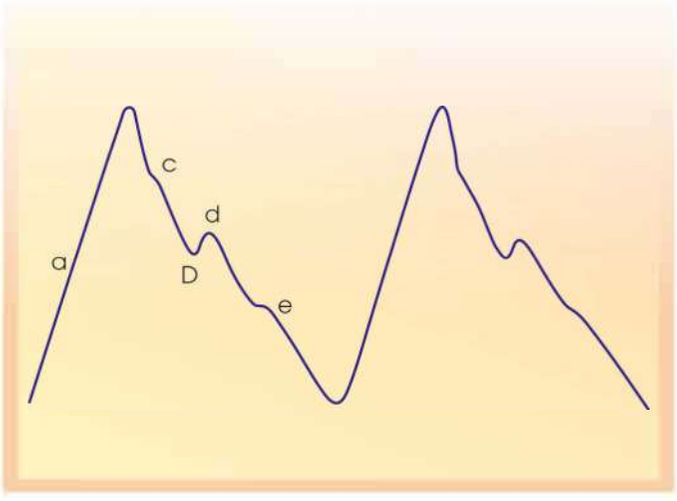

Fig. 38.3: Tracing of radial pulse. a—primary wave; c— pre-dicrotic wave; d—dicrotic wave; e—post-dicrotic wave

Significance

A large primary wave is generally due to:

1. A large stroke volume.
2. Slow heart rate.
3. Low peripheral resistance.

A small primary wave is due to:

1. Small output.
2. Rapid heart rate.
3. High peripheral resistance (i.e. high blood pressure).
4. Stiffness of the vessel walls.

The downstroke becomes more abrupt in subjects with low diastolic pressure. It becomes more sloping in cases with high blood pressure.

Clinical Features of Radial Pulse

While examining pulse, the following features are to be noted:

1. Rate means the frequency of pulse per minute. Normally, it corresponds to heart rate. Increased pulse rate is called tachycardia and diminished pulse rate is called bradycardia.
2. Rhythm indicates whether the beats are equidistant or not.
3. Volume means the rise of the pulse wave above the diastolic level. Other factors remaining constant it varies as the stroke volume.
4. Tension is the approximate measure of the systolic pressure. It is determined by noting the amount of pressure required to obliterate the pulse wave. It is examined with three fingers placed side-by-side on the radial artery. The proximal finger adjusts the pressure, the middle finger remains stationary and only feels the appearance and disappearance of the pulse wave; while the distal finger applies a constant maximum pressure to stop retrograde pulsation.

Special Varieties of Pulse

Depending on the variations of the above features, various types of pulse waves are clinically described. The following types are of special interest:

1. *Sinus arrhythmia:* The frequency of the pulse increases during inspiration and falls during expiration. Sometimes it is found in children. It is due to alteration of the vagal tone during respiration.
2. *Water-hammer pulse:* The rise and fall are steep and abrupt without any dicrotic notch or wave. This is typically found in aortic incompetence.
3. *Pulsus alternans:* Here, the pulse is alternately large and small. It is found in serious myocardial damage.
4. *Pulsus paradoxus:* The volume and frequency is more during expiration than during inspiration, i.e. reverse of sinus arrhythmia.
5. *Weak pulse:* A weak pulse at the radial artery generally indicates that the quantity of blood ejected by the left ventricle to the arteries with each beat is less than normal.

From the above discussion, it will be noted that examination of pulse is of great clinical importance. From it, the condition of the heart, of the arteries, the extent of blood pressure, etc. may be known.

Atrial Fibrillation and Atrial Flutter

Atrial Fibrillation

It is a clinical condition in which normal coordinate contraction of the atrium is replaced by in coordinate contraction of the individual fibres characterised by irregularly irregular pulse. The incomplete contraction of the atrial muscle is at the rate of 400 to 600 per minute. Only a part of the atrial impulses pass through the AV bundle to the ventricle due to long refractory period of the conducting tissue. So the ventricular rate is not so fast. Again, some of the ventricular beats are so weak that they are not transmitted to the radial pulse. The apex beat therefore does not correspond with the pulse rate. This is called the pulse deficit. Atrial fibrillation is frequently seen in mitral stenosis, thyrotoxicosis, myocardial disease, etc. The underlying process in the production of atrial fibrillation was explained by 'circus movement' theory but the recent evidence goes against this theory.

Atrial Flutter

It is a clinical condition in which there is marked acceleration of the coordinate contraction of the atria. The atria contract at the rate of 180 to 360 per minute. The usual rate is about 300 per minute. The ventricular rate is not so fast as all the impulses from the atria are not transmitted to the ventricles due to longer refractory period of the junctional tissues. Atrial flutter is also seen in mitral stenosis, myocardial disease, etc. The underlying process in the production of atrial flutter was explained by the 'circus movement' theory but the recent evidence goes against this theory.

EXAM-ORIENTED QUESTIONS

Short Notes

1. Define circulation time and explain the factors affecting circulation time.
2. Explain clinical significance of circulation time.
3. Describe the normal characteristic of pulse.
4. Discuss the pulse pattern in various pathological conditions.

Chapter

39

Regional Circulation

INTRODUCTION

Circulation in a particular region or organ is directly proportional to the degree of local activity. It is adjusted on two lines: (1) By regulating the general circulation and (2) by adjusting the local blood vessels. Generally, vasodilatation of an active part is accompanied with vasoconstriction of other inactive parts. In this way, blood is shifted from the inactive to the active regions without any fall of general blood pressure.

The following regional circulations are of special physiological importance: (a) Coronary circulation, (b) cerebral circulation, (c) pulmonary circulation, (d) hepatic circulation, (e) splenic circulation, (f) renal circulation, (g) capillary circulation, (h) cutaneous circulation, (i) skeletal muscle circulation. They are briefly described below.

CORONARY CIRCULATION

Anatomical Considerations

Arterial Supply

1. Blood supply to the heart is mainly carried out by the two coronary vessels, e.g. right and left coronary arteries arising from the sinuses behind the cusps of the aortic valve at the root of the aorta.
2. Both the arteries are patent throughout the cardiac cycle as the eddy currents keep the cusps of the valve away from the orifices of the arteries.
3. The left coronary artery gives off two main branches: the anterior descending and the left circumflex branch. The latter runs along the atrioventricular groove to the left and proceeds downwards as the posterior descending branch. The anterior descending branch runs downwards up to the apex.
4. The right coronary artery gives out several descending branches on both ventricles (Figs 39.1 and 39.2).
5. Predominance of the coronary artery supply is seen in about 50% of human heart by the right coronary artery; and in about 20% of human heart by the left coronary artery and in about 30% of cases by both coronary arteries.
6. The right and left coronary arteries break up into a large number of capillaries.

Venous Drainage

The venous blood from the myocardium is chiefly returned through two systems: *Superficial venous system and deep venous system*.

1. The *superficial venous system* lies beneath the epicardium and consists of (a) the coronary sinus, draining mostly the blood from the left coronary artery, partly from the right coronary artery, and ends ultimately in the posterior part of the right atrium; (b) the great cardiac vein, draining blood from the left heart, ends in the coronary sinus; (c) the anterior cardiac veins which are generally 2 to 3 in number, draining blood mostly from the right heart and partly from the left heart, ends directly into the right atrium.
2. The *deep venous system* arises within the substance of the myocardium and ends directly into the cavity of the right heart by the thebesian, luminal and sinusoidal vessels.

Anastomosis

Normally, the coronary arteries are end arteries though the functional anastomoses are present and become active under unphysiological state. These anastomoses are between (a) branches of one coronary artery with that of the other, (b) thebesian vessels and cavity of the heart, (c) arterioluminal and arterio-sinusoidal vessels with the cavity of the heart, and (d) extra cardiac anastomoses (Fig. 39.3).

METHODS OF STUDY

The coronary blood flow and the oxygen usage of the heart can be studied by the Pitot tube, orifice metre, electromagnetic flow metre, thermostromuhr and also,

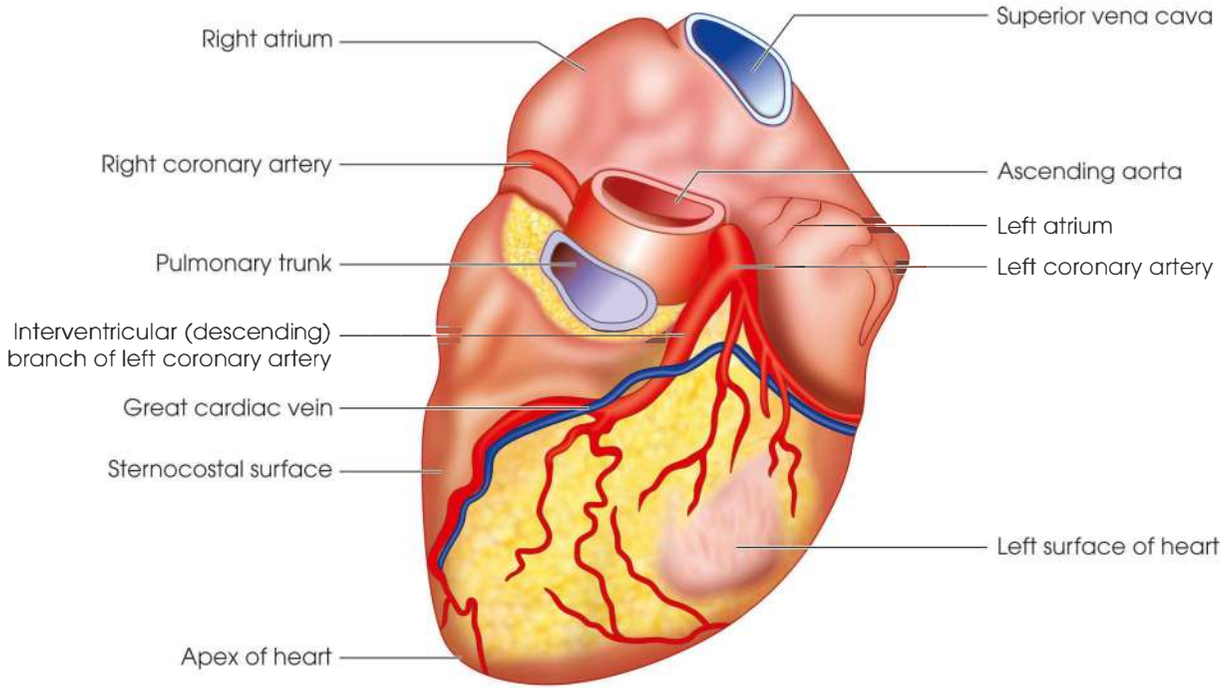

Fig. 39.1: The heart showing the disposition of the left coronary artery and its branches (left surface)

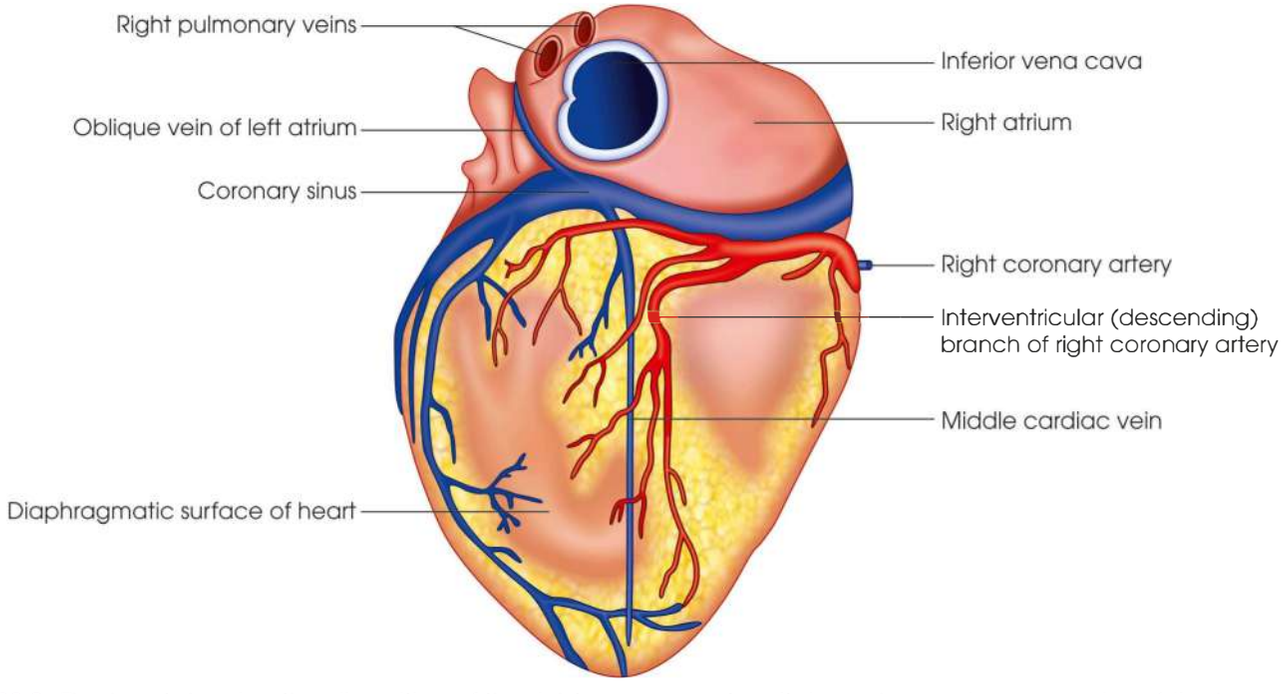

Fig. 39.2: The heart showing the disposition of the right coronary artery, its branches and coronary sinus (diaphragmatic surface)

by the use of nitrous oxide gas. Nitrous oxide method (Fick principle) gives almost accurate values. The subject inhales a mixture of 15% of nitrous oxide and air for 10 minutes. The amount of nitrous oxide taken up per minute is determined. Several samples of blood are taken from an artery and from a catheter introduced into the mouth of the coronary sinus at intervals during inhalation of nitrous oxide by the subject and their nitrous oxide content is determined. The arteriovenous difference of nitrous oxide is then calculated. Coronary inflow is then determined with the help of Fick principle:

$$= \frac{\text{Quantity of nitrous oxide inhaled per minute}}{\text{Different of nitrous oxide content of arterial and venous blood}}$$

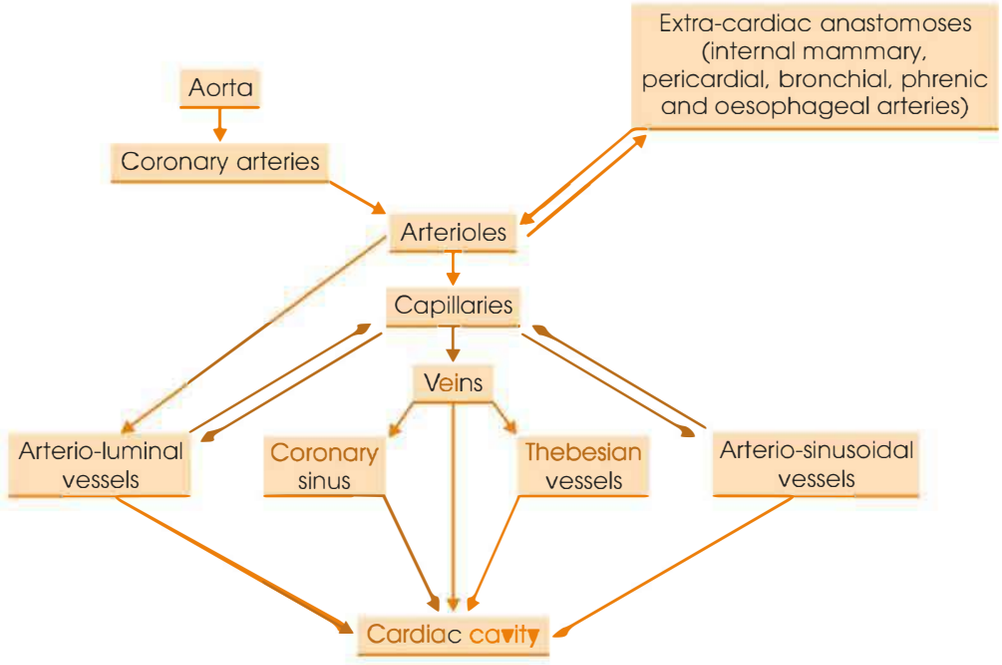

Fig. 39.3: Coronary circulation along with anastomoses channels (schematic representation)

Normal Values

1. During rest for each 100 gm of the left ventricle, the left coronary inflow varies from 65 to 85 ml per minute. The arteriovenous O_2 difference is very high and is about 10 to 15 ml per 100 ml. So, the extraction of O_2 by the cardiac muscle is very high.
2. During heavy exercise minute volume increases about ten times, and coronary inflow also raises ten folds to near about 2 litres. The O_2 usage of the heart is very high. The blood in the anterior cardiac veins or in the coronary sinus shows 20% saturation with O_2. During exercise, heart consumes about 250 ml of O_2 per minute (same as the resting O_2 consumption of the whole body). The arteriovenous O_2 difference of heart is about 10–15% (skeletal muscle—5–6%). This is equivalent to a minute flow of about 2 litres of blood through the coronary vessels.

VARIATIONS OF CORONARY INFLOW DURING DIFFERENT PHASES OF CARDIAC CYCLE

Ventricular action affects coronary circulation in two ways: (a) By altering the aortic pressure, (b) by exerting a variable degree of compression on the coronary vessels. The following phases are seen (Fig. 39.4).

Coronary Inflow

Left Coronary Artery

1. During isometric contraction phase, coronary inflow sharply falls and reaches minimum and even falls and reaches below the zero level due to backflow. Because, the aortic pressure is at

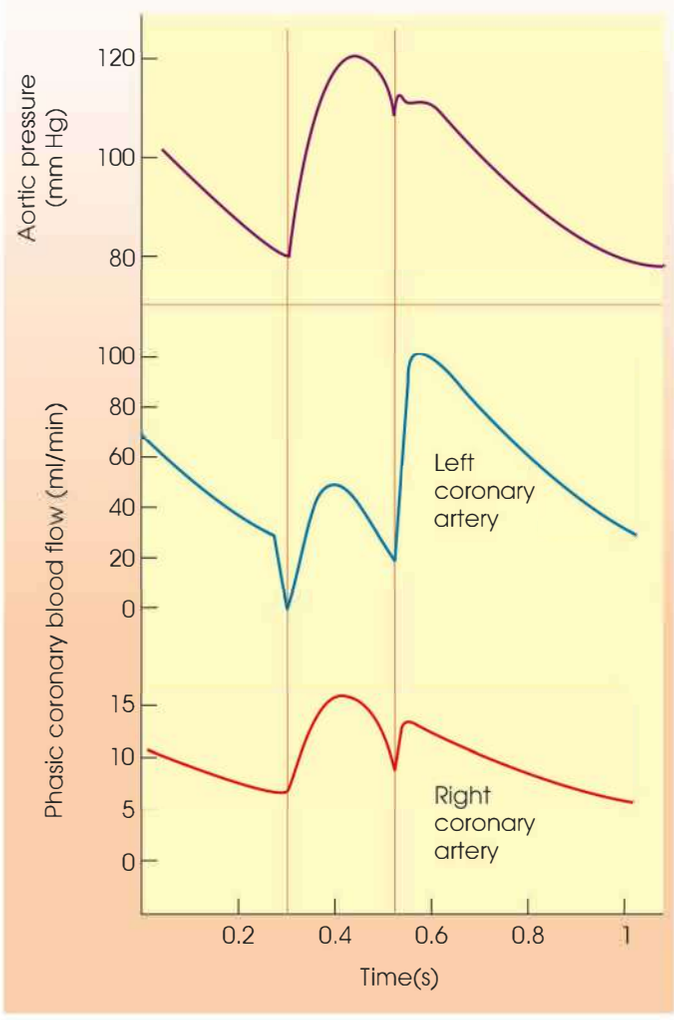

Fig. 39.4: Diagram showing phasic coronary inflow

minimum and the compression on the coronary vessels is maximum.
2. During reduced ejection phase, the coronary inflow again falls, because, the aortic pressure is falling and the compression is continued.
3. During isometric relaxation phase, the coronary inflow sharply rises, because, the aortic pressure is fairly high and the compression is minimum. Maximum coronary filling takes place during this phase due to fall of coronary vascular resistance.
4. During rapid ventricular filling phase, the coronary inflow continues to rise but slowly, because, the relaxation of the cardiac musculature continues and the vessels open up further.
5. In the later part of diastole, the coronary inflow slowly diminishes, because, the aortic pressure is falling and the coronary vessels are stretched due to filling of heart and the consequent elongation of cardiac muscle.

Right Coronary Artery

1. During isometric contraction phase, the coronary inflow sharply falls and rapidly rises again during the maximum ejection phase and falls during the reduced ejection phase.
2. In the isometric relaxation phase, the coronary inflow rises but not so steeply like the left coronary inflow. So, greater coronary inflow takes place in diastole than systole due to less compression of the coronary vessels during relaxation of the cardiac muscle and diminution of intramural tension. The left coronary inflow during systole is affected much as the pressure differential between aorta and left ventricle becomes 1 mm of Hg. But the right coronary inflow during the systole is not so affected as such pressure differential is 95 mm of Hg.
3. In the coronary sinus, the outflow of blood gradually rises from the isometric contraction phase and reaches its peak during protodiastolic phase and then gradually falls.

FACTORS INFLUENCING CORONARY CIRCULATION

The amount of blood passing through the coronary vessels is directly proportional to the work done by the heart. Blood flow can be adjusted on two principles: (1) By adjusting the lumen of the coronary vessels, (2) by adjusting the mean aortic pressure. The aortic pressure acts as the driving force. The lumen of the vessels may be altered in two ways (a) through the vasomotor centre (directly or reflexly) and (b) by directly acting on the vessels. The following factors modify coronary circulation, through one or both the above principles:

1. ***Mean aortic pressure:*** It is the chief motive force for driving blood into the coronary vessels. Any alteration of aortic pressure will therefore cause parallel changes in coronary circulation.
2. ***Role of cardiac output:*** Obviously, the coronary inflow is directly proportional to the cardiac output. Increased output raises coronary inflow in two ways: (a) By raising the aortic pressure, (b) by reflex inhibition of the vagal vasoconstrictor tone.
3. ***Metabolic factors:*** With the increased metabolism of heart, the O_2 requirement is increased and the circulation is greatly increased. There is a causal relationship between the myocardial metabolic activity, oxygen consumption and coronary blood flow. In the normal heart, blood oxygen content of coronary sinus is low under a variety of physiological conditions which supports the view of metabolic regulation of coronary blood flow (CBF) by reactive hyperaemia. Adenine nucleotide may be responsible for this reactive hyperaemia.
4. ***CO_2 and O_2:*** If O_2 requirement of the heart is increased then the coronary circulation is increased. Furthermore, if the O_2 supply to the heart muscle is decreased then the coronary flow is increased. But if the O_2 is supplied more than it is required, then the coronary circulation is decreased.

   Similarly, CO_2 stimulates the coronary flow. During asphyxia or inhalation of CO_2, concentration in the blood is increased; coronary flow is also increased at the first stage in order to maintain the total O_2 requirement of the cardiac muscle.
5. ***Effect of ions:*** K^+ in low concentration dilates the coronary vessel, whereas K^+ in higher concentration constricts. Calcium in therapeutic doses increases the flow and O_2 consumption of the cardiac muscle.
6. ***Polypeptides:*** Bradykinin has vasodilating effect on coronary vessels. Angiotensin II is an active octapeptide which causes arteriolar constriction in the skin, kidney, brain and also in coronary vessels.
7. ***Adenine nucleotides:*** Adenine nucleotides have been known to be potent coronary vasodilators. ATP and ADP are equally potent vasodilators. AMP is a bit-weaker vasodilator than those of ATP and ADP. ATP is not permeable to cell membrane, but adenosine can easily pass through the cell membrane.

   Possible metabolic regulation of coronary blood flow by adenosine has been presented schematically by Berne (1963) (Fig. 39.5).
8. ***Cardiac sympathetic and parasympathetic nerves***
   a. Stimulation of cardiac sympathetic fibres from the stellate ganglion or the ganglion itself

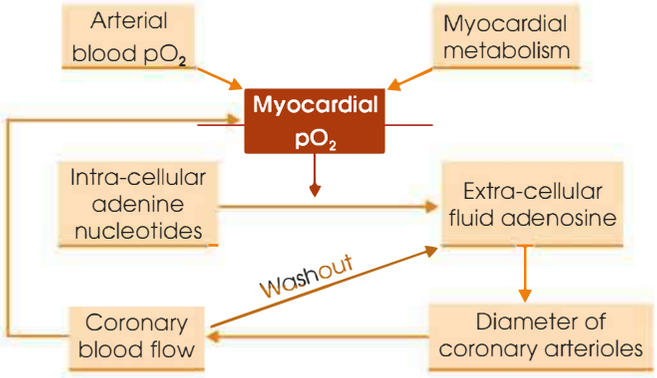

Fig. 39.5: Metabolic regulation of coronary blood flow

produces increased coronary inflow. This is mainly due to the influence of the cardiac sympathetic activity effect on coronary blood vessels resulting in release of norepinephrine; which causes vasodilatation of the coronary vessels and increases the coronary inflow. The acute metabolic changes in the cardiac muscle due to increase of cardiac work following sympathetic stimulation is possibly the cause of increased coronary flow.

b. Furthermore, the mediator released at sympathetic postganglionic endings of the cardiac muscle following stimulation increases the O_2 consumption and this state causes the cardiac muscle hypoxia, a condition favourable for increasing blood flow through reactive hyperaemia.

c. Similarly, the vagal stimulation, produce; vasodilatation of the coronary vessels through liberation of acetylcholine.

9. ***Heart rate:*** When the heart rate is increased, minute cardiac output, aortic blood pressure may increase but the stroke volume decreases.

   a. The phasic coronary inflow and O_2 consumption per beat decrease, but minute coronary flow and O_2 consumption per minute are increased.

   b. With the increase of heart rate, O_2 requirement of the heart muscle is increased and is maintained normally through increase of minute flow.

   c. It has been observed that with the increase of heart rate, the extra-vascular resistance is increased, but the intra-vascular resistance is actually decreased causing a decrease of resistance, hence increase of minute flow.

10. ***Hormones:*** Thyroid: In thyrotoxicosis metabolism is increased along with increased coronary inflow and O_2 consumption per minute. In hypothyroidism, the flow is decreased which is possibly related with the altered metabolism of the cardiac muscle.

    Adrenaline and noradrenaline: These cause increased coronary inflow along with increased O_2 consumption per minute. These produce the relaxation of the coronary vessels by acting on the β-receptor of the vessel. Nicotine also increases the coronary flow through the liberation of noradrenaline. Relaxation of coronary blood vessels is prohibited by β-adrenergic blockers.

    Pitressin: It causes increased coronary resistance and diminution of coronary inflow. This decreased flow is presumably due to direct vasoconstrictor effect on the coronary vascular bed. This vasoconstrictor effect is not due to metabolic effect or due to increase of intra-cellular and extra-cellular K^+ values. It possibly constricts the vessels at arteriolar level.

    Acetylcholine: It increases coronary inflow due to dilatation of the coronary vessels. This increases the flow.

11. ***Temperature:*** With the rise of body temperature, the metabolism is increased and for the maintenance of normal O_2 need the coronary circulation is increased. But with the fall of body temperature, as in the case of hypothermia, the coronary circulation is greatly decreased along with the decreased metabolic need of the cardiac muscle.

12. ***Muscular exercise and excitement:*** As mentioned above, coronary inflow is adjusted according to the work done by heart. During heavy exercise, the inflow rises about ten times. This is due to the fact that almost all the factors which increase coronary inflow come into action during muscular exercise, viz. O_2 lack, CO_2 excess, increased H-ion concentration, metabolites, increased temperature, adrenaline secretion, raised blood pressure, etc. During excitement, as the heart rate is increased, the coronary blood flow is also increased greatly, although the diastolic phase is decreased.

13. ***Anaemia:*** In anaemia, the coronary flow is increased sharply in order to maintain the normal O_2 need of the cardiac muscle, because the O_2 carrying capacity of the blood is decreased under such state. The increase in the coronary flow is related partly to the decreased viscosity of blood and mostly to the active vasodilatation (reactive hyperaemia) resulting from (a) anaemic hypoxia and (b) metabolic hypoxia due to compensatory increased heart rate.

14. ***Intra-ventricular pressure:*** The increase of intra-ventricular pressure also alters the coronary flow. The increase of the right ventricular pressure (due to mitral stenosis, emphysema, atelectasis, etc.) affects coronary flow greatly as this pressure is reflected in the coronary venous bed of the right

ventricle. In case of the left ventricle, the coronary flow through this side is initially increased with the rise of intra-ventricular pressure (due to coarctation of the aorta or increase of peripheral resistance by any means). Under such state the left ventricular workload is increased greatly and ultimately the heart muscle is hypertrophied and coronary flow is gradually decreased. Ultimate fate of the heart is the degeneration of cardiac muscle fibres leading to congestive heart failure.

15. ***Transfusion:*** During transfusion, ventricular load is increased due to rapid venous return; and the systolic and diastolic heart size, ventricular stroke volume and work and also arterial blood pressure are all increased. Under such state the heart rate is decreased causing greater increase of stroke volume, diastolic pause and thus the coronary flow per beat and per minute is increased.
16. ***Extra-vascular pressure:*** This is an important determinant of the coronary flow as the coronary vascular resistance is increased due to rhythmical compression of the myocardial vessels during contraction. Viscerocardiac reflex: The coronary flow is markedly altered during visceral distension and it is often encountered in a patient with ischaemic heart disease. Anginal pain, following a meal in such cardiac patient, is the consequence of decreased coronary flow.

CIRCULATORY STATUS OF THE CARDIAC MUSCLE UNDER CERTAIN DISEASED CONDITIONS

Aortic Stenosis

In aortic stenosis, there is initially increase of coronary flow along with increase of coronary perfusion. With the stenosis of the aorta, the intra-ventricular pressure of the left ventricle is increased and the left heart has to work against increased resistance. Consequently there is hypertrophy of the left ventricle with decrease of coronary flow if the stenosis is maintained for some time.

Pulmonary Hypertension

Pulmonary hypertension may be the cause of mitral stenosis, emphysema, atelectasis, pneumonia, etc. Under such state the right ventricular pressure is greatly increased and this increase of intra-ventricular pressure causes decrease of coronary flow of the right heart because the arteriovenous pressure difference is greatly decreased. Pulmonary hypertension may be associated with right ventricular hypertrophy and ultimately leading to right heart failure.

Aortic Insufficiency

Patients suffering with aortic insufficiency are always associated with anginal pain. This anginal pain is due to coronary insufficiency. This coronary insufficiency is mostly due to decrease of aortic pressure and also of central coronary diastolic pressure.

Mitral Stenosis

In mitral stenosis, an obstruction is exerted during flow of blood from left atrium to left ventricle. If this state is prolonged then pulmonary hypertension may happen and coronary flow of the right heart is greatly affected.

Aortic Coarctation

It is the condition when a vessel remains constricted or narrowed and thereby the blood flow through the vessel is decreased. In coarctation of the thoracic aorta, workload of the heart is increased as the heart has to work against the resistance. The venous return by way of the inferior vena cava may be decreased but the total venous return may be unaffected only by opening up of other branches of the aorta. Thus, in case of mild form of coarctation, the cardiac output and at the same time coronary flow may be maintained but if the constriction is in severe form and the blood flow through the aorta is severely affected then the venous return, cardiac output and the coronary flow are greatly decreased. As the heart has to work against increased resistance, there is possibility of left ventricular hypertrophy along with decreased coronary flow. Ultimate fate of, it is the congestive heart failure.

Hypertensive Cardiovascular Disease

In essential hypertension having no organic changes in the heart and blood vessels, the mean coronary flow remains unaltered although the systemic pressure remains elevated. But in hypertension having coronary atherosclerosis or arteriosclerosis, the elastic behaviour of the coronary vessels are altered, the flow may be affected greatly, although the systemic pressure remains at higher level.

Ischaemic Heart Disease

Ischaemic heart disease is the condition when cardiac muscle suffers from coronary insufficiency. This coronary insufficiency is due to major cause of (a) sclerosis of the coronary vessels and (b) coronary occlusion by thrombosis. In both the cases, if these are not so severe then the mean coronary flow is maintained through the development of anastomosing channels in-between the affected vessels and the unaffected vessels. In such case, the affected area (ischaemic area) gets blood supply through backflow. If the subject maintains a normal well-adjusted life and the heart is not given any unusual workload then the management or rehabilitation of this patient is possible through the newly adjusted circulatory machineries. But further

cardiac attack or any increased workload of the heart becomes an unmanageable episode of such patient because readjustment becomes impossible.

CORONARY SPASMS AND INTERCORONARY REFLEXES

Coronary spasms have been described to be the major cause of acute myocardial infarction.

Pathological Physiology of Angina Pectoris and Acute Myocardial Infarction

Ischaemia is the cause of pain of angina pectoris and of acute myocardial infarction. Angina pectoris is thus a symptom of cardiac ischaemia but not a disease. The anginal pain is often associated with disproportionate blood supply according to myocardial requirement. In ischaemic heart disease the patient often complains this pain following a heavy meal or a physical exertion or an excitement. The pain is due to accumulation of metabolites produced during coronary ischaemia. The metabolites stimulate the nerve endings. But the actual stimulus at the nerve endings that may cause the pain is not yet known. Sir Thomas Lewis has described it the P factor which has some characteristics with lactic acid. Predisposing cause of myocardial infarction is the coronary obstruction due to atherosclerosis. The obstruction may be due to gradual shortening of the vessels itself by the deposition of lipid in the intima or by the locally formed thrombus. If the ischaemia is prolonged then it may lead to irreversible changes of necrosis.

Applied Physiology

Role of angiogenesis and vasculogenesis: The vascular endothelial growth factor (VEGF) is a signal protein produced by cells that stimulates vasculogenesis and angiogenesis. It helps restoring the oxygen supply to tissues especially in hypoxic conditions. The normal function of VGEF is to create new blood vessels in embryonic life, development of new blood vessels post injury, following exercise, and as new vessels collateral circulation formed the blood can freely flow bypassing blocked vessels thus preventing cardiovascular damage.

CEREBRAL CIRCULATION

Anatomical Considerations

Key Points

1. Blood enters the cranium through four vessels, two internal carotid and two vertebral arteries. The last two combine to form the basilar artery which divides into two posterior cerebral arteries. Each internal carotid artery divides into two branches: The middle and anterior cerebral arteries. Intercommunicating branches unite the six arteries on two sides forming the circle of Willis (Fig. 39.6). These vessels supply the different parts of brain.
2. Brain has a rich blood supply. The grey matter is more vascular than the white matter. The total capillary length per cu mm of the grey and white matter is about 1 m and 200 mm, respectively.
3. The cerebral vessels are not strictly end arteries. They freely anastomose. The free anastomosis in the circle of Willis makes an adequate distribution of blood to the different parts of the brain tissue and this is the only contributory help during emergency. A red cell from one lobe can easily pass to another lobe.
4. Venous blood drains into large cerebral sinuses, e.g. the superior sagittal, inferior sagittal, cavernous, straight, etc. all of which ultimately unite to form the two transverse sinuses, which become continuous with the two internal jugular veins.

METHOD OF STUDY: NITROUS OXIDE METHOD: FICK PRINCIPLE

The subject inhales a mixture of air and 15% of N_2O. Blood samples are collected from a peripheral artery and from the jugular vein at frequent intervals during the period when the subject inhales the mixture. N_2O content of the arterial and venous blood is determined. Cerebral blood flow per minute is determined from the arteriovenous difference of N_2O and the partition coefficient for N_2O between blood and brain. Results are recorded in millilitre per 100 gm of brain tissue per minute.

Vasomotor Supply

The sympathetic carries vasoconstrictor fibres. Their stimulation causes an average reduction in diameter between 7 and 8%. The dilator fibres pass in the vagus nerve and the VIIth nerve. The vagus nerve carries the afferent fibres and the great superficial petrosal branch of the facial nerve carries the efferent fibres. Hence, stimulation of the VIIth nerve or that of the central cut end of the vagus nerve increases the diameter to about 22%. Since, the flow of liquid through a capillary tube is directly proportional to the fourth power of its radius, a dilatation of 22% will increase the flow by about 150%. There is evidence that vascularity of a particular part of brain increases during the activity of that part and may be associated with vasoconstriction of the other parts of brain.

Normal Values of Cerebral Circulation

1. The ***average blood flow*** of normal subjects in resting condition is 54 ml per 100 gm of brain tissue per minute. Taking the weight of brain as 1,400 gm, total cerebral blood flow is 750 ml.
2. ***Blood pressure*** in the large cerebral arteries is 100 mm of Hg systolic and 65 mm of Hg diastolic. In the

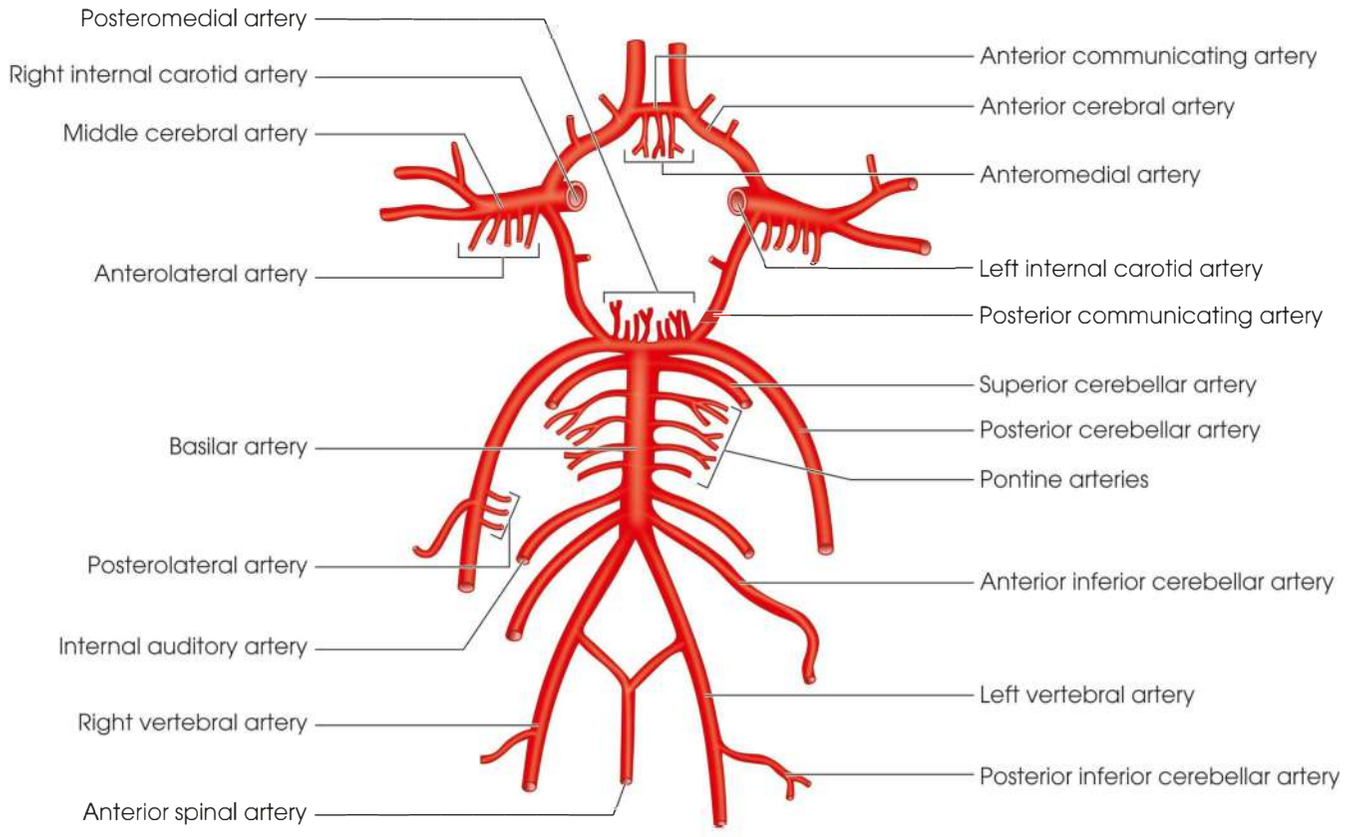

Fig. 39.6: Arrangement of cerebral blood vessels and formation of the circle of Willis

capillaries, it is about 13 mm of Hg. It rises during inspiration and falls during expiration. But forced expiratory efforts, especially with closed glottis, raise the pressure by 30–50 mm of Hg.

3. The ***total oxygen consumption*** of the brain is 50 ml per minute. Hence, the rate of oxygen consumption is fairly high. Arteriovenous O_2 difference is 6.2 ml% (arterial: 19 ml%, jugular: 12.8 ml%).
4. ***Respiratory quotient (RQ) is unity***, showing that carbohydrates are mainly used (probably only as galactose).
5. ***Cranial circulation time*** (carotid to jugular) is 3 seconds (radioactive method). The capillaries of the cerebral vessels, choroid plexus, etc. are less permeable. As a result of this less permeable crystalloid elements of the blood cannot pass into the cerebral tissue spaces. This is called blood–brain barrier (Fig. 39.7).

REGULATION OF CEREBRAL CIRCULATION

Cranium being a rigid box, the amount of blood in it, at any moment, cannot rise to any considerable degree. Only slight rise may take place by compressing the veins and displacing a little cerebrospinal fluid (CSF). Hence, blood supply to the brain can only be increased by raising the velocity of blood flow through it. Broadly speaking, cerebral blood vessels have got no well-organised vasomotor control and the circulation can be maintained by (a) adjusting the general blood pressure through the sino-aortic mechanism, (b) regulating the lumen of the cerebral vessels.

EXISTENCE OF AUTO-REGULATION OF CEREBRAL BLOOD FLOW

Key Points

In normal individual the auto-regulation of cerebral blood flow as if sub-serves a homeostatic function. As the increased CO_2 tension of the blood abolishes the auto-regulatory mechanism; it is quite likely that two factors may play. One is the myogenic response of smooth muscle which is effective with the increase of pressure and the other, the metabolic factors involving the tissue tensions of the respiratory gases, to which the cerebral arterioles are highly sensitive.

FACTORS CONTROLLING CEREBRAL CIRCULATION

There are so many factors that may control the cerebral circulation. The main factors that generally operate are the driving force is the difference between the mean arterial blood pressure (MABP) and the internal jugular pressure (IJP), and the cerebrovascular resistance (CVR). So, the cerebral blood flow (CBF) is directly proportional to driving force (the difference between

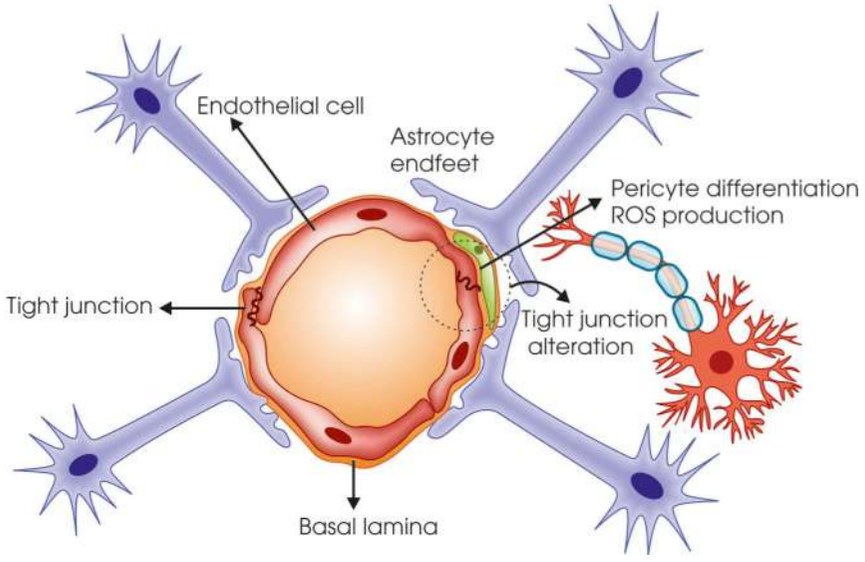

Fig. 39.7: Protoplasmic astrocyte showing blood-brain barrier

the mean arterial blood pressure and the internal jugular pressure) and inversely proportional to the cerebrovascular resistance, i.e.

$$CBF = K \frac{\text{Driving force}}{\text{Cerebrovascular resistance}}$$

Driving Force or Arterial Pressure Head

This factor depends upon the cardiac output and peripheral resistance. The arterial blood pressure is the sole determinant of driving force and internal jugular pressure has a little role under normal condition. But in case of positive g, this internal jugular pressure plays an immense role. In human beings with centrifugal accelerative stress, the cerebral circulation is still maintained for several seconds though the arterial blood pressure at head level is practically nil. This maintenance of pressure under such state is due to simultaneous fall of pressure in the internal jugular vein.

In normal individual having well-organised homeostatic control, any alteration of the systemic pressure will have practically no effect on the cerebral blood flow but if the homeostatic control fails, then hazard may occur. Sometimes cerebral haemorrhage or senselessness occurs possibly due to absence of this homeostatic control. Intrinsic factors play only when pressure drops further and the flow is maintained by lowering the cerebrovascular resistance.

CEREBROVASCULAR RESISTANCE

The cerebrovascular resistance depends upon the following factors:

1. **Intracranial pressure:** Rise of intracranial pressure (brain tumour, meningitis, etc.) reduces the blood flow, by mechanically compressing the cerebral capillaries and veins. Cerebrovascular resistance has got mostly a linear relationship with intracranial pressure. The cerebral blood flow has got negative correlation with increased intracranial pressure; however, this correlation only comes into play until pressure exceeds 500 mm of H_2O.
2. **Viscosity of blood:** In anaemia, the viscosity of blood is decreased due to fall of haematocrit value and hence the increase of flow. But in case of polycythaemia vera the flow is decreased as the viscosity is higher due to raised haematocrit value.
3. **Diameter of the cerebral blood vessels:** Cerebral vascular resistance has got inverse relationship with the diameter of the blood vessels. The diameter of the cerebral blood vessels are altered under the following factors, viz. neurogenic factors, CO_2 and O_2 level in blood, neurohormones, etc.
4. **Carbon dioxide tension:** Increased CO_2 tension raises the flow. It causes cerebral vasodilatation by local action but general vasoconstriction through vasomotor centre (VMC) and sino-aortic reflexes. Cerebral blood flow increases by 75% after inhalation of 5–6% of CO_2.
5. **O_2 lack:** It increases cerebral circulation by local vasodilatation as well as by general vasoconstriction (VMC, sino-aortic reflex, etc.) and thus raises blood pressure.
6. **Excess O_2:** It causes reduction of cerebral blood flow provided the concomitant hypocapnia is prevented.
7. **Adrenaline and noradrenaline:** The adrenaline increases the cerebral blood flow through vasodilatation but the noradrenaline decreases the cerebral blood flow through profound vasoconstriction. There are other drugs which may modify the cerebral circulation. Ethyl alcohol in therapeutic doses has

got no significant effect but, in severe alcoholic intoxication it may increase the cerebral blood flow. This effect is possibly due to effect of accumulated CO_2 in the blood.

8. **Papaverine:** It is a good cerebral vasodilator.
9. **Xanthine** group of drugs have got some vasoconstrictor effects. Aminophylline in therapeutic dosage produces vasoconstriction. Caffeine has got similar effects. Nicotinic acid injected intravenously, though produces facial vasodilatation but does not produce any cerebral vasodilatation. Though histamine produces cerebral vasodilatation yet it is not so effective as it simultaneously produces systemic vasodilatation causing fall of systemic blood pressure.

Clinical conditions: In essential hypertension, the cerebral circulation remains unaltered though the vascular resistance is greatly increased. But in case of cerebral arteriosclerosis, the cerebral blood flow is diminished due to increased cerebrovascular resistance and loss of elasticity of the blood vessel wall.

PULMONARY CIRCULATION

Anatomy of Circulation

Blood enters lungs through two sources; pulmonary artery and bronchial arteries. The cross-sectional area of the pulmonary artery is same as that of the aorta, but it is more elastic and distensible. Through the pulmonary artery venous blood of the right ventricle goes to the lungs for oxygenation. It also carries nutrition to the pulmonary tissues. Bronchial arteries, originating from the aorta, carry oxygenated blood for the nutrition of bronchi and bronchioles (Fig. 39.8). The pulmonary artery breaks up into wide arterioles and capillaries which form a rich network surrounding the alveoli where gaseous exchange takes place. The bronchial artery also breaks up into capillaries which partly join the alveolar network but is mostly returned through bronchial veins and partly through azygos vein. The branches of the bronchial artery are also distributed to the bronchial glands and walls of the bronchioles as well as respiratory bronchioles and thus form capillary plexuses which drain blood into the pulmonary venules (Fig. 39.9). The oxygen supply to the pulmonary tissues comes from the deoxygenated blood as well as from the oxygenated blood.

Method of Recording Pulmonary Arterial Pressure

With the help of the cardiac catheter, it is inserted through the systemic vein into the right atrium and then into the right ventricle and then into the pulmonary artery from where the pulmonary arterial pressure can be recorded.

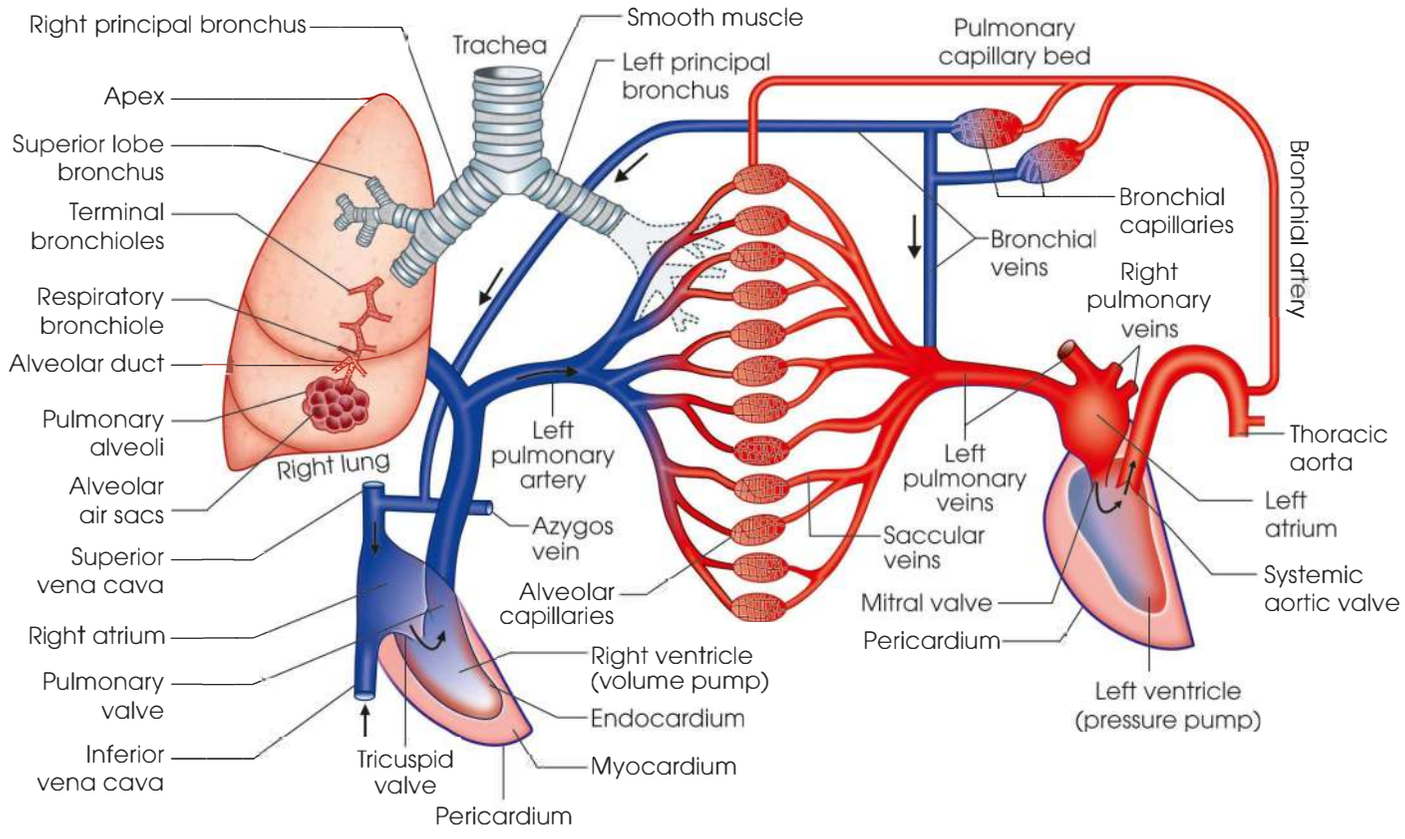

Fig. 39.8: Vascular supply showing the pulmonary circuit, and the bronchial arteries and veins

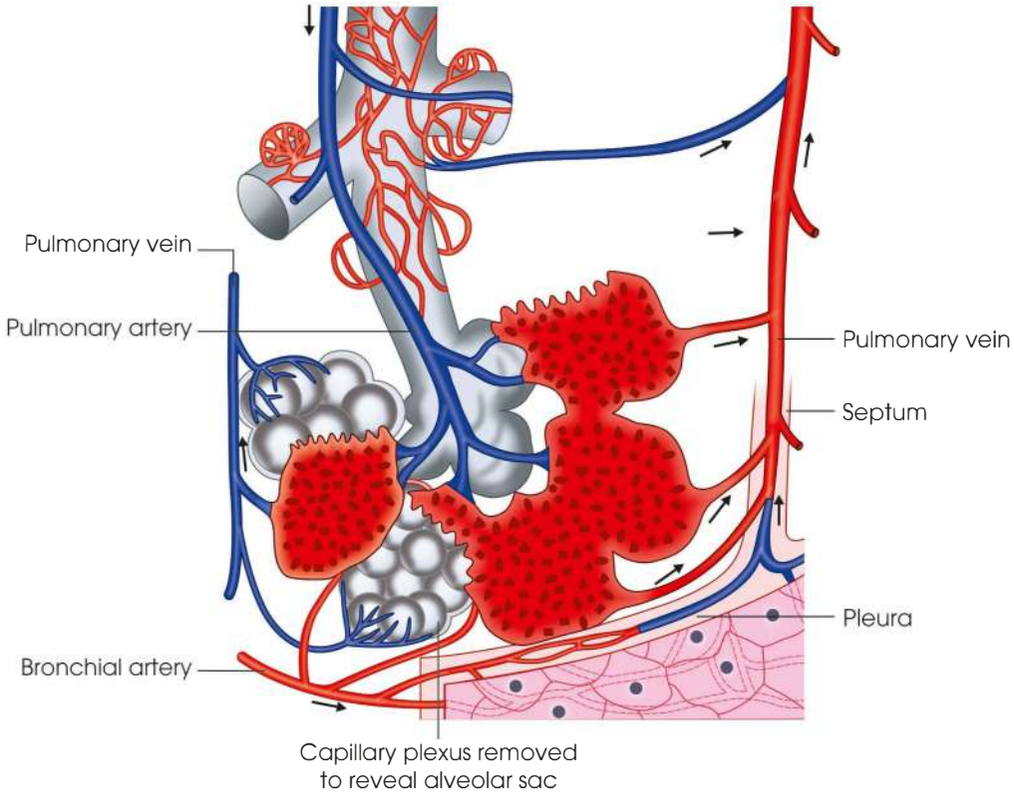

Fig. 39.9: Intrapulmonary anastomoses in between the pulmonary artery and bronchial artery

Vasomotor Supply

The sympathetic supply is from upper thoracic segments and the parasympathetic supply from the vagus. Results of stimulation of nerves are variable. Stimulation of the chemoreceptors of the carotid body causes diminution of the pulmonary vascular resistance which disappears after section of the vagus and the sympathetic, so pulmonary circulation is under reflex control.

Normal Values of Pulmonary Circulation

1. **Minute blood flow:** It is the sum total of right ventricular output and blood flow through the bronchial arteries. It approximates around 4–5 litres.
2. **Blood pressure:** The systolic pressure in the human pulmonary artery is about 22 mm of Hg (right ventricle—about 22 mm of Hg). The diastolic pressure is about 10 mm of Hg (right ventricle 0 to 1 mm of Hg). With each heartbeat the pressure rises in the pulmonary artery and thus causes pulsation in these vessels. The pulmonary capillary pressure is about 8 mm of Hg. The pulmonary venous pressure is about 5 mm of Hg (left atrium—approximately 4 mm of Hg).
3. **Circulation time:** 3–9 seconds (ether).

FUNCTIONS OF PULMONARY CIRCULATION

1. **Gas exchange:** It is the main function of the pulmonary circulation. Through circulation, the mixed deoxygenated blood is passed through alveolar capillaries and thus gaseous exchange in between the alveolar air and alveolar capillaries takes place. Due to gaseous exchange, blood that passes through alveolar capillaries gets proper amount of O_2, and gets rid off proper amount of CO_2.
2. **Filter:** The fine pulmonary blood vessels act as a filter. It traps the emboli that pass through the pulmonary capillaries. Thus, filter prevents from reaching and blocking the vessels in the brain and heart.
3. **Nutrition:** Pulmonary circulation maintains the nutrition of the lung tissue.
4. **Fluid exchange:** Pulmonary capillary pressure is very low which tends to pull fluid from the alveoli and thus any fluid accumulation in the alveoli is speedily absorbed into the blood.
5. **Reservoir for left ventricle:** Left ventricular output is fully dependent upon the return of blood from the pulmonary bed to the left atrium. So any alteration of the pulmonary haemodynamics will alter the left ventricular function.

CONTROL OF PULMONARY CIRCULATION

The amount of blood passing through lungs follows the same general principles as elsewhere. It depends upon the following factors:

1. **Output of the right ventricle (mechanical factor):** It depends upon the force and frequency of contraction and the degree of venous return. Cardiac output may

increase 3 or 4 times but the pulmonary arterial pressure does not rise appreciably.

2. **Resistance of the pulmonary bed:** It depends upon the following factor: (a) Lumen of the pulmonary vessels—hypoxia produces pulmonary vasoconstriction. Increased CO_2 tension in the blood constricts the pulmonary blood vessels. It has been observed that if one lung is ventilated with a mixture of CO_2 and O_2, and the other lung with air then most of the blood is shifted to the normally ventilated lung so as to protect the body from hypercapnoic effect. This is auto-regulation of pulmonary blood flow.
3. **Adrenaline and noradrenaline** also produces vasoconstriction. Acetylcholine also dilates the pulmonary blood vessels but the degree of vasodilatation is mostly dependent upon pre-existing tone of the smooth muscle of the pulmonary blood vessel. Serotonin, a humoral substance originating from disintegrating platelets or by secretory products of chromaffin tissues also constricts the pulmonary blood vessels.
4. **Role of respiration:** During inspiration, the pulmonary bed enlarges, capillary pressure falls to about 2 mm of Hg and, therefore, more blood enters the lungs. This is caused by elongation of the capillaries due to stretching and their dilatation due to negative pressure and probably vasomotor effect. Thus, during inspiration, lungs can hold about 10% of total blood volume. During expiration, reverse changes take place, pressure rises to about 4 mm of Hg and lungs can hold only about 6% of the blood volume. But total pulmonary vascular resistance increases during both maximal inflation and forced deflation of the lungs. Measurement of vital capacity acts as a guide. A fall shows pulmonary congestion.
5. **Nervous control:** The pulmonary blood vessels have got both parasympathetic and sympathetic nerve supply. But there is still doubt that these nerve supplies have got any major physiological role in maintenance of normal circulation.
6. **Reflex control:** The pulmonary circulation is also modified through reflexes originated due to stimulation of baroreceptors and present in the sino-aortic areas. Stimulation of baroreceptors in the carotid sinus and aortic arch produces reflex vasodilatation in the pulmonary vascular bed, whereas stimulation of chemoreceptors in the aortic bodies or carotid bodies produces pulmonary vasoconstriction.

PECULIARITIES OF PULMONARY CIRCULATION

Pulmonary artery carries deoxygenated blood and pulmonary veins carry oxygenated blood.

1. **Filtration of fluid:** In systemic capillaries, filtration of fluid takes place into the tissue space, but, nothing such happens in the lungs. The purpose is obvious. Filtration would cause collection of liquid in the alveoli and retard oxygenation of blood. The mechanism is also obvious. In the pulmonary capillaries the colloidal osmotic pressure (25 mm of Hg) is much higher than the blood pressure. In the systemic capillaries it is just the reverse. Hence, no filtration in the lungs. Pulmonary congestion, from any cause, will increase the local blood pressure leading to filtration and causing oedema of lungs.
2. **Filtration of emboli:** The fine pulmonary capillary acts as a filter that traps emboli from reaching and blocking the blood vessels of heart, brain or other organs.
3. **Ventricular output of both sides remain same:** Blood enters lungs through pulmonary and bronchial arteries. Blood is returned from the lungs through similar two channels; the pulmonary veins (oxygenated blood) and bronchial veins (reduced blood). One may think that the reduced blood instead of being returned through the bronchial veins could have easily joined the alveolar capillaries, become oxygenated and be returned through the pulmonary veins to the left heart. But in that case the venous return to the left heart would be more than the output of the right heart. This discrepancy may lead to heart failure. Only that amount of blood which was expelled by the right heart will return to the left heart, so that the output of the two ventricles may remain same.
4. **Pulmonary vascular blood resistance:** Thus, blood flow through the two systems—pulmonary and systemic, are equal. The pulmonary vascular bed is a low, resistance circuit, whereas the systemic one is a high resistance circuit. This is not actually true, because a certain amount of systemic blood, flowing through the bronchial arteries, do not drain into bronchial veins and right atrium, but drain into pulmonary veins and left atrium. Some amount of systemic blood, flowing through the coronary arteries, return directly to the cavity of the heart through the thebesian vessels.
5. **Pulmonary vascular bed:** It has to supply blood to one type of tissue, whereas the systemic one has to supply blood to different types of tissues.
   a. Pulmonary vascular bed is relatively short distensible with large calibre and can accommodate a large volume of blood (blood reservoir).
   b. As the pulmonary vascular bed is exposed to subatmospheric pressure, the pulmonary pressure and flow are altered during inspiration and expiration.
   c. As the pulmonary vascular bed is short and distensible, blood flow is not fully dependent upon neurogenic control; and mechanical factor

of the right heart plays an important role in maintenance of normal blood flow.

d. Local actions of CO_2 and low O_2 on the pulmonary vascular bed are of vasoconstrictions which are just the reverse in case of the systemic one.

Though the pulmonary vascular bed has got both sympathetic and parasympathetic innervations, yet its role in the maintenance of circulation is less important than the systemic one.

EFFECT OF RESPIRATION ON THE SYSTEMIC BLOOD PRESSURE

Generally systemic blood pressure falls during inspiration and rises during expiration. This is due to increased capacity of the pulmonary vascular bed during inspiration holding a larger volume of blood and thus momentarily reducing the return of blood to the left heart. Because during inspiration, pulmonary vascular resistance is greatly reduced as the intrathoracic pressure is below the atmospheric pressure. So at the first phase of inspiration, aortic pressure is decreased and at the last phase of inspiration as well as with the onset of expiration the systemic pressure is increased because the venous return to the left heart is gradually increased at the later phase of inspiration and towards the early phase of expiration.

PULMONARY VASCULAR REFLEX

There are baroreceptor areas in the pulmonary arch of aorta and when these receptors are stimulated, reflexly alter the systemic blood pressure, heart rate and capacity of the peripheral blood vessels. An increase in the pulmonary arterial pressure produces reflex bradycardia, hypotension and increase of blood flow in the splanchnic bed. These responses are abolished following divisions of the vagus nerves:

- Reflexes from the pulmonary vascular bed may participate in the regulation of blood volume. Increased blood volume in the thoracic cavity reflexly produces diuresis through the inhibition of the antidiuretic hormone (ADH) secretion.
- Intravenous injection of starch grain, multiple emboli may produce rapid shallow breathing. This reflex respiratory response is due to the stimulation of receptors in the pulmonary vascular bed and can be abolished following section of the vagi.

PULMONARY DEPRESSOR CHEMOREFLEX (PAINTAL, 1955)

Intravenous injection of phenyl diguanide induces bradycardia and hypotension. It has been claimed that these reflex effects are initiated through the stimulation of pulmonary deflation receptors. Likewise serotonin, starch grain, multiple emboli stimulate these deflation receptors and induce pulmonary depressor chemoreflex.

CIRCULATORY STATUS IN DIFFERENT CARDIOPULMONARY DISEASES

Mitral Stenosis

It is the condition when the orifice of the valve is narrowed by fusion of the cusp margins. The cusps become rigid and thickened. The chordae tendineae hold the valve in a fixed position and the opening becomes narrowed. The whole ring looks like a narrow rigid funnel. Due to narrowing of the opening, a resistance is offered for blood flow from the left atrium to the left ventricle. The left atrium is dilated and thickened due to accumulation of blood within itself. Furthermore, the great problem that arises due to this is the occurrence of pulmonary hypertension. With the obstruction of blood flow from the left atrium to the left ventricle, the left atrial pressure is tremendously increased causing decrease in pulmonary driving force. So, the consequence is the decrease in pulmonary flow, right ventricular hypertrophy or cor pulmonale ultimately leading to right heart failure.

Emphysema

It is the condition in which there is enlargement of the air spaces distal to the terminal bronchiole. Obstruction to expiration due to oedema, inflammatory changes or mucus in the bronchi may cause emphysema. In emphysema, pulmonary vascular resistance is greatly enhanced causing obstruction to blood flow. Ultimately, the pulmonary hypertension and then right heart failure may occur. The increase of pulmonary vascular resistance is the consequence of destruction of blood vessels. Poor exchange of air in the emphysematous alveoli may also cause increase of vascular resistance by accumulated CO_2 in the blood.

Pulmonary Embolism

In pulmonary embolism either massive or diffuse, the blood flow is greatly affected due to blockade of pulmonary blood vessels by free-moving clot (embolus).

Atelectasis

It is the condition when a lung or a part of a lung remains in a collapsed state. In pneumothorax that is when the chest cavity is opened to atmospheric pressure. In such condition, the pulmonary vascular resistance is greatly increased causing the decrease of pulmonary blood flow. In natural atelectasis, the alveoli not only constrict, blood vessels lining the alveoli are also constricted causing vascular detachment with the rest of the lung. This is a safety mechanism of the lung so that the ill-ventilated lung is prevented from supplying major quantities of blood.

Removal of Lung

In case of pneumectomy or lobectomy, pulmonary haemodynamics remain unaltered so long the subject maintains a sedentary life. But if the subject performs heavy work then due to increase of cardiac output, the pulmonary pressure may be increased drastically because in such subject the pulmonary reserve is very low.

Diffuse Sclerosis of Lung Vessels

In sclerosis of the pulmonary blood vessels the elasticity as well as the distensibility of the lung vessels is decreased. Pulmonary vascular capacity is also affected greatly. In such case, if subjects do not have such heavy or even mild work so that the cardiac output is increased, then there should have no trouble. In late or extreme stages of sclerosis, the pulmonary blood flow is greatly decreased and pulmonary hypertension along with the right heart hypertrophy may be happened.

Pulmonary Fibrosis

It is the cause of formation of fibrous tissue during healing of the wound. The generalised fibrosis happens in lung tissue following recovery from tuberculosis, bronchopneumonia, pneumoconiosis, gas poisoning, etc. Under such conditions the pulmonary vascular resistance is increased. Thus, obstruction to blood flow may be offered causing pulmonary hypertension in late stage.

HEPATIC CIRCULATION

Vascular Arrangement

Blood enters the liver through two sources: (1) portal vein, and (2) hepatic artery (Fig. 39.10). The hepatic artery is not an end artery and has got anastomotic channels with the portal vein at several levels of the interlobular regions (Fig. 39.11). The hepatic artery transfuses the portal vein as well as the sinusoid at a higher pressure head. The portal vein is originated from two vessels: (1) The superior mesenteric vein draining blood from the mesenteric bed and (2) the splenic vein draining blood from the spleen. The left lobe of the liver gets blood from the spleen and the right lobe of the liver gets it from the superior mesenteric vein.

Though the major blood supply is made by the portal vein, yet the hepatic artery ligation may or may not be compatible with survival but it is depending mostly on the level of the ligation and species. The portal vein and the hepatic artery have anastomotic connections with the sinusoid.

The sinusoid is a smooth-walled cylindrical tube and is lined by phagocytic cells (Kupffer). It is guarded by inlet and outlet sphincters at their terminals and these sphincters play an important role in the regulation of flow and storage of blood.

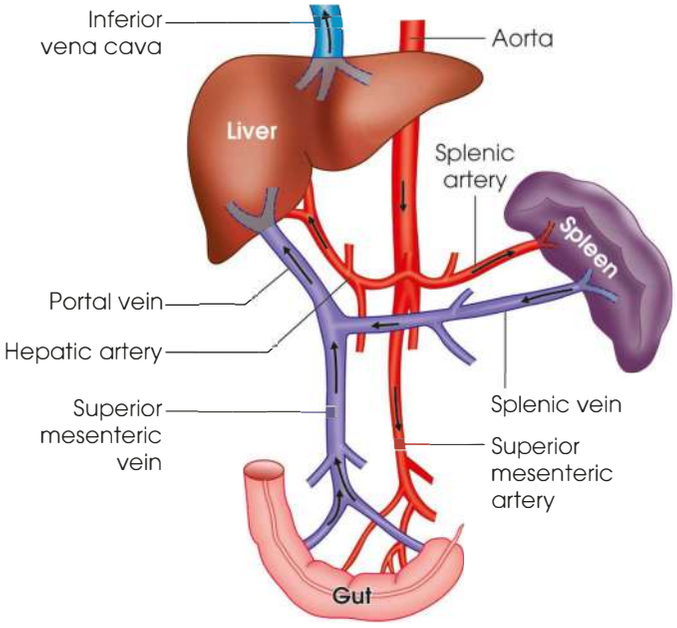

Fig. 39.10: Macro-anatomy of the hepatic circulation

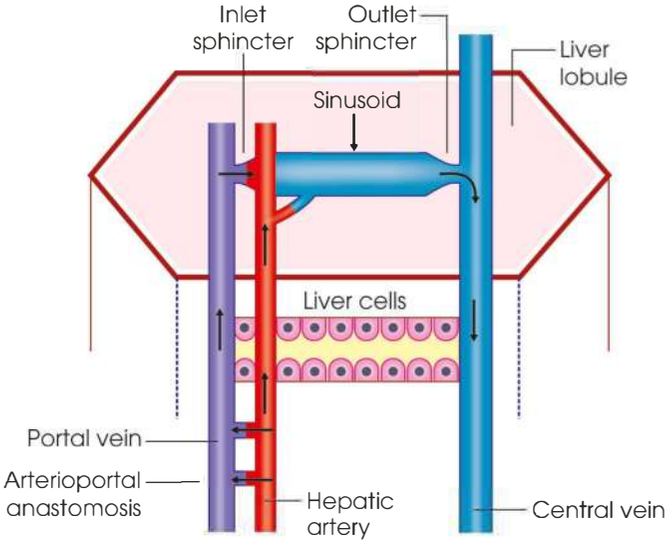

Fig. 39.11: Micro-anatomy of the hepatic circulation in the liver lobule (after Block, 1956)

Vasomotor Supply

Constrictor fibres are carried in the splanchnic (sympathetic) fibres and stimulations of the splanchnic nerves cause vasoconstriction in the arterioles and other intrahepatic vessels. The vagus has got no role on the hepatic circulation and there is still doubt about the existence of any vasodilator fibres in the liver.

Normal Values of Hepatic Circulation

1. **Hepatic blood flow:** The portal area can hold about a quarter of the total blood volume. The average minute flow is about 100 ml per 100 gm of the liver tissue. The hepatic artery supplies about one-third

to one-fourth of the total blood flow and 40% of O_2 requirement. The portal vein supplies two-thirds to three-fourths of the total blood flow, 60% of O_2 requirement and major part of nutrition.

2. **Hepatic blood pressure:** Pressure in the portal vein is about 8–10 mm of Hg. Measured directly at operation in man, it comes to about 14–22 mm of H_2O. This indicates that in the hepatic capillaries the pressure must be much lower (about 2 mm of Hg). Otherwise such a large amount of blood could not pass through it.

FACTORS MODIFYING HEPATIC CIRCULATION

The principles are same as elsewhere. Hepatic circulation can be adjusted either by altering the lumen of the local vessels or by adjusting the rate of blood flow through them. The following factors regulate hepatic circulation:

1. CO_2 and H-ion concentration changes, metabolites, O_2 lack, etc. alter the lumen of the vessels and adjust the flow as elsewhere.
2. Respiration helps in two ways. During inspiration (a) intra-thoracic pressure falls and intra-abdominal pressure rises, so that blood is sucked in by the thorax and pumped out by abdomen, and thus portal flow rises. (b) Diaphragm descends and exerts a massaging action on the liver, thus pushing out more portal blood towards the heart.
3. Contraction of the spleen and movements of the intestine also help in driving blood through the liver. Systemic blood pressure: Effects of systemic pressure on hepatic circulation are as follows: (a) When pressure falls, O_2 supply to the liver decreases; (1) due to less blood flow through the hepatic artery, and (2) due to less oxygen being carried by portal blood which is more reduced owing to slow circulation. (b) Rise of systemic pressure will cause opposite effects. The principle is that a rise of systemic pressure, without constriction of the splanchnic vessels, will raise the hepatic flow. Opposite changes will reduce. (O_2 tension in the centre of the liver lobule is much lower than in the tissues elsewhere. These cells are therefore easily injured by poisons, congestion, etc.)
4. Neurogenic factors: It has been discussed earlier that the sympathetic stimulation changes the hepatic blood flow by increasing resistance of the hepatic blood vessels. But the role of vagi on the hepatic blood flow is uncertain.
5. Vasomotor reflex control: Heymans and others (1930, 1931) have shown that the volume of liver is increased following increase of pressure in the carotid sinus and the aortic arch. They have also described the importance of liver as blood depot for the maintenance of blood supply to the vital organ like the heart and brain during haemorrhage.
6. Adrenaline and noradrenaline: Adrenaline has got both constrictor and dilator effects but the nature of effect is depending upon the concentration of adrenaline given. In higher concentration it has got constrictor effect whereas in lower concentration has got dilator effect. Noradrenaline has got constrictor effect.
7. Effect of posture: Due to changes of posture from the supine to the upright positions, the hydrostatic factor (pgh) may decrease the blood pressure of the upper part of the body but the splanchnic bed compensates it reflexly through the redistribution of blood from its own area to the upper part of the body (mainly the heart and brain) by increasing vascular resistance. If the splanchnic nerves are sectioned then this autotransfusion process does not take place.
8. Effect of exercise: Muscular exercise decreases the splanchnic blood flow by redistributing blood to the active muscle and brain. The reduction of splanchnic blood flow is caused by increased vascular resistance. Haemorrhage: Following haemorrhage the blood flow to the liver is decreased but the vascular resistance in the splanchnic bed remains unaltered and normal flow is resumed within an hour. Increased arteriovenous oxygen difference across the splanchnic bed actually maintains the constant oxygen supply of the liver during haemorrhage. In severe massive haemorrhage, the liver blood flow is curtailed so greatly that the compensation becomes inadequate resulting hepatic hypoxia. This hypoxic state of the liver actually determines the irreversibility of the fatal haemorrhagic shock.
9. Visceral distension: Distension of the stomach due to intake of food reduces the hepatic blood flow probably by increasing the blood volume in the spleen and mesenteric blood vessels.

Clinical conditions: In cirrhosis of liver, the hepatic vascular resistance is increased and in order to maintain the normal blood flow the portal venous pressure is also increased (also due to decrease of blood flow). But the ultimate fate is the accumulation of fluid in the peritoneal cavity causing dropsy. In congestive heart failure, the hepatic blood flow is also decreased greatly.

PECULIARITIES OF HEPATIC CIRCULATION

1. Hepatic circulation is a portal system. Blood has to pass through two capillary networks—at first, the splanchnic and then the hepatic.
2. Although blood enters liver through two vessels (portal vein and hepatic artery), yet the outflow from the liver is only through the hepatic vein. There is no vein corresponding to the hepatic artery (contrast with pulmonary circulation).
3. The pressure difference between portal vein and portal capillaries are not very high, yet a large amount of blood passes through the liver. Mechanical factors,

mentioned above, help. Union between the radicles of the hepatic artery and portal vein possibly adds motive force to the latter.

4. Filtration in the capillary area elsewhere takes place, because capillary pressure (32 mm of Hg) is more than colloidal osmotic pressure (25–30 mm of Hg). But in the liver, the osmotic pressure is same, while the capillary pressure is almost zero.
5. In the liver, blood comes into direct contact with the hepatic cells to some extent. This may partly explain above point.

SPLENIC CIRCULATION

Spleen (lien) is the largest lymphoid tissue in the body and specialised, been-shaped organ for filtering blood. It is a highly vascular haemopoietic organ situated in the left hypochondrium directly beneath the diaphragm, above the left kidney and descending colon, behind the fundus of the stomach and weighing about 150 gm in adult. It also plays an important role in the metabolism and defense mechanism of the body. There are no afferent lymphatic vessels. The anatomy and physiology of spleen has been covered with Chapter on Lymphatics.

Control of Blood Flow

Pressure/flow relationship of the spleen indicates that the resistance is greatly decreased during increase of perfusion pressure. This decrease in resistance is considered to be the cause of opening of the vascular sinuses. Factors that alter the splenic blood flow are described below:

1. **Neurogenic factor:** Neurogenic factors seem to have an important role in the regulation of blood flow in the spleen. Stimulation of the splanchnic nerve causes rapid and intensive rhythmical contraction of the spleen along with decrease of flow. During contraction, it gives into the general circulation in a considerable amount of blood.
2. **Reflex control:** Stimulation of baroreceptors in the carotid sinus and aortic arch baroreceptors causes reflex modification of the splenic volume, splenic weight and splenic circulation. Profound increase or the splenic volume following increased pressure in the isolated carotid sinus of dogs has been observed by Heymans (1929). Reflex dilatation of the splenic vessels is possibly the cause of this response. On the contrary during increase of pressure in the sinus causes reflex decrease of splenic volume through the activation of the sympathetic nerve.
3. **Humoral control:** Adrenaline and noradrenaline both decrease the splenic volume. Splenic rhythmical contraction is increased following administration of adrenaline or noradrenaline. It is claimed that these hormones play an important role in emptying mechanisms of spleen. The noradrenaline helps by arteriolar constriction whereas the adrenaline helps by increasing the venous outflow through venular dilatation or through contraction of the splenic smooth muscle. Acetylcholine and methacholine generally increase the arterial and venous outflow. The volume of the spleen is also slightly decreased. But topical application of acetylcholine, adrenaline, noradrenaline and histamine use arteriolar constriction.
4. **Haemorrhage:** During haemorrhage, when the systemic blood pressure falls, the contractions of the spleen along with arteriolar constriction, decrease of splenic weight and increase of venous outflow are observed due to reflex sympathetic activity through the stimulation of chemoreceptors.
5. **Exercise:** During exercise, the contraction of the spleen is increased. This is due to redistribution of blood from the inactive area to the active area. Increased sympathetic activity during exercise is the possible cause.
6. **Distension of viscera:** The splenic vein and the superior mesenteric veins provide the major blood supply to the liver through the portal vein. Thus, the splenic venous drainage is through the portal vein. So, if there is any distension of liver vascular bed due to increase of sinusoidal resistance or due to pathological conditions then splenic drainage may be affected. Besides this, recently it has been observed that if the hollow viscera like stomach is distended then redistribution of blood occurs there. Under such state, the hepatic blood flow is decreased whereas splenic and mesenteric blood flow are increased.

RENAL CIRCULATION

The kidneys are not only an essential secretory organs, but they also play an important role in circulatory dynamics. The renal circulation will be discussed when kidney function is discussed. About 1,300 ml of blood flows through the kidneys per minute due to relatively low resistance in the renal circulation although a large fraction of cardiac output flows through them. The kidneys can markedly influence the general circulation by the secretion of renin. The renal vessels act under the control of sympathetic and participate in general circulatory adjustments. Due to haemorrhage or diminished cardiac output or during exercise, renal vasoconstriction elevates the resistance and diminishes renal blood flow.

CAPILLARY CIRCULATION

Histology

The average length of a capillary is 0.5–1 mm, average diameter, 8 µm or often less than a red cell, average velocity of flow, 0.5–1 mm per second. Capillaries are lined by a layer of the flat endothelial cells bound

together by an intra-cellular cement substance. A basement membrane surrounds capillary endothelial cells. A pericapillary sheath forms the outer wall of the capillary. In the pericapillary sheath there are branched Rouget cells or pericytes representing modified muscle cells. Zweifach has shown various types of arrangements of the capillaries in the muscle and nail-bed (Fig. 39.12). The arterioles are connected with metarterioles. Metarterioles lead into thoroughfare (preferential) channels which resemble a large capillary and are connected with venules. The true capillaries are shorter in length and led from the metarterioles and arterioles into venules. At the root of each capillary there is a pre-capillary sphincter which adjusts the flow of blood whereas in the thoroughfare (preferential) channel there is no such sphincter and the blood flow cannot be adjusted through it.

METHODS OF STUDY OF CAPILLARY CIRCULATION

Capillary circulation can be studied as follows: It may be watched in the web of frog's foot or omentum, etc. under a microscope with suitable light arrangement. Projection of the some on a screen with the help of an epidiascope is very helpful for demonstration.

Vasomotor Supply

Sympathetic constrictor fibres pass to the Rouget cells. Antidromic dilator fibres are also present.

Peculiarities of Capillary Circulation

1. ***Direction of flow:*** There is no fixed direction of capillary flow. Blood may be found to pass in opposite directions through two capillaries running side by side.
2. ***Nature of the stream:*** In the arterioles, the blood stream can be divided into two parts: (a) Axial stream consisting of red cells mainly and (b) peripheral stream consisting of plasma and stray leucocytes. But in the capillaries, due to narrow lumen, only a single file of cells can pass through. Often a red cell may be found to be folded upon itself and squeezing somehow through the capillaries.
3. ***Capillary tone:*** In the resting condition the majority of the capillaries remain collapsed. During activity they open up and increase the vascularity of the part. In the muscles, the blood supply may increase by 750 times during activity.
4. ***Independent contractility of the capillaries:*** The capillaries can adjust their diameters independent of arterioles and venules. Raised venular and arteriolar pressure cannot distend the capillaries if the tone is high. Ordinarily, however, the capillary lumen changes in the same direction as the arterioles and venules.
5. ***Capillary pressure: Method of recording intracapillary pressure:*** Landis method of recording intracapillary pressure is the most satisfactory one. A fine micropipette is introduced into the capillary loops of the skin of the nailbed and observed through a binocular microscope. It is connected by fine tubes containing citrate solution to a mercury manometer by which the pressure may be recorded. In man, the average pressure at the arterial end is about 32 mm of Hg, at the venous end 12 mm of Hg, and at the summit 20 mm of Hg. There is a difference of 5–10 mm of Hg between systolic and diastolic pressure, at the arterial end. Hence, the flow is pulsatile there. But at the venous end it is continuous.
6. ***Capillary pressure is affected by the following factors***
   - *Heart level:* When lowered below the heart level, the capillary pressure rises proportionately. But if the part is raised above the level of heart, there is a slight fall in the arterial side of capillary pressure but none on the venous side.
   - *Venous pressure:* Venous obstruction raises capillary pressure.
   - *Condition of arterioles:* Dilatation of arterioles (without fall of general blood pressure) increases

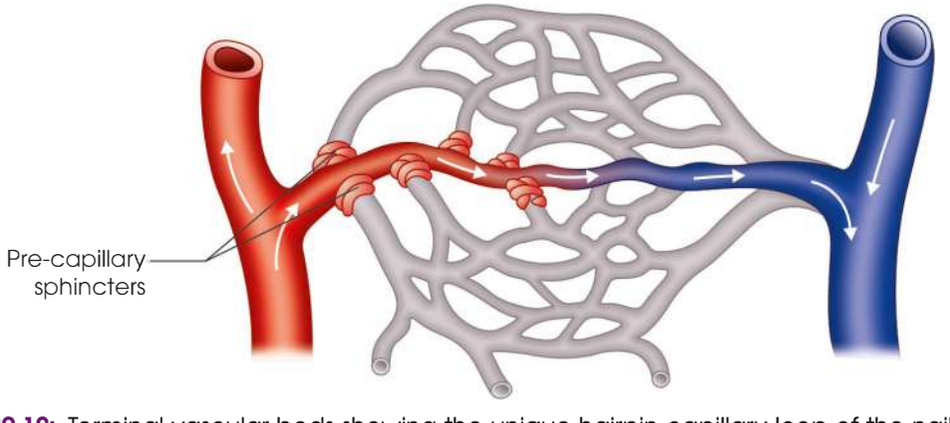

Fig. 39.12: Terminal vascular beds showing the unique hairpin capillary loop of the nailbed

both arterial and venous capillary pressure (from 40–60 and 10–40 mm of Hg, respectively). Under such conditions, capillary pulsation is seen. This is due to the fact that, systemic blood pressure is more directly transmitted to the capillary area. General vasodilatation, with marked fall of blood pressure, will have opposite effects. Constriction of the arterioles beyond the optimum limit may raise systemic blood pressure but reduces capillary pressure and flow.

- *Visceral characteristics:* In the viscera, capillary pressure differs according to the nature of local mechanism. For instance, in the renal glomeruli, it is about 75 mm of Hg, in the intestinal capillaries about 10–20 mm of Hg, in the liver about 2 mm of Hg, etc.

CONTROL OF CAPILLARY CIRCULATION

The extent of capillary flow is directly proportional to the activity of the locality. The adjustment of vascular supply to local needs can be carried out by adjusting the general blood pressure and arteriolar tone on the one hand and by number of the patent capillaries and their diameters on the other. Apart from their independent contractility, the capillaries respond to chemical, hormonal, physical and nervous stimuli. They are as follows:

Chemical stimuli: CO_2 excess, increased H-ion concentration, O_2 lack, non-acid dilators (metabolites) histamine, adenylic acid and certain other products of tissue activity dilate the capillaries.

Hormonal stimuli: Adrenaline, noradrenaline and pituitarian not only constrict the arterioles but also the capillaries.

Physical stimuli: Application of heat or strong light (including ultraviolet and infrared rays) dilates the vessels and increases the vascularity of the part due to direct action of the rise of temperature capillaries and probably also due to histamine liberation. Cooling constricts the arterioles and capillaries. Freezing produces damage of the tissues and liberation of H-substances which causes redness, flare and wheal.

Nervous stimuli: Nervous control of capillaries is carried out in two ways:

Through the vasoconstrictor nerves which reduce the capillary lumen by causing contraction of the Rouget cells. Their inhibition will naturally produce dilatation.

Axon reflex (antidromic vasodilators): Irritation of the skin produces vasodilatation in the locality. This response persists even when the posterior root is severed either proximal or distal to the posterior root ganglion, but not when the peripheral nerves are allowed to degenerate. This shows that, the dilatation is not due to the direct action of any substance on the blood vessels but through the intact peripheral sensory nerves (probably nocifensor system). Since no nerve cell is involved in the process, the reflex arc is completed by the two branches of the same sensory fibre. Through one branch, the impulse is received and through the other, it is transmitted back to the peripheral vessels causing vasodilatation. The impulse is called antidromic impulse and the reflex is known as the axon reflex.

Interchange in the Capillary Area (Fig. 39.13)

At the arterial end of the capillary loop, blood pressure is about 32 mm of Hg and colloidal osmotic pressure about 25 mm of Hg. Hence, filtration takes place and water, nutrition, salts, etc. pass out into the tissue fluid. At the venous end, blood pressure is only 12 mm of Hg and colloidal osmotic pressure is raised due to concentration of proteins. Hence, water and crystalloids are again reabsorbed. In this way capillary circulation and interchange go on.

CUTANEOUS CIRCULATION

Anatomy of Cutaneous Circulation

Vascular architecture of the skin has the general pattern of the capillary circulation. For the most part, papillae contain capillary blood vessels and nerve endings. The arteries that supply the skin, originate from richly anastomosing irregular plexus (first plexus) of the deepest part of the corium (dermis). From this cutaneous arterial plexus, the single arteriole arises and ascends through the corium and forms the second plexus just below the dermis. Capillaries arising from this plexus supply the hair follicles and papillae of the dermis. The arterioles also ascend towards the superficial layer and form the third plexus in the subpapillary region of the dermis. Every papilla gets capillary network from this plexus.

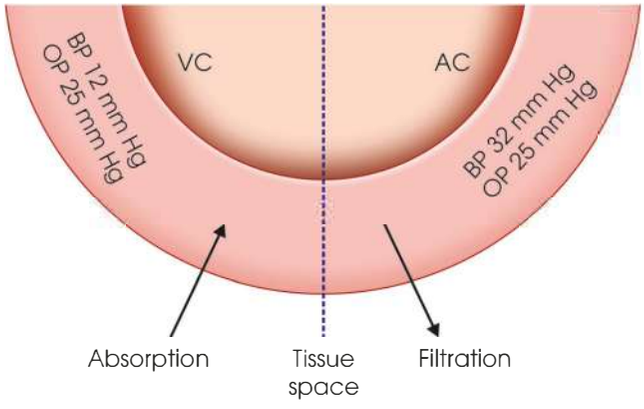

Fig. 39.13: Illustrates the mechanism of exchange in the capillary bed. AC—arterial side of capillary loop, VC—venous side of capillary loop, BP—blood pressure, OP—osmotic pressure. On the arterial side BP > OP hence filtration. On the venous side OP > BP hence absorption

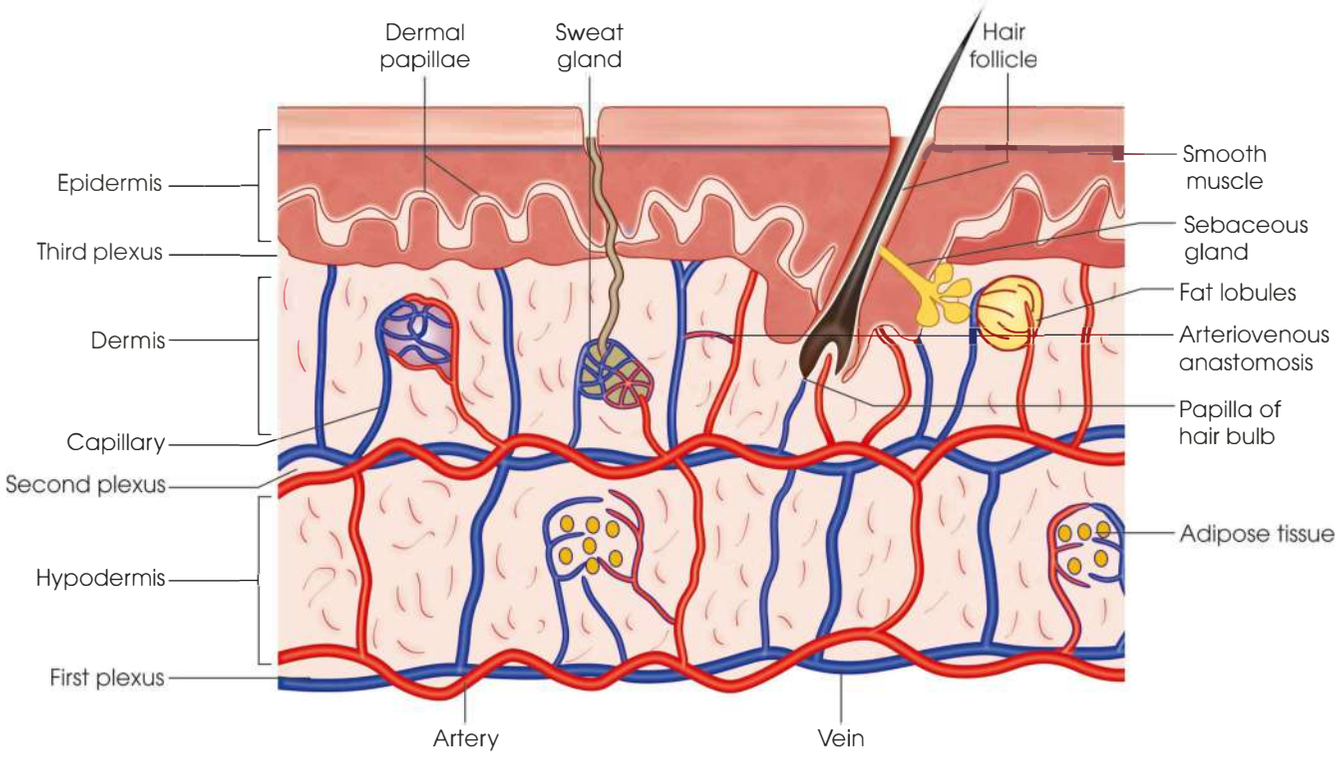

Fig. 39.14: Diagram represents three plexuses at different levels of the skin and by means of arteriovenous anastomoses and precapillary sphincters; blood can be routed into or away from the capillary beds

The arterial limb of the capillary loop ascends in the papillae and turns back to form the venous limb. The venous limb then reaches the base of the papillae and joins with the venous limb of the neighbouring loops to form the collecting venule. The collecting venules then anastomose with one another and form the so-called sub-papillary venous plexus. This sub-papillary venous plexus runs horizontally at the base of the papillae and drains in the deeper veins (Fig. 39.14).

Rate of Blood Flow

Rate of blood flow alters under different conditions like haemorrhage, temperature of the surroundings, metabolic activities of the body, emotional and physical stress of the body. Under ordinary cooled atmosphere, the blood flow is about 0.30 L/sq m of the body surface or total about 400–450 ml per minute. This value is increased during exercise or decreased during fall of surrounding temperature.

REGULATION OF BLOOD FLOW: NERVOUS CONTROL

Considering the temperature control as the main function of the skin, the blood flow through the skin is mainly maintained through the nervous mechanism rather than through auto-regulatory mechanism.

1. **Role of hypothalamus:** Hypothalamus, being the autonomic centres for the sympathetic and the parasympathetic, plays an important role in controlling blood supply of the skin. Besides this, the temperature-regulatory centre is situated at the pre-optic region of the anterior hypothalamus which modifies the skin blood flow in altered body temperature, through the activation of either the sympathetic vasoconstrictor or sympathetic vasodilator pathway. If the above temperature-regulatory centre is heated, then vasodilatation along with sweating occurs and on the contrary if the same centre is cooled, then vasoconstriction along with cessation of sweating occurs.
2. **Sympathetic adrenergic vasoconstrictor pathways:** These fibres secrete noradrenaline at their nerve endings and when excited, on exposure to cold, profound vasoconstriction occurs. These vasoconstrictor effects are most powerful in the hands, lips nose, ears, etc.
3. **Sympathetic cholinergic vasodilator pathways:** These fibres produce vasodilatation by liberating acetylcholine at their endings. When the body temperature is increased, these fibres are activated causing vasodilatation and sweating. The sympathetic nerve supply to human fingers exerts a tonic dilator influence on vessels through the release of acetylcholine.

VASCULAR RESPONSE OF SKIN

White line: In a normal subject, if a light stroke is applied on the skin, a white line appears exactly on the

area stroked, after a brief latent period. It persists for a minute or two and then gradually fades away. It occurs even when nerves are cut and allowed to degenerate. Hence, it is due to local constriction of capillaries as a response to the mechanical stimulus.

Triple response: In subjects with hypersensitive skin, a moderate stroke elicits the following triple response:

- *Red line:* A red line appears on the line of stroke after a short latent period, caused by vasodilatation independent of nerves, because it occurs even after section and degeneration of nerves.
- *Flare:* After 15–30 seconds, a red flush or flare appears and spreads beyond the area. It is not obtained when peripheral nerves degenerate. It is caused by arteriolar dilatation through antidromic axon reflex.
- *Wheal:* A local oedema develops in the area of stroke and spreads a little beyond, reaching its maximum in about 5 minutes. It is caused by escape of fluid from blood vessels resulting from increased permeability of the latter.

All evidences suggest that triple response is due to the action of histamine, which is liberated in the area as a result of stroke. The red line is due to dilatation of capillaries and venules caused by the direct action of histamine on them. The flare is due to vasodilatation brought about by the action of histamine on the nerves, thus eliciting axon reflex. The wheal is due to increased permeability caused (1) by stagnation of blood flow, and (2) by the action of histamine on the capillaries.

Direct effect of cold on the skin: If cold is directly applied on the skin then vasoconstriction occurs. If a finger is immersed in cold water then vasoconstriction occurs very promptly but fluctuation in blood flow occurs in most cases due to alternate vasoconstriction and vasodilatation. This vasodilatation is the cause of local axon reflex and occurs as a protection against cold injury.

AUTO-REGULATION OF SKIN BLOOD FLOW

The local auto-regulation mechanisms in the skin like other tissues are linked with the nutritional aspects during emergency.

Normal Colour of the Skin

Colour of the skin is mostly dependent upon the blood flow through the capillary loops and sub-capillary plexus. Diameter and degree of engorgement of superficial blood vessels actually determine the depth or intensity of the skin colour. Light and pale colour of the skin is due to the constriction of blood vessels. Intense scarlet colour is due to increased blood flow and dilated vessels. Deep blue colour is due to decreased blood flow and dilated vessels.

SKELETAL MUSCLE CIRCULATION

Vascular Arrangement in Skeletal Muscle

Blood vessels and also nerves enter the muscle at neuromuscular hilus, which is often located, at the half length of the muscle. Arteries, after entering the substance of the muscle, branch freely 'along the perimysium and forms numerous anastomoses, small arteries are given off at regular interval from this network and again finer arteries come out and cause free anastomosis of secondary cubical network. From the threads of the secondary network, smallest arteries or terminal arterioles generally branch off transversely to the long axis of the muscle fibre and at fairly regular intervals of 1 mm. Finally, these arterioles open up into capillary network that runs parallel to the long axis of the muscle fibre and with frequent transverse linkages forming a delicate oblong mesh. The venules are intercalated regularly between arterioles and follow almost exactly the course of arterioles and arteries. The veins have got valves which direct the blood to flow towards the heart. The muscles usually possess a rich capillary blood supply. A large man having a muscle mass of 50 kg, possesses about 2,000 capillaries/mm^2. Total length of the capillary of such muscle will be 100,000 km (62,000 miles). Krogh (1929) has described that during rest approximately 100 capillaries/mm^2 remains open but during exercise as 3,000 capillaries/mm^2 are open up (Fig. 39.18). Muscle blood vessels are comprised of large elastic vessels which may convert the pulsatile flow into smooth steady flow.

There are two sets of resistance vessels; one is the pre-capillary, mostly the arterioles and other is the post-capillary which are mainly small veins. These vessels actually offer the major resistance to blood flow. There are capacitance vessels which are the veins and have a little effect on resistance but have got influence on cardiac output. Arteriovenous anastomosis is present in skeletal muscle. Microscopic studies on the circulation of rat skeletal muscle show many arteriovenous communications. In resting state most of the flow is through these anastomoses and to the muscle fibres proper. Functional importance of arteriovenous anastomosis is not clear.

RATE OF BLOOD FLOW THROUGH MUSCLE

Resting muscle blood flow is about 7–9 ml/100 gm tissue. During exercise, the blood flow is tremendously increased. It may be increased more than 100 ml/100 gm tissue. During exercise, nearly all the capillaries are open up and for this reason the flow is increased. During rest, only 3–4% capillaries remain open.

During rhythmic muscle contraction, the steady blood flow to the muscle is greatly affected and flow becomes intermittent, i.e. flow increases during

relaxation and decreases during contraction. The cause of decreased blood flow during contraction is due to compression of blood vessels.

Basal Tone of Arterioles

In resting state, muscle blood vessels, particularly the arterioles, exhibit a tremendous basal vasomotor tone. This basal tone is considered to be due to sympathetic supply to muscle. Because blocking of the sympathetic nerves may reduce the vascular resistance.

CONTROL OF SKELETAL MUSCLE BLOOD FLOW

1. **Autoregulation of blood flow:** In isolated and denervated muscle preparation, blood vessels exhibit good auto-regulation when perfused at controlled arterial pressure. The tremendous increase of blood flow that occurs following onset of exercise is presumably due to local vasodilatation of arterioles. It has been described that during exercise, skeletal muscle exhibits local autoregulation which is probably due to local increased need of O_2. Berne (1963, 1968) has described a metabolic regulation of blood flow in relation to O_2 need of the cardiac muscle. He has described that blood flow in the muscle is metabolically controlled when the O_2 content of the venous blood is decreased. This increased blood flow takes place during such condition through reactive hyperaemia.
2. **Nervous control:** In skeletal muscle, the sympathetic nerve has got dual functions. It has got vasoconstrictor and vasodilator function. The blood vessels receive both sympathetic adrenergic vasoconstrictor fibre and sympathetic cholinergic vasodilator fibre. The vasoconstrictor fibres when stimulated cause profound decrease of blood flow through the liberation of noradrenaline.
3. **Reflex control of blood flow:** The skeletal muscle blood flow is reflexly controlled under certain conditions of the body.
   a. *Sino-aortic baroreceptor reflexes:* During systemic rise of blood pressure, the baroreceptors of carotid sinus and aortic arch are stimulated causing withdrawal of vasoconstrictor activities of the sympathetic vasoconstrictor fibres. On the contrary on withdrawal of baroreceptor activity by bilateral carotid occlusion (BCO), the vasoconstriction occurs in the muscle. This vasoconstriction may be abolished by a receptor blockades or by sympathetic nerve denervation. But this effect is unaffected by atropine.
   b. *Carotid and aortic chemoreceptor reflexes:* During hypotension or in haemorrhage the carotid and aortic bodies are stimulated causing systemic rise of pressure by the decrease of blood flow to the muscle and splanchnic bed. If the sympathetic nerves to the muscle are cut, then this decrease of blood flow no longer occurs.
   c. *Thoracic aorta baroreceptor reflexes:* Gruhzit and others (1953) have observed the reflex vasodilatation of the limb muscle following stimulation of the mechanoreceptors of the wall of the thoracic aorta. Cardiopulmonary receptor reflexes: In human beings, vasodilatation may occur only in the forearm if the lower limbs in a recumbent subject are elevated. It is the result of shifting of blood from limbs to the thorax by stimulation of the receptors in the cardiopulmonary low-pressor area, so that the sympathetic vasoconstrictor tone is altered.
   d. *Neurohormones:* Acetylcholine has got dilator effects on the skeletal muscle blood vessels. Intra-arterial administration of acetylcholine has got no effect if atropine is administered previously. Adrenaline has got both vasodilator and vasoconstrictor effects because it activates both α and β receptors of the skeletal muscle blood vessels. Noradrenaline, on the other hand, has got only vasoconstrictor effect and activates only the α-receptors of the blood vessels.
   e. *Blood pH:* A decrease or an increase of pH has practically no effect in innervated muscle in the forearm blood flow. If the sympathetic nerves are blocked then an increased or decreased pH may alter the blood flow.
   f. *Nucleotides:* Studies on adenosine, AMP, ADP and ATP show arteriolar vasodilatation but relative role of these substances in the skeletal muscle blood flow during reactive hyperaemia has to be further explored. Polypeptides. (a) Bradykinin is a potent vasodilator of skeletal muscle as evidenced from plethysmography studies. (b) Angiotensin administered intravenously produces renal vasoconstriction and some extent the splanchnic bed resistance is increased on and muscle vascular bed remains unaffected. This effect causes marked shift of blood from the viscera to the muscle bed (Brod et al, 1968).
   g. *Ions:* Increased blood levels of potassium and magnesium elicit arteriolar dilatation. These ions produce their effects by acting directly on the vascular smooth muscle.
   h. *Other metabolites:* Citrate, acetate, pyruvate also produce arteriolar dilatation. Lactic acid also has got similar effect.
   i. Hypoxia, increased CO_2 tension, lactic acid, bradykinin, histamine, acetylcholine, adenosine triphosphate, adenylic acid and potassium ions have been considered to be the determinant of muscle circulation during exercise hyperaemia.
   j. *Exercise:* Muscle blood flow during exercise has also been considered (vide muscle physiology).

With the onset of muscular work, the metabolic need of O_2 is increased tremendously and such need is made adequately through activation of cardiovascular, respiratory and neuroendocrine processes along with local modification of muscle blood circulation. If these mechanisms fail then the anaerobic processes prevail, causing accumulation of lactic acid in the muscle and blood and ultimately leading to exhaustion or fatigue.

The blood circulation that increases during exercise is possibly due to reactive hyperaemia caused through metabolites acting directly on the vascular smooth muscle or indirectly through axon reflex. It has been considered that the hyperaemia which is produced by metabolites is mostly dependent upon combinations of several factors instead of one single factor.

In the working muscles, local changes of pH and composition of interstitial fluid cause opening up capillaries and arterioles that are not already dilated by the sympathetic vasodilator activities. Extra-cellular K^+ concentration is so much increased during muscular exercise that this can account for a major part of the vascular dilatation accompanying muscular activity.

Note

Foetal circulation is covered in detail in section of reproduction.

EXAM-ORIENTED QUESTIONS

Essay

1. Discuss the characteristics, mechanism and regulation of coronary circulation.
2. Discuss the characteristics, mechanism and regulation of pulmonary circulation.
3. Discuss the characteristics, mechanism and regulation of cerebral and hepatic circulation.

Short Notes

1. Hepatic circulation
2. Muscular circulation
3. Capillary circulation

Chapter

40

Physiology of Exercise

INTRODUCTION

Muscular Exercise

Work physiology is a branch of science which deals with the physiology of man at work.

Physical exercises and activities promote physical and psychological well-being in an individual.

There are mainly three types of physical exercises.

1. *Aerobic exercise:* These exercises are targeted to improve cardiovascular endurance. The aerobic exercise includes brisk walking, swimming, cycling, rowing, skipping rope, etc. These exercises adapt the larger group of muscle to utilize more oxygen then the resting state.
2. *Anaerobic exercise:* These exercises are targeted towards improving strength and resistance and tone in muscles. These exercises improve bone strength and also help in developing better balance and coordination during exercise. The muscle strengthening exercise includes pull ups and push ups, biceps, triceps and other limb muscles curls using dumbbles and lunges. The aerobic exercises include sprinting, weight training, interval training, high intensity interval training, etc.
3. *Flexibility exercises:* These exercises mainly stretch and lengthen muscles. It helps to improve joint flexibility and muscles limber. Flexibility exercises also improve the range of motion which brings down the chance of injury.

Volitional muscular movement or exercise is a cortical phenomenon and is under the 'feedback' control of cerebellum (vide functions of the cerebellum in nervous system). During muscular exercise, the energy needs of the muscle are increased tremendously and these energies are met with through increased metabolism of the active muscle. With the increase of metabolism, the oxygen needs of the muscle are increased and these requirements are met with by causing oxygen-rich RBC more available to the active site. This happens by (a) *increasing heart rate*, (b) *increasing cardiac output*, (c) *increasing venous return*, (d) *decreasing rate of blood flow in the inactive muscle*, (e) *increasing vascular resistance in time inactive muscle*, (f) *redistribution of blood from non-vital organs* (splanchnic bed) to vital organs (brain and heart) and active site, (g) *increasing blood pressure*, (h) *increasing pulmonary ventilation*. Other bodily functions that are altered during exercise are the (a) *blood cell changes*, (b) *kidney functions*, (c) *body temperature regulation*, (d) *digestive functions*, (e) *water and salt balance*, (f) *endocrine function*.

These show that during exercise cerebral cortex mobilises several functions only to cope with the high degree of metabolism that is brought about by the active muscles. Exercise induced physiological changes are discussed below.

CHANGES IN CARDIOVASCULAR SYSTEM

Heart and exercise: Prolonged and systematic exercise causes enlargement of the heart, and this happens only to cope with the excessive workload imposed upon the heart during work. There is a lot of misunderstanding that prolonged exercise may cause dilatation of the heart similar to that happens in heart disease. But the hypertrophy of the heart in athletes is caused by physiological processes. The nature of processes is similar to the hypertrophy of skeletal muscle resulting from systematic exercise. Thus, hypertrophied athletic heart is more powerful, efficient and capable of greater increase in stroke volume but the dilated diseased heart is less efficient and has a limited capacity for work.

1. Heart Rate Changes during Exercise (Fig. 40.1)

The acceleration of the heart is observed immediately following exercise. It has been observed that the heart rate is increased slightly even before onset of exercise and it is presumably due to influence of the cerebral cortex on the medullary cardiac centre. A short rise of heart rate is observed at first minute of exercise but after

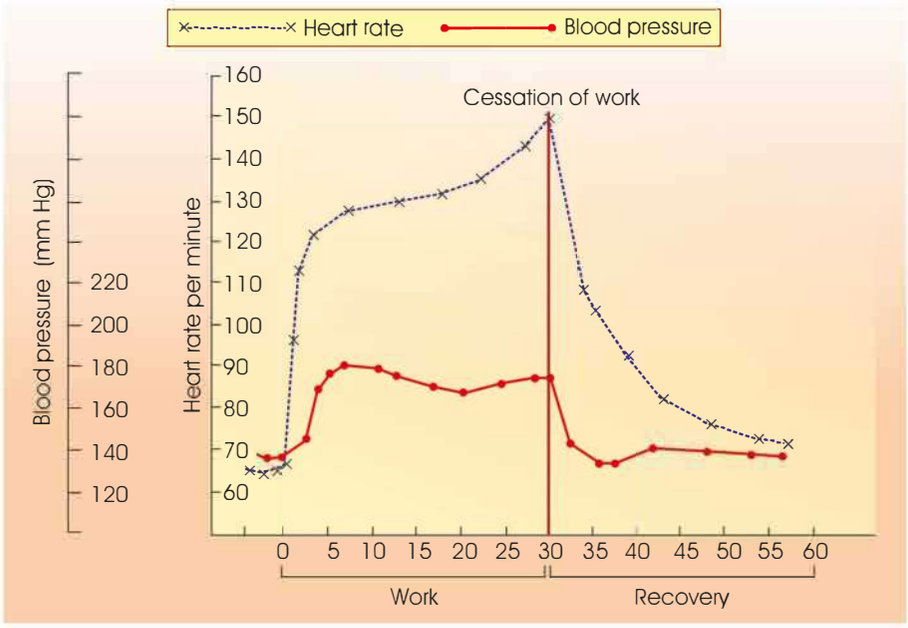

Fig. 40.1: Diagrammatic tracing of cardiovascular response during work and recovery

that this rate of rise is slight decreased. Within 4 to 5 minutes of exercise the maximal rise is more or less achieved. A 'plateau' is observed if the exercise is further continued. But the time is variable from individual to individual and even with different degrees of work load. In athletes, the rate of rise of the heart will be slower. Besides these, maximal heart rate that is reached during exercise and the rapidity with which the maximal value is attained depends upon several factors which are (a) emotional, (b) environmental temperature and humidity, and (c) physical conditions of the subjects.

Initial rise of heart rate (anticipatory heart rate) just before exercise is due to the influence of cerebral cortex and other higher brain centres. With the onset of exercise the rise of heart rate may be due to (a) reflexes originating in the receptors of moving joints or contracting muscle, (b) stimulation of chemoreceptors in muscles by the acid metabolites, (c) sympathetico-adrenal activation causing section of much larger amounts of epinephrine in the blood, (d) rise of body temperature and regarding the return of heart rate to initial resting level depends upon the intensity of work-load and also on the physical condition of the individual. The rapidity with which the heart rate returns to the resting level following cessation of exercise is considered as a test for physical fitness. In trained individual or in physically fit person the recovery period is very short.

Cardiac output: During exercise the cardiac output is greatly increased. In trained athletes, it may achieve a maximal output of 30 litres per minute, at an O_2 uptake of 4 litres per minute but in non-athletes, the output may be average 22 litres at an O_2 uptake of 3.3 litres per minute. The cardiac output during exercise is the result of the increase in stroke volume and heart rate.

Venous return: Venous return is greatly increased during exercise for the following reasons:

a. Milking or massaging action of skeletal muscles during exercise, the alternate contraction and relaxation of the muscle act as a booster pump for flowing blood towards the heart. Due to presence of valves in the veins, the blood is squeezed out from the vein towards the heart during contraction and allowed to fill blood during relaxation of the muscle. This pumping mechanism depends upon intensity and type of exercise.

b. *Respiratory movements:* Respiratory movements exert a sucking effect over the right heart and great veins so that greater venous return may occur. Visa fronte is the consequence causing of the above effect during respiratory effort. During inspiration the thoracic cavity is enlarged causing fall of intrathoracic pressure. This fall of intrathoracic pressure as well as increase of pressure on the anterior abdominal wall due to descent of diaphragm cause rapid return of blood into the heart. Expiration has got the opposite effect.

c. *Contraction of limb veins:* It is claimed that limb veins undergo reflex vasoconstriction during exercise thus facilitating rapid venous return to the heart.

Blood pressure: Blood pressure is raised with the onset of exercise. There may be an anticipatory blood pressure due to nerve impulses originating from the cerebral cortex to the medullary cardiac and vasoconstrictor centres. Other factors that may participate in the rise

of blood pressure during exercise are due to activation of sympathetico-adrenal systems causing shifting of blood from the splanchnic beds to the other parts of the body.

So, the rise of arterial blood pressure during exercise is due to (a) increase of cardiac output, causing greater distension of aorta and large arteries, (b) increase of heart rate and (c) compensatory vasoconstriction in the non-active organs (splanchnic beds and skin) and vasodilatation in the active organs so as to perfuse the active organs with a greater pressure. The nature of blood pressure rise cannot be generalised because the pressure changes mostly depend upon the type, speed and duration of the activity and also of the physical condition of the subject.

2. Circulatory Status during Exercise

During exercise, the circulation is adjusted in such a way that the active muscles as well as the vital organs get blood supply to a greater proportion than that of the inactive organs and the non-vital organs.

a. It has been observed that the active muscle gets more blood supply during exercise and the circulation is increased more than about 30 times (Fig. 40.2). This greater supply is due to decrease of vascular resistance caused by locally accumulated metabolites. During exercise sudden lack of O_2 caused the increased accumulation of CO_2, lactic acid, adenosine, intracellular K^+ and histamine. These substances may cause hyperaemia (reactive hyperaemia) and thus the resistance to blood flow is decreased.
b. *Coronary blood flow:* As the workload of the heart is increased tremendously during exercise, the coronary flow is increased accordingly to its own nourishment, otherwise hypoxia may prevail. So, in moderate exercise, coronary flow is increased according to the O_2 requirement of the cardiac muscle. But in severe exercise, the coronary flow may be increased no doubt, but the cardiac muscle due to tremendous increase of heart rate, will fail to maintain its O_2 according to its need and the subject may feel anginal pain.
c. Pulmonary circulation during exercise is increased in proportion to the increase in venous return to the heart. But with the increase of pulmonary circulation, the pulmonary arterial pressure is insignificantly increased possibly due to distensibility of its blood vessels.
d. Blood flow to the brain is relatively under normal state and remains mostly unaltered during exercise.
e. During exercise the blood flow in the active muscle, lung, heart is increased, but the same in the abdominal organ, kidneys and in the skin (initially) is greatly decreased due to compensatory vasoconstriction. This happens possibly through the chemoreceptor reflex initiated by the accumulated metabolites during exercise so as to cause redistribution of blood from abdominal organs to the exercising muscle, heart, lung and skin (later stage).
f. Skin blood flow is initially decreased but as the work is continued and the body temperature is increased the skin blood flow is also increased only to eliminate excess heat produced by the contracting muscle.

3. Changes in Respiration

Pulmonary ventilation: *Increase in ventilation:* Pulmonary ventilation in moderate exercise is always linear with the amount of O_2 absorbed. It has been observed that a linear relationship between the workload and the pulmonary ventilation is maintained until the workload is so increased that the steady state is not achieved. It has been so observed that the pulmonary ventilation is increased without appreciable increase in O_2 consumption with graded increase of workload. This

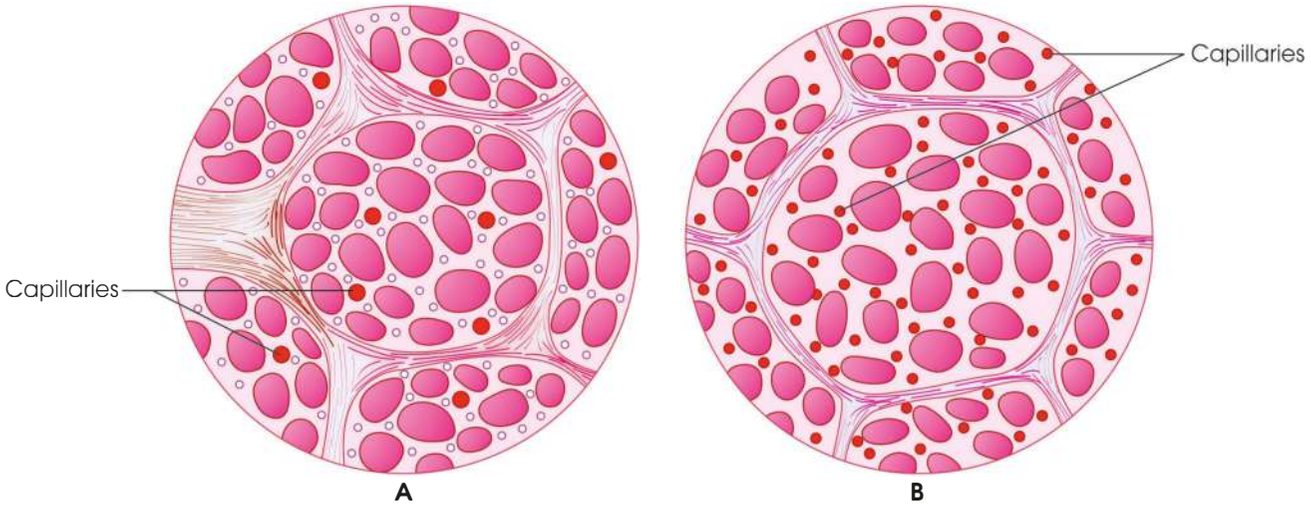

Fig. 40.2A and B: Represents the circulatory status of the muscle during rest and exercise. (A) Smaller number of capillaries in respect to a muscle bulk present at rest; (B) Greater number of capillaries opened up in the same muscle during exercise

shows that pulmonary ventilations do not limit the workload but the O_2 consumption limits workload because the cardiac output is not increased accordingly. It then indicates that with the increase of workload, pulmonary ventilation is still capable of further adjustment when the cardiovascular system has reached to its limit.

Breathing: *Increase in rate and depth of respiration:* With the increase of O_2 demand during exercise the rate and depth of respiration are increased. These effects may be due to (a) direct effect (centrogenic) of increased CO_2, produced by the working muscle, on the respiratory centre, (b) indirect effect (reflexogenic) of metabolites on respiratory centre through chemoreceptors (carotid body and aortic body), (c) proprioceptive impulses from the joints of the extremities, (d) stimulation of chemo-receptors present in pulmonary artery, (e) increase of temperature causing rise of breathing by (1) direct stimulation of respiratory centre, (2) stimulation of cortical centre or carotid and aortic bodies, and (3) other reflex pathways.

None of the above factors is solely responsible for increase in breathing during exercise and possibly multifactors are responsible for it. Other factors that may take part in increasing the respiration during exercise are the impulses from the cortex and adrenaline liberated into the blood.

Nature of second wind: During violent exercise initially there is developed a frequent feeling of distress but if the exercise is further continued then this sense of distress is replaced by a sense of great relief, which is called second wind. The distresses that precede the second wind are; (a) breathlessness, (b) throbbing of the head, (c) fluttering and irregular pulse, (d) muscle pains, and (e) feeling of sensation of constriction around the chest. With the onset of second wind, the facial distress disappears and breathlessness other than discomforts disappear. The subject feels a sense of comfort. The second wind should not be confused with the steady state which is the condition in which oxygen need and oxygen supply are balanced in such a way that the lactic acid and oxygen debt do not take place. The physiological basis of second wind is not clear and it is a complex phenomenon in which different types of physiological adjustments take place.

Oxygen Exchange during Exercise

Oxygen exchange during exercise: The alveolar and arterial pO_2 remains normal during entire period of exercise. The arterial pO_2 is maintained due to proportionate increase ventilation inspite of rapid utilization of oxygen by the exercising muscles. With the increase of cardiac output during exercise, the velocity of blood flow is not increased but the volume flow of blood in each minute is increased and this permits oxygen exchange in the lungs quite adequately so as to bring about normal saturation of blood with O_2 so long as the oxygen uptake does not exceed 4 litres per minute. In moderate exercise the amount of lactic acid formed, can be fully disposed of the muscle buffers and the greater amount of oxygen supplied by increased blood flow. But in severe exercise the rate of oxygen supply lags behind and lactic acid rapidly accumulates (Table 40.1). It enters the blood stream and causes acidosis. Thus, in severe exercise the lactate content of blood may go up to 100–200 mg% (10–20 mg% resting). In order to dispose of these excess metabolites and lactic acid extra amount of O_2, is needed. This is called oxygen debt (energy debt). During the first few minutes of recovery period, the lactate content of plasma or muscle does not diminish-showing that O_2 is used or the resynthesis of creatine phosphate and ATP during recovery period. The oxygen used for this purpose is called galactic acid debt. Galactic acid debt is paid at a much faster rate than the lactic acid debt.

RQ: It varies according to the degree of exercise. In moderate exercise RQ varies from 0.85 to 0.89, i.e. nearly same as in rest. If exercise be prolonged with a high fat diet, RQ falls to 0 7. But in severe exercise (a) the RQ of excess metabolism (i.e. gaseous exchange over and above the resting) during the period of exercise rises above unity and may go up to 1.5 to 2. This is due to the fact that the H-ion concentration is much greater in this case than in moderate exercise—being partly due to excess CO_2, and partly to the entry of lactic acid from the muscles. Lactic acid combines with bicarbonates liberating more carbonic acid. Hence, respiration is much more stimulated in this case and more CO_2 is liberated without any corresponding oxygen utilisation. Thus, RQ rises above unity. The extent to which it rises above unity is a measure of the severity of the exercise and extent of lactic acid formation. (b) Late in the recovery period, RQ falls to 0.5, because respiration is depressed and CO_2 is retained to form the lost bicarbonates. This CO_2 comes from the burning of the lactic acid, part of Na—lactate in the tissues and combines with the Na of the lactate now set free.

4. Blood Cell Changes during Exercise

RBC count is increased in the early part of the exercise and it is probably due to haemoconcentration. This haemoconcentration is the cause of transfer of fluid from the blood to the tissues. But with more prolonged exercise the fluid passes back to the blood causing haemodilatation and RBC count is thus lowered. In strenuous exercise there may happen haemolysis. WBC count is increased significantly at any type of exercise. It has been claimed that the greater rise of the WBC count is related with the degree of stress produced with the exercise. In less fit person the rise is more than in athletes under the same workload. Specific gravity of blood in a muscular activity is increased and it varies directly with the RBC.

Table 40.1: Distinction between moderate exercise and severe exercise

Changes	*Moderate exercise*	*Severe exercise*
• **Cardiovascular**		
– General heart	Heart rate, venous return, cardiac output—increase VMC stimulated—vasoconstriction. Blood pressure raised	Same but more
– Local	Increased vascularity. Slow blood flow. Blood more extensively reduced	Same but more
• **Respiration**		
– Pulmonary ventilation	Increases proportionally but comes to normal quickly after stopping exercise	Remains high for a long time after exercise to supply oxygen debt
– RQ	Almost normal 0.85–1.20	High—1.5–2.0 later on falls—0.5
• **Blood**		
– Cell count	Rises	Rises
– Acidosis	Present—only for H_2CO_2	More—for H_2CO_2 and latic acid
– Alkali reserve	Reduced	More reduced
– Locate	Normal (10–20 mg%)	Raised (100–200 mg%)
– O_2 content	Less than normal	Further reduced
– Cori cycle	Nil	Mobilised
• **Urine**		
– Volume	Less	Less
– Reaction	Acid	Acid, later alkaline
• **Skin**		
– Volume	Constriction, later dilatation to help heat loss	Same
– Sweat	Reduced, later increases	Same
• Oxygen debt	Nil	Present
• Second wind	Possible	Not possible
• Steady state	Possible	Not possible
• Fatigue	Delayed	Early
• Duration	Can be continued for a long time	For short periods only
• Recovery	Quick	Delayed

5. Body Temperature

Normally the body temperature is balanced by the rate of the heat production and heat loss. During exercise the heat production is greatly increased and when simultaneous heat loss does not happen then body temperature is raised. In exercise, the body temperature is raised and it is possibly due to the failure of temperature-regulating centre to maintain the normal body temperature.

6. Body Fluid Changes during Exercise

During exercise acute dehydration is a great problem because there is rapid loss of water through sweating and expired air. In acute exercise there is haemoconcentration due to (a) shifting of fluid from the blood to the tissue spaces, (b) sweating, and (c) rapid expiration. But if the exercise is continued then water loss is partly checked and dehydration may be minimum presumably by the return of fluid from the tissue spaces to the blood. In chronic exercise as in the case of athletes or in subjects undergoing systematic exercise, there appears no permanent shifts of body fluid. Along with water loss there is excessive loss of salt which deteriorates work performance. If the fluid and salt losses are adjusted by taking water along with salt during the period of exercise then the work performance may be increased. But it is not generally taken as there is a general belief of the coaches and of the trainers that drinking is harmful during exercise.

7. Kidney Function in Exercise

Alteration in kidney functions that are generally observed during exercise is presumably due to shunting of blood from the kidneys and other abdominal organs to the vital organs and the active muscle. The renal blood flow is considerably decreased during exercise and this decrease of flow is maintained as long as an hour following cessations of exercise:

Rate of urine formation is greatly diminished due to (a) excessive reabsorption of fluid from the renal tubles with the help of anti-diuretic hormone (ADH) secreted by the posterior pitutary and (b) decreased renal blood flow.

Furthermore, the kidneys excrete excessive acid metabolites that are accumulated in the blood during exercise. Besides these, there is often transient proteinuria with exercise. After strenuous exercise there is accumulation of albumin in the urine; and has been considered to be the cause of increased permeability of

these substances in the glomerular capillaries. Some are of opinions that the increased activity of the kidney tissues may alter the kidney functions during exercise. Often Masch haemoglobinuria, a condition in which physical exertion causing formation of red urine containing haemoglobin is observed. This condition is mostly due to intra-vascular breakdown of RBC during strenuous exercise. Haemoglobin generally appears 1–3 hours after severe exercise. Exercise induced myoglobinuria is often encountered after severe exercise.

8. Digestive System

Strenuous exercise inhibits both the motor and secretory functions of the stomach. Campbell and others (1928) have described that moderate exercise inhibits gastric secretion as well as motility of the stomach but the lighter exercise (walking) helps in gastric juice secretion and also in emptying of the stomach. But after exercise the motility of the stomach and secretion of gastic juice are increased considerably. This shows that the depressed motility and gastic juice secretion during strenuous exercise are balanced by the increased motility of the stomach and gastric juice secretion after the cessation of activity.

9. Endocrine Status

Many endocrine hormones are released during exercise. They exert their physiological effects and also help in long-term adaptation to exercise induced stress.

a. The catecholamines, epinephrine and norepinephrine are released during exercises. They mobilize glucose from liver and fatty acids from adipose tissue to supply energy source to the exercising muscle.
b. Growth hormone utilization during exercise is increased. It has been observed that during exercise, mobilization of depot fat is increased by the growth hormone secreted in larger quantity. It is claimed that the liberation of growth hormone or STH appears mostly responsible for mobilization of depot fat when the exogenous carbohydrate is not made available during exercise.
c. It has already been discussed that the ADH secretion is increased during physical exercise. This secretion of ADH actually combats against dehydration during exercise.
d. The secretion of endorphin also increases during the exercise. Endorphin reduces exercise stress and also post-exercise pain.
e. Glucagon is released during prolonged exercise and it promotes glycogenolysis and lipolysis.

AEROBIC TRAINING

The adaptation to stress induced in exercise is gradually achieved and the person does not fell fatigued due to the endurance training.

Endurance training enhances various physiological functions and thereby the person fitness status is achieved.

1. The mitochondria oxidizing activity increases thereby enhanced oxidative phosphorylation activities. The enzyme activities of NADH dehydrogenase, cytochrome oxidase and succinate dehydrogenase are increased by aerobic training.
2. The training enhances blood flow to skeletal muscle and also promote opening of more number of capillaries. The increased capillary density in exercising muscle along with increased transit time of circulating blood helps in more extraction of oxygen from blood to the tissue. Training increases oxygen carrying capacity of the blood, increase VO_2 and improve oxygen extraction as well as delivery to the tissue.
3. The general adaptation to exercise reduces maximal heart rate, increases stroke volume and also increases cardiac output.

Thus, training enhances endurance and promotes better cardio-respiratory responses to exercise thereby improving cardio-respiratory fitness in an individual. Regular exercise lowers body fat mass and helps to reduce obesity, improves and enhances parasympathetic response in our body and decreases sympathetic tone, stimulates osteoblastic activity and prevents osteoporosis, improves sleep, promotes physiological and psychological well-being and strengthens immunity.

ROLE OF NUTRITION IN EXERCISE

Adequate nutrition is vitally important during exercise. The correct ratio of macronutrients and micronutrients supplementation are needed to aid the body with the recovery process following strenuous exercise. The resistance training should be complemented with balance diet intake program. The consumption of a protein-rich meal promotes muscle hypertrophy by stimulating myofibrillar muscle protein synthesis (MPS) and inhibiting muscle protein breakdown (MPB). Protein intake helps in gaining muscle strength.

Over-exercising and Ill Effects on Health

The over-exercising undermines exercise performance largely. The unaccustomed over-exertion of muscles during strenous exercise leads to rhabdomyolysis (damage to muscle). Over-training syndrome (OTS) lowers immune resistance and there is increased incidence of upper respiratory tract infection (URTI). The increased occurrence of URTIs has also been found to be associated with high volume/intensity training, as well as with excessive exercise (EE). Over-exercising in females may lead to amenorrhea (missing of menstrual period). Such females should ensure no excessive exercise training session.

EXAM-ORIENTED QUESTIONS

Essay

1. Discuss the physiological changes in human body during exercise.

Short Notes

1. Metabolic changes during exercise
2. Muscular blood flow during exercise
3. Respiratory quotient

Chapter

41 Applied Cardiovascular Physiology: Haemorrhage, Heart Failure, Hypotension, Hypertension and Shock

HAEMORRHAGE

Definition: Escape of blood from ruptured blood vessels is called haemorrhage.

Effects of haemorrhage: The effects of haemorrhage depend upon the amount and rapidity of loss of blood, and the efficiency of the compensatory power of the subject. Severe uncontrolled haemorrhage may lead to circulatory collapse and death. If haemorrhage is moderate (about 5–15 ml per kg body weight) and the subject is healthy, compensatory mechanism restores normal circulation and prevents circulatory collapse.

Haemorrhage produces the following disturbances: (a) Blood volume is diminished. (b) Blood pressure—due to decreased blood volume cardiac output is diminished and there is reflex vasoconstriction. The vasoconstriction of blood vessels leads to increased peripheral resistance. In addition, venous constriction along with vasoconstriction propels more blood towards the heart. The blood supply to the skin, splanchnic area, muscle, etc. decreases due to constriction of arterioles. The pressure in the capillaries also decreases and less amount of fluid is transuded from the blood to the tissue spaces. Although the capillary pressure falls, but there is no change in the osmotic pressure and so fluid from the tissue spaces enters the blood and keeps the blood volume almost constant. If the haemorrhage occurs slowly the blood pressure level may be maintained for a considerable time. In case of severe haemorrhage, there is fall of cardiac output and diminution of peripheral resistance, dilatation of the vessels of the skeletal muscles. All these lead to profound fall of blood pressure. The heart rate is markedly diminished. There is diminution of cerebral blood flow which results in sudden loss of consciousness or fainting. (c) Heart rate is increased to maintain cardiac output. (d) Respiration increases both in rate and depth. Anoxia develops due to less haemoglobin. (e) Pallor of the skin occurs due to diminution of blood flow in the skin. (f) Cold sweat: Skin is cold and there is less evaporation of sweat. (g) Excitement is caused by anoxia. (h) Diminution in urine flow occurs due to impaired renal circulation.

Role of Atrial and Ventricular Receptors and the Sino-aortic Baroreceptors

The discharge of impulses from the receptors in the atria and ventricles is not dependent upon the mean systemic pressure, but upon the venous return to the heart. In haemorrhage there is diminution of venous return, stroke volume and pulse pressure. Due to the reduction of venous return the inhibitory effect of these afferents on arteriolar constriction is reduced. The resultant effect is the increase of peripheral resistance and consequently there is some improvement of the venous return. Again, stimulation of the sino-aortic baroreceptors is dependent on pulse pressure. In haemorrhage due to fall of pulse pressure the stimulation of the baroreceptors is diminished. So, there are vasoconstriction and tachycardia due to reflex chemoreceptor's influence following haemorrhage. Tachycardia further reduces the stroke volume and pulse pressure.

Compensatory Changes After Haemorrhage

1. **Changes in the cardiovascular system:** The immediate need is to maintain the blood pressure. To attain this need the following changes take place:
   Heart rate rises to maintain cardiac output—caused by low blood pressure, O_2 lack, CO_2 excess, etc. through central and sino-aortic mechanism.
   General vasoconstriction—caused by effects of (a) O_2 lack, (b) CO_2 excess, etc. directly on the vasomotor centre as well as reflexly through the chemoreceptors of the sino-aortic nerves. (c) In the experimental animal it has been observed that after severe bleeding the blood pressure falls much below normal. The chemoreceptors of the sino-aortic nerves bring about reflex vasoconstriction and exert their influence to maintain the mean systemic pressure in the bled animal. But after section of the vagi reflex

vasoconstriction disappears and the blood pressure falls still further.

Adrenaline and noradrenaline secretion—caused by reflex chemoreceptor's activity and excitement leading to further vasoconstriction and increase of heart rate.

Contraction of spleen and mesenteric blood vessels—expelling stored corpuscles into circulation.

Liver blood volume—the volume of blood supplied to liver is also decreased and blood is diverted towards the general circulation do as to compensate the blood loss following haemorrhage.

2. **Changes in respiration:** Respiration increases both in rate and depth and thus total pulmonary ventilation rises, caused by low blood pressure, anoxia, CO_2 excess, etc. by central as well as sino-aortic process. Its purpose is to supply more oxygen.
3. **Renal changes:** The volume of urine decreases due to fall of blood pressure, renal vasoconstriction and decreased blood supply to the kidney. But the filtration fraction is found to be more than normal, showing that the efferent glomerular vessels undergo a selective constriction. Besides these, angiotensin II that is formed due to interaction of plasma globulin substrate (α_2-fraction), and renin (secreted from the juxtaglomerular region of ischaemic kidney) has got the effect. This renin-angiotensin system also stimulates the secretion of aldosterone and also glucocorticoids from the adrenal cortex. Aldosterone by maintaining electrolyte balance (Na^+ retention and K^+ excretion through the kidneys) restores blood volume. Under such state vasopressin (antidiuretic hormone) is also secreted from the posterior pituitary and this helps further in restoration of blood volume through excretion of less urine. Due to less urine formation, nitrogen retention takes place in the blood and may lead to azotemia or uraemia. In case of prolonged hypotension there may occur severe renal tubular damage (lower nephron nephrosis).
4. **Restoration of blood:** The lost blood is regained. At first the fluid, then the plasma proteins and lastly the red cells and haemoglobin are restored. The brief details are as follows:
   - *Fluid:* The first change in the indrawing of water from the tissue spaces even before the haemorrhage is over. Due to low blood pressure, the capillary pressure falls much below the colloidal osmotic pressure of plasma. Hence, instead of normal filtration, water from the tissue spaces is drawn in and thus blood volume starts rising. A definite increase of plasma volume takes place after one hour, as shown by dilution of plasma proteins and haemoglobin. In 24 hours plasma volume is fully restored and even may rise above normal to compensate for the low red cell count. During this period the subject feels thirsty and takes a lot of water. This is a great help for restoring blood volume.
   - *Proteins:* Plasma proteins are replenished in two stages: (a) The stored proteins (labile protein) are mobilised within a few hours from the tissues and organs, mainly from liver. (b) They are also actively manufactured by the liver. During this regeneration, a sequence is seen—at first the fibrinogen, then the globulin and lastly the albumin is regenerated. Provided the blood is adequate, the lost plasma proteins are fully restored in a few days.
   - *Red cells:* Red cells are rapidly manufactured by the red marrow after a few days. Anoxia stimulates the marrow. Full restoration of the red cells and haemoglobin requires 3 to 5 weeks time, if the diet is adequate.

Clinical aids: The best remedy lies in whole blood transfusion, because it fully supplies all the materials lost. Plasma transfusion comes next. Dried plasma can be preserved and may be used after suitable dilution.

Summary of Compensatory Reactions in Haemorrhage

(a) Venoconstriction, (b) vasoconstriction, (c) increased secretion of epinephrine, norepinephrine, vasopressin, aldosterone and glucocorticoids, (d) rapid rate of thoracic pumping and skeletal muscular pumping (occasionally), (e) increased flow of interstitial fluid in the capillaries, (f) secretion of erythropoietin, (g) increased plasma protein synthesis, and (h) tachycardia.

HEART FAILURE

It is a pathological condition in which cardiac output is insufficient to meet the oxygen, metabolic and all functional requirements of the tissue thus altering homeostasis equilibrium. The common causes of heart failure (Fig. 41.1) are myocardial infarction, cardiac arrhythmias, hypertension, coronary artery diseases, congenital heart diseases, valvular heart disease, myocarditis, etc. The clinical manifestations in heart failure patients include shortness of breath (dyspnoea), exercise intolerance, fatigue, rapid and irregular heartbeat (arrhythmias), altered sensorium, swelling (oedema) in legs, ankles and feet, persistent cough with expectoration of white or pink blood-tinged phlegm, lack of appetite, nausea, etc.

Types of Heart Failure

The types of heart failure are:

Left-sided heart failure: In left-sided or left ventricular (LV) heart failure, the left side of the heart is unable to pump adequate blood in systemic circulation and this causes transudation of fluid into air spaces leading to pulmonary oedema. The patient may present with signs and symptoms of dyspnoea, dyspnoea in lying down position (orthopnoea), paroxysmal nocturnal dyspnoea and frothy sputum.

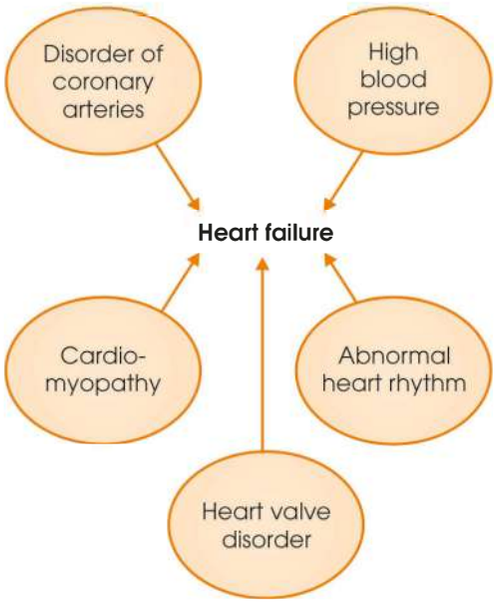

Fig. 41.1: Causes for heart failure

There are two types of left-sided heart failure.

1. *Systolic failure:* It is due to failure of left ventricle to contract normally due to which sufficient amount of blood cannot be pumped into circulation.
2. *Diastolic failure:* The left ventricle loses its ability to relax normally due to which there is insufficient filling of blood during the resting period between each beat.

Right-sided heart failure: The failure of the right ventricle to pump adequate blood produces venous congestion and increased systemic venous pressure. Rise in venous pressure leads to transudation of fluid into interstitial spaces leading to oedema in the dependent part like limbs, feet and sacral region, increased jugular venous pressure, hepatosplenomegaly, ascites and portal hypertension.

Congestive heart failure: The backward failure which manifests as right and left ventricular failure is also called congestive heart failure as it leads to congestion in venous system and increases in venous pressure. The right heart failure follows the left heart failure and together they manifest signs and symptoms of congestive heart failure like rise in jugular venous pressure, ascites, oedema over feet and sacral region, dyspnoea, orthopnoea, paroxysmal nocturnal dyspnoea and frothy sputum (due to irritation of diaphragm).

Role of Compensatory Mechanisms in Heart Failure

1. Sympathetic activation of the heart causes release of epinephrine/norepinephrine which increases heart rate, increases cardiac contractibility and produces peripheral vasoconstriction. The arterial vasoconstriction increases arterial pressure.
2. Hormonal response: The decreased renal perfusion interpreted by juxtaglomerular apparatus as hypovolaemia. The sympathetic stimulation along with hypovolaemia causes an increase in renin release from the kidney. Renin converts angiotensinogen in the plasma to angiotensin I, which by action of converting enzyme is converted to angiotensin II, which causes aldosterone release and this promotes sodium and water absorption (via ADH secretion) along the tubules and increases the blood volume. Angiotensin II also has direct vasoconstrictor actions on blood vessels, which contributes to the increase in systemic vascular resistance. These compensatory mechanisms may restore CO to near-normal. But, if excessive the compensatory mechanisms can worsen heart failure because:
   - Vasoconstriction: It increases the resistance in the arteries against which heart has to pump (there is increased afterload) and may therefore decrease cardiac output.
   - Sodium and water retention increases fluid volume which increases preload. If cardiac muscle is overstretched there will be decreased strength of contraction and decrease CO.
   - Excessive tachycardia due to sympathetic stimulation decreases the diastolic filling time thereby decreasing the ventricular filling leading to decreased stroke volume and cardiac output.

Management and Prevention

1. The management of heart failure depends on the cause. Some of the common medications used in management of heart failure are: Angiotensin-converting enzyme (ACE) inhibitors, angiotensin II receptor blockers, beta-blockers, diuretics, etc.
2. The suggested lifestyle changes you can make to help prevent heart failure include cessation from smoking and intake of alcohol, balance diet, effective stress management and regular exercise.
3. Heart failure is a chronic disease needing lifelong management.

HYPOTENSION

Orthostatic Hypotension

It is also known as postural hypotension. The blood pressure lowers down abruptly on standing from sitting or lying down position. The person may experience dizziness or even they may faint. The symptoms last for a few seconds to a few minutes after standing. The signs and symptoms of orthostatic hypotension are: Feeling of light headedness or dizziness, blurry vision, confusion, nausea, sinking feeling and fainting (syncope).

Pathophysiology: The blood pools down in lower limb after standing. This decreases the venous return and the cardiac output. The physiological response of decreased blood pressure is via stimulation of baroreceptors which sends signal to cardiac centres stimulating sympathetic response thus increasing the heart rate and the blood pressure. In patients of orthostatic or postural hypotension some aetiology interrupts with the process of physiological response of baroreceptors in rectifying lowered blood pressure.

The common causes of orthostatic hypotension are: Dehydration, postprandial hypotension after meals, heart disease (heart valve disease, heart failure), endocrine disorders (adrenal insufficiency—Addison's disease), nervous system disorders (Parkinson's disease, multiple system atrophy, pure autonomic failure and amyloidosis), etc. The complications experienced in orthostatic hypotension are fainting (syncope) and stroke. The complication due to orthostatic hypotension can be prevented by intake of medication for underlying pathological causes, altered lifestyle pattern such as use of adequate salt in diet, balance diet intake (low-carbohydrate meals), adequate fluid intake, regular exercises (exercising the calf muscles before sitting up), wear compression stockings or abdominal binders, etc.

HYPERTENSION

The sustained increased in arterial blood pressure leads to hypertension. The risk factors in causation of hypertension mainly are smoking, alcohol, excessive salt intake and obesity. The chronic rise in blood pressure leads to chronic renal diseases, coronary artery disease, heart failure, stroke, retinopathy, etc.

The hypertension is classified as primary or essential hypertension and secondary hypertension.

Signs and Symptoms

Most of the patients with hypertension do not report with symptoms and are diagnosed on routine health check up. The hypertensive patients may present with complains of headache, vertigo, altered vision or light headedness. The hypertensive patient may develop complications such as retinopathy, neuropathy or nephropathy.

Primary hypertension: The arterial blood pressure is more than 150/ 90 mm Hg in primary hypertension. The most commonly occurring type of hypertension is primary hypertension. It is a complex disorder in which etiological causes cannot be ascertained. It may show genetic pre-disposition, another factors which may predispose a patient to primary hypertension are sedentary life style, obesity, smoking, alcohol addiction, high intake of fats in meals, high intake of salt in food, anxiety induced stress, etc. Primary hypertension may get controlled by life style modification, if not the patient may require to intake medication.

Secondary hypertension: There is known underlying cause.

The causes of hypertension are:

1. Diabetes complications (diabetic nephropathy).
2. Glomerular disease.
3. Polycystic kidney disease
4. Renovascular hypertension.
5. Aldosteronism.
6. Cushing's syndrome.
7. Pheochromocytoma (tumour in an adrenal gland)
8. Hypothyroidism or hyperthyroidism
9. Hyperparathyroidism.
10. Coarctation of the aorta.
11. Sleep apnoea.
12. Obesity.
13. Pregnancy (pregnancy-induced hypertension or pre-eclampsia).
14. Medications and supplements. Medications birth control pills, antidepressants and drugs used after organ transplants can cause or aggravate high blood pressure.

Malignant hypertension: The blood pressure increases as higher up to 260/150 mm Hg in malignant hypertension. When high blood pressure occurs suddenly and drastically the patient is termed to be having malignant hypertension. Malignant hypertension may occur in patient as a complication of primary or secondary hypertension. The common complications observed in malignant hypertension are stroke, seizures, renal failure, blindness, myocardial infarction, angina, etc.

Isolated systolic hypertension: It may be caused by underlying conditions such as stiffness of arteries, heart valve problems or in hyperthyroidism. Isolated systolic hypertension can lead to complications such as stroke, heart disease and chronic kidney disease. As arteries become stiff; it results in a high systolic blood pressure and a normal diastolic blood pressure.

White coat hypertension: When blood pressure is taken in a clinical setting in a hospital the patient's blood pressure is noted to be increased, but outside the hospital setup the blood pressure is normal. Anxiety caused in sensitive patient on visit to a doctor might be the underlying cause.

Resistant hypertension: The patient is unresponsive to any medications in resistant hypertension. Hypertension is considered to be resistant if three medications (anti-hypertensive drugs) fail to successfully treat the underlying condition. Around four medications may be necessary to treat resistant hypertension.

Pathophysiology of Hypertension

The various underlying mechanism involved in patho-physiological manifestation of hypertension includes:

1. The essential hypertension may get established with advancement of age as the cardiac output falls and peripheral resistance increases. The atherosclerotic changes with structural narrowing of small arteries and arterioles in old age or in patients of hyperlipdaemia leads to increased peripheral resistance causing hypertension in patients.
2. The increased peripheral resistance in hypertension may be due to abnormalities in the intra-renal renin-angiotensin system and/or abnormalities of the sympathetic nervous system.
3. The decreased peripheral venous compliance increases venous return, increased cardiac preload and eventually diastolic dysfunction.
4. The underlying cause of secondary hypertension has well established etiological cause for the disease.

Compensatory mechanism: The baroreceptor reflex, chemoreceptor reflex, capillary fluid shift mechanism, stretch reflex, Cushing reflex and renin-angiotensin-aldosterone mechanisms play important role in regulation of blood pressure.

Management of hypertension: The initial line of management includes lifestyle modification and intake of balanced diet (appropriate salt intake). If the lifestyle changes fail to control blood pressure the drug therapy is initiated.

Drugs used for the treatment of hypertension are:

1. *ACE inhibitors (angiotensin converting enzyme inhibitors):* Inhibit the conversion of angiotensin I (inactive) to its active form angiotensin II. Examples are ramipril, enalapril, and captopril, etc.
2. *Angiotensin II receptor antagonist:* Angiotensin II receptor antagonism produces vasodilatation and hence lowers the blood pressure. Examples are losartan, valsartan, etc.
3. *Calcium channel blockers:* They inhibit the movement of calcium in the muscle cells of the heart and arteries. They decrease the force of the cardiac contraction and relax the muscle cells in the walls of the arteries. Examples are amlodipine, nifedipine, verapamil, diltiazem, etc.
4. *Alpha receptor blockers:* They produce vasodilatation and lower blood pressure. Examples are tamsulosin or alfuzosin, etc.
5. *Diuretics:* Thiazides are preferred diuretics for treating hypertension, because their diuretic effect is accompanied by a reduction in peripheral resistance and further reduces blood pressure. Example: Hydrochlorothiazide.

SHOCK

It is a syndrome in which profound and widespread reduction of effective tissue perfusion leads initially to reversible, and then if prolonged, to irreversible cellular dysfunction, irreversible multi-organ failure and death.

The complex clinical syndrome encompassing a group of conditions with variable haemodynamic manifestations

- Generalised inadequacy of blood flow to the systemic organs.
- Inadequate perfusion of tissue throughout the body and hypoperfusion lead to decreased delivery of oxygen and nutrients to the tissue and slow removal of accumalated metabolites.
- Tissue hypoxia shifts metabolism to anaerobic pathways with production of lactic acid.

Shock index (SI): Heart rate divided by systolic blood pressure gives the value of shock index. It is an accurate diagnostic measure. In physiological condition the observe value is between 0.5 and 0.8. Increase in this ratio alerts the probable progressive stage of shock.

Types of Shock

1. **Hypovolemic shock:** It occurs due to intra-vascular fluid volume loss due to haemorrhage, fluid depletion or sequestration. The non-haemorrhagic causes of hypovolemic shock are: Vomiting, diarrhoea, bowel obstruction, pancreatitis, burns, etc. The haemorrhagic causes of hypovolemic shock are: GI bleed, trauma, massive haemoptysis, aortic aneurysm rupture, ectopic pregnancy, post-partum bleeding, etc.
2. **Cardiogenic shock:** There is impairment of heart pump; the causes of cardiogenic shock are acute myocardial infarction, sepsis, myocarditis, myocardial contusion, aortic or mitral stenosis, cardiomyopathy, acute aortic insufficiency.
3. **Obstructive shock:** There is obstruction to normal CO and diminished system perfusion. The causes of obstructive shock are: Tension pneumothorax, intrathoracic tumours, cardiac tamponade, constrictive pericarditis, massive pulmonary emboli, venae cava obstruction, coarctation of aorta, etc.
4. **Distributive shock:** There is pathologic redistribution of intra-vascular fluid volume. The causes are:
   a. *Septicaemia:* It is endotoxic, secondary to specific infection such as perforated duodenum, strangulated bowel, etc. in which there is release of bacterial toxins which get absorbed into systemic circulation via the bloodstream.
   b. *Anaphylactic shock:* IgE mediated severe systemic hypersensitivity reaction characterized by multisystem involvement. There is generalised arteriolar dilatation and increased capillary permeability leading to fall in blood pressure.
   c. *Neurogenic shock*: There is loss of peripheral vasomotor control from spinal injury, or similar

phenomenon of vasovagal syncope, postural syncope, micturition syncope, cough syncope, carotid sinus syncope, deglutition syncope or neurocardiogenic syncope.

Clinical Features of Shock

1. Fall in systolic blood pressure to value less than 90 mm Hg.
2. Rapid and thready pulse.
3. Rapid shallow breathing
4. Tachycardia
5. Urine output less than 20 ml/hour: Oliguria
6. Cool, pale and wet skin.
7. Marked muscular weakness
8. Intense thirst due to loss of extra-cellular fluid.
9. Hypothermia (except septic shock)
10. Disorientation

Stages of Shock

1. **Non-progressive (compensated) stage:** The normal compensatory mechanisms of the circulation can return cardiac output and blood pressure to normal levels. The compensatory mechanisms involved in this stage are:
   - *Baroreceptor reflexes:* The sympathetic stimulation constricts arterioles in most parts of the body and venous reservoirs and protects and restore the coronary and cerebral blood flow.
   - Angiotensin-aldosterone and anti-diuretic hormone (ADH) release in response to hypovolemia produces vasoconstriction, water and salt retention by the kidneys.
   - Absorption of fluid from interstitial fluid and GIT and increased thirst and water intake restores blood volume.
2. **Progressive stage:** The shock eventually results in death; if no medical intervention or therapeutic measures are taken. As circulatory system begins to deteriorate the decreased contractility of heart is followed by decreased coronary blood flow and this vicious cycle continues and shock worsens. When circulation collapses the reduce flow of blood to vasomotor centre depresses the centre that it becomes progressively less active and eventually inactive leading to vasomotor failure. The sludged blood causes blockade of very small vessels. Progressive shock causes ischemic tissues to release toxins like histamine, serotonin, tissue enzymes that causes further deterioration of the circulatory system. The intestines hypo-perfusion leads to endotoxin formation and absorption of toxic substances into circulation producing septic shock. The vasodilatation in precapillary bed, cardiac depression and generalised cellular deterioration (↑K^+, ↓ATP, release of hydrolases) leading to multiorgan failure.
3. **Irreversible stage:** All forms of therapy are inadequate and eventually the patient dies. Despite therapy the circulatory system continues to deteriorate.

The processes which eventually lead to death in irreversible stage of shock are (Fig. 41.4):

- Marked hypoxic tissue damage
- Endothelial dysfunction, the adhesive molecules, neutrophils, macrophages accumulates producing inflammatory response.
- Microcirculation failure → plasma proteins leak to interstitium
- Progressive acidosis
- Advanced disseminated intravascular coagulation

1. **Hypovolemic shock** (Fig. 41.2)**:** It is due to a decreased circulating blood volume in relation to total vascular capacity. Hypovolemic shock is caused by dehydration, excessive sweating, fluid loss in severe diarrhoea or vomiting, excess loss of fluid by the kidneys, inadequate intake of fluids and electrolytes, destruction of the adrenal cortices, with the loss of aldosterone, external haemorrhage due to trauma or internal haemorrhage such as GIT bleed. The signs and symptoms in hypovolemic shock include cold, clammy skin, dry mouth, parched skin, sweating, decreased urinary output and tachycardia. Oxygenation, and volume replacement is always a necessary component of treatment. Isotonic crystalloids and colloids for hypovolemia and whole blood, packed RBCs and isotonic crystalloids for haemorrhage are administered.
2. **Cardiogenic shock** (Fig. 41.3)**:** The failure of the cardiac pumping activity which impairs cardiac functioning eventually leads to cardiogenic shock. The causes for cardiogenic shock are acute myocardial

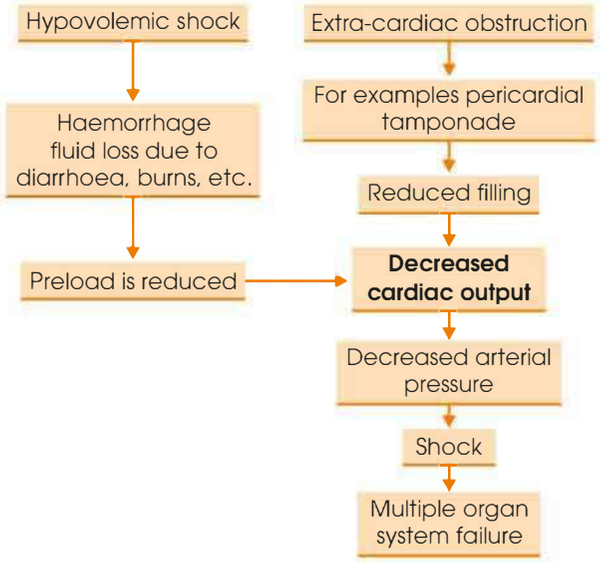

Fig. 41.2: Hypovolemic shock and obstructive shock leading to multi-organ failure

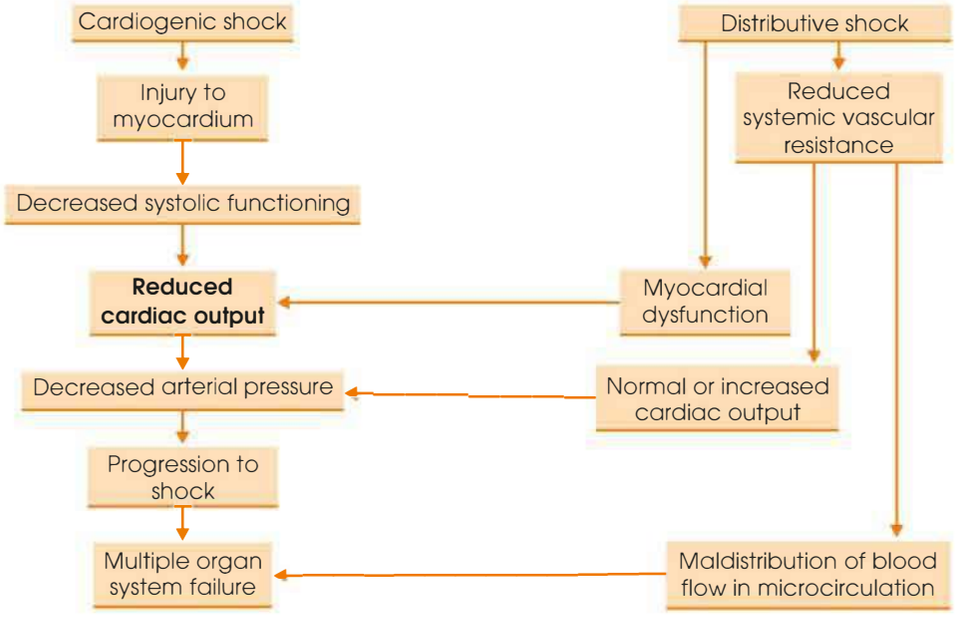

Fig. 41.3: Cardiogenic shock and distributive shock leading to multi-organ failure

The loss of effective circulating blood volume leads to hypovolemic shock. The signs and symptoms observed are:

- Decreased blood flow and tachycardia lead to rapid, weak, thready pulse.
- Cool and clammy skin due to vasoconstriction.
- Sympathetic nervous system stimulation and acidosis leading to rapid and shallow breathing.
- Livedo-reticularis (cold and mottled skin) especially extremities due to inadequate perfusion of the skin
- Hypothermia, increased thirst and dry mouth

infarction, myocarditis, aortic or mitral stenosis, acute aortic insufficiency, atrial myxoma, dysrhythmia, cardiomyopathy, aortic dissection, aortic stenosis, mitral stenosis, ventricular septal defect, etc. The cardiac output decreases with diminished blood volume due to decreased vasomotor tone, there is decreased blood pressure followed by self-perpetuating cycle then ensues (vicious cycle): Metabolic acidosis and reduced coronary perfusion further impairing ventricular function and predisposing to the development of arrhythmias

- The clinical signs seen in cardiogenic shock are oliguria (decreased urinary output), increased jugular venous pressure, and evidence of third heart sound and pulmonary oedema. The principle lines of management in cardiogenic shock are stabilizing blood pressure with vasopressors, inotropes and fluids infusion and treating pulmonary congestion with diuretics and venodilators.
- The general line of management includes monitoring of vitals, oxygen support, supplementing nitrodilators (coronary vasodilatation), morphine, etc. (pain management), pressor support (dopamine or dobutamine), definitive warranted therapy depending on the underline pathology (fibrinolytic therapy, coronary artery bypass graft, ventricular assist device, cardiac transplant, etc.).

3. **Obstructive shock:** It causes obstruction to normal cardiac output and diminishes system perfusion. It directly impair diastolic filling of the right ventricle and indirectly impair right ventricle by obstructing venous return. It increases ventricular afterload. The common causes are pericardial tamponade, constrictive pericarditis, tension pneumothorax, acute pulmonary hypertension, intrathoracic tumours, massive pulmonary emboli (>2 lobar arteries with >50% occlusion), aortic dissection and saddle embolus. The general management guidelines in obstructive shock are managing the airway, breathing and circulation and cater appropriate medical therapy or surgical interventions (needle decompression for tension pneumothorax, pericardiocentesis in cardiac tamponade, heparin (thrombolytics) in pulmonary embolism, valve surgery for aortic stenosis, etc.).
4. **Distributive shock** (Fig. 41.3)**:** There is loss of peripheral vascular resistance. They are of three types: Septic shock, anaphylactic shock and neurogenic shock.
   a. *Septic shock:* Septic shock is an immediate life-threatening syndrome initiated by micro-organisms, their toxins, or both, that have invaded the bloodstream. The common causes are peritonitis, gangrenous infection and pyelonephritis. The clinical features of septic shock are high fever, marked vasodilatation (inflammation), normal or increased cardiac output (vasodilatation),

disseminated intra-vascular coagulation (haemorrhages occur into many tissues such as GIT). The inflammatory mediators which released in septic shock are IL-1 and TNF: PGE_2, leukotrienes and NO. They produce vascular relaxation, increased endothelial permeability and decreased myocardial contractility. If bacterial infection progress to septicaemia it leads to release of bacterial toxins in circulation, producing circulatory deterioration and prolong tissue hypoperfusion causing eventually leading to death. The management includes maintenance of airway, oxygenation, IV fluids (crystalloids), pressor support (dopamine, norepinephrine), antibiotics, etc.

b. *Anaphylactic shock and histamine shock:* Release of histamine in immune type reactions, causing venous dilation, arteriole dilation and increased capillary permeability. There is IgE mediated release of mediators from tissue mast/basophils. The patients management include maintenance of airway, oxygenation, IV fluids (crystalloids, epinephrine (IM or IV), anti-histaminic, steroids, beta-agonist, aminophylline and pressor support (dopamine, dobutamine or epinephrine).

c. *Neurogenic shock:* There is loss of peripheral vasomotor control and sudden loss of vasomotor tone. It may be due to side effects of deep general anaesthesia, spinal anaesthesia or due to brain damage. Management include maintenance of airway, oxygenation, cardiopulmonary resuscitation, pressor support (dopamine, dobutamine or epinephrine) and shifting to spine centre for further management.

Physiological process in shock: The acute circulatory insufficiency leads to mismatch between blood volume and volume of vascular bed leading to tissue hypoperfusion. The inadequate oxygen delivery to meet metabolic demands results in generalised tissue hypoperfusion and metabolic acidosis. The inadequate systemic oxygen delivery activates autonomic responses to maintain systemic oxygen delivery.

1. **Cardiac reflexes:** The baroreceptor reflexes, chemoreceptor reflexes, and ischaemia within the medulla oblongata initiate strong sympathetic responses that result in intense vasoconstriction and increased heart rate and blood pressure.

   Cushing reflex: The ischaemia is counteracted by Cushing reflex (increase in intracranial pressure constricts cerebral arteries leading to cerebral ischaemia and cerebral ischemia induces hypertension (sympathetic response) which produces reflex bradycardia.
2. **Sympathetic nervous system:** Sympathetic stimulation releases norepinephrine, epinephrine, dopamine and cortisol. This leads to vasoconstriction, increase heart rate and increase of cardiac contractility and this increases the cardiac output. The physiological goal to maintain cerebral and cardiac perfusion is aided by the vasoconstriction of splanchnic, musculoskeletal, and renal vasculature.
3. **Renin-angiotensin axis:** Renin released in response to hypoxia and decrease circulatory blood flow via angiotensin aldosterone axis results in a greater rate of angiotensin II formation, causing vasoconstriction and increased aldosterone release from the adrenal cortex. Aldosterone causes water and sodium retention from kidney. This increase the blood volume and blood pressure.
4. **Stretch relaxation:** As the blood volume decreases, the stress-relaxation response of blood vessels causes the blood vessels to contract and helps sustain blood pressure.
5. **Anti-diuretic hormone:** In addition, ADH is released from the posterior pituitary gland and enhances the retention of water by the kidneys.
6. **Capillary fluid shift mechanism:** The fluid shift mechanism causes the water to move from the interstitial spaces and the intestinal lumen to circulation in order to restore the normal blood volume. An intense sensation of thirst increases water intake, also helping to elevate normal blood volume.
7. **Vasodilators:** Hypoxia-induced vasodilatation may be direct (inadequate O_2 to sustain smooth muscle contraction) or indirect via the production of vasodilator metabolites. The vasodilators released in response to reduced tissue perfusion and accumulation of metabolic products. Examples are lactic acid, inorganic phosphates and potassium ions in muscle, adenosine in coronary circulation, hydrogen ions in cerebral circulation, etc.

   Overriding of compensatory mechanism

   Lactic acidosis: The progressive hypoxia leads to systemic metabolic lactic acidosis that overcomes the body's compensatory mechanisms.

   Cellular responses to decreased systemic oxygen delivery: This leads to ATP depletion affecting ion pump function, produces cellular oedema and hydrolysis of cellular membrane and cellular death.

The lactic acidosis, cardiovascular insufficiency and increased metabolic demands further cause the progression of shock and eventually lead to cardiac depression, respiratory distress, renal failure, disseminated intra-vascular coagulation and multiple organ failure.

Physiology of Treatment in Shock

Management of shock is carried out depending on type and cause. The basic management in different types of shocks are mentioned in the text above. But the general guidelines in treatment of shock are:

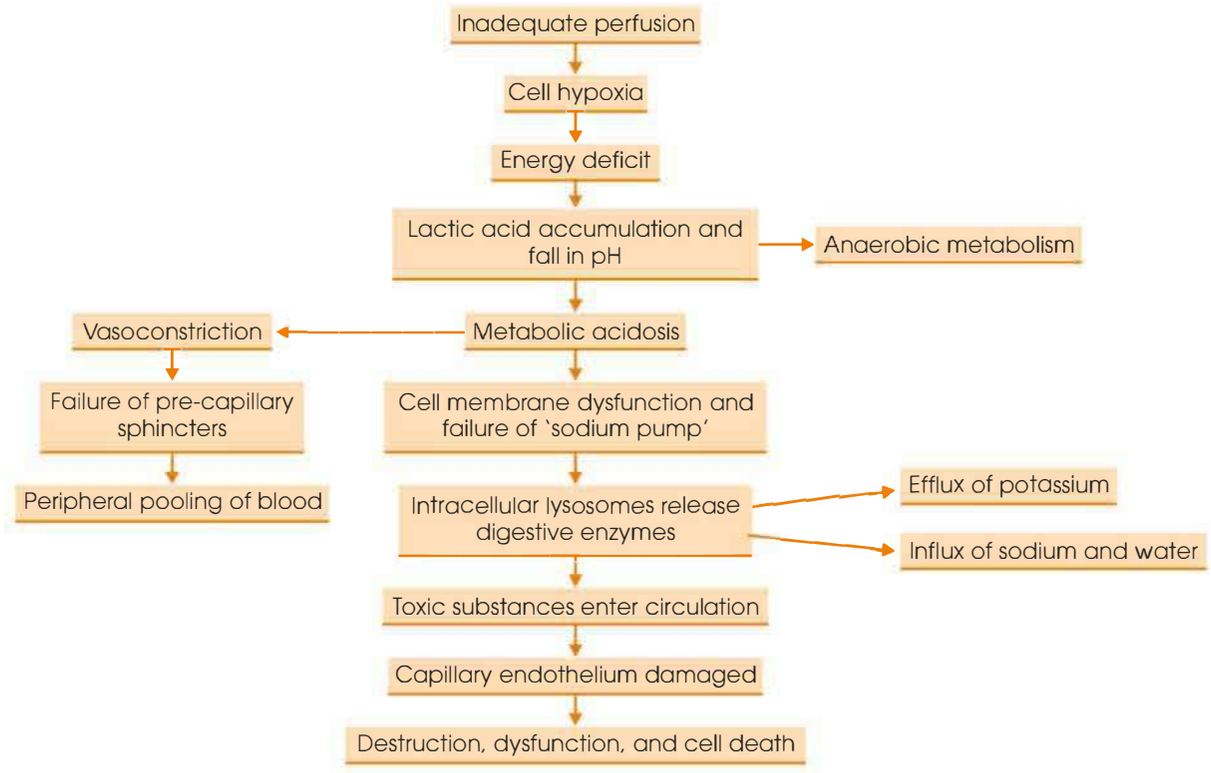

Fig. 41.4: Progression of shock to end stage

Airway management, oxygen support, replacement therapy with blood and plasma transfusion, dextran solution as a plasma substitute, sympathomimetic drugs (simulate sympathetic system).

EXAM-ORIENTED QUESTIONS

Essay

1. Discuss the causes, pathophysiology and management of haemorrhages.
2. Discuss the causes, pathophysiology and management of heart failure.
3. Discuss the causes, pathophysiology and management of hypertension.
4. Describe the stages of shock and the compensatory mechanism in different types of shock.

Short Notes

1. Orthostatic hypotension
2. Define shock and classify the types of shock.

CLINICAL CASE SCENARIO

Cardiovascular System

Q1. Enlist a few clinically associated with systolic murmur and diastolic murmur.

Ans. The systolic murmurs are observed in mitral and aortic stenosis, mitral regurgitation, aortic regurgitation, etc.

The diastolic murmurs are observed in mitral and tricuspid stenosis, aortic regurgitation or pulmonary regurgitation.

Q2. A patient aged 45 years reported with blood pressure of 150 (systolic)/100 (diastolic). Doppler investigation revealed stenosis of renal arteries. What is the diagnosis? Enlist a few systemic diseases producing increase blood pressure.

Ans. The patient is suffering from secondary hypertension due to renal disease. The other systemic condition which may lead to secondary hypertension are glomerular nephritis, coarctation of aorta, Cushing syndrome as a result of excessive cortisol secretion, polycythemia, phaeochromocytoma (tumour of adrenal medulla), adrenal cortex tumour (Cons syndrome), hyperthyroidism, etc.

Q3. A 25-year-old female reported with history of giddiness and fainting on sudden standing on a few earlier occasion. What is probable cause? Enlist a few causes for the same.

Ans. The patient is diagnosed as a case of orthostatic hypotension. This may be seen in certain clinical conditions in patients on sympatholytic drugs, patient of syphilis and diabetes mellitus having developed neuropathy, patients of autonomic insufficiency, etc.

Q4. A patient had severe traumatic injury due to car accident. The patient had excessive soft tissue injury. The clinician diagnosed the case as traumatic injury secondary to crush syndrome. Explain the condition and cause for the traumatic shock.

Ans. The damage cause due to soft tissue injury is referred to as crush syndrome. The loss of blood into injured area produces hypovolemia and leads to a condition of shock and as it is secondary to trauma it is referred as traumatic shock.

Q5. A 50-year-old male complained of sudden chest pain and was admitted in the casualty. His vitals and systemic examination was normal. His ECG showed ST elevation. He was given sorbitrate orally and ST elevation was resolved. What is the diagnosis? What are the precipitating factors? Explain the physiological basis of the findings.

Ans. The diagnosis is Prinzmetal's angina. This many a time occurs spontaneously and is characterized by transient ST-segment elevation that may spontaneously resolve or resolves with glyceryl trinitrate without further progressing to myocardial infarction. These attacks can be precipitated by an emotional stress, hyperventilation, exercise or exposure to cold.

Q6. A 26-year-old male complained of sudden onset palpitation episode with chest discomfort. His ECG showed wide complex tachycardia. The patient was diagnosed as Wolff-Parkinson-White (WPW) syndrome with atrial fibrillation. What is this condition due to? What is the primary line of management?

Ans. Wolff-Parkinson-White (WPW) syndrome is the presence of an accessory pathway between the atrium and the ventricle, which predisposes to development of tachyarrhythmias and sudden cardiac arrest in patients. Immediate synchronized defibrillation should be delivered in patients with WPW syndrome and atrial fibrillation. Electrophysiology study with radiofrequency ablation of the accessory pathway is the first-line therapy.

Q7. A 62-year-old male reported with complaints of excessive sweating, tachycardia, raised JVP, central cyanosis, tachypnoea, basal respiratory crackles and pitting ankle oedema. He was diagnoses as patient of congestive cardiac failure. Explain the physiological basis of congestive cardiac failure.

Ans. Congestive heart failure (CHF) occurs when the left ventricle cannot pump out the amount of blood entering the ventricle due to ventricular damaged or even secondary to circulatory disorders. The blood begins to congest in the lungs leading to pulmonary oedema. As the airways are obstructed by the fluid it rescues the intake of air into the lungs and the effort of breathing increases. It leads to severe dyspnoea and as the condition progresses, this congestion will eventually cause the right ventricle to fail. The delivery of oxygen and nutrients to the systemic cells is disrupted, and the by-products of metabolism (such as CO_2) are no longer eliminated effectively causing toxins to accumulate and cause cell death and ultimately lead to congestive cardiac failure.

Important Cardiovascular Research Study: Framingham Heart Study

The Framingham study is one of the most impressive medical works in the 20th century. During the 1st half of the century, there was a steady increase in deaths attributed to heart disease. However, the causes of coronary heart disease were speculative.

1. National Heart Institute (now National Heart, Lung, and Blood Institute [NHLBI]) supported the 1st collection of information from a community cohort study.
2. Between the period 1948 and 1951, 1980 men and 2421 women were enrolled in an observational study in Framingham and Massachusetts.

3. The first public report of this long-term study, "Factors of risk in the development of coronary heart disease—six-year follow-up experience"; was published in 1961.
4. The study revealed that high blood pressure, smoking, and high cholesterol levels were major factors in heart disease. From this report, the concept of cardiovascular risk factors were identified.

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First Cardiac Catheterization and Future Development of Technique

1. Werner Forssmann, a young surgical resident, performed the 1st documented human cardiac catheterization on himself in Germany in 1929. He anaesthetized his left elbow, inserted a catheter into his antecubital vein, and confirmed the position of the catheter tip in the right atrium by use of radiography. His aim was to identify a safe and effective way to inject drugs for cardiac resuscitation.
2. Cournand and Richards used cardiac catheter as a diagnostic tool to measure right-heart pressures and cardiac output in 1941. For their landmark work, they shared a Nobel Prize in medicine with Forssmann in 1956.
3. Mason Sones performed selective coronary arteriography in more than thousand patients in 1958. This development initiated a period of rapid growth in coronary arteriography then application in clinical practice.

Open-heart Surgery

1. **1849:** Wilfred Bigelow and his team performed open-heart procedures using hypothermia in animals.
2. **1953:** John Lewis performed the 1st successful closure of an atrial septal defect in a 5-year-old girl patient. He used the open-heart hypothermic technique.
3. **1953:** John Gibbon used heart-lung machine, which offered additional protection to vital organs during the repair of an atrial septal defect.
4. **1956:** Walton Lillehei and his team corrected pure mitral regurgitation with suture plication of the commissures under direct vision.
5. **1964:** Vasilii Kolessov, a Russian cardiac surgeon, performed the 1st internal mammary artery-coronary artery anastomosis.
6. **1967:** René Favaloro used a saphenous vein autograft to replace a stenotic segment of the right coronary artery.
7. **1968:** Use of the internal mammary artery instead of the saphenous vein for bypass grafting.

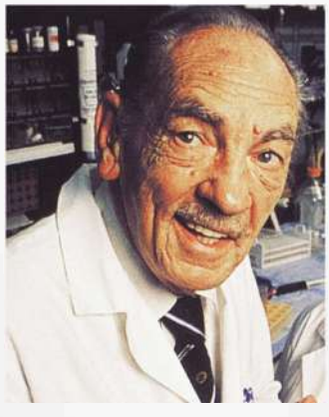
Today, coronary artery bypass grafting has become one of the most common operations and is performed all over the world.

Recent Advances in Cardiovascular Research

James Black, Gertrude Elion and George Hitchings were awarded the 1988 Nobel Prize in physiology or medicine for their work leading to the development of propranolol and cimetidine which had become a boon for patients of hypertension and gastric ulcers. **Sir James Whyte Black** developed propranolol a beta blocker used for the treatment of heart disease. Black was also responsible for the development of cimetidine, a H_2 receptor antagonist, a drug used in a similar manner to treat stomach ulcers. **Gertrude Belle Elion** apart from a Noble Prize winning feat developed a multitude of new drugs using innovative research methods that helped in development of the drug for treatment of AIDS Zidovudine. **George Herbert Hitchings** was an American doctor and was also well known for his work on chemotherapy.

Sir James Whyte Black
1924–2010

Gertrude Belle Elion
1918–1999



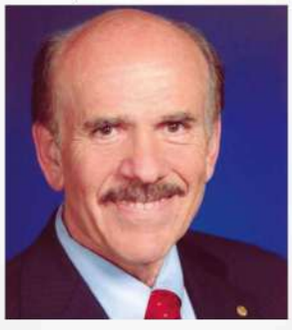
George Hitchings
1905–1998

Robert F Furchgott, Ferid Murad and Louis J Ignarro

The Nobel Prize in physiology or medicine in 1998 was awarded to Robert F. Furchgott, Louis J. Ignarro

Robert F Furchgott
1916–2009

Ferid Murad
1938



Louis J Ignarro
1941

and Ferid Murad (Whiting, United States) for their discovery findings on nitric oxide (NO) as a signaling molecule in the cardiovascular system. Nitric oxide plays an important role in endothelial homeostasis through its anticoagulant and vasodilating properties. Research study have also proven that NO can exert antiatherosclerotic actions. Nitrates used in treatment of coronary artery disease by indirectly increasing NO bioavailability.

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Section

V

Respiratory System

Chapter

42

Functional Organization of Respiratory System

Definition: Respiration is the process by which oxygen from the lungs is carried by the blood to the tissues; and carbon dioxide formed in the tissues by metabolic activity is carried by the blood to the lungs and is expired out.

The process of respiration involves four stages:

1. Ventilation means the passage of air in and out of lungs during inspiration and expiration respectively.
2. Intra-pulmonary gas-mixing or distribution of oxygen-rich inspired air with the air already present in the lungs.
3. Diffusion which means gas transfer across the alveolo-capillary membrane due to tension gradient.
4. Perfusion means flow of adequate quantity of blood through the lungs so that the diffused gases are carried away.

Throughout the body, the function of an organ is reflected in its structure, this is true of the lung in particular.

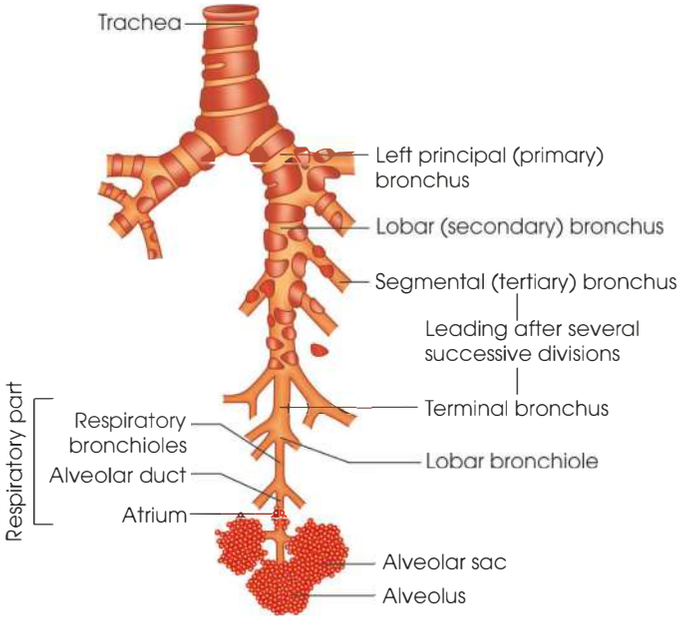
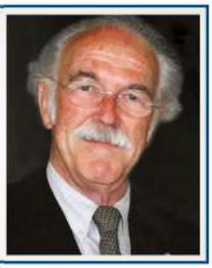


Fig. 42.1: Broncho-pulmonary structure

Ewald R Weibel is a Swiss biologist was one of the first scientists to describe the endothelial organelles. He was awarded the Marcel Benoist Prize in 1974. He is well known for his classification of lung lobes.



Structure of the Respiratory Tract

1. Upper respiratory tract extends from the upper nares to the vocal cord.
2. Lower respiratory tract extends from the vocal cord to the alveoli.

TRACHEA

The trachea (windpipe) is a cartilaginous and membranous tube, about 10 or 11 cm long. It is not quite cylindrical, being flattened posteriorly, its external diameter from side to side is about 2 cm in the adult male and 1.5 cm in the adult female. It is kept patent by incomplete C-shaped rings of cartilage on its anterolateral wall, which keeps air tubes open. There are plenty of seromucous glands in the submucous coat innervated by the vagi. Mucous gland along with goblet cells traps dust in the inspired air and air is moistened by contact with moist lining of serous glands. The cilia beat spontaneously at the rate of 20 times per minute and by their movement drive out mucus-trapped foreign particles and bacteria towards the mouth from the respiratory passage, the velocity of movement being 24 mm per minute. The ciliary movement is independent of nerves but dependent in the presence of atmospheric oxygen in the respiratory tube.

FUNCTIONS OF RESPIRATORY TRACT

The infolding of nasal mucous membrane over the turbinate's offers an area of contact approximately 160 sq cm for the atmospheric air flowing through the nose. The inspiratory air is modified during its passage through the nose as described below:

1. **Filtering effect:** Lung clears the dust particles in following way:
   a. It is cleared of dust particles
   b. Large dust particles are caught by the hairs at the nostrils.
   c. Air can flow uninterruptedly through the zigzag passage of the nasal cavity caused by the turbinates but smaller particles suspended in the air due to their momentum are precipitated on the surface of the mucous membrane of the nose and are caught in mucus. This has been described as 'turbulent precipitation' and particles up to the size of 6 µm are caught in this way and are moved towards pharynx by ciliary action of the nasal epithelium when they are expectorated or swallowed. This size is smaller than the size of red blood cells.
   d. Particles between 1 and 5 µm in diameter get precipitated on the wall of the smaller bronchioles (gravitational precipitation) where they evoke fibrotic reaction ('coal-miners' disease).
   e. Particles of size 1 µm or less in diameter diffuse into the alveoli and adhere to the wall of the alveoli.
   f. Particles smaller than 0.5 µm remain suspended in the alveolar air and are usually expired out. This size of the particles of cigarette smoke is about 0.3 µm. Most of them diffuse into the alveoli where about 1/3rd get caught in the alveolar fluid the remainder is expired out. Entrapped particles in the alveoli are removed by macrophages or provoke growth of fibrous tissue in the alveolar septa.

   An excess of particles provokes fibrous tissue reaction causing permanent disability.

   Role of ciliated epithelium: The cilia of trachea and bronchi beat towards the pharynx and propel the mucus with the entangled foreign particles with a velocity of one centimetre per minute towards the pharynx. They thus help materially to keep the respiratory passages free from foreign particles.
2. **Air-conditioning effect:** The inspired air is brought to near about body temperature and is humidified before it is permitted to enter the deeper air passages. Cold and dry air coming in direct contact with the lungs such as after tracheotomy will cause serious lung crusting and infection. It has been estimated that the inspired air is brought to within 3% of body temperature during its passage through the nose and it is almost completely saturated with water vapour before it is delivered to the lungs.
3. **Cough reflex:** 'Cough' is a protective reflex by means of which respiratory passages are kept free from foreign matter. Patients, in whom cough reflex is lost, get drowned in their own secretion.
   - An instrument called 'Cofulator' has been designed to stimulate cough reflex in these patients.
   - Sensory stimulus necessary for this reflex is either physical or chemical irritation of the respiratory tract. The larynx and the area of bifurcation of the trachea are most sensitive areas. The afferent fibres run in the vagus to the medulla.
   - At the commencement of the act, a deep inspiration is taken, the epiglottis is then closed and the vocal cord closes the laryngeal opening tightly thus entrapping about 2.5 litres of air into the lungs.
   - The abdominal muscles then contract forcefully pushing firmly the diaphragm against the distended lungs. Internal intercostal and other expiratory muscles contract forcefully causing the pressure within the lungs to rise about 100 mm Hg or more.
   5. The epiglottis and vocal cord then open suddenly and thus the lung air under pressure comes out with explosive violence with a velocity of about 160 km or 100 miles per hour. This act thus helps in clearing out the obnoxious irritating agents from the respiratory passages.
4. **Sneeze reflex:** It is very much like cough reflex but here the zone of afferent stimulus is in the nose and the afferent pathway is in the trigeminal nerve. A large volume of air is drawn into the lungs and the uvula is depressed so that the expired air passes mostly through the nose and partly through the mouth.

RESPIRATORY UNITS (Fig. 42.2)

Respiratory units consist of:

1. Respiratory bronchioles
2. Alveolar ducts
3. Respiratory atrium
4. Pulmonary alveoli, the walls of these being so thin that gas exchange (Fig. 42.2) may take place between the air contained in these tubules and the capillaries on their wall.

Each terminal bronchiole opens into a thin-walled respiratory bronchiole of equal diameter which communicates with some alveoli situated on its wall. However, for the most part each respiratory bronchiole opens into several alveolar ducts, these latter open into dilated spaces called (pulmonary) atrium which again communicate with many pulmonary alveoli. Each alveolus is a thin-walled sac filled with air measuring

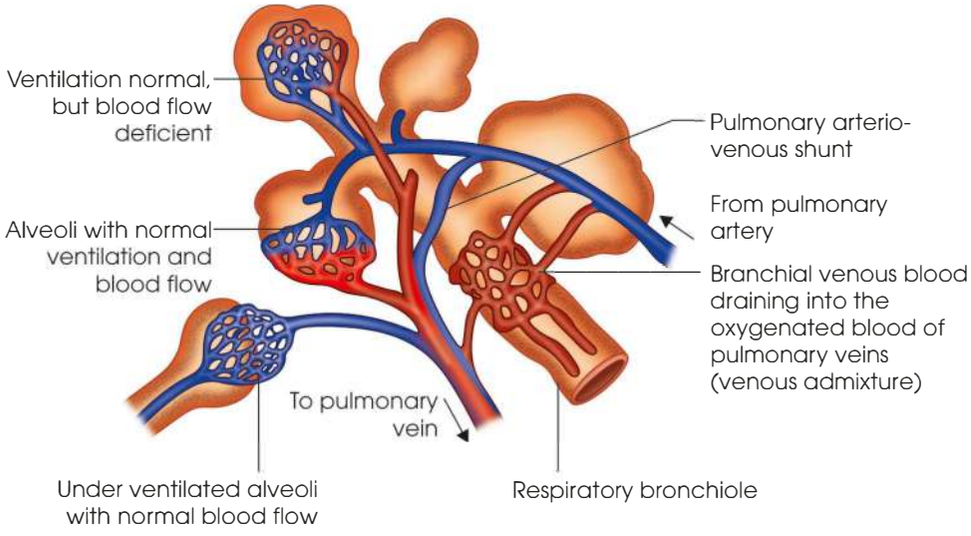


Fig. 42.2: Ventilation and perfusion (blood flow) of primary respiratory lobule

from 75 to 300 µm in diameter. The capillaries of the pulmonary blood vessels ramify around the walls of the alveoli. The alveolar tissue (parenchyma) contains fibres of elastin and collagen and the fluid lining the alveoli has surface tension. As a result, the lung is elastic and is held expanded by keeping the pressure around it (intrapleural pressure) lower than alveolar pressure.

Broncho-pulmonary Anastomosis

On the wall of the respiratory bronchioles the venous blood from the bronchial circulation via the bronchial arteries which arise from the aorta, drains directly into the arterial blood of pulmonary veins. Venous admixture also takes place by direct shunts between the branches of pulmonary artery and pulmonary vein.

Pulmonary Alveoli (Fig. 42.3)

Pulmonary alveoli are polygonal in shape and are packed so tightly that some have no distinct separate walls and communicate with adjacent alveoli by minute pores. They are lined by a thin layer of squamous epithelium which is separated from the endothelium of the pulmonary capillaries by a homogeneous basal lamina which together with small amount of connective tissue constitutes interalveolar septa. Scattered in between the alveolar epithelial cells are found isolated cuboidal cells or great alveolar cells which are characterised by microvilli on their free surface.

Alveolar cells are also known as pneumocytes. Pneumocytes are cells lining the lungs alveoli. There are two types of alveolar cells: Type I alveolar cells and type II alveolar cells.

Type I alveolar cells are squamous type; comprising of cytoplasmic plates relatively devoid of organelles; and are involved in the gas exchange between the alveoli and blood. They cover approximately 90–95% of the alveolar surface.

Type II alveolar cells are granular and roughly cuboidal and secrete pulmonary surfactant. Surfactant, a lipoprotein complex composed of phospholipids and four surfactant proteins known as hydrophilic proteins SP-A and SP-D and hydrophobic proteins: SP-B and: SP-C. The main lipid component of surfactant, dipalmitoylphosphatidylcholine reduces surface tension and thus helps in keeping the alveoli open and prevents their collapse. When the lung tissue is damaged the type II alveolar cells undergo cellular division, forming more type I alveolar cells.

Chevalier L Jackson (1865–1958) was an American pioneer in laryngology and also referred as the "father of endoscopy". The delineation of the bronchopulmonary segments was made by Chevalier Jackson and John Franklin Huber.

Functions of Respiration

1. **Gas transfer:** Transfer of O_2 from the alveoli to the venous blood and CO_2 in the opposite direction.
2. **Regulation of pCO_2 of blood:** The most important function of respiration is to keep the arterial pCO_2 at 40 mm Hg which is essential for many vital functions of the body.
3. **Regulation of pH of blood:** By the reversible reaction H_2CO_2 equilibrium.
4. Excretion of certain volatile gases, e.g. chloroform, ether, ammonia, etc.
5. **Pumping action:** The rhythmic movement of the diaphragm and chest wall causes rhythmic alteration of pressure in the abdomen and chest cavity. This assists in drawing blood from the lower part of the body to the abdomen and then to chest and thus helps in maintaining venous inflow to the heart.

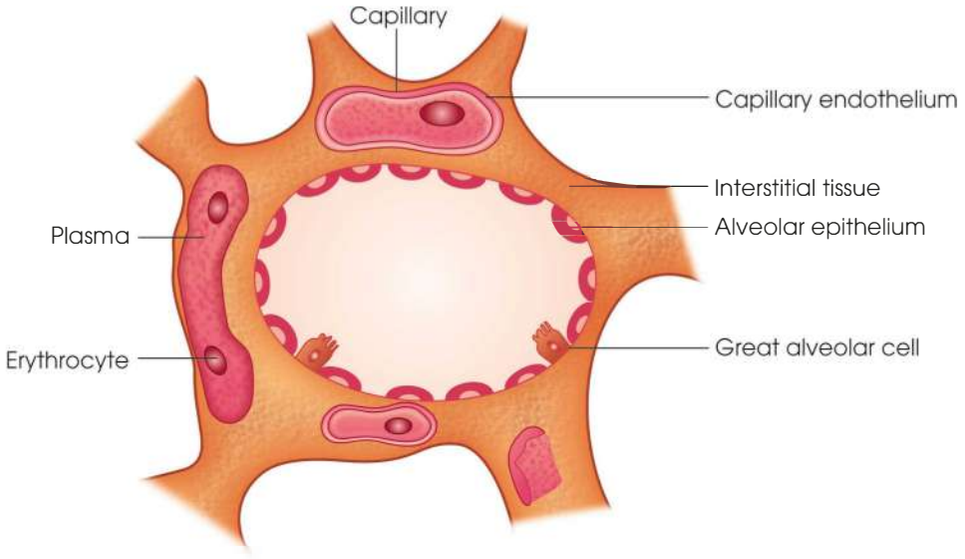


Fig. 42.3: Pulmonary capillary in the alveolar air

Apart from the respiratory functions, the non-respiratory functions of lung are:

1. Filtration of dust particles and inhaled antigens: Explained earlier under title of functions of respiratory tract.
2. Warming and humidification of air: Explained earlier under title of functions of respiratory tract.
3. Olfaction: The nerve endings in roof of nasal cavity are sensitized on exposure to different odorants from the external environment. The impulse travel down to olfactory cortex and aids in identifying different odours.
4. Phonation: Larynx has two vocal cords which lines the glottis. Pitch of the sound could be varied by the altering the size of the glottis, which occurs by contraction and relaxation of the laryngeal muscles. The air movement in lung passage helps in improvement of voice.
5. Reservoir for left ventricle: As pulmonary vascular bed is a low pressure system, it can occupy a large volume of blood. It stores around 0.5 litres of blood; serving as a reservoir for left ventricle.
6. Filtration of blood at the pulmonary capillaries: As blood passes through the small calibre of the pulmonary capillaries the air bubbles, cell debris, emboli and fat globules get trapped in the pulmonary vessels and are filtered thus preventing their entry into the systemic circulation and thrombo-embolic episodes.
7. Metabolic functions of the lungs: Lungs participate in uptake or conversion of chemical substances. The chemical substances formed in lungs and releases for local use are surfactant, histamine, serotonin, leukotrienes, platelet activating factor, prostaglandins, etc. The lower airways are lined by a large number of neuro-endocrine cells. These cells are responsible for the secretion and release of bradykinin, prostaglandins, serotonin, substance P, heparin and histamine. Lung also participates in conversion of angiotensin I to angiotensin II and the catabolism of bradykinins, adrenaline and noradrenaline. Waste products and metabolites are excreted via the lungs as volatile gases (e.g. ethanol, acetone).

PLEURAL CAVITY AND INTRA-PLEURAL PRESSURE

The lungs are covered by visceral pleura which are reflected over the inner aspect of the chest wall enclosing a potential cavity between its two layers known as pleural cavity. The two layers, parietal and visceral are kept moist and lubricated by a few millilitres of a mucopolysaccharide containing fluid in the intra-pleural space so that during respiration the lungs with visceral pleura surrounding it glide smoothly over the parietal pleura (Fig. 42.4).

Intra-pleural Pressure

Normal intra-pleural pressure, that is pressure in the pleural cavity is negative and amounts to –2.5 mm Hg at the end expiratory position. This means that the lungs are not completely collapsed and that alveoli remain partially inflated even after complete expiration.

The factors responsible for negative intrapleural pressure are:

Elastic recoil: Due to presence of elastic fibres the lung tissue has a continuous tendency to recoil away from the chest wall. The tendency naturally increases during inspiration with inflation of the alveoli.

Surface tension of the intra-alveolar fluid: Due to intra-molecular attraction of the surface layer of the fluid lining the alveoli, they have got a tendency to collapse. This collapsing force of the millions of the alveoli

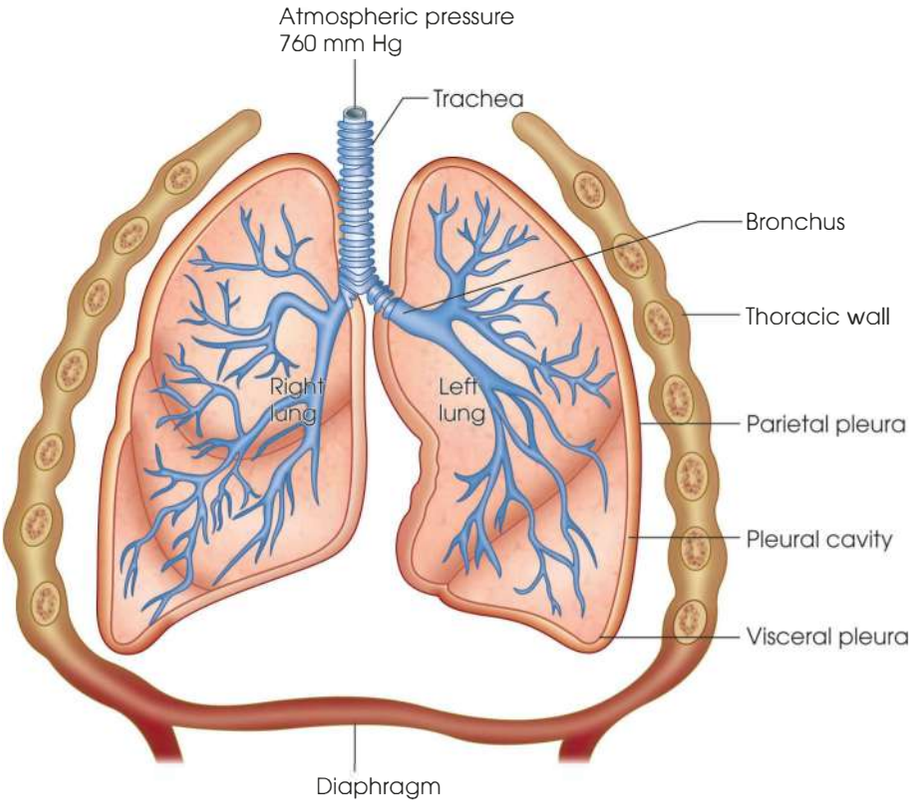


Fig. 42.4: Pleural cavity (diagrammatic representation)

produces a summated effect resulting in tendency of the whole lung to recoil away from the chest wall.

In fact, about two-thirds of the recoil tendency of the lungs is attributable to the surface tension phenomenon.

Surface Tension at the Fluid–Air Interface within the Alveoli and the Role of Surfactant

1. It was observed by von Neergaard that the pressure required to inflate the air-filled lungs was higher than when it was filled with normal saline. Alveoli are minute spherical bodies, not necessarily of the equal size, lined by the thin layer of fluid and filled with air.
2. The surface tension at the liquid air interface is high and prevents, its expansion whereas in lungs filled with physiological saline the surface tension is absent so that they expand readily.

   In a spherical bubble, the tension of its wall (T) tends to collapse the bubble whereas the pressure of air within (P) tends to expand it. The relationship between these two opposing forces in equilibrium is given by the equation:

   $P = 2 \times T/r$ where r is the radius of the bubble. Naturally, large bubbles (alveoli in this context) have got lower tension than smaller ones and if communication exists between the two the smaller bubbles (alveoli) will empty into the larger ones.
3. Further as the alveoli become smaller during expiration, the surface tension (T) increases and tends to collapse the alveoli. This is prevented by surfactant because of its surface tension reducing properties.
4. Alveolar surfactant is a lipoprotein with dipalmityl lecithin as an important component. It is secreted by the lamellar bodies of the great alveolar cells lining the alveoli. This substance forms a lining for the interior of the alveoli and increases their surface tension during expansion, i.e. inspiration and decreases their surface tension during expiration. Surfactant, therefore, not only prevents collapse of the alveoli during expiration but also prevents emptying of smaller alveoli into larger ones, thus ensuring stabilising effect on the respiratory process.

Pressure Changes in the Pleural Cavity and its Relation to Volume Changes in the Lungs

In reference to pressure changes in the pleural cavity and volume changes in the lungs it has already been mentioned that; the intra-thoracic pressure (intra-pleural pressure) at the resting stages is slightly negative (2.5 mm Hg).

1. With the enlargement of the thoracic cage in all its diameters during inspiration, the intra-pleural pressure becomes still more negative and at the end of inspiration in quiet breathing becomes about–6 mm Hg. This inspiratory increase in negative pressure in the pleural cavity is reflected in the pressure within the lungs (intra-pulmonary pressure) which normally is euqal to atmospheric pressure

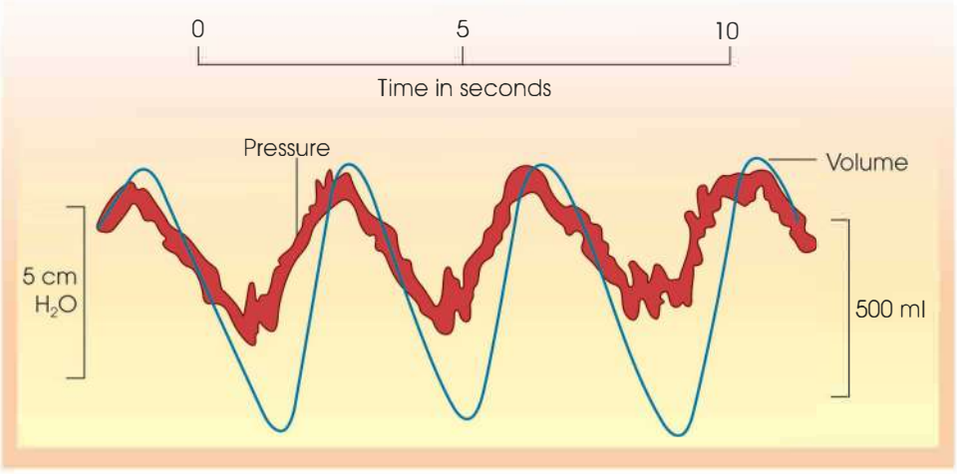


Fig. 42.5: A simultaneous tracing of tidal volume and intra-oesophageal pressure in a normal resting subject

(normal = 760 mm Hg) at rest but falls to about–1 mm Hg below atmospheric pressure (i.e. 759 mm Hg at the end of inspiration. Air, therefore, rushes in from the atmosphere to the lungs causing inflation of the lungs during inspiration. Negative intra-pleural pressure is thus primarily responsible for inspiratory inflow of air into the lungs. It is also responsible for keeping the patency of the airways.

2. Expiration is usually a passive process due to relaxation of the inspiratory muscles. The intra-pleural pressure rises to its resting value with the diminution in size of the thoracic cage, the lung collapses and the intra-pulmonary pressure rises above the atmospheric pressure till at the end-expiratory resting stage it becomes equal to the atmosphere.
3. In forced inspiration and expiration, the pressure variations in pleura and the lungs are considerably exaggerated. In forced expiration with closed glottis, the intra-pulmonary pressure may go up to + 40 mm Hg. It is possible to record intra-pleural pressure with a fine polythene tube attached to a thin-walled balloon lying in the lower-third of the oesophagus. The diagram shows intrapleural pressure tracing synchronous with volume tracing during respiratory cycle in a normal subject (Fig. 42.5).

Note

The time lag between volume tracing and pressure tracing. The pressure change occur fraction of a second earlier than the volume changes. The undulations on the pressure tracing with intraoesophageal balloon are due to pressure variations resulting from heart beat.

EXAM-ORIENTED QUESTIONS

Essay

1. Discuss the respiratory and non-respiratory functions of lungs.

Short Notes

1. Functions of respiratory tract
2. Mechanism of breathing
3. Intra-pleural pressure
4. Respiratory units

Chapter

43

Mechanics of Breathing

INTRODUCTION

The expansion of the lungs during inspiration follows the enlargement of the thoracic cavity in all its diameters by the contraction of the respiratory muscles. These are mentioned below.

Diaphragm

A dome-shaped muscle separates the abdominal wall from the thoracic cavity, with vertically running fibres at the periphery and horizontally running fibres at the centre ending in the central tendon. It is innervated by phrenic nerves (C3, C4 and C5). The muscle is attached behind to fixed structures like vertebrae; and by front to the sternum and is attached to mobile structures like the lower ribs along the sides.

Mechanism of Inspiration

1. It commences by contraction of the diaphragm; thereby the central tendon of which moves downwards increasing the vertical diameter of the chest.
2. At the same time the lower ribs are moved somewhat upwards increasing the lateral and anteroposterior diameter of the chest.
3. The descent of diaphragm is accompanied by displacement of abdominal contents downwards associated with relaxation of the abdominal muscles. It has been estimated that during quiet breathing the diaphragm is displaced downwards by about 1.5 cm and the area of the diaphragm being 350 sq cm the whole volume of inspired air during a single quiet breathing 500 ml can be accommodated by downward movement of the diaphragm only (Fig. 43.1).

Mechanism of Expiration

During expiration the diaphragm relaxes, its dome moves upwards aided by contraction of the abdominal muscles pushing the diaphragm upwards. This results in expulsion of air from the lungs during expiration (Fig. 43.1).

Intercostal Muscles

The normal breathing is predominantly diaphragmatic.

Key Points

1. The relative proportions between diaphragmatic and intercostal contribution perhaps vary with different age groups and among different individuals. For example, the infant's ribs are commonly horizontal. Both the external and intercoastal groups of muscle fibres originate and insert on adjacent ribs.
2. The muscle fibres of the external intercostal group run forwards and downwards and those of the internal intercostal group run backwards and downwards from rib to rib (Fig. 43.3).
3. The cephalad motion of the ribs indicates that the upper ribs are fixed relative to the lower ones. When the contraction of the abdominal muscle fibres fixes the lower ribs during cough or forced inspiration the rib cage moves downwards.
4. These intercostal muscle fibres are innervated by intercostal nerves (T_1 to T_{11}). Electromyography studies depict that the intercostal muscles become active during quiet inspiration, forced inspiration and forced expiration. Normal quiet expiration is due to passive relaxation of the intercostal muscles and is not attended with any electrical variation.

Motion of the Ribs during Inspiration and Expiration

During inspiration: The ribs are almost semicircular bones which articulate with the vertebrae at one end and with the sternum or coastal cartilages at the other end. During inspiration the sternum is elevated and pushed forwards due to the elevation of the shaft of the ribs while the sternal ends are lower than the vertebral ends during expiration. This is caused due to the contraction of external intercostal muscles and results in an increase in the anteroposterior diameter of the chest.

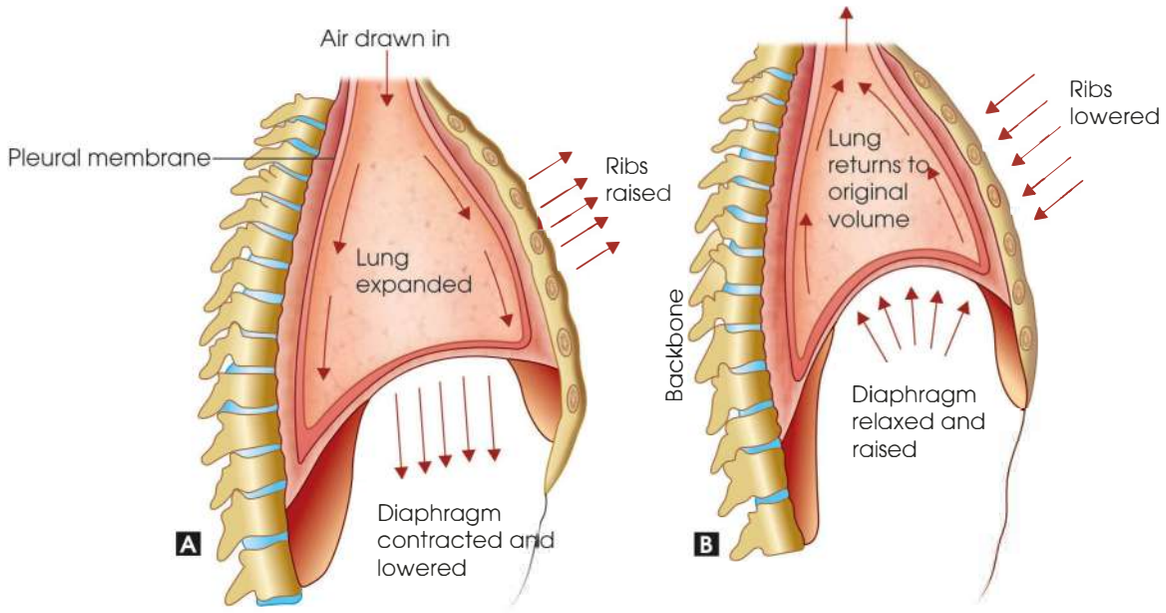


Fig. 43.1A and B: Position of the diaphragm (from the side) during active inhalation of air (inspiration) in (A) and passive inhalation of air (expiration) in (B)

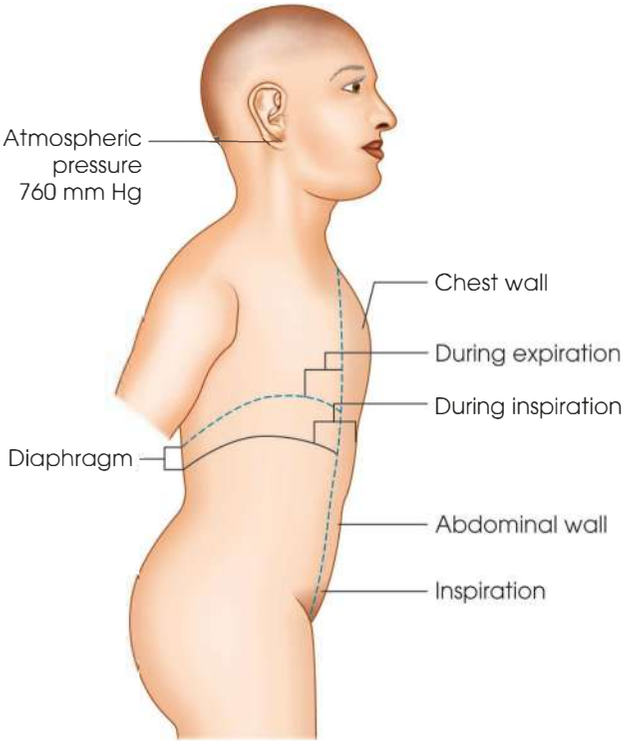


Fig. 43.2: The changes in position of the abdominal wall and the diaphragm

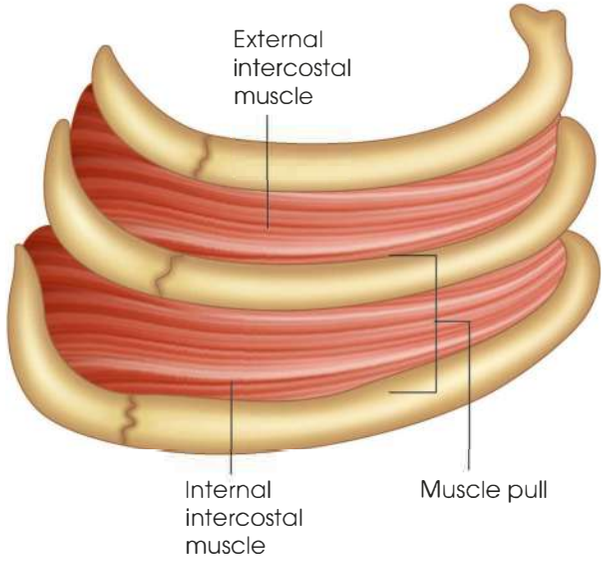


Fig. 43.3: Diagram showing action of external and internal intercostal muscles during respiration

Bucket handle motion: Rib motion includes the rotation of the ribs around an axis joining two points of attachment at the vertebrae and the sternum (bucket handle motion). This rotation causes an increase in transverse diameter of the thorax (Fig. 43.5).

During expiration: Depression of the shaft of the ribs during expiration causes the opposite effect (Fig. 43.4 and 43.5). During expiration the intercostal muscles which are responsible for movement of the ribs and sternum as described above relaxes, the size of the thorax decreases in anteroposterior and in transverse diameter and the lung volume consequently decreases. The changes in position of the diaphragm and thorax during inspiration and expiration are represented diagrammatically in the model as shown in Fig. 43.1 and 43.2.

Key Points

1. The rib motion is not equal throughout the thorax. The upper ribs are more nearly horizontal than the lower ribs and are arcs of smaller circles.

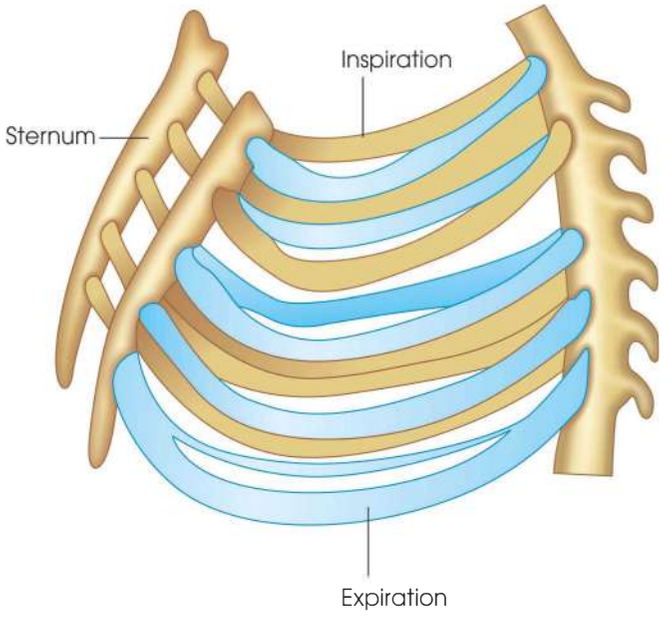


Fig. 43.4: Positions of ribs and the sternum (schematic representation)

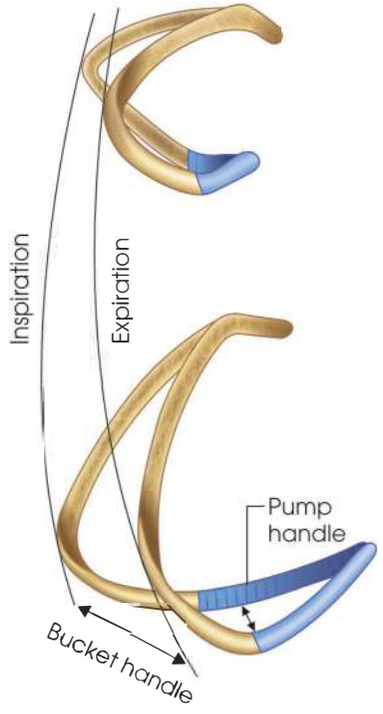


Fig. 43.5: Two kinds of movements of the ribs in respiration; pump handle in which the anterior end of the downward sloping rib is raised and bucket handle in which the middle of the rib is moved upwards and outwards about an anterior–posterior axis

2. In inspiration the lower ribs are affected by displacement of all the superior ribs. Their motion is somehow, the sum of the narrowing of many interspaces rather than only the adjacent one. Due to such differences, the changes in the lateral diameter of the thorax are greater in the lower chest than in the upper chest.
3. The contribution of costal motion to breathing depends on the force of intercostal contraction and on the position of the ribs prior to the intercostal contraction.

Accessory Muscles of Respiration

During forced inspiration and expiration many other muscles besides the diaphragm and intercostal muscles come into play to assist respiration. Most important of this group are the scalene and sternocleidomastoids of the anterior neck muscles which stabilise the first ribs and upper sternum during forced inspiration. The abdominal muscles contract violently during forced expiration and thus act as accessory muscles. During violent respiratory effort even the platysma and all other muscles of the trunk take part in respiration.

RESISTANCE TO BREATHING

The air as it enters the lungs meets with two types of resistances; (a) elastic resistance and (b) viscous resistance.

Elastic Resistance

Elastic resistance is exerted by the elastic tissue of the lung parenchyma and surface tension of intra-alveolar fluid. Elastic tissues of the lungs obey Hooke's law which means that change in volume of lungs is directly proportional to the force applied that is to intra-pleural pressure.

Lung compliance has been defined as the change in lung volume in litre per centimetre change of H_2O pressure. This is measured under static conditions to avoid frictional resistance to air flow which occurs during movement of air. After a known volume of air has been inspired and respiratory movement is 'zero'—the change in intra-pleural pressure is noted. The change in lung volume (Δv) divided by change in pressure (ΔP) is the measure of the lung compliance or stretchability.

Normal value ranges from 0.09 to 0.26 L/cm H_2O. Diminution in the lung compliance indicates increased rigidity of the lungs. The lung compliance decreases in fibrosis, hydrothorax, pneumothorax, chronic restrictive lung diseases, etc. The increased lung compliance is seen in emphysema, a chronic obstructive lung disease due to diminished elasticity of collagen and lung tissue.

Viscous or Non-elastic Resistance

This is the resistance offered to the air flow into the lungs due to friction between moving column of air and the branching bronchial tubes which get narrower and narrower at each branching (airway resistance) and also the frictional resistance between the muscle fasciculi of the chest wall and tissues. Unlike the elastic resistance which is to be measured only when air flow

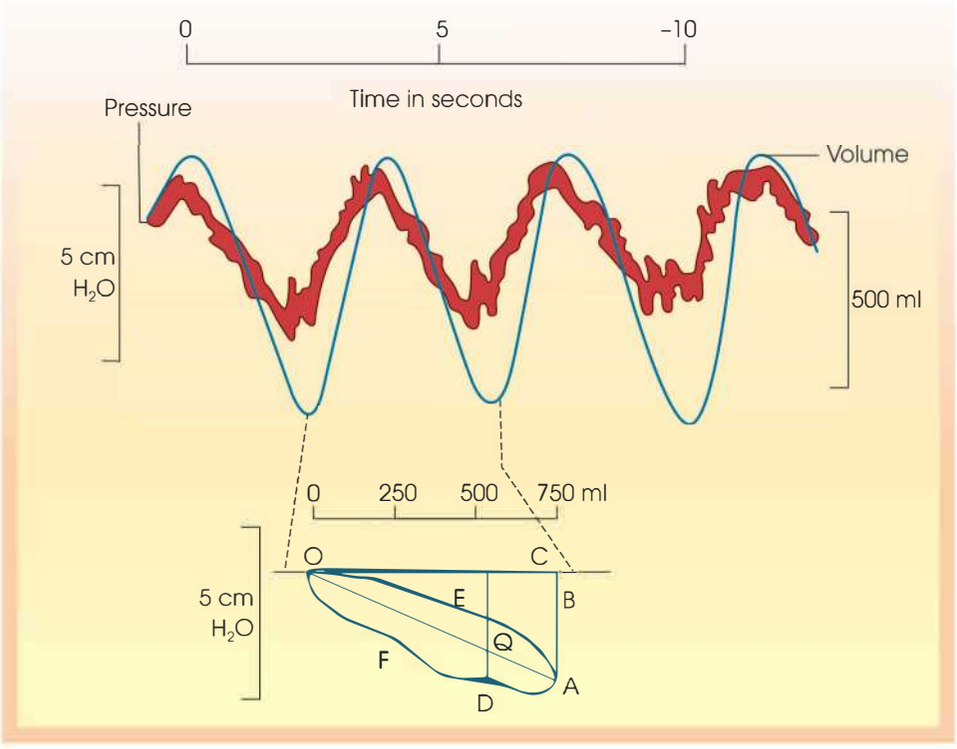


Fig. 43.6: The intra-pleural pressure is recorded by an intra-oesophageal balloon. Note that (i) the fall in intra-pleural pressure occurs fraction of a second earlier than change in volume (i.e. inspiration). (ii) The small waves on the pressure tracing are due to variation of intra-pleural pressure from heartbeat

is 'zero' frictional resistance comes into play during air flow and can be measured only during active air flow. It is also to be noted that the flow of air in the bronchial system is turbulent and the turbulence increases during Broncho-constriction. Turbulence is responsible for increase in viscous resistance—the relationship between the 'resistance' and 'rate of flow', i.e. velocity is given below: The relationship between frictional resistance (R) and velocity (V) of air flow is given by the formula:

$$R = K \times V$$

where K is a density dependent constant and 'V' is the velocity of air flow. Note that if the velocity of air-flow is doubled the resistance increases 4 times. Typical asthma patient breaths slowly and any attempt to breath rapidly increases their distress.

The diagram (Fig. 43.6) has been constructed by integration of the volume of air moved with that of intrapleural pressure from moment to moment during one respiratory cycle.

Line AO of the 'ellipse' OFAE represents elastic resistance. The elliptical nature of the pressure–volume curve is due to frictional resistance to air flow. The area OFAB is the total work of inspiration. The area OAB is work done against elastic forces. Area OFA is work against viscous resistance during inspiration.

The energy OAB stored in the lungs during inspiration is available to do the work OAE necessary to overcome the frictional resistance during expiration. The pressure exerted against 'viscous' or non-elastic resistance (DQ) is obtained by subtracting the elastic pressure (CQ) from the total pressure (CD) exerted at any point of the respiratory cycle. At the point C this is:

$$CD–CQ = DQ$$

Work of Lungs

It can be calculated that the lungs perform work of the order of 0.4 kg-m per minute during quiet breathing of which 60% is spent to overcome the elastic resistance and about 40% is spent to overcome the non-elastic or viscous resistance.

EXAM-ORIENTED QUESTIONS

Essay

1. Describe the mechanism of respiration.

Short Notes

1. Muscles of respiration
2. Elastic and non-elastic resistance in lungs
3. Accessory muscles of respiration

Chapter

44

Pulmonary Volumes and Capacities (Spirometry)

INTRODUCTION

Ventilation (the business of getting gas to and from the lung alveoli) constitutes the first part of respiratory process and ventilation has the dimensions of both volume and time. Ventilation occurs as a result of a pressure difference between the alveolar and oral ends of the airways. Ventilation can be measured with a spirometer which consists of an inverted bell-jar over a double-walled chamber with water in-between the two walls as shown in Fig. 44.1. The bell-jar is attached to counterweight and to a writing point which writes over a moving kymograph (recording) drum. The subject breaths in and out from the air enclosed on the surface of water below the bell-jar and the respiratory movement can be recorded on the moving drum and its various components may be analysed.

Normal ventilation in an adult (75 kg) is about 6 litres per minute with a respiratory rate of 12 breaths per minute and a tidal volume of 500 ml. In a resting day-old infant about 25 kg, normal ventilation is about 500 ml per minute at a respiratory rate of 33 breaths per minute and a tidal volume of 15 ml. Thus, an adult ventilates about 100 ml/minute/kg body weight

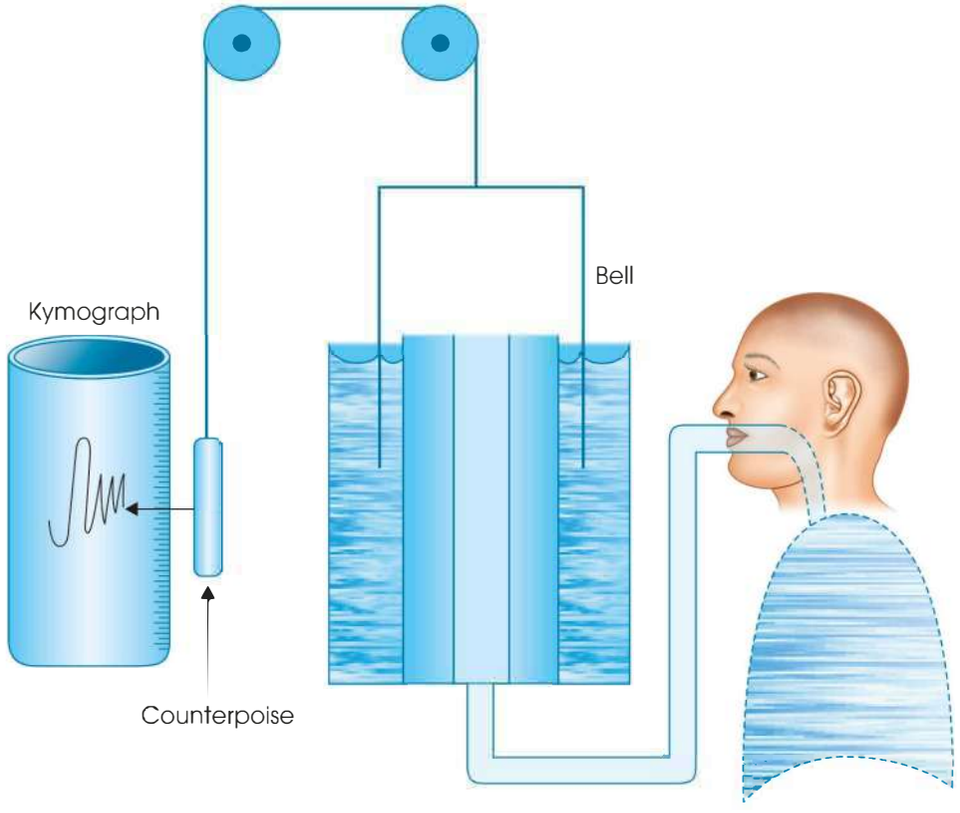


Fig. 44.1: Recording spirometer. Inspiration causes the spirometer bell to descend and the writing lever to move upwards on the chart. The chart is calibrated so that measurements of lung volume change can be made

whereas an infant ventilates two times more than adult's breaths.

Pulmonary ventilation = Tidal volume × Respiratory rate

Air in lungs may be sub-divided (Fig. 44.2) at different points into four different volumes and four different capacities. These are defined below.

LUNG VOLUMES

Tidal Volume (TV = 500 ml)

The TV (or resting tidal volume, RTV) is the volume of air breathed in and out during quiet respiration (about 500 ml).

Respiratory Minute Volume (RMV)

By definition RMV can be obtained by multiplying tidal volume by respiratory rate per minute and is approximately equal to 500 ml × 12 = 6 L/min. The value of course may vary widely and is increased considerably during exercise when both the tidal volume as well as respiratory rates is increased. At rapid rates of breathing, a person usually cannot sustain a tidal volume more than about one-half of his vital capacity.

Inspiratory Reserve Volume (IRV)

The air that can be breathed in by maximum inspiratory effort after an ordinary inspiration is the inspiratory reserve volume. It amounts to 2.0 to 3.3 L.

Expiratory Reserve Volume (ERV)

The air that can be breathed out by maximum expiratory effort after an ordinary expiration (about 1 L) is the expiratory reserve volume.

Residual Volume (RV)

The RV is the amount of air which remains in the lungs after maximal expiration. It can only be expelled out from the lungs by opening the chest and allowing the lungs to collapse (average 1.2 L). The volume of air which enters the alveoli with each breath is equal to the TV minus the dead space volume.

LUNG CAPACITIES

Inspiratory Capacity (IC)

The maximum volume of air that can be inspired from the end-expiratory position is the inspiratory capacity. IC = TV + IRV = about 3.5 L.

Functional Residual Capacity (FRC)

The volume of air remaining in the lungs after a quiet expiration is the functional residual capacity. FRC = RV + ERV (about 2.2 L).

Total Lung Capacity (TLC)

The volume of air that the lung can hold after a maximum possible inspiration is the total lung capacity. TLC = IC + FRC (about 5.7 L).

Vital Capacity (VC)

It is the volume of air that can be breathed out by maximal expiratory effort after a maximum inspiration. By definition it amounts to IC + ERV = (3.5 + 1) litres = about 4.5 L. Vital capacity in normal adult is in-between 3 and 5 L. The exact amount of vital capacity depends on age, sex and size of the individual. It also shows a racial variation.

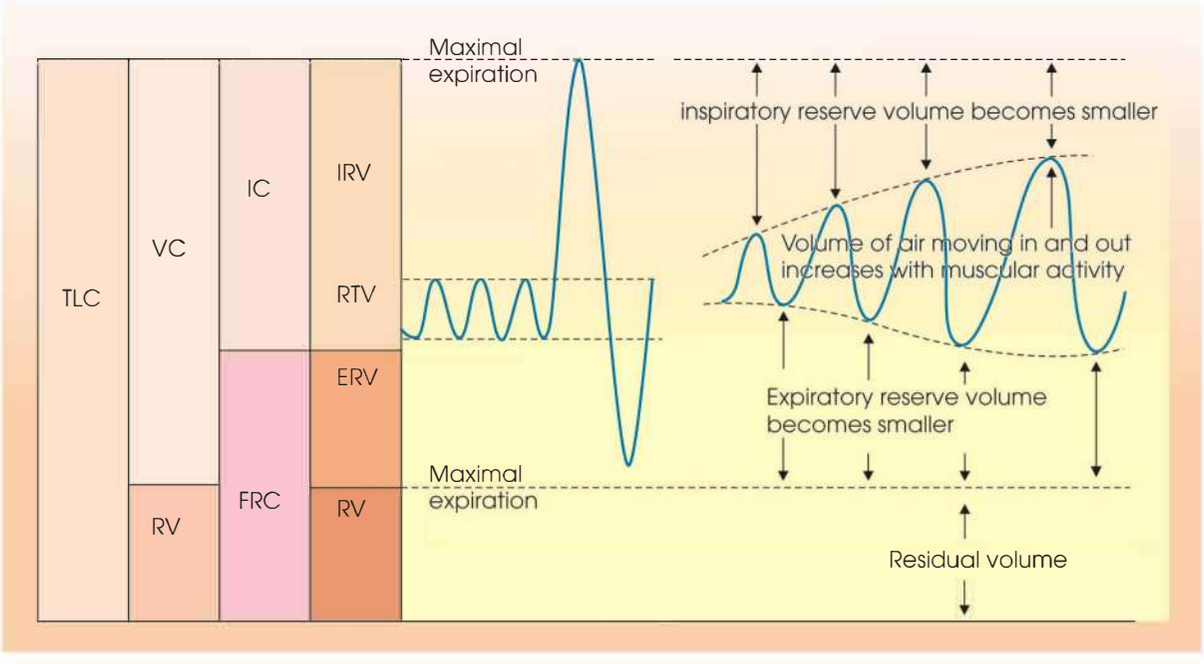


Fig. 44.2: A schematic representation of the subdivisions of lung volumes and capacities obtained by a spirometer

The causes for variation in vital capacity are as follows:

1. Vital capacity diminishes with age and is always lower by more than 10% in old people.
2. Vital capacity is higher by 30–40% in the athletes compared to the subjects with sedentary disposition.
3. Vital capacity is less in supine position than in standing position because the intra-thoracic blood volume diminishes in standing posture, and the diaphragm can move downwards more easily than in supine position.
4. Vital capacity diminishes in conditions associated with weakness of the muscles of respiration, or when the movement of the thoracic cage is restricted or in space occupying defects of the chest. Increased rigidity of the lungs (diminished compliance) as may occur during pulmonary congestion, emphysema, chronic asthma or bronchitis will also cause diminished vital capacity.

Significance of vital capacity as a respiratory function test is indeed limited because it is a static test for lung function. However, day-to-day assessment of vital capacity say in a patient with paralysis of respiratory muscles (respiratory poliomyelitis) is of prognostic significance.

Maximum breathing capacity (MBC): It is also called as maximum ventilation volume (MVV). The maximum amount of air that can be inhaled or exhaled within one minute is the maximum voluntary ventilation (MVV). It should be preferably carried out for 15-second time period and then extrapolated to a value for one minute expressed as litres/minute. Average values for males and females are 140–180 and 80–120 L per minute respectively. The determination of maximum breathing capacity (MBA) is an exacting procedure and it is the often not possible to perform it on debilitated patients.

Forced Expiratory Volume (FEV)

It is the volume of air, forcibly breathed out after a deep inspiration in a given time. Useful information regarding respiratory status of a patient can be obtained by analysis of a single forced expiration after a maximum inspiration on a rapidly moving kymograph. A normal subject can expire 80% or more of his vital capacity in the first second (i.e. FEV1%). Patients with obstructive disease of lung will expire proportionately much less within the first second (i.e. FEV1%) of expiration (Fig. 44.3).

Functional Residual Capacity (FRC)

During quiet breathing the pulmonary ventilation is achieved almost entirely by muscles of inspiration. The end-expiratory position of the lungs represents the 'resting position' during which all the muscles relaxed. As mentioned earlier, the volume of air remaining in the lungs at this level represents 'functional residual capacity' and measures about 2300 ml in an adult male. It provides the air in the alveoli to aerate the blood between the two consecutive respiratory acts, thus preventing marked rise all in the concentration O_2 and CO_2 in the intervals of respiration. Functional residual capacity (FRC) and functional residual volume (FRV) increase in all conditions associated with over-distension of the lungs, e.g. in emphysema. Quantitative measurement of residual volume and functional residual capacity is not possible by spirometer. Some of the special techniques employed for this purpose are described below:

a. **N_2 wash-out technique:** The man inspires pure O_2 (N_2 free) and expires into a Douglas bag for a 7-minute period. During this period all the N_2 present in his lungs is washed away by inspired O_2. The total volume of expired air in the Douglas bag can be determined by a gas-meter. The volume of expired air multiplied by N_2% gives the quantity of N_2 washed off from the depth of the lungs. Since the lung air contains about 80% of N_2—the volume of functional residual air can be easily calculated.

   Example: Suppose the volume of expired air over the 7-minute period = 50,000 ml and the concentration of nitrogen in the collected gas is 4%.

   Total quantity of N_2 washed off from lungs is: 50,000 × 0.04 = 2000 ml. Therefore the functional residual capacity

$$\frac{2000 \times 100}{80} = 2500 \text{ ml}$$

b. **Body plethysmograph** (Fig. 44.4)**:** It is simply as air-tight tank in which the subject is placed. He inspires the air within the tank and then expires forcefully against a manometer. The expiratory pressure is recorded. The rise of pressure within the chest decreases the volume of gas in the lungs (Boyle's law) and the volume of the chest also decreases. Thus, the subject's body occupies less space within the plethysmograph. A volume recorder records the amount of the reduction which is equal to the degree of compression of gases in the lungs which together with pressure recorded will give the volume of gas in the lungs. Inspiratory capacity is then recorded and deducted from the former value to give functional residual volume (FRV).

Spirometry (Fig. 44.5)

The spirometry test is conducted to evaluate pulmonary functions. The test can be performed using spirometer. The graphs displayed as spirograms of computerised pulmonary function profile show a volume-time curve represented by time (seconds) along the X-axis and lung

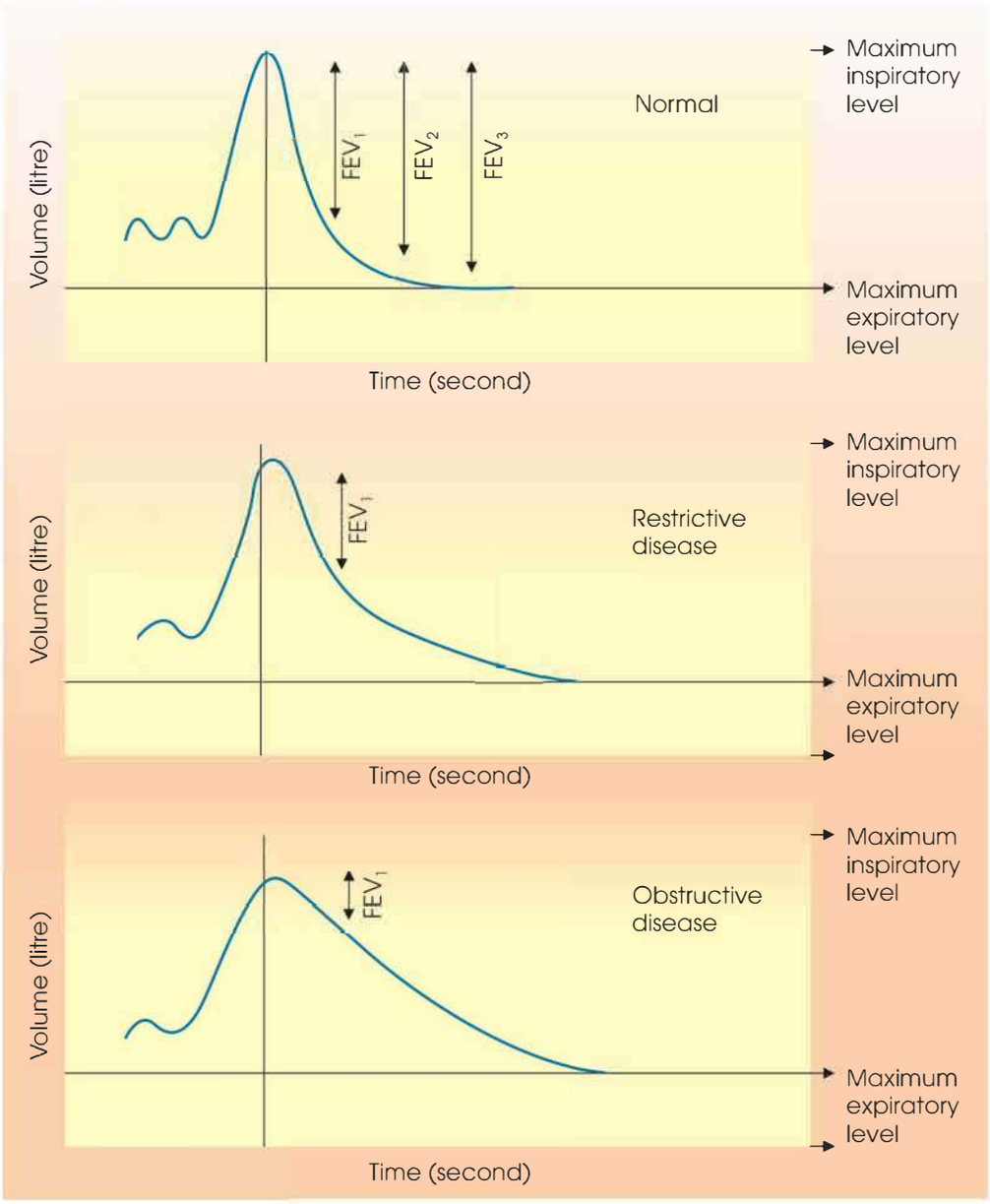


Fig. 44.3: Forced expiratory volume

volume (litres) along the Y-axis. Similarly, a flow-volume loop earmarks airflow on the Y-axis and the total volume of air on the X-axis.

Procedure: The patient is accommodated to understand the manoeuvre of performing the test and way of exhaling air into the sensor. The patient is then asked to take the deepest breath, and then asked to exhale into the sensor completely and forcefully for. Soft nose clips are applied and may be used to prevent air escaping through the nose. The computerized readings are obtained for lung capacity.

Limitations of test: The manoeuvres are dependent on patient cooperation and effort. As results are dependent on patient cooperation, values may be underestimated in uncooperative patient. Moreover the test cannot be deployed in unconscious or heavily sedated patients.

Indications for pulmonary function test are: It help in diagnosis of various functional abnormalities and help in ascertaining whether the disease is obstructive or restrictive type, identify the probable cause of respiratory signs and symptoms, screening of unsuspected respiratory ailment, to verify effectiveness of bronchodilator treatment, and to assess ventilatory functions and gaseous exchange across lung.

The abbreviations, those are commonly used in the spirometric measurement, are given in Table 44.1.

The most common parameters measured in computerized spirometry are vital capacity (VC), forced vital capacity (FVC), forced expiratory volume (FEV) at timed intervals of 0.5, 1.0 (FEV_1), 2.0, and 3.0 seconds, forced expiratory flow 25–75% (FEF 25–75%) and maximal voluntary ventilation (MVV).

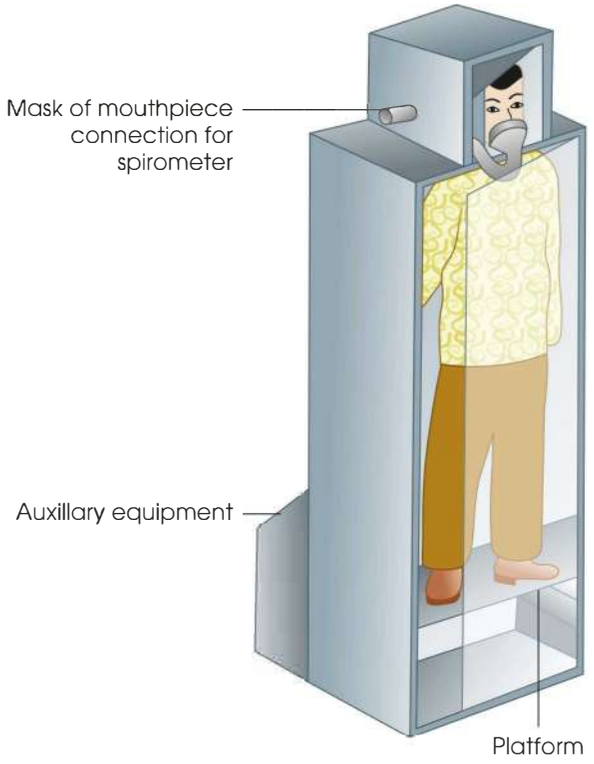


Fig. 44.4: Body plethysmograph in which an air-tight box fitted with devices for measuring different parameter of 'lung volume' airways resistance and pulmonary blood flow

Fig. 44.5: Computerised spirometer

Forced Vital Capacity (FVC)

Forced vital capacity (FVC): It is the volume of air that can forcibly exhale out after full inspiration. It is expressed in litres.

Forced Expiratory Volume in 1 (FEV_1) Second

It is the volume of air that can be forcibly exhaled out in one second, after full inspiration. The FEV_1 value in healthy subject depends primarily on sex and age, in normal individuals the FEV_1 value between 80% and 120% of the average value are observed.

FEV_1/FVC Ratio (FEV_1%)

It is the ratio of FEV_1 to FVC. The FEV_1% is observed to be less than 80% in obstructive lung diseases. The common causes of obstructive diseases are bronchial asthma, COPD, chronic bronchitis and emphysema. The decreased value of FEV_1 is due to increased airway resistance to expiratory flow. The premature closure of airway in expiration may lead to decreased FVC. The decreased value of FEV_1 leads to decreased FEV_1%.

Forced Expiratory Flow (FEF)

It is the flow speed of air during the mid-portion of a forced expiration. It is reported at usual intervals 25%, 50% and 75% of FVC (FEF 25%, FEF 50% and FEF 75%). The FEF 25–75% value is generally noted for interpretation of forced expiratory flow. The FEF 25–75% value is the mean of the flow during an interval at specified fractions of FVC, e.g. FEF 25–75%. The maximal mid-expiratory flow (MMEF) is the peak of expiratory flow derived out of the flow-volume curve and measured in litres per second.

Peak Expiratory Flow (PEF)

It is the maximal flow speed of air achieved during the maximally forced expiration initiated at full inspiration. It is expressed as litres per minute. It measures the airflow through the bronchi. It indicates the degree of obstruction in the airways. It is measured with help of peak flow meter. The normal expected value depends on gender, age and height of an individual. It is decreased in obstructive lung disorders, such as asthma, COPD or cystic fibrosis.

Breathing Reserve (BR)

Breathing reserve = MBV (maximum breathing capacity) – RMV (respiratory minute volume) = about 136 L/min. When the breathing reserve falls to 60–70% of MBC dyspnoea will result.

APPLIED PHYSIOLOGY: OBSTRUCTIVE AND RESTRICTIVE LUNG DISEASE

Obstructive lung diseases are due to airway obstruction and there is restricted expiration. FEV_1% is the ratio of FEV_1 to FVC. The FEV_1% is observed to be less than 80% in obstructive lung diseases. The common causes of obstructive diseases are bronchial asthma, COPD, chronic bronchitis and emphysema. The decreased value of FEV_1 is due to increased airway resistance to expiratory flow. The premature closure of airway in expiration may lead to decreased FVC. The decreased value of FEV_1 and FVC thereby decreases the FEV_1%.

Restrictive lung diseases are due to restricted lung expansion and there is restricted inspiration and expiration. In restrictive diseases the FEV_1 and FVC are both reduced proportionally and the FEV_1/FVC (FEV_1%) value may be normal or even increased as a result of

Table 44.1: Abbreviations commonly used in the spirometric measurement

Terms	*Symbols*	*Descriptions*
Vital capacity	TC	Maximal volume of air exhaled after forced inspiration (includes TV, IRV and ERV)
Tidal volume	TV	Volume of air inhaled or exhaled during quiet breathing
Inspiratory reserve volume	IRV	Maximal air that can be inhaled after a quiet inspiration
Expiratory reserve volume	ERV	Maximal air that can be breathed out after quiet expiration
Residual volume	RV	Volume of air remaining in lungs after full expiration
Inspiratory vital capacity	IVC	Maximal volume of air inhaled after full expiration
Forced expiratory volume per time interval in seconds	FEV	Volume of air exhaled in a given period during a complete forced expiration (FVC)
Maximal expiratory flow rate	MEFR	Volume of air exhaled per second measured between 230 ml and 1,200 ml volumes of the forced expiratory spirogram
Maximal mid-expiratory flow	MMEF	Volume of air per second exhaled during middle half of expired volume of forced expiratory spirogram
Maximal voluntary ventilation	MVV	Maximum breathing capacity—litre/minute. Subject can breathe with maximal voluntary effort (actual measurement for twelve seconds)

decreased lung compliance. The common restrictive lung diseases are interstitial diseases (pneumonia), fibrosis of lungs (asbestosis, silicosis), pneumothorax, malformations (kyphosis, scoliosis) and fracture.

EXAM-ORIENTED QUESTIONS

Essay

1. Describe the lung volume and capacity. Draw a well-labeled diagram depicting lung volumes.

Short Notes

1. Tidal volume
2. Inspiratory reserve volume
3. Expiratory reserve volume
4. Functional residual capacity
5. Maximum breathing capacity
6. Forced expiratory volume in 1 second
7. Forced expiratory volume %
8. Obstructive and restrictive lung disorder

Chapter

45

Alveolar Ventilation and Gases Exchange in Lung

INTRODUCTION

Respiratory 'Dead Space'

The respiratory tract is so designed that during inspiration its upper part up to the level of terminal bronchiole is filled with atmospheric air. The first part of the expired air, therefore, is also atmospheric air from the non-respiratory part of the lungs, the second part is a mixture in which the percentage of atmospheric air gradually decreases and that of the alveolar air from the respiratory part of the lungs gradually increases and the last part of the expired air is pure alveolar air.

Definition

The air which remains confined in the upper respiratory tract with each inspiration and is not available for gaseous interchange constitutes what is known as 'dead space air'.

Anatomical dead space: The air which does reach the gaseous exchange area but remains in the respiratory passage such as nose, pharynx and trachea amounts for the anatomical dead space. It amounts roughly to 150 ml. It increases marginally with ageing.

Physiological dead space: In an ideal lung all the alveoli are evenly ventilated and adequately perfused with exactly the required amount of blood. But such an ideal condition is seldom obtained. In fact, in normal subjects the apical alveoli are ventilated adequately but have got a poor blood supply. Most of the ventilation in these alveoli, therefore, is wasted and produced partial dead space effect and attribute for the physiological dead space.

In diseased conditions the situation may be worse—there may be many alveoli which are hyperventilated but have very poor blood flow. These are, therefore, partially dead space areas.

Measurement of Dead Space Volume

The Fowler's method is deployed to measure the anatomical dead space using nitrogen washout technique.

Nitrogen Meter Method

The subject takes a deep breath of oxygen and then expires through a N_2 meter which records graphically the percentage of N_2 in the expired air from moment to moment. The first part of expired air contains 'zero' % of N_2 because it is pure O_2 from dead space. The second part of the curve shows rising N_2 concentration because it is a mixture of O_2 from the dead space and N_2 from alveolar air. In the last part of the tracing the N_2 percentage level off and remains more or less steady because it is pure alveolar air and in an ideal lung all the alveoli are contracting synchronously unloading their N_2 content in the expired air at a uniform rate. In Fig. 45.1, the steady N_2 level is shown to be at 60%.

If the total volume of expired air be 500 ml and the dotted area represents the alveolar air and the hatched area be dead space air:

$$\text{The dead space} = \frac{\text{Area of hatching} \times \text{Total air expired}}{\text{Area of dots} + \text{Area of hatching}}$$

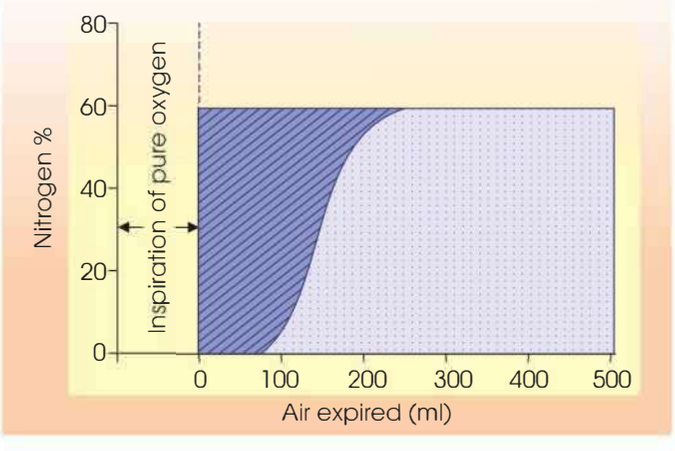


Fig. 45.1: Continuous tracing of the changes in the nitrogen concentration in expired air following a pure inspiration of O_2. This record is used in calculating dead space

Let us assume that the area of dots = 70 sq cm and the area of hating = 30 sq cm. Since the total volume of expired air = 500 ml, the dead space air

$$= \frac{30}{30+70} \times 500 \text{ or } 150 \text{ ml}$$

In healthy individual all the alveoli are functional and thus the anatomical and physiological dead space are almost equal. In case of partially functional or non-functional alveoli the physiological dead space will be ten times the anatomical dead space and may amount to 1–2 litres.

INTRAPULMONARY GAS-MIXING OR EVEN DISTRIBUTION OF INSPIRED AIR

The inspired air enters the alveoli in the form of a cone. At the time of phase reversal, vibrations are set up in the wall of the alveoli due to elastic recoil and cause the inspired air to be evenly distributed throughout the mass of alveolar air so that gaseous inter-changement may take place satisfactorily. In diseased conditions associated with loss of elasticity there occur uneven distributions of inspired air which may lead to impairment of respiration.

Methods of Detection of Uneven Ventilation

Uneven ventilation can be detected with N_2 meter.

1. In a subject with ideal lungs the N_2 concentration in the expired air soon reaches a steady level and in the example cited it was 60% after the dead space air was cleared off.
2. Patients with alveoli not contracting synchronously due to loss of elasticity in many of them will show a rising phase of N_2 concentration in the expired air instead of a horizontal line.
3. The difference in N_2 concentration between expired volume of 750 ml and 1250 ml should not exceed 1% in normal subjects whereas in emphysema patients the difference is much higher between these two levels of expired ventilation (Fig. 45.2).
4. It was mentioned previously that breathing pure O_2 for a 7-minute period washes off all the N_2 from the alveolar air and that advantage is taken of this fact in estimation of FRV of the lungs.

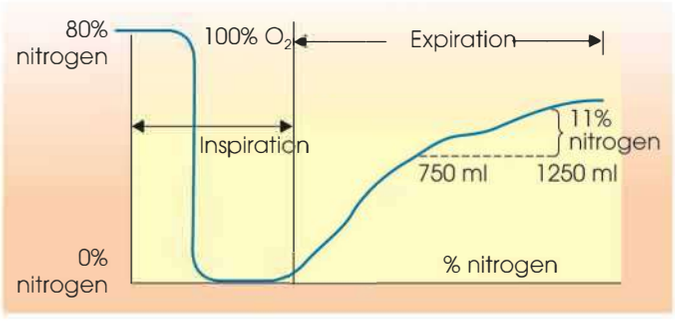


Fig. 45.2: N_2 meter record in an abnormal subject measuring the single breath

5. In a patient with uneven ventilation, N_2 remains confined here and there in the diseased alveoli which have lost their elasticity to varying degree. In such cases, even after O_2 breathing for a 7-minute period, the air on forced expiration will contain 2% or more of N_2 indicating defective intra-pulmonary gas mixing.

The increase in nitrogen concentration is measured for a 500 ml sample of the alveolar gas.

ALVEOLAR AIR

Alveolar air represents the air located in the respiratory part of the lungs which take part in gas exchange with the blood in the pulmonary capillaries. Alveolar air, therefore, is a physiological quantity and does not represent the air located strictly in the anatomical alveoli.

It assures about 3000 ml and is the most important part of the air in the respiratory system since it is primarily responsible for oxygenation of venous blood and unloading the venous blood of adequate quantities of CO_2. With a tidal volume of 500 ml; about 350 ml of oxygen-rich atmospheric air mixes with the alveolar air to replenish the oxygen lost from alveolar air by absorption with the venous blood.

Method of Collection of Alveolar Air

Haldane and Priestley's Method (Fig. 45.3)

A tube, about 1.22 m (4 feet) long, is taken. It is fitted with a mouthpiece at one end. A side tube is attached very near to the mouthpiece and is attached to a sampling tube. Through the mouthpiece, the subject makes forcible expiration twice—at first (1) after normal inspiration and then (2) after normal expiration. This is necessary because the alveolar air varies slightly in composition in different phases of respiration. Through the sampling tube, two samples of air are drawn in from the last part of the expired air, while the subject closes the mouthpiece with his tongue at the end of expiration.

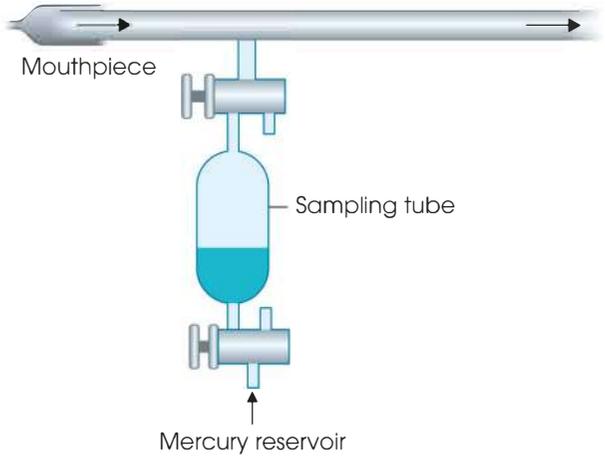
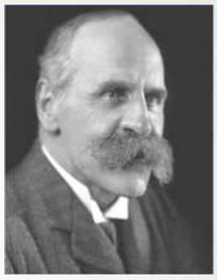


Fig. 45.3: Appropriate apparatus for collection of alveolar air as designed by Haldane and Priestley

45

Haldane, together with Priestley, discovered that the respiratory reflex was triggered by an excess of carbon dioxide in the blood, rather than a lack of oxygen in 1906. John Scott Haldane was a Scottish physiologist well known for his intrepid self-experimentation and discovered many unknown facts about the human body and the nature of gases.



John Scott Haldane
1860–1936

Otis–Rahn Method

A second method of automatic collection of alveolar air has been described by Otis and Rahn method and is shown in Fig. 45.4. During inspiration the end expiratory air of the previous expiration from the neighbourhood is drawn into the balloon by that no dead space air may contaminate the alveolar air collected into the balloon.

Method of Analysis of Alveolar Air

The classical method is to analyse alveolar air with Llyod's modification of Haldane's gas-analysis apparatus, which is used for analysis of respiratory gases. A measured quantity of air is admitted in the apparatus and its volume is accurately noted. The gas is then passed over caustic potash solution and its volume is noted again. The diminution in volume is due to absorption of CO_2 and from the difference between the original reading and the second reading the percentage of CO_2 in the alveolar air may be calculated. The gas is now passed through alkaline pyrogallol solution which absorbs oxygen. From the reduction in volume the percentage of O_2 in the alveolar air can be calculated.

Composition of Alveolar Air

The composition of inspired air, normal expired air and alveolar air and also the tension of different gases are given in Table 45.1.

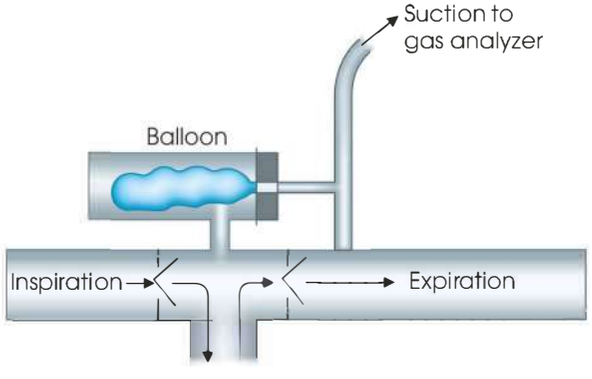


Fig. 45.4: Diagram shows combined respiratory value and Otis-Rahn alveolar air sample

Table 45.1: Composition of gases in inspired, expired and alveolar air

Gases (in vol %)	*Inspired air vol %*	*Expired air vol %*	*Alveolar air vol %*
Oxygen	20.94	16.4	14.2
Carbon dioxide	0.04	4.0	5.5
Nitrogen	79.02	79.6	80.3

The increase in the percentage of nitrogen in the expired air is not real but relative. This is because the volume of the expired air is slightly less than that of inspired air, which is caused by the fact that the amount of CO_2 evolved is less than the amount of oxygen absorbed. Consequently, in the percentage composition, nitrogen shows a relative rise.

Further the composition of expired air varies depending on metabolic activity so that the composition of the alveolar air is kept as constant as possible.

Partial Pressure of Gases in Inspired Air, Expired Air and Alveolar Air

In a gas mixture, the pressure exerted by a particular gas is directly proportional to the percentage composition of the same gas in the mixture. Supposing in a mixture, oxygen constitutes 20% then oxygen will exert 20% (i.e. one fifth) of the total pressure. Table 45.2 shows the partial pressures of the various gases.

It will be noted that the alveolar air differs in composition from that of the inspired (atmospheric) air.

The reasons for this difference are:

1. Only a part of the *alveolar air* is replaced by inspired air as explained previously.

   There is continuous absorption of O_2 from the alveolar air by pulmonary venous blood—the alveolar air, therefore, is poorer in oxygen. CO_2 is added continuously to the alveolar air by the pulmonary venous blood—the alveolar air, therefore, is richer in CO_2.
2. The *inspired air* is dry but gets saturated with water vapour during its passage through the respiratory tract. Since some of the space in the alveoli is now occupied by water vapour, the space available for other gases is diminished.
3. *Expired air:* It has been noted that part of the expired air ('dead space' air) is atmospheric air

Table 45.2: Partial pressures of various gases

Barometer 760 mm Hg / *Gases*	*Partial pressure (in mm Hg)* *Inspired air*	*Expired air*	*Alveolar air*
Oxygen	158.25	116.2	101.2
Carbon dioxide	0.30	28.5	40.0
Nitrogen	596.45	568.3	571.8
Water vapour	5.00	47.0	47.0
Total	**760.00**	**760.0**	**760.0**

rich in O_2 and poor in CO_2. As expiration progresses the expired air becomes a mixture of 'dead space' air and alveolar air; and that the last part of the expired air is pure alveolar air. The expired air, therefore, is richer in O_2 but poorer in CO_2 as compared to alveolar air.

Method of collection of expired air: Expired air is collected in a Douglas bag (Fig. 8.14) over a certain period of time. The sample of air is taken out from the side tube and analysed with the help of Llyod's modification of Haldane gas analysis apparatus.

4. *Tension of gases in the alveolar air:* In the steady state the total tension of gases including water vapour in the alveoli is equal to the ambient barometric pressure.

Key Points

a. At sea-level the barometric pressure is 760 mm Hg. The alveolar air is saturated with water vapour which exerts a tension of 47 mm Hg at body temperature irrespective of the barometric pressure.

b. With these data in view it is possible to calculate tension of O_2 and CO_2 in the alveolar air provided the percentage composition of these gases in the alveolar air is known according to the 'law of partial pressure'. This states that the tension of a particular gas in a gas mixture is proportional to its percentage composition.

c. Thus, in atmosphere the pressure of O_2 is roughly 1/5 of 1 atmosphere = 153 mm Hg approximately. The atmospheric air, however, is dry. In calculation of tension of gases in the alveolar air, the pressure of water vapour (47 mm Hg) is to be deducted from the total pressure of gases in the alveoli which amounts to 760 mm Hg at sea-level.

- Thus, total pressure in the alveoli = 760 mm Hg
- Pressure of H_2O vapour = 47 mm Hg
- The pressure of $O_2 + CO_2 + N$ = 713 mm Hg
- Percentage of O_2 in the alveolar air = 14.2 vol %
- Tension of O_2 in the alveolar air = 14.2% of 713 = 101 mm Hg (approximately)

d. Similarly, tension of CO_2 in the alveolar air is 5.5% of 713 = 39.2 mm Hg or 40 mm Hg (approx.). Of the 4 gases present in alveoli ($O_2 + CO_2 + N_2 + H_2O$ vapour) the N_2 and H_2O vapour are neither taken into the blood stream nor added to alveolar air and as such the sum of partial pressure of these gases is constant in the alveoli. This of course, means that the sum of partial pressure of O_2 and CO_2 is constant in the alveoli under steady state of metabolism and amounts roughly to 140 mm Hg at sea-level.

e. A subject, therefore, can alter the pO_2 of his alveolar air only at the expense of this alveolar pCO_2 which is rather limited because the alveolar pCO_2 cannot be reduced from normal 40 mm Hg to below 24 mm Hg.

Method of Measurement of Alveolar and Arterial pCO_2

Diffusion of CO_2 is so rapid that alveolar pCO_2 is always equal to arterial pCO_2. Indirect bloodless method has also been evolved by Campbell, etc. all and gives reliable result.

Procedure

1. Arterial pCO_2 can be measured by direct electrochemical method using a CO_2 sensitive electrode through an arterial puncture needle.
2. An anaesthetic bag is filled with about 1 litre of O_2 and the subject breaths and re-breaths into this bag for 1½ minutes. During this period there occurs an almost perfect 'lung bag' equilibrium so that the pCO_2 of the bag air is almost equal to pCO_2 of the alveolar air.
3. However to get an accurate result, the subject takes rest for at least 2 minutes and then re-breaths into the bag once again for 20 seconds during which period there occurs fine adjustment between the mixed venous pCO_2 (P_vCO_2) and the bag air. Recirculation of the blood and consequent higher pCO_2 value is avoided by keeping the second re-breathing period limited to 20 seconds.
4. After the end of the experiment the gas in the bag is analysed and its pCO_2 is calculated. This is equal to mixed venous pCO_2. The arterial pCO_2 is always 6 mm Hg lower than the venous pCO_2.

Effect of Voluntary Hyperpnoea on Alveolar Air

Hyperpnoea flushes out the alveoli with air so that the CO_2 content and CO_2 tension of the alveolar air and so of the arterial blood is diminished. This causes depression of respiration and may lead to temporary apnoea. During the depressed respiratory phase CO_2 builds up again till the alveolar pCO_2 attains its normal value and breathing is resumed. The alveolar pO_2 rises in the early phase when the pCO_2 is depressed but this has no effect on arterial saturation, which is normally 95% saturated.

After normal inspiration breath can be held for 30 to 50 seconds. This is normal 'breath-holding time'. During breath-holding time the percentage of CO_2 and pCO_2 in the alveolar air gradually rises and the percentage of O_2 and pO_2 in the alveolar air gradually falls. At the breaking point the O_2 content of the alveolar air is about 8% (pO_2 = 57 mm Hg) and its CO_2 content is about 7% (pCO_2 = 50 mm Hg). It will be noted that during apnoea there occurs a more significant fall in

percentage and tension of oxygen in the alveolar air than rise of CO_2 content and tension. This is due to different shape of the dissociation curves of the two gases, so that removal of a given quantity of oxygen from blood will cause a more significant fall in oxygen tension than elevation of CO_2 tension when the same volume of CO_2 is added.

1. Further CO_2 being highly soluble most of it gets dissolved in body fluids and less is available for elevation of alveolar pCO_2. Here the two stimuli of O_2 lack and CO_2 excess interact with each other in stimulation of respiration.
2. Inhalation of O_2 before voluntary apnoea will prolong the breath-holding time by 15 to 20 seconds. This is due to: Absence of O_2 lack stimulus during breath-holding period. The sensitivity of respiratory centre to CO_2 excess is less in the absence of oxygen lack.
3. Voluntary hyperpnoea before breath holding will wash off CO_2 from the alveolar air and the breath-holding time will be increased considerably so that the subject may develop cyanosis due to O_2 lack before the alveolar pCO_2 rises sufficiently to stimulate the respiration. This shows that CO_2 excess plays a more significant role in stimulating respiration than oxygen lack.
4. If a gas mixture with CO_2 and O_2 in quantities similar to that at the termination of the breath-holding time is re-breathed by a person at the time of breaking point he can hold his breath for a further period in spite of diminished oxygen and elevated CO_2 in the lungs. This indicates other factors besides O_2 lack and CO_2 excess play an important role in precipitation respiratory movement.
5. During the period of apnoea a larger volume of O_2 is removed than the volume of CO_2 added to the lungs. This causes a sense of discomfort and distress. Further, holding the chest in a fixed position stimulates afferents from the respiratory muscles which are obviated by the re-breathing experiment cited above. The afferent stimuli originating in the chest muscles, therefore, constitute the third important factor in determining the breaking point after breath-holding.

Effects of High Altitude on Alveolar Oxygen

At high altitude the barometric pressure falls and so the tension of gases in the inspired air and also in the alveolar air falls. Water vapour exerts a tension of 47 mm Hg at all altitudes and CO_2 is continuously exerted from the body into the respiratory alveoli. As a result of this adverse combination, the pO_2 of the alveolar air falls at high altitude. Further the fall of alveolar pO_2 is disproportionately low because of extremely low O_2 tension of tissues, O_2 is absorbed very quickly from the alveoli. The formula for calculating alveolar pO_2 at different altitudes is:

$$\text{Alveolar } pO_2 = \frac{PB - pCO_2 - 47}{5} - pO_2 \text{ loss}$$

(pO_2 loss is the oxygen pressure decrease caused by oxygen uptake into the blood and the value 47 is the vapour pressure of water.)

There occurs a light fall in alveolar pCO_2 due to hyperventilation but this cannot compensate for O_2 deficit at high altitude. Table 8.3 gives alveolar pCO_2 and pO_2 at different altitudes along with barometric pressure (PB) and percentage saturation of arterial blood with oxygen.

It may be noted that an altitude of 15 km or 50,000 feet the barometric pressure is only 87 mm Hg. This therefore, is the total pressure of gases and water vapour in the alveoli, the latter accounts for 47 mm Hg leading only (87–47) = 40 mm Hg for O_2, N_2 and CO_2. Since pCO_2 is 24 mm Hg, only 16 mm Hg of pressure is distributed between N_2 and O_2 present in the ratio of approximately 4:1. That being so on theoretical ground, one would expect that the alveolar pO_2 at this altitude would be about 3 mm Hg. But due to increased uptake of O_2 by grossly anoxic tissues at this altitude the alveolar pO_2 falls to 1 mm Hg only. Even if pure O_2 is inhaled and all the N_2 in the alveoli is replaced by it, the pO_2 in alveoli would be increased to 16 mm Hg only. Oxygen, therefore, must be administered under pressure to sustain life at this altitude.

Factors Controlling Alveolar pCO_2

Two important factors are:

1. Metabolic rate, i.e. production of CO_2 in the body. The higher metabolic rate the greater will be the $paCO_2$.

Table 45.3: Alveolar pCO_2 and pO_2 at different altitudes along with barometric pressure and % saturation of arterial blood with gases

Attitude	*PB (mm Hg)*	*pCO_2 (mm Hg)*	*pO_2 (mm Hg)*	*Saturation of arterial blood with oxygen (%)*
0	760	40	104	97%
3 km or 10,000 feet	523	36	67	90%
6 km or 20,000 feet	349	24	40	70%
12 km or 40,000 feet	141	24	8	5%
15 km or 50,000 feet	87	24	1	1%

2. Alveolar ventilation: The greater the alveolar ventilation the lower will be the $paCO_2$ and the higher will be paO_2.

Alveolar pO_2 and Venous Admixture

There is normally a difference between pulmonary capillary pO_2 (pCO_2) and arterial pO_2. Alveolar pO_2 which normally is in near equilibrium with pulmonary end-capillary pO_2 therefore, is higher than arterial pO_2. The difference is explained by the fact that under normal conditions some blood passes from the venous side to the arterial side avoiding the lungs and without being exposed to alveolar O_2. For example, thebesian veins draining directly into the left heart, the bronchopulmonary anastomosis already mentioned. It has been estimated that about 6% of venous blood pumped by the right heart by-passes the lungs altogether and thus reduces the pO_2 of the pulmonary venous blood. This is called shunt effect and by definition it is anatomical shunt-contributing to venous admixture.

Physiological Shunt

Even under physiological conditions the millions of alveoli of the lungs are not adequately ventilated and some of them may be completely non-ventilated. Blood flowing through the non-ventilated alveoli will not be oxygenated at all and so will contribute to venous admixture effect. Similarly, venous blood flowing through poorly ventilated alveoli will not be adequately oxygenated and thus will produce the same effect as noted above. It is possible that both hypoventilated and hyperventilated alveoli may be present in the same lung but because of the fact that normally ventilated alveoli can effect almost complete saturation of haemoglobin with oxygen, the defective oxygenation of venous blood perfused through hypoventilated alveoli cannot be compensated by perfusion of hyperventilated alveoli.

Anatomical shunt: When the blood supplying the lung tissues passes via the bronchial arteries and returns back to the pulmonary veins without undergoing any gas exchange and this forms the anatomical shunt. As some of the coronary veins drain directly into the left ventricle of the heart apart from anatomical shunt, the arterial pO_2 is slightly lower than the alveolar pO_2.

Normal true physiological shunts: Under physiological conditions such as in case of normal true shunts of bronchial veins and thebesian veins in which the mixed venous blood bypass the lung and are carried over to the arterial stream and also when normal virtual shunts due to slight deviation of ventilation–perfusion ratio from the ideal value. In pathological conditions the mixed venous blood enter the arterial circulation through an abnormal anatomical right-to-left shunt due to a congenital cardiac anomaly, and an abnormal virtual shunt from the alveoli having a low ventilation–perfusion ratio.

From quantitative aspect, therefore, physiological shunt is larger than anatomical shunt. It is the commonest cause of hypoxia in respiratory diseases.

The concept of anatomical and physiological shunts is analogous to the concept of anatomical and physiological dead space. Normally alveolar ventilation is about 4 litres/minute and about 5 litres of blood (cardiac output) is perfused through the alveoli per minute. The average ventilation–perfusion ratio for the whole lungs, therefore, is 4/5 or 0.8. Increase in physiological dead space is due to absence or diminution of blood flow through well-ventilated alveoli, the condition, therefore, is attended with increase in ventilation–perfusion ratio. The reverse is true in increase in 'shunt' or venous admixture effect when the ratio of ventilation–perfusion is decreased.

EXAM-ORIENTED QUESTIONS

Short Notes

1. Anatomical dead space
2. Physiological dead space
3. Physiological shunt
4. Composition of alveolar air
5. Partial pressure of gases in inspired air, expired air and alveolar air
6. Methods of detection of uneven ventilation
7. Effect of voluntary hyperpnoea on alveolar air

Chapter

46

Ventilation and Perfusion in Lungs

DIFFUSION

Diffusion means movement of a substance from an area of high concentration to an area of low concentration. In the present context the diffusion of O_2 from alveoli to pulmonary capillaries and of CO_2 in the reverse direction is to be considered. N_2 being metabolically inert may be left out of discussion.

The following points are to be noted in this connection:

1. Gases in the alveoli are dissolved in small quantity of alveolar fluid and are in equilibrium with partial pressure of the respective gases in alveolar air.
2. Gases in the blood of pulmonary capillaries are also dissolved in water of the plasma where these exert a tension.

The average values of tension of O_2 and CO_2 in these two areas are given in Table 46.1.

Table 46.1: Average tension of O_2 and CO_2 in alveoli and venous blood

	pO_2	pCO_2
Alveoli	100 mm Hg	40 mm Hg
Venous blood	40 mm Hg	46 mm Hg

Diffusion, therefore, takes place in the direction shown by the arrows through the alveolo-capillary membrane (Fig. 46.1) which consists of:

1. Alveolar epithelium—thin epithelial cells together with its basement membrane.
2. Thin interstitial space between (1) above and (3) below.
3. Capillary endothelium together with its membrane.

The alveolo-capillary membrane consisting of all these layers is very thin—the average thickness being about 0.5 µm. This membrane is freely permeable to respiratory gases and thus ensures rapid diffusion of O_2 and CO_2 through them in the direction shown by the arrows from the point of high pressure to the point of low pressure. The total surface area of the alveolo-capillary membrane is approximately 70 sq m in an adult, indicating the large area available for diffusion. The total amount of blood in the lungs at any moment is about 150 ml. This small quantity of blood spread over this large area naturally facilitates diffusion. Further the diameter of the pulmonary capillaries is about 8 µm: The red blood cell membrane, therefore, touches the capillary wall during their passage through

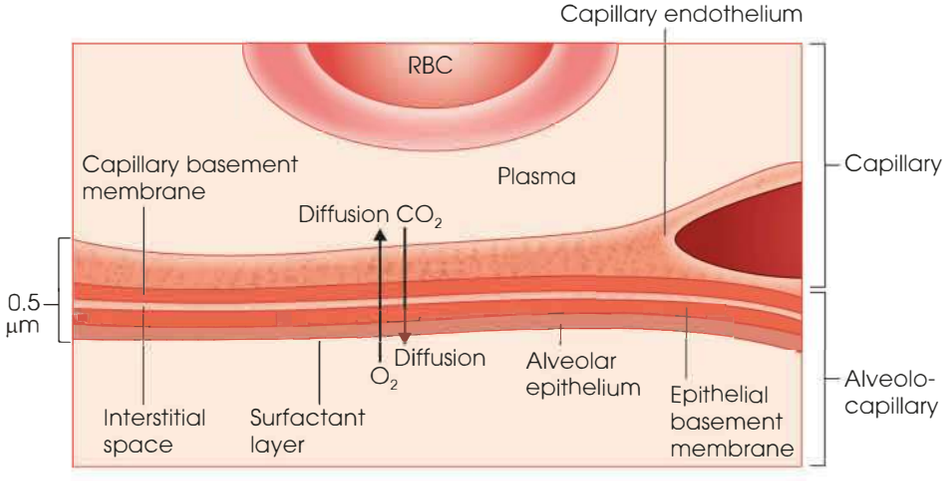


Fig. 46.1: Alveolo-capillary membrane showing the O_2 diffusion

the lungs and the gas molecules get almost direct access to the RBC without passing through significant layer of plasma—a fact which also accelerates the process.

Factors Controlling Diffusion

Rates of diffusion of a gas between two given points depends not only on the tension gradient between the points but also on the distance between the two points, the surface area available for diffusion, the temperature and the characteristic properties of the gas itself. The most important properties of a particular gas which determines their rates of diffusion are; (a) solubility and (b) molecular weight.

Solubility: Solubility of a gas in water is indicated by its solubility coefficient which may be defined as millilitres of gas (STPD) dissolved in 1 ml of water per atmosphere of pressure. When a liquid is exposed to a mixture of gases at a particular temperature—the pressure exerted by an individual component of gas will be related to its percentage-composition (law of partial pressure). And the quantity dissolved in the fluid will be regulated by it. The pressure of the dissolved gas in the fluid will be same as the partial pressure of the same gas in the atmosphere to which it is exposed and with which it is in tension equilibrium. The solubility co-efficient of important gases at 0°C and 1 atmosphere of pressure are listed in Table 46.2.

This means that N_2 is half as soluble in water as O_2 under standardised condition of temperature and pressure. Undoubtedly, CO_2 is most highly soluble of all the respiratory gases.

Molecular weight: The second important property which determines the rate of diffusion is the molecular weight of the gas. The rate of diffusion of a gas at a particular temperature and pressure gradient is inversely related to the molecular weight (MW) of the gas and directly related to other factors, such as temperature.

The tension gradient of the gas can be evaluated, and tension gradient remaining constant the rate of diffusion of a gas = $\frac{S}{MW}$ where S is the solubility coefficient and MW is the molecular weight. The diffusion coefficient of a gas has been defined as the volume in millilitres of a gas which will diffuse 0.001 mm distance over a sq cm of surface per minute at a pressure of 1 atmosphere. A more practical way of consideration of the problem is to assume the diffusion coefficient of O_2 to be 1 and compare it with those of other gases of respiratory importance as given below:

$O_2 = 1.0$
$CO_2 = 20.3$
$N_2 = 0.53$
$CO_2 = 0.81$
Helium = 0.95

Table 46.2: Solubility coefficient of important gases; at 0°C and 1 atmospheric pressure

Gases	*Solubility*
O_2	0.024
CO_2	0.57
CO	0.018
N_2	0.012
Helium	0.008

Pulmonary Diffusing Capacity for O_2 (DO_2)

DO_2 is the ratio of O_2 transfer in millilitres per minute (VO_2) from the alveolar gas to the capillaries divided by the pressure in mm Hg required for this transfer.

$$DO_2 = \frac{VO_2}{(p_aO_2 - p_CO_2)}$$

p_CO_2 indicates the average pressure of O_2 in the pulmonary capillary which changes from moment-to-moment due to gas transfer in a non-linear manner and to obtain an accurate figure involves mathematical calculation and measurements of complicated nature.

For all practical purposes, therefore, CO diffusing capacity is measured and the result is multiplied by 1.23. The affinity of haemoglobin for CO is so great that with small concentration of all the CO remains combined with haemoglobin so that the PCO of plasma is practically 'zero'.

The above equation, therefore, is simplified to:

$DCO = \frac{VCO}{p_ACO}$ and these can be measured with a CO analyser.

In a normal person DO_2 at rest is approximately 15 to 20 ml of O_2/minute/mm Hg pressure difference between alveolar air and mean capillary pO_2.

This may be increased from 3 to 4 times during muscular exercise due to increase in the surface area for diffusion from opening up of new capillaries in alveoli and dilatation of the existing capillaries. In various diseases, e.g. interstitial fibrosis, pulmonary collagenosis there occurs; an increase in resistance to diffusion of O_2 from alveoli to the capillaries. These diseases together constitutes alveolo-capillary block syndrome.

Diffusion of CO_2

CO_2 is much more soluble than O_2 and its diffusibility is at least 20 times higher than that of O_2. An alveolo-arterial pCO_2 difference never occurs and in alveolo-capillary block syndrome retention of CO_2 in the blood does not occur but hypoxia is always prominent.

PERFUSION

The term 'perfusion' simply means blood flow and in this context of respiration it means blood flow through the lungs. It is approximately equal to 5 L/min. Since the alveolar ventilation is about 4 L/min the ratio

$$\frac{\text{Alveolar ventilation}}{\text{Perfusion}} = 0.8 \text{ for the whole lung.}$$

Since the pulmonary circulation is a low pressure system in the erect posture the blood flow through the apex is much less than that in the base of the lungs due to effect of gravity. Since there occurs no remarkable change in ventilation from base to apex of the lungs the ventilation–perfusion ratio (V_A–Q) is high (about 3.3) at the apex and is low (about 0.6) at the base. The apical alveoli, therefore, receive more air than blood (dead space effect) and some ventilation is wasted. The alveoli at the base of the lungs receive more blood than air (shunt effect) and some blood remains unsaturated with oxygen. In the supine posture this regional difference due to effect of gravity is lost and is replaced by low V_A–Q ratio in the posterior part and a high V_A–Q ratio in the anterior part.

EXAM-ORIENTED QUESTIONS

Short Notes

1. Alveolar ventilation
2. Pulmonary diffusing capacity for O_2 (DO_2)
3. Perfusion

Chapter

47

Transport of Oxygen and Carbon Dioxide in Blood

INTRODUCTION

Oxygen Transport

It is observed that about 98% of oxygen is present in the blood in chemical combination with haemoglobin and only 0.3 ml in arterial blood (about 2%) is in physical solution in water of the plasma. However, this small quantity in physical solution is responsible for tension of oxygen in the plasma.

The O_2 content of the arterial and venous blood and relevant data are given in Table 47.1.

Key Points

1. It is the oxygen tension in the blood which is responsible for transfer of this gas from alveolar air to the venous blood across the alveolo-capillary membrane and also for transfer of this gas from arterial blood to the tissue fluid across the capillary membrane.
2. Further, it is the tension of oxygen in plasma which controls the amount of oxyhaemoglobin present in the blood.

O_2 Capacity, O_2 Content and Percentage Saturation of Haemoglobin

1. **O_2 capacity:** Fully saturated each gram of normal haemoglobin contains 1.34 ml of oxygen in blood. Thus, 1 gm of haemoglobin when fully saturated will combine with 1.34 ml of O_2.* Since the blood contains approximately 15 gm of Hb per 100 ml, the oxygen content of the blood when fully saturated will be about $15 \times 1.34 = 20$ ml. This is called O_2 carrying capacity or oxygen capacity of the blood. Naturally the O_2 capacity depends upon the amount of haemoglobin in blood. Incidentally, the most accurate method of estimating haemoglobin content of blood is to determine the oxygen capacity and divide the result by 1.34.
2. **Saturation of haemoglobin:** If the blood is agitated with air so that the Hb gets fully saturated with O_2 the result will indicate oxygen carrying capacity. Percentage saturation of Hb with

$$O_2 = \frac{O_2 \text{ content}}{O_2 \text{ capacity}} \times 100$$

Example: Thus, if in a particular sample of blood the O_2 content = 19 volume % and the O_2 capacity = 20 volume %. The percentage saturation of Hb = 19/20 × 100 = 95%. If in another sample of blood the O_2 content is 15 volume % and capacity is 20 volume %. The saturation of Hb will be 75%. If the same blood contained 10 volume of O_2 per 100 ml, the saturation would be 50%.

Dissociation Curve for Haemoglobin

When fully saturated, each gram of haemoglobin binds 1.34 ml of oxygen. The degree of saturation is co-related to the oxygen tension (pO_2), which normally ranges from about 35 mm Hg in veins to about 100 mm Hg in arterial blood. The relation between oxygen tension and haemoglobin oxygen saturation is described by the oxygen-dissociation curve of haemoglobin.

The curve is constructed by placing blood in containers provided with stopcock at either end (called 'tonometer') and filled with oxygen of known tension. After equilibration the percentage saturation of haemoglobin in the tonometer is determined and

Table 47.1: The content of oxygen in arterial and venous blood and relevant data

	O_2 content	*Oxy-Hb*	*O_2 in solution*	*pO_2*
Arterial blood	19.3	19	0.3	100 mm Hg
Mixed venous blood	14.2	14	0.2	40 mm Hg

*Incidentally, the most accurate method of estimating haemoglobin content of blood is to determine the oxygen capacity and divide the result by 1:34.

plotted against different oxygen tension. About 5 ml of blood is put in tonometer of 250 ml capacity and rotated for 20 minutes in horizontal position in a water bath at 37.5°C. The percentage saturation of haemoglobin at different tensions for human blood is summarised in Table 47.1.

The dissociation curve for O_2 (Fig. 47.1) is obtained by plotting the percentage saturation of Hb against pO_2.

Nature of the Curve

1. The curve shows a steep rise in percentage saturation of haemoglobin with O_2 between 0 and 40 mm Hg O_2 tension.
2. No significant increase in percentage saturation of Hb occurs between O_2 tension of 60 mm Hg (90% saturation) and 100 mm Hg (100% saturation).
3. The first part of the curve (between pO_2 zero and 40 mm Hg) is nearly vertical the last part of the curve (from pO_2 60 mm Hg to 100 mm Hg) is almost horizontal and the whole curve is sigmoid in shape.

Explanation of the Sigmoid Shape of the Curve

The adult haemoglobin in normal individuals contains two alpha chains and two beta chains. Each haemoglobin molecule has a haem prosthetic group which contains an atom of iron (Fig. 47.2). Each molecule of globin consists of 4 polypeptide chains to each of which is attached a molecule of haem with Fe^{2+} capable of combining with one molecule of oxygen, thus each haemoglobin molecule has the capacity to carry four oxygen molecules.

As soon as one of the molecules of haem combines with O_2 the other three molecules in the same polypeptide complex of the globin acquires a great affinity for O_2 and rapidly combines with it. This rapid oxygenation of haemoglobin in the initial phase of its exposure to O_2 is known as allosteric activation and explains the sharp rise in the dissociation curve in the lower range of O_2 tension and also the gradual levelling off of the curve at higher range as the haemoglobin reaches the near saturation point (Table 47.2).

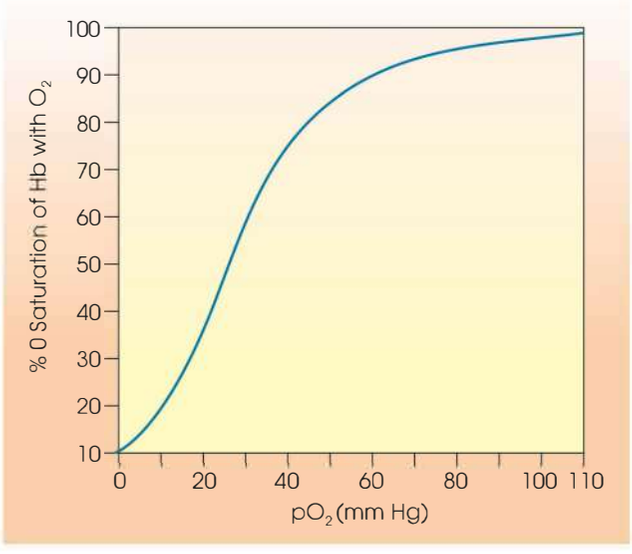


Fig. 47.1: Oxygen haemoglobin dissociation curve at 40 mm Hg, CO tension, pH 7.4, temperature 38°C

Factors Influencing the Shape of the Curve (Fig. 47.3)

1. *Bohr effect:* In presence high of pCO_2, the curve is deviated to the right, which means CO_2 favours dissociation of oxy-haemoglobin (oxy-Hb) and release of O_2. This reaction is most pronounced in the middle range of the curve at about pO_2 40 mm Hg and is helpful in release of O_2 in the tissues where the pCO_2 is high and pO_2 is about 40 mm Hg at rest.
2. *Increased pH:* Acidity in the blood shifts the dissociation curve to the right. During exercise lactic acid and other organic acids produced in the active tissues favour release of oxygen from oxy-Hb. It is known that increased CO_2 is the cause of increased pH. Effect of CO_2 shifting the dissociation curve to the right, therefore, is perhaps non-specific in nature.
3. Increased temperature shifts the dissociation curve to the right (Fig. 47.4). The phenomenon also favours release of oxygen in the active tissues with increased blood flow and metabolic activity leading to rise in temperature.
4. *DPG (2,3-diphosphoglycerate):* DPG favours the dissociation of HbO_2 to Hb and O_2 ($HbO_2 \rightleftharpoons Hb + O_2$) and shifts the dissociation curve to the right. The altitude hypoxia and consequent alkalaemia favour this reaction.

Advantages of the Sigmoid Shape of the Curve

1. The shape of the curve is such that at normal alveolar pO_2—the haemoglobin of the blood leaving the lungs is almost completely saturated. Any further increase in alveolar pO_2 under normal circumstances, e.g. O_2 inhalation is of no advantage in so far as percentage saturation of haemoglobin is concerned.
2. The flat upper part of the curve indicates that relatively a little reduction in percentage saturation of haemoglobin occurs unless the oxygen tension of the alveolar air falls below 60 mm Hg.
3. The steep slope of the oxygen dissociation curve at lower range of oxygen tension indicates that there occurs rapid breakdown of oxy-Hb and consequent release of O_2 in the oxygen tension (40 to 20 mm Hg) prevailing in the tissue.

Increase CO_2, pH, temperature and DPG all favour rapid release of oxygen in the tissues during activity.

Note

The composition of oxygen and carbon dioxide in arterial and venous blood is given in Table 47.4.

Velocity of the Reaction

In the lungs the time taken for 50% saturation of the haemoglobin is only 0.07 second. Since the RBC takes

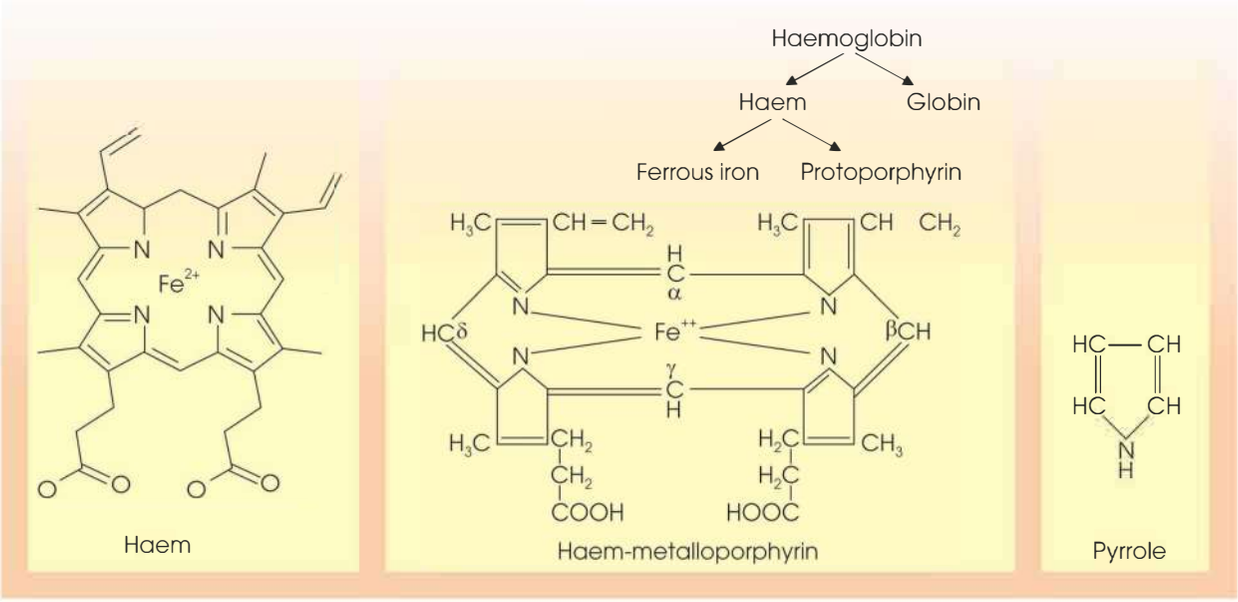


Fig. 47.2A: Structure of haemoglobin molecule and pyrrole

Table 47.2: Percentage saturation of haemoglobin for different tension for human blood

Oxygen tension (pO) in mm Hg	*Percentage saturation of haemoglobin*
10	15%
20	40%
30	60%
40	75%
50	85%
60	90%
70	95%

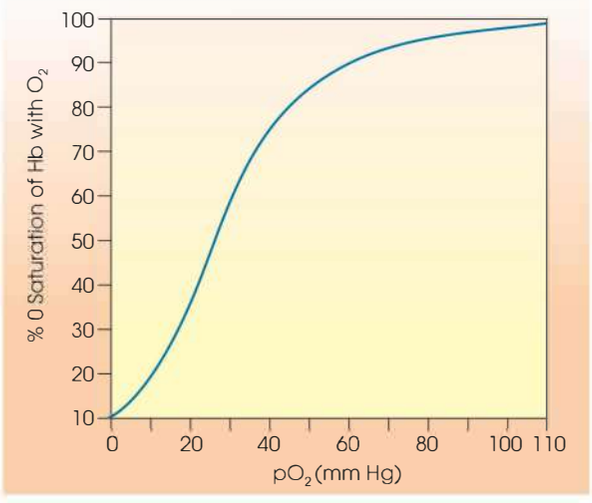


Fig. 47.2B: Oxygen haemoglobin dissociation curve at 40 mm Hg, CO_2 tension, pH 7.4, temperature 38°C

0.5 second to pass a lung capillary, enough time is available for complete oxygenation of haemoglobin.

In the tissues the reverse reaction $HbO_2 \rightleftharpoons Hb + O_2$ takes place much more quickly, time taken for 50% desaturation of haemoglobin being only 0.038 second.

Delivery of O_2 in the Tissues in Anaemia (Table 47.3)

Table 47.3 gives approximate relevant values of oxygen in blood in normal subjects and in a patient with anaemia (Hb content of blood = 7.5 gm of or 50% of normal).

The first-line of compensation is, therefore, affected by drawing upon the reserve volume of O_2 from the mixed venous blood as a result of which the venous blood gets more unsaturated. However, in spite of the greater coefficient of O_2 utilisation the tissues fail to get adequate amount of O_2 as shown in the last column.

Coefficient of O_2 utilisation = (measured in%)

$$= \frac{\text{Arterio-venous } O_2 \text{ content difference}}{\text{Arterial } O_2 \text{ content}} \times 100$$

$$\text{Normal value} = \frac{5}{20} \times 100 = 25\%$$

$$\text{In anaemia} = \frac{4}{10} \times 100 = 40\%$$

Due to increased quantity of 2, 3-diphosphoglycerate within the RBC in anaemia, the dissociation curve shifts to the right which favours release of oxygen in the tissues. The third method of compensation is increased cardiac output which ensures increased blood flow (and oxygen supply) to the tissues.

Oxygen Exchange in the Lungs (Fig. 47.5 and Table 47.5)

1. The tension of O_2 in the alveolar air is 100 mm Hg and that of the dissolved O_2 in the plasma of the mixed venous blood is only 40 mm Hg. Due to the tension gradient O_2 diffuses rapidly from the alveolar air to the mixed venous blood increasing the quantity and also tension of O_2 in the plasma in the venous blood. Rise of O_2 tension of the mixed venous blood

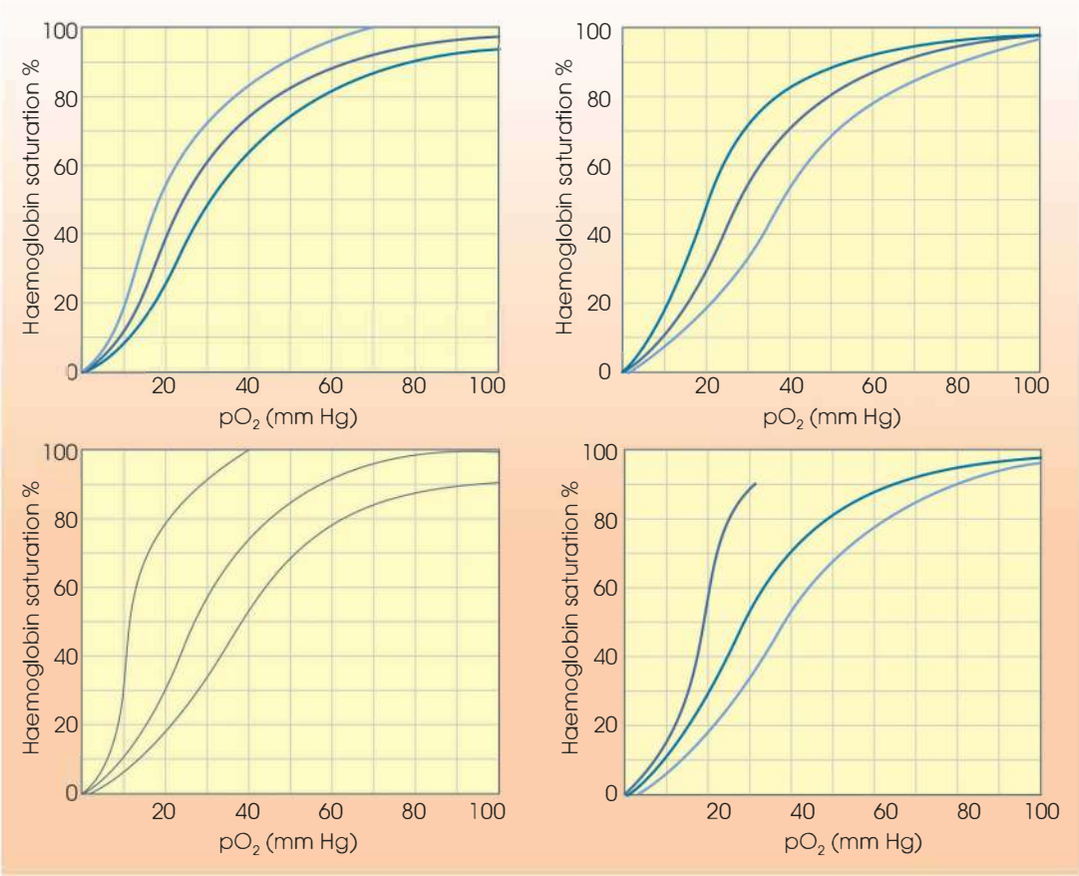


Fig. 47.3: Shift of oxygen dissociation curves due to various factors

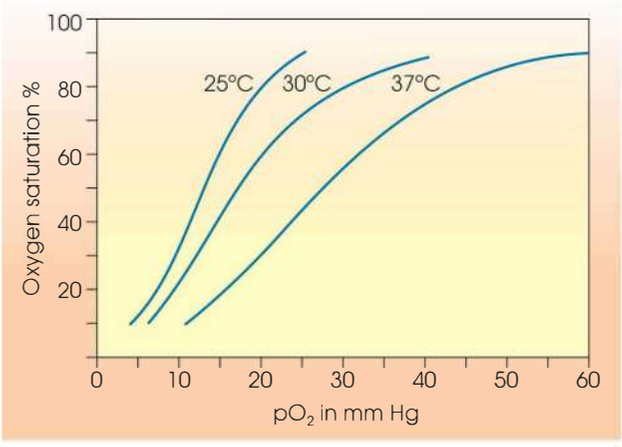


Fig. 47.4: The effect of temperature on the oxygen dissociation curve of oxy-Hb of human blood at pH 7.4 (from data P. Astrup, K. Engel, N. Severinghous and E. Munson (1965) Scandinavian, Journal of Clinical and Laboratory Investigations).

is followed by rapid increase in O_2 saturation of the haemoglobin.

2. The oxygenated blood leaves the pulmonary capillaries in tension equilibrium with alveolar air, that is, with O_2 tension of 100 mm Hg and with haemoglobin 98% saturated with oxygen. The term mixed venous blood indicates sample of venous blood from the right heart or pulmonary artery. The actual gas exchange in the lungs takes place between the gases dissolved in alveolar fluid and plasma water.

Oxygen Transport in the Tissues (Fig. 47.5)

1. Oxygen tension in the tissue fluid is low and is about 40 mm Hg during 'rest'. The arterial blood enters the tissue capillaries with an oxygen tension of 100 mm Hg and with haemoglobin 98% saturated with oxygen. So, the O_2 diffuses from the plasma to

Table 47.3: Relevant values of oxygen in normal subjects and in patients of anaemia

Hb content	*Arterial blood*		*Mixed venous blood*		*Arterio-venous O_2 content difference*
(gram %)	O_2 content	% saturation	O_2 content	% saturation	
15	20	98	15	75	5
7.5	10	98	6	30	4

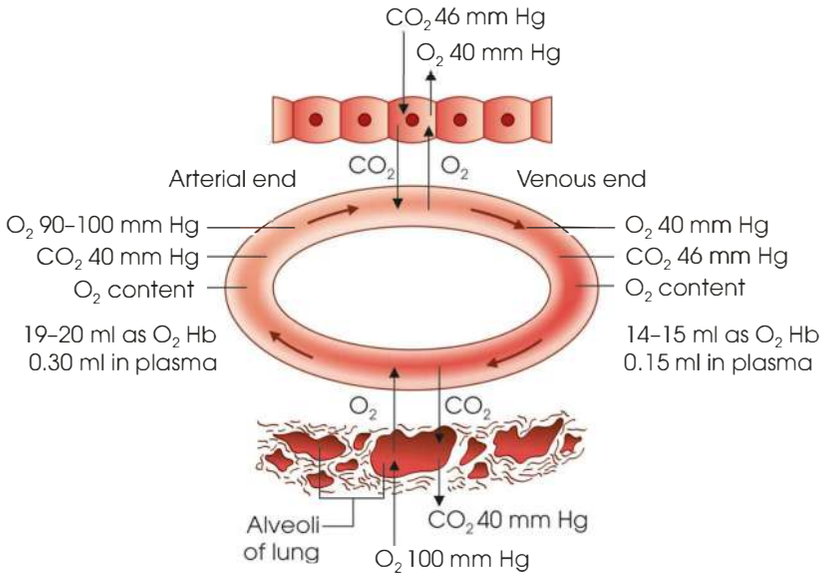


Fig. 47.5: Carriage of O_2 in tissue and lung

Table 47.4: Composition of oxygen and carbon dioxide in venous blood and arterial blood

Gases	Venous blood		Arterial blood	
	Total vol %	In simple solution vol %	Total vol %	In simple solution vol %
Oxygen	01	19.0	0.30	
Carbon dioxide	52.1	2.7	48.3	2.40

Table 47.5: Tension of gases in venous blood and arterial blood

Gases	Tension (in mm Hg)	
	Venous blood	Arterial blood
Oxygen	40	90–100
CO_2	46	40

the tissue fluid due to tension gradient between the two fluids.

2. As O_2 diffuses the amount of O_2 in solution decrease and the tension of O_2 in the arterial blood falls. This results in desaturation of haemoglobin which gives oxygen firstly to the plasma from where it goes to the tissue space—the guiding force being the tension gradient.
3. It has already been mentioned that the reaction is very rapid and so the blood leaves the tissues with oxygen in tension equilibrium with tissue fluid that is 40 mm Hg and consequent 75% saturation of haemoglobin with oxygen.

CARBON DIOXIDE TRANSPORT

CO_2 Content and Tension of Blood

Arterial blood contains about 48 volume % of CO_2 and venous blood 52 volume % of CO_2 per 100 ml, tension of CO_2 being 40 mm Hg and 46 mm Hg in the arterial and venous blood respectively. CO_2 is carried in blood in: (a) Physical solution and (b) as chemical compounds. Table 47.6 summarises the CO_2 content in arterial and venous blood.

Both the corpuscles and plasma carry CO_2, the former carries about one-fourth of the total.

CO_2 in Physical Solution

1. Roughly about 5% of CO_2 is carried in physical solution in blood. In solution, CO_2 exists mostly as H_2CO_3 according to the following equation.

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3$$

Since the reaction is reversible, it is clear that H_2CO_3 always remains in equilibrium with small amount

Table 47.6: CO_2 content in arterial and venous blood

	Arterial blood			Venous blood		
	RBC	Plasma	Whole	RBC	Plasma	Whole
In solution	0.8	1.6	2.4	0.9	1.8	2.7
Bicarbonate	9.8	33.1	42.9	10.5	35.2	45.7
As carbamino-compound	2.0	1.0	3.0	2.6	1.1	3.7
Total	12.6	35.7	48.3	14.0	38.1	52.1

of molecular CO_2 gas which is responsible for CO_2 tension of blood.

2. The ingress of CO_2 into the blood in the tissue capillaries is not accompanied by any significant increase in H^+ concentration because of the effective 'buffering' action of the blood and of haemoglobin in particular. The buffering property of haemoglobin is due to imidazole group of histidine linked with haem which contains an ionisable H^+ ion. On oxygenation, histidine parts with its H^+ ion thus act as an acid. On reduction the H^+ ion is mostly linked with N and thus acts as a weaker acid.
3. Reduced Hb, therefore, is potentially H^+ ion acceptor. 1 mmol of reduced Hb can accept 0.7 mmol of H^+ ion without any change of blood reaction. It has been suggested that the H^+ of the NH_2 radical of haem linked valine is also oxylabile like that of histidine and thus confers upon haemoglobin its remarkable buffering property. This explains why venous blood with reduced Hb contains more CO_2 than arterial blood at a given pCO_2 and the dissociation curve of CO_2 for venous blood is situated at higher level.

CO_2 as Bicarbonate

It is clear that more than 80% of the CO_2 is carried as bicarbonate in the blood and that the major fraction of the carbonate is present in plasma. However, most of the bicarbonates found in plasma are primarily formed within the RBC and then shifted to plasma.

Mechanism of Formation of CO_2

1. We have seen that hydration of CO_2 occurs in the blood and the mechanism can be represented as $CO_2 + H_2O \rightleftharpoons H^+ + HCO_3$. Further the H^+ is buffered by reduced Hb within the RBC and by plasma buffers outside the RBC. The reaction can be represented $H^+ + HCO_3 + HbO_2 \rightleftharpoons HHb + HCO_3^- + O_2$ (liberated in the tissues). HHb represents H ion buffered by reduced Hb).
2. The negatively charged HCO_3 left over in the reaction is neutralised by available bases in the blood. The most abundant available base in plasma being Na^+ the HCO_3^- in the plasma combines with Na^+ to form $NaHCO_3$.
3. The most abundant base within the RBC being K^+, $KHCO_3$ is formed in that location. The reaction $H_2O + CO_2 \rightleftharpoons H^+ + HCO_3^-$ is accelerated tremendously due to presence of a specific enzyme carbonic anhydrase* within the RBC. Concentration of HCO_3^- within the RBC, therefore, becomes very high within a short time and attains a level much higher than that in the plasma.
4. Now since the red cell membrane is impermeable to positively charged ions, some HCO_3^- from within the RBC migrates into plasma and some Cl^- from the plasma migrates within the RBC in exchange of HCO_3. This is allied 'chloride shift' phenomenon (Fig. 47.6) and was discovered by Hamburger in 1927. Thus, the concentration of HCO_3^- in plasma becomes higher due to migration of this ion from within the RBC according to the law of Donan's equilibrium.

Hamburger described the chloride shift, the process by which red blood cells exchange bicarbonate for chloride in red blood cell. He quantified the process of phagocytosis by incubating neutrophil granulocytes with carbon particles, and measuring the uptake.

Hartog Jakob or Hartog Jacob Hamburger
1859–1924

Note

To be more accurate the changes may be explained as follows: The concentration of HCO_3^- ion disproportionately increases in the red cells and the tonic balance between the plasma and corpuscles is disturbed. Depending upon the peculiar property of the red cell membrane, HCO_3^- ion comes out of the cells and chlorine ion moves into the cell till the balance is restored. The ionic interchanges can be explained easily on the principle of Donan equilibrium. The balance of anions and cations between plasma and corpuscles is maintained according to the following equations.

$$\frac{HCO_2^- \text{ (cells)}}{HCO_2^- \text{ (plasma)}} = \frac{Cl^- \text{ (cells)}}{Cl^- \text{ (plasma)}} = \frac{H^+ \text{ (cells)}}{H^+ \text{ (plasma)}}$$

Whenever this ratio is disturbed, ionic interchanges will take place till the proper balance is restored. Chloride shift takes place according to this principle.

CO_2 Transport in the Tissues

1. Tension of CO_2 in the tissue fluid is 46 mm Hg (at rest) and that in arterial end of the capillary is 40 mm Hg. CO_2, therefore, diffuses from the tissue space to the capillary due to tension gradient. It may be recalled that the coefficient of diffusion of CO_2 is very high and in spite of the rather low tension

*Carbonic anhydrase is an enzyme with mol wt of 30,000 and containing one atom of Zn per molecule. It is found in the RBC, in renal tubules, in gastrointestinal mucosa, pancreas, salivary glands, liver, brain and many other tissues. It is inhibited by heavy metals, cyanide, sulphonamide and certain other drugs. Acetazolamide (Diamox) is a particular type of sulphonamide which inhibits this enzyme in the kidneys and thereby promotes diuresis.

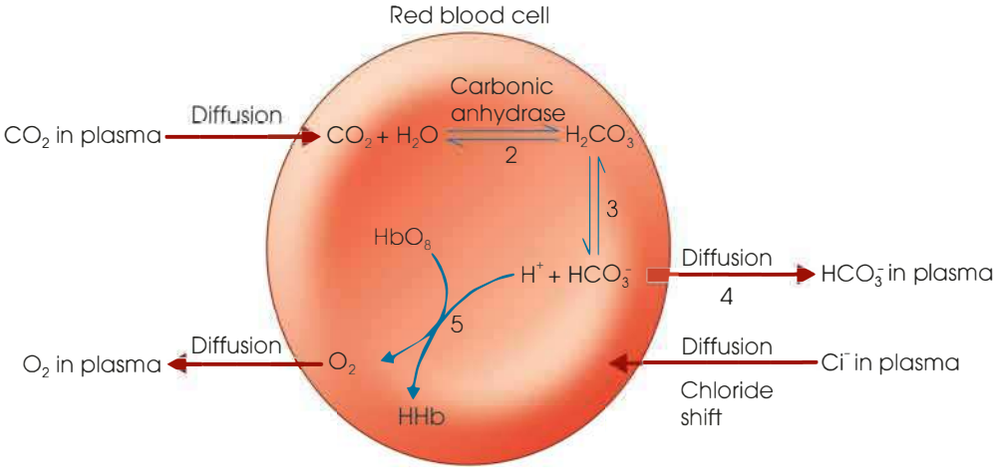


Fig. 47.6A and B: Chloride shift

gradient rapid and complete diffusion of CO_2 occurs till tension equilibrium is established.

2. When equilibrium is established the plasma changes may be summarised:
   - Some free molecular CO_2 in solution, which is responsible for CO_2 tension.
   - Some CO_2 in solution in H_2O as H_2CO_3.
   - Some CO_2 buffered as $NaHCO_3$.
   - Some CO_2 as carbamino-protein.

Since formation of H_2CO_3 is accompanied by formation of almost equivalent quantities of $NaHCO_3$ the ratio bicarbonate/sodium bicarbonate is a little disturbed and the pH of the blood remains almost unaffected.

CO_2 Transport in the RBC

CO_2 diffuses rapidly and the reaction $CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3$ takes place 13,000 times quicker than in plasma because of the presence of the specific enzyme carbonic anhydrase.

Carriage of CO_2 as Carbamino-compound

Key Points

1. CO_2 combines directly with free amino group of the globin and forms carbamino-compounds which may be represented as follows:

$$Hb - NH_2 + CO_2 \rightleftharpoons Hb - NHCOO + H^+$$

This occurs independent of carbonic anhydrase and therefore the reaction is not inhibited by cyanides. CO_2 is not initially changed to H_2CO_3 and the reaction happens at a very rapid rate in the tissues where haemoglobin is desaturated.

2. Carbamino-compounds are also formed by direct union of CO_2 with plasma proteins but since the concentration of haemoglobin within the RBC is high, a larger fraction of carbamino-compounds are carried within the RBC than in the plasma. Further an increase in PCO_2 does not increase the formation of carbamino-compounds since increased PCO_2 means increased H^+ concentration which leads to formation of $HbNH_3$. This does not react with CO_2 directly.
3. Oxygenation of haemoglobin inhibits carbamino-compound formation because oxy-Hb by virtue of its greater acidity H^+ which blocks carbamino-compound formation. Reduced Hb, on the other hand, favours formation of carbamino-compound. In the tissues oxy-Hb is also being converted

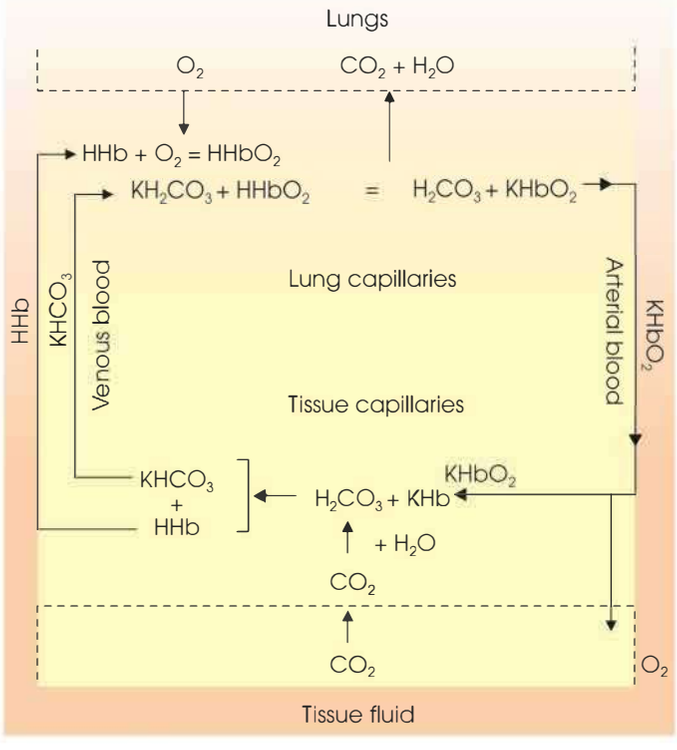


Fig. 47.7: Schematic representation showing inter-relation between carriages of O_2 and of CO_2

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simultaneously to reduce Hb; which is a weaker acid in comparison with the former and has got a weaker hold on the base (K^+) available within the RBC. If oxy-Hb is represented symbolically by HbO_2 its 'salt' with K (potassium) may be symbolised as $KHbO_2$. With the ingress of CO_2 and reduction of oxy-Hb the following changes occur:

$$CO_2 + H_2O \rightleftharpoons H^+ + HCO_3^- + KHbO_2,$$
$$\rightleftharpoons HHb + KHCO_3 + O_2 \text{ (literated)}$$

The $KHbO_3$, of course, ionises to $K + HCO_3^-$. Large quantities of carbamino-haemoglobin are also formed which ionises liberating H^+ which are neutralised by imidazole group of histidine and β-amino group of valine of the globin polypeptide chains.

4. As already explained the HCO_3^- from within the RBC is exchanged for Cl^- of the plasma according to the law of Donan's equilibrium (chloride shift). The chloride shift mechanism prevents accumulation of large amount of HCO_3^- within the RBC and thus prevents shifting of its pH to the alkaline side.

O_2 Transport in the Lungs (Fig. 47.7)

CO_2 diffuses from plasma (pCO_2 = 46 mm Hg) to the alveolar air (pCO_2 = 40 mm Hg) and so the H_2CO_3 breaks down liberating further amount of CO_2.

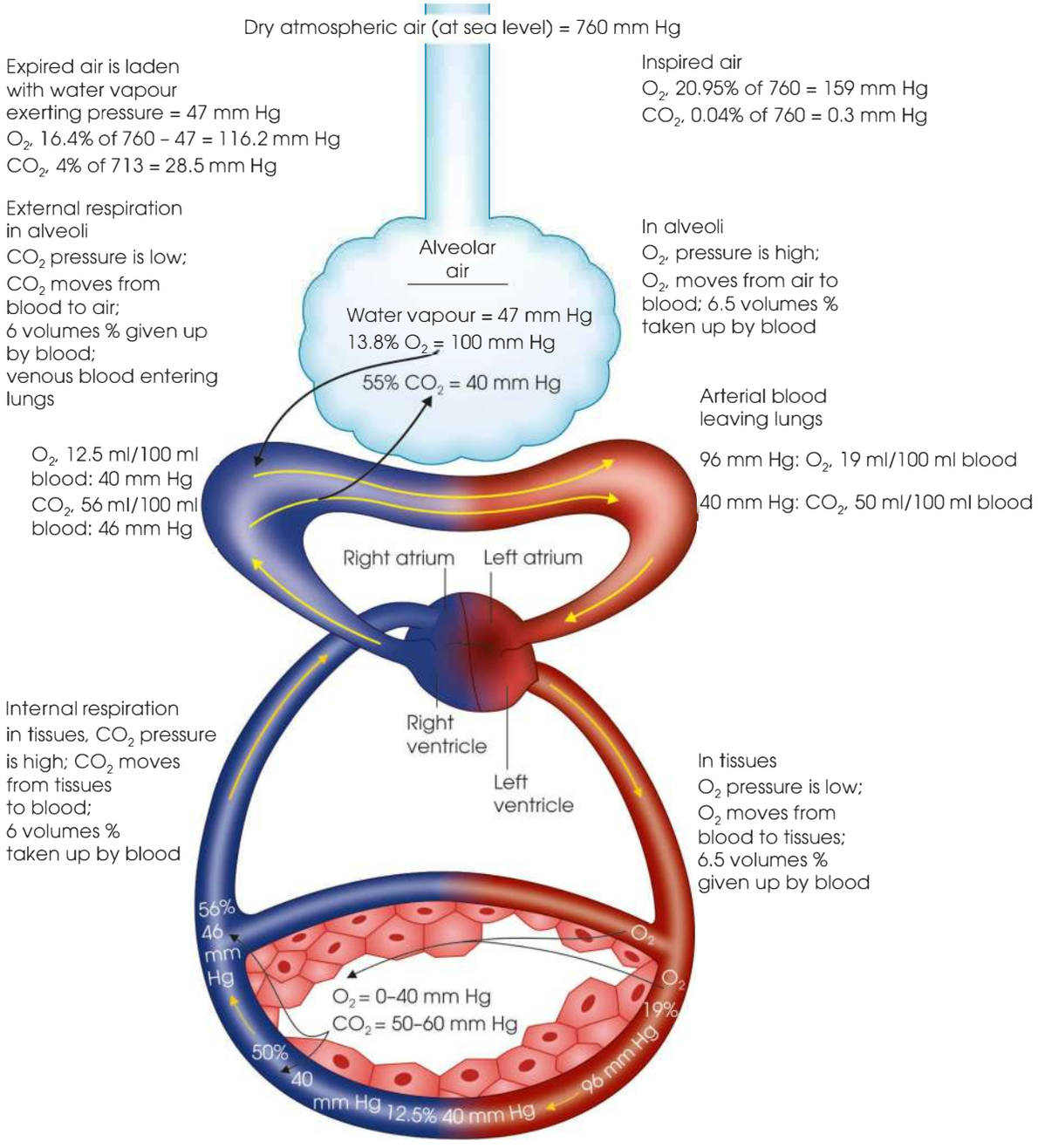


Fig. 47.8: Mechanism of gas transfer in the lung (schematic representation)

Simultaneously oxy-Hb is formed which being a stronger acid snatches off K within the RBC from its combination with acid ions and form $KHbO_2$. Reversed chloride: Sn if occurs and the HCO_3^- ion enters the RBC and is broken down 4 berating ferther amount of CO_2 which passes from RBC to plasma and then to lungs

$$KHHb + HCO_3^- + O_2 \rightleftharpoons H_2CO_3 + KHbO_2$$

$$H_2CO_3 \rightleftharpoons H_2O + CO_2$$

Oxygenation of haemoglobin also breaks down carbamino-compound liberating further quantity of CO_2. The mechanism of gas transfer between the lungs → blood → tissues → venous blood and back to the lungs has been summarised in Fig. 47.8.

Carbon Dioxide Dissociation Curves

Conversely, oxygenated blood has a reduced affinity for carbon dioxide. The oxygenation of blood in the lungs displaces carbon dioxide from haemoglobin which increases the removal of carbon dioxide. This property is the Haldane effect.

Conversely, a decrease in carbon dioxide provokes an increase in pH, which results in haemoglobin picking up more oxygen (Bohr's effect which state that haemoglobin's oxygen binding affinity is inversely related both to acidity and to the concentration of carbon dioxide).

With a comparative study of the CO_2 contents of reduced blood, oxygenated blood, bicarbonate solution and water, under different pressures of CO_2, important facts have been known regarding the behaviour of CO_2 of blood under different physiological conditions. The results when plotted in the form of curves, constitute the CO_2 dissociation curves (Fig. 47.9). The following facts are seen:

1. In a vacuum, CO_2 content of blood is nil.
2. At any given CO_2 tension, reduced blood takes up larger amount of CO_2 than oxygenated blood. The reduction of blood in the tissue capillaries increases the degree of CO_2 uptake from the tissues.

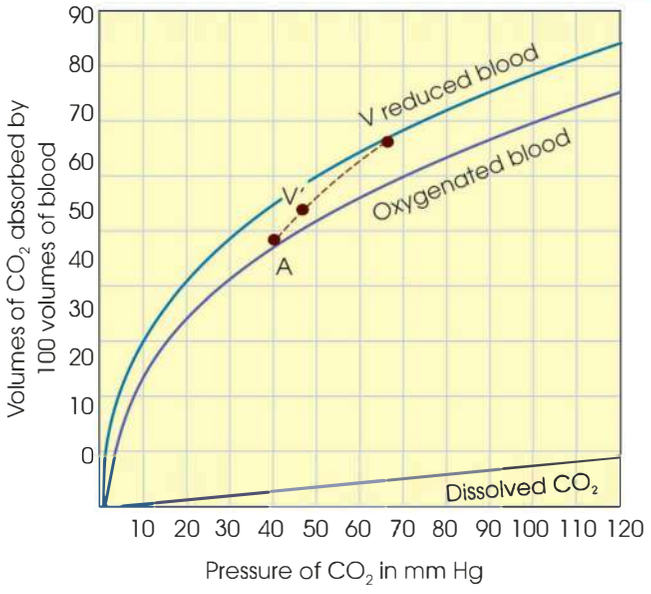


Fig. 47.9: CO_2 dissociation curves of blood. Continuous curves: Upper curve of reduced blood, Lower curve of oxygenated blood. A—arterial point (CO_2 = 40 mm Hg), V—fully reduced point (CO_2 = 64 mm Hg), V¹—mixed venous point (CO_2 = 64 mm Hg). Straight line: Dissolved CO_2, i.e. CO_2 in physical solution

3. Oxygenation of blood causes evolution of CO_2. This happens in lungs.
4. As the CO_2 tension is increased, the total amount of CO_2 taken up by blood also rises. As the CO_2 tension falls, CO_2 content also diminishes.

EXAM-ORIENTED QUESTIONS

Essay

1. Discuss the mechanisms of oxygen transport across lung.
2. Discuss the mechanisms of carbon transport across lung.

Short Notes

1. Oxygen dissociation curves
2. Carbon dioxide dissociation curves

Chapter

48

Regulation of Respiration

INTRODUCTION

The normal rate or respiration in an adult is 14 to 18 per minute with a tidal volume of about 500 ml. The rate and depth of respiration (pulmonary ventilation per minute) is adjusted according to requirement of the body. For instance during muscular exercise metabolic activities are increased and the demand for oxygen by the working muscles is increased. At the same time a large amount of carbon dioxide is produced by the muscles and it becomes necessary to eliminate it. Thus, in muscular exercise the respiration is increased both in rate and depth. In fact, the increase in minute ventilation is directly proportional to the magnitude of muscular exercise. On the other hand, respiration is depressed during sleep when the metabolic rate is low. Since the rate and depth of respiration can be accurately adjusted to metabolic needs of the body there must be an efficient mechanism for its regulation.

Like other physiological functions of the body the act of respiration, too, is controlled by a special group of nerve cells in the brain stem, which constitutes the 'respiratory centre'. The activity of this centre is controlled by many factors which may be classified under two headings:

1. Nervous factors
2. Chemical factors

Respiratory Centre

Respiratory centre consists of a widely scattered group of nerve cells in the reticular formation of the pons and medulla (Fig. 48.1) which may be divided into four major areas.

Pneumotaxic Area

It is located in the upper pons. It controls the activity of the two lower centres. Section at 'M' (Fig. 48.1) produces apneustic type of respiration characterised by prolonged inspiratory cramps. It has subsequently been established that section of vagal afferents together with section at 'M' is necessary for typical apneustic respiration.

Apneustic Area

Located in the lower pons, so named because when isolated from the influence of pneumotaxic centre it leads to apneustic type of respiration mentioned above. Section at 'N' (Fig. 48.1) leads to gasping type of respiration from the medullary respiratory centre isolated from the influence of other parts of respiratory centre.

Bötzinger Complex

Bötzinger complex is a group of neurons which are located in the rostral ventrolateral medulla, and ventral respiratory column. It is located caudally to the facial nucleus and ventral to nucleus ambiguous in medulla. The Bötzinger complex plays vital role in controlling breathing pattern and also for responses in hypoxia. The Bötzinger complex consists primarily of glycinergic neurons which inhibit respiratory activity. During the respiratory cycle phases Bötzinger complex generates post-inspiratory activity and augments expiratory activity.

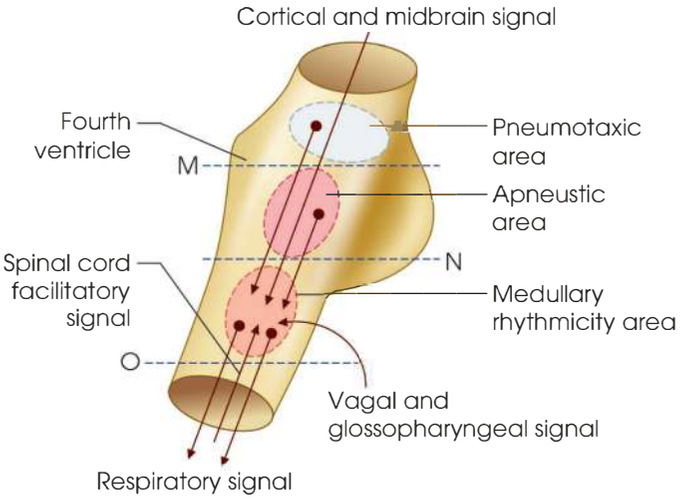


Fig. 48.1: The three-part of respiratory system located in the brain stem

Medullary Area

Section at 'O' (Fig. 48.1) produces stoppage of respiration because the connection between the medullary respiratory centre and the motor neurons of the phrenic and intercostal nerves are severed. Medullary respiratory centre located near the lower part of the floor of the fourth ventricle (Fig. 48.2) consists of two parts:

1. *Inspiratory centre:* Ventromedially placed
2. *Expiratory centre:* Dorsolaterally placed.

The above conclusions were based on stimulation experiments. Subsequent careful studies with microelectrodes confirmed the existence of two sets of neurons (viz. inspiratory and expiratory) but they are comingled with each other, so that all the inspiratory or expiratory neurons are not collected together to form a 'centre' in the way it is usually understood. The stimulation study revealed that the:

1. Inspiratory and expiratory centres have got 'to-and fro' connection and reciprocally inhibit the activity of each other, so that stimulation of the inspiratory centre is attended simultaneously with inhibito-expiratory effect (Fig. 48.3).
2. The medullary respiratory neurons have got an intrinsic rhythmicity of their own. If the medullary respiratory centre is isolated from impulses of higher parts by rostral transection 'gasping' type of respiration occurs (hence the name 'gasping centre').
3. The inspiratory centres, have got a basic rhythmicity of their own.
4. The pre Bötzinger complex is a premotor respiratory network critical for inspiratory rhythm generation.

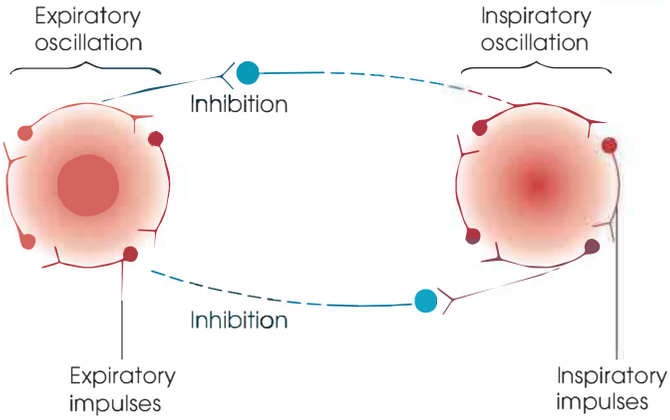


Fig. 48.3: Diagram depicting the dotted inter-neuronal connection between the expiratory and inspiratory centres responsible for alternate excitation and inhibition of inspiratory and expiratory centres

Quick recap: Neural respiratory centres

Neurons which control respiration are located bilaterally along the medullo-pontine region and are of three types:

a. Dorsal respiratory group of neurons are located in medulla in the nucleus of the tractus solitarius. They receive sensory input from the vagus and glossopharyngeal nerves.
b. Ventral respiratory group of neurons are located in the ventrolateral part of the medulla in the nucleus ambiguus and nucleus retroambiguus.
c. Pneumotaxic centre lies in the superior pons in the nucleus parabrachialis and the apneustic centre in the lower pons.

The normal respiration occurs due to co-ordinated activity of the respiratory centres mentioned above.

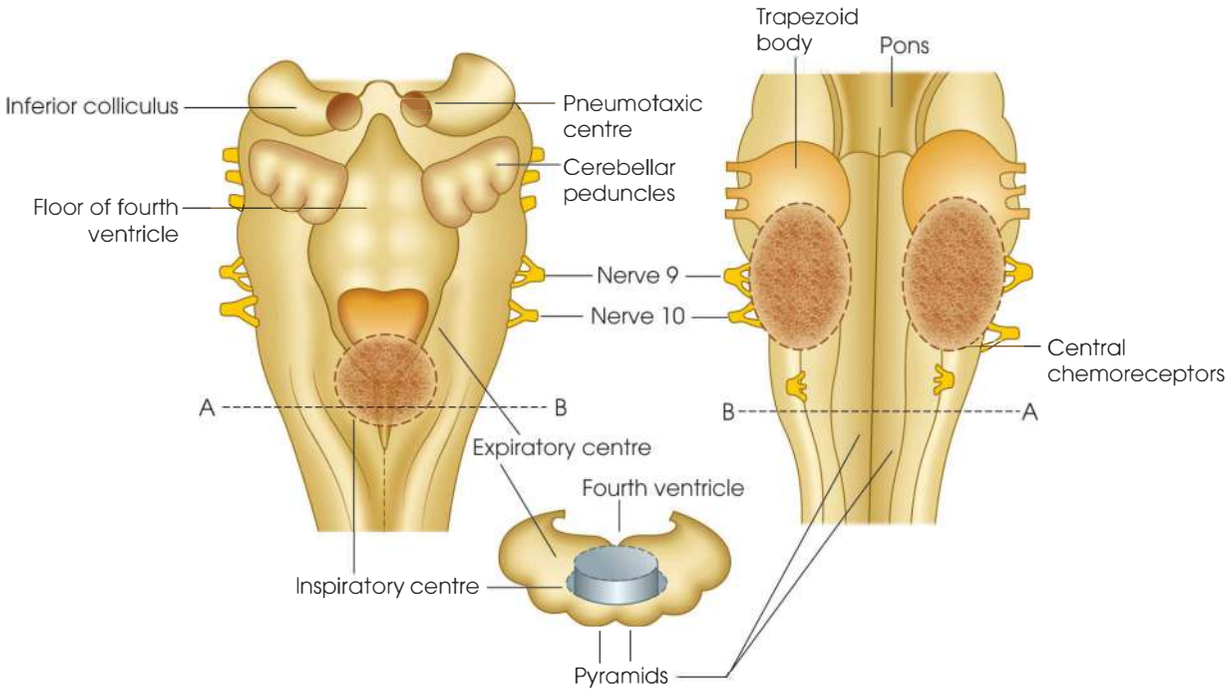


Fig. 48.2: Diagram of the medulla and pons in the cat's brain stem showing areas involved in control of respiration after electrical stimulation with microelectrodes connected to an amplifier

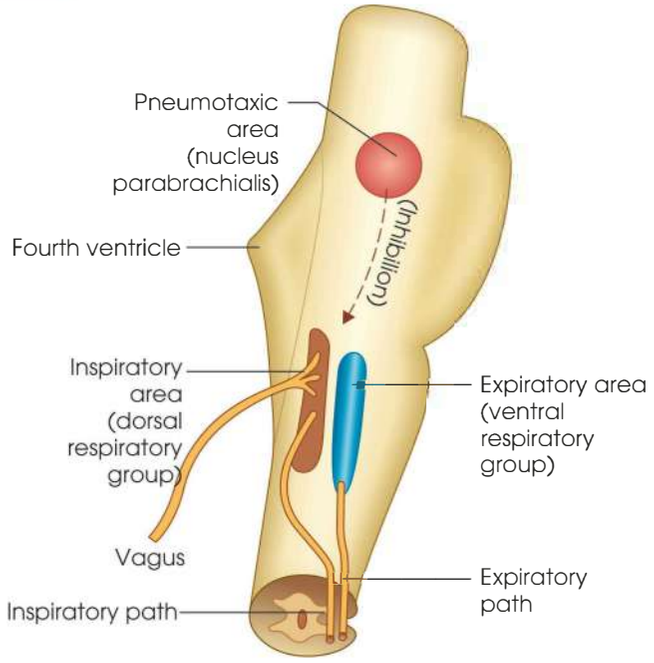


Fig. 48.4: Organisation of the respiratory centre

Mechanism of Rhythmic Respiration (Fig. 48.4 and 48.5)

Inspiration commences by discharge of impulses from apneustic centre to the inspiratory centre. Apneustic centre at the same time sends impulses to the pneumotaxic centre of upper pons. This centre sends excitatory impulses to the expiratory centre which inhibits the inspiratory centre. The inspiratory centre at the same time receives inhibitory impulses from the vagal stretch afferents stimulated by lung inflation during inspiration. Being influenced by the double negative feedback mechanism—the activity of the nerve cells of the inspiratory centre stops and expiration commences passively. The cycle is repeated automatically at the end of expiration because the vagal stretch afferents are no longer active and the reciprocal inhibition of inspiratory centre from expiratory centre comes to an end after the stoppage of activity of the expiratory centre.

NERVOUS CONTROL OF RESPIRATION

Role of Abdominal Muscles in Respiration (Fig. 48.6)

During normal respiration the abdominal muscles are inactive. They become active during:

1. Exercise and other conditions leading to hyperpnoea (increase in rate and depth of respiration).
2. During positive pressure breathing (for example, during anaesthesia when a pump is attached to an intra-tracheal tube).
3. Due to diminution in blood volume in certain low pressure areas of circulatory system.

The atrial mechanoreceptors (and probably receptors in other parts of circulatory system) normally maintain reflexly the tone of the abdominal muscles. The afferent fibres run in the vagus and are separate from those concerned in Hering-Breuer reflexes. Centre for these reflexes is a group of nerve cells separate from those of respiratory centre and has been named as abdominal compression centre (ACC) or abdominal muscle tonus centre. This centre is influenced by the adjoining respiratory centre in reflexes (1) and (2) and in reflexes from the sino-aortic zone, but is affected directly by reflexes from low pressure areas of cardio-vascular system. Amongst the latter the atrial mechanoreceptors have been extensively studied. Fall in pressure in the atria causes increased tonicity of abdominal muscles thereby diminishing the blood volume in the vessels of the abdomen and increasing the blood volume in the atria. Rise in atrial pressure has got opposite effect.

Besides respiration, the ACC also reflexly modifies the tone of the abdominal muscles in such a way that the intra-thoracic blood volume remains unaltered and an effective cardiac output is maintained.

Role of Reflex in Respiration

A. The Hering-Breuer Reflexes

These reflexes prevent over distension of the lungs during inspiration and collapse of the lungs during expiration. The former reflex is known as Hering-Breuer inflation reflex and the latter is Hering-Breuer deflation reflex. The Hering-Breuer reflex is not active in adult humans at rest, but may play a role when tidal volume exceeds 1 litre.

Inflation reflex: The receptors for these reflexes are 'stretch receptors' located in the lungs, mainly the bronchi and bronchioles. They are stimulated during inspiration when the lungs become stretched. The afferent impulses travel in the vagi and are relayed in 'tractus solitarius' before they reach the respiratory centre where they exert an inspiration-inhibiting effect. The inspiration is thus cut off in time and expiration commences (Fig. 48.7).

Section of the vagi abolishes the inflation reflex when the inspiration gets unduly prolonged and the chest is held up in inspiratory position at a time when no air is entering the lungs.

Deflation reflex: The receptors may be described as compression receptors located probably in the alveolar septa. They are stimulated during expiration and reflexly inhibit expiration and reciprocally stimulate inspiration. The afferent fibres are carried in the vagi. The deflation is thus mediated by a separate set of receptors and carried by a separate set of fibres in the vagi. It may be mentioned that during normal expiration the stretch receptors become unstretched resulting in cessation of inhibitory impulses to the inspiratory centre. Since this centre is tonically active, inspiration commences as soon as inspiration-inhibiting impulses resulting from lung inflation ceases.

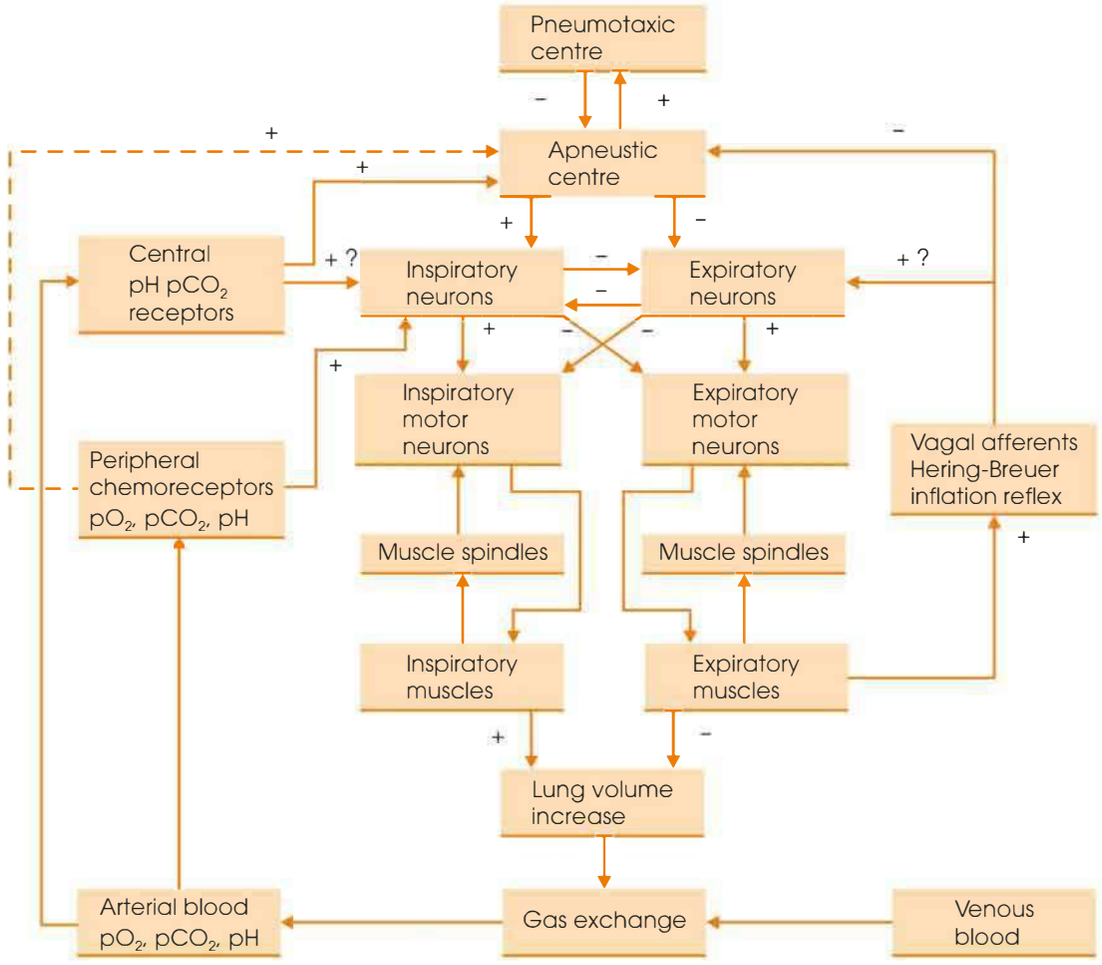


Fig. 48.5: Diagram depicting major mechanism concerned with the control of respiration and the regulation of alveolar ventilation

The inspiration-exciting effect of 'deflation reflex' becomes obvious in condition, such as pneumothorax, hydrothorax, etc. where the degree of collapse of the lungs is more severe and results in stimulation of the respiratory process.

The physiological effect of Hering-Breuer reflexes is to maintain the extent of lung inflation so that the tidal volume tends to fall within a useful range. Indirectly the respiration rate is also adjusted so as to maintain adequate level of pulmonary and alveolar ventilation.

B. Irritant Receptors

The rapidly adapting irritant receptors are located in the airway epithelium. They respond to noxious stimuli such as cold air, dust or smoke. Vagus nerves innervate them and result in bronchoconstriction and hyperpnoea.

C. Impulses from Ascending Tracts of the Spinal Cord (Fig. 48.7)

It has been mentioned that the respiratory centre consists of a specialised group of cells in the reticular formation. The cells of the reticular formation receive impulses from the collaterals of the ascending tracts. It is believed that those sensory impulses from different parts of the body play an important facilitatory role in maintaining normal respiration. In a patient with depressed respiratory centre respiration may be stimulated by peripheral stimulus of any kind, such as slapping the skin. In fact, it is the practice to slap the newborn baby to establish the first respiratory cycle of life.

Stimulation of any afferent nerve causes reflex alteration in breathing, pain fibres are especially potent in this respect, and stimulation of cold fibres such as application of cold water on the face has got well-known excitatory effect on respiration. Heat receptors located on the skin and in the hypothalamus are stimulated by warm blood during exercise and fever and produce hyperventilation.

D. Muscular Proprioceptors

Muscular proprioceptors send afferent impulses to the respiratory centre and markedly stimulate respiration during muscular exercise. Proprioceptors of the

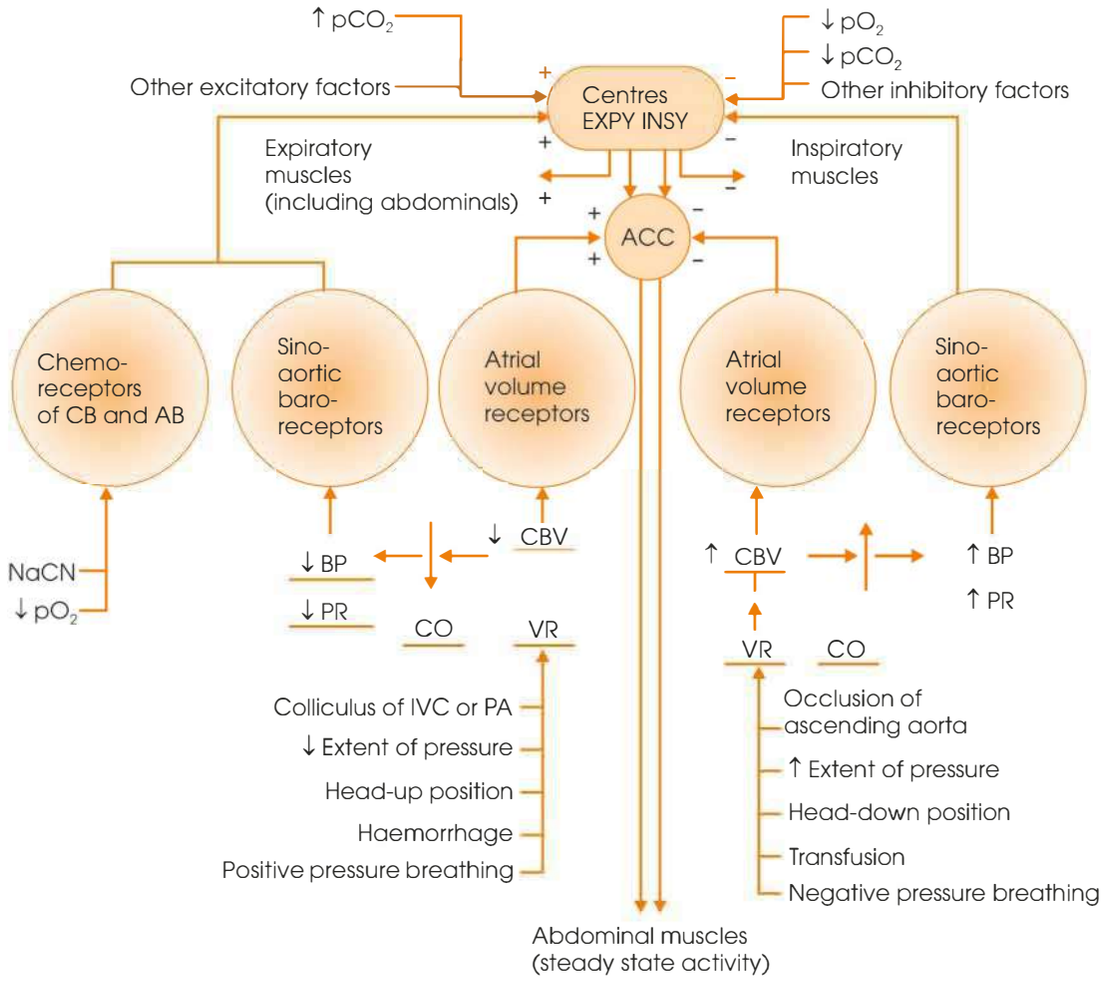


Fig. 48.6: Schematic representation of main factors and mechanisms concerned with altering the level of activity of abdominal muscles in respiratory to cardiorespiratory changes. The left side of the diagram depicts excitatory influences which affect the action of abdominal muscles either through the respiratory centre or more directly. The right side illustrates inhibitory changes which promote a decrease in the activity of abdominal muscles. EXPY—expiratory centre; INSY—inspiratory centre; ACC—abdominal compression centre (abdominal muscle tonus centre); BP—blood pressure (arterial); PR—peripheral resistance; CO—cardiac output; CBV—central blood volume; VR—venous return; IVC—inferior vena cava (post-caval vein); PA—pulmonary artery; – inhibitory effect; + excitatory effect.

respiratory muscle profoundly modify the respiratory movement of the next cycle. Rise in pressure in right atrium and the great veins reflexly augment respiration during muscular exercise and cardiac failure.

E. Deglutition

Glossopharyngeal nerve contains afferent fibres which inhibit respiration during second stage of deglutition.

F. Juxtapulmonary Receptors: J Reflex

Juxtacapillary ("J") receptors: These receptors are located in the alveolar walls along the pulmonary capillaries. They respond to chemicals injected into the pulmonary circulation. They carry impulses via non-myelinated fibres in the vagus to the medulla. Stimulation of juxtacapillary ("J") receptors results in rapid shallow breathing or apnoea.

G. Hiccup

Hiccup is due to reflex spasm of the diaphragm associated with spasm of the laryngeal muscles arising from irritation of sensory nerve endings of the gastro-intestinal tract.

H. Cough Reflex

Coughing is a reflex phenomenon caused by irritation of the mucous membrane of the respiratory tract. It is an important protective reflex in which the irritant material is got rid off from the respiratory tract. At the beginning of the act a deep inspiration is taken, the glottis is then closed and violent expiratory effort is made against a closed glottis causing great rise in intra-thoracic pressure. The glottis is then opened suddenly causing expulsion of the 'cough'—the droplets are ejected with the velocity of a jet plane.

I. Sneezing Reflex

Stimulation of somatic sensory nerves of the nose inhibits respiration and may cause reflex sneezing. The sneeze reflex is similar to cough reflex except that the afferent limb is the trigeminal nerve from the nasal mucosa and while on forceful expiration, the uvula is depressed allowing air to leave through the nose, and the eyes are closed.

J. Reflexes from Sino-aortic Zone

A sudden rise of pressure in this area causes reflex slowing in the rate and amplitude of breathing. If the pressure rise is considerable in experimental animals, apnoea may result (adrenaline apnoea). If the rise in blood pressure is maintained for some time an 'escape' occurs and respiration commences again. This is due to adaptation of the receptors and rise in pCO_2 during apnoea stimulating respiratory centre. Fall in blood pressure stimulates respiration reflexly through the chemoreceptors of the sino-aortic zone.

The reflex slowing of respiration during the rise of blood pressure depresses the 'pump' effect on the abdominal venous reservoirs so that less blood enters the heart and cardiac output is also depressed. Fall of blood pressure is associated with hurried respiration so that more blood is pumped from the abdomen to the thorax which causes an increase of cardiac output. The important role played by abdominal muscles in these responses can be easily understood and is controlled by 'abdominal compression centre' under the influence of respiratory centre.

K. Baroreceptor Reflex

Stimulation of the aortic and carotid body baroreceptors by an increase in blood pressure results in hypoventilation.

L. Distension of Pulmonary Vascular Bed

In experiments where the pulmonary vascular bed was completely isolated but for its nerve supply distension of the vessels caused rapid shallow breathing.

This was abolished by vagotomy proving its reflex nature. This explains rapid shallow breathing in patients suffering from pulmonary congestion.

VOLUNTARY CONTROL OF RESPIRATION

The cerebral cortex can exert a voluntary effect over respiration. If any individual feels restless in stuffy closed room, his cerebral cortex may extend a command on respiration and the individual may voluntarily take deep breaths.

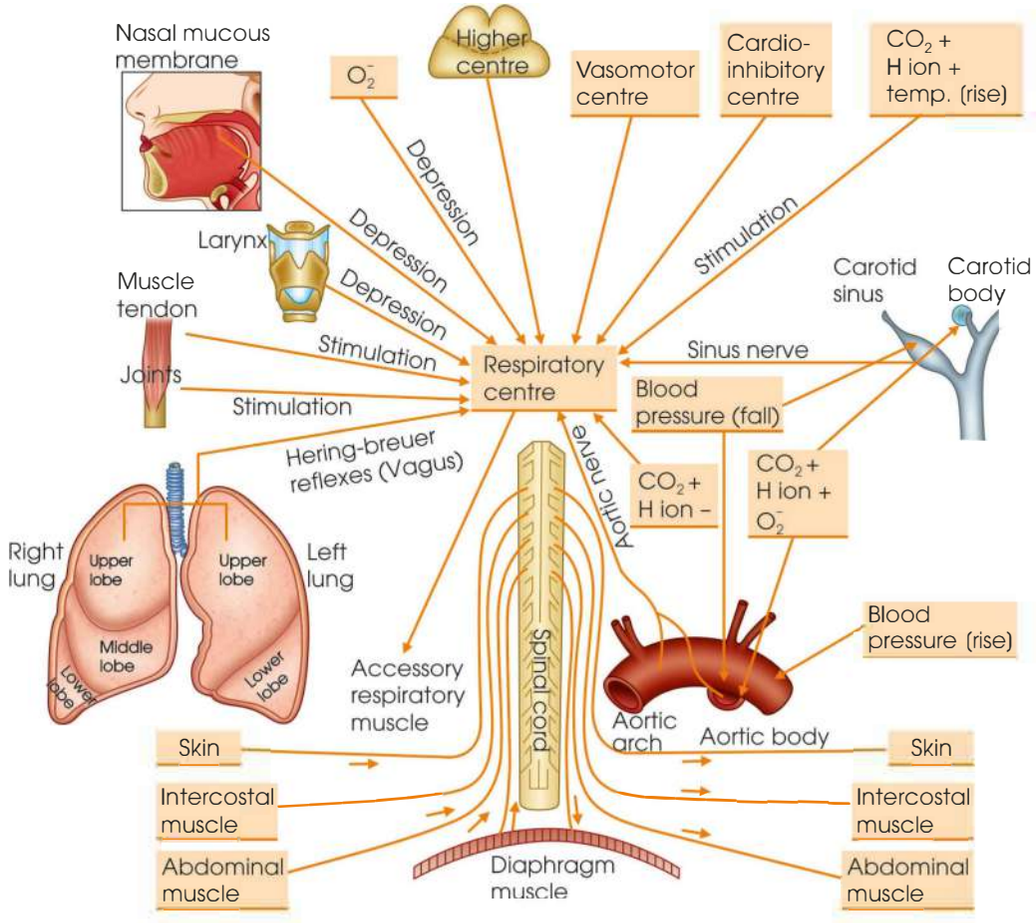


Fig. 48.7: Schematic representation of regulation of the respiratory centre. + means excess;—means less

CHEMICAL CONTROL OF RESPIRATION

CO_2 and Respiration

The powerful stimulant action of CO_2 on respiration has been well established. CO_2 sensitive cells (chemoreceptors) are located in two places:

1. Centrally in the medulla.
2. Peripherally in the carotid and aortic bodies.

1. Central chemoreceptors

- These cells are located bilaterally on the ventral aspect of the medulla and are placed superficially anterior to the point of entry of the glossopharyngeal and vagus nerves. They are highly sensitive to CO_2 change and to change in pH of the blood and also of cerebrospinal fluid (CSF).
- Rise in pCO_2 or fall in pH stimulates these chemoreceptors primarily which again stimulates secondarily the cells of the respiratory centre increasing the volume and frequently of respiration.

Mode of action

1. CO_2 by itself has a little direct stimulant action on respiration. The most potent source of stimulation is rise in H^+ concentration which occurs whenever there is arise in pCO_2 according to the equation $CO_2 + H_2O \leftrightarrow H^+ + HCO_3^-$. H^+ cannot diffuse easily from blood to the brain or to CSF but CO_2 can diffuse freely in either direction.
2. A rise in H^+ concentration of the blood therefore will have a delayed access to the chemoreceptor cells whereas a rise in CO_2 concentration of the blood will be followed very soon by rise in CO_2 concentration of the CSF and tissue fluid in the brain. The H^+ liberated in CSF by dissociation of $H_2CO_3^-$ will have immediate and direct access to the medullary chemoreceptor stimulation of respiration. The H^+ liberated in brain tissues will be 'buffered' in the first instance by the acid–base buffers of the brain and its action on the chemoreceptors will consequently be delayed (Fig. 48.7). It may be noted that there is no significant amount of 'buffers' in the CSF.
3. The initial-stimulating effect of CO_2 on respiration declines after a few minutes. This is due to the fact that HCO_3^- is transported activity from the blood to the CSF and thus the H^+ of the CSF is neutralised to a considerable extent. This explains the diminution of stimulant effect on respiration of CO_2 which is prompt at the onset but declines to about one-fifth of the original effect after about 2 days.
4. From the quantitative point of view CO_2 is more potent a respiratory stimulant than H^+ and further the action of both these factors is very conspicuous in the neighbourhood of their normal value. Thus, the respiratory stimulant effect of CO_2 is most prominent in the neighbourhood of pCO_2 range of 40 to 45 mm Hg and that of H^+ in the range of pH 7.45 to 7.35.

2. Peripheral Chemoreceptors

Chemoreceptor cells are located in carotid and aortic bodies and reflexly stimulate respiration when the concentration of CO_2 of the blood flowing through them is increased. The chemoreceptors of the aortic bodies and carotid bodies are directly sensitive to pO_2, and trigger immediate response when pO_2 falls below 60 mm Hg. The chemoreceptors of the aortic bodies and carotid bodies are also sensitive to pCO_2 and pH, but the effect is not so pronounce as in cases of those of the central chemoreceptors. The chemoreceptors response is conducted from the carotid bodies via the glossopharyngeal nerves, and in the aortic arch is conducted to the medulla via the vagus nerves. This results in increased alveolar ventilation via action on the dorsal respiratory group. The increase in ventilation is considerably damped by fall in pCO_2 during normal gaseous exchange, but in case if pCO_2 does not fall or pH is restored by renal compensation within a few days, then the increase in ventilation is sustained.

Role of central versus peripheral chemoreceptors in normal respiratory drive

Arguments have been raised regarding the relative importance of the central versus peripheral chemoreceptors in normal respiratory drive. It appears that the central receptors are more important in this respect—the peripheral receptors playing a secondary supporting role in conditions of emergency. In animals, after chronic denervation of the carotid and aortic bodies the resting minute volume (RMV) of respiration falls to 80% of normal and consequently the arterial pCO_2 rises by 10 mm Hg. It is, therefore, reasonable to conclude that 80% of the resting respiratory drive of respiration is due to central chemoreceptors and 20% due to peripheral chemoreceptors.

Oxygen Tension and Breathing

This has been studied by making the subject breath O_2 and N_2 mixture containing different proportion of oxygen.

1. Normal atmospheric air contains 20.93% of O_2. The amount of oxygen in the inspired gas mixture can be reduced to about 12% in many individuals without any appreciable change in ventilation.
2. However, at levels of 10% of O_2 in the inspired air the respiration is stimulated with simultaneous reduction of $paCO_2$ as a result of hyperventilation. The low $paCO_2$ has a depressant action on respiration. It is clear, therefore, that respiration-stimulating effect of O_2 lack will be enhanced if suitable measures are taken to prevent the fall of $paCO_2$ by adding appropriate amount of CO_2 to the inspired air.
3. Further a subject breathing low concentration of O_2 will have a low paO_2, a condition analogous to the situation of a subject at high altitude where the low paO_2 is due to fall in barometric pressure. It can be

calculated that the $paCO_2$ of a subject at 5.5 km (18,000 feet) is the same as that of a subject at sea-level breathing 10.5% of O_2. In both the resultant hyperventilation will lead to fall in $paCO_2$ and alkalosis, which is corrected later by the kidneys. It is known that fall in $paCO_2$ by 4 mm Hg causes apnoea and alkalosis depresses respiration. The respiration under these conditions is maintained entirely by O_2 lack or a re-adjustment of sensitivity of respiratory centre to CO_2.

4. In acute exposure to high altitude respiratory drive is maintained by O_2 lack only whereas in persons residing at high altitude for some time the respiratory centre becomes sensitive to low pCO_2.

Mode of Action of Oxygen Lack

1. The respiration stimulating effect of O_2 lack is seen only in intact animals. In an animal with denervated carotid sinus O_2 lack will depress breathing. It has been proved by various workers and is a universally accepted fact that direct action of O_2 lack on respiratory centre is depression of respiration—the respiration-stimulating effect of O_2 lack is a reflex one through the sino-aortic chemoreceptors.
2. The discharge of impulses from the chemoreceptor cells increases with the fall in oxygen tension of the arterial blood—the maximum effect is observed between arterial pO_2 of 60 mm Hg and 30 mm Hg. This is range in which percentage saturation of blood with oxygen falls almost perpendicularly (dissociation curve) and consciousness is lost very soon.
3. The carotid and aortic bodies are most highly vascular structure in the body. Consequently, the arterio-venous oxygen content difference is very low. In hypotension with blood pressure near about 60 mm Hg the blood flow through these structures becomes very slow and sluggish and the arterio-venous O_2 content difference becomes high indicating low pO_2 within the chemoreceptor cells. Under these conditions there occurs reflex stimulation of respiration (and also vasoconstriction) which tends to restore the blood pressure to near normal value.
4. In conclusion it may be said that under normal conditions the level of pulmonary ventilation is controlled by three chemical agencies:
   a. Rise in arterial pCO_2
   b. Fall in arterial pO_2
   c. Decrease in arterial pH.
5. Any one of these factors may play a major role in driving respiration. It seems that above a certain range (approximately 75 mm Hg pO_2 of inspired air)—O_2 lack has no effect on respiration, similarly below a certain range (approximately 33 mm Hg pCO_2)—CO_2 has no effect on respiration. Under ordinary circumstances the effect of above three factors are additive but one of three factors is enough to augment ventilation in the absence of other two. For example, in metabolic acidosis there is great increase in H^+ concentration of the blood, the pO_2 being normal and pCO_2 below normal. However, the RMV is greatly augmented in this condition.

VENTILATION DURING EXERCISE

Chemical Factors

It has been postulated that the three chemical factors, viz. O lack, CO excess and increased H ion concentration play an important role in augmenting the ventilation during muscular exercise. However, it has not been possible to demonstrate any significant alteration in arterial pCO_2, pO_2 or pH in muscular exercise which means that the chemical factors have got a little role in initiating or maintaining the hyperventilation of exercise. On the other hand, it has also been observed that inhalation of O_2 during muscular exercise reduces the RMV significantly. This can only be explained on the presumption that hypoxic drive is certainly a factor in exercise hyperventilation, even though the arterial pO_2 does not fall below its normal value. This is due to change in sensitivity of the arterial chemoreceptors to pO_2 and it has been suggested that these become hypersensitive to normal pH and pCO_2 as well.

Nervous Factors Influencing Respiration

1. Cerebral Cortex

During transmission of impulses to the muscles involved in exercise the pyramidal tract gives collaterals to the respiratory centre as well as to the vasomotor and cardiac centre, which are specialised parts of the reticular formation. This explains the anticipatory rise in RMV, actually before the exercise commences. It has been shown that at the commencement of exercise the alveolar ventilation may rise to a level so as to reduce the alveolar pCO_2. Very soon, however, large amounts of CO_2 formed by the exercising muscles restore the alveolar pCO_2 to normal level, and the alveolar ventilation is stabilised according to the alveolar pCO_2.

2. Proprioceptive Impulses from the Muscles

In an anaesthetised person passive movement of the limbs increases the RMV. It is reasonable to conclude, therefore, that during muscular exercise proprioceptive impulses from the muscles and the body acts on the respiratory centre and augments respiration.

The nervous factors, viz. direct stimulation from cerebral cortex and reflex proprioceptive stimulation from the muscles perhaps, play more important role in initiating and maintaining the appropriate level of ventilation during exercise so that the muscles get an adequate supply of O_2 and the excessive CO_2 and lactic acid formed are disposed off. Any slight deviation from

the appropriate level is rectified by the chemical factors. Rise in body and blood temperature during exercise also stimulates respiration through the hypothalamus.

PERIODIC BREATHING

This means periods of hyperventilation followed by apnoea repeated in succession. Two types of periodic breathing have been described:

1. *Cheyne-Stokes breathing:* In which the period of apnoea is followed by a phase of gradually rising ventilation followed by gradual fall and the cycle is repeated, each phase lasting about 30 seconds.
2. *Biot's breathing:* It consists of runs of several normal respiration followed by apnoea.

Cheyne-Stokes Breathing (Fig. 48.8)

Cheyne-Stokes breathing sometimes occurs in normal children or adults during sleep or at high altitude. Diseases in which Cheyne-Stokes breathing occurs are:

1. Elevated intra-cranial pressure from any cause, e.g. brain tumour, meningitis, etc.
2. Hypoxia from heart failure or pulmonary disease.
3. Uraemia and other toxic conditions in which the respiratory centres are depressed.

Mechanism of Cheyne-Stokes Breathing

Cheyne-Stokes breathing is associated with rise of blood pressure sometimes in the apnoeic phase and sometimes later. Rise of intracranial pressure causes hypoxia of respiratory centre as well as vasomotor centre, the former is depressed and the latter is stimulated, the depression of respiratory centre is followed by apnoea.

In Cheyne-Stokes breathing the respiratory centre is less sensitive to CO_2. Arterial pCO_2 of 40 mm Hg produces augmentation of ventilation to only about 50% of the expected value. Anoxic stimulation of ventilation reflexly through the sino-aortic zone perhaps plays a role but denervation of the sino-aortic area does not abolish the Cheyne-Stokes type of respiration. Administration of O_2 improves metabolism of nerve cells of the respiratory centre and administration of CO_2 stimulates respiratory centre. So, both the procedures abolish the abnormal type of breathing.

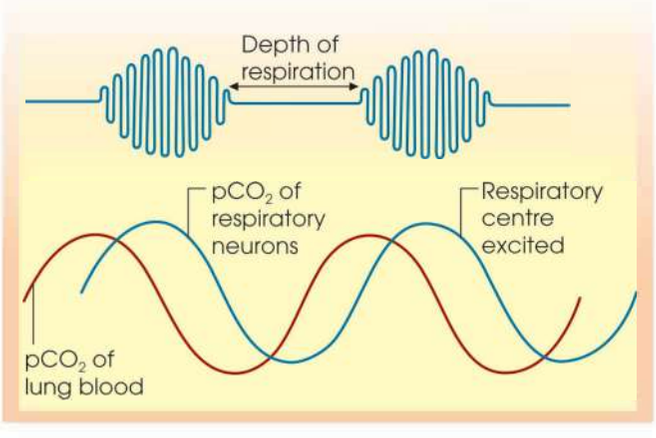


Fig. 48.8: Periodic Cheyne-Stokes breathing

Dr John Cheyne
1777–1836

William Stokes
1804–1878

Cheyne-Stoke respiration is known after John Cheyne and William Stokes, the physicians who first described it in the 19th century.

EXAM-ORIENTED QUESTIONS

Essay

1. Describe the nervous regulation of respiration.
2. Describe the chemical regulation of respiration.

Short Notes

1. The Hering-Breuer reflexes
2. Cheyne-Stokes breathing
3. Biot's breathing
4. Periodic breathing

Chapter

49 Hypoxia

DEFINITION

Hypoxia is a condition characterised by inadequate or decreased supply of oxygen to the tissues because of extrinsic reasons. The term 'Hypoxia' is used synonymously with hypoxia.

Classification of Hypoxia

1. **Hypoxic type of hypoxia or arterial hypoxia:** This type is characterised by low oxygen tension in the arterial blood.
2. **Anaemic type of hypoxia:** This type is characterised by low oxygen content of the blood due to diminished quantity of haemoglobin or non-functionating haemoglobin.
3. **Stagnant hypoxia or hypokinetic hypoxia:** This type is characterised by decreased rate of blood flow through the tissue. The oxygen content and tension of the arterial blood is normal but that of the venous blood is abnormally low due to sluggish circulation.
4. **Histotoxic hypoxia:** This means inability of the tissues to utilise oxygen and occurs in cyanide poisoning. Anoxaemia is a term which means diminished oxygen in the blood.

Anoxic Hypoxia or Arterial Hypoxia

The main cause is diminished pO_2 of the arterial blood which, of course, results in diminished saturation of haemoglobin with oxygen. The total oxygen content of the arterial blood, therefore, is lower than normal, since the O_2 tension in the tissues is normally low (40 mm Hg), it may be that in spite of diminished pO_2 the arterial blood may unload its normal quota of oxygen to the tissues resulting in lower pO_2 and low oxygen saturation of haemoglobin in the venous blood than would occur normally (Table 49.1).

O_2 content of the mixed venous blood is reduced from 15 volume % to 12 volume %. The final value of O_2 in the mixed venous blood, of course, depends on degree of hypoxia.

Causes of Arterial Hypoxia

Diminished O_2 tension in the inspired air may occur due to:

1. Admixture with foreign gases like CO or CH_4, etc. at the bottom of the mines.
2. High altitude due to low barometric pressure—the partial pressure of O_2 in the alveoli is diminished. Diseased conditions in which the ventilation/perfusion ratio (normal 0.8) is disturbed. This may be due to ineffective ventilation (dead space effect), e.g. in advanced pulmonary emphysema, asthma, obstruction of air passages, or due to venous-admixture effect as may occur in pneumonia or septal defect of the heart.
3. Diseased conditions in which there occurs defective diffusion across the alveolo-capillary membrane, e.g. pulmonary oedema or alveolo-capillary block syndrome.

Anaemic Hypoxia

The characteristic feature of this type of hypoxia is diminished quantity of functioning haemoglobin in blood. The oxygen content of blood, therefore, is

Table 49.1: O_2 content of the mixed venous blood is reduced from 15 volume to 12 volume %. The final value of O_2 in the mixed venous blood, of course, depends on degree of hypoxia

	Arterial blood			*Venous blood*		
	pO_2	*O_2 content*	*O_2 saturation*	*pO_2*	*O_2 content*	*O_2 saturation*
Normal	100	19.3	95%	40	15	75%
Arterial hypoxia	60	17.2	90%	30	12	60%

diminished proportionately to the reduction of haemoglobin but the oxygen tension of blood and percentage saturation of haemoglobin with oxygen is normal.

Causes

1. *Anaemia* from any causes.
2. *CO poisoning:* Here a large amount of haemoglobin remains combined with CO and as such is not available for oxygen carriage.
3. *Altered haemoglobin*, e.g. methaemoglobin found after poisoning with chlorates, nitrites, ferricyanide and acetanilide, etc.

Anaemic hypoxia, is of moderately severe intensity, is attended with increased cardiac output, that is increased blood flow through the tissues so as to compensate to some extent for the diminished O_2 content of blood.

Stagnant Hypoxia or Hypokinetic Hypoxia

The characteristic feature of this condition is diminished blood flow through the tissues due to sluggish circulation. The cardiac output is low but the O_2 content and tension of the arterial blood is normal. Due to sluggish circulation after the tissues abstract normal amount of O_2 they need—the venous blood is considerably reduced, with its pO_2 much less than normal. Consequently, the pO_2 of the tissue fluid and tissues in general is reduced which has got a damaging influence on the tissues.

Myocardial infarction is an example of localised hypoxia resulting in loss of function which leads to generalised hypoxia and worsening of the ischaemia of the heart.

Causes of Stagnant or Hypokinetic Hypoxia

1. Congestive cardiac failure
2. Haemorrhagic shock
3. Obstruction to venous return.

Histotoxic Hypoxia

This type of hypoxia is due to poisoning with cyanides or sulphide in which the respiratory enzymes are inhibited and so the tissues cannot utilise O_2 of the blood which contains adequate amount of O_2 at normal tension.

Altitude Hypoxia

Hypoxia at high altitude is due to low barometric pressure. The percentage composition of atmospheric air in so far as O_2 and N_2 concentration is concerned is same at all altitude but due to fall in barometric pressure at high altitude there occurs a disproportionate fall in pO_2 of the alveolar air and consequent percentage saturation of haemoglobin with oxygen.

Respiratory Changes in Hypoxia: Acute and Chronic at High Altitude

Hypoxia of gradual onset occurs in mountaineering and the respiratory responses of a subject to such type of hypoxia differs to some extent depending on whether the subject is conditioned for high altitude atmosphere (acclimatised subject) or ascending to high altitude for the first time and is unacclimatised for the high altitude environment.

Respiration in Altitude Hypoxia in Unacclimatised Subjects

1. Breathing 14% O_2 in N_2 (simulated altitude 3 km or 10,000 feet) produces practically no change in respiration. When the oxygen in the inspired air is brought down to 10% (simulated altitude about 5.5 km or 18,000 feet) there occurs 8% increase in ventilation rate over the resting value. Respiration is doubled when the respired air contains 8% oxygen in nitrogen. Hypoxia, therefore, is not so effective a stimulant to respiration as is excess of CO_2.
2. Anoxic stimulation occurs reflexly through the chemoreceptors of the sino-aortic area. The anoxic hyperventilation, however, is associated with fall in alveolar and arterial pCO_2 and with (H^+) in blood, and also in the cerebrospinal fluid and interstitial fluid of the brain which bath the CO_2 sensitive chemoreceptor cells which form part of the respiratory centre complex of the brain stem. This causes depression of respiration.
3. However, if the anoxic stimulus is sufficiently intense the chemoreceptor reflexes from the sino-aortic area assumes a prepotent role in maintaining hyperventilation in spite of the fall in pCO_2 and (H) resulting from anoxic hyperventilation.
4. Further, it has been proved that the chemoreceptor cells of the respiratory centre become hypersensitive to CO_2 and (H^+) in presence of oxygen deficiency (Fig. 49.1) which indicates that the respiratory stimulant effect of CO_2 is significantly greater when the alveolar pO_2 is maintained at a steady value of 40 mm Hg than when it is kept at its normal value of 100 mm Hg. The slope of V/PCO_2 line increase as the alveolar pCO_2 is lowered. Hypoxia, therefore, increases the sensitivity of respiratory chemoreceptors to CO_2 and (H^+).

Respiration in Subjects Exposed to Hypoxia of Long Duration (Acclimatised Subjects)

The above discussion applies to person exposed to hypoxia for the first time (acute hypoxia).

1. The respiratory pattern of subjects exposed to hypoxia for a long time or who are permanent residents of high altitudes (acclimatised subjects) is shown by the lower curve in the same diagram. As

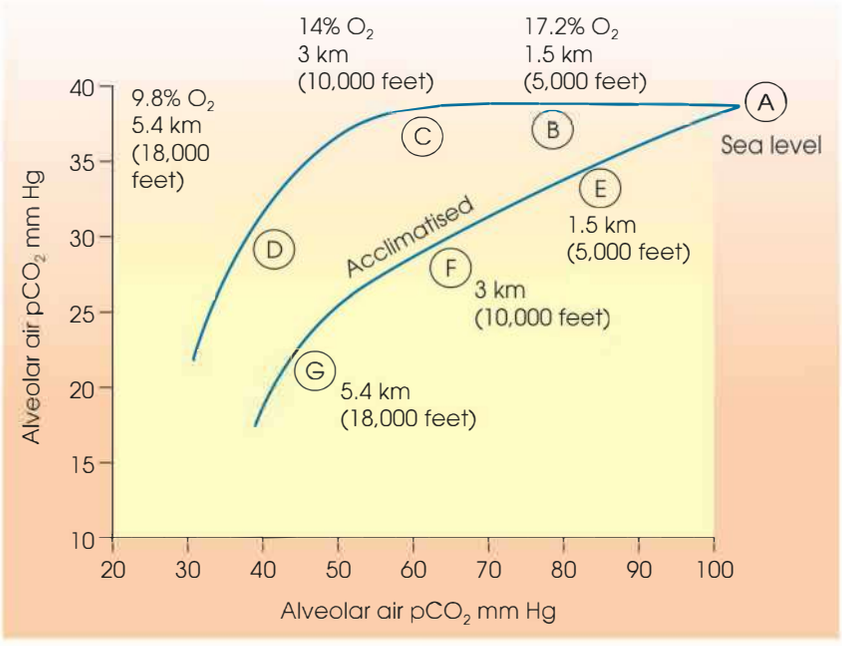


Fig. 49.1: The alveolar air gas tensions of subjects exposed to chronic and acute hypoxia

can be seen from the diagram the alveolar pCO_2 value of an acclimatised subject begins to fall from an altitude of 1.5 km or 5000 feet upwards and is always lower than that of an unacclimatised subject. Thus, at an altitude of 5.4 km or 18,000 feet the alveolar pCO_2 value of an acclimatised subject is only 22 mm Hg as compared to 30 mm Hg of an unacclimatised subject. This of course, is due to the fact that the acclimatised subjects hyperventilate more than his unacclimatised friend in spite of the consequent fall in alveolar pCO_2. The alveolar pO_2 rises consequently with obvious advantage for better oxygenation of venous blood.

2. CO_2 being highly soluble and diffusible the changes in pCO_2 of the blood is more or less the same as that in the cerebrospinal fluid (CSF) but the (HCO_3) in the CSF is controlled independently and that active transport of HCO_3^- occurs from the CSF to blood in hypoxia so that the fall in (HCO_3) is always more marked in the CSF than the blood in cases of low arterial and CSF pCO_2. Thus (H^+) of the CSF and interstitial fluid of the brain is quickly brought back to normal level so that the chemoreceptors of the brain stem influencing the respiratory centre can function properly in spite of the fall in pCO_2 of alveolar air and arterial blood.
3. Thus, 4 subjects were studied at an altitude of about 3.8 km or 12,500 feet. In 24 hours, the pCO_2 of the arterial blood fell on an average from 40 to 30 mm Hg and the pH increased from 7.43 to 7.48. The pCO_2 of the CSF fell to the same extent, i.e. by 10 mm Hg but there was no increase in pH of the CSF. This was due to remarkable fall in (HCO_3) of the CSF to the extent of 5 mEq/L compared to about 2 mEq/L in blood. The stabilisation of (H^+) in the CSF and tissue fluid of the brain was thus achieved by active transport of (HCO_3) from the CSF to the cerebral capillaries. The sensitivity of the bulbar chemoreceptors acting on the respiratory centre was thus unaffected in spite of fall in pCO_2 of the CSF and brain.
4. It may be summarised, therefore, that during acclimatisation the low pCO_2 of the CSF and interstitial fluid of the brain resulting from anoxic hyper-ventilation is counteracted by active secretion (HCO_3) from the CSF. and tissue fluid of the brain to the blood so that the (H^+) of the CSF and brain remains unaltered and that the activity of the respiratory chemoreceptors is not depressed.
5. After some time, however, kidneys also play an important role in adjustment of pH of the blood and CSF by excreting urine with high pH value. The advantage of this hyperventilation is obvious because it enables to maintain high alveolar pO_2 and ensures more effective oxygenation of venous blood. In residents of high altitude—the lung volume is increased so that the lungs can hold more air. Further the alveolar wall is stretched out facilitation gaseous interchange. More pulmonary capillaries open up increasing the surface area for diffusion.

Oxygen Therapy in Hypoxia

Three methods are commonly employed in administration of oxygen. These are:

1. **Nasal catheter:** It is possible to raise the alveolar pO_2 from its normal value 100 to 600 mm Hg by this method.
2. **Oxygen tent:** The patient is put in an atmosphere enriched with oxygen. The alveolar pO_2 usually goes up to about 300 mm Hg.

3. **Oxygen mask:** Either pure oxygen on high concentration of oxygen is breathed through a mask. Sometimes a specially designed mask (BLB mask) is used in which part of the oxygen in the expired air is utilised for rebreathing.
   - In altitude hypoxia or hypoxia due to presence of foreign gases in the atmosphere at high concentration administration of pure O_2 is of great value and offers absolute protection except when the altitude is very high and O_2 must be delivered through a pressure mask.
   - In hypoxia due to alveolar hypoventilation breathing, 100% O_2 can increase the alveolar concentration of oxygen 5 times the normal value and thus is extremely beneficial.
   - In alveolo-capillary block syndrome oxygen therapy is extremely beneficial because the rise in concentration of oxygen in the alveoli raises the diffusion gradient and thereby facilitates diffusion of oxygen across the diffusing membrane.
   - In anaemic hypoxia, the functionating haemoglobin is low but the oxygen saturation of functionating haemoglobin is normal and pO_2 of plasma is normal. Oxygen therapy, therefore, is of limited benefit because only the O_2 in solution can be increased by O_2 therapy, the O_2 content is a little affected.
   - In hypokinetic hypoxia, the O_2 content and tension of arterial blood leaving the lung is normal and so O_2 inhalation is of limited value. However, the normal amount of O_2 in solution in arterial blood is 0.3 ml per 100 ml. This can be increased to 1.8 volume of O_2 per ml of blood when the alveolar pO_2 is raised to 600 mm Hg by O_2 inhalation. This means only an increase of about 10% of O_2 content at high pressure and may mean the difference between life and death.
   - In histotoxic hypoxia, the enzymes are paralysed so that the tissues fail to pick up oxygen from blood therapy, therefore, are of no avail.

Danger of Oxygen Therapy in Hypoxia

In patients suffering from chronic hypoxia, the respiration is maintained by reflex stimulation of the sino-aortic bodies and the sensitivity of the chemoreceptors to CO_2 is diminished. Sudden relief of hypoxia by oxygen therapy may produce hypoventilation with hypercapnia or hypercarbia and unconsciousness which may lead to death.

DYSPNOEA

Dyspnoea means difficulty in breathing associated with a sense of distress. It is to be differentiated from hyperpnoea which simply means hyperventilation, as occurs in muscular exercise, and is usually not associated with sense of distress, unless, of course, the exercise very severe.

The factors responsible for dyspnoea may be classified into three categories.

a. *Alteration in chemical composition of blood:*
   1. Hypoxia
   2. Hypercapnia or hypercarbia
   3. Increased H ion concentration.

b. *Excessive work of the respiratory muscles to inflate and deflate the lungs so as to provide adequate ventilation:*
   1. Diminished lung compliance, e.g. in congestion, fibrosis, etc.
   2. Abnormalities in chest wall and diaphragm.
   3. To provide increased demand for O_2 in diseases associated with high metabolic rate.

c. *Psychogenic dyspnoea:* Due to impulses from higher centre.

Dyspnoea due to Alteration in Chemical Composition of Blood

1. O_2 lack from causes enlisted under arterial hypoxia
2. Hypokinetic hypoxia interferes with transport of respiratory gases due to sluggish circulation as occurs in cardiac failure.
3. Anaemic hypoxia, if severe, will lead to O_2 lack in the body.

Factors Affecting Diffusion of Gases Across the Alveolo-capillary Membrane (Alveolo-capillary Block Syndrome)

Stimulation of respiration leading to dyspnoea by the factors mentioned above occurs reflexly via the sino-aortic mechanism. It is believed that the Hering-Breuer reflex also becomes overactive in these conditions, which also augment respiratory rate and supplements the discomfort of dyspnoea. The CO_2 excess and respiratory acidosis occurs in alveolar hypoventilation associated with decrease in ventilation/perfusion ratio. The causes listed above are usually not associated with CO_2 retention because CO_2 diffuses at least 20 times more rapidly than O_2.

Metabolic acidosis will also produce dyspnoea by stimulating respiratory centre both directly as well as reflexly in the same way as CO_2 excess.

Excessive Work of the Respiratory Muscles

Obstruction of the larynx or bronchi as in diphtheria or asthma will produce dyspnoea due to excessive work of the respiratory muscles to overcome the resistance. O_2 lack and CO_2 excess due to hypoventilation will also add to the discomfort of dyspnoea by reflex and direct effect on respiratory centre. Reduced distensibility of the lungs as occurs in oedema, congestion, fibrosis, and inflammation of the lungs. Due to diminished

distensibility of the lungs, i.e. diminished compliance, the respiratory muscles are to work hard to maintain adequate ventilation through the rigid alveoli. The Hering-Breuer reflex is said to be overactive in these cases and so accelerate the rate of respiration.

Applied Physiology

1. Dyspnoea on exertion or even at rest in advanced cases is the commonest symptom of heart disease, e.g. mitral stenosis. It has been proved to be not due to arterial hypoxia or hypercapnia. Engorgement of pulmonary capillaries leading to diminished—distensibility of lungs is the prime cause of cardiac dyspnoea. When pulmonary oedema supervenes, hypoxia will complicate the picture. It has been mentioned elsewhere that distension of pulmonary capillaries causes rapid shallow breathing.
2. Conditions which cause limitation of movement of the diaphragm or chest wall will lead to distressed breathing resulting from undue effort of the muscles of respiration. In emphysema, for instance, the elasticity of the lungs is lost and the chest is held up in inspiratory position during rest. The diaphragm is fixed in an elevated position. Inspiration can, therefore, be effected only by unusual effort of the inspiratory muscles. Expiration is also effected by active contraction of the muscles of expiration.
3. In diseases associated with high metabolic rate, e.g. thyrotoxicosis there is an increased demand for O_2 in the tissues and also an increased demand for elimination of CO_2. This demand is increased during muscular effort. In such patients the respiratory muscles are to work more to meet the increased demand for oxygen and such these patients are liable to be dyspnoea on slight exertion.
4. In severe diabetic acidosis, Kussmaul breathing, or air hunger may be observed in comatose subject.

Psychogenic or Emotional Dyspnoea

Many persons of neurotic disposition are apprehensive that they may not be able to get sufficient quantity of air if they enter a overcrowded room. These persons develop 'air hunger' on entering a crowded room, e.g. cinema hall and sometimes hyperventilates to such an extent that they develop alkalosis and tetany in a crowded place or rather 'stuffy' atmosphere.

EXAM-ORIENTED QUESTIONS

Essay

1. Define hypoxia. Classify hypoxia and discuss the cause for the same. Add note on oxygen therapy in hypoxia.
2. Discuss the pattern of respiration in subjects exposed to hypoxia of long duration (acclimatised subjects).

Short Notes

1. Factors affecting diffusion of gases across the alveolo-capillary membrane (alveolo-capillary block syndrome)
2. Anaemic hypoxia
3. Anoxic hypoxia
4. Stagnant hypoxia
5. Histotoxic hypoxia
6. Oxygen therapy in hypoxia
7. Dysponoea

Chapter

50

Compressed Air Sickness

DYSBARISM (CAISSON DISEASE)

Divers, who work under water, generally work in caissons. A caisson is a steel chamber, filled with compressed air in which the divers are placed and sunk in deep waters. Thus, the workers are exposed to air at high pressure.

1. At the outset, there may not be much discomfort. Temporary dizziness, noises in the ears frequency of micturition, slowing of respiration and slowing of pulse rate may occur.
2. But when the subject comes out of the chamber and returns to normal atmospheric pressure (i.e. during rapid decompression) serious effects appear which may set in immediately or after some time. In milder cases, a sensation of pain is felt. It is felt especially in the joints, so that the subject adopts an attitude of flexion.
3. In severe cases there may be: Cyanosis, small running pulse showing circulatory failure, paralysis (usually of the legs), unconsciousness, which may lead to death. The phenomenon is explained as follows: The air being breathed under high pressure, the amounts of oxygen and nitrogen, carried in physical solution in plasma are much more than normal.
4. When the pressure is reduced during decompression this extra amount can no more be held in physical solution and the gases come out in the form of bubbles. The oxygen is rapidly used up by the tissues and does not cause much harm. But the nitrogen bubbles cannot be disposed of in this way and may block the capillaries in any locality (causing gas embolism).
5. It may accumulate in the heart and be frothed up in the blood stream. This prevents the cardiac action and leads to circulatory failure. The pain is due to local gas embolism. The unconsciousness and paralysis are due to bubbles of nitrogen in the nervous system. Nitrogen being about five times more soluble in fats than in water and the nervous system being especially rich in lipids (especially the medullary sheaths), bubbles of nitrogen evolve in the central nervous system and are particularly prominent at the junction of white and gray matter. So that the nervous system is very easily affected in dysbarism (caisson disease).
6. The percentage reduction of pressure is more important than absolute reduction. Thus, a sudden reduction to less than 45% of original pressure in which the subject was placed will lead to dysbarism. Thus, a diver originally exposed to a pressure of 8 atmospheres can be subjected to quick decompression of 4 atmospheres without any harmful effect. After some time the pressure may be reduced to 2 atmospheres and then after equilibration at this pressure can be brought back to 1 atmosphere.
7. Obviously dysbarism may occur in rapid ascents to high altitude. About 9 km or 30,000 feet are the critical altitude where symptoms of dysbarism are first felt because the barometric pressure here (226 mm Hg) is less than 45% of that at sea level. This can be avoided by inhalation of pure O_2 before the flight so that the system gets practically N_2-free. Oxygen inhalation is to be continued during the flight.

The pressure within the Russian space-ships is kept at about 1 atmosphere in a hermitically sealed cabin containing N_2 and O_2 in the same proportion as these are present in the atmosphere. The space-ships move through perfect vacuum and any leak in the cabin either in the door or in the wall will cause dysbarism. In fact, two Russian astronauts lost their lives due to this cause because they failed to lock the cabin door properly during their return journey. In order to prevent the occurrence of this disease, decompression should be very gradual and if symptoms have already appeared the subject should be immediately exposed to compressed air.

EXAM-ORIENTED QUESTION

Short Note

1. Dysbarism (caissons disease)

Chapter

51

Respiration in Abnormal Conditions

ASPHYXIA

Definition

Improper aeration of blood, if continued for some time in an intact animal, produces a series of pathological manifestations and ultimately death. These manifestations are collectively called asphyxia.

Classification

1. **General:** Such as, by occlusion of the trachea, pneumothorax, etc.
2. **Local:** As by ligature of blood vessels supplying a particular locality.

The phenomenon of asphyxia has been divided into three stages, each stage showing characteristic features.

Essential Conditions of Asphyxia

1. There must be both CO_2 excess as well as O_2 lack
2. Animal must be intact (if the sino-aortic nerves are cut, typically asphyxia will not be obtained).
3. The improper aeration must be continued (Fig. 51.1).

The phenomenon of asphyxia has been divided into three stages, each stage showing characteristic features. The whole phenomenon, from the onset to death, takes about 5 minutes. The stages, their manifestations and their causes are given in Table 51.1.

HYPERPNOEA

The term hyperpnoea means increased breathing. Any rise in the quantity of air breathed per minute is called hyperpnoea.

Causes

1. Voluntary
2. Impulses from the cerebral cortex to the respiratory centres, e.g. emotion.
3. Impulses from the hypothalamus.
4. Reflexly by the stimulation of nerves of general sensation (pain, heat, cold, etc.) in the skin.
5. All conditions where metabolic rate is very high demanding more oxygen supply and producing

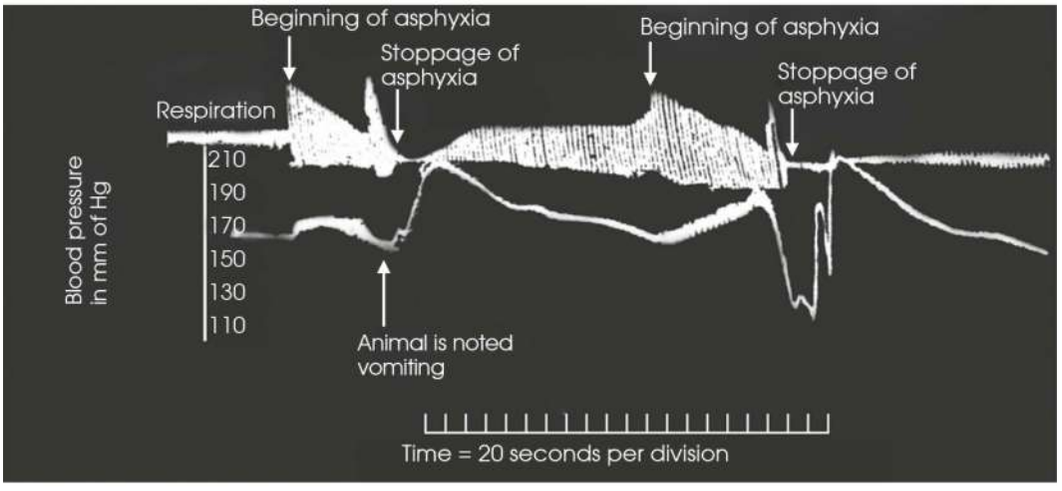


Fig. 51.1: The tracing shows the increase of blood pressure in intact animal during artificial asphyxiation of anaesthetised cat

more CO_2 for elimination, for instance muscular exercise.

6. Factors causing dyspnoea will evidently cause hyperpnoea.

Voluntary hyperpnoea, being of special interest, is discussed in detail below.

Effects of Voluntary Hyperpnoea

When a person, at bodily and mental rest, goes on breathing deeply and quickly (causing over ventilation) for about 3 minutes and then stops, the following changes are found to take place:

1. Breathing remains suspended for a period (apnoea). This is due to the washing-out of excess CO_2 and consequent reduction of CO_2 in the arterial blood. After this period of apnoea, periodic breathing (alternate breathing and apnoea) may follow for some time and then normal breathing is restored.
2. Alkaosis, due to washing out of excess CO_2, blood becomes more alkaline.
3. Increased excretion of alkaline urine containing bicarbonates. This is due to failure of the renal tubular cells to provide sufficient H^+ due to washing-out excess CO. Normally secretion of H^+ and absorption of HCO^- are interdependent. In the absence of secretion of H^+, HCO^- and Na are eliminated in the urine. Presence of keto acids in the urine.
4. Skin vessels are constricted and the skin is white and cold.
5. Increase in cardiac output and a slight rise of blood pressure.
6. Dizziness and paraesthesia of the extremities. The consciousness may be dulled. The development of the cerebral symptoms is due to hypoxia which causes cerebral vasoconstriction.
7. Tetany (may be due to alkalosis). This is due to failure of renal tubular cells to secrete H^+. As a result NH_3 manufactured by the renal distal tubular cells cannot form ammonium salts. Keto acids as such are eliminated in the urine.

ORTHOPNOEA

With severe congestive cardiac failure dyspnoea occurs even at rest. The patient feels more comfortable in the sitting position than when lying down. This condition is called orthopnoea. The subject passes the whole day and night, being propped up by pillows or sleeping on an orthopnoeic table. Many theories have been advanced to explain the relative comfort in upright position. Some of the theories are as follows:

1. Removal of the weight of the enlarged liver and other abdominal viscera and thus facilitating the descent of the diaphragm.
2. In the sitting position there is greater drainage of blood from the medulla and consequently better blood flow through the respiratory centre.
3. The vital capacity in the upright position is about 25% more than in the recumbent position.
4. Better drainage of blood from the lungs and consequent relief of the pulmonary congestion. This increases the dispensability of the lungs and thus renders the Hering-Breuer reflex less sensitive.

Cyanosis

Cyanosis is a clinical condition in which the skin and mucous membranes assume a bluish colour. It may be local or general. When general, it is best seen at the lips, nose, cheeks, ears, hands and feet. It is caused by an alteration in the character of blood circulating in the capillaries. Hence, when blood is pressed out of the part, cyanosis temporarily disappears.

It depends upon the absolute amount of reduced haemoglobin present in the blood. It may also be due to the presence of other haemoglobin derivatives having a darker colour (e.g. methaemoglobin and sulphaemoglobin). At least 5 gm of reduced haemoglobin must be present (i.e. 5 × 1.34 ml = 6.7 ml of oxygen should be less per 100 ml of blood) before cyanosis can be produced. Consequently, in an anaemic subject containing less than 5 gm of haemoglobin (i.e. below 33% Hb) cyanosis is not possible.

Factors Causing Cyanosis

1. Inadequate oxygenation of blood in the lungs due to:
   a. Less O_2 in the air as in high altitude.
   b. Diseases of the lungs.
   c. Collapse of the lungs.
   d. Obstruction of the trachea and bronchus.
   e. Heart failure.
   f. CO poisoning, etc.
2. Admixture of venous and arterial blood: This takes place in certain congenital heart diseases, where there is direct communication between right and left sides of the heart, e.g. patent interventricular septum, patent foramen ovale.
3. Greater reduction of oxy-Hb:
   - Local chilling.
   - Venous obstruction: This will retard the local circulation and allow more time for greater reduction of haemoglobin.
   - High metabolic rate and thereby higher degree of oxygen utilisation of a part occur.

Applied Physiology

The common respiratory disorders are:

Asthma is caused due to exposure to certain triggering factors (allergens such as pollen, dust mites; cold air; cigarette smoke, etc.) which lead to broncho-constriction.

Acute bronchitis: It is produced due to inflammation of the bronchial passages due to bacterial and viral infections. The common symptoms are cough, dyspnoea, malaise, fever, etc.

Chronic bronchitis: It is a chronic obstructive pulmonary disease. This condition is caused due to chronic exposure to air pollutants, smoke dust, coal dust, etc. The disease is characterised by history of cough with sputum due to excess mucus production in the lower respiratory tract.

Atelectasis is a condition in which there is incomplete expansion of lung tissues due to blockage of the airways or compression of the alveolar sacs. It may occur due to chronic bronchitis or as a result of blocked bronchial passages due to excessive mucus secretion. The compression atelectasis occurs when lung tissue gets externally compressed by air, fluids or a tumour. This may result due to prolonged inflammation of respiratory airways and bronchioles. There is abnormal dilation of the bronchus or bronchi. It is most frequently associated with chronic respiratory disease, infections, cystic fibrosis, tumour growth or exposure to respiratory toxins.

Emphysema: It is the abnormal, permanent enlargement and destruction of the air spaces distal to the terminal bronchioles. It may be inherited due to α_1-antitrypsin deficiency or caused secondary to chronic smoking and dust exposure. The common signs and symptoms are dyspnea upon exertion, wheezing, cough and may lead to right heart failure or progress to respiratory acidosis

Chronic obstructive pulmonary disease: It may manifest as combination of chronic bronchitis, asthma and emphysema. It is cause due to chronic smoking, prolonged exposure to environmental pollutants, coal dust, sand dust, etc. It may also occur genetically due to α_1-antitrypsin deficiency. The common signs and symptoms are dyspnoea, wheezing, mucous sputum expectoration, cyanosis, peripheral oedema, tachyponea, etc.

Respiratory distress syndrome of newborn: Respiratory distress in the newborn is most commonly caused by a lack of surfactant in the lungs. The surfactant covers the surfaces of the alveoli and provides surface tension that prevents the thin-walled alveoli from collapsing. It occurs in infants who are born preterm due to which there lung do not produce adequate surfactant leading to collapse of alveoli and produces respiratory distress.

Adult respiratory distress syndrome (ARDS) is a syndrome associated with destruction of alveolar membranes and their related capillaries. It may occur as a result of direct injury to the lungs or as a result of dramatic decreases in blood flow to the lung. The common causative factors are septicaemia, uraemia, secondary to drowning, inhalation of toxic gases or agents, trauma, widespread pneumonia, etc.

EXAM-ORIENTED QUESTIONS

Essay

1. Define asphyxia. Discuss the stages of asphyxia, manifestations and its causes.

Short Notes

1. Factors causing cyanosis
2. Hyperpnoea
3. Orthopnoea

Chapter

52

Artificial Respiration or Resuscitation

Indication: In any condition where respiration fails but heart continues to beat, application of artificial respiration is indicated.

Principle: The purpose of giving artificial respiration is as follows:

1. By maintaining the vitality of the nerve centres, as well as that of the heart, is maintained. Artificial respiration also helps to maintain circulation. It is expected that after some time, the respiratory centres will start functioning spontaneously.
2. During artificial respiration, the alternate inflation and deflation of lungs reflexly stimulate the respiratory centres, and thus help them to take up their own spontaneous rhythm.

METHODS OF ARTIFICIAL RESPIRATION

Manual Methods

Schafer's Method (Fig. 52.1)

The subject is laid in prone position and a small pillow is placed underneath the chest and epigastrium. The head is turned to one side. The operator kneels down by the side of the subject facing towards his head. Two hands are placed on the two sides of the lower part of the chest and then the operator slowly puts his body weight leaning forwards and pressing upon the loins of the subject. Intra-abdominal pressure rises, the diaphragm is pushed up and air is forced out of the lungs. After this the operator releases the pressure and comes back to his original erect position. The abdominal pressure falls, diaphragm descends and air is drawn in. These movements are repeated about twelve times a minute (roughly the normal rate of respiration). By this means it is possible to have a total pulmonary ventilation of 6,500 ml per minute, and this amount is sufficient for complete aeration of blood. The advantage of this method is that the patient being in the prone position, mucus or saliva comes out of the mouth and cannot obstruct his airways.

Laying casualty's face downwards with head turned to one side, arms bent and forehead resting on his hands with neck extended and kneeling to one side of casualty's hips facing his head; and placing hands flat on the small of casualty's back just above the top of pelvic bones, thumbs almost touched each other in the midline, fingers being spread over the loins and pointing towards the ground (Fig. 52.1A). Sitting on heels and swinging body slowly forward from knees being kept arms straight and hands in place all the time only applying gentle pressure by the body weight. Forcing air out of the lungs (expiration) to be maintained this position for two seconds counting 'one, two' (Fig. 52.1B). Relaxing the pressure by swinging quietly and gradually backwards onto heels allowing inspiration and counting three, four, five, in three

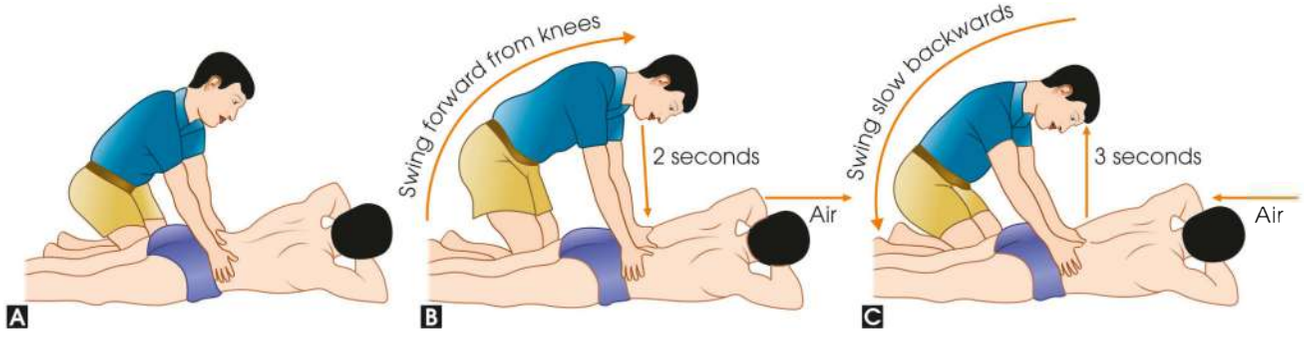


Fig. 52.1A to C: Schafer's manual method for artificial respiration

seconds before swinging forwards again to first movement being kept arms straight and hands in place all the time (Fig. 52.1C). Until the last few years Schafer's prone pressure method of artificially inflating the lungs was most widely practised. But now-a-days either back-pressure arm-lift method of Holger-Nielsen's method or the mouth-to-mouth method is practiced preferably.

Sylvester's Method

The subject is placed in supine position. The operator stands or kneels at the head end and holds the two arms of the subject. The operator then raises the subjects hands above his head and then folds the hands back upon the chest, compressing the chest wall at the same time. Such movements alternately increase and decrease the thoracic cavity, thus drawing in and pushing out air from the lungs. This method is most commonly used in the operation theatre or in other accidents. The tongue should be kept pulled out and the mucus from the mouth cavity should be wiped out from time to time. The rate is same as in Schafer's method. In drowning cases, the water in the lungs must, at first, be driven out, by holding the subject upside down or revolving the subject by holding his legs. After this the subject should be given artificial ventilation. Respiratory level and volume during the artificial method has been presented in Fig. 52.2C.

Holger-Nielsen Method (Fig. 52.2)

The subject is placed in the prone position with the arms abducted at the shoulders and elbows remaining flexed. The face is turned to one side and rests on the hands. The mouth is cleaned after wiping out mucus, fluid, etc. from it. The operator kneels down in front of the subject facing towards the head. Two hands are placed on the two sides of the back of the chest with the thumbs and fingers spread apart. Then the operator puts his body weight leaning forwards upon the subject's back. This compresses the chest and helps in expiration. The subject's arms forwards by holding them above the elbows. This helps in natural inspiration. This process is repeated about 10–12 times a minute. The respiratory level and volume during this artificial method have been presented in Fig. 52.2B.

Mouth-to-mouth Method (Fig. 52.3)

The subject is laid in the supine position with extended head. The operator sits by the side of the subject's head. The operator holds the lower jaw of the subject by one thumb and index-finger and clamps the nostrils with the other thumb and index-finer. The operator then keeps his mouth over the subject's mouth and exhales forcibly which causes inflation of the lungs and thorax. The operator then takes off his mouth and the process is repeated 10–20 times per minute. It is positive pressure breathing. The respiratory level and volume during this artificial method have been presented in Fig. 52.4.

Eve's Rocking Method

The patient is tied on a stretcher. The head and feet are alternately tilted through an angle of 45°. Eight or nine movements are carried out per minute, 7 seconds for each movement—4 seconds head down and 3 seconds feet down. When the head is down, the weight of the abdominal viscera presses against the diaphragm, so that air is pushed out of the lungs (expiration).

When the feet are down, diaphragm descends and air is drawn into the lungs (inspiration). This method is useful aboard ship when a hammock can be used.

Instrumental Method

Instead of a human operator, machineries are used. The advantage is that it can be carried on for good length of time, whereas the human operator is likely to be fatigued. The machines generally work on two principles:

1. Negative pressure breathing by alternately compressing and relaxing the chest wall
2. Positive pressure breathing by introducing air or oxygen directly into the lungs—intermittently or continuously.

Some of the methods working on the first principle are mentioned below.

Drinker's Method (Fig. 52.5)

In this method the patient is placed in an airtight chamber, the head remaining outside. By mechanically driven pumps, the pressure in the chamber is alternately lowered and raised. When the pressure is lowered the chest swells up and air is drawn into the lungs. When the pressure is raised chest becomes compressed and air is pushed out. In this way, artificial ventilation may be continued for any length of time. These methods are very useful in cases where prolonged artificial respiration is necessary, such as in morphine poisoning, in paralysis of the respiratory muscles, as in poliomyelitis, pneumothorax, etc. (The so-called iron lungs are an instrument working on this principle.)

Resuscitator (Fig. 52.6B)

This apparatus forces air through the mask that fits over the patient's face; into the lungs of the patient during the positive pressure cycle and then either allows air to flow out the lungs during the remainder of the cycle or pulls the air out by negative pressure. Resuscitator commonly has safety valve which prevents the positive pressure from rising normally about +14 mm Hg and the negative pressure from falling below–9 mm Hg.

In the Newborn Baby

Artificial respiration is necessary for those newly born babies, whose respiration is delayed. The methods and principles followed in such cases are as follows:

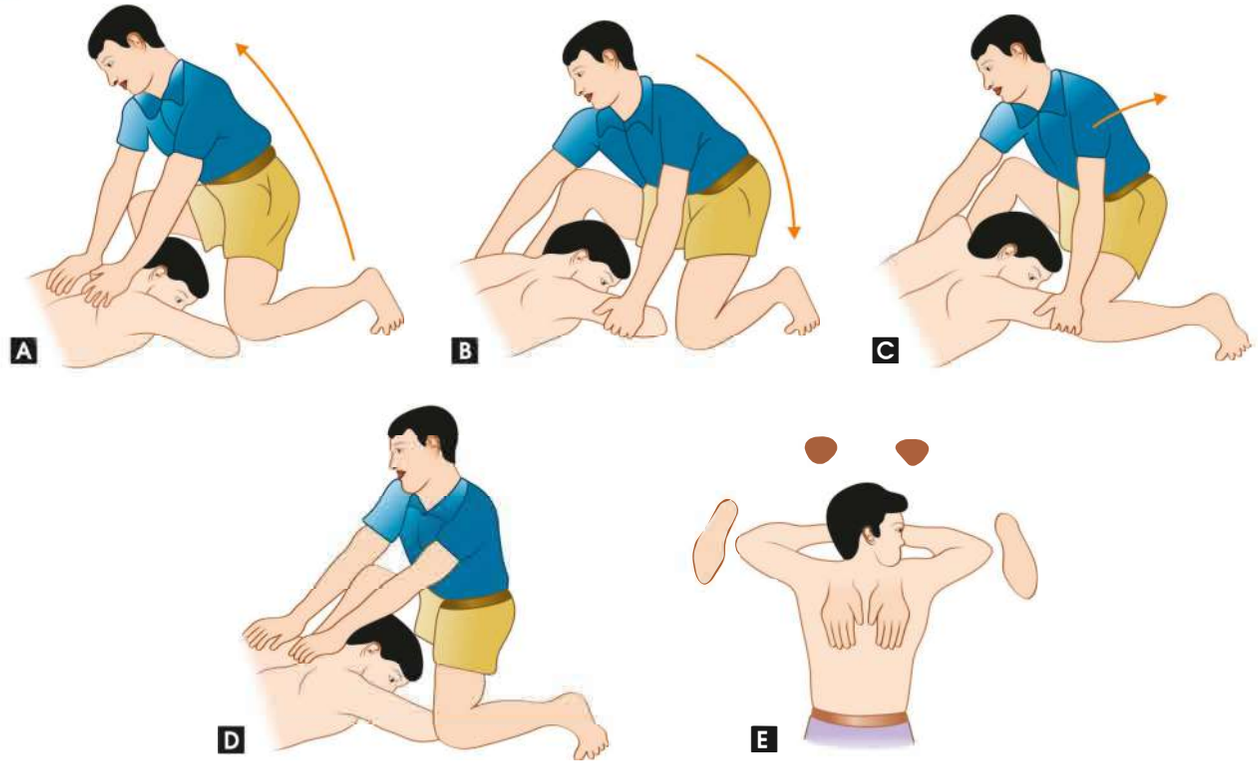


Fig. 52.2A to E: Back-pressure arm-lift method or Holger-Nielsen's manual method for artificial respiration. (A) Rocking forward quietly with arms straight and applying light pressure to back by weight of the upper part of body only counting for two seconds, 'one', 'two' (expiration). (B) Rocking back with arms straight releasing pressure gradually and sliding hands to elbows of casualty counting 'three' for one second. (C) Raising and pulling casualty's arms until tension being felt for two seconds counting 'four', 'five' causing inspiration. (D) Placing hands over casualty's shoulder blading with thumbs being touched in the midline and arms straight. Then lying casualty's arms down and placing hands on his back as in figure A for one second counting 'six'. (E) Position of casualty's and operator— laying casualty with face downwards and turning to one side arms bent and forehead rested on his hands being nose and mouth unobstructed and neck extended; and kneeling at his head being placed one knee near casualty's head and one foot alongside his elbow and hands being placed over casualty's shoulder blades with thumbs touching in the midline and fingers spreading out and being kept arms straight. (If arms being injured placing them by the sides of body doing the complete procedure but inserting hands under casualty's shoulders and raising them for inspiration. If arms and chest both injured do arm to be raised and lowered by inserting hands under casualty's shoulders.)

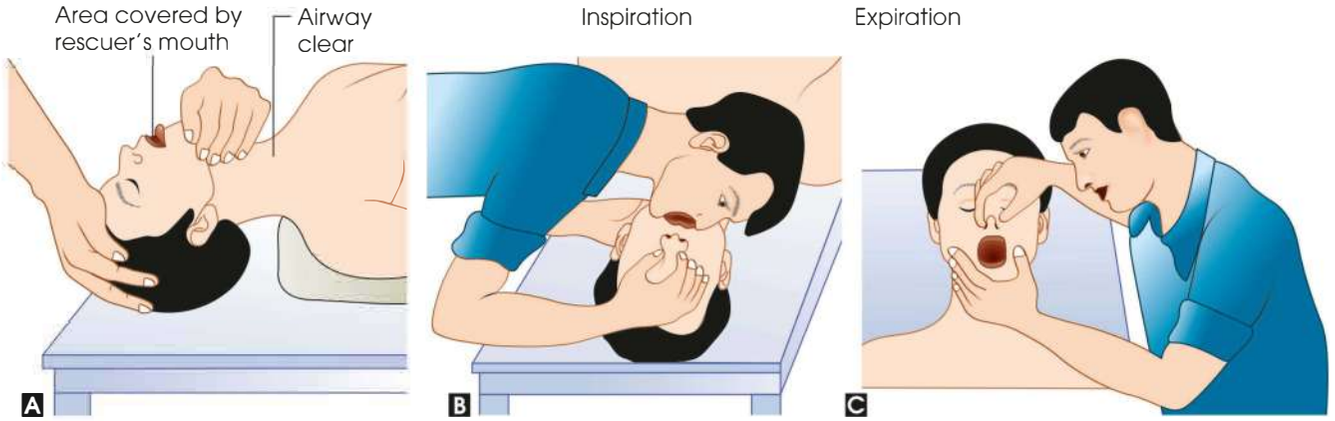


Fig. 52.3A to C: Schematic representation of mouth-to-mouth artificial ventilation. (A) The patient is laid flat on his back on the patient's shoulders some soft article is placed so that the patient's head falls well back. Standing (or kneeling) opposite to the patient's head the rescuer presses with one hand on the crown of the patient's head gently so that the head is fully extended. Other hand is placed on the patient's jaw so that the jaw is drawn well forward. (B) Breathing in deeply the rescuer's mouth is placed over the patient's mouth while pinching the patient's nose to block the nasal orifice. The rescuer breaths out forcibly into the patient's lungs. (When the patient is child or baby the rescuer should breathe delicately.) (C) The rescuer expires passively and the jaw is kept drawn well forward so that the patient's air passage is always kept patent

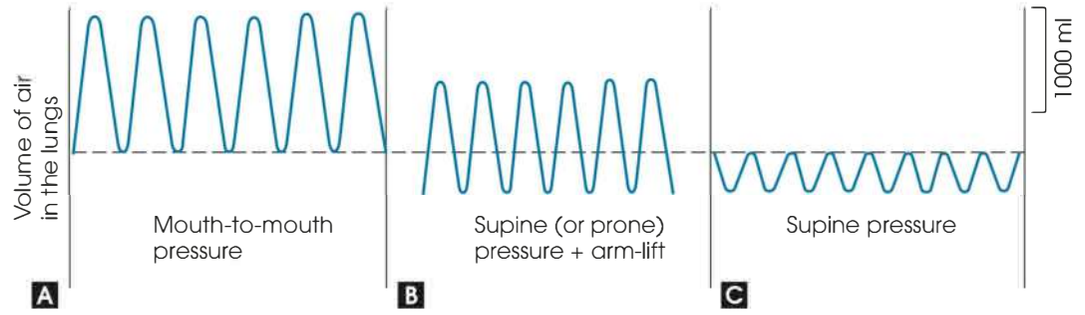


Fig. 52.4A to C: Graphical representation of pressure of amount of gas during some artificial ventilation showing cessation of respiration in the dotted line

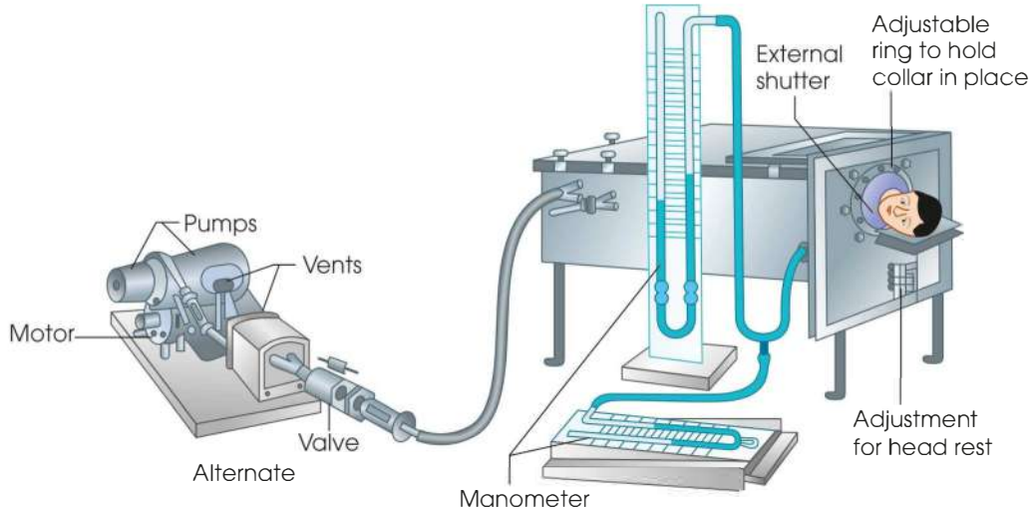


Fig. 52.5: Drinker's respiration (so-called iron lungs)

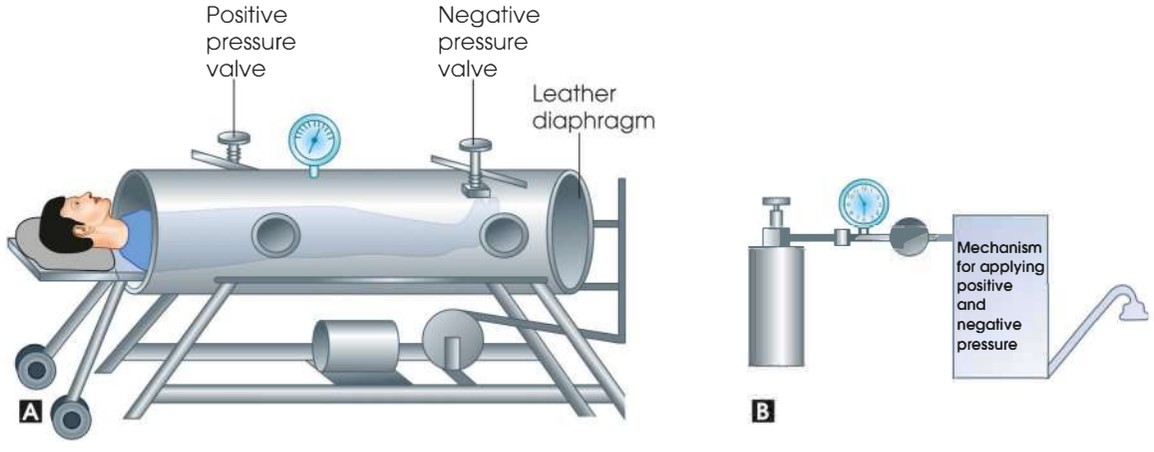


Fig. 52.6A and B: (A) Tank respirator; (B) Resuscitator

1. Holding the baby upside down (to allow more blood to go to the brain) and patting on the back (reflex stimulation).
2. In the maternity hospitals various other methods are employed working on these principles: Pumping CO_2 through the nostrils or mouth into the lungs of the child. This is usually done by forcibly blowing through the mouth of the child after closing its nostrils. This is supposed to raise the CO_2 tension of blood and stimulate respiratory centres. Mouth-to-mouth resuscitation is quite effective in infants.

EXAM-ORIENTED QUESTIONS

Essay

1. Discuss the indications for artificial respiration. Describe the various methods of artificial respiration.

Short Notes

1. Schafer's method
2. Holger-Nielsen method
3. Drinker's method
4. Mouth-to-mouth respiration
5. Resuscitator

Chapter

53

Acclimatisation

Compensatory Changes at Moderately High Altitude

The term acclimatisation means the adjustment of the human body to suit the climate at a higher altitude. When the ascent is slow and to a moderate height (between 3000 and 4250 metres or 10,000 and 14200 feet) as in mountaineering, various compensatory processes are mobilised to combat the injurious effect and the subject is ultimately adapted to the rarefied atmosphere. Some of the changes are immediate and others are a little delayed.

Changes in Acclimatisation

Changes in the bone marrow: The red marrow proliferates as a result of hypoxia. The yellow marrow may be transformed into red marrow.

Changes in respiration: The respiratory centre becomes hypersensitive to pCO_2 due to long continued hypoxia. In chronic acclimatisation, e.g. in persons residing at high altitude the hypersensitiveness of the respiratory chemoreceptors are more marked than in acute acclimatisation.

Barometric pressure is 440 mm Hg at an altitude of 4260 metres or 14200 feet. So, the O_2 pressure in the alveoli is 14% of (440 – 47) 393 mm Hg, i.e. about 56 mm Hg.

Vital capacity of lungs increases in those people who live at higher altitude for long.

Diffusing capacity for O_2 through the alveolo-capillary membrane increases.

The factors responsible for this change are:

1. Expansion and dilatation of pulmonary capillaries.
2. Increased blood volume.
3. Increased lung volume.
4. Elevated pulmonary arterial pressure.
5. The apical part of the lungs which is normally under-perfused is adequately perfused during anoxic acclimatisation.

Changes in circulation: The right heart hypertrophies so that blood can be effectively pumped through the expanded capillary bed of the lung. There occurs temporary increase in cardiac output. Blood flow through the heart, brain, muscles and other organs is increased at the expense of blood flow through the skin and kidneys. The blood capillaries of animals exposed to hypoxia for a long time get dilated to accommodate the extra blood which is brought in more intimate contact with the tissues.

Changes in blood: 2, 3-DPG content of RBC increases.

In acclimatisation the haemoglobin content of the blood increases due to hypoxia, the PCV rises and also the blood volume increases often as much as 20 to 30% resulting in total increase of circulating haemoglobin by 50 to 90%.

Cellular acclimatisation: Mitochondria and certain cellular oxidative enzyme systems increase in animals exposed to hypoxia for a long time. It is presumed that in human beings too intracellular enzyme systems develop in such a way that oxygen can be utilised more effectively.

Changes in urine: Kidneys excrete alkaline urine. Urea content is more and ammonium salts, less. In other words, the ammonia coefficient falls. There is less excretion of acid in the urine. These are attempts to combat alkalosis resulting from hyperventilation.

NATURAL ACCLIMATISATION OF NATIVES RESIDING AT HIGH ALTITUDE

Natural acclimatisation occurs in natives born and brought up at high altitude. Highest altitude at which permanent acclimatisation is possible perhaps about 5,500 metres or 18,000 feet (Peruvian Andes).

The natives of these places have got a short body structure with a large-sized chest giving a high ratio to ventilatory capacity to body mass. The right heart is usually hypertrophied with rather high pressure in the

pulmonary artery so as to fill up effectively the expanded pulmonary capillary system.

The mechanism of O_2 transfer in these natives is also highly effacious and has been compared with that of an unacclimatised subject in the accompanying diagram.

The dissociation curve for the person residing at high altitude is shown in Fig. 53.1.

Low pO_2 (40 mm Hg) of the arterial blood compared to 100 mm Hg of the person at sealevel. In spite of the low pressure, the O_2 content of the arterial blood is higher than that of the person at sealevel because of high haemoglobin content. Venous pO_2 of the high altitude natives is only 10 mm Hg lower than those of the sea level dwellers despite the low arterial pO_2. The fact is that the oxygen consumption of the high landers is usually higher in comparison to that of sea level residents there cannot be any doubt the tissues of the naturally acclimatised person can utilise oxygen more effectively.

In Peruvian Andes coal mines are situated at an altitude of about 5800 metres or 19,000 feet whereas the workers in the mine live with their families in a township at an altitude of about 5,500 metres or 18,000 feet. It has not been possible to persuade the workers to live permanently at about 5,800 metres or 19,000 feet in neighbourhood of the mines. They complain that at that altitude they do not keep good health and that they cannot sleep well at night, do not relish their food and that they develop also sexual weakness.

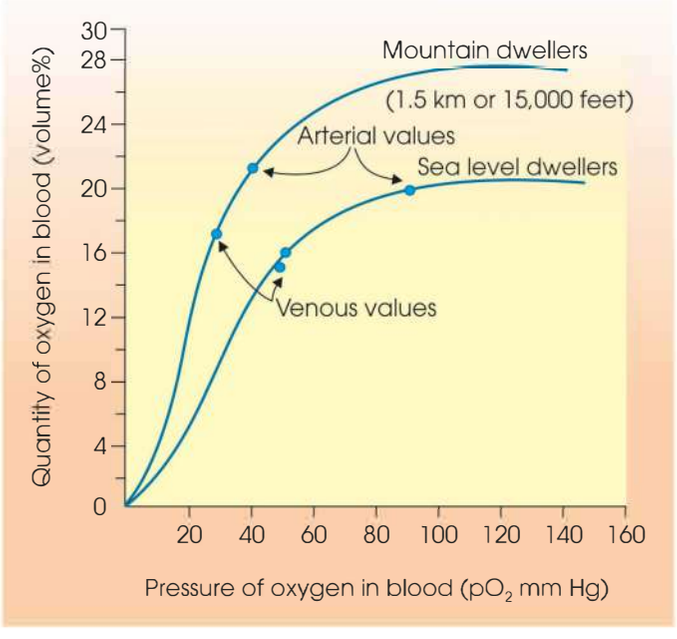


Fig. 53.1: Oxygen-dissociation curves for blood of high-altitude and sea level dwellers, showing the respective arterial pO_2 and venous pO_2 and oxygen contents as recorded in their native surroundings

About 5,500 metres or 18,000 feet altitude may be taken to be the limit of human tolerance and the altitude where permanent acclimatisation is possible. The natives born and brought up here are superior to the best acclimatised low landers in the following respects:

1. The chest-size is increased whereas body-size is somewhat decreased giving a high ration of ventilatory capacity to body mass.*
2. The right heart of these persons is considerably hypertrophied to provide a high head of pressure in the pulmonary arteries so that blood can circulate through a greatly expanded pulmonary capillary system.
3. The RBC count and so the haemoglobin content and oxygen capacity of the blood is high so that even though the arterial pO_2 is low the O_2 content of the arterial blood is higher than those living at lower altitudes.
4. The O_2 content of the mixed venous blood is also higher though its percentage saturation is only slightly less than those living at lower altitude.
5. In all probability acclimatisation also occurs at cellular level the exact nature of which is not properly understood. It is, however, known that the power to utilise oxygen in the natives of high altitude is higher and that the efficiency of muscular work of these persons is by no means less than those living at sea level.

ACUTE MOUNTAIN SICKNESS

Sometimes a well-acclimatised mountaineer may develop acute illness due to failure of compensatory adjustments to high altitude. The following effects are noticed:

1. The red cell mass and PCV are elevated considerably.
2. Pulmonary arterial pressure becomes very high and the right heart failure supervenes.
3. The peripheral arterial pressure begins to fall and death occurs due to acute pulmonary oedema which aggravates the anoxic situation. Most of the patients recover if brought promptly to a lower altitude and oxygen is administered.

Maj. Joyal, a veteran mountaineer, and the founder principal of the Himalayan Mountaineering Institute at Darjeeling died of acute sickness as described above.

MOUNTAINEERING

Mr. Tenzing Norgay's ascent of Everest (about 8,825 metres or 29,028 feet) along with Mr. Edmund Hillary provided a great impetus to mountaineering and it was to commemorate this signal success, a special institute named as Himalayan Mountaineering Institute was established in Darjeeling in 1954. With the setting up of this institute, mountain-climbing in India has become

*The same has been observed in Tenzing Norgay and other mountaineer Sherpas of Himalayan Maintaining Institute.

a source for scientific studies. Physiologists have studied different physiological changes which occur at high altitude. The heights at which symptoms of altitude sickness actually start or physiological changes which manifest themselves are not common to all men. Generally within 3,050 metres (10,000 feet), there is no additional O_2 necessary. It has however been noticed that in most cases breathing becomes laboured at about 3600 metres (12,000 feet). At about 5,500 metres (18,000 feet), there is definite painting and other symptoms of altitude sickness or mountain sickness appear and additional O_2 is necessary. It is not possible to maintain steady acclimatization beyond 5,500 metres and often deterioration occurs at an altitude of about 6,600 metres (22,000 feet) and pure O_2 must be inhaled continuously. It has been observed in the aptitude tests of flyers that acclimatisation is easiest between the ages of 30 and 40 years. In these ages there is increased of general efficiency, better circulation and less violability.

Mere physiological adaptation is not enough, psychological adaptation plays a vital part. Mental make-up, loss of sleep, deteriorating physical condition and the rarefied atmosphere are contributory factors. Beside these there are nutritional problems, namely total intake of calories, the fluid balance, etc. have to be taken into account. The effect of extreme cold is another very important factor in mountaineering. Abrupt changes in weather cause abnormalities in regulation of circulation and of body temperature. Sometimes this stress along with exertion of mountain climbing causes fatal circulatory crisis. The mountaineers who succumbed during ascent developed headache, nausea, vomiting, difficulty in breathing, pain in the chest, rapid pulse, high body temperature, ultimately loss of consciousness, etc.

ATMOSPHERE HIGH ALTITUDE PHYSIOLOGY

The atmosphere surrounding the earth is divided into various layers according to their physical characteristics:

Troposphere: It is the lowest and the most dense layer in direct contact with the surface of the earth and extending upwards for about 5 miles on the equatorial region and 10 miles over the poles. About 50% of the weight of the atmosphere is due to weight of the air in the first 3.5 miles in the troposphere. The density of air in this region ensures easy respiration, and also helpful in aeroplane flight and act as a protective layer for ultraviolet and other radiations from space. Further, most of the meteors are burnt into ashes by friction with this dense layer.

Tremendous amount of heat is generated during take off and landing of the spaceships due to friction and special devices are employed for temperature regulation during journey of spaceships through this layer. The troposphere contains minute particles of dust and also water vapours in varying proportions which condense to form cloud. Rain, storms and thunderstorms are characteristically located in troposphere only. The atmosphere gets colder and colder as one ascends higher and higher so that at the upper level of troposphere or the lower level of stratosphere the temperature is about –5°C.

1. *Stratosphere:* Commences where troposphere end and extends for about 50 miles. Stratosphere is iso thermal—the average temperature being –55°C and is free from any air turbulence. This region is ideally suited for aeroplane flight.
2. *Ionosphere:* Extends for a distance of about 200 miles above the stratosphere. This layer is so-called because molecules of gases here exist in an ionised state due to action of ultraviolet light. The temperature of the particles is about 3000°C and they move with tremendous velocity. The particles however are so few that a spacecraft moving through this zone do not come in contact with them and the temperature of the craft is regulated by radiation it receives from the sun and the heat it re-radiates into the space. This layer is rich in ozone and is responsible for reflection of shortwave radio to distant places. This is the most important layer in protecting us from the harmful effect of cosmic rays.
3. *Exosphere:* It is the atmosphere extending for about 600 miles above ionosphere and merging imperceptibly into the space.

HYPOXIA

Problem Faced by Aviators

Hypoxia: It has also been shown that above an altitude about 3 km or 10,000 feet the alveolar pO_2 falls rapidly accompanied with desaturated of arterial blood with oxygen. At an altitude of about 12 km or 40,000 feet the arterial blood is only 5% saturated with oxygen. The fall in alveolar pO_2 is due to the following factors:

1. Fall in inspired air pO_2 is dependent on fall in barometric pressure.
2. Occupation of alveolar space by N_2 which is an inert gas and enters the alveoli in quantities about 4 times that of oxygen during each inspiration.
3. CO_2 which is produced metabolically and exerts a tension of 24 mm Hg at high altitude due to hyperpnoea.
4. Water vapour which exerts a tension of 47 mm Hg at all altitude. It will be seen, therefore, that the pCO_2 + pH_2O amount to 71 mm Hg and is always to be deducted from the total available gas pressure in the alveoli which is equal to the ambient barometric pressure. The gas pressure remaining after deduction will be disturbed t air (but not the CO_2 or H_2O vapour) can be replaced by oxygen inhalation and thereby the alveolar O_2 and N_2 of which about only 14% will

Table 53.1: Gives the barometric pressure, the values of alveolar pO_2 and arterial O_2 saturation at different altitudes with and without inhalation of oxygen

Altitude (feet)	*Bar. Pr. (mm Hg)*	*Alvoelar O_2 without O_2*	*Tension (mm Hg) with O_2*	*Art. O_2 without O_2*	*Sat. (vol%) with O_2*
0	760	104	673	97	100
3 km or 10,000	523	67	436	90	100
6 km or 20,000	319	40	262	70	100
9 km or 30,000	226	21	139	20	99
12 km or 40,000	141	8	58	5	87
15 km or 50,000	87	1	16	1	15

be pO_2 and the remaining PN_2. Nitrogen in the alveolar of the alveolar air at that altitude.

Figure 53.2 shows the value of arterial oxygen saturation at different altitudes, without oxygen (left hand) and with O_2 (right hand curves).

The data and the curve indicate that inhalation of pure O_2 will keep the arterial blood 90% saturated with oxygen up to an altitude of 11.7 km or 39,000 feet. It then falls rapidly so that at an altitude of 14 km or 47,000 feet the arterial blood is only 50% saturated inspite of O_2 inhalation. 50% arterial saturation is the point where consciousness is soon lost. Without oxygen this limit is reached at 7 km or 23,000 feet. The rule in aviation, therefore, is that with supply of pure oxygen one can ascend up to an altitude about 12 km or 40,000 feet. Above that point a pressurised cabin will be necessary.

Radiation Hazards

About 20% of the sun's rays are filtered by the atmosphere before they reach the earth. Brightness of the sun increases, therefore, by 1/5th at the upper atmosphere and from above the earth appears less distinct for 2 reasons:

1. One is looking at a less bright object from a more bright area.
2. Reflection of light from the atmosphere blocks the vision of the horizon.

It has already been mentioned that most of the UV radiations and cosmic rays are absorbed by the upper atmosphere. The cosmic particles consist mostly of electrons and protons and are aggregated in two zones, viz. Van Allen's inner belt and outer belt respectively. The inner belt extends at an altitude from 300 to 3000 miles (about 480 to 4825 km) around the equator and consists mostly of high energy protons and electrons and since its energy level is high it is not possible to protect a spacecraft from its penetrating effect. The outer belt is situated between 6000 and 20,000 miles (7650 and 32200 km) around the equator and consists almost entirely of electrons.

These belts are to be avoided during space travel. For orbiting round the earth the space craft should be kept below at altitude of 300 miles or 480 km that is the inner radiation belt (Fig. 53.3) of Van Allen. For interplanetary travel, the spaceship should leave the earth through the polar escape route.

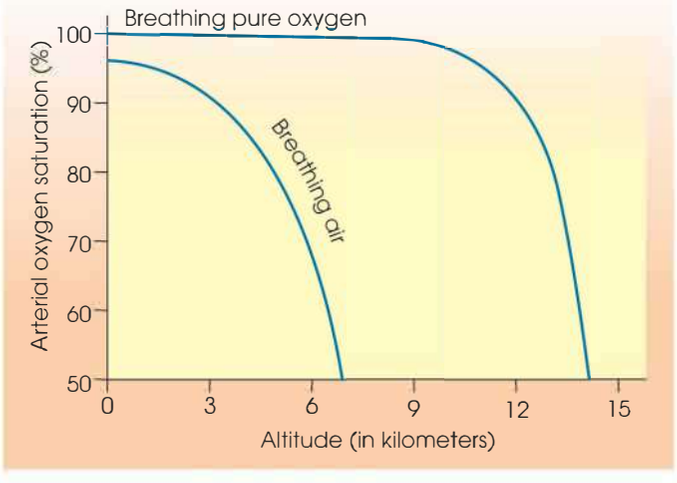


Fig. 53.2: Effect of low atmospheric pressure on arterial oxygen saturation when breathing pure oxygen on the alveolar pO_2 at different altitudes

Dysbarism at High Altitude (Decompression Sickness)

It may occur in aviators during rapid ascent to an altitude of 9 km or 30,000 feet in an unpressurised aircraft. Since nowadays, pressurised planes are always used especially during high altitude flight decompression

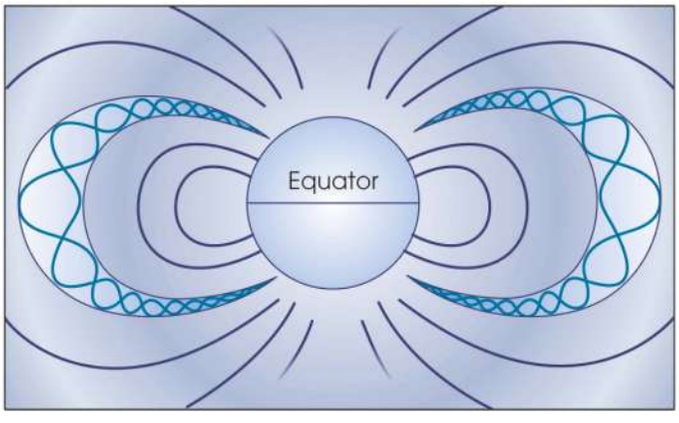


Fig. 53.3: Hazardous radiation belts of Van Allen around the earth

sickness ordinarily should not occur in aviators unless the aircraft is damaged leading to sudden failure of cabin pressure at an altitude of about 9 km or 30,000 feet. Parachute jumping from that altitude or above may also cause decompression sickness and the aviators usually wash off the N_2 in their system as much as possible by taking several deep breaths of oxygen, before the jump. In fact, nowadays, parachute jump is also affected in a sealed capsule with adequate oxygen to prevent dysbarism.

Explosive Decompression

Sudden decompression from sealevel pressure to low barometric pressure of an altitude of 15 km or 50,000 feet or over do not cause any damage to the system and with a little practice human beings can tolerate this explosive decompression without much discomfort. Air in the cavities, e.g. the middle ear is expelled through the eustachian tube, the air in the colon is expanded and is expelled out as flatus, air in the stomach is belched out, air in lungs escapes through the open glottis. Subsequent X-ray examination of the chest reveals partial atelectasis in some parts of the lungs in a few subjects. Boiling of body fluids occurs on exposure to an altitude of 63,000 feet where the barometric pressure is less than the water vapour pressure in the lungs (47 mm Hg). About 2% of the body weight is lost during the 3-minute time the subject remains alive.

Acceleratory Forces

1. *Linear acceleration:* At the commencement of flight simple linear acceleration and the termination of flight linear deceleration occurs.
2. *Angular acceleration:* During turning on plane or 'loop' formation or dive bombing operation.

The force of centrifugal acceleratory force (F) during turning of an aeroplane is given by the formula:

$$F = \frac{mv^2}{r}$$

Where m mass of the plane, v is the velocity of turning and r is the radius of curvature of the turn. It may be noted that the force of angular acceleration increases with the square of the velocity, i.e. if the velocity is doubled the force will increase 4 times. The force also varies directly as the sharpness of the turn $(1/r)$.

Acceleratory Force 'G' Unit

When a man is sitting on his chair the intensity of the force exerted on his seat by the weight of his body resulting from the pull of gravity called 1G. If the force with which he presses against his seat becomes 4 times his normal weight such as during pull out from a dive—the force acting on his seat and body is equal to 4G.

During outside loop formation the pilot is to be held down to his seat by belt and a force of negative G acts upon his body from foot to head direction. The magnitude of this force may be –1G or –2G, etc. depending on its intensity.

Effect of Centrifugal Acceleratory Forces

Circulation: Blood by virtue of its fluid nature can be easily translocated along the long axis of the body. When the human body is subjected to positive 'G' force acting from head to foot direction, the blood is translocated to lower parts of the body resulting in rise of pressure of veins of the lower limb and stagnation of blood in that situation. When the centrifugal acceleratory force is +4G or over the venous pressure in the lower limbs in the standing position is approximately 400 mm Hg and stagnation of the blood in the lower parts of the body results in fall of inflow to the heart followed by diminished cardiac output and consequently the blood pressure falls to a very low level. Figure 53.4 shows the relationship between the 'G' force and blood pressure. It may be seen that with a force of +4G acting on the body the systemic blood pressure falls to 40 mm Hg. This causes at first retinal anaemia leading to 'blackout' followed almost immediately by cerebral anaemia leading to unconsciousness. Such a situation is faced by pilots during 'pull out' of the dive bombing operations and can be avoided by adopting appropriate posture (knee and hip bent position) and by wearing proper type of suits called anti-G suits which maintain a firm pressure on the lower

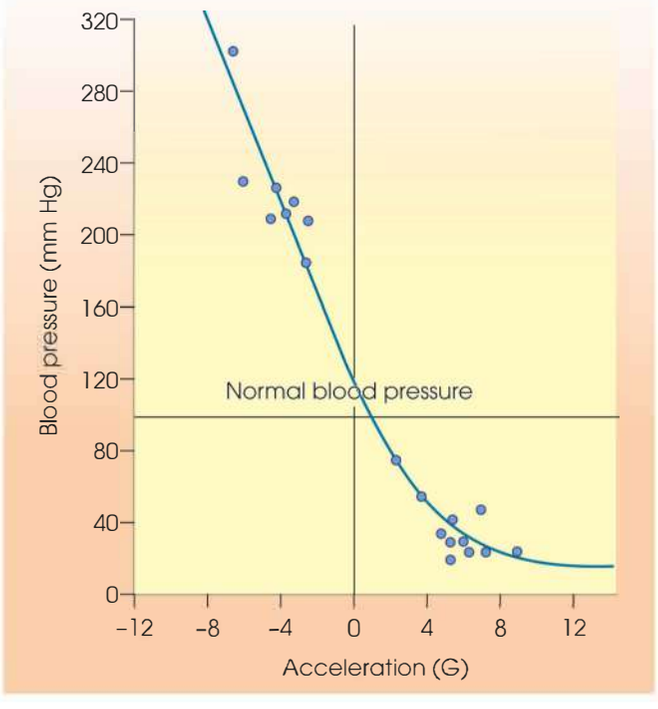


Fig. 53.4: Effect of angular acceleratory forces on arterial pressure measured of the heart

limbs and abdomen preventing accumulation of blood in these areas. However, dive bombing the consciousness is soon regained and the vision is restored to normal. Mental confusion persists for some time during which the plane may go out of control.

Extremely high positive acceleratory forces, e.g. +20G lasting for fraction of a second will cause fracture of vertebrae. Since negative G forces act from foot to head direction blood is translocated towards the brain and as shown in Fig. 53.2. The blood pressure at the heart level may be as 400 mm Hg with a force of –12G acting for a few seconds. This causes extreme bradycardia from baroreceptor reflexes. Rupture of the subarachnoid vessels in spite of severe hypertension occurs much less frequently than expected because translocation of CSF also occurs towards the head region which buffers the effect of hypertension on cerebral blood vessels. Extreme congestion of the blood vessels of the eye with temporary 'red out' is a common feature. Mental confusion is likely to last for some time after the negative G forces have ceased to act. Transverse G forces acting through the anteroposterior axis of the body will not produce any ill-effect ordinarily. Thus, a force of 15 to 25G acting for many seconds or a force of 100G acting for fraction of a second can be tolerated easily. When very large acceleratory forces are to be faced the astronauts should place himself in a semi-reclining or lying position.

Protection of Body against Centrifugal Acceleratory Forces

Posture

If the aviator compresses his abdominal muscles to an extreme degree and then leans forwards with the knees flexed and drawn up pooling of blood in the large vessels of the abdominal and legs can be prevented to some extent and onset of blackout delayed.

'Anti-G' suit: It prevents pooling of blood to lower part of the body. Theoretically, a pilot with a suit of water can withstand large acceleratory forces both positive and negative because the movement of blood. Suits have been devised which prevent pooling of blood to the lower part of the body by applying positive pressure to the legs and abdomen. It has been said that the Germans lost the last Great War because they failed to devise appropriate 'anti-G' suits.

Effect of Liner Acceleratory Forces on the Body

The problem of toleration of tremendous linear acceleratory forces develops during blast-off acceleration and landing deceleration of the spaceships. A force of about +9G develops during the first stage booster and +8G during the second stage. It is not possible for human body to withstand these acceleratory forces in the standing position. Astronauts, therefore, assume a semi-reclining position transverse to the direction of acceleration. It is possible to tolerate this force for as long as 5 minutes or longer the G force acts through the transverse axis of the body.

Deceleration during landing poses another difficulty. It must be accomplished much more slowly from high velocities and deceleration should commence at a distance of 10,000 miles (about 16,000 km) for a space craft travelling at a speed of 100 Mach (a speed possible in interplanetary space travel).

Sense of Position and Equilibrium

The utricle and saccule and the 3 pairs of semicircular canals are primarily concerned in perception of the sense of position of the head in relation to space and trunk and also in angular rotation of the head in difference direction. These organs reflexly adjust posture and equilibrium by altering the position of the body in relation to the position of the head through their elaborate connection with vestibular nuclei, flocculonodular lobe of the cerebellum and the red nucleus, reticular formation and medial longitudinal fasciculus. These organs have been described elsewhere.

The sensory epithelium of the utricle and saccule is known as macula and consists of rows of sensory hair cells embedded in gelatinous mass and pulled by calcareous particles in different directions depending on different position of the head. They are, therefore, typical gravity receptors and fail to function in zero-G state. Further, they are not stimulated unless the head leans forward by at least 10 degree or backward by 25 degrees. These receptors, therefore, are very inefficient and their evolution lags far behind the requirement of rapid change in position demanded by modern aircrafts. Aircrafts, therefore, are provided with appropriate instruments to keep the aviator informed about the rapid ascent and descent, during fight. This is, of course, more important during night flight.

The sensory epithelium of the semicircular canals is simulated during angular rotation of the head due to inertia of the endolymph. They, however, are stimulated at the beginning and at the termination of rotatory movement and remain inactive during rotation. Further, the rotational movement must exceed 2° per second to stimulate effectively the semicircular canal mechanism. These organs, therefore, are also very inefficient organs for perception of rotatory movement demanded by modern aircraft manoeuvres. The aviator again must depend on his instruments for proper appreciation of this movement during flight.

Parachute Jump

When a aviator jumps with a parachute he falls with an accelerated velocity of 16 feet manoeuvres per second. However, as his velocity of fall increases the air resistance also increases because the atmosphere becomes denser and denser. After about 12 seconds the

decelerator forces due to atmosphere counter balances the acceleratory forces due to gravity and by the time the aviator has fallen by about 4.25 km or 1400 feet, he acquires a constant rate of fall, of about 55 metres or 175 feet per second. This is called terminal velocity (Fig. 53.5).

By this time either the parachute opens spontaneously or is opened voluntary by the aviator by pulling on the ripcord. With usual size parachute the terminal velocity is reduced to about 6 m or 20 feet per second and force of impact is actually the same as experienced by one jumping from a height of 7 feet and in untrained subjects is severe enough to cause fracture of the leg bones and the pelvis.

1. An aviator jumping from a very high altitude will certainly be unconscious immediately after fall due to acute hypoxia and will regain his consciousness when he has descended to an altitude of about 7 km or 23,000 feet. His mental confusion will persist for a few seconds but if he is provided with a parachute with automatic opening device he will have safe landing on the earth.
2. Another hazard of parachute landing is injury from the damaged plane. Usually the tail-end of the plane hits the head of jumper causing serious injury. To avoid this hazard nowadays, pilots of military planes are provided with a device so that they enclose themselves in a sealed capsule filled with oxygen. The capsule is fitted with the seat of the pilot which is ejected upwards by pressing a lever so that the pilot lands safely after the damaged plane has cleared off and also is protected from hypoxia and cold.

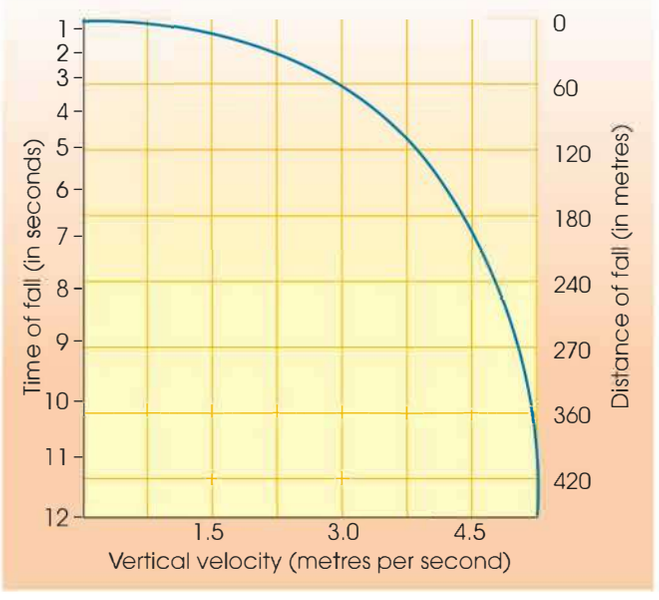


Fig. 53.5: Velocity of fall of a human body from a high altitude, showing the attainment of a terminal velocity. Deceleratory forces associated with parachute jump (from Armstrong's Principles and Practice of Aviation Medicine—The Williams Co. 1943).

Weightlessness in Space (Zero-G State)

An astronaut in an orbiting satellite experiences weightlessness because a component of the force applied to his spaceship is utilised to counteract the effect of gravity and another component is used to keep the spaceship in the orbit. Weightlessness may also occur in journey to the moon or other planets because there exists in space an area where the gravitational pull of the earth is counterbalanced by that of the moon or other planets acting in opposite direction. An astronaut in weightless state simply floats in his cabin and it becomes a problem for him to take food or to dispose his excreta in the desired way because these substances like the astronaut himself have got a tendency to float and move in any direction because they have no weight. It is an engineering problems to device means by which the astronaut may keep himself steady and can work for control of the spaceships.

Biological problems of 'zero-G' state are:

1. Translocation of body fluids within the body because absence of gravity causes loss of hydrostatic pressure.
2. Loss of muscle tone, which is normally reflexly maintained by gravity receptors. This, of course, diminishes strength of muscular contraction and results in clumsy and often useless voluntary movement.

Prolonged Stay in Space

Prolonged stay in space results in:

1. Diminished maximum cardiac output.
2. Decreased capacity to do work.
3. Decreased PCV.
4. Decreased blood volume.
5. Osteoporosis—due to decalcification.

The changes are most marked during the first week of stay in space and fortunately are not progressive. These changes can be seen in any patient with prolonged stay in bed. These changes can be avoided to a considerable extent by vigorous exercise programme during space journey.

EXAM-ORIENTED QUESTIONS

Essay

1. Define acclimatization. Discuss the compensatory changes at moderately high altitude.

Short Notes

1. Acute mountain sickness
2. Parachute jump
3. Radiation hazard at high altitude
4. Weightlessness in space (zero-G state)
5. Protection of body against centrifugal acceleratory forces
6. Natural acclimatisation of natives residing at high altitude.

Chapter

54

Underwater Physiology

INTRODUCTION

It is known that the hydrostatic pressure of water increases by 1 atmosphere for each 33 feet or about 10 metres increase in depth of water so that a person submerged to a depth of 10 metres will be exposed to a pressure of 2 atmospheres (atmospheric pressure + hydrostatic pressure). Similarly, a diver at a depth of 66 feet or about 20 metres will be exposed to a pressure of 3 atmospheres and one at 100 feet or 30 metres depth to 4 atmospheres, in this case 1 atmospheric pressure is due to air and the rest due to water.

The gases in his lungs, gastrointestinal tract, air sinuses, etc. which were originally at 1 atmospheric pressure will be compressed in accordance with Boyle's law. According to this law, if the volume of a given mass of gas is reduced, the molecules are brought closer together and the rate of bombardment upon a unit surface increases, the increase becoming manifest as a rise in pressure. For example; at a depth of 10 metres below the sea the volume of air in his lungs will be half that at sea level. The respiratory gases will, therefore, be at high pressure in the lungs, in the blood and in the tissues with undesirable side effects. The three gases of primary concern in respiration are N_2, O_2 and CO_2. Helium is sometimes used in place of nitrogen in respiratory gas mixture. The effect on body of these gases under high pressure is mentioned below.

NITROGEN

A diver beneath the sea breaths compressed air—the pressure of which must be equal to the atmospheric pressure plus the hydrostatic pressure of the column of water below which the diver is working. Nitrogen, if breathed at high pressure for some hours, leads to varying degrees of 'narcosis' which increases as the depth increases and so the pressure of the gas increases. At greater depths there occur mental confusion and muscular weakness, till at a depth of 300 feet (about 90 metres) or more (10 atm) the diver becomes comatose. The mechanism of nitrogen narcosis is perhaps due to interference with transmission of nerve impulses in the brain and nerve tissues in general which are exceedingly rich in lipids. Since nitrogen is highly soluble in lipids, the nerve tissues get saturated with nitrogen which interferes with excitability and transmission of nerve impulses.

OXYGEN

High pressure of oxygen has got deleterious effect on nervous system. Breathing oxygen at a pressure of 2280 mm Hg (3 atm) will lead to convulsion and coma in most persons within an hour. At lower pO_2, numbness and pins and needles sensations, restlessness and irritability, muscular twitching, nausea are common symptoms. Muscular exercise greatly accelerates the appearance of toxic symptoms in divers exposed to high pO_2.* Figure 54.1 shows the tolerance curve of persons doing moderate amount of work at different depths breathing pure oxygen. The curve shows that the time limit for safety is 23 minutes at 12 metres or 40 feet, 45 minutes at 9 metres or 30 feet, and 1½ hour at 6 metres or 20 feet depth. However, the toleration curve is rough guides only because of great inter-individual and intra-individual variation.

Chronic oxygen poisoning may occur by inhaling pure O_2 at normal atmospheric pressure, and is characterised by congestion and oedema of respiratory passages. The epithelium of the bronchi and alveoli are damaged by direct contact with 100% oxygen for more than 12 hours at a stretch. The lung tissues are damaged locally due to disruption of some of the essential elements. The damage in this case is limited to lung tissue and respiratory passage epithelium—the brain

*During the last great war elaborate experiments were done to perfect respiratory gas mixtures for divers operating for "human torpedoes", 'x-crafts' and 'frogmen' (Fig. 54.2). It was soon realised that pure oxygen can be used only for very shallow diving. It was further observed that diluting the oxygen with nitrogen will allow the divers to work at a depth double that was possible with the use of oxygen only.

and other tissues are protected by haemoglobin which buffers oxygen. When the pO_2 rises above 2 atmospheres—the haemoglobin buffer mechanism fails—the pO_2 of all the tissues rises and acute poisoning symptoms with convulsions and coma precipitated.

Mechanism of Oxygen Poisoning

Oxygen at high pressure inactivates enzymes containing sulfhydryl (SH) groups in the Krebs tricarboxylic acid cycle. Conversion of pyruvic acid to acetyl coenzyme A and of α-ketoglutaric acid to succinyl-CoA requires lipoic acid as a co-factor, which contains two SH groups. Oxygen at high tension inactivates the lipoic acid complex and thus prevents the formation of high energy phosphate bond from oxidation of pyruvic acid via the Krebs cycle.

Carbon Dioxide

The pCO_2 in the alveoli depends solely on metabolic activity and is independent of the depth of dive. Thus, if the tidal volume is adequate and the design of the diving gear is perfect, CO_2 should not accumulate in the body. In certain types of respiratory apparatus, however, the expired CO_2 accumulates and is rebreathed by the diver. If the pCO_2 rises to about 80 mm Hg in the breathing apparatus compensatory hyperpnoea develops and the blood pCO_2 is kept near normal. Any further rise in pCO_2 of the respiratory apparatus cannot be compensated by hyperpnoea and alveolar and blood pCO_2 begins to rise. This results in depression of respiration, lethargy, finally coma and death from respiratory acidosis.

HELIUM

Helium is often used as a substitute for N_2 by the deep sea divers because of the following advantages:

1. Unlike N_2 it exhibits no narcotic effect even at depths below 650 feet (about 200 metres).
2. Because of its low density it offers comparatively a little airway resistance and as such is easier to breath.
3. Because of its low atomic weight it diffuses more rapidly than nitrogen through the body tissues and also is removed more quickly from the body during decompression after a long dive.
4. Helium is less soluble than N_2 in the body fluids which reduce the quantity of bubbles than can form in the tissues of the diver during decompression after a long dive.

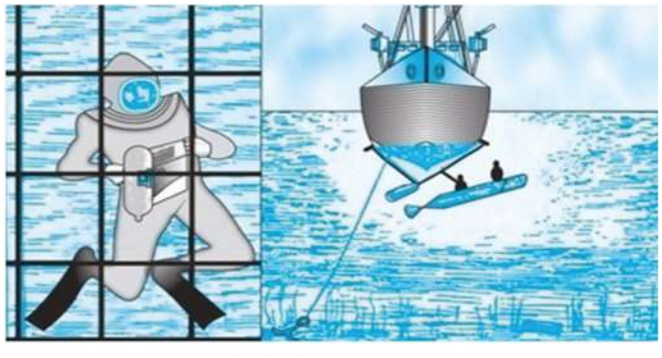


Fig. 54.1: Deep sea diving

Underwater Respiration

Breathing through tubes extending up to the surface becomes more and more difficult as the diver descends downwards. Thus, at a distance of only 5 feet below the surface breathing through a tube becomes very difficult because the hydrostatic pressure of water at this depth will be higher than 100 mm Hg so that an external pressure of about 860 mm Hg is acting around the chest of the diver. The pressure at the inlet of the tube on the surface of water is 760 mm Hg. It is difficult to make respiratory effort against this gradient. It is, therefore, necessary to supply air or other breathing mixture through tubes at a pressure equal to the external pressure applied to the body.

It is clear that at a depth of (say) 33 feet the volume of gases in the lungs will be that of the normal and to maintain adequate tidal volume double the amount of tidal level is to be pumped into prevent CO_2 retention. Thus, if tidal volume is 0.5 litre at rest on surface, at a depth of 33 feet under water, 1 litre of air is to be pumped in at each stroke. Since in the situation under discussion the environmental pressure around the diver is 2 atmospheres the partial pressure of the gases in the alveoli will also be approximately doubled. The blood gas tension will initially remain unchanged since blood is incompressible. The alveolar pCO_2 will, therefore, be higher than that of the venous blood coming to the lungs and CO_2 will diffuse from the lungs to the blood. To prevent the accumulation of CO_2, hyperventilation will be necessary, before and during the diving operation. Oxygen, with much pO_2 in the lungs will always pass from the lungs to the blood. However, during rapid ascent there is a chance of pO_2 being lower in the lungs than in the blood and so oxygen may diffuse in the reverse direction (i.e. from blood to lungs) producing acute hypoxia and unconsciousness.

Associated hazards are those affecting the circulation and blood pressure. At a depth of 33 feet the absolute systolic blood pressure will be (1520 + 120) 1640 mm Hg. In order to see clearly under water it is necessary to wear a face mask. If goggles only are worn the blood pressure in the blood vessels of the eye will be greater than that of the pressure of air trapped in the goggles and conjunctival haemorrhage will occur. At greater depths the eyes may bulge out with serious injuries unless masks are worn so that the environmental pressure outside the eyeball counteracts the high level of absolute blood pressure in the orbital and retinal blood vessels.

A mixture of 98% helium and 2% oxygen has been found useful for divers working at depths between 400 and 1000 feet. At this range of great pressure nitrogen is poisonous and only 1 to 2% O_2 in the breathing mixture is enough to maintain sufficient oxygen tension in the alveoli. Inspite of the advantages of helium noted above, at this concentration it changes the quality of the voice and makes it high pitched and squeaky and speech communication becomes difficult, particularly on telephone with the surface. Temporary inhalation of nitrogen is helpful in restoring the quality of the voice. Aquanauts may come out of especially designed underwater craft and work for long period at the ocean floor. Helium under pressure conducts the body heat rapidly and as such maintenance of body temperature is an additional problem under these conditions.

Squeeze occurs during rapid descent when volume of all the gases in the body becomes greatly reduced due to increased pressure outside the body. 'Squeeze' affects the lungs very adversely and even if a diver commences to dive with a full inspiration, his chest begins to cave in at a depth of about 100 feet. The only way to avoid it is to breath additional volume of air during descent. During rapid ascent there is risk of overexpansion of the lungs and if the glottis kept closed the pressure of air in the lungs may go up to such an extent as to cause rupture of the pulmonary capillaries. This may be followed by the air embolism and depth.

Decompression sickness has already been described. It has been pointed out that release of nitrogen bubbles dissolved in body fluids and especially in the nerve tissues are responsible for the symptoms which may in some instances be very severe and lead to death. The only means by which decompression sickness can be avoided is slow and gradual decompression, remembering that in cases of prolonged dive under the sea most of the nitrogen remains in solution in fats and lipids and is released very slowly on decompression. Obviously the time required for decompression depends on two factors:

1. The depth to which the diver has descended.
2. The time he has been exposed to the heavy atmosphere.

Decompression tables have been prepared by US Navy to control the process. For instance a diver who has worked at a depth of 190 feet for 1 hour will need decompression in different stages lasting 3 hours.

SCUBA DIVING

SCUBA (self-contained underwater breathing apparatus) or aqualung is now popularly used by professionals as well as sport divers. The hazards of decompression sickness and inert gas narcosis occur. Unconsciousness and collapse may also supervene. Lung rupture and consequent air embolism may ensue if the divers ascend without exhaling. Under these circumstances the much expansion of volume of gas in the lungs causes the alveolar walls to tear and as a result air escapes into the pulmonary veins and thence to the cerebral and coronary circulation. SCUBA works on 'open-circuit' principle which means that the expired air is expelled into the sea water. 'Close-circuit' apparatus has also been developed where the O_2 of the expired air is used over and over again after removal of the CO_2 by CO_2 absorbent. They resemble the anaesthetic Boyle's apparatus without the anaesthetic gas. The limitation of the close-circuit system is intolerance to pure oxygen. The safe rule is not more than 30 minutes at 30 feet depth with oxygen alone.

In Fig. 54.2, air cylinders are made of special alloy and are light but can withstand light pressure of compressed air. First stage valve reduces the pressure from the tank to a constant flow pressure level. Inspiratory and expiratory demand value allows air to be inspired with very slight negative pressure and then to be expelled into the sea. Shallow water black-out

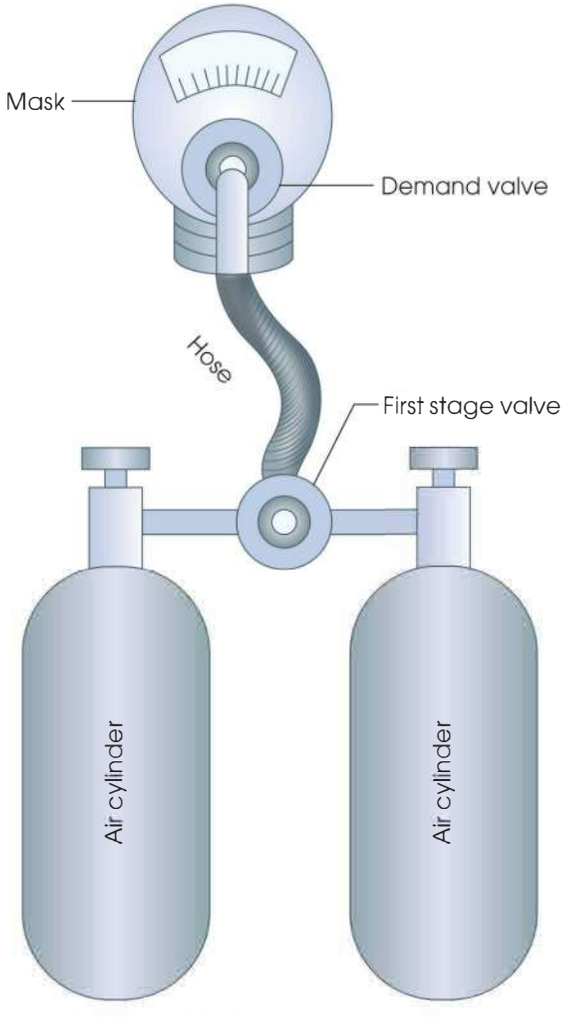


Fig. 54.2: The SCUBA apparatus of aqualung

is generally happened in relatively shallow water, viz. swimming pool where the victim becomes unconsciousness during ascent from a breath-hold dive. As he ascends the ambient pressure falls, and the alveolar, therefore, arterial, pO_2 becomes so low that the subject loses consciousness. The subject can stay submerged for a long time until the arterial pO_2 falls to a low level and stimulates breathing. A diver may hyperventilate before a dive to reduce the ventilatory dive caused by CO_2 accumulation.

EXAM-ORIENTED QUESTION

Short Notes

1. Under water respiration
2. SCUBA diving

Chapter

55

Vocalisation

INTRODUCTION

Two separate processes are involved in vocalisation:

1. Phonation.
2 Articulation.

The whole process is dominated and co-ordinated by speech centre in the cerebral cortex, damage to which will lead to permanent aphasia.

Phonation is due to vibration of vocal cords which are folds stretched along the lateral wall of the larynx between thyroid and arytenoid cartilages. These are positioned by and their tension is regulated by the intrinsic muscles of the larynx.

The relevant intrinsic muscles (Fig. 55.1) and their mode of action are:

1. Thyroarytenoid muscle is made of many small slips of muscle fibres controlled separately by different nerve fibres. The contraction of these muscle fibres independently of each other is responsible for control of shape of vocal cord, thick or thin or with sharp and blunt edge during phonation of different types (Fig. 55.2).
2. Posterior crico-arytenoid muscle pulls the arytenoid cartilages away from thyroid cartilage and thereby stretches the vocal cords increasing their tension.
3. The transverse arytenoid muscle pulls the arytenoid cartilages together and thus draws the vocal cords towards each other.

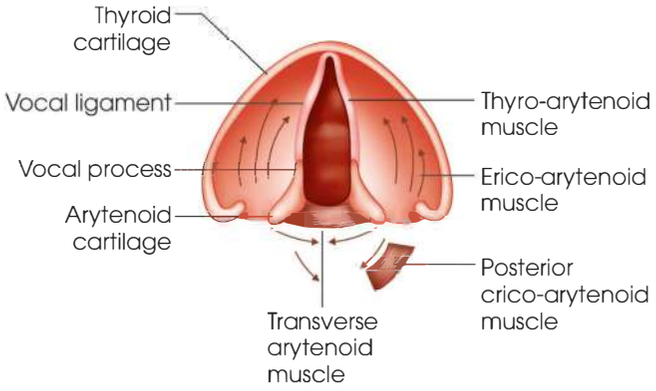


Fig. 55.1: Diagrammatic representation of the basic structure of the larynx showing that each vocal cord is stretched between the thyroid cartilage and an arytenoid cartilage which helps phonation

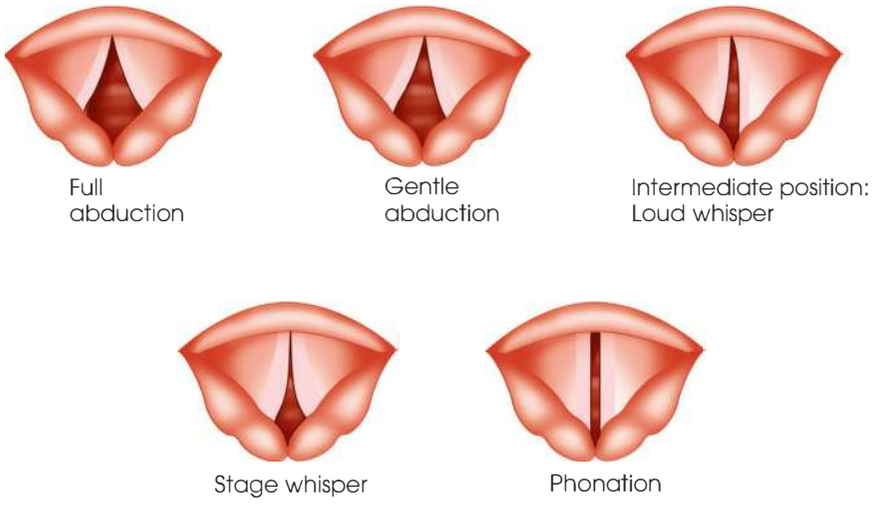


Fig. 55.2: Some of positions and shapes of the vocal cords during different types of phonation

4. The lateral crico-arytenoid muscle pulls the arytenoid cartilages forwards and this increases the gap between the vocal cords to allow easy respiration.

The vocal cords vibrate laterally because the pressure of air from below pushes the vocal cords apart. The rapid flow of air between their margins creates a 'negative pressure' which approximates the cords towards each other. The cycle is then repeated.

Changes in frequency of vibration of vocal cords are effected by:

1. Alteration in tension of the cords
2. Alteration in thickness of the vibrating edge.
   a. Tension of the vocal cords is regulated by intrinsic laryngeal muscles described above, e.g. posterior crico-arytenoid and transverse arytenoid muscles which increase and lateral crico-arytenoid muscles which decrease the tension of the vocal cords.
   b. Thickness of the vibrating edge is regulated by the muscle fibres of the thyro-arytenoid muscles. It has been mentioned that groups of muscle fibres in this muscle may contract independent of each other—one group producing thin edge and the other group producing thick edge of the vocal cords.
   c. Extrinsic muscles of the larynx also have got a role to play.
   d. Elevation of the larynx increases the tension and depression of the larynx decreases the tension of the vocal cords.

ARTICULATION AND RESONANCE

The three major organs for articulation are:

1. Lips
2. Tongue
3. Soft palate.

The buccal cavity, the nose and nasal sinuses, the pharynx and the chest cavity—they all act as resonators and determine the quality of the voice. Thus, if the nose is blocked due to cold—the quality of voice is appreciably altered.

EXAM-ORIENTED QUESTIONS

Short Notes

1. Changes in frequency of vibration of vocal cords
2. Processes are involved in vocalisation

RESPIRATORY SYSTEM

Q1. An infant died immediate by after birth due to pulmonary insufficiency. The cause of death was certified as hyaline membrane disease. Discuss the cause of death in such disease.

Ans. The deficiency of the surfactant leads to hyaline membrane disease. The surfactant forms a layer between the air and the fluid thereby reducing the surface tension. The main protection action of surfactant is it prevents alveoli from collapsing. The increased surface tension, collapsed alveoli and pulmonary oedema lead to respiratory insufficiency and death.

Q2. A clinician noted the findings as Cheyne-Stokes respiration observed. What is Cheyne-Stokes respiration? What are the physiological and pathological causes for the same?

Ans. The repeated sequence of gradual outset of apnoea followed by normal respiration is known as Cheyne-Stokes respiration. The physiological causes of Cheyne-Stokes respiration are during sleep in any normal person, high altitude or as a result of voluntary hyperventilation. The pathological causes are secondary to uraemia, heart failure and brain injury.

Q3. A 40-year-old male patient developed anoxia due to cyanide poisoning. What is the cause for the same?

Ans. The anoxia due to cyanide poisoning is due to inhibition of respiratory enzymes due to which tissues are unable to utilize oxygen of the blood which contains adequate amount of oxygen at normal tension.

Q4. A patient of mitral stenosis reported with dyspnoea on exertion. What are the causes for the same?

Ans. The engorgement of pulmonary capillaries leading to diminished distensibility of lung is the primary cause of cardiac oedema.

Q5. A patient of congenital cyanotic heart disease (patent inter-ventricular septum) developed sign of cyanosis? What is the cause for the condition?

Ans. There is admixture of venous and arterial blood because of direct communication of right side and left side of heart leading to signs of cyanosis.

RECENT ADVANCES

Chronic Intermittent Hypoxia Alters Local Respiratory Circuit Function at the Level of the pre-Bötzinger Complex

The chronic intermittent hypoxia (CIH) is commonly observed in various breathing disorders such as obstructive sleep apnoea (OSA) and apnoeas of prematurity. The pre-Bötzinger complex is a pre-motor respiratory network and vitally important for inspiratory rhythm generation. Electrophysiological recordings in experimental study from brainstem slices revealed that CIH enhanced burst-to-burst irregularity in period and/or amplitude. There was individual fidelity among pre-Bötzinger neurons, and altered transmission from pre-Bötzinger complex to the hypoglossal motor nucleus resulting in increased transmission failure to XII nerve. It was observed that CIH increased the degree of lipid per oxidation in the

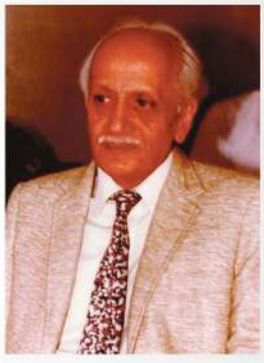
pre-Bötzinger complex. Treatment with the anti-oxidant reduced CIH-mediated irregularities on the network rhythm and improved transmission of to the XII nerve. The CIH promotes a pro-oxidant state which is responsible for altered rhythmogenesis originating from the pre-Bötzinger complex and changes the local rhythm generating circuit which can lead to intermittent transmission failure to the hypoglossal nerve.

REFERENCE

Alfedro J Gracia, Sebastien Zanella, Tatiana Dashevskiy, Shakil A Khan, Maggie A Khuu, Nanduri R Prabhakar and Jan Ramiro Ramirej. Chronic Intermittent Hypoxia Alters Local Respiratory Circuit Function at the Level of the pre-Bötzinger Complex. Front Neurosci. 2016;10:4.

Biographical Memoirs of Fellows of the Royal Society. 52: 251–262.

Notable contributions in respiratory physiology



Autar Singh Paintal
1925–2004

Professor Paintal made significant contributions towards understanding of visceral sensory mechanisms and their reflex effects. He is known worldwide for the discovery of the volume receptors of the atria and J-receptors of the lungs. He also proved that stimulus for the aortic chemoreceptor was oxygen chiefly. Professor Paintal had named the receptor as 'juxta-pulmonary capillary receptor' and called it as 'J-receptor'. He also developed single-fibre technique for recording afferent impulses from individual sensory receptors.

Section

VI

Gastro-intestinal Tract

Chapter

56

General Outline of Digestive System

INTRODUCTION

Anatomical Consideration

The human digestive canal is a long muscular tube consisting of the following parts from above downwards—the mouth (guarded by lips and teeth), tongue, pharynx, oesophagus, stomach, small intestine, large intestine, rectum and anal canal. The ducts of the salivary glands open into the mouth. The proximal end of the stomach (i.e. its junction with oesophagus) is guarded by the cardiac sphincter. The distal end of stomach is guarded by the pyloric sphincter. The small intestine begins after pyloric sphincter and consists successively of the following subdivisions: Duodenum, jejunum and ileum (Fig. 56.1). The duodenum receives food from the stomach.

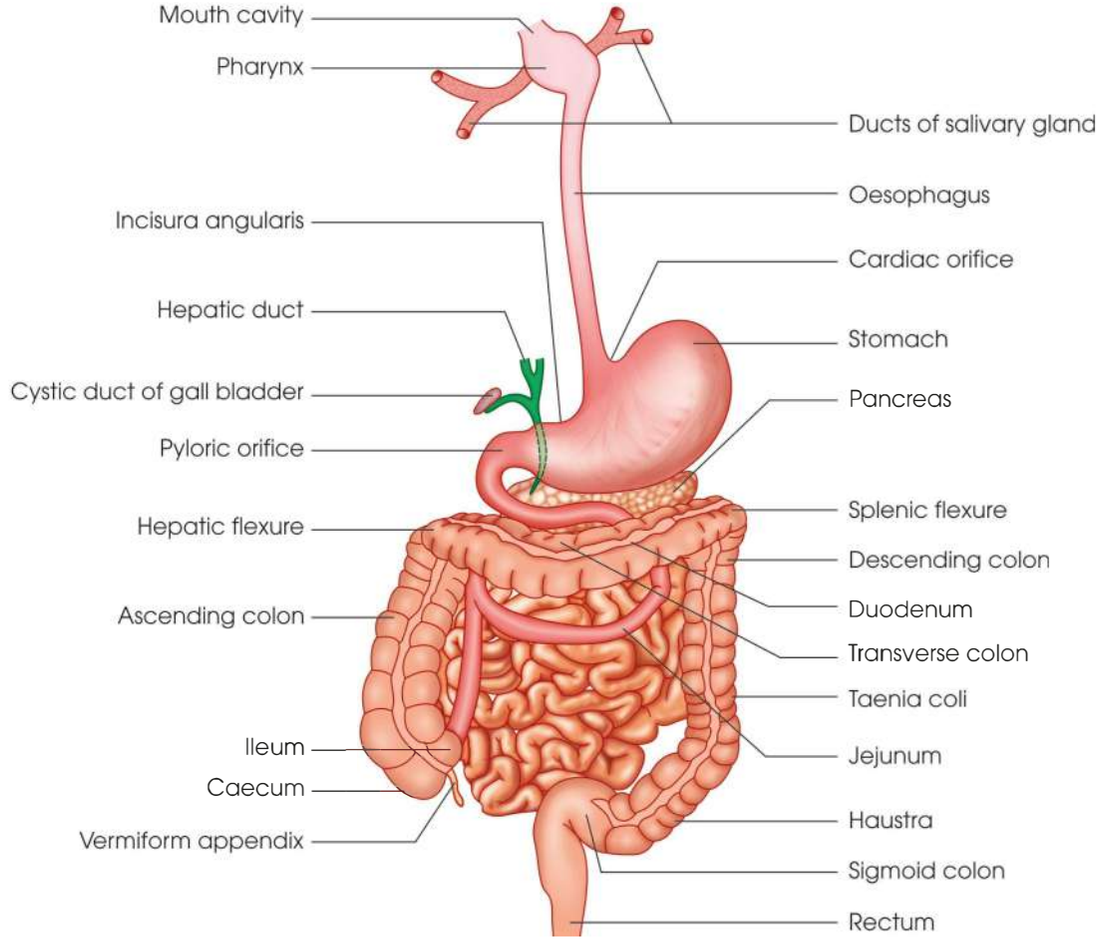


Fig. 56.1: Digestive tract

Where the duodenum joins the jejunum, connective tissue and muscle fibres (ligament of Treitz) thicken the mesentery. The bile duct and pancreatic duct jointly open in it through ampula of Vater. In humans, the small intestine is about 6 metres or 20 feet long and the large intestine is about 1.5 metres or 5 feet long. The great length of the small intestine provides enough time and surface area, so that digestion and absorption of foodstuff may be complete.

The small intestine opens into the next part—the large intestine. The opening between them is guarded by iliocolic sphincter. In the large intestine water is absorbed and the faeces become formed. The large intestine opens into the last part—rectum and anal canal. The latter opens outside through the anal orifice. Peritoneum is a serous membrane and lines the interior of the abdominal cavity. The parietal outer layer is in contact with the body wall and the visceral (inner) layer envelops the abdominal organs. The mesentery is the continuation of the peritoneum and extends to the small and large intestines from dorsal body wall. The lesser omentum of the stomach attaches to the liver and the greater omentum hangs from the greater curvature of the stomach over the intestine to the colon as an apron. In this fold fat may accumulate.

Histological Structure

The wall of the alimentary canal (Fig. 56.2) from the oesophagus to the anal canal consists of typical four encircling layers or tunics from outside inwards:

1. Tunica adventitia or serosa (fibrous outer coat) carries large blood vessels and nerves. When the organ is covered by the peritoneum, this layer is called serosa; otherwise tunica adventitia.
2. Tunica muscularis consists of a double layer of smooth muscle of which the inner sheet lies circularly and the outer sheet runs longitudinally. Nerve and vascular plexuses lie between the layers. This muscular layer controls the diameter of the intestine and propels its contents towards the anus.
3. Tunica submucosa is a loose areolar connective tissue layer which contains large blood and lymphatic vessels, nerves and glands in certain portions.
4. Tunica mucosa (mucous membrane) consists of; (a) stratified squamous or single columnar epithelial layer, (b) lamina propria which is a delicate areolar connective tissue layer and is normally infiltrated with lymphocytes and lymph nodules, (c) muscularis mucosa is a thin layer of smooth muscle and forms the boundary between mucous membrane and submucosa.

Innervation of the Digestive Tract

The nerve supply consists of (a) an intrinsic part which is represented by nerve cells and fibres originating and located in the intestinal wall itself and (b) an extrinsic portion which is represented by vagal fibres and postganglionic fibres of the sympathetic.

Key Points

1. The intrinsic neural structures supply the smooth musculature of the alimentary canal except the striated muscle fibres of the mouth, the upper part of the oesophagus and external anal sphincter. The intrinsic mechanism consists of a series of plexuses which are composed of large or small groups of nerve cells and bundle of nerve fibres.

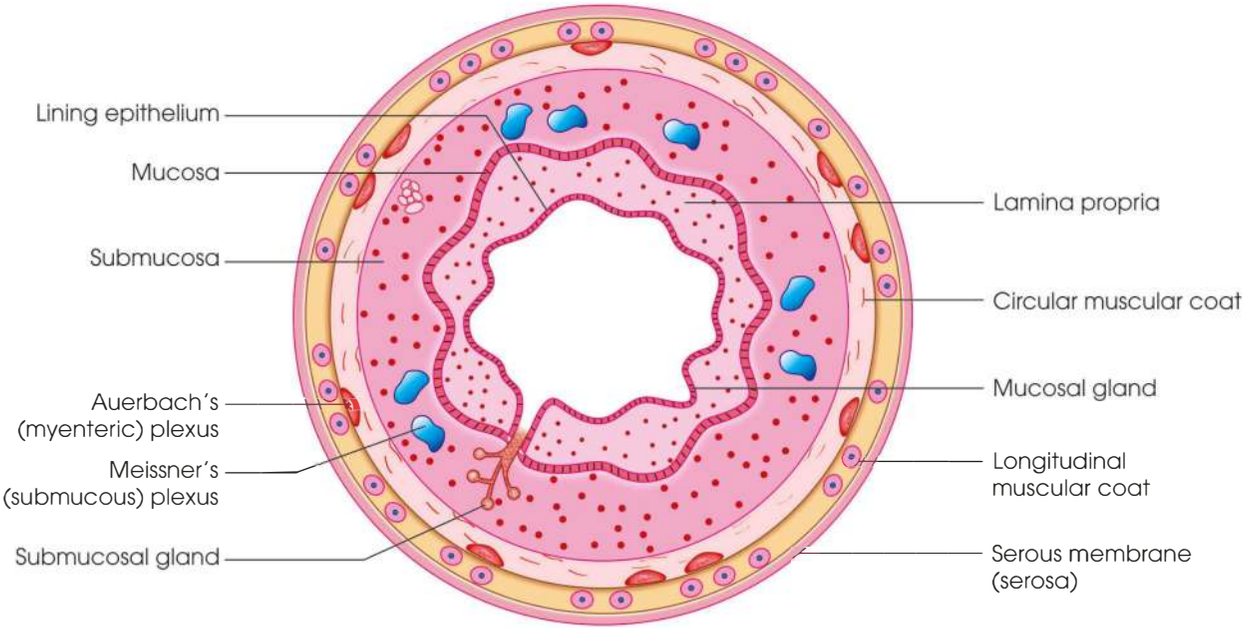


Fig. 56.2: Cross-section of the digestive tract

Two intramural plexuses (Fig. 56.3) are located in a narrow space (a) between longitudinal and circular muscle layers of the intestinal wall (myenteric plexus of Auerbach) and (b) between circular muscle layer and submucous (submucous plexus of Meissner). The myenteric plexus originates in the medulla oblongata from the ventral part of the brain stem as a collection of neurons. The vagus nerve further carries the axons to the gastro-intestinal tract. The myenteric plexus physiologically functions as a part of the enteric nervous system (digestive system).

The enteric nervous system can function independently, but normal gastro-intestinal digestive function requires communication between this intrinsic system and the central nervous system. The parasympathetic and sympathetic fibres connect either the central and enteric nervous systems or connect the central nervous system directly with the gastro-intestinal tract. The enteric nervous systems contain numerous neurotransmitters and they are employed in its functioning. The neurotransmitters of primary importance in functioning of enteric nervous system include acetylcholine, dopamine, and serotonin. The neuropeptide substance P which is also found in gut facilitates smooth muscle contractions, and other tissue responses.

Details of extrinsic nerve supply

2. The extrinsic nerve supply acts to regulate the intrinsic neuromuscular mechanism that determines the movements of the digestive tube. However, the intrinsic neural mechanism is modulated by extrinsic nerves supplied by both divisions of the autonomic nervous system.
3. The sympathetic fibres which are branches of the splanchnic nerves originating in the coeliac (superior mesenteric) ganglion, do not enter into synaptic junction with the cells of the intra-mural myenteric and submucous plexuses. But they appear to branch in the muscular layers to form intra-muscular plexuses and finally to terminate on the smooth muscle fibres. The sympathetic also appear to supply blood vessels within the intestinal wall.

   The pre-ganglionic para-sympathetic fibres are derived from (a) the cells in the medulla oblongata and (b) the sacral segments of the spinal cord. Those fibres originating from the medulla oblongata are mainly parts of vagal ones and supply muscles of the stomach, small intestine and upper half of the large intestine. The vagal fibres make synaptic connections with the intra-mural plexuses. The pre-ganglionic fibres from the sacral spinal cord reach the pelvic ganglion (hypo-gastric plexus) by way of the pelvic nerves and post-ganglionic fibres supply the lower half of the large intestine and the rectum. The internal anal sphincter contraction is mediated and maintained via stimulation of sympathetic fibres from the superior rectal and hypo-gastric plexuses. Whereas the external anal sphincter is supplied by somatic (motor) nerve fibres (Fig. 56.4).
4. The sympathetic fibres are excitatory for ileocaecal and internal anal sphincters, and smooth muscle fibres of the muscularis mucosa throughout the whole gastro-intestinal canal and help to increase the number of folds in the tract, and are inhibitory for the remaining musculature. The para-sympathetic system is excitatory for all the musculature except the sphincters where it is usually inhibitory (Fig. 56.4).

Note

The two nervous systems are antagonistic but so far as chyme is concerned, they are complimentary.

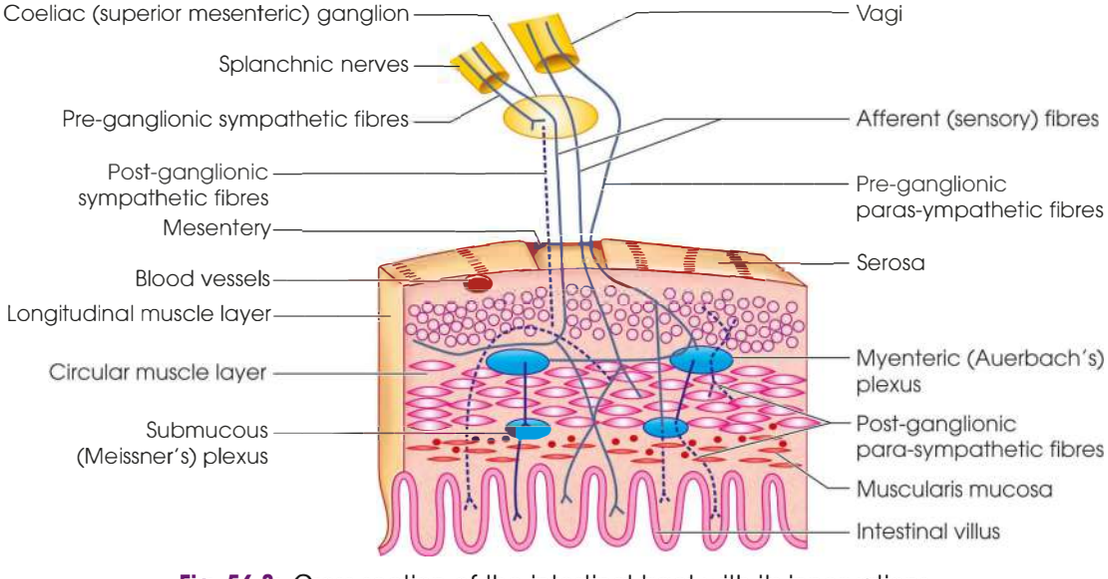


Fig. 56.3: Cross-section of the intestinal tract with its innervations

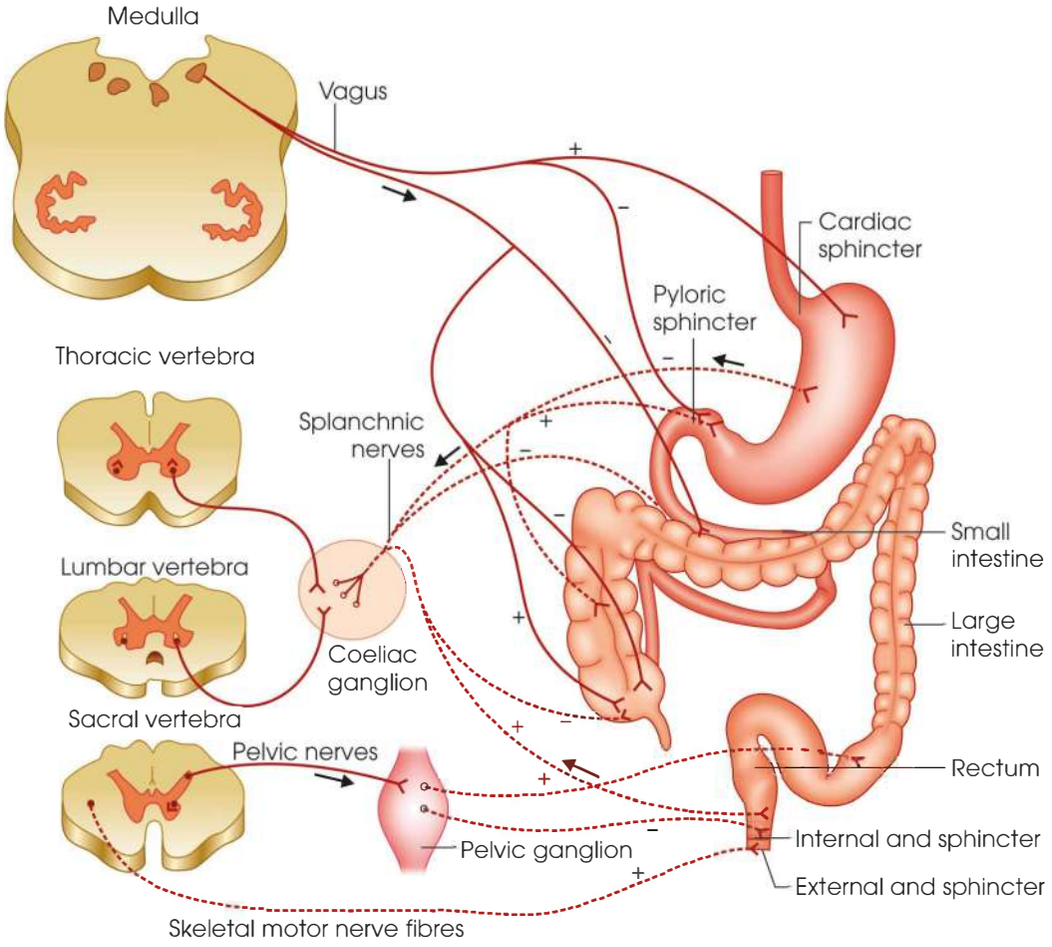


Fig. 56.4: Innervations of the alimentary canal. Plus indicates excitation and minus indicates inhibition

Functions of the Digestive System

The human digestive system serves the following functions:

1. Ingestion of food.
2. *Digestion of food:* The partial digestion of the food commences here in stomach. The churning action of the stomach muscles breaks down the food physically. The stomach further releases acids and enzymes for the chemical breakdown of food. The protein breakdown is by enzyme pepsin.
3. Secretion of various digestive juices.
4. Absorption of water, salts, vitamins and end products of food digestion.
5. *Excretion:* Heavy metals, toxins, certain alkaloids, etc.
6. *Movements:* Certain types of movements are present in the whole of the gastro-intestinal tract. The functions of these movements are to facilitate admixture of food with digestive juices, to propel food onwards, to help blood and lymphatic circulation through the intestinal wall. Defecation is also due to the movements of the large intestine.
7. *Erythropoiesis:* Stomach manufactures a substance called the intrinsic factor. The extrinsic factor is vitamin B_{12}; the intrinsic factor interacts with it and helps in the absorption of the extrinsic factor. The extrinsic factor promotes the maturation of the erythroid cells. Pernicious anaemia is associated with gastric lesion. Regulates blood reaction (*vide* under blood reaction): The alimentary canal takes part in the regulation of blood reaction.
8. *Regulates blood sugar (vide under blood sugar):* It takes part in the regulation of blood sugar.
9. *Maintains water balance:* The phenomenon of thirst is an important function of the digestive tract by which the fluid balance of the body is maintained.
10. *The histological peculiarities of different parts of the digestive canal are as follows:* Mouth and oral cavity—which are lined by stratified epithelium.

Histology of Tongue

Tongue (Fig. 56.5) is made up of three elements; epithelium, muscles and glands.

Epithelium: The epithelium is stratified and non-cornified. Two types of special structures are seen on it; the papillae (Fig. 56.6) and the taste buds. The taste buds (Fig. 56.7) are the sense organs of taste (*vide* under special senses). These buds are lined by stratified

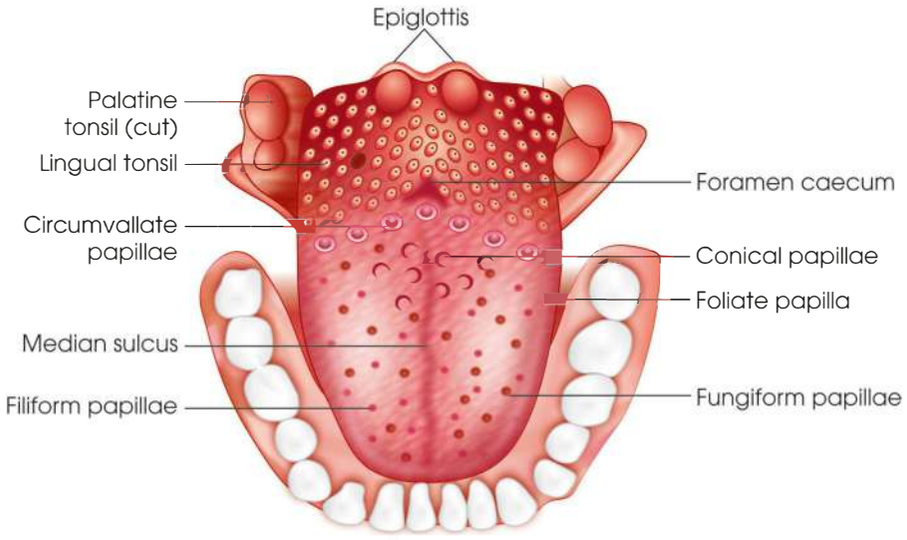


Fig. 56.5: Dorsal position of the tongue

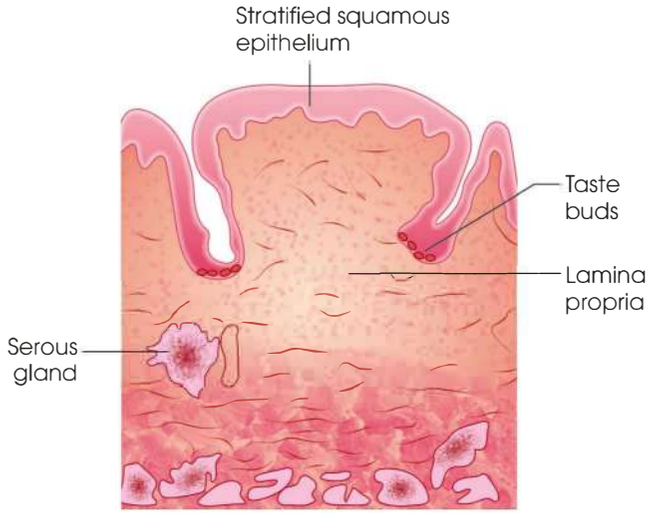


Fig. 56.6: Histological structure of circumvallate papillae

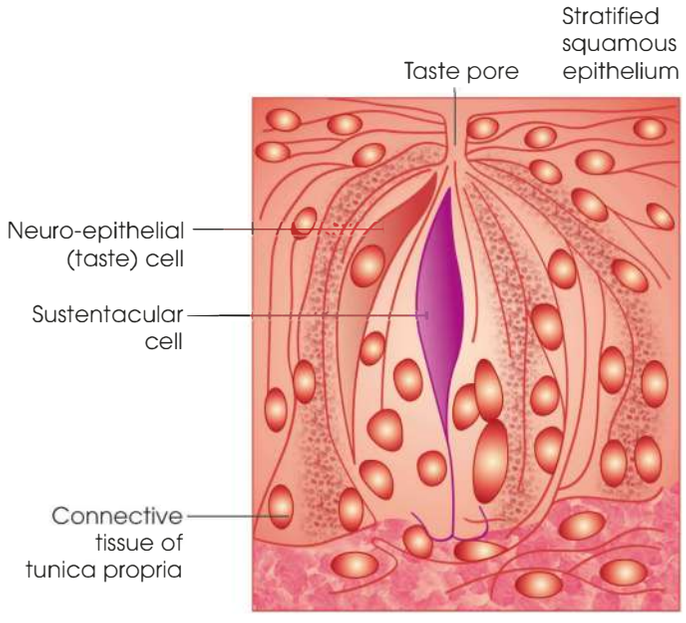


Fig. 56.7: Simplified taste bud

squamous epithelium and are flask-like with a wide bottom. A taste pore pierces the short and narrow neck of each taste bud. The taste bud possesses melon-shaped and frequent supporting (sustentacular) cells and also scanty, narrow and long neuro-epithelial (taste) cells to its outer ends. The supporting cells are spindle-shaped and their ends surround a small opening, the inner taste pore. The taste cells vary from 4 to 20 per taste bud. They have slender rod-shaped form with a nucleus in the middle, and on the free surface short taste hairs which project freely into the lumen of the pit. These cells are responsible for detection of taste which is to be dissolved in saliva for proper sensation.

The papillae are minute projection of the mucous membrane and are as follows:

- Circumvallate papillae are large and can be easily seen with the naked eyes. They are only 10–12 in number situated at the back of the tongue and arranged in the form of a 'V' with its limbs opening anteriorly. At the apex of the angle there is small invagination, foramen caecum. A circumvallate or vallate papilla (Fig. 56.6) consists of a central-rounded elevation, surrounded by the non-cornified stratified squamous epithelium on lamina propria. External to this groove the mucous membrane is raised and is known as vallum.
- Fungiform papillae having a flat-rounded head-like fungus are covered by the non-keratinising squamous epithelium on the fibrous lamina propria, tip being broader than the base (Fig. 56.8). Circumvallate and fungiform papillae carry taste buds. The fungiform papillae are rich in blood vessels and hence have a marked red colour.
- Filiform, also known as conical papillae due to presence of conical pointed cap with keratinising squamous epithelium on the lamina propria (Fig. 56.9). In man, this cap consists of epithelial filaments.

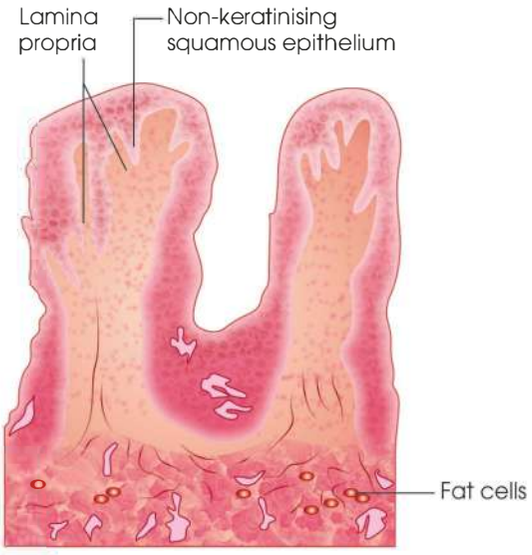


Fig. 56.8: Histological structure of fungiform papillae

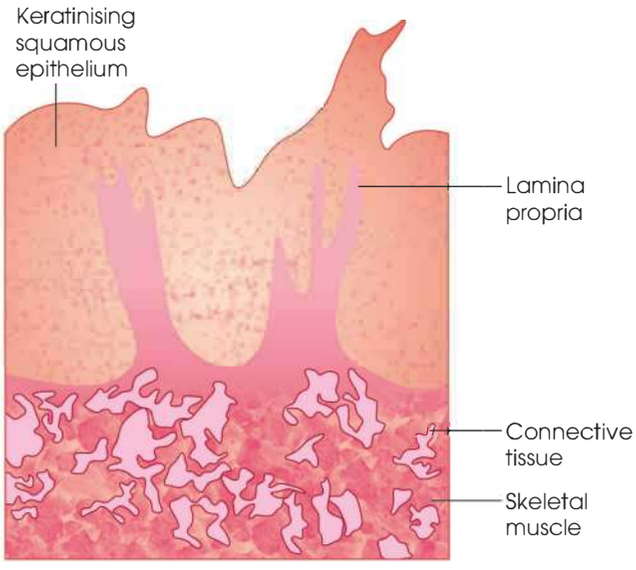


Fig. 56.9: Histological structure of filiform papillae

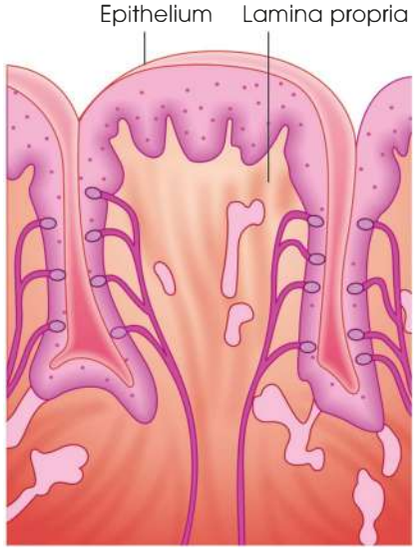


Fig. 56.10: Histological structure of foliate papillae

- Conical (conic) papillae, situated at the dorsum of the tongue, are scattered among the filiform papillae and similar to them, but they are shorter than the filiform.
- Foliate papillae, found on lateral margin of the posterior part, are arranged in several transverse folds (Fig. 56.10). In man, they are rudimentary. The filiform and conic papillae possess tactile sensitivity (perceive touch) and all the other papillae are gustatory.

GLANDS

The glands are small and scattered. They are of three types:

1. Mucous glands
2. Serous glands
3. Lymph nodes (glands)

The glands of the last variety are very prominent at the posterior part of the tongue, and are collectively known as the lingual tonsil.

Nerve Supply

The sensation of taste is carried by the chorda tympanic branch of the facial nerve (anterior two-thirds of tongue) and the glossopharyngeal nerve (posterior one-third). The general sensations of touch, pain, temperature, pressure, etc. are carried by the trigeminal nerve. The muscles are supplied by the hypoglossal nerve.

Functions

Tongue serves the following functions:

- *Mastication:* It helps in the act of chewing.
- *Deglutition:* It helps in the act of swallowing.
- *Taste:* Sub-serves taste and general sensation.
- *Speech:* Essential for speech.
- Secretion of mucus and of serous fluids with which it keeps the mouth moist.

SALIVARY GLANDS

There are three pairs of salivary glands; parotid, submaxillary or submandibular, and sublingual. One of each pair remains on one side and opens into the oral cavity. The parotid gland opens upon the inner surface of the cheek opposite the second upper molar tooth, by a single duct called the duct of Stensen. The submaxillary gland similarly opens by Wharton's duct upon the floor of the mouth on the side of the frenulum of the tongue. The sublingual gland, on the other hand, opens by several fine ducts, upon the floor of the mouth by the side of the frenulum. These are called the ducts of Rivinus (Fig. 56.11).

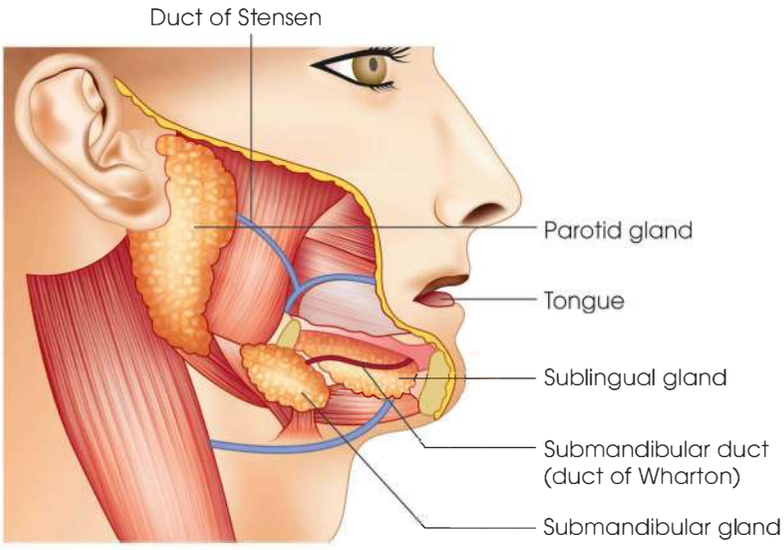


Fig. 56.11: Anatomical positions of the salivary glands

Histology of Salivary Glands

Key Points

1. Salivary glands are typical racemose glands, consisting of lobules which are made up of alveoli and the interlobular septum. Each alveolus is enclosed by a basement membrane upon which the gland cells are arranged.
2. The gland cells may be of two types: Serous and mucous, and accordingly the glands may be of two types. The parotid gland (Figs 56.12 and 56.16) is composed entirely of serous cells. The sublingual gland (Figs 56.14 and 56.15) is predominantly of the mucous type, whereas submandibular or submaxillary gland (Figs 56.13 and 56.17) is mixed, but in man, predominantly of the serous type. Each serous cell has a rounded nucleus towards the base of the cells.
3. In the mixed glands, the serous cells remain compressed at one side of the alveolus in the form of a crescent being sandwiched between the basement membrane and the more numerous mucous cells, lining the alveolus. These crescentic elements are called the demilunes or crescents of Giannuzzi.
4. The serous cells towards the apices contain numerous fine granules called zymogen granules which are more opaque and appear darker in sections than the mucous cells.
5. The mucous cells have flattened nuclei towards the base and have got less chromidial substance. These cells contain coarser and less numerous granules called mucinogen granules. In addition, myoepithelial basket cells described above are present.
6. The secretion of the serous glands is thin, watery, poor in solid, but rich in enzymes, such as the starch-splitting enzyme, amylase, also known as ptyalin. The secretion of the mucous glands is thick and viscid containing much mucus.

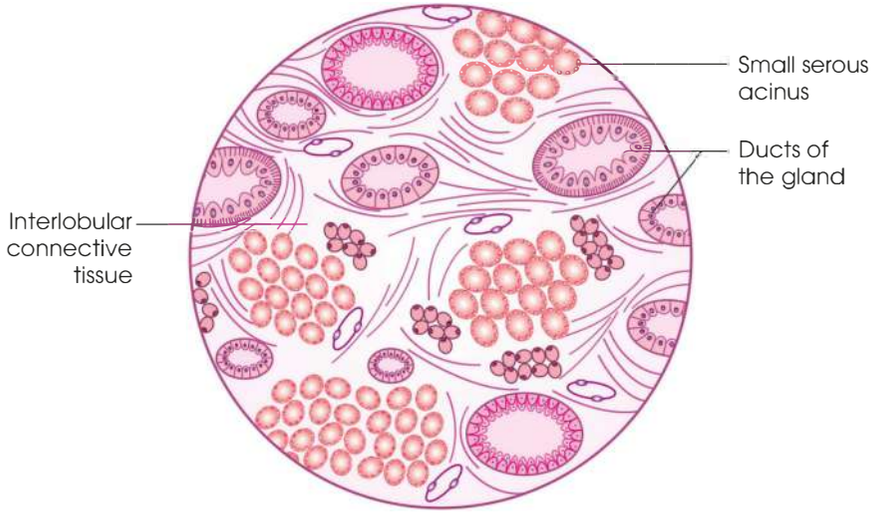


Fig. 56.12: Histological structures of the parotid glands

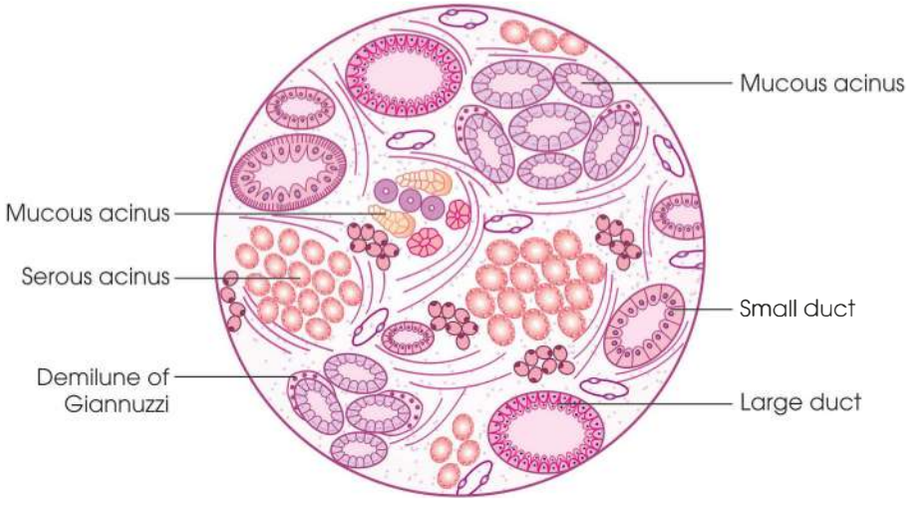


Fig. 56.13: Histological structures of submaxillary (submandibular glands)

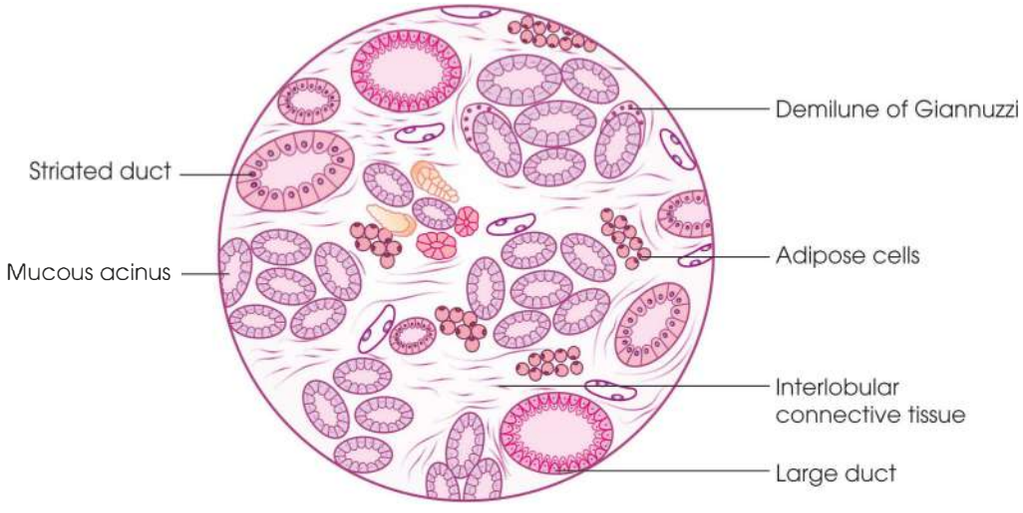


Fig. 56.14: Histological structures of sublingual glands

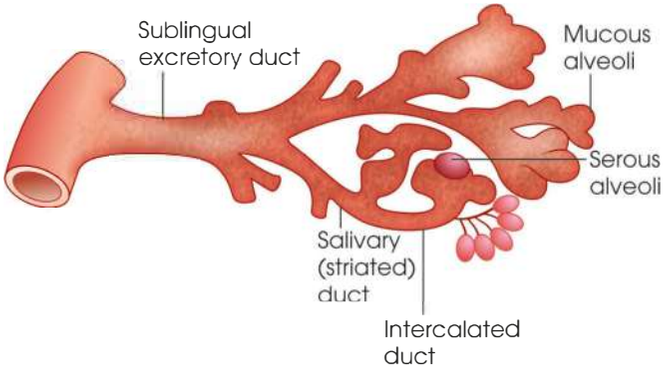


Fig. 56.15: Structures of sublingual gland

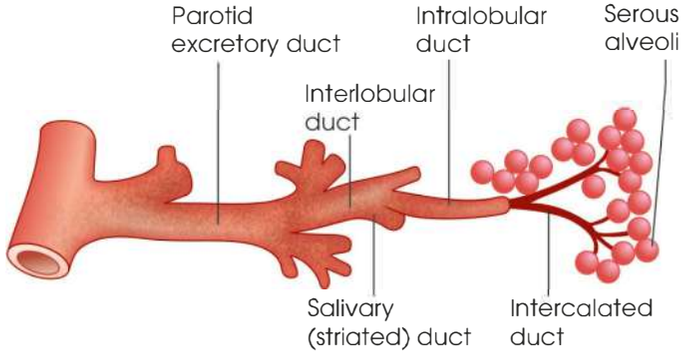


Fig. 56.16: Structures of parotid gland

The secretion of the glands is conveyed through various salivary ducts. The ducts also modify the composition of the saliva.

7. There are three types of ducts, e.g. excretory, striated and intercalated ducts composed of four different types of cells. The intercalated ducts lead away from the alveoli and are lined by cuboidal epithelium. The cells are practically filled by large nucleus, cytoplasm is scanty and few if any granules.
8. The intra-lobular ducts are lined by a two-layered epithelium, composed of columnar superficial layer and a flattened deep layer. At the termination the lining of the duct changes to a layered stratified squamous epithelium.

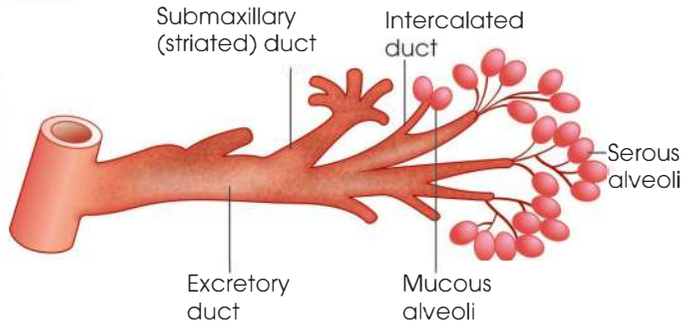


Fig. 56.17: Structures of submaxillary gland

9. In addition to these three pairs, there are small accessory buccal glands scattered throughout the mucous membrane and containing cells chiefly of the mucous type. It has been observed that the histological structure of salivary glands is influenced by endocrine secretion of thyroid and sex hormones.

PHARYNX OR THROAT CAVITY

It is a musculomembranous tube whose constricted end, ends in the esophagus. Its divisions from above downwards are nasal, oral and laryngeal. The mucous membrane lines the pharynx. Its walls are provided with sensory receptors. These receptors are sensitive to mechanical stimulation and are essential in the mechanism of swallowing. When liquid or food stimulates these touch receptors, a complex reflex of swallowing is initiated but when these sensory areas are anaesthetized, swallowing becomes difficult.

Other than functions of transmission of air from nose or mouth to the larynx and production of voice, pharynx serves as a channel to transport food from mouth to esophagus. During deglutition or swallowing, closure of mouth and nasopharynx effectively shuts off the pharynx from outside atmosphere. Dilatation of closed pharynx by contraction of pharyngeal muscles results in development of a slight negative pressure. When this effect is combined with thrust, food is pushed downward and onward in the oesophagus. Pharyngeal reflex may be absent in hysteria and glossopharyngeal nerve lesions.

OESOPHAGUS

Histology

Key Points

1. The mucous membrane of it consists of stratified squamous epithelium. Just under the mucous membrane, there is another very thin layer of plain muscle, called muscularis mucosae.
2. Inner to which lie the submucous coat layer. It consists of collagenous and elastic fibres.
3. Together with the muscularis mucosae, the submucous layer forms numerous longitudinal folds which result in the irregular form of the lumen in cross-section. During swallowing of food, these folds are smoothed out.
4. Outside this, remains the muscular coat consisting of two layers; the outer longitudinal and the inner circular layer. In the upper third of oesophagus (Fig. 56.18), the muscles are voluntary, in the middle third both voluntary and involuntary, in the lower third only plain muscle is found.
5. Lastly comes the outermost covering consisting of fibrous tissue, called the tunica adventitia.

Functions

It receives food from the pharynx and passes it onto the stomach by a series of peristaltic contraction.

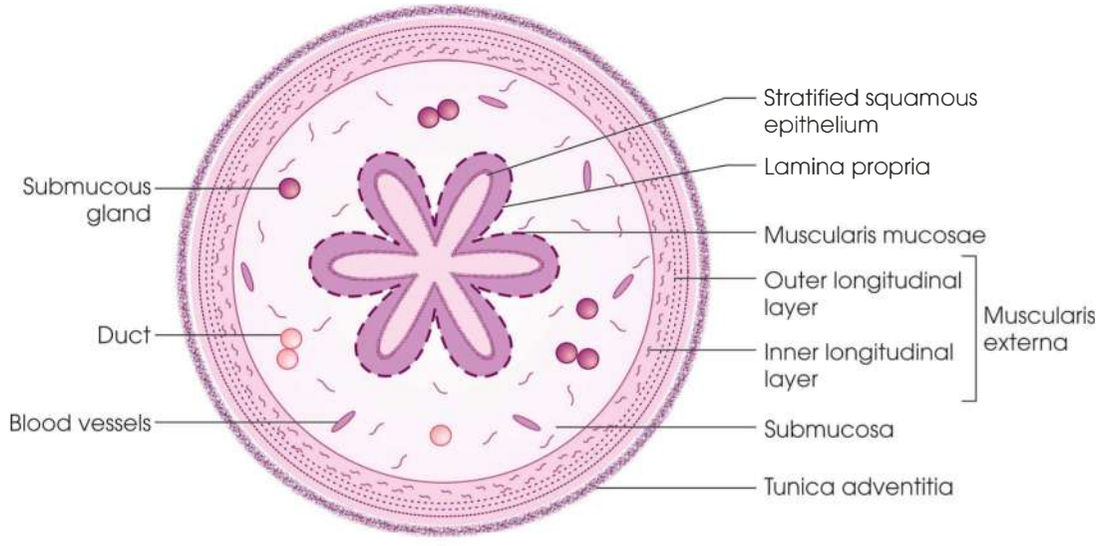


Fig. 56.18: Transverse representation of upper third of the oesophagus

STOMACH

The shape of the normal stomach is generally like the letter J. Sometimes the long axis may be slanting from left to right (*steer horn* type) or it may be even horizontal. The junction of the oesophageal mucosa with that of the stomach is abrupt. The oesophago-cardiac line of junction is irregular or zig-zag and is often referred to as the Z or ZZ line.

At the pylorus, the mucous membrane of the stomach makes junction with that of the duodenum. The capacity of the average stomach is about 1.12–1.70 liters (2–3 pints).

It can be subdivided into three parts: The fundic, the body and the pylorus, each of which contains a particular type of gland.

The cardiac area is the zone, 1 to 4 cm wide that guards the esophageal orifice, also known as cardiac sphincter.

- The fundic area is the largest area of the stomach accounting for 60 to 80% of total mucosal surface, interposed between the cardiac and the pyloric areas. The lower part of fundic area is separated from the pylorus by a sharp angle on the lesser curvature; called the incisura angularis.
- The junction of the pyloric and fundic area is not sharply demarcated and is frequently known as the transitional zone.
- The pylorus is limited on the left by the incisura and on the right by the pyloric sphincter. The circular fibres of pyloric sphincter guards against backflow of small intestinal contents into the stomach. The pyloric area is about 15% of the total gastric mucosal area. It is subdivided into two parts: (a) The pyloric antrum (pyloric vestibule) which is the short, comparatively wider, proximal chamber, and (b) the pyloric canal which is narrow tubular passage, about 3 cm long, ending in the pyloric sphincter (Fig. 56.19).

Histology of Stomach

Key Points

1. Histologically, stomach consists of the same four layers but with certain characteristic differences. The outer serous coat consists of peritoneum.
2. The muscular coat consists of three layers: The outer longitudinal, the middle circular and the inner oblique layer.
3. Next comes; the submucous coat.
4. Then comes; the layer of muscularis mucosae and a supporting stroma of connective tissue. This layer of muscle also consists of an outer longitudinal and an inner circular layer.
5. Finally comes; the mucous membrane which is thrown out into large folds called rugae when the stomach is empty and these folds tend to disappear when the stomach is distended. Hence, this folded arrangement of the mucous membrane is a great protective device to prevent damage from stretch.
6. The surface of the gastric mucosa is constantly covered by a thick layer of tough tenacious mucus secreted by the surface epithelial cells, the layer varying in thickness from 0.5 to 2.5 mm.
7. Beneath the surface layer of epithelium is located simple tubular glands which remain arranged like parallel tubes opening upon the surface. These glands secrete gastric juice. Histologically, as well as functionally, the glands (Fig. 56.20) are not all same. They differ in different parts of stomach but they are all tubular in structure extending to the muscularis mucosae where they terminate in a blind

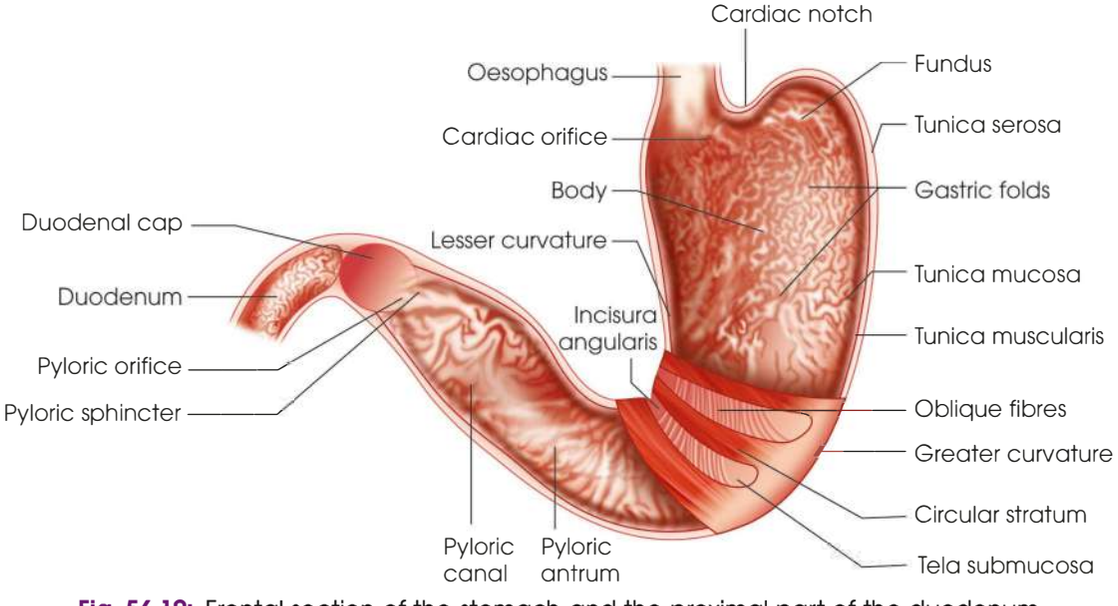


Fig. 56.19: Frontal section of the stomach and the proximal part of the duodenum

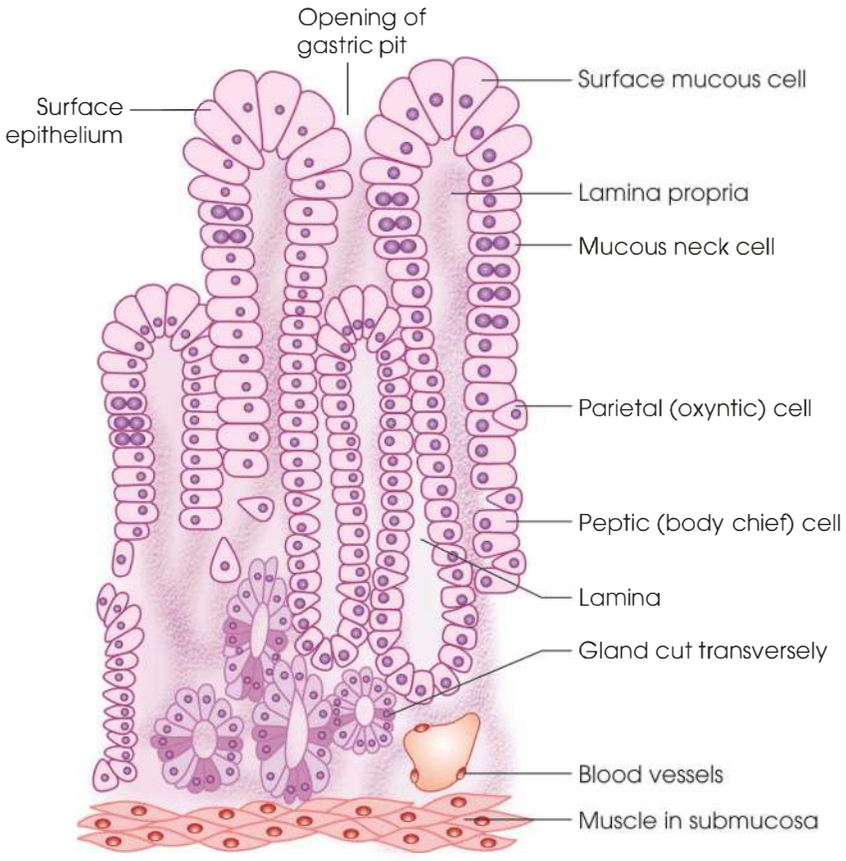


Fig. 56.20: Tubular glands in the mucosa of the stomach (body)

bulbar end, known as the fundic of the gland. The main tubular part is the body. The neck of the gland connects the body to the isthmus which communicates with the gastric crypt.

8. The glands are located near cardiac orifice, fundus, body of stomach and pyloric region.
   - *Near the cardiac orifice:* The cardiac glands which are short and tortuous are mostly surface mucous cells and occupy a small zone around the orifice and secrete mucus although a few pepsinogen cells are also present.
   - *In the fundic and body of stomach:* The fundic glands tend to be straight, slender structures with narrow lumen. The following types of cells are found to compose these glands:
     - The mucous neck cells: They secrete mucus which is soluble and have been found to constitute a part of soluble mucinogen droplets of the gastric juice under electron microscope.
     - Chief (zymogenic) cells in the body of the glands or the peptic cells: These cells are basophilic and secrete pepsin and contain zymogen granules which are precursors of pepsin. Histologically, the cells appear as a typical protein secreting one. In addition to pepsinogen, the chief cells probably produce gastric rennin, and a gelatin-splitting enzyme, known as gelatinase.
     - Oxyntic (parietal) cells: They are found pressed at the bed by chief cells and are oval in shape. They secrete hydrochloric acid (HCl). The cytoplasm of oxyntic cells is stained with acid dyes. Under electron microscope, secretory canaliculi are found to be lined by microvilli from the apical border, deep into cells.
     - Argentaffin (Kulchitzky or enterochromaffin) cells: These cells are responsible for the secretion of vasoconstrictor serotonin and are present in fundic glands.
   - *In the pyloric region:* Here the glands are fewer in number and shorter in length. They are devoid of both peptic and oxyntic cells. They are entirely lined by cells, resembling the mucous cells, as found in the neck of the fundic glands and secrete alkali and mucus.

The combined secretion of the above glands forms the gastric fluid. High concentration of ribonucleoprotein is present in the enzyme-producing cells.

It is to be noted that the secretion of the body and the fundic of the stomach is acid in reaction, and rich in enzyme and chlorides, whereas the secretion of pylorus is alkaline in reaction, rich in mucus and poor in enzyme and chloride. The surface lining of the stomach consists of columnar cells and goblet cells (mucus-secreting).

Functions of Stomach

1. ***Mechanical functions***
   - Stomach receives food material and acts as a reservoir of food.
   - The movements of stomach help in the proper mixing of food with the digestive juices and also help to propel the food into the duodenum.
   - The ingested solid food is gradually broken down by forceful muscle contractions in the lower end of the stomach. This helps in producing small food particles which are suitable to enter the duodenum, where processes of nutrient absorption begin.
2. ***Secretion:*** Stomach secretes gastric juice (mainly HCl and pepsin) which acts as a digestive fluid (Fig. 56.21).
3. ***Digestion:*** With the help of gastric juice, stomach digests protein up to peptone stage. It also digests fats to some extent with gastric lipase. Gastric rennin coagulates milk but this is of significance in young mammals. HCl causes some hydrolysis of foodstuffs.
4. ***Absorption:*** Small quantities of water, alcohol, glucose and certain drugs are absorbed from the stomach.
5. ***Excretion:*** Stomach excretes certain toxins (such as those of uraemia), certain alkaloids like morphine and certain other substances.
6. ***Stimulatory functions:*** Stomach manufactures two chemical substances which act as stimulants. They are as follows:
   - *Gastrin:* This substance is manufactured by the pyloric mucous membrane and is a true peptide hormone. It acts as a stimulant for gastric secretion. This is responsible for the second phase of gastric secretion.
   - *Castle's intrinsic factor:* The normal gastric juice as well as the gastric mucous membrane contains an intrinsic factor. The extrinsic factor is vitamin B_{12} taken along with food and the intrinsic factor interacts with it and helps in its absorption. Vitamin B_{12} exerts great influence on the normal development and maturation of red blood cells. There is also release of enterogastrone (enteron, gastron, chalone) from the mucosa of the upper small bowel which inhibits gastric secretions. The intrinsic factor, as suggested by Castle, is an enzyme like unidentified substance secreted by the stomach. It is present in the gastric juice as well as in the gastric mucous membrane. The optimum pH for its action is 7 and it is inactivated at temperature above 45°C. It does not necessarily run parallel with the amount of HCl or pepsin in the gastric juice. So that in some cases the intrinsic factor may be present even if there be no HCl or pepsin and vice versa. The site of formation of the intrinsic factor varies in human beings is the parietal cell of the stomach.
7. ***Reflex function:*** Various reflexes are initiated from the stomach. They are as follows:
   - *Gastro-salivary reflex:* Irritation of stomach stimulates salivation.
   - *Gastroilial reflex:* About half an hour after meal, increased peristaltic movements occur in the last part of ileum.
   - *Gastrocolic reflex:* Mass peristalsis is initiated in the colon about half an hour after taking food.

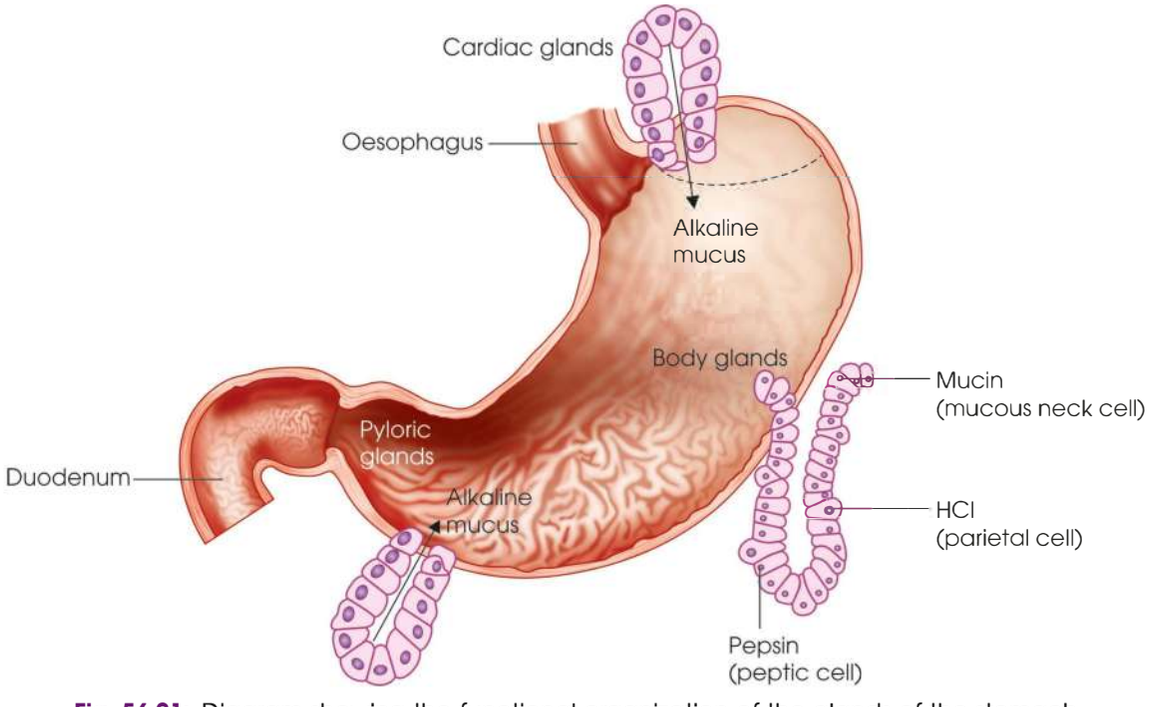


Fig. 56.21: Diagram showing the functional organization of the glands of the stomach

SMALL INTESTINE

From outside inwards, the arrangement of the layers is same, i.e. the serous coat, the muscular coat, composed of outer longitudinal and inner circular, the submucous coat, the layer of muscularis mucosa consisting of outer longitudinal and inner circular layers and lastly the mucous membrane. The mucous membrane consists of the following three characteristic features:

1. The simple tubular glands lined by columnar epithelium secrete intestinal juice through the openings between the villi, known as the crypts of Lieberkuhn. In much of the small intestine, especially in the jejunum, the mucosa is thrown into the circular folds, called folds of Kerckring (Fig. 56.22). These folds encircle the lumen and serve to increase the surface area of the lumen.
2. The villus is a slender finger-like fold of the mucous membrane projecting outward from the surface. The surface of the mucous membrane is covered with numerous such projections and the structures of villi in the different parts of the small intestine are leaf-shaped in the duodenum, rounded in the jejunum and club-shaped in the ileum. The function of the villi is to absorb the different foodstuffs. Their number is about 20–40 per sq mm. In the jejunum the number is less; in the ileum the number is more. Hence, the chief function of jejunum is secretion, while that of ileum, absorption. Each villus consists, at its centre, of a blind lymph vessel called the lacteal. It contains a milky fluid rich in fat, hence the name. Outside the lacteal is the loose highly vascular submucous coat. Then comes the superficial mucous layer which is composed of tall columnar epithelial cells and a few goblet cells (Fig. 56.23). The free end of each columnar cell, next to the lumen of the intestine, has a specialised cuticular border, resembling the brush border of certain renal tubular cells. The brush border of the surface epithelial cells of the villi is composed of microvilli of about 1 µm in length and 0.1 µm in width. The filaments of plain muscles, derived from muscularis mucosae, remain attached to the submucous coat of the villus all round the lacteal. The movements of the villi are due to the contraction of these muscular strips.

   A schematic structure of microvilli: The columnar epithelium shows continuity of parent cell membrane and intercellular space. Inset B (Fig. 56.23) shows functional organization in cross-section of the villus showing epithelial cells and basement membrane. Inset C (Fig. 56.23) shows cross-section of the intestinal tract.
3. *Lymphoid tissue:* This is another characteristic feature of the mucous membrane. The lymphoid tissue may remain in single isolated forms or in the form of aggregated islands, especially in the ileum, known as Peyer's patches. Among the columnar absorbing cells, are goblet cells that secrete mucus. The specialised cells which have the property of reducing ammoniacal silver to metallic silver are called argentaffin (Kulchitzky) cells. The cells are found mostly near the base of straight tubular intestinal glands or crypts of Lieberkuhn. The cells are low columnar in shape with nuclei which are larger and clearer than those of neighbouring cells. The cytoplasm contains granules which are always infranuclear in position. Other cells with a large acidophilic nucleus known as Paneth cells are found only in the small intestine at the base of crypts of Lieberkuhn and are possibly concerned with the

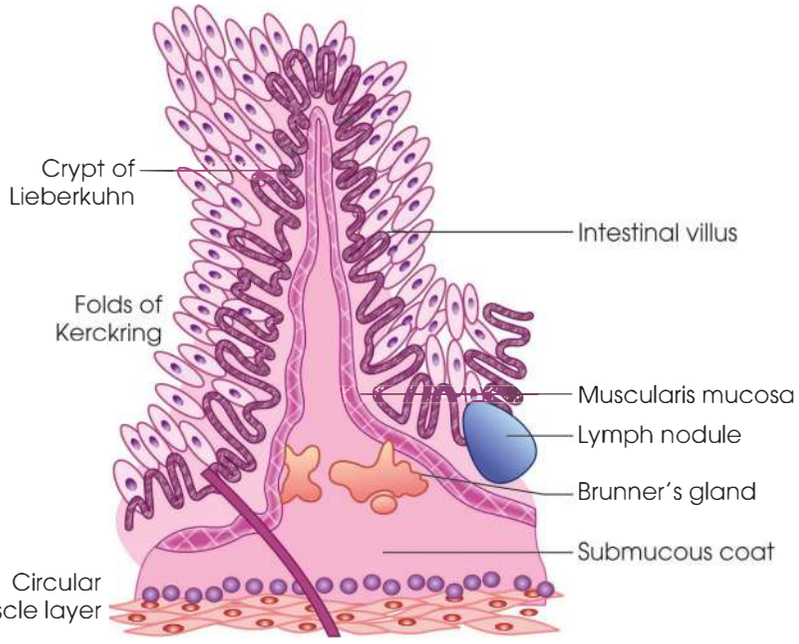


Fig. 56.22: Portion of the small intestinal wall showing folds of Kerckring, villi and glands of Brunner

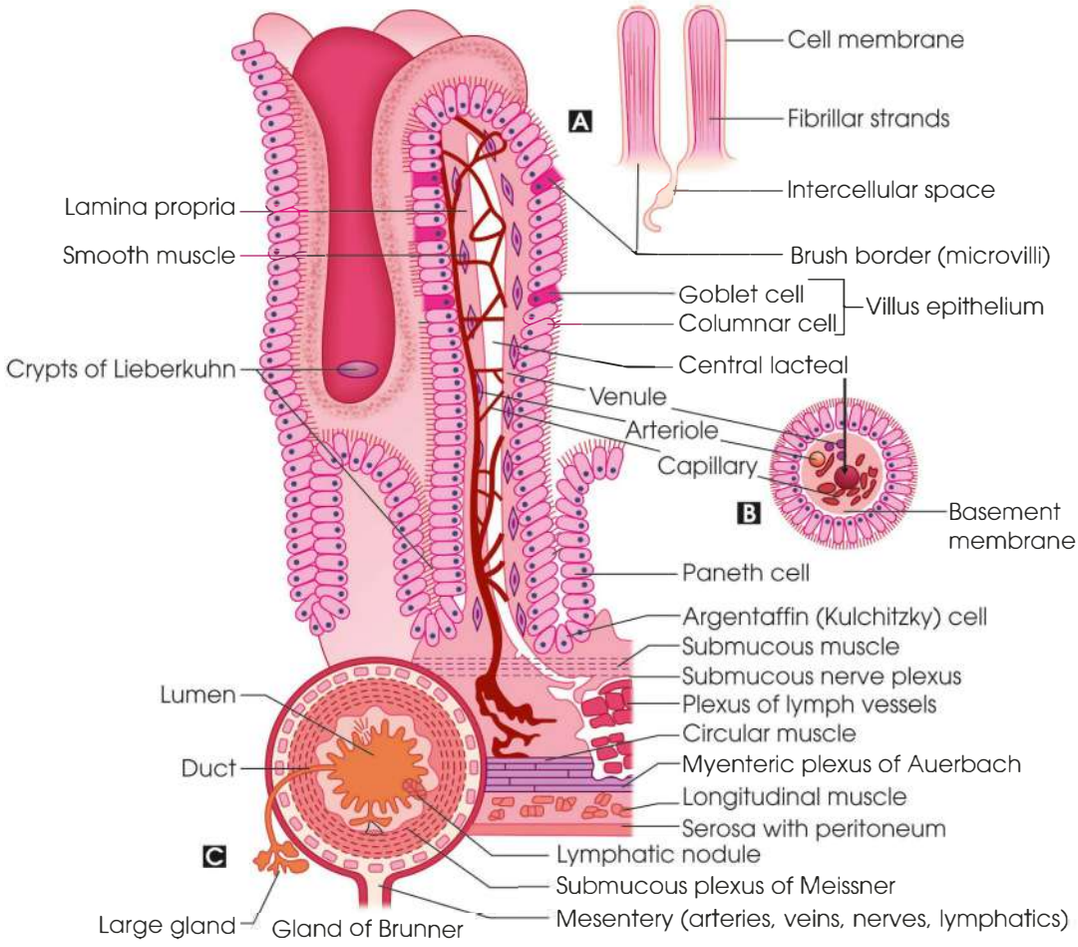


Fig. 56.23A to C: Structure of intestinal villus and adjacent crypts of Lieberkuhn

secretion of protein. In addition, there are also found granular argyrophil cells. In the duodenum, in addition to these general features, certain glands are present in the submucous coat under the muscularis mucosae. These glands open on the surface of the mucous membrane and are called duodenal digestive (Brunner's) glands.

Vermiform Appendix

It is a blind outgrowth of the caecum which in man is too short to have any functional activity. Lymphoid tissue is very much prominent here, like that of palatine tonsil. Hence, it is sometimes called abdominal tonsil. Due to shape it is labile to bacterial invasion and so, inflammation. Surgical removal of this organ is often required.

LARGE INTESTINE

The longitudinal muscle layers become especially collected into three bands passing along the whole length of the large gut; and are called the taeniae coli. The circular muscles become thickened at intervals giving rise to a series of pouch-like dilatations. There is no villus in the mucous membrane which is thrown into large folds. The glands are less in number, lined by columnar cells and large number of the mucus-secreting goblet cells. In the anal region, the mucous membrane is thrown into longitudinal folds, the rectal columns of Morgagni.

Rectum

Histologically, the rectum is similar to the large intestine except that the taeniae coli fan out and enclose the tube in an outer longitudinal layer of uniform thickening. The rectum functions as a reservoir for semisolid faeces and rectal valves help to support the faecal mass as the rectum fills.

Anal Canal

This canal is maintained by normal tonus of the puborectalis muscle. In defaecation, the muscular sling relaxes and the rectum becomes straight to permit easy passage of stool. The internal anal sphincter consists of a thickening of circular layer of smooth muscle. The external anal sphincter is a ring of skeletal muscle in an anal triangle. When the vein becomes varicosed at the junction of the rectum and at the anal canal, internal and external haemorrhoids (piles) occur respectively.

BRIEF SUMMARY OF THE CHIEF IDENTIFYING FEATURES OF DIFFERENT PARTS OF THE DIGESTIVE TRACT

1. **Oesophagus:** Stratified epithelium, two layers of muscles which may be voluntary or involuntary, outer fibrous covering.
2. **Stomach:** Thick mucous membrane, numerous glands—all of the same height, oxyntic cells, three layers of muscles, outer serous coat.
3. **Small intestine:** Villi alternating with crypts of Lieberkuhn, two layers of muscles, serous coat.
4. **Duodenum:** Same as above as well as Brunner's glands.
5. **Ileum:** Similar as above, presence of Peyer's patches as well.
6. **Jejunum:** Absence of either Brunner's glands or Peyer's patches.
7. **Large intestine:** Absence of villi, numerous goblet cells, taeniae coli.

EXAM-ORIENTED QUESTIONS

Essay

1. Describe the various parts of digestive tract and discuss their functions.

Short Notes

1. Tongue
2. Oesophagus
3. Achalasia cardia
4. Functions of the digestive system
5. Functions of the stomach
6. Functions of the small intestine
7. Functions of the large intestine

Chapter

57

Digestive Juices

INTRODUCTION

There are five digestive juices; saliva, gastric juice, pancreatic juice, succus entericus (intestinal juice) and bile. The necessity for so many digestive juices is that:

a. Juice does not contain all the enzymes necessary for digesting all the different types of foodstuffs. For instance, saliva contains only carbohydrate-splitting enzymes; whereas gastric juice contains both fat and protein splitting enzymes but none acting on carbohydrates.
b. The second reason is that, one particular digestive juice cannot digest a particular type of food up to completion. It will digest only up to a certain stage and then, the products will be handed over to the next digestive juice for further digestion. In this way digestion is completed. For instance, gastric juice digests protein up to the stage of peptone, pancreatic juice carries the digestion of peptone further up to lower peptide. The latter is digested completely up to amino acids by succus entericus.
c. Moreover digestive juices is that, their reactions are acidity alkaline status not all same. Saliva is slightly acid, gastric juice is strongly acid, but pancreatic juice is strongly alkaline. This, more or less, alternate acid and alkaline reaction, prevents any serious alteration of blood reaction. This is a special device for maintaining blood reaction constant.

The composition and functions of various digestive juices are mentioned below.

SALIVA

Characteristics

1. *Total amount:* 1,200–1,500 ml in 24 hours. A large proportion of this 24-hour volume is secreted at meal time, when secretory rate is highest.
2. *Consistency:* Slightly cloudy, due to the presence of cells and mucin.
3. *Reaction:* Usually slightly acid (pH 6.02–7.05). On standing or boiling it loses CO_2 and becomes alkaline. This alkaline reaction causes precipitation of salivary constituents, as tartar on the teeth or calculus in salivary duct.
4. *Specific gravity:* 1.002–1.012.
5. *Freezing point:* 0.07–0.34°C.

Composition

- **Water:** 99.5%
- **Solids:** 0.5%

1. *Electrolytes:* 2–21 mmol/L of Na, 10–36 mmol/L of potassium, 25 mmol/L of bicarbonate, 0.08–0.5 mmol/L of magnesium, 5–40 mmol/L of chloride, 1.2–2.8 mmol/L of calcium, 1.4–9 mmol/L of phosphate and iodine (concentration is more than that of plasma, but depends of dietary iodine intake).
2. *Antibacterial compounds:* Thiocyanate, secretory immunoglobulin A and hydrogen peroxide.
3. *Epidermal growth factor*
4. *Enzymes:* α-amylase, lingual lipase, kallikrein, proline rich proteins, and minor enzymes such as NAD(P) H dehydrogenase, superoxide dismutase salivary acid phosphatases A+B, N-acetylmuramoyl-L-alanine amidase, glutathione transferase, class 3 aldehyde dehydrogenase and glucose-6-phosphate isomerase. Antimicrobial enzymes like lysozyme, immunoglobulin A and lactoferrin are also present in saliva.
5. *Mucus*
6. *Cells:* There are about 6–8 million human and 500 million bacterial cells per ml of saliva.
7. *Opiorphin* (a pain-killing substance)
8. *Salivary agglutinins* which agglutinate bacteria are also present in saliva.

Functions

1. ***Mechanical functions***
   - It keeps the mouth moist and helps speech. Decrease in salivary secretion as occurs after nervousness, causes impairment of speech.
   - It helps in the process of mastication of the foodstuff and in preparing it into a bolus, suitable for deglutition. Here, saliva also acts as a lubricant.
   - Constant flow of saliva washes down the food debris and thereby does not allow the bacteria to grow. In acute fevers, where the salivary secretion is inhibited, the food debris is not properly washed away and the bacteria multiply. These collect as the sordes at the root of the teeth and upon the tongue.

   It is to be noted that the mechanical functions of saliva are its chief functions in human beings, and is mainly contributed by mucin one of its main constituents.
2. ***Digestive functions:*** Saliva contains two enzymes:
   - *Ptyalin:* Splits starch up to maltose in the following manner (Table 57.1).

     Maltase (in traces) converts maltose into glucose.
   - Salivary lingual lipase has a pH 4.0 and its get activated after reaching the acidic environment of the stomach.
3. ***Excretory functions:*** Saliva excretes urea, heavy metals (Hg, Pb, Bi, As, etc.), thiocyanates, certain drugs like iodide, etc. Alkaloids, such as morphine, antibiotics, such as penicillin, streptomycin, etc. are also excreted in the saliva. The excretion of ethyl alcohol by the salivary gland has promoted the recommendation that such a test should be used for medicolegal purpose. [It also excretes certain virulent micro-organisms, such as the virus of hydrophoboea, acute anterior poliomyelitis, mumps, etc.]
4. ***Helps in the sensation of taste:*** Taste is a chemical sensation. Unless the substances are in solution, the taste buds cannot be stimulated. Saliva acts as a solvent and is thus essential for taste.
5. ***Helps water balance:*** Saliva keeps the mouth moist. When moisture is reduced in the mouth, certain nerve endings at the back of the tongue are stimulated and the sensation of thirst arises. When body water is lost (sweating, diarrhea, etc.)—saliva is reduced and thirst is felt. The subject feels the necessity of drinking water and thus water balance is restored.

Table 57.1: Action of ptyalin

		Bolied starch
		↓ *ptyaline*
(Blue colour with iodine)	.. ..	Soluble starch
		↓
(Red colour with iodine)	.. ..	Erythrodextrin and maltose
		↓
(No colour with iodine)	.. ..	Achrodextrin and maltose
		↓
(No colour with iodine)	.. ..	Isomaltose and maltose

6. ***Helps heat loss:*** This is mainly found in animals (dog, sheep, etc.). When they become hot or excited more saliva is secreted causing greater heat loss.
7. ***Buffering action:*** Mainly bicarbonate and to a lesser extent phosphate and mucin present in saliva act as buffers. There is an increase in bicarbonate concentration during food intake.
8. ***Bacteriolytic action:*** The bacteriolytic action is mediated via antimicrobial agents such as immunoglobulin A and lysosomal action. The other antimicrobial enzymes that kill bacteria are salivary lactoperoxidase and lactoferrin.
9. ***Others***

   Kallikrein enzyme presents in saliva cleaves to high-molecular weight kininogen to form bradykinin which is a vasodilator.

   Endothelial growth factor (EGF): Saliva contains EGF which aids in the maintenance of oroesophageal and gastric tissue integrity.

   Opiorphin found in saliva has pain killing effect. It prevents the breakup of enkephalins, which are the natural opioids in the spinal cord and thus opiorphin extends the duration of enkephalin effect thereby relieving pain.

GASTRIC JUICE

Composition

The average composition of human gastric juice is as follows:

- **Water:** 99.45%.
- **Total solids:** 0.55%.

1. *Inorganic:* 0.15% (NaCl, KCl, $CaCl_2$, calcium phosphate, magnesium phosphate, bicarbonate, etc.).
2. *Organic:* 0.40%.
3. *Mucin:* It is of two types. Soluble mucin from the mucous neck cells and the insoluble mucin from the surface epithelium of stomach.
4. *Intrinsic factor*
5. *Enzymes:*
   i. Pepsin.
   ii. Other proteolytic enzymes of the gastric juice are: Cathepsin, gastricin, parapepsin I and II.
   iii. Gastric rennin: It is absent in adult human beings, may be present in infants (but it is of more significance in young mammals and it coagulates milk in the stomach, moreover, it is also important in the digestive processes of infants as it prevents the rapid passage of milk from the stomach. Rennin in the presence of calcium changes irreversibly the casein of milk to a paracasein which is further acted upon by pepsin).
   iv. Gastric lipase
   v. Other gastric enzymes are present in minute amounts and are lysozyme, gelatinase, urease,

carbonic anhydrase. The gelatinase is a very useful enzyme for the digestion of gelatin.

6. *HCl:* It mainly activates pepsinogen to pepsin. It can kill bacteria that have entered stomach along with the food.

Characteristics

1. *Total quantity:* About 500–1,000 ml per meal (1,200 ml–1,500 ml per day).
2. *Reaction:* Strongly acid.
3. *Free HCl:* 0.4–0.5%
4. *Total acidity:* 0.45–0.6%. It includes free HCl, as well as HCl combined with proteins. It also includes other acids, such as lactic acid. As ordinarily examined, the gastric contents show a lower acidity (0.15% to 0.25% HCl), because the HCl is partly neutralised by mucin and other substances.
5. *pH:* 0.9–1.5
6. *Specific gravity:* 1.002–1.004.
7. *Freezing point:* 0.59°C.

Functions

1. The enzyme pepsin, with HCl, digests proteins up to the stage of peptone.
2. Rennin also known as chymosin. This is of more significance in young mammals and it coagulate milk in the stomach, moreover it is also important in the digestive processes of infants in human; as it prevents the rapid passage of milk from the stomach. Rennin in the presence of calcium changes irreversibly the casein of milk to a paracasein which is further acted upon by pepsin.
3. Gastric lipase digests fat to some degree.
4. HCl acts as an antiseptic and causes some hydrolysis of all the foodstuffs.
5. *Excretion:* Toxins, heavy metals, certain alkaloids, etc. are excreted through gastric.

PANCREATIC JUICE

Characteristics

1. *Total quantity:* A bout 500 ml per meal. About 1,500 ml in 24 hours.
2. *Reaction:* Alkaline
3. *Specific gravity:* 1.010 to 1.030
4. *pH:* 8.0–8.3 (in dog).

Constituents

1. *Inorganic constituents:* The distinguishing chemical characteristic is its high bicarbonate content. The principal bases are sodium and potassium. Small amounts of calcium, magnesium and zinc are also present.
2. *Organic constituents:* Enzymes. The enzymes of pancreatic juice are trypsinogen, chymotrypsinogen, procarboxypeptidase, nucleotidases (ribonuclease and deoxyribonuclease), elastase, collagenase, pancreatic lipase, lecithinase, cholesterol esterase and amylase.

Its composition varies according to the means used to cause secretion. Table 57.2 shows the difference between secretin juice and pilocarpine juice.

Functions

1. *Digestive action:* Digestion of proteins: Enteropeptidase converts trypsinogen and chymotrypsinogen into the trypsin and chymotrypsin. These activated enzymes convert polypeptides to tripeptides, dipeptides and amino acids.
   Digestion of carbohydrates: Pancreatic amylase helps in the conversion of starch (polysaccharides) which is not acted upon by salivary amylase to disaccharides.
   Digestion of fats: Bile salts help lipase in conversion of fats to fatty acids and glycerol. This is achieved by decreasing the size of the globules which result in increased surface area.
2. *Neutralizing action:* It being alkaline, neutralizes almost equal volume of gastric juice.

SUCCUS ENTERICUS

Intestinal juice, in pure form, is difficult to collect because it is mixed up with bile and pancreatic juice. It can be collected from fistula preparations, such as Thiry fistula, Thiry-Vella modification and Mann-Bollman fistula.

Characteristics

1. *Total quantity:* Roughly about 1–2 liters in 24 hours. [Accurate measurement is not possible due to the great length of the small intestine.]
2. *Specific gravity* (*sp. g*)*:* 1.010.
3. *Reaction:* Faintly acid to faintly alkaline.
4. *pH:* Varies from 6.3 to 9.0 average 8.3.

Composition

- **Water:** 98.5%
- **Solids:** 1.5%

1. *Inorganic:* 0.8% salts of sodium, potassium, calcium and magnesium with that of chloride, bicarbonate

Table 57.2: Difference between secretin and pilocarpine juices

Constituents	*Secretin juice from 3 dogs Sp. g.—1.014*	*Pilocarpine juice*
Alkalinity: number of ml of (N/10) NaOH equal to 10 ml juice	12.7	5.5
Total solid in 100 ml	1.58 gm	6.4 gm
Total proteins in 10 ml	0.5 gm	4.8 gm
Ash in 100 ml	0.96 gm	0.3 gm
Chloride in 100 ml	0.30 gm	0.27 gm
Total nitrogen	—	0.74 gm

and phosphate. The bicarbonate concentration is higher than it is in the blood or interstitial fluids.
2. *Organic:* 0.7%
3. *Activator:* Enteropeptidase (previously known as enterokinase). It activates trypsinogen into trypsin.
4. *Enzymes*
5. *Mucin*

Intestinal Juice Enzymes

Proteolytic

1. **Erepsin:** A mixture of enzymes containing dipeptidases (break down dipeptides into amino acids) and amino peptidases (remove terminal amino acid containing free NH_2 group from polypeptides).
2. Several enzymes acting on the different fractions of nucleic acid, such as nuclease, nucleotidase and nucleosidase.
3. **Arginase:** Acts on arginine producing urea and ornithine.

Carbohydrate Splitting

1. *Amylase:* Found in traces, acts on starch and dextrin.
2. *Sucrase (invertase):* Digests cane sugar.
3. *Maltase:* Acts on maltose.
4. *Isomaltase*
5. *Lactase:* Breaks down lactose.

Fat-splitting: Lipase

Other enzymes: Alkaline phosphatase, cholesterol esterase, lecithinase, etc.

Key Points

1. The enzymes are mainly secreted in the gastric mucosa or in the pancreas.
2. Most of these digestive enzymes are actually intracellular and are present in the juice only because cells desquamate.
3. Enteropeptidase and amylase are highly soluble and diffusible and are present in the succus entericus. As regards other enzymes they are mostly present in the epithelial cells. Peptidases (erepsin), lactase, maltase, sucrose (invertase) and lipase are found in the intestinal epithelium as well as in the shed cells present in the juice.
4. Proteases, nuclease, phosphatase and arginase are present in the scrapings of the mucous membrane only. These scrapings also show the presence of all the enzymes mentioned above.

From this it can be concluded that the enzymes discussed above digest the foodstuffs in three ways

1. *Soluble enzymes:* Enteropeptidase and amylase freely exert their action on trypsinogen and starch respectively.
2. The shed cells breakdown in the succus entericus, set free their insoluble enzymes which digest polypeptides, disaccharides and fats.
3. Those insoluble enzymes which remain in the intestinal mucosa and found only in the scrapings exert their actions of the corresponding substrates during their transit through the epithelium, in the course of absorption.

Functions

Intestinal juices complete the process commencing in pancreatic juice, e.g. the trypsin exists in pancreatic juice in the inactive form trypsinogen which is activated by the intestinal enterokinase in intestinal juice. Trypsin then activates other protease enzymes. It thus digests polypeptides into amino acids, completing protein digestion. It also contains digestive enzymes, mucus, substances to neutralize hydrochloric acid of the stomach, hormone and erepsin.

BILES

Introduction

Bile is both a product of secretion as well as of excretion of the liver. Minute droplets of bile collect inside the tiny vacuoles of the liver cells and are discharged into the bile capillaries through the intracellular canaliculi. The primary bile capillaries start between hepatic cells as blind tubules. They join together repeatedly and form bigger channels and ultimately come out of liver as the right and left hepatic ducts. The two ducts unite and form the common bile duct, which enter into the duodenum, through the ampulla of Vater. Through the same ampulla also the pancreatic duct opens. From the upper part of the common bile duct commences the cystic duct, which ends in the gall bladder (Fig. 57.1).

Formation of bile by the liver is an active process, but entry of bile into the duodenum is intermittent and takes place only after meal. This necessarily indicates that bile must be stored somewhere. Gall bladder acts as the chief storehouse. The common bile duct also stores some bile.

Composition of Bile

Bile is a complex fluid containing various substances, some of which are merely waste products undergoing excretion, whereas others are products of secretion serving important physiological functions. In the gall bladder bile is concentrated five to ten times and its alkalinity is reduced (*vide* 'gall bladder'). The composition, as estimated by different observers, varies widely. The usual composition and the characters are as follows.

Characteristics

1. *Total quantity:* 500–1,000 ml daily. On the average about 700 ml.
2. *Sp. gravity:* 1.010–1.011 (gall bladder bile 1.026–1.040).

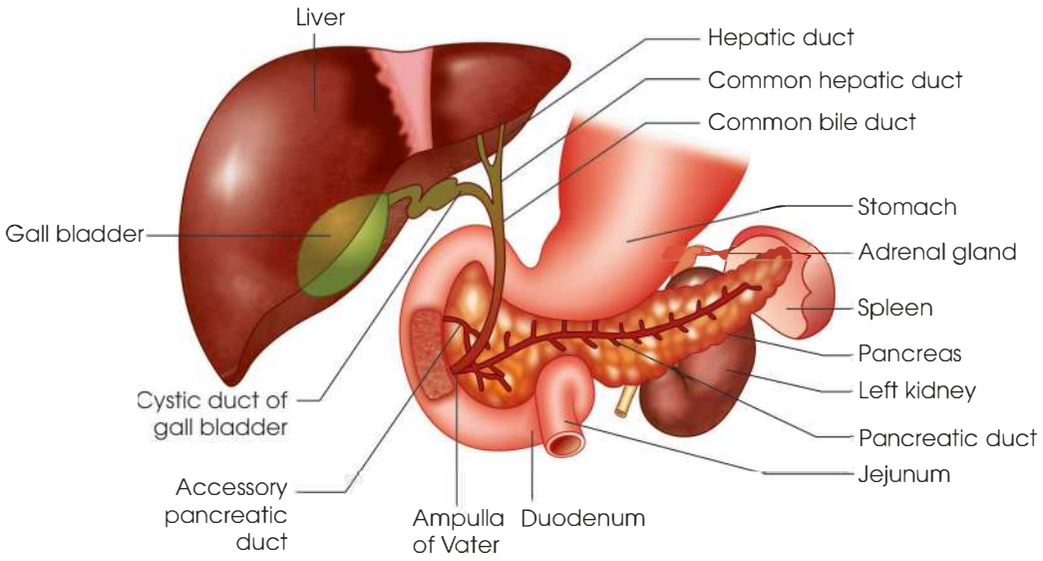


Fig. 57.1: Biliary system, pancreas and pancreatic duct

3. *Colour:* Human bile is yellowish-green. [In the carnivore, it is golden-yellow, due to the presence of more bilirubin. In herbivore, the colour is green, due to more biliverdin.]
4. *Taste:* Bitter
5. *Consistency:* Viscid, mucoid liquid.
6. *Reaction:* Liver bile is definitely alkaline, pH 7.7 (some hold pH 8.0–8.6). Gall bladder bile is neutral or slightly alkaline (pH 7.0–7.6) or slightly acidic (pH 6.8). That of dog and cat, definitely acidic (pH 5.6).

Composition

- Total solids, 2–11%.

The chief constituents are:

1. *Inorganic salts:* Chlorides, carbonates and phosphates of Na, K and Ca and $NaHCO_3$. The total base is equivalent to about 170 ml of (N/10) NaOH per 100 ml of liver bile (300 ml % in gall bladder bile).
2. *Bile salts:* Sodium taurocholate and sodium glycocholate. These are the most important constituents of bile and are synthesized by the liver (secretion).
3. *Bile pigments:* Of which bilirubin and biliverdin are the chief.
4. *Cholesterol, lecithin* and traces of fatty acids, soaps, etc. Cholesterol is probably an excretory product, because its amount in bile varies with its level in blood. It is kept in solution by the hydrotropic action of bile salts. Average composition of human bile is given in Table 57.3.

Table 57.3: Average composition of human bile

Constituents	*Gall bladder bile%*	*Liver bile (bile ducts)%*
Water	89.0	98.0
Solids	11.0	2.0
Inorganic salts	0.8	0.75
Bile salts	6.0	0.9
Mucin and pigments	3.0	0.4
Cholesterol	0.38	0.06
Fats, fatty acids, etc.	0.82	0.07

Functions of Bile

Bile is essential for life. Although it does not contain any enzyme, yet, it acts as a very important digestive juice. Its importance is so much that, life cannot be maintained without it. If a cannula is inserted in the common bile duct and all bile is collected outside, it is seen that the dog develops various abnormalities of bone, anaemia, and lack of nutrition and eventually dies (Whipple). Bile serves the following functions:

a. **Digestion:** Bile is essential for the complete digestion of fats and to some extent of proteins and carbohydrates. This action is due to the presence of bile salts, which act in the following ways: By reducing surface tension, so that fats are converted into an emulsion. The fine globules of fat, due to their innumerable number, render a larger surface area for the enzyme (lipase) to act. Due to this the process of digestion is quickened.

Activating action: The bile salts, by virtue of the cholic acid radical, act as a specific activator for different lipases. [That this action is not due to emulsification is proved by the fact that, although emulsification is unnecessary for the digestion of water-soluble triacetin by pancreatic lipase, yet the action of the enzyme is accelerated by bile salts.]

Solvent action: Bile acts as a good solvent. Due to this property, it serves as a good medium for the interacting fats and fat-splitting enzymes.

b. **Absorption:** Bile helps in the absorption of various substances. This is also due to presence of bile salts. The following things are absorbed with the help of bile:
Fats: Bile is essential for fat absorption. This is carried out in two ways:
   1. Hydrotropic action: By this property the insoluble fatty acids, cholesterol, calcium, soaps, etc. are made readily soluble in the watery contents of intestinal canal. In this way they are made easily diffusible and thus suitable for absorption. [This action is brought about by the combination of these substances with bile acids. Fatty acids, cholesterol and many such insoluble substances make loose compounds with desoxycholic acid. Such compounds are soluble in water and are called micelles.]
   2. Bile salts reduce the surface tension of the absorbing epithelium, increase their permeability and thus facilitate absorption of iron, calcium and probably other mineral constituents of diet. Vitamins: Bile salts help in the absorption of lipid-soluble vitamins A, D, E and K; and pro-vitamin carotene.

c. **Excretion:** Certain substances are excreted through bile, for instance:
   1. Some metals like copper, zinc, mercury, etc.
   2. Toxins, bacteria, etc.
   3. Bile pigments. [A portion of these pigments is then excreted in the faeces and in urine in various forms.]
   4. Cholesterol and lecithin are probably chiefly excretory products.

d. **Laxative action:** Bile salts stimulate peristalsis. When introduced directly into the colon it stimulates peristalsis of these parts.

e. **Choleretic action:** Bile salt acts as its own stimulant. Bile salts are the choleretic substances. They are absorbed from intestine, carried to liver and stimulate further bile secretion. The taurocholate is stronger in this respect than the glycocholate.

f. **Bile helps to maintain a suitable pH** of the duodenal contents and thus helps the action of all the enzymes. Bile is an important source of alkali for neutralising the hydrochloric acid entering the intestine from stomach. Lecithin and cholesterol, present in bile, also help in some ways: First, they are treated as food and are reabsorbed. Secondly, they act as adjuvant to bile salts in the process of emulsification of fats (but on the whole they are regarded as excreted products).

g. **Mucin of bile** acts as a buffer and a lubricant.

h. **Regurgitation of bile** in the stomach helps to neutralise gastric acidity and thus prevents the injurious effect of acids on gastric mucosa.

From the above it will be evident that bile is important not only as a digestive juice but for also various other purposes.

BILE SALTS

Variety and Chemistry

In the human bile there are two bile salts almost in equal proportion. They are:
1. Sodium taurocholate
2. Sodium glycocholate.

These are the sodium salts of taurocholic and glycocholic acids respectively system. They are also present in the bile in free form. Cholic acid forms soluble compounds with many insoluble substances such as fatty acids, higher alcohols, etc. Upon this property depends the hydrotropic action of bile salts.

$C_{23}H_{39}O_3$. $CO.NH.CH_2CH_2.SO_3H$
(Taurocholic acid)

$C_{23}H_{39}O_3$. $CO.NH.CH_2COOH$
(Glycocholic acid)

Synthesis of Bile Salts

Site for synthesis: The bile salts are synthesized by the liver. Nearly among 6 gm of bile salts are formed by the liver.

Mechanism of synthesis: Cholesterol is the precursor of bile salts. The source of this cholesterol is mainly from diet apart from that which is synthesized by liver during process of fat metabolism. Cholesterol forms cholic acid and chenodeoxycholic acid which combine mainly with glycine and taurine. Glycine is a simple amino acid which is synthesised in the body. Taurine is derived from sulphur-containing amino acid, cysteine. Glycocholic and taurocholic acids are formed by the combination of glycine and taurine with cholic acid respectively.

The reaction is represented as follows:
1. Cholic acid + CoA + ATP = Cholyl CoA + AMP + PPi
2. Cholyl CoA + Glycine = Cholyl glycine conjugate + CoA. The second enzyme similarly catalyses conjugation with taurine forming cholyl taurine conjugate. Because of the alkalinity of bile a major part of the conjugated bile acids forms salts with sodium or potassium, the glycocholates or taurocholates.

The salts of this acid including sodium are secreted in the bile.

Enterohepatic Circulation and Fate of Bile Salts

Bile salts are mostly (80–90%) reabsorbed from the intestine and are re-excreted in the bile. A number of other substances are excreted in the bile along with bile salts and most of them act as choleretics. This cyclical migration of the bile salts is called enterohepatic circulation. At each cycle a small part is lost in the faeces, which makes about 10–20% of the total amount. Normally, liver synthesizes only this lost amount to keep up the usual quantity.

Functions of Bile Salts

Most of the important functions of bile are due to the presence of bile salts. It will be seen that, as the bile salts run in the enterohepatic circulation, they serve some important functions at each step. The functions may be summarised as follows step by step:

1. While present in bile (i.e. in liver and bile channels): Bile salts keep insoluble cholesterol in solution (by hydrotropic action). The normal ratio between cholesterol and bile salts in the bile, varies from 1:20 to 1:30. When this ratio falls to 1:13 cholesterol is precipitated and forms gallstones. Biliary stasis and infection of gall bladder are the factors contributing the formation of gallstone.
2. While present in the intestine: They sub serves the following function: Digestion of fats and to some extent of other foodstuffs. This is done in three ways: first, by reducing surface tension of water, fat is emulsified, thus rendering larger surface area for the enzymes to act; secondly, by activating the lipases directly and thus increasing their actions; and thirdly, due to their presence, bile acts as a good solvent for the otherwise insoluble substances, viz. fatty acid and water-insoluble soaps, and in this way, acts as a suitable medium facilitating the interaction between fats and the fat-splitting enzymes.
3. Absorption of (a) fatty acids, monoglycerides, cholesterol and other lipids; (b) iron, calcium and probably other minerals, and also of (c) the fat soluble vitamins—A, D, E and K; and provitamin carotene.
4. Increase peristalsis of both small and large intestine and thus acts as a laxative.

BILE PIGMENTS

Chemistry and Varieties

A number of pigments is present in bile. The two chief pigments are bilirubin (golden-yellow) and biliverdin (green). Bilirubin ($C_{33}H_{36}N_4O_6$) is the chief pigment of human and carnivorous bile. Biliverdin ($C_{33}H_{36}N_4O_8$) is the oxidation product of bilirubin. It is present chiefly in the bile of birds and of herbivorous animals. Biliprasin is supposed to be an intermediate product formed during oxidation of bilirubin into biliverdin. Bilicyanin (blue), bilifuscin (red) and choletelin (yellow) are three other pigments formed by the successive oxidation of biliverdin. They are found in the gallstones. The bile pigments are porphyrin compounds and constitute about 15–20% of the total solids of the liver bile. [They can be detected by Gmelin's test.]

Origin and Formation

The old and worn-out red blood cells disintegrate and are removed from the circulation by the cells of the reticuloendothelial system; the bone marrow appears to be the most active site. Haemoglobin is released and by degradation, opening of the porphyrin ring system occurs. The degraded compound is known as verdohaemoglobin or choleglobin. In the next stage it is broken down into protein and haem. Protein is broken down into amino acids which enter the general amino acid pool of the body. The iron present in the haem remains stored in the body as ferritin and haemosiderin which help in the formation of new haemoglobin. The rest of the haem is converted into yellow pigment bilirubin which is oxidized into green pigment biliverdin or the green pigment biliverdin is formed first which by reduction forms the yellow pigment bilirubin. Biliverdin reductase is the enzyme which catalyses the reduction of biliverdin to bilirubin. These are also derived to some extent from myohaemoglobin.

A schematic representation of bile pigment formation is given in Fig. 57.2. It does not represent proved steps of the reactions, but only attempts to summarize the facts and supplies possible pathways.

Red Blood Cell Lysis

The oxidation and reduction take place by transference of hydrogen from the substrate and NAD/NADH or NADP/NADPH system. The bilirubin then probably combines with albumin of the plasma. When it enters the liver cells, plasma albumin is separated from bilirubin. In the liver cells and to lesser extent in kidney

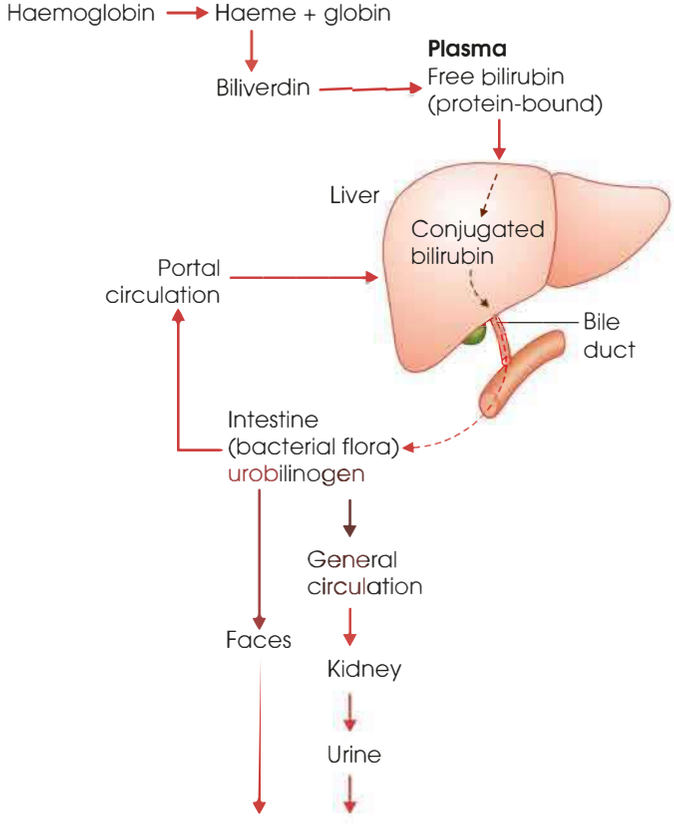


Fig. 57.2: Bilirubin formation and circulation

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cells, it is conjugated with glucuronic acid (UDP glucuronic acid) and forms monoglucuronide and diglucuronide. In hepatic bile these are bound in addition with protein and in gall bladder bile with lipoprotein, cholesterol and bile acids. The reaction is catalyzed by glucuronyl transferase. Some bilirubin is also esterified by sulphuric acid as bilirubin sulphate.

Site of Formation, Circulation and Fate

Liver (Kupffer cells), spleen and bone marrow, being the chief seats of the reticuloendothelial system, take the main part in bilirubin formation. Blood leaving the spleen and bone marrow has a much higher bilirubin content than the arterial blood. Normal blood serum contains traces of bilirubin which on the average amounts to about 0.5–0.8 unit*. It is to be noted that bilirubin, as it is present in blood (haemobilirubin), is not the same as that present in bile (cholebilirubin). Haemobilirubin remains combined with serum albumin and cholebilirubin remains in combination with glucuronic acid.

Haemobilirubin is called free (or unconjugated bilirubin while cholebilirubin is called bilirubin glucuronide.

The main differences between the two bilirubins are summarised below in Table 57.4.

After passing through the hepatic cells, conjugated bilirubin and biliverdin enter bile channels and then into the intestine along with bile. In the intestine following changes take place: *bilirubin → mesobilirubin → mesobilirubinogen → stercobilinogen*. On being exposed to air stercobilinogen is further oxidized into yellowish-brown stercobilin and is responsible for the normal colour of the faeces. Nearly half of the amount of total bile pigments; are getting excreted in the faeces; this varies from 40 to 280 µg per day.

The remaining part of stercobilinogen is reabsorbed from the intestine and is carried back to liver. Under normal conditions, this reabsorbed stercobilinogen is almost fully re-excreted in the bile. A trace of stercobilinogen may fail to pass through the liver, and is excreted in the urine. This excretory product is named as urobilinogen which is quickly oxidized into urobilin by the air after the urine is voided (Fig. 57.3). It is believed that some urobilinogen passes directly to the kidney escaping the liver for excretion (not shown in Fig. 57.3). Urobilinogen is identical with stercobilinogen

Table 57.4: Difference between haemobilirubin and cholebilirubin

Blood bilirubin (haemobilirubin)	*Bile bilirubin (cholebilirubin)*
1. van den Bergh's reaction is indirect or delayed direct	1. van den Bergh's reaction is immediate direct
2. Cannot be readily oxidised	2. Easily oxidised
3. Can be extracted with chloroform	3. Cannot be extracted
4. Warmth does not alter the quality of the van den Bergh's reaction	4. Warming changes the reaction. Serum giving direct van den Bergh's reaction will give indirect reaction after short warming
5. The molecules are larger and do not pass through a collodion membrane (because it remains combined with serum albumin)	5. The molecules are smaller and can be easily dialysed through a collodion membrane (remains as Na bilirubinate)
6. Renal threshold is much higher and is about 16–18 units (i.e. unless the concentration rises so high in the blood none will be found in the urine)	6. Renal threshold is only 4 units (4 units = 1 µg in 50,000 ml of serum)

van den Bergh's reaction

This test helps in detection of bile pigment in blood serum. There are three types of reactions:

- Direct
- Biphasic
- Indirect

Two types of solutions are used:

- ***Solution I:*** Sulphanilic acid (0.1 gm), concentrated HCl (1.5 ml), distilled water (100 ml)
- ***Solution II:*** Sodium nitrate (0.5 gm) and water (100 ml)

25 ml of No. I solution is mixed with 0.75 ml of No. II solution—*Diazo reagent*. 1 ml of serum is taken in a small test tube. To it equal amount of *Diazo reagent* is added and any one of the following reactions may occur:

- *Direct reactions*
  - Immediate or prompt: A bluish-violet colour immediately appears (within 10–30 sec).
  - Delayed: Reddish colour appears which gradually becomes violet and this takes from 5 to 15 minutes or even half an hour.
- *Biphasic reaction:* A reddish colour appears promptly and after much longer time becomes violet.
- *Indirect reaction:* Af first 1 ml of serum is treated with 2 ml of 95% alcohol. It is shaken and centrifuged. 1 ml of supernatant fluid is taken and to it 0.25 ml Diazo reagent is added. A reddish-violet colour appears immediately.

In *Jaundice* there is excessive accumulation of bile pigments in blood which causes yellowish discolouration of skin, mucous membrane and conjunctiva. There are *three* types of jaundice:

***One unit of bilirubin** is equivalent to a concentration of 1 mgm of the pigment in 200.000 ml of serum.

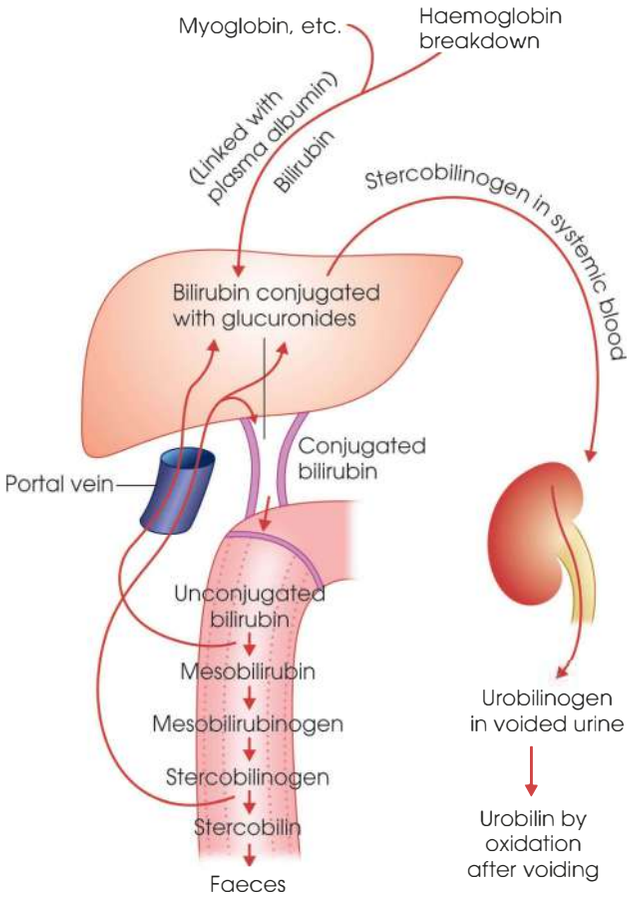


Fig. 57.3: Pathways of bile pigment excretion

and urobilin is identical with stercobilin. Normally faecal excretion of bile pigments varies from 50 to 250 µg per day, only 1–2 µg being excreted through urine.

When liver is damaged, urobilinogen reabsorbed from the intestine, fails to pass through the liver cells and appears in the urine in a larger amount. Under such conditions, urine contains considerable amounts of urobilinogen and urobilin. Presence of urobilinogen in urine in excess, therefore, indicates functional deficiency of liver.

EXAM-ORIENTED QUESTIONS

Essay

1. Discuss the characteristics, composition and functions of saliva.
2. Discuss the characteristics, composition and functions of gastric juices.
3. Discuss the characteristics, composition and functions of intestinal juices.
4. Discuss the characteristics, composition and functions of bile salts and bile acids.

Short Notes

1. Functions of saliva
2. Functions of stomach
3. Functions of bile
4. Bile pigments

Chapter

58

Mechanism of Secretion of Various Digestive Juices

INTRODUCTION

There are five digestive juices, viz. saliva, gastric juice, pancreatic juice, succus entericus (intestinal juice) and bile, secreted from salivary, gastric, pancreatic, intestinal and hepatic glands respectively, which are poured in the alimentary canal at its different levels successively from oral to aboral side. The term, mechanism of secretion is meant by (a) how the glands respond to secrete by the stimuli and how its flow and composition are maintained, (b) how the glands modify their secretion as regards their flow and composition by various types of stimuli and site of stimulation.

The types of stimuli are generally: (a) nervous, (b) chemical; (i) hormonal and (ii) other chemicals (diet, etc.), (c) mechanical.

Types of Stimuli

Nervous stimuli: The stimuli, e.g. touch of food on the tongue or other parts of the GI tract, sight of food, smell of food, etc. act on the sensory nerve endings and thereby nervous impulses are generated which pass via the central nervous system to trigger the corresponding motor nerve of the gland into action and the secretion takes place. The whole process of secretion is reflex process.

The salivary centre consists of superior and inferior salivary nuclei in the reticular formation of the medulla.

Nerve Supply of Salivary Glands

The salivary glands receive double nerve supply—both from the sympathetic and the parasympathetic.

Key Points

1. The parasympathetic fibres to the submaxillary (submandibular) and sublingual glands arise from the superior salivary nucleus (dorsal nucleus of the VIIth cranial nerve) in the medulla as nervous intermedius and by-passing the geniculate ganglion descend downwards through the facial (VIIth cranial) nerve and then through the chorda tympanic branch of the facial nerve.
2. The chorda tympanic nerve descends downwards and reaching the cavity of the mouth meets the lingual nerve. Then the secretory fibres leave the lingual nerve and end in the submaxillary (submandibular) ganglion (Langley's ganglion in animals).
3. From the submaxillary ganglion the postganglionic fibres arise and reach the submaxillary and sublingual glands and supply them with secretory and dilator fibres.
4. The parasympathetic or bulbar fibres to the parotid gland arise from the inferior salivary nucleus (dorsal nucleus of IXth nerve) in the medulla and descend downwards through the glossopharyngeal (IXth) nerve and being separated as the tympanic branch pass through the tympanic plexus and then through the lesser superficial petrosal nerve end ultimately in the otic ganglion. From this the postganglionic fibres arise and reach the parotid gland through the auriculotemporal branch of the fifth nerve to supply it with secretory and dilator fibres.
5. The sympathetic fibres to all these glands are derived from first and second thoracic segments of the spinal cord and come out through the first three or four anterior thoracic nerve roots and end in the superior cervical ganglion.
6. The postganglionic fibres arise from this ganglion, pass along the walls of the arteries and supply all the salivary glands (Fig. 58.1).
7. The sympathetic fibres are believed to end in the serous gland or in the serous part of the mixed gland and supply vasoconstrictor fibres to vessels of glands and myoepithelial cells of the duct.

Significance of Double Nerve Supply

Each glandular cell is supplied by two sets of nerves. The sympathetic carries vasoconstrictor fibres; hence their stimulation will cause vasoconstriction in the

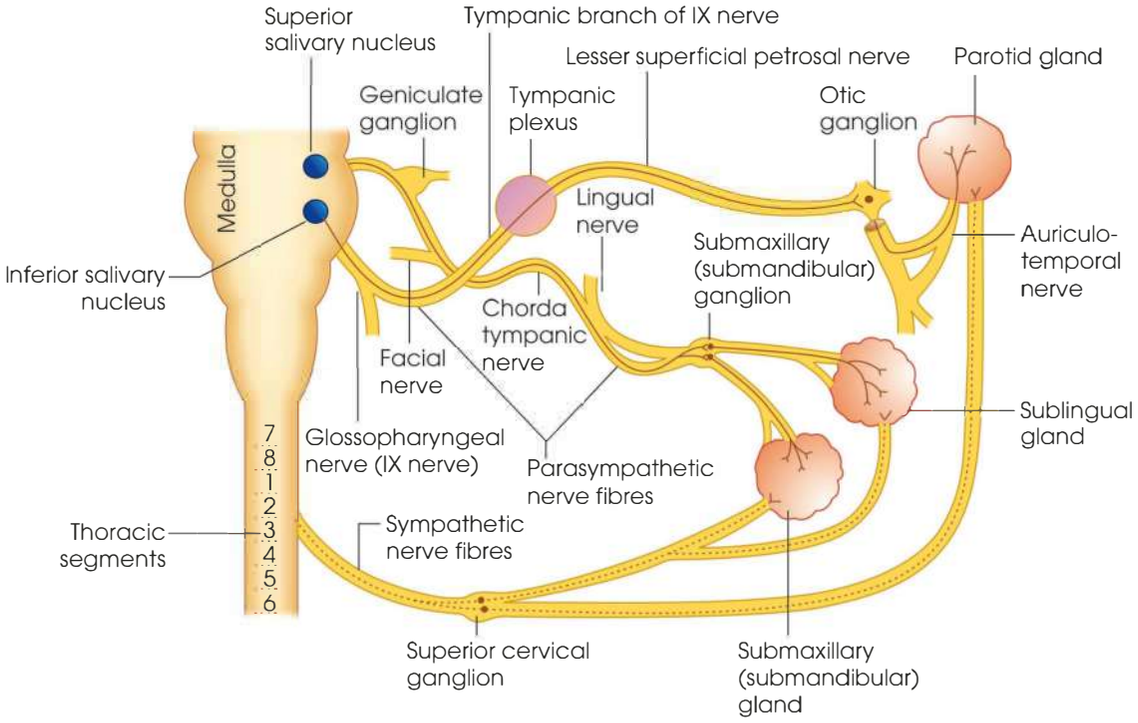


Fig. 58.1: Parasympathetic and sympathetic innervations of the salivary glands

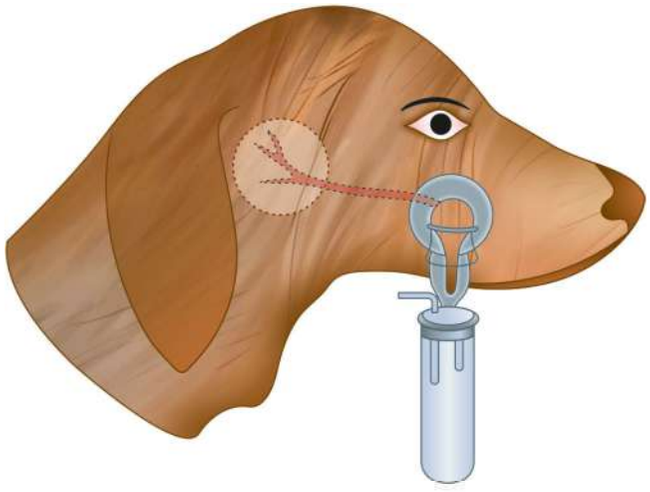


Fig. 58.2: Dog's parotid gland with fistula (schematic representation). The funnel along with a test-tube is being fixed on the cheek for collection of saliva at the opening of the glandular duct which is brought out to the exterior

gland and produce consequently less amount of saliva which becomes necessarily thick. Para-sympathetic fibres will cause vasodilatation thus increasing the amount of saliva, which becomes necessarily thin.

Conditioned Reflex

The existence of this reflex is proved by the fact that even the sight or smell of food can stimulate salivation, although no food is actually given. Various conditioned stimuli can be established which can produce salivation. Pavlov used to sound a gong just before giving food to the animal. After continuing this procedure for some days, it was seen that only the gong sound was sufficient to cause salivation even when no food was given. The gong sound here acts as the conditioned stimulus. The amount of saliva secreted can be assessed by creation of fistula along with parotid gland in dogs (Fig. 58.2).

Unconditioned Reflex

For this reflex, food should actually be given to the dog. The sensory stimulus for this reflex may arise from various sources as follows:

From the mouth: This is the chief place from which the normal unconditioned stimulus for salivation arises. The act of chewing, the sensation of taste, the irritation caused by the presence of food upon the mucous membrane of mouth—all these act as the sensory stimuli which reflexly produce salivation (Fig. 58.3). Here the effector is the salivary gland, the afferent path is represented in the trunks of the chorda tympani, the pharyngeal branches of the vagus and glossopharyngeal nerves, and the lingual, buccal and the palatine branches of the trigeminal nerve, the efferent path is the secretory fibres of chorda tympanic nerve with another peripheral relay station and its centre is the medulla.

Oesophago-salivary reflex: The sensory stimulus may arise from the oesophagus. When the food passes down the oesophagus, salivation is stimulated to some extent. Pathological conditions of oesophagus, such as ulcer, cancer, or the presence of a foreign body in the

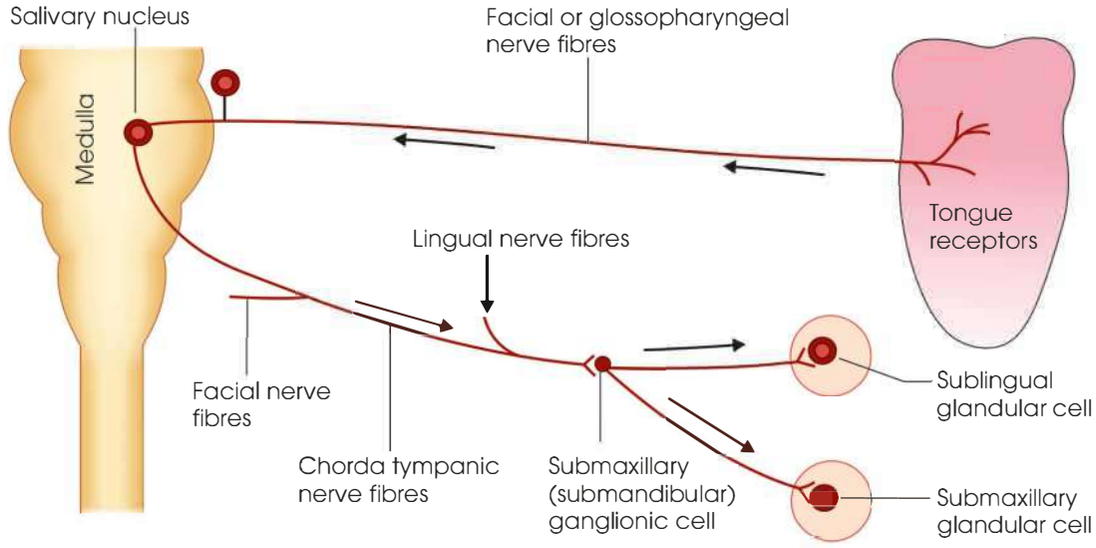


Fig. 58.3: Reflex pathways for the secretion of saliva

oesophagus, stimulates salivation. If the distal end of cut oesophagus is stimulated, salivation occurs. If the vagi are divided, the reflex is abolished. The purpose of this reflex seems (a) to provide enough saliva necessary to wash away the irritating substance, and (b) swallowing of saliva will set up peristalsis like movement of oesophagus which is likely to drive on the irritant. Oesophageal movement cannot be initiated by mechanical irritation but only when something is swallowed.

Gastro-salivary reflex: The stimulus may arise from the stomach. Irritation of stomach stimulates salivation. When food is introduced in the stomach of a sleeping dog (to avoid psychic effects), salivation takes place after about 20 minutes. This is also seen in many irritating conditions of stomach, for instance, gastritis, gastric cancer, etc. Increased salivation, before vomiting, is a typical example.

From other viscera: It is possible that stimulus for salivation may arise in other viscera also. For instance, in pregnancy increased salivation occurs. It is believed that the sensory stimulus arises from the distended uterus.

Mechanical Effects of Mastication

As the food is chewed, the contractions of the muscles of mastication help to press out the saliva accumulated in the ducts and acini of the glands. Hence, mastication acts not as a real stimulus but through its mechanical effect.

Reflex Control of Rate of Flow and Composition of Saliva

The receptor-centre-efferent system has got discriminating power so it can govern the salivary secretion, i.e. rate of flow and composition depend on the nature and intensity of the stimulus (e.g. food).

Spontaneous Secretion

The continuous secretion of saliva without any known stimulus is termed as spontaneous secretion. Although its mechanism is not known; but the acetylcholine may be the factor which is constantly secreted by the parasympathetic post-ganglionic nerve endings in small amount. Since atropine cannot check and cyanide or other metabolic poisons stop this type of secretion, so it is indicated that this is related and dependent to metabolic functions.

Adaptability of Salivary Reflex

The saliva secreted from the gland varies in both quantity and quality with the physical and chemical nature of the substances stimulating the secretion. The salivary gland does not secrete as a unit but different sets of epithelial cells of the gland contribute different components of secretion and their local productivity depends upon the intensity of excitation coming from the salivary centre. The afferent nerves are also different groups which carry impulse of specific nature and stimulate the different components of salivary centre which is a compound structure consisting of several parts and these in turn excite reflexly and selectively the different epithelial groups for appropriate types of secretion.

Disturbances of Salivary Secretion

The salivary secretion may be under certain conditions:

1. When decrease or absent called hyposalivation.
2. When increase called hypersalivation.

Hyposalivation

1. *Temporary:* Emotional state, e.g. anxiety, fear, fever and obstruction of the duct due to calculi (sialolithiasis).

2. *Permanent:* Aptyalism is rare but when occurs is due to congenital hypoplasia or absence of the gland.
3. *Xerostomia:* This is commonly seen in any acute stressful conditions in which patients complain of dryness of mouth. This is attributed to sympathetically induced decreased secretion of saliva in various stress induced pathological diseases.

Hypersalivation

Sialorrhea: The continuous and persistent increase in salivary secretion leads to sialorrhea. It is also caused in various conditions such as:

- Pregnancy
- Neoplasm of the mouth, tongue, carious tooth, oesophagus, stomach and pancreas
- Ulceration of oesophagus and stomach, spasm of stomach
- Neurological disorder, e.g. parkinsonian disease and schizophrenia.

Mechanism of Gastric Secretion

Gastric juice is a mixture of secretions of the different types of glands found in the stomach. As a whole gastric juice is acid in reaction, but when collected separately, it is found that the body and fundic secrete an acid juice, while the pyloric part secretes an alkaline juice. During fasting stomach secretes at a variable rate of 10–60 ml per hour. This juice is rich in mucus, poor in acid and contains pepsin. It probably acts as a mild antiseptic against swallowed bacteria. After meal gastric secretion is stimulated. On the average 500 ml of gastric juice is secreted per meal. The secretion starts almost immediately after food, reaches its maximum between one and a half to two hours, then gradually declines and comes to the fasting level after three to four hours.

Experiments

The mechanism of gastric secretion has been chiefly studied on animals. Some direct evidence has been obtained in man, from cases of accidental gastric fistula through which gastric juice could be collected. In man, another method is often applied known as fractional test meal. This method is commonly adopted for investigating gastric functions in man at bedside. In animals, two very important experiments have been done for investigating the mechanism of gastric secretion: (1) The experiment of sham feeding, (2) The preparation of Pavlov's pouch.

1. Sham Feeding (Fig. 58.4)

The oesophagus of a dog is exposed and divided in the middle of the neck and the two cut ends are brought to the surface. When the dog swallows food, the latter comes out through the upper cut end and does not enter the stomach (Fig. 58.4).

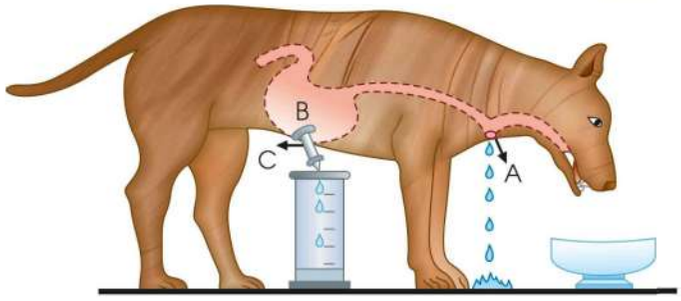


Fig. 58.4: Sham feeding. A: Opening of the divided oesophagus through which swallowed food is dropping out. B: Body of the stomach with fistula through which gastric juice is coming out. C: Cannula in the fistula

This experiment is very important to prove whether the food can stimulate gastric secretion even before entering stomach.

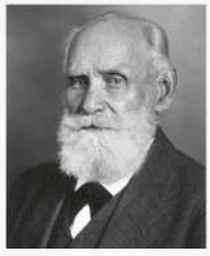
2. Pavlov's Pouch (Fig. 58.5)

It is a small diverticulum prepared from the body of the stomach and representing about one-eighth of the whole stomach. The pouch is prepared in such a way that its inner end is shut off from the main cavity of the stomach by two layers of mucous membrane while the outer end opens outside through an wound in the abdominal wall.

During the surgical procedure least injury is done to the vessels and nerves, so that the pouch secretes a juice identical with that secreted by the body of the stomach. This preparation has got the following advantages:

1. Pure gastric juice can be collected from this pouch unmixed with food. This is a great help in studying the variations of gastric secretion—both in quality and quantity—as may be produced by different stimuli.
2. It is found in dogs, that the juice secreted by the pouch is always a constant fraction of the total amount of juice secreted by the main stomach. From this the total secretion can be found out.

Pavlov was a Russian physiologist known primarily for his work in classical conditioning.



Ivan Petrovich Pavlov
1849–1936

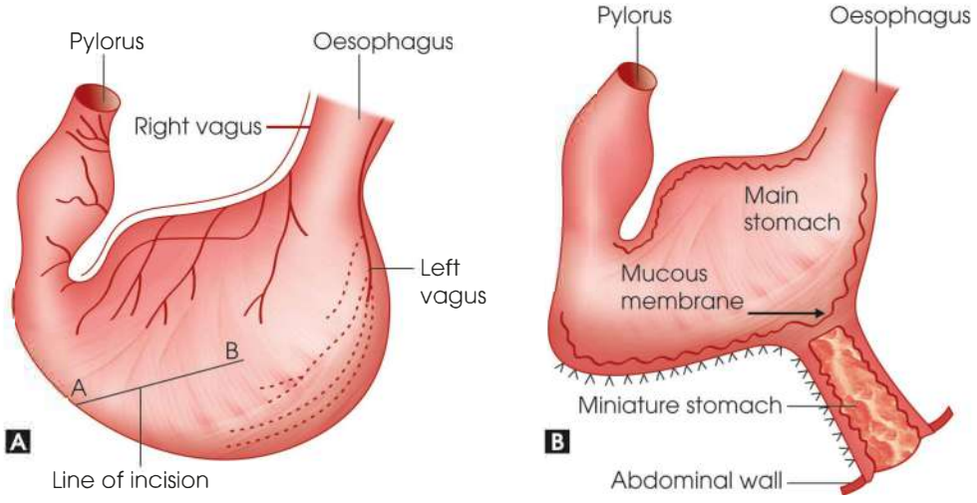


Fig. 58.5A and B: (A) Preparation of Pavlov's pouch for the study of gastric secretion where an incision is made that leaves the innervation intact; (B) Final stage after healing showing abdominal wall through which Pavlov's pouch is opening outside

Mechanism of Secretion

From such studies it is found that gastric secretion can be divided into different phases:

1. The *nervous phase* (cephalic phase due to the arrival of impulse in the brain, i.e. head = cephalic)
2. The *gastric phase*
3. The *intestinal phase*

   The gastric and intestinal phases may be collectively called the *chemical phase*, because in these two phases the working stimuli are chemical substances
4. *Interdigestive phase*.

   Each of these phases is briefly described below.

Nervous Phase

A pouch of Pavlov is prepared in a dog (and upon the same animal oesophagus is divided), as done in the experiment of sham feeding. The food, swallowed by the animal, comes out through the cut end of the oesophagus and does not enter stomach. In spite of it, it is found that the stomach secretes pepsin and HCl after a latent period of about 5–10 minutes and continues for as long as one and half hours. When the vagi are cut this secretion fails to occur.

- Stimulation of the vagus produces a secretion rich in pepsin and HCl also some mucus, the most powerful action is possibly on acid secretion. It stimulates gastrin secretion. Gastric releasing peptide is present at vagal nerve endings and when released cause increases in gastrin secretion.
- Stimulation of the sympathetic nerves, supplying the stomach causes vasoconstriction, but its effects on gastric secretion are not constant.
- Hypothalamus exerts undoubted influence upon gastric secretion. Stimulation of hypothalamus increases gastric secretion by augmenting vagal activity. Hypoglycaemia has similar effect mediated in an identical way. Experimental lesions of hypothalamus have been found to produce gastric haemorrhages, erosions and even perforations. It is believed that some such lesion may be associated with the causation of gastric ulcer.

These show that the initial phase of gastric secretion is a reflex process and this type of secretion is called appetite juice by Pavlov. On further analysis it is seen that two types of reflexes are involved in it.

- *Unconditioned reflex:* The sensory stimulus for the unconditioned reflex arises in the mouth during chewing and swallowing of the food. The sensory nerves are the fifth, seventh and ninth cranial nerves. The motor nerve is the vagus.
- *Conditioned reflex (psychic reflex):* The existence of conditioned reflex is proved by the fact that sight or smell of the accustomed food stimulates gastric secretion. Various other conditioned stimuli can be established which can arouse gastric secretion even when no food is actually given to the dog, i.e. without the contact of food in the mouth. The sensory nerves are those of special senses, viz. vision, smell and hearing. Motor nerve is the vagus.

The Appetite Juice

It has got the following character. It is rich in pepsin, acid in reaction and contains mucus. The composition of appetite juice is constant and does not vary with the type of food.

The quantity varies with the intensity of appetite. The secretion of psychic juice may be inhibited by shock, fear, anxiety, etc. In animals, it forms a considerable part of the total gastric secretion but in man the quantity is probably much less and is not essential. Its importance lies in the fact that it helps to initiate the second phase of gastric secretion.

Conclusion: Cephalic Phase

The sight, smell, thought, or taste of food brings over cephalic phase of gastric secretion. The neurogenic signals that produce the cephalic phase of gastric secretion originate from the cerebral cortex especially from the appetite centres of the amygdala and hypothalamus. The signals are carried to the dorsal motor nuclei of the vagi and then via the vagus nerve impulses are conducted to the stomach. Cephalic phase accounts for about 20% of the gastric secretion and is mainly associated with eating a meal. This enhanced secretory activity by the thought or sight of food forms the conditioned reflex. When appetite as in disease condition, in fever, etc. is depressed the cephalic reflex is inhibited. The cephalic phase stimulates enterochromaffin cells to secrete histamine and HCl in the stomach, G cells to increase gastrin circulation and chief cells to release pepsinogen.

Gastric Phase (Hormonal)

At the end of sham feeding, gastric secretion elicited by cephalic phase dies away. But if food enters the stomach, further secretion of gastric juice takes place. The gastric phase of secretion is mediated by local and vagal reflex response to distension and also by the hormone gastrin released by the mucosa of the pyloric area.

Thus, when the stomach is completely denervated, this secretion is not affected. This proves that this secretion is addition to a nervous reflex and mechanical irritation of food on the gastric mucosa is due to a chemical stimulus. By further experiments, it has been proved that a chemical excitant is actually operating in this phase and is called gastrin (Fig. 58.6).

The following experimental facts can be put forth to uphold the gastrin theory:

1. Acid extract of pyloric mucosa, on injection, stimulates gastric secretion.
2. At the height of gastric secretion, a substance is found to be present in the venous blood of stomach which can excite gastric secretion.
3. After resection of the pyloric part of stomach this phase of gastric secretion is greatly reduced.
4. When coagulated egg albumin, raw meat, undigested starch or fat, is introduced into the stomach through the gastric fistula or through the oesophagotomy wound of a sleeping dog (to avoid secretion of psychic juice), no secretion takes place. This proves that these substances neither have any mechanical effect nor carry the necessary chemical stimulus. But when meat extracts, liver extracts and partly digested meat, egg-white, etc. be introduced in the stomach, gastric secretion is stimulated.
5. It has been demonstrated that stimulation of the vagus causes release of gastrin. Vagal stimulation of the parietal cell occurs via M3 cholinergic receptors and via the release of histamine and gastrin from enterochromaffin-like cells and G-cells regulates acid and gastrin release. This

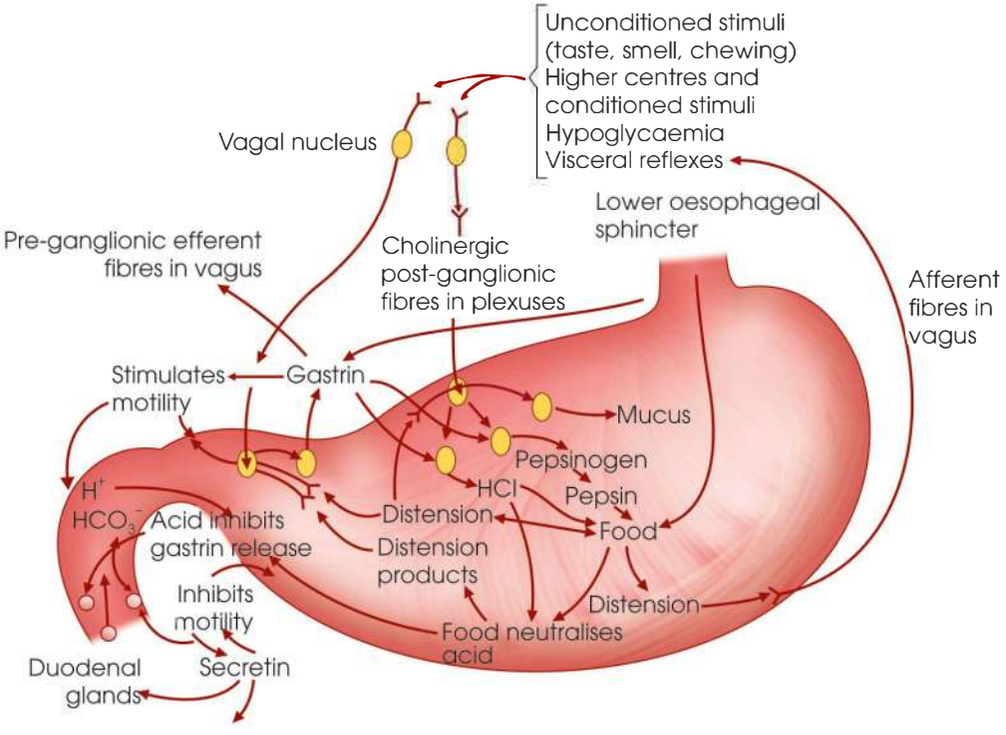


Fig. 58.6: Action of hormones controlling gastric secretion (schematic representation). From physiology of the digestive tract by Horace W. Davenport, Year Book Medical Publishers, Inc: Used by permission

gastro-intestinal hormone is also liberated through a local reflex mechanism mediated through cholinergic nerves other than the vagus.

Nature and Action of Gastrin

It is polypeptide in nature, two gastrins; gastrin I and II, differing in amino acid sequence have nbeen isolated. Both of them stimulate gastric secretion. On injection: (a) Gastrin stimulates gastric secretion—which is rich in acid but poor in pepsin, (b) it stimulates bile secretion, (c) it also stimulates pancreatic secretion to a slight extent.

Conclusion: Gastric Phase

From these observations it can be concluded that gastrin is manufactured by the pyloric mucosa by the products of protein digestion. This substance enters the blood stream, brought back to the gastric glands and stimulates their secretion.

The gastric phase of secretion constitutes the main part of gastric juice and continues for about three hours. Unlike psychic juice, this part of secretion and varies in quality and quantity according to the type of foodstuff. The variations are as follows:

Response to food

1. Meat increases both the quantity and the HCl content.
2. Fat inhibits secretion both in quality and quantity. (It also inhibits the movements of stomach.) This depressing effect may be due to a chemical substance called enterogastrone. The inhibitory effects of fats are more strongly exerted from the duodenum than from the stomach.
3. Water, tea, coffee, spices, condiments, vegetable juices, etc. stimulate gastric secretion.
4. Mechanical distension of stomach by gas, such as with aerated waters, stimulates gastric secretion (and movements).
5. Emotional stress also may delay stomach emptying for many hours.

Intestinal Phase

Key Points

1. It was observed that the presence of certain food substance in the small intestine excites gastric secretion. The latent period is 2–3 hours but continues for 8–10 hours. When water, meat extract, peptone and partly digested proteins, etc. enter the duodenum in the process of digestion or are directly introduced into the duodenum (through a duodenal fistula), this secretion occurs. When these parts are completely denervated this phase of gastric secretion is not affected. This proves that it is due to a chemical stimulant, a hormone or secretagogue absorbed with the food from the intestine, the exact nature of the stimulus is not known.
2. Gastric secretion can also be inhibited by the presence of certain substances in the duodenum. For instance: (a) Introduction of alkali directly into duodenum inhibits gastric secretion, (b) presence of fats in the duodenum inhibits gastric secretion (both the gastric and intestinal phases). This inhibitory action of fat is due to the liberation of an intestinal hormone called enterogastrone. It inhibits gastric secretion and gastric motility. Such an inhibitory agent has been detected in the blood of fat-fed animals and has been extracted from the intestinal mucosa.
3. Urogastrone is another inhibitory substance similar to, but not identical with enterogastrone. It has been isolated both from the urine of a normal male and from that of a pregnant woman. It exerts a specific inhibitory effect on gastric secretion (for this reason its therapeutic use in the treatment of gastric ulcer has been recommended). Its role in the normal process of gastric secretion is not known.*

Interdigestive Phase

Hydrochloric acid secretion has been found to take place at regular intervals, even in fasted man and dog. They all act by stimulating the nucleus of the vagus. It has been observed that both hormonal and nervous mechanisms are involved in such secretion, the latter being mediated through the vagus. Recently, it is believed that the interdigestive phase is a part of intestinal phase and partly due to spontaneous secretion of saliva.

Hormones on Gastric Secretion

Hormones secreted by different endocrine glands influence gastric secretion.

1. Glucocorticoids secreted by adrenal cortex (stimulated by ACTH) increases acid and pepsin secretion by the stomach but decreases the mucus secretion, and thus make it more susceptible to ulceration.
2. Epinephrine and norepinephrine, on the other hand, decrease gastric secretion.
3. Hypophysectomy causes characteristic changes in the chief cells of the gastric glands, consisting of a decrease in the size of nucleus and loss of most of

*Certain other factors influence gastric secretion, such as: (a) Blood sugar—low blood sugar stimulates and high blood sugar depresses gastric secretion. They act by stimulating the nucleus of vagus. (b) Insulin stimulates gastric secretion by reducing blood sugar. (c) Amino acids—certain amino acids are glycine, alanine, glutamic acid, etc. when given intravenously, increases gastric secretion.

Heidenhain did extensive research concerning the secretory and absorption processes of glands. He studied the stomach's gastric glands and the processes it used to produce pepsin and hydrochloric acid.

Rudolf Peter Heinrich Heidenhain
1834–1897

the pepsinogen granules. Secretion of hydrochloric acid is also reduced.

4. Serotonin, possibly a hormone secreted by certain enterochromaffin cells in the intestinal mucosa, inhibits gastric secretion particularly that activated reflexly or by cholinergic drugs.
5. Insulin acts through its effect on glucose metabolism and has an effect on the gastric glands similar to that of stimulation of the vagi. Release of gastrin is reduced by insulin.

Effects of Various Chemicals and Drugs on Gastric Secretion

Numerous chemical agents and various drugs affect gastric secretion.

1. Histamine is a powerful stimulant of gastric secretion. It is thought that it acts directly on the parietal cells.
2. Histalog, an analog of histamine, is also a powerful gastric stimulant.
3. Caffeine and alcohol are strong secretory stimulants, producing a juice of high acidity and rich in mucin. Para-sympathetic agents, such as acetylcholine, mecholyl, etc. are secretory stimulants.
4. Secretory depressants are also known. Alkali and acids depress gastric secretion. Belladonna, atropine, hyoscine, etc. are secretory depressants.

Interrelation between the Different Phases

The phases of gastric secretion are closely interrelated. The appetite juice initiates gastric digestion, having a powerful digestive action on protein. Secondly due to the presence of food in the stomach gastrin is liberated which stimulates the gastric phase of secretion. Intestinal phase starts when stomach empties into duodenum. It probably ensures maximum gastric digestion of food. The value of enterogastrone lies in the fact that it stops rapid emptying of stomach and thus keeps fatty food for a longer time in the duodenal region, so that it may be properly mixed up with bile and pancreatic juice. A very small amount of gastric juice is secreted during the interdigestive phase.

Investigation of Gastric Secretion in Man

The methods which are commonly adopted for investigating gastric secretion in man are called fractional test meal or gastric analysis.

The procedure is as follows: The subject is given diet on the previous evening. In the next morning the patient is made to swallow a thin flexible rubber tube known as the stomach tube (Ryle's tube, Lyon's tube or some other modification (Fig. 58.7). The tube has got three markings on it. When swallowed up to the first mark coinciding with incisor teeth (about 30 cm or 12 inches from the end), the end is near the cardiac end of the oesophagus; if up to the second mark the end is within the stomach; when up to the third mark the end has entered the duodenum.

During gastric analysis, the subject swallows the tube up to the second mark. The resting contents of the stomach are aspirated out and are preserved. Then the patient takes about a pint of oatmeal gruel, the stomach tube remaining swallowed as it was. Every fifteen minutes a sample of about 20 ml is drawn out and the procedure is continued for three hours. Altogether thirteen samples are obtained, the resting contents being the first sample.

Each sample is then tested for the following:

1. ***Free HCl:*** Normally the free HCl of the resting contents lies between 1.5 and 2.0 mEq or 54–60 µg (34.46 µg = 1 mEq) of HCl. After the gruel is taken the acidity is reduced by dilution. The free HCl then steadily rises and becomes maximum 40–50 mEq of HCl in the second hour. Then it gradually declines. When bile enters due to regurgitation, gastric acidity is reduced (Fig. 58.9). In gastric ulcer the value increases up to 3 times.
2. ***Combined acidity:*** This includes HCl combined with protein, mucus, etc. as well as organic acids such as lactic acid, produced by fermentation. Normally it varies from 10 to 55 mEq of HCl. In hypochlorhydria or achlorhydria the rate of fermentation is more, so that, level of combined acidity becomes high.
3. ***Total acidity:*** This is the sum total of free HCl organic acids, combined acid and acid salts.

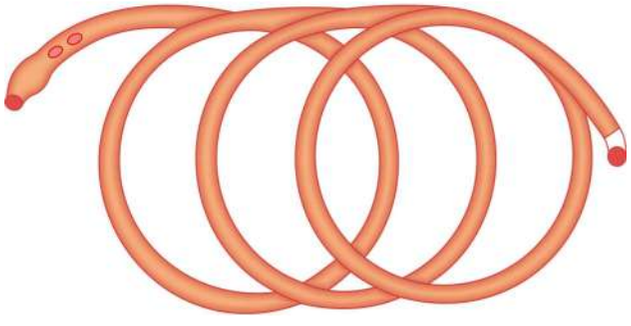


Fig. 58.7: Ryle's stomach tube

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4. ***Total chloride:*** This includes free HCl, combined HCl and inorganic chlorides. Its importance lies in the fact that the free acid level is always disturbed by the entry of bile, but the total chlorides remain unaffected. Hence, estimation of total chlorides, along with estimation of free acidity, will give more correct information about the secreting capacity of stomach.
5. ***Starch and sugar:*** Sugar is produced by salivary digestion of starch. Presence of sugar and starch indicates that stomach has not yet completely emptied. Their absence, therefore, indicates the emptying time. Normally, they are not found from the tenth or eleventh sample.
6. ***Bile:*** Presence of bile as indicated by yellow or green colour of the stomach contents shows duodenal regurgitation. It also indicates that pyloric sphincter has opened and gastric emptying has begun. Generally, bile first appears in the second hour.
7. ***Blood:*** It is not a normal constituent. Its presence shows ulcer, cancer or other haemorrhagic conditions of stomach. In case of ulcer the blood might be bright red or brown in colour and in case of cancer it is brownish-black.
8. ***Lactic acid:*** Derived mainly from fermentation of carbohydrate when there is a fall in the gastric hydrochloric acid. Hence, if free HCl is low, lactic acid will be high.
9. ***Mucus:*** Excess of mucus indicates an irritated condition of the stomach (gastritis, etc.).
10. ***Presence of pepsin:*** It indicates the functional condition of the peptic cells.
11. In addition to this, microscopic examination of each sample is carried out for blood cells, epithelial cells, tumor cells, bacteria, etc.

Taking these facts into account a normal gastric analysis curve will be as shown in Fig. 58.8. It will be seen from there that, this test not only gives an idea of the secreting capacity of stomach but the degree of motility (to be obtained from the emptying time), opening time of pylorus, duodenal regurgitation, etc. can be also known from it. In certain pathological conditions, characteristic variation of the curve is seen, viz. in gastric cancer and pernicious anaemia there will be achlorhydria, in duodenal ulcer the curve will be high 'climbing' type and so on.

To make a complete investigation of gastric functions only fractional test meal is not enough; radiological examination after barium meal has also to be performed. This will show the size, shape, motility, emptying time, presence of ulcer, etc. in the stomach.

Other Functional Tests

Other functional tests of stomach are as follows:

Histamine test of gastric secretion: Histamine is a strong stimulant for the oxyntic cells. Only 0.5 mgm histamine chloride, injected subcutaneously, will stimulate gastric secretion at the rate of 200 ml per hour. In those patients who show achlorhydria with ordinary gastric analysis, this histamine test is performed in order to see the condition of the oxyntic cells. If

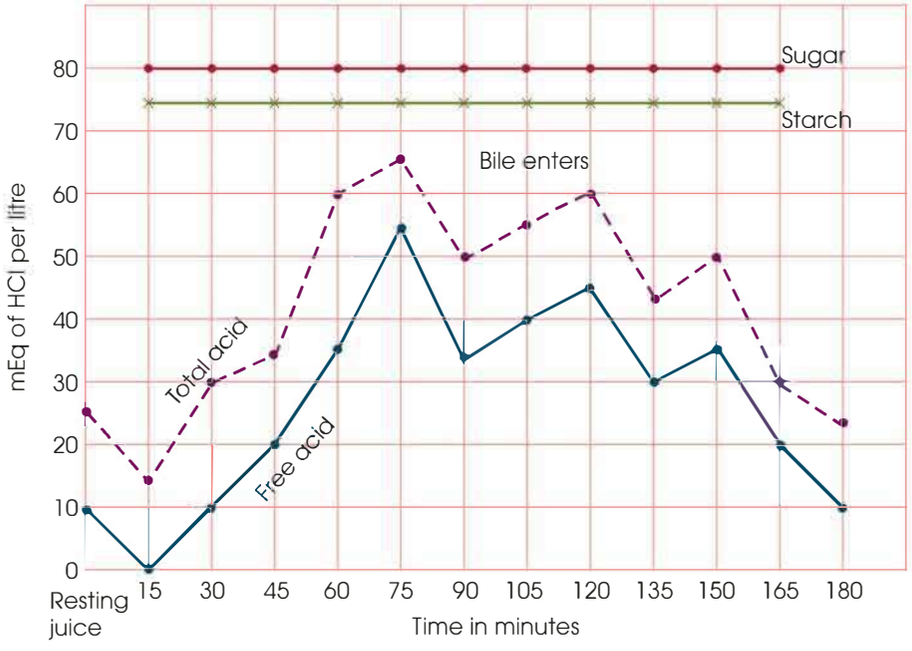

Fig. 58.8: Fractional test meal in a normal subject. Showing free HCl (maximum 55 ml), total acidity (maximum 65 ml) entry of bile (7th sample—1½ hour), presence of starch and sugar. Absence of starch and sugar in the 12th sample shows that the emptying time of stomach is 2 hours 45 minutes

performed in a normal subject, it shows the maximum secretory capacity of the oxyntic cells. Negative response indicates atrophy of oxyntic cells. Nowadays Pentagastrin or same type of drug which enhances gastric acid output is injected subcutaneously. The specimen is collected every 15 minutes for one hour. The maximal acid output (MAO) is determined by titrating each of the four specimens collected during an hour and the results is averaged. The average is used to determine the millimoles of hydrogen ion produced per hour.

Insulin test of gastric secretion: Insulin reduces blood sugar which in its turn, stimulates vagus and thereby excites gastric secretion. A positive insulin test is proof of the presence of intact vagal fibres but a negative result is less conclusive since some subjects with intact vagi fail to secrete in response to insulin. However, the test is effective in most cases. Seven units of insulin given subcutaneously produce marked secretion of gastric juice (which is rich in HCl and pepsin content) although reduction of blood glucose by insulin to moderate degree causes inhibition of secretion. The secretion takes place after a latent period of 40 minutes. This test also shows the secretory capacity of stomach. Since the response does not occur in absence of vagus so the absence of gastric secretion following insulin induced hypoglycaemia is a test for the vagal denervation.

Investigations which are commonly employed nowadays

Oesophagogastro-duodenoscopy: Most of the acute gastric problems are treated empirically. However, a direct visualisation of the stomach is conducted by upper endoscopy examination known as EGD (or oesophagogastro-duodenoscopy). It is indicated in case of chronic symptoms of nausea, vomiting, abdominal pain, gastro-intestinal bleeding, etc.

Gastric tissue biopsy: A specimen (biopsy) of the stomach mucosa may be warranted in case of chronic gastritis or infections (*Helicobacter pylori*) or carcinoma of stomach. Further tests are required for confirmation of *Helicobacter pylori* infection.

ORIGIN AND CHARACTER OF THE IMPORTANT CONSTITUENTS OF GASTRIC JUICE

Hydrochloric Acid

Key Points

This is secreted by the oxyntic cells (parietal cells.) They are present in the fundic and the body of the stomach. They contain canaliculi from which the HCl is secreted by active transport into the stomach. The enzyme hydrogen potassium ATPase (H^+/K^+ ATPase) transports the H^+ against a concentration gradient and thus aid in HCl secretion. The secretions of parietal cells are mediated via histamine, acetylcholine and gastrin.

Synthesis (Fig. 58.9)

The hydrochloric acid is synthesized in stomach as detailed below:

1. The dissociation of water into H^+ and OH^- occurs in the parietal cells. The H^+ is secreted for potassium (K^+) in the canaliculi with the aid of hydrogen potassium ATPase (H^+/K^+ ATPase).
2. The potassium ions are transported into the cell from the extracellular fluid by Na^+/K^+ ATPase. They leak into the lumen of canaliculus but are drawn back into the cell by the hydrogen potassium ATPase.
3. The low intracellular sodium in the parietal cell due to the operational Na^+/K^+ ATPase along the basolateral membrane leads to sodium reabsorption from the lumen of canaliculus.
4. The OH^- ions in the parietal cells (the OH^- ions formed in step 1 due to H^+/K^+ ATPase) combines with CO_2 in the parietal cells to form HCO_3^- in the presence of carbonic anhydrase.
5. The bicarbonate ion (HCO_3^-) is exchanged for a chloride ion (Cl^-) on the basal side of the cell and the bicarbonate diffuses into the venous blood. These chloride ions are secreted in the canaliculus via chloride channels. The H^+ ions along with chloride ions (Cl^-) form hydrochloric acid in the canaliculi.

The parietal cells secrete hydrochloric acid in response to three types of stimuli:

1. Histamine which is released from enterochromaffin cell stimulates HCl secretion and this effect is mediated via H_2 histamine receptors which increases intracellular cAMP level, which increases protein kinase A; protein kinase A phosphorylates proteins involved in the transport of H^+/K^+ ATPase causing resorption of K^+ ions and secretion of H^+ ions.
2. Acetylcholine stimulates HCl secretion via M3 receptors.
3. Gastrin via CCK2 receptor.

The effect of acetylcholine and gastrin is mediated via increased intracellular calcium level. Acetylcholine directly stimulates parietal cells to increase acid secretion. Gastrin by stimulating histamine release from enterochromaffin cells cells stimulates acid secretion. Somatostatin is also secreted by endocrine cells of the gastric epithelium. It inhibits acid secretion by direct effects on parietal cells, and also by inhibiting release of histamine and gastrin.

Note

The hydrochloric acid in the canaliculi denatures ingested protein and activates pepsinogen an endopeptidase for further protein digestion.

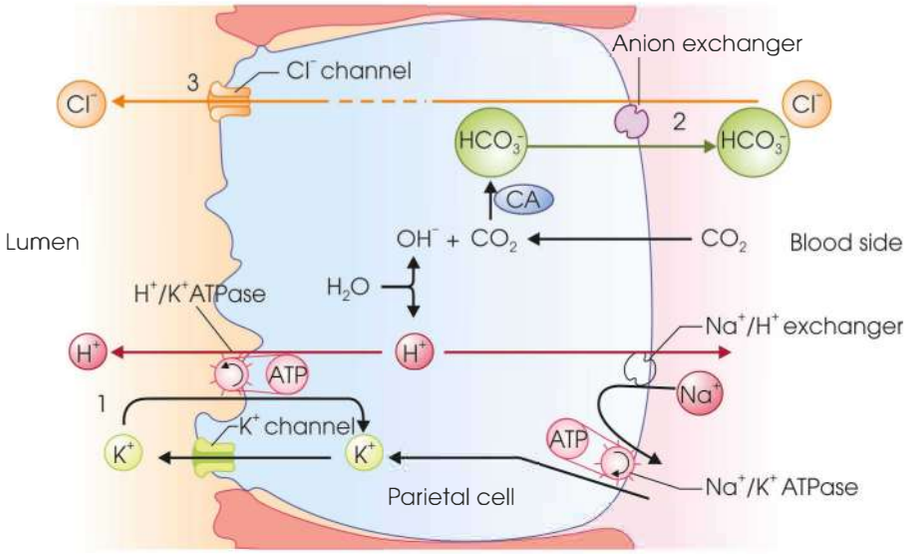

Fig. 58.9: Mechanism of secretion of hydrochloric acid by parietal cell (diagrammatic representation)

APPLIED PHYSIOLOGY: PEPTIC ULCER

Peptic ulcer is the excoriated area of stomach or intestinal mucosa caused by the digestive action of gastric juices particularly hydrochloric acid. The basis of its pathophysiology lies in the imbalance between the degree of protection by the gastro-duodenal mucosal barrier and the amount and rate of gastric juice secretions. Other protective mechanism in stomach is carried by the neutralisation effect of the gastric juices by the alkaline secretions of small intestines. The bicarbonate secretion from pancreas especially neutralises the effect of pepsin and hydrochloric acid, thereby preventing digestion of mucosa.

The causes of peptic ulcers are:

1. Hypersecretion of hydrochloric acid which may be due to smoking, alcohol addiction, high consumption of chilly containing foods, stressful lifestyle and so also in disease conditions such as Zollinger-Ellison syndrome (gastrin secreting tumour of pancreas) and frequent ingestion of non-steroidal anti-inflammatory analgesic drugs containing salicylic acid, etc.
2. Infection by *Helicobacter pylori*. This bacterium disrupts the gastro-duodenal mucosal barrier and increases secretion of gastric juices. It penetrates through the barrier. It releases ammonium which increases HCl secretion from gastric mucosa and it is also responsible for liquefaction of gastro-duodenum mucosa.

Drug Management of Peptic Ulcer

1. Treatment by antibiotic for *Helicobacter pylori* infection.
2. Administration of aluminium hydroxide gel which provides a protective layer of coating and aids in ulcer healing.

Robert Zollinger observed and reported regarding the unusual cases of peptic ulceration in the presence of pancreatic tumours and he along with EH Ellison first postulated a causal relationship between these findings.

Robert Milton Zollinger, MD
1903–1992

3. Suppression of acid secretion by prescribing H_2 blockers as cimetidine and ranitidine.
4. Use of proton pump inhibitor such as omeprazole which also decreases the hydrochloric acid secretion. The proton pump inhibitors irreversibly block the H^+/K^+ ATPase or the gastric proton pump of the gastric parietal cells. The proton pump is the final step in gastric acid secretion, and secretes H^+ ions into the gastric lumen, and this action is blocked by the proton pump inhibitor.
5. Use of prostaglandin analogues in patients of peptic ulcer due to over ingestion of non-steroidal anti-inflammatory drugs.

Lifestyle modifications with balanced diet, regular exercise, quitting of alcohol and smoking and by using stress relaxation techniques one can prevent the incidence of peptic ulcer.

Pepsin

1. It is manufactured by peptic cells. In the resting cells it is present in the form of zymogen granules as pepsinogen. During secretion these granules disappear. Pepsinogen has been isolated in pure form.

2. Pepsinogen is converted into active pepsin by any solution having acidity stronger than pH 6.
3. Under normal conditions, HCl of gastric juice activates pepsinogen into pepsin. But any other inorganic acid also do it. In the process of activation, about 15% of the nitrogen content of the pepsinogen molecule is lost.
4. Pepsin is inactive in neutral or alkaline medium, but it is highly proteolytic in strong acid solutions. On the average, the optimum pH lies between 1.5 and 2.
5. The optimum pH varies with the nature of protein to be acted upon. For instance, for albumin and globulin it is about 1.5, for casein 1.8, for gelatin 2.2 and so on.
6. Pepsin is protein in nature. It acts upon proteins and converts them up to peptone, but not to free amino acids.
7. Pure crystalline pepsin can also act like rennin and coagulate milk.
   - Another proteolytic enzyme cathepsin (optimum pH 4) has also been found in the gastric juice.
   - Human blood and urine contain uropepsinogen derived from the stomach. The uropepsin of strongly acid urine has proteolytic property.

Mucin

Soluble or transparent mucus is secreted by the cells of the pyloric and cardiac glands, and also by the mucous neck cells of the fundic glands. It is a constituent of pure gastric juice (dissolved mucin). Mucin is a glycoprotein. It serves certain important functions:

1. It acts as a buffer and has a high acid-combining power. Thus, it reduces high gastric acidity and prevents injury to the gastric mucosa.
2. By forming a coating on the mucous membrane it protects the latter from the injurious effects of the gastric juice.
3. It also acts as a lubricant.

Intrinsic Factor

A heat-labile mucoprotein is secreted from parietal cell of stomach and is responsible for the proper absorption of vitamin B_{12} (extrinsic factor). Absence of this causes pernicious anaemia. Secretion of this factor takes place before HCl or pepsin.

Neuropoietic Factor

As pernicious anaemia is often associated with subacute degeneration of spinal cord, it has been suggested that the stomach might produce a factor responsible for nutrition of the nervous system.

Pancreas

The pancreas is both an endocrine as well as exocrine gland. It is devoid of distinct connective tissue capsule and is covered by a fine layer of the loose tissue which passes into the gland as septa and subdivides the gland into many lobules.

Key Points

1. The exocrine component portion of the pancreas is a compound tubular gland (Fig. 58.10).
2. The terminal secretory portions of this gland are known as acini or alveoli which are tubular and somewhat convoluted and secrete pancreatic juice. These acini resemble those of serous (serozymogenic*) alveoli of salivary glands and do not contain myoepithelial cells.
3. The main excretory duct of the gland is the duct of Wirsung, which extends the entire length of the gland, giving out several intra-lobular ducts or intercalated ducts.
4. The duct of Wirsung opens in company with the common bile duct into the ampulla of Vater, which opens into the second part of the duodenum. An accessory pancreatic duct, duct of Santorini, is present.
5. Within the lumina of many acini, one or more cubical cells are lying in contact with the apices of the secreting cells. These cells are known as centro-acinous (-acinar) cells. The cytoplasm of this cell does not possess any secretory granules. In each secretory cell, there are two well-marked zones—an inner (apical) zone towards the lumen and the outer (basal) zone towards the basement membrane. In the inner zone there are numerous course zymogen granules and their number are varying with the functional activity of the cell. Their number are diminished during digestion but increased during rest (Fig. 58.10).
6. The basal zone contains the nucleus as well as basophilic or chromophilic substance. Electron microscopic structure shows highly developed rough-walled endoplasmic reticulum and a supranuclear Golgi apparatus. Surface membranes are studded with ribosome granules which give basophilic staining. In resting stages the granular zone gradually increases up to three-fourths of the cell, but during activity the granular zone gradually diminishes in size.
7. The pancreatic excretory duct is also similar to that of the salivary gland. Larger ducts contain elastic fibres and plain muscles which, when contract, may prevent the flow of juice into the duodenum. Near the duodenum small mucous glands are seen in the lamina propria.

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*It refers to the cells whose serous secretions are known to contain an enzyme.

Development

Pancreas develops as an outgrowth of the endoderm of the small intestine and ultimately becomes hollowed out to form the alveoli or acini. The islets of Langerhans develop as buds from the ducts and remain as solid clumps of cells. During development in most cases, the connection with the duct vanishes and the islets become completely isolated. But in a few cases, the connections persist as solid chords.

Mechanism of Pancreatic Secretions

The pancreatic secretion consists of two phases:
1. Nervous phase 2. Chemical phase.

Nervous Phase

Pancreatic secretion starts 1–2 minutes after taking food. When the vagi are cut, the secretion is abolished. Vagi are the motor nerves of pancreas are proved by the fact that when they are stimulated, increased pancreatic secretion takes place. This vagal secretion is rich in enzyme but has very little effect on bicarbonate concentration. Acetylcholine is the mediator and para-sympathomimetic drugs, e.g. pilocarpine, are also effective in stimulating pancreatic secretion. The response is blocked by atropine. Inhibition of secretion may also be obtained by stimulating adrenergic nerves producing vasoconstriction and constriction of ducts. Thus, stimulation of sympathetic nerves may decrease secretion of pancreatic juice by reducing the blood flow through the organ and flow of juice through the duct.

Chemical Phase

The cells of intralobular ducts secrete water and bicarbonate and the enzymes are secreted by acinous cells. It has already been noted that the rate of secretion of pancreatic juice rises sharply when the gastric contents enter duodenum. This is the onset of chemical phase.

The different components which stimulate pancreatic secretions are: (a) Secretin, (b) cholecystokinin pancreozymin, (c) hepatocrinin stimulates liver to secrete bile. (d) enterocrinin stimulates release of intestinal juice (Fig. 58.11).

The first two which are related to the pancreas are described below:

Secretin: Secretin which is a major peptide hormone is made up of 27 amino acid residues. Its molecular weight is 3055. It is rapidly destroyed by pepsin and trypsin in alkaline or neutral medium. It remains stable in acid solution. Injecting secretin intravenously; the flow of pancreatic juice increases (Fig. 58.12). This juice is watery, rich in bicarbonate but poor in enzyme.

Cholecystokinin pancreozymin: Cholecystokinin was previously called *pancreozymin*. It is synthesized and

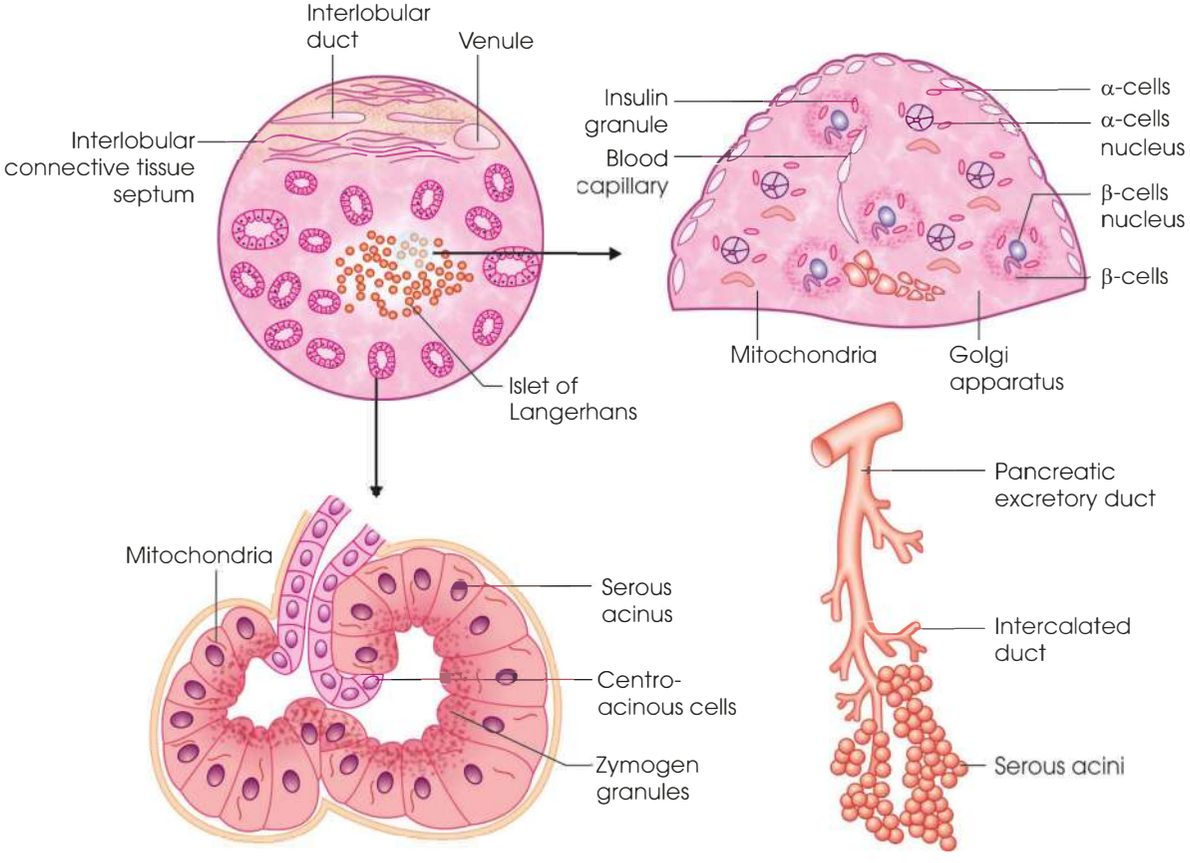

Fig. 58.10: Pancreas showing exocrine and endocrine components (diagrammatic representation)

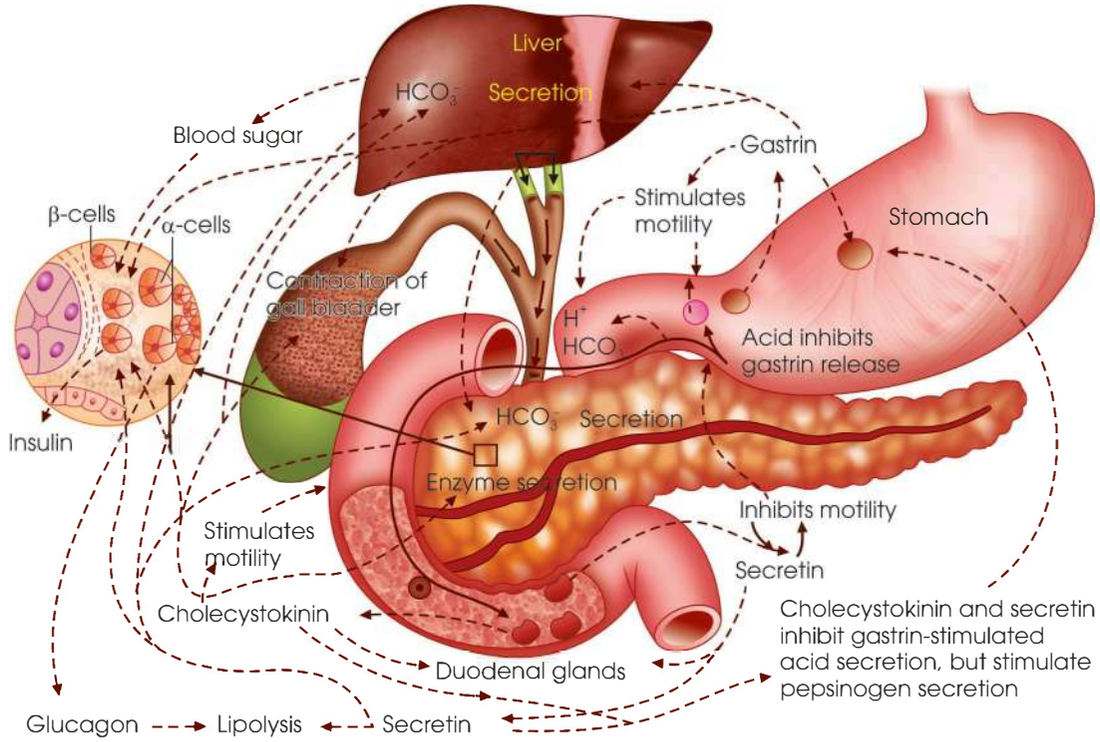

Fig. 58.11: Action of hormones controlling of pancreatic and hepatic secretions (schematic representation) from physiology of the digestive tract, by Horace W. Davenport, New Book Medical Publishers, Inc. used by permission

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secreted by enteroendocrine cells in the duodenum, the first segment of the small intestine. But it is now clear that it is a single hormone and is secreted from the mucosa of the upper small intestine when foodstuffs (especially dilute acids, lipids, fatty acids, peptones, etc.) come in contact with it. Cholecystokinin hormone has got action on the contraction of the gall bladder; it also releases stored bile into the intestine, and stimulates the secretion of pancreatic juice by secreting enzyme-rich pancreatic juice.

Influence of Various Foodstuffs on Pancreatic Secretion

It has been observed that pancreatic secretion remains high for about three hours and then declines. This is due to the fact that stomach is completely emptied within three hours when mixed meal is ingested so contact of gastric chyme is lost with the mucosa of proximal part of the small intestine.

Pancreatic juice is found to vary in quality and quantity with different types of food. Meat stimulates a secretin type of response, i.e. large volume, more alkali and fewer enzymes. Fat elicits a vagal type of response, i.e. moderate volume, low alkali and rich in enzyme. Bread produces a mixed type of response.

Pancreatitis

Acute pancreatitis: It is an inflammatory condition of pancreas in which trypsin activation leads to autodigestion of the pancreatic tissue. The common causes of acute pancreatitis may be due to chronic consumption of alcohol, hypercholesteremia with increased triglyceride levels, side effects of drugs like sulphonamide and tetracycline; contusion and laceration injury over abdomen, etc. The increased level of serum amylase confirms its diagnosis. The patients are treated with analgesic, antibiotic and IV fluids; further intervention is carried out only under complications.

Chronic pancreatitis: The failure to complete recover from acute episode of pancreatitis may progress towards chronic inflammatory changes in pancreas leading to steatorrhea (deficiency of pancreatic lipase secretion) and malabsorption causing malnutrition in these patients.

Mechanism of Bile Secretion (Fig. 58.13)

The phenomenon of bile secretion by the liver is an active and continuous process, but its entry into the duodenum is intermittent, and takes place only during digestion. Contraction of the gall bladder starts a self-sustaining mechanism for expulsion of bile and indirectly stimulates bile secretion. After digestion is complete, the sphincter of Oddi closes, breaking the cycle. About 800–1,000 ml of bile is secreted by an average adult per day. The total amount bears a direct relation with the body weight, the rate of secretion being 15 ml per kg of body weight per day. The rate of secretion increases markedly about one hour after food,

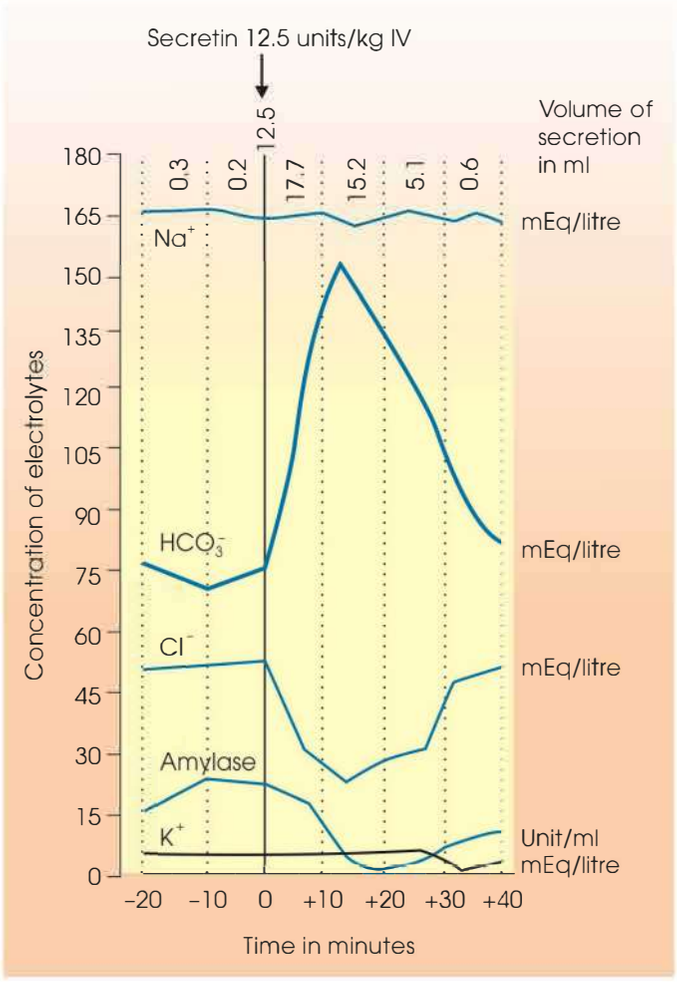

Fig. 58.12: Graphical tracing of effects of a single dose of secretin on composition and volume of the pancreatic juice (after Ganong)

reaches its maximum between 2 and 5 hours and then declines. At the end of intestinal digestion and absorption it comes down to the normal resting rate.

Mechanism of Secretion

Key Points

Bile secretion is practically independent of nervous influence. The normal stimulus for bile secretion is almost wholly chemical in nature.

Any agent which increases the flow of bile into the intestine is known as a cholagogue. A choleretic is an agent which increases the output of bile from the liver without necessarily changing its constitution, and a hydrocholeretic cause an increase in the volume of bile without corresponding increase in bile solids.

The following substances are found to stimulate bile secretion

1. *Bile salts:* These are the most powerful choleretic. They act as the main stimulus in the normal process of bile secretion during digestion. Bile salts absorbed from the intestine are carried to the liver and re-excreted. This is known as enterohepatic circulation. The taurocholic acid is more powerful than glycocholic acid. When they are given by mouth or injected intravenously marked increase of bile secretion takes place. They increase the amount of bile as well as the total solids.
2. *Influence of foodstuff:* Fats and proteins stimulate bile secretion, carbohydrates have no such effect. The exact process by which fats and proteins stimulate bile secretion is not known. It has been noted that bile secretion increases about one hour after meal, remains high for about 2–5 hours and then declines.
3. *Hormone:* Secretin increases bile flow. Similarly, hepatocrinin may be a specific liver hormone present in intestine released by the action of food increasing bile flow.

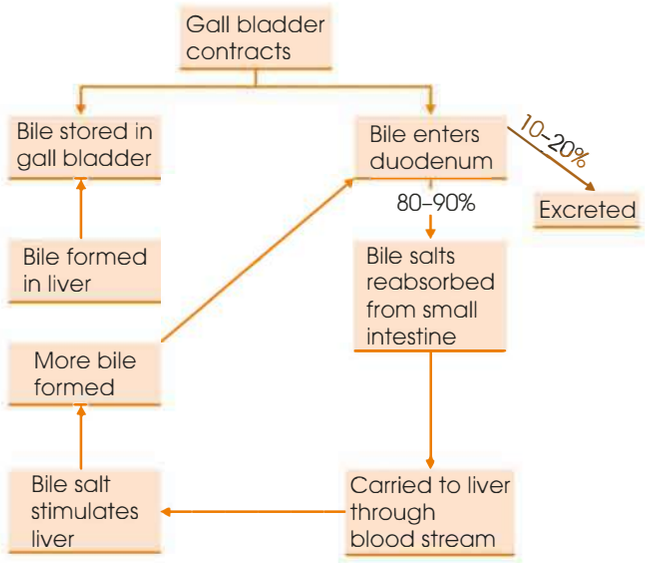

Fig. 58.13: Block diagram showing regulation of bile secretion

Storage of Bile

The gall bladder is the chief place for bile storage. The common bile duct, which is shut off from duodenum by the sphincter of Oddi, may hold some bile. When the pressure of bile in the common bile duct rises to about 70 mm of water, bile begins to pass into gall bladder. The gall bladder has got a special capacity of absorbing water and slightly the inorganic salts (Fig. 58.14). Due to this property it can concentrate bile nearly ten times. The average capacity of gall bladder is about 50 ml since bile is ten times concentrated; 50 ml of bladder bile is equivalent to 500 ml of duct bile. Thus, gall bladder acts as a very efficient reservoir. It can store large amount of bile without any appreciable rise of pressure.

Movements of gall bladder: In humans it can be studied by administering an X-ray opaque substance

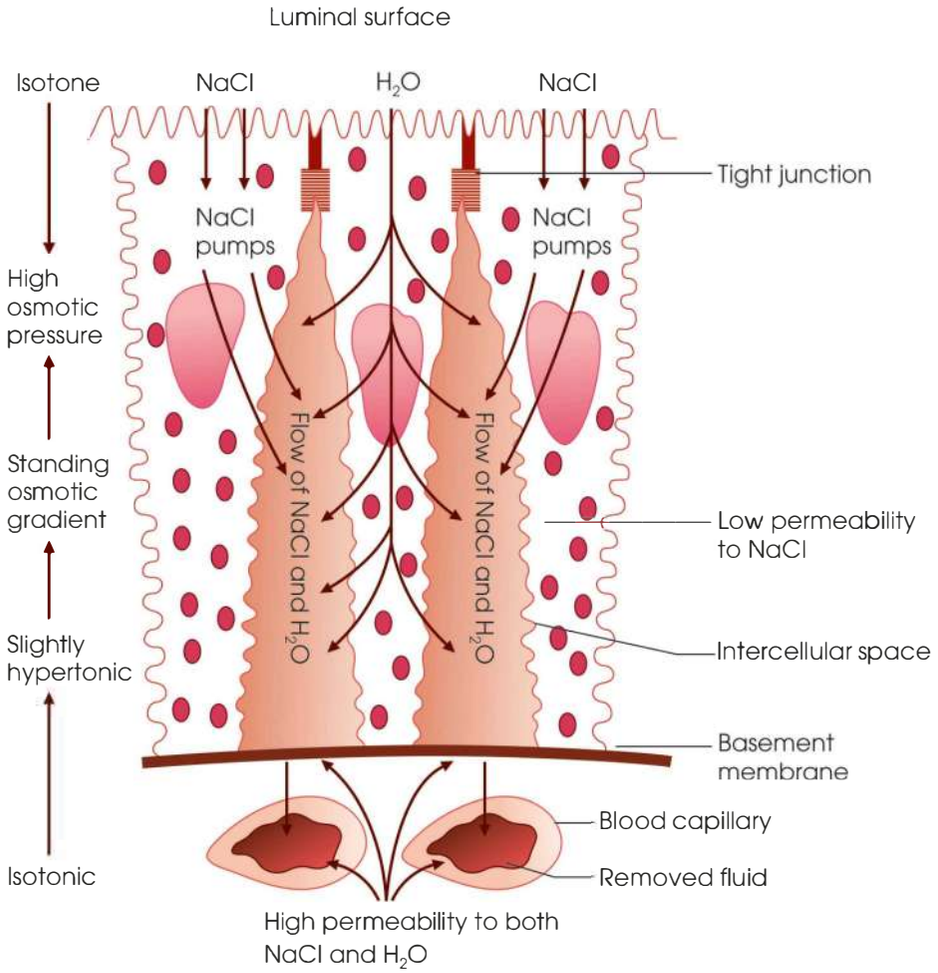

Fig. 58.14: Mechanism of fluid transport by the gall bladder

tetra-iodophenolphthalein, which is excreted through bile. The movements are then observed under X-ray. Under fasting conditions gall bladder shows weak and irregular contractions which are not strong enough to expel bile in the duodenum. After food, active contractions begin in about 5–30 minutes. The contraction continues intermittently till gall bladder is empty. The normal emptying time varies from 2 to 5 hours.

MECHANISM OF EXPULSION OF BILE

Experiment

In humans it can be studied by giving tetra-iodophenolphthalein (which is excreted in bile and is opaque to X-ray) and then noting the alteration of size of gall bladder under X-ray cholecystography. During fast there is no expulsion of bile, because the gall bladder contractions are weak and the tone of the sphincter is high. When food is seen or taken, contraction of gall bladder starts and at the same time, the resistance of the sphincter is reduced. The pressure in the bile duct rises to about 200 mm of H_2O and bile is momentarily expelled. This expulsion of bile is probably assisted by the contraction of the ampullary part of the common bile duct. (Under normal conditions the sphincter can withstand a pressure of about 100–120 mm of H_2O.) After 24 hours fasting or with a carbohydrate diet the resistance may become very high, as great as 300 mm of H_2O. Usually, the pressure in the gall bladder is about 100 mm of H_2O during fasting. After food there is immediate lowering of the sphincter resistance and strong contraction of gall bladder starts. During contraction of gall bladder the pressure may be raised to about 250 mm of H_2O.

Once expulsion starts, it goes on intermittently at frequent intervals for the next few hours as long as digestion and absorption continue. After this, expulsion ceases.

Mechanism

It is obvious that for expulsion of bile, two things are required:

1. Increased pressure of bile.
2. Relaxation of the sphincter of Oddi.

Increased pressure of bile can be brought about by (a) increased rate of secretion of bile, (b) contraction of gall bladder. This latter is brought about by stimulation of the vagus (both conditioned and unconditioned

reflexes), high fat and protein diet, cholecystokinin and salts like magnesium sulphate, calomel, etc. Relaxation of the sphincter is also brought about by the same factors (*vide* under 'movements of gall bladder') from these facts it will be obvious that expulsion of bile takes place only during digestion and absorption of food. This is due to the fact that it is during these processes that the various factors, which stimulate contraction of gall bladder and relaxation of the sphincter, come into operation.

Factors Controlling Movements of Gall Bladder

Control of gall bladder seems to be due to two factors: (1) Reflex, (2) chemical.

Reflex Control of Gall Bladder

Gall bladder is richly supplied with the vagus and the sympathetic. Stimulation of the vagus causes contraction of gall bladder and relaxation of the sphincter of Oddi. Stimulation of the sympathetic exerts opposite effects. (Conflicting results have been found by different observers.) It is believed that during digestion reflex stimulation of gall bladder occurs. The stimulus for the reflex may arise in the mouth during eating or due to the presence of food in the duodenum and probably also in the stomach. It has been shown that entry of acid into the duodenum reflexly stimulates contraction of gall bladder and relaxation of the sphincter of Oddi.

Chemical Control of Gall Bladder

- *Effect of foodstuffs:* Fatty foods, particularly cream, egg-yolk, etc. are the most effective stimuli. Proteins also stimulate but to a less extent. Carbohydrates have no such effect. Acids are also strong stimulants.
- Cholecystokinin hormone has got action on the contraction of the gall bladder; it also releases stored bile into the intestine, and stimulates the secretion of pancreatic juice by secreting enzyme-rich pancreatic juice.
- *Action of drugs*: Adrenaline, histamine, Pitressin, etc. stimulates the smooth muscle of the gall bladder, whereas morphine, ergotamine, atropine, etc. are inhibitory.

Functions of Gall Bladder

Functions of gall bladder can be summarised as follows:

1. It is an efficient storehouse of bile.
2. It absorbs water and concentrates bile about 10 times.
3. It absorbs inorganic salts from bile to some extent and reduces the alkalinity of liver bile.
4. It excretes cholesterol to some degree.
5. It secretes mucus, which is the main source of mucin in bile.
6. It helps in equalization of pressure within the biliary duct system.

Applied Physiology

Gall Bladder Diseases

Gall stones: These may be due to:

1. *Cholesterol gall stones:* Precipitation of cholesterol in gall bladder.
2. Over absorption of bile acids from bile.
3. Inflammation of the epithelium of gall bladder which affects the absorptive characteristic of the gall bladder.
4. Over absorption of water from the bile
5. Calcium bilirubinate stones.
6. Decreased gall bladder emptying.

The patients may exhibit symptoms of pain only when there is obstruction of the duct. The pain is acute spasmodic type.

Gall stones can be confirmed by ultrasound scan. A magnetic resonance imaging (MRI) scan can also help in identifying gall stones in the bile ducts. A cholangiography is performed after injecting the dye into blood stream followed by taking an X-rays in which the gall stone and the block is identified. The block can be removed by removing the gall stone using an endoscope. This technique is known as an endoscopic retrograde cholangio-pancreatography (ERCP).

The removal of gall bladder surgically helps in management of gall stone and this surgical procedure is known as cholecystectomy.

MECHANISM OF SECRETION OF SUCCUS ENTERICUS (INTESTINAL JUICE)

Very little secretion of succus entericus takes place during fasting. The flow starts about one hour after a meal. During the first two hours the rate of secretion is slow, but it increases in the third hour, persists for some time at a high level and then, as intestinal digestion and absorption are completed, the rate gradually declines and finally ceases.

Mechanism

Under normal conditions, presence of food in the intestine sets up the mechanical irritation which reflexly stimulates the secretion of succus entericus through local nerves. Vagal stimulation or injection of acetylcholine or pilocarpine stimulates secretion of succus entericus. On stimulation of sympathetic (splanchnic) nerves secretion of succus entericus is inhibited, but cutting the nerves results in a marked increase of secretion. This secretion, known as paralytic secretion, is increased by physostigmine and inhibited by atropine. It is, therefore, dependent upon some cholinergic mechanism in the intestine. In general, parasympathomimetic drugs increase paralytic secretion and sympathomimetic drugs tend to inhibit it. It is found that products of digestion of some foodstuff—particularly proteins, stimulate secretion of succus entericus,

even when the parts are completely denervated. It is, therefore, a chemical effect. (Food, therefore, stimulates in two ways—first, by mechanical irritation and secondly, by chemical means.)

Enterocrinin

Nasset and his associates have discovered the presence of a hormone in the intestinal mucosa which when injected stimulates intestinal secretion even when all the nerves are severed. This hormone is called enterocrinin. Presence of a second hormone in crude enterocrinin preparation has also been suggested responsible for the release of intestinal enzymes.

Key Points

1. Separate regulatory control, both chemical and nervous, exists for Brunner's gland.
2. Vagal stimulation increases the secretion while the chemical (hormonal) agent responsible for increased secretion has been named as duocrinin.
3. Secretin and glucagon also stimulate secretion.
4. The colonic secretion is rich in mucus and aqueous secretion is scantly. Often it is viscous and opalescent. The reaction is alkaline. Aqueous phase is reabsorbed which is predominant here and the secretion becomes more viscous. The secretion is reflex in nature. The centre is situated in the lumbar spinal cord. The vagi supply the proximal colon, and stimulation produces increased secretion. On the other hand, stimulation of the sympathetic diminishes secretion.
5. The appendix secretes a significant volume of fluid spontaneously or in response to pilocarpine in man, rabbit and chimpanzee. Its secretion is drained freely and continued indefinitely. But if lumen is obstructed, there is a rise in intraluminal pressure and focal ischaemic necrosis occurs, followed by rupture and may lead to infection in this area. Remedy lies in appendicectomy.

Applied Physiology

Malabsorption Syndrome

Intestine is principally involved in aetiology of malabsorption syndrome. The various intestinal diseases leading to malabsorption are gluten enteropathy (coeliac disease), tropical sprue, radiation induced enteritis, etc. Protein malnutrition, vitamin malabsorption and impaired carbohydrate and fat absorption are the critical problem associated with malabsorption syndrome.

Blind Loop Syndrome

The surgically created blind loop in patients may progressively lead to this syndrome and the observed symptoms include generalised weakness, angular cheilitis, red beefy tongue and other signs of vitamin B_{12} deficiency, macrocytic anaemia and stetorrhoea.

Interactions of Gastro-intestinal Hormones (Figs 58.7 and 58.12)

1. As gastrin stimulates acid secretion, it probably combines with specific receptor sites on the effector cells. When the concentration of gastrin is high, many receptor sites are occupied and when this concentration is low, few receptor sites are occupied. This relationship between receptor sites and hormones is dynamic.
2. Cholecystokinin and gastrin have the competition for binding on the same receptor of the secreting cell due to the presence of same C-terminal sequence in them (active group), so they have same spectrum of activities. One inhibits the other as competitive inhibition of enzymes and the exhibition of the action of hormone will be noted whose concentration is higher than the other. Gastrin along with cholecystokinin are additive in stimulations of the pancreas and each of these potentiates the stimulating effect of secretion.
3. Secretin does not possess the same structure as that of gastrin and cannot inhibit histamine-stimulated acid secretion. When secretin exerts its inhibitory activity on acid secretion, it combines with closely related, but different receptor site and hence is non-competitive. During inhibition of acid secretion and motility by secretin, the pepsinogen secretion is greatly stimulated. Cholecystokinin and secretin does not inhibit pepsinogen secretion.
4. The inhibitory action of chyme in the duodenum upon acid secretion was attributed to enterogastrone. But when the existence of cholecystokinin (pure) and secretin was proved, much of the action of enterogastrone was observed to be a property of these two hormones.
5. The inhibitory activity of acid in the duodenum is the inhibitory action of secretin. A rapid fall in the histamine-stimulated acid secretion is due to the presence of fat in the duodenum, but not the property of cholecystokinin or secretin. Therefore, it may be presumed that there is another hormone to be isolated. Moreover, the nervous reflex action mediates some of the inhibitory effects of fat and acid.

Metabolic Effects of Gastro-intestinal Hormones (Fig. 58.12)

There is a role of gastro-intestinal hormones in the metabolic effects. The release of insulin from the β-cells of the islet of Langerhans is stimulated by gastrin, cholecystokinin and secretin and in addition the effect of rising blood glucose concentration in releasing insulin is enhanced by circulating secretin.

Adenyl cyclase activity and lipolysis in the adipose tissue are stimulated by secretin and glucagon due to their similar structure. In these respects, secretin is more potent than glucagon.

Summary of Secretions of the Various Digestive Juices

Salivary Secretion

Salivary secretion: It is purely a reflex process; two types of reflexes present; conditioned and unconditioned. Conditioned reflex is proved by the fact that even sight and smell of food stimulate secretion. Other conditioned stimuli can be established. The stimulus for unconditional reflex arises chiefly in the mouth.

Gastric secretion: Experiments: Sham feeding and Pavlov's pouch.

Phases

Three phases: (1) Cephalic (or nervous), (2) gastric, and (3) intestinal.

Cephalic phase: Starts immediately after taking food. It is a reflex process involving both conditioned and unconditioned reflexes.

Gastric phase: Starts half an hour after the entry of food in the stomach. The stimulus is chemical. The chemical substance is manufactured by the pyloric mucous membrane from some products of protein digestion and is known as gastrin. Gastrin enters blood stream carried to the gastric glands and stimulates their secretion independent of all nerves.

Intestinal phase: Starts when food enters the duodenum. It is small in amount and is independent of nerves; consequently, and the stimulus is chemical. Presence of fat in the duodenum inhibits gastric secretion.

Pancreatic secretion

Two phases: (1) Nervous, (2) chemical

Nervous phase: Starts 1–2 minutes after taking food. The reflex is purely unconditioned.

Chemical phase: Starts when stomach empties into duodenum. This is due to hormone-like substances called secretin and cholecystokinin pancreozymin. The secretin fraction stimulates the secretion of water, alkali and other salts of pancreatic juice; whereas, increases the enzyme content, promotes gall bladder contraction and stimulates bile secretion.

Bile secretion

Mechanism: Bile secretion by liver is active and continuous, total amount 800–1,000 (15 ml per kg body weight) per day. The rate of secretion is influenced by the chemical stimuli such as: Bile salts: Chief choleretics.

Phases

Three phases: (1) Cephalic (or nervous), (2) gastric, and (3) intestinal. The three phases are closely interrelated.

Humoral mechanism of secretion of GIT

The various humoral (chemical) factors controlling the different secretions in the gastro-intestinal tract may be summarised as follows:

For gastric secretion

Gastrin: Responsible for gastric phase, a chemical substance may be a hormone or secretagogue: Responsible for intestinal phase and enterogastrone: Inhibits gastric secretion.

For pancreatic secretion

Secretin: Main chemical stimulus, cholecystokinin pancreozymin and foodstuffs.

For succus entericus

Products of protein digestion, enterocrinin and duocrinin.

For bile secretion

Bile salts: Chief stimulus, fat and protein food, hepatocrinin acting on liver cells.

Cholecystokinin: Stimulates contraction of gall bladder.

EXAM-ORIENTED QUESTIONS

Essay

1. Describe the mechanism of secretion of saliva.
2. Describe the mechanism of secretion of stomach.
3. Describe the mechanism of secretion of pancreas.
4. Describe the mechanism of secretion of small intestine.
5. Describe the mechanism of secretion of large intestine.
6. Describe the mechanism of secretion of bile and discuss the functions of bile.

Short Notes

1. Gall bladder disease
2. Malabsorption syndrome
3. Mechanism of secretion of Brunner's gland
4. Factors controlling movements of gall bladder
5. Blind loop syndrome
6. Interactions of gastro-intestinal hormones

Chapter

59 Movements of Alimentary Canal

INTRODUCTION

The presence of muscular tissue in the alimentary canal indicates that some sort of movements must be present in the gastro-intestinal tract. As a matter of fact, the alimentary canal does move, its different parts showing different kinds of movements.

Reason for movement: If one recollects the chief functions of the digestive tract, such as digestion, secretion, absorption, defaecation, etc. it would be evident that, unless some kind of movement is present in the gastro-intestinal tract, the latter will not be able to perform its functions properly. For instance, movements are necessary to propel the food mass onwards, in order to bring it in contact with the different digestive juices and also for thorough mixing. Movements are also necessary to ensure active circulation through the walls of the gut, so that secretion and absorption may proceed rapidly. Lastly, movements are essential for the process of defaecation. All these considerations show that movements are essential requisites for the proper functioning of the digestive tract.

Facts about Movement

The broad facts about the movements are as follows:

Classification: There are many varieties of movements, such as peristalsis, antiperistalsis, mass peristalsis, segmentation, pendular, tonus rhythm, etc.

Functionally, they can be put into two groups:

1. *Translatory movements:* These types of movements travel onwards and propel the food mass, viz. peristalsis, antiperistalsis, and mass peristalsis.
2. *Stationary movements:* These movements remain localised and do not move onwards, viz. segmentation, tonic contraction, etc. Translatory movement is present in every part of gastro-intestinal tract but not the stationary movements.

Cause of Movements

1. Peristaltic and antiperistaltic movements are neurogenic, i.e. depending on nerves.
2. Segmentation or pendulum is myogenic, i.e. depending on muscles only.

Relation with Degree of Activity

Movements are directly related to the degree of activity of the part. Greater the degree of activity greater will be the variety and rate of movements. Small intestine, being the most active part, shows the maximum varieties of movements.

Characteristics: Each type of movement has the following characteristics:

Nature, *frequency* (i.e. its number per minute), *rate of propagation* (if it is translatory), *cause* (neurogenic and myogenic) and *functions*.

Deglutition

Deglutition or swallowing is probably a reflex phenomenon. Higher centres facilitate this reflex. Once aroused, the swallowing centres in the medulla evokes the complete act of swallowing by discharge through six nuclei and the motor neurons. This reflex act occurs in three stages: (1) First or oral, (2) second or pharyngeal, and (3) third or oesophageal (Fig. 59.1).

1. First Stage

The first stage consists of the passage of material through the oral cavity into the pharynx which is under voluntary control. Due to the contraction of the mylohyoid, styloglossus and hypoglossus muscles, upward and backward movements of the tongue occur and the bolus of food which remains on the upper surface of the tongue is thrown back through the pillars of the fauces into the pharynx. During this phase mastication ceases and respiration is inhibited reflexly.

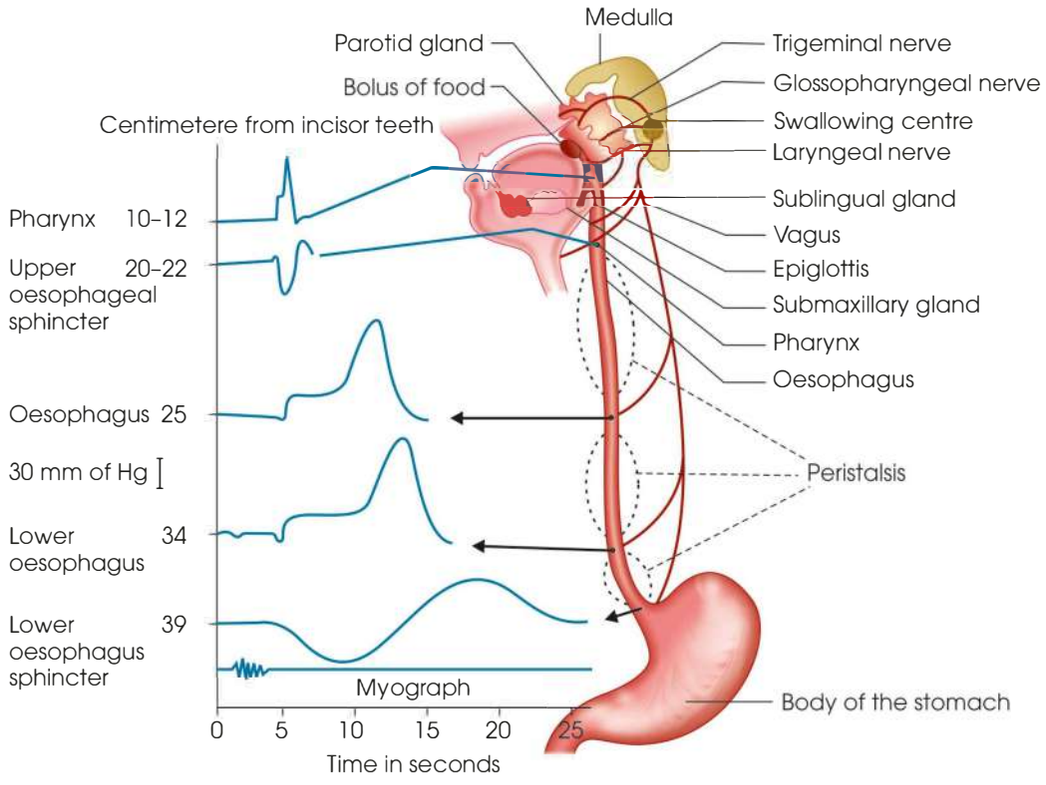

Fig. 59.1: Swallowing mechanism with pressure changes in the pharynx and oesophagus

2. Second Stage

It consists of passage of bolus from the pharynx into the oesophagus which is reflex process and known as swallowing reflex.

Key Points

1. The contact of food material with the pharyngeal and peripharyngeal structures initiates and completes reflexly the second as well as third stage of deglutition. These reflexes are inhibited and abolished by cocainisation.
2. The soft palate is elevated and the nasopharynx is closed off from the rest of the pharynx.
3. There are elevation and forward movements of the larynx along with the elevation of the hyoid bone.
4. The vocal cords are adducted and there is momentary stoppage of respiration and speech. With the entrance of bolus in the pharynx, contraction of superior pharyngeal constrictor occur inducing rapid pharyngeal peristaltic wave (primary peristaltic wave) which moves down the pharynx, propelling the bolus in front of it. The wall and structure of hypopharynx are elevated to engulf the oncoming bolus.
5. The oesophagus, which was kept closed, until now by the contraction of cricopharyngeus muscle, relaxes as the bolus approaches the oesophagus and thus the bolus enters the oesophagus and the pharynx reopens.

Mechanism of Protection of Airway during the Passage of Food through the Pharyngeal Crossroads

There are four possible outlets from the oral pharynx through which food may be expelled: (a) Back into the mouth, (b) up into the nasopharynx, (c) forward into the larynx, and (d) downward into the oesophagus.

The swallowing reflex is so coordinated that food passes only in one of these possible paths, namely into the oesophagus. Return in the mouth is prevented by the high pressure (even 100 cm of H_2O) developed in this area (posterior half of oral cavity) due to forceful contraction of the tongue against hard and soft palates. Combined actions of tensor veli palatini and levator veli palatini muscles stiffen the soft palate which presses against posterior pharyngeal wall and passage into nasopharynx is prevented. Entrance of food into the respiratory tract is prevented by inhibition of respiration.

3. Third Stage

It consists of passage of bolus through the pharyngo-oesophagus junction (i.e. upper oesophageal sphincter), body of the oesophagus and oesophagogastric junction (i.e. lower oesophageal sphincter). The terminal part of the second stage and the first part of third stage cannot be differentiated, because both are same and one. The upper oesophageal sphincteric mechanism is

dealt here along with remaining portion of the third stage.

1. Upper oesophageal sphincter is 4 cm in length and usually is located between 15 and 20 cm from the incisor teeth in the region of cricopharyngeus muscle at the level of cricoid cartilage which is characterised by a short zone of high intraluminal pressure (basal-resting pressure 20 to 30 cm of H_2O above the atmospheric pressure). Immediately after the onset of deglutition there is brief increase in the high resting pressure (lasting only a few tenths of a second) followed by marked fall below the resting pressure and there is simultaneously rise of pressure in the pharynx due to arrival of peristaltic wave caused by contraction of the superior and middle pharyngeal constrictor. Thus, considerable pressure gradient forms from the pharynx across the sphincter. As soon as the bolus reaches the sphincteric junction the previously relaxed fibres of the upper sphincter contract and propagate the peristaltic contraction and pressure gradient into the upper oesophagus.
2. Body of the oesophagus: The primary peristaltic contraction originating in the pharynx already mentioned passes over the pharyngoesophageal junction and continues into the oesophagus driving the bolus onward maintaining the pressure gradient. Secondary peristaltic contraction also appears in the oesophagus as a result of distension of its wall due to bolus (local) which progresses down-like the primary peristaltic contraction and the pressure wave produced by this contraction is similar to final pressure wave of the swallowing pressure pattern. The secondary peristaltic contraction brings over sequential contraction of circular muscle of the oesophageal body, and this produces a peristaltic wave which pushes the food content towards the stomach and relaxation and opening of the lower oesophageal sphincter.

Tertiary oesophageal contraction: This type of contraction has also been observed which occurs irregularly and locally and is observed in the lower oesophagus mainly.

- *Lower oesophageal sphincter:* The distal 2–5 cm of oesophagus possess a characteristic motor function: Deglutition pressure pattern observed in the oesophagus does not propagate into this area.
  - The body of oesophagus and its terminal part react reciprocally to cholinergic and anti-cholinergic stimulation. The patterned activation of the preganglionic neurons in the dorsal motor nucleus of the vagus occurs as response to oesophageal peristaltic contractions and central mechanism further influence the inhibitory and excitatory neurons in the oesophageal myenteric plexus. The excitatory nerves release acetylcholine and substance P while the inhibitory nerves act by releasing nitric oxide and vasoactive intestinal peptide.
  - The mean resting maximum pressure in this region was greater than the pressure in the fundic of stomach is 10.7 cm of H_2O at the end of expiration and 3.5 cm of H_2O at the end of inspiration.
  - As the peristaltic contraction in the body of oesophagus progresses and approaches the lower oesophagus, the lower oesophageal sphincter relaxes like the cricopharyngeus muscle at upper oesophageal junction and the pressure gradient between the lower oesophagus and lower oesophageal sphincter is formed so that oesophageal content is emptied into the sphincteric area. Then the sphincteric area contracts slowly and the sphincteric content is emptied into the stomach.

Common Disturbances in the Swallowing

1. **In the first stage:** Inflammation of any oral structure, paralysis of tongue and congenital defects of oral structure result difficulty of swallowing.
2. **In the second stage:** Acute pharyngitis, tonsillitis, poliomyelitis, diphtheria result difficulty in deglutition.
3. **In the third stage:** Diffuse spasm in the oesophagus producing dysphagia and cardiac pain, achalasia (lower sphincter does not relax), scleroderma (lower part of oesophagus generally in spam), chalasia (lower sphincter remains relaxed condition, inducing gastro-oesophageal reflux).
   - *Reflux esophagitis:* Incompetent lower esophageal sphincter leads to reflux of acid gastric content into oesophagus leading to reflux esophagitis.
   - *Achalasia cardia:* In this condition, the tone of the esophageal sphincter is high in some individuals which lead to incomplete opening of oesophagus during deglutition. The food accumulates in the oesophagus and it gets largely dilated. The myenteric plexus is inadequately developed in these patients. The condition can be treated by pneumatic mechanical dilatation procedure or by loosening the sphincter surgically by myotomy.

Nervous Mechanism

The afferent impulses reach the swallowing centre through the glossopharyngeal and trigeminal nerves and internal branch of the superior laryngeal, recurrent laryngeal and oesophageal branch of the vagus. The efferent nerves are trigeminal, facial, vagus and hypoglossal which regulate the actions of pharynx, larynx and oesophagus musculature.

The movements of the important parts of the gastrointestinal tract are briefly described as follows.

Movements of Stomach

Types of movements of the stomach are described below.

In Empty State

In the fasting condition three types of movements are seen in the stomach:

Type I contraction or tonus rhythm: Rhythmic variations of tone occur at the rate of about 3 per minute.

Type II contractions or hunger contractions: At intervals, a series of strong contractions occur involving the whole stomach and lasting for about 30 seconds. Since these contractions are associated with the sensation of hunger, they are called hunger contractions.

Type III contraction is also known as an incomplete tetanus. They are usually observed at the end of a period of gastric motor activity and are followed by a period of quiescence. They occur rarely in man.

After a Meal

Two different kinds of movements are seen in two halves of the stomach after the entry of food into it. It can be studied best under X-ray after barium meal. The pyloric part has a different kind of movements than the fundic and body of the stomach. Three kinds of movements occur in the full stomach:

- Peristaltic waves
- Systolic contractions of the terminal antrum
- Diminution in the size of the fundic and body.

Movements of the Fundus and Body

The weak peristaltic constrictor wave which is also known as mixing wave starts in mid to upper portion of the stomach and progress towards the antrum once every 15–20 seconds. This peristaltic wave follows the basic electrical rhythm (BER) consisting of electrical slow waves in the stomach. It starts in the longitudinal muscle of greater curvature as a pacemaker and passes over the body, antrum and pylorus at a rate of 3 per minute and the frequency and rate of progress are influenced by nervous and humoral factors. Rate of progress is 1 cm per sec in the muscle of body and the duration of wave at a point is 1.5 sec, which is accelerated in the antrum to 3–4 cm per sec. The excitatory wave spreads from longitudinal to circular muscle. Consequently irregular band of contraction, 2 cm wide, moves as excitation wave over the body and the antrum following BER. The wave deepens as the digestion proceeds and duration of wave increases.

Hunger Contractions

In state of fasting or when stomach is empty for long hours ingestion of food; not only generates peristalsis but also hunger contractions. They are rhythmic peristaltic contraction of body of stomach. The hunger contraction pangs may appear nearly after 12–18 hours of empty stomach.

Movements of Pylorus

The terminal antrum and the pylorus contract with the arrival of wave in their region known as systolic contraction. Due to the viscous property of gastric contents and pressing of gastric contents into the terminal part of antrum by the peristaltic wave, the antral pressure rises which overcomes the pressure barrier in the pylorus and thus antral contents passes into the duodenum. The passage of chyme into the duodenum is stopped suddenly by the contraction of pylorus. Since the contraction of last antral portion continues and the pressure rises to about 10–25 mm of Hg, the antral contents are forcibly moved in oral side and not in aboral side, i.e. in the stomach, as the pylorus (pyloric sphincter) prevents it. Thus, the reflux of chyme helps in mixing the food with gastric juices. Finally the terminal part of antrum and pylorus relax and continue to relax till the next peristaltic wave reaches the antrum.

Cause of the Movements

The movements in the two parts of the stomach are myogenic in nature and predominantly depend on the integrity of the local nervous system (Auerbach's plexus).

Control of Pyloric Sphincter (Fig. 59.2)

In the fasting condition the pyloric sphincter remains relaxed. When food enters, the sphincter closes. It then opens at intervals. The time of opening varies with different conditions. For instance, with water and liquid

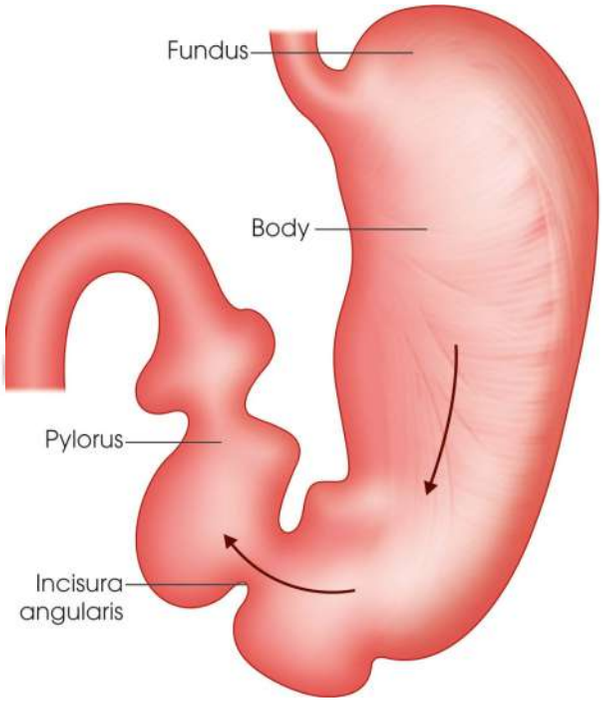

Fig. 59.2: Constriction waves are seen on both sides of this incisura, passing towards the pyloric sphincter

diet, the sphincter almost immediately opens; but with a mixed diet the opening starts at least 15 minutes (average half an hour). The time of opening depends on (a) the normal motility of the stomach, (b) quality and consistency of the food, (c) stage of digestion and possibly some other factors.

Other Factors Influencing Control of Pyloric Sphincter

These are:

1. *pH:* The sphincter is regulated by the pH of the gastric and duodenal contents. High acidity on the gastric side opens it, whereas that on the duodenal side closes it.
2. *Amino acid, glucose, fat or fatty acid concentration:* The proteose-peptone concentration of the gastric content is supposed to control the sphincter. As gastric digestion proceeds, the proteose-peptone concentration rises and when it arrives at a certain level, pylorus opens. Some quantity is thereby expelled into the duodenum and thereby fall in concentration rises again due to further protein digestion and the cycle repeats. Similar reaction of sphincter is seen in case of glucose but reciprocal reaction occurs in case of fat or fatty acid.
3. *Osmotic pressure:* The osmotic pressure of the gastric content is another factor that controls the sphincter. When the osmotic pressure of the gastric content approaches that of the constituent of the plasma, the sphincter opens. As digestion proceeds, number of dissolved particles increase, so that osmotic pressure rises. When it goes up to the requisite level, the sphincter opens.
4. *Tonus:* Gastrin increases tonus whereas enterogastrone or urogastrone inhibits tonus, so intraluminal pressure increases and decreases, respectively.

It may be concluded that the opening and closure of the pyloric sphincter depend on several factors.

Emptying of Stomach

The type of foodstuffs and their consistency determine the emptying time of stomach. Water almost immediately passes out; liquid food is emptied more rapidly than solid. Larger meals have necessarily longer emptying time. A small amount is being evacuated out at interval of every 20 seconds. The emptying of the stomach is regulated by the accumulation of evacuated substances in the intestine. Fats are retained for the longest time, because they inhibit gastric movements. Fats act through the hormone enterogastrone released into the blood from the mucous membrane of the intestine. Carbohydrates leave early. Proteins are intermediate. Products of protein digestion act mainly through the vagi enterogastric reflex. In animals, mechanical irritation or distension of the duodenum is followed by inhibition of peristalsis and this is due to enterogastric reflex mediated also through the vagi. With a normal mixed diet the stomach completely empties in about three and a half hours. With a rich fatty diet about four and a half hours.

Role of the Vagus and the Sympathetic

Stomach is supplied by both vagus and sympathetic. In general sympathetic nerve stimulation inhibits gastric movements and constricts the sphincter, while vagus stimulates movements and relaxes the sphincter.

Role of Hormones and other Chemicals

Adrenaline has the same effect as sympathetic. Pituitrin stimulates gastric contractions. Insulin (as well as low blood sugar) increases gastric movements. Enterogastrone inhibits movements. High gastric acidity is generally associated with a hypermotile stomach, whereas low acidity with a hypomotile stomach.

Dumping Syndrome

The patients of gastrectomy experience generalised weakness, sweating, giddiness and fatigue about two hours after a meal. This is due to hypoglycaemia produce by rapid entry of food into intestine where there is faster absorption of glucose producing increased blood glucose level, faster secretion of insulin and thereby hypoglycaemia which leads to sweating, weakness and dizziness.

Vomiting (Emesis)

Vomiting is a reflex which serves to relieve the upper GI tract by forcible expulsion of gastric contents through the mouth. This may occur either because the contents are irritating or because the organs themselves or the nerves that supply them are more irritable than normal. Excessive distension and compression or irritation of the intestine, appendix, bile ducts and other abdominal viscera can also initiate this movement. This is a reflex movement (Fig. 59.3).

Sequence of Events

A number of events takes place during vomiting, more or less, in the following order:

1. *Nausea:* At the onset a feeling of nausea is experienced, followed by excess salivation.
2. *Glottis becomes closed and the nasopharynx is also shut off by raising the soft palate:* The purpose is to prevent the entry of any vomited material into the trachea and nose.
3. *The body of the stomach, the cardiac sphincter and the oesophagus relax:* The pylorus contracts and presses its contents into the relaxed stomach. Weak contraction and anti-peristalsis may take place in the stomach but they are not important, because vomiting normally takes place even if stomach is replaced by a bladder. Pyloric sphincter remains closed.

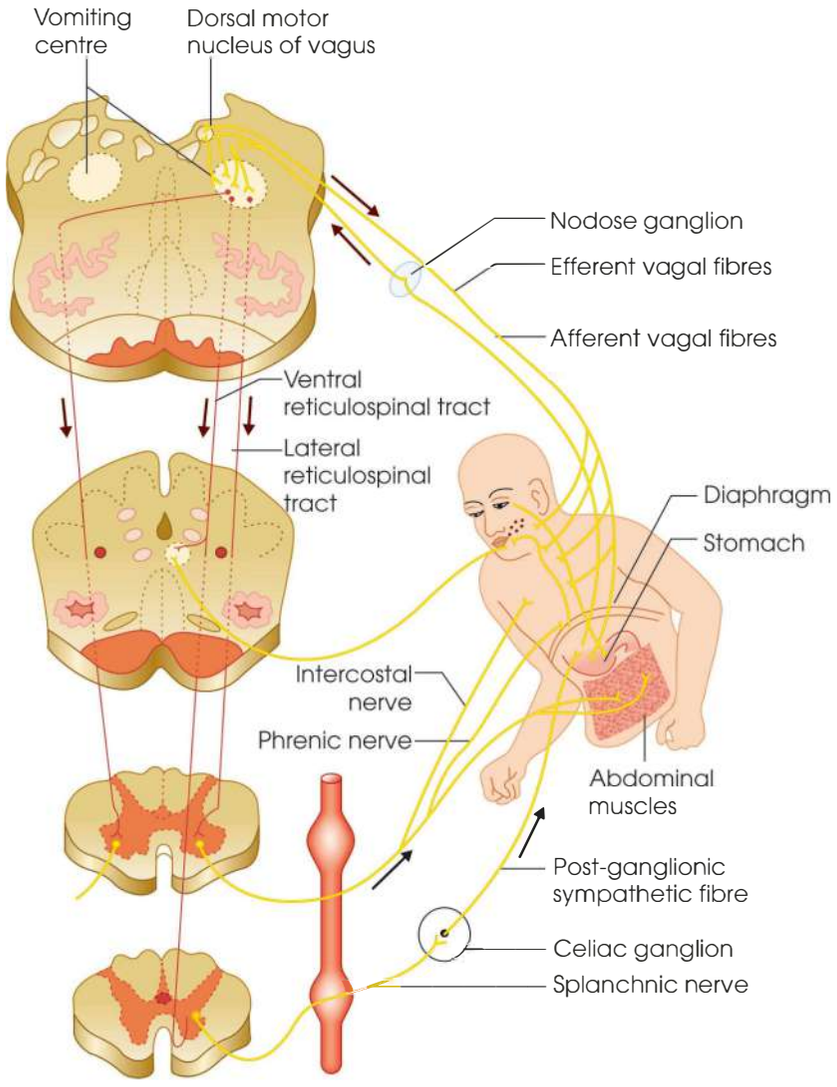

Fig. 59.3: Vomiting reflex. Diagram showing viscerovisceral and viscerosomatic reflexes initiated from irritation of the stomach, the afferent impulses being carried in the vagus. The actions involved are: (a) Elevation of soft palate, (b) larynx and hyoid drawn forward. (c) salivation and opening of mouth, (d) closure of glottis, (e) relaxation of oesophagus, (f) opening of cardia, (g) flaccid relaxation of stomach, (h) constriction of lower end of stomach, (i) inhibition of normal respiration, (j) forced inspiration, (k) Sharp, spasmodic contraction of diaphragm and abdominal muscles, (l) characteristic posture—flexion of waist, clenched fists, etc.

4. *Intra-abdominal pressure sharply rises:* This is caused by retching during which expiratory, abdominal muscles and diaphragm contract. These contractions increase the intra-abdominal pressure so that the relaxed stomach is forcibly compressed. This increased intra-abdominal pressure is the chief motive force of vomiting. Gastric contents are pressed into oesophagus and then from the latter ejected out through the mouth. Anti-peristalsis may take place in oesophagus helping ejection.
5. *The process continues till the stomach is empty:* Towards the end diaphragm ascends (relaxes) and the expiratory muscles contract. Glottis being closed it raises the intra-pulmonary pressure and compress the oesophagus. This helps to expel the last remnants of the vomitus from the oesophagus.

Mechanism

Vomiting is a reflex process. Straightforward vomiting is governed by a vomiting centre which is situated in the dorsal part of the lateral reticular formation of the medulla lying ventral to the solitary tract and its nucleus. It forms one of the components of the complex visceral centres which include salivation defaecation and vasomotor centre and vestibular nuclei. So, there is consistent relationship of vomiting with salivation defaecation, respiration and vaso-motion. This centre can be directly stimulated (central vomiting) by certain drugs (apomorphine, etc.), certain toxins (such as those of uraemia), increased intra-cranial pressure (brain tumor, asphyxia, meningitis, etc.) and such others. It can be reflexly stimulated in various ways.

There are two pathways by which the vomiting centre is affected:

1. Nervous path and 2. vascular path

The nervous path lies in the various afferent pathways coming from various organs especially digestive tract. The most sensitive part is in the first part of duodenum. The afferent impulses may also arise in the throat (tickling sensory nerves V and IX), stomach (irritation), intestine or other organs outside the gastrointestinal tract like heart, kidney, uterus or semicircular canals. Chemical substances (emetics) pass via body fluids and act on chemo-sensitive area called chemoreceptor trigger zone in the floor of IVth ventricle and cause vomiting. Destruction of this area results loss of response when emetics placed directly on the receptor site in chemoreceptor trigger zone. The efferent impulses—both excitatory and inhibitory, are carried in the phrenic chiefly and vagus and sympathetic.

The commonest cause of vomiting is gastric irritation and its purpose is to drive out the irritant from the stomach.

Projectile Vomiting

The central vomiting caused by stimulation of central nervous system (e.g. trauma and tumour in brain, irritation in meninges, etc.) is projectile type having a little feeling of nausea and absence of participation of voluntary muscles.

Persistent Type of Pernicious Vomiting of Pregnancy

In this type the excitability of the centre is increased by metabolic disturbances (e.g. carbohydrate starvation and dehydration with ketosis).

Note

Loss of water, and both H^+ and Na^+ ions due to excessive vomiting might be harmful. Emetics are substances that cause vomiting (such as drinking of sodium chloride and warm water).

Movements of Small Intestine

The following types of movements are found in the human small intestine: (1) Rhythmic segmentation or Ludwig's pendulum, (2) peristalsis. Each type is briefly described below.

Rhythmic Segmentation

Nature: These are rings of contraction occurring at regular space of intervals in which a portion of the intestine is divided into segments. The contraction is followed by relaxation. The contraction takes place at the site of maximum distention. It can be studied under X-ray after barium meal. The opaque column of barium meal becomes broken into several small segments. At the next moment each of these segments is subdivided by a fresh batch of contractions, the previous group having disappeared in the mean time. The halves of the adjacent segments so divided run together and form new segments. These are again subdivided and thus the process goes on (Fig. 59.4). According to Friedman, there are two types of segmenting contractions in the duodenum: (1) One type consisted of a contraction localised in a segment less than 2 cm and was eccentric in appearance. (2) The other was concentric and consisted of a local contraction involving a segment usually longer than 2 cm and uniform circumferentially.

Frequency

Key Points

1. In animals the groups of contractions succeed at the rate of 20–30 per minute. In man, the rate is slower. The frequency is inversely proportional to the distance from the stomach.

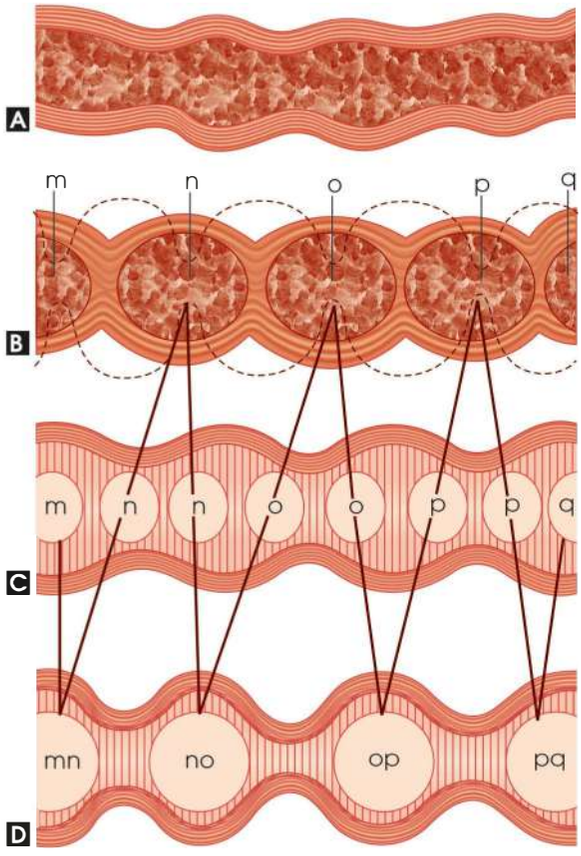

Fig. 59.4A to D: Diagram representing the process of rhythmic segmentation in a segment of the small intestine and sequence of events from A to D. A quiescent loop of the small intestine in A, suddenly divides itself by contraction of its circular muscle fibres in a good number of segments as in B. Only after a few seconds, each of segments divides into two parts as in C. Afterwards each of the halves as m, etc. which are formed from previous segments unite with neighbouring halves as n, etc. formed from another segment and thus forms a new segment as mn, etc. in D, of the equal size as the original B

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2. A cyclic changes in the electrical potential occurred in the duodenum known as basic electrical rhythm (BER) originates near the entrance of the bile duct and moves down the duodenum. In the duodenum it is about 17 per minute, in the ileum it is about 12 per minute. In addition, an irregular burst of spike potential superimposed on BER appears in an electrical record. So, the contraction is segmental and not peristaltic since spike potential and contractions do not proceed more than a few centimetres. The duration of electrical cycle is about 3.5 sec and hence the rhythm is 17–18 per minute.
3. The frequency variation at different region is due to gradient in the physiological properties, viz. rhythmicity, irritability, variation in latent period and drug susceptibility. Muscular contraction occurs at intervals of some multiple of 3.4 seconds and does not travel vary far along the intestine. The vagi (Fig. 59.5) and the splanchnic nerves (Fig. 59.6) regulate the activity of the intestine, and adrenal medulla in psychic conditions, but action of these nerves is reversed in the control of ileocaecal sphincter.

Cause

These are the most fundamental movements of the intestine and are due to outstanding property of smooth muscle that is rhythmicity. Circular muscle is responsible for the most visible movement. They are myogenic in nature and are independent of all nerves.

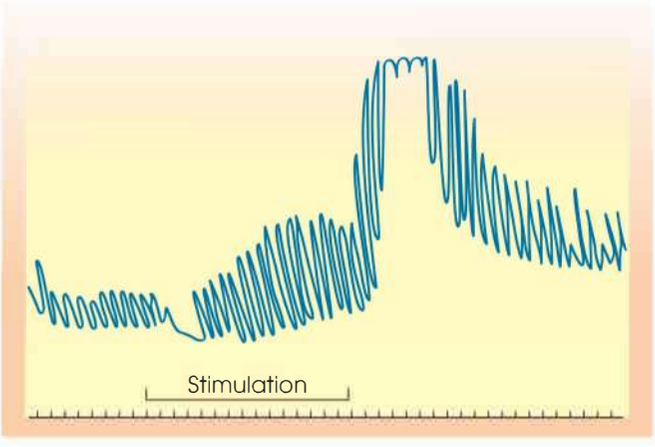

Fig. 59.5: Tracing representing effect of stimulation of the vagus on intestinal contraction

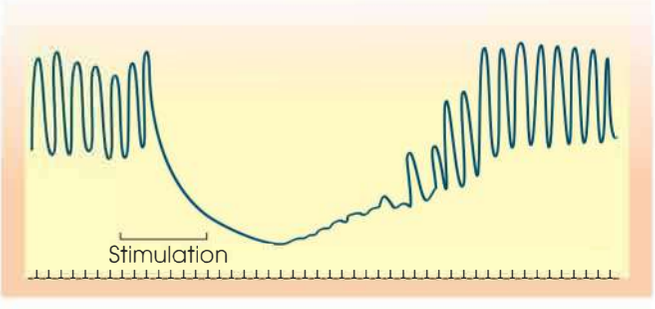

Fig. 59.6: Graphical representation of effect of stimulation of both splanchnic nerves on intestinal contraction (diagrammatic)

Functions

Segmentation movement does not cause forward passage of food materials. It helps (1) in digestion due to proper mixing of food with enzymes of digestive juices, (2) in absorption due to (a) constantly changing the layer of fluid in contact with mucosa, (b) change in pressure, (3) in the improvement of intestinal circulation.

Peristalsis

Nature: Peristalsis is described to be a composite wave, consisting of a wave of relaxation followed by a wave of constriction. It is a translatory movement and travels down the gut in an aboral direction (away from the mouth). Bayliss and Starling have demonstrated that a stimulus applied to a given point on the intestinal wall causes contraction above and relaxation below the stimulated point (Figs 59.7 and 59.8). This is a local reflex of smooth muscles and their intrinsic plexuses. This is called the *law of intestine* or *myenteric reflex*. It is suggested that peristalsis depends on this reflex.

Usually two types of contraction, viz. peristaltic and rhythmic segmenting are present simultaneously, the former is superimposed on the latter and responsible for rise into the tone level of intestinal muscle without any interruption in the rhythm of the segmenting contraction. Peristaltic wave travels for varying

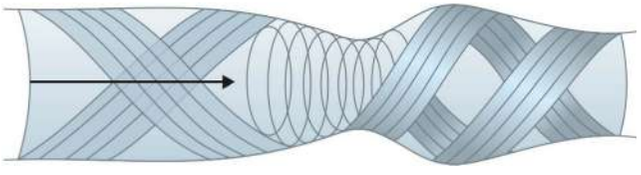

Fig. 59.7: Diagram showing helical arrangement of smooth muscle in the small intestine with longitudinal helix being incomplete and tighter than normal and circular helix being complete

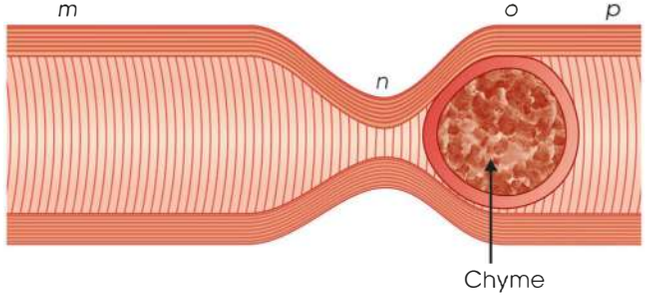

Fig. 59.8: Diagram showing peristalsis. Due to presence of chyme at *o* a local stimulation is provided making the circular muscle at *p* to relax and at *n* to contract and thus propelling the material down the intestine

distances—in some cases for three centimetres and other a few metres depending on the intensity of stimulus. Segmental contractions sometimes recur frequently maintaining its character and travel aborally as peristaltic movements. A peristaltic wave induced by strong stimulus may sweep over the entire length of small intestine what is called rush wave or peristaltic rush.

The peristaltic waves move aborally and not orally and are due to the gradient of rhythmicity, conductivity and irritability. The impulse arises in the most irritable point and travels in the less irritable, i.e. the aboral side and not in the oral side due to the long refractoriness.

Causes of Peristalsis

Peristalsis depends on both nervous and chemical factors. The vagi and sympathetic have got influence on peristaltic movements. Stimulation of vagus increases and that of sympathetic inhibits peristalsis. Vagotomy on the other hand decreases the peristaltic activity only to a minor extent. The local nerve plexus (Auerbach's plexus) helps in the co-ordination of peristaltic movements. Distension of the intestine, normally caused by presence of food, causes peristaltic movements due to a stretch reflex called myenteric reflex. Reflex inhibition of whole of the small intestine may take place due to stretching of lower part of the small intestine (such as intestine and intestinal reflex) or stretching of gall bladder, urinary bladder, etc. Presence of local nerve plexus (myenteric plexus) is required for this and the afferent receptors which are present in the mucous membrane of the intestine. Liberation of 5-hydroxytryptamine (serotonin) from the enterochromaffin cells is a possible mediator in this reflex action. Role of a basic polypeptide, substance P as a mediator has also been suggested.

Role of Endocrines

Hormones also exert great influence. Pituitrin excites the movements, as also thyroxine. Adrenaline inhibits the movements.

Gastro-ileal Reflex

This is a special manifestation of peristaltic movements in the ileum. Peristalsis is generally very sluggish in the last part of the ileum. But after a meal, brisk peristalsis is set up reflexly in this region. This is called gastro-ileal reflex. The purpose is to drive out the ileal contents into caecum and thus making room for fresh supply.

Functions

1. Chief function is the propagation of the food onwards.
2. Other functions are same as of segmentation movement.

Ileocaecal Sphincter

The ileum and colon is separated by a barrier called ileocaecal sphincter, situated at the junction of the two, which normally remain closed (Fig. 59.9). Its functions are to delay the passage of chyme from the small intestine and to prevent regurgitation of material from the colon into the small intestine, thus facilitating more digestion and absorption. Gastro-ileal reflex and enterogastric reflex exert influence of activity in one part of the digestive tract on that of another part. The former reflex results increased motility of last part of ileum and relaxation of ileocaecal sphincter due to incoming of food into the stomach and this ileocaecal content is present in the caecum through the sphincter. The latter reflex results relaxation of stomach due to reaching of chyme into the caecum. The latter space is so adjusted by these two reflexes that when food residue of meal reaches into the caecum, the other meal enter into the stomach. The closure of the sphincter is due to the myenteric reflex of caecum which is initiated by mechanical stimulation of the caecal mucosa by the chyme.

Movements of Villi

Villi possess independent movements of their own. These movements are due to the contraction of strips

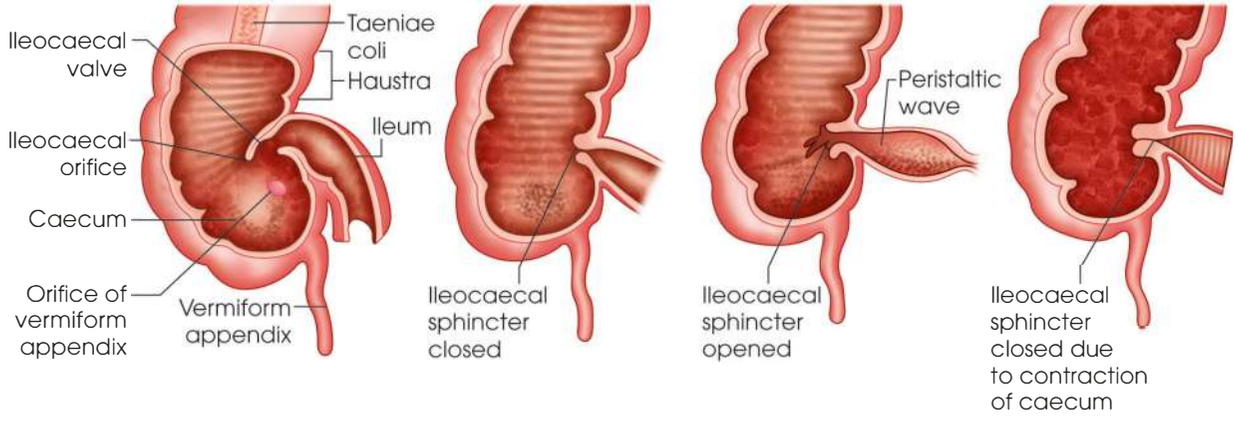

Fig. 59.9: Ileocaecal valve after removing the anterior wall of terminal ileum and caecum which prevents regurgitation of caecal contents in the ileum (diagrammatic representation)

of muscle fibres which proceed from muscularis mucosae and get attached to the sides of the villus.

Generally, *two kinds of movements are seen:*

1. *Side-to-side movement:* In this movement the villi bend to one side which may be in any direction. This is caused by the contraction of muscle strip on that side. The function of this movement is to help admixture and also to help absorption by bringing the villi in contact with different portions of food mass. During active digestion these movements are very rapid.
2. *Pumping movements:* In this movement all the muscular strips around the villus contract simultaneously and the villus shorten in length as a whole. At the height of digestion and absorption this movement takes place at the rate of about 1–6 per minute. The function of this movement is the increase of flow of blood and lymph.

Beside these movements, the villus performs protecting action on the inflammation due to the contraction of muscular is mucosae as a result of contact of sharp foreign body in the intestinal contents.

Factors Controlling Movements of Villi

1. Mechanical irritation of food acting through the local nerve plexus.
2. *Neural stimuli:* Stimulation of the splanchnic nerve augments the movement of villi. Stimulation of the sympathetic nerves causes contraction of the muscularis mucosae. The nerves are adrenergic and respond to sympathomimetic drugs. The muscularis mucosae also respond to parasympathomimetic agents, e.g. acetylcholine with contraction. They are depressed by atropine and nicotine.
3. *Chemical stimuli:* Some products of food digestion, especially some amino acids, as well as yeast extracts (vitamin B) act as strong stimulants.
4. *Villikinin:* This is a hormone that can be extracted from the intestinal mucosa. It is believed that during active digestion villikinin is absorbed through blood and stimulates the movements of villi.

It will be seen from the above that all these factors operate during digestion of foodstuffs. Hence, during digestion the movements of villi are very brisk. During fasting they are very slow. Movements of villi cease during asphyxia, loss of blood, circulatory failure, etc.

Applied Physiology

Adynamic Ileus

Injury to abdomen following abdominal surgery or over handling of intestine during surgery may lead to decrease movement of intestine producing paralytic ileus. It occurs because of enhance discharges of adrenergic fibres in splanchnic nerves.

Movements of Large Intestine

The colon receives mixed residues of food which have escaped digestion and absorption in the stomach and small intestine, i.e. undigested or indigestible food residues, whatever remains of digestive juices including large amount of H_2O and the fluid that has been swallowed or secreted and has escaped absorption in the small intestine in liquid form. The colon extracts mainly H_2O and other substances to some extent, viz. glucose, amino acids, NaCl, drugs, etc. from this mixture and convert it to a solid form of faecal masses to be evacuated through the anal canal by means of its different movements which may be divided into two classes: (1) Stationary and (2) translatory.

Stationary Movements

These are localised movements having no absolute forward movement of masses and are responsible for the agitation of the colonic contents and extraction of H_2O and other substances from liquid mixture of food residues received in the colon which appear to be performed by the four types of movements successively.

1. Segmenting contraction, as seen in small intestine.
2. Haustral contraction (bulging of wall of colon between the teniae).
3. Kneading movements (fairly large segment contract while adjacent segment relaxes and followed by contraction and relaxation in reverse phase).
4. Finally by means of peristalsis and anti-peristalsis.

These movements occur principally in the ascending and transverse colon for the maximum absorption of H_2O in this area. Each type of these movements not necessarily occurs in every species of animal.

Translatory Movements

The second class of movements includes:

1. Peristalsis
2. Mass peristalsis which propels the colonic contents anal wards.

1. Peristaltic Movement

In colon is the same as that of small intestine except in frequency. The power of contraction is greater in the descending colon due to dry and hard character of the material to be moved. In this type of movement, integrity of myenteric plexuses is not required.

2. Mass Peristalsis

It is also of propulsive nature but is not true peristalsis since it involves simultaneous contraction of large segments of the colon. This occurs generally when the stomach is filled with food (unconditioned) or as reflex process called gastrocolic reflex which may be also due

to conditioned stimulus. This gastrocolic reflex may be preceded by gastro-ileal reflex. This movement serves to empty of the contents of the proximal colon into the more distal portion and finally into the rectum giving rise to feeling or desire to defaecate.

Functions of Large Intestine

Large intestine serves the following functions:

1. **Absorption:** Water absorption and formation of stool is one of the chief functions of large intestine. Daily about 350 gm of fluid chyme is passed into the large gut and about 135 gm of moist faeces is produced on average.
   - About 60–80% of water is absorbed here.
   - Sodium is absorbed.
   - Glucose: Isolated large intestine absorbs glucose at the rate of 6 gm per hour. 5% glucose solution is suitable for administration per rectum in the human subject.
   - Certain drugs, e.g. some anaesthetics are absorbed.
   - Amino acids are also absorbed.
   - Absorption in the proximal colon is better than in the distal.
2. **Excretion:** Heavy metals like bismuth, mercury, arsenic, etc. are excreted through the large gut. The diffusible substances present the bolus may be excreted if the concentration of these substances in the colon is lower than the blood. Due to the basis of which, this part of intestine may be used as artificial kidney for removal of body waste product whose kidneys is in trouble provided the concentration of these substances in the intestine is kept lowered by withdrawing them from it constantly. When they are injected subcutaneously they appear in the faeces.
3. **Secretion:** The goblet cell of the large intestine secretes mucus which acts as a lubricant. Mechanical irritation stimulates some watery secretion. The secretion of the large intestine has a distinct alkaline reaction (pH 8.4), but normal stool has an acid reaction due to acids produced by bacterial action.
4. **Synthetic functions:** Bacterial flora of the large intestine synthesizes vitamin K, folic acid and some other members of vitamin B complex. Large amounts of vitamin B_{12} are also synthesised but they are not absorbed.
5. **In large intestines**
   - Fats are converted to lower fatty acids and glycerol.
   - Choline is converted to a toxic product called neurine.
   - Proteins are converted into amino acids, ammonia, etc.
   - By decarboxylation amines are produced:
6. **Bacterial digestion:** Large intestine is the seat of growth of various types of micro-organism or bacteria. Whether they are useful or not, is not definitely known. But the following facts indicate that they may be useful to some extent. These bacteria are very rich in cytochrome. Some observers believe that it is from the dead body of these bacteria that the human body derives a large part of its cytochrome requirement. Some people suggest that the normal flora of the large intestine prevent the growth of other pathogenic bacteria and thus serve a very useful purpose.

Movements: One great function of the large intestine is its capacity to move. Its mass peristalsis is essential for defaecation.

RATE OF PROGRESS OF BARIUM MEAL

The barium meal (Fig. 59.10) starts leaving the stomach almost immediately after it has been swallowed. It passes steadily through the duodenum and very rapidly through the jejunum, where it may be found after one hour. In the ileum its progress gradually becomes slower. It reaches the last part of ileum in about three and a half hours. By this time stomach should be completely empty. In the caecum it is found after four and a half hours, hepatic flexure—6-6½ hours, splenic flexure—nine hours, descending colon—eleven hours, iliac colon—twelve hours, pelvic colon—eighteen hours. Evacuation occurs usually between 16 and 24 hours. The first part of the meal is found in the stool at this time but the last traces may persist normally up to 48 hours. These time relations are important for radiological examination of the gastro-intestinal tract, and also for testing the degree of constipation in the subject. For the latter purpose, the subject is given charcoal biscuits and the time of appearance of charcoal in the faeces and its complete clearing is noted. From this the degree of constipation can be judged.

Applied Physiology—Large Intestine

Irritable bowel syndrome (IBS): There is no underlying pathological cause behind the condition and the patients of IBS complain of abdominal pain. Stress, emotional instability, food induced hypermotility are the common condition in which IBS is observed.

Hirschsprung's disease: It is due to congenital absence of aganglionic plexus; particularly myenteric and submucosal plexus in distal part of colon and rectum. Anal sphincter does not relax completely during defaecation leading to rectal filling and distension in colon. It can be treated surgically.

Constipation: The decreased intestinal motility leads to stagnation of chyme in large intestine and this leads to indrawing of water from chyme and this hardens the stool and patients complain of strain and incomplete evacuation during defaecation.

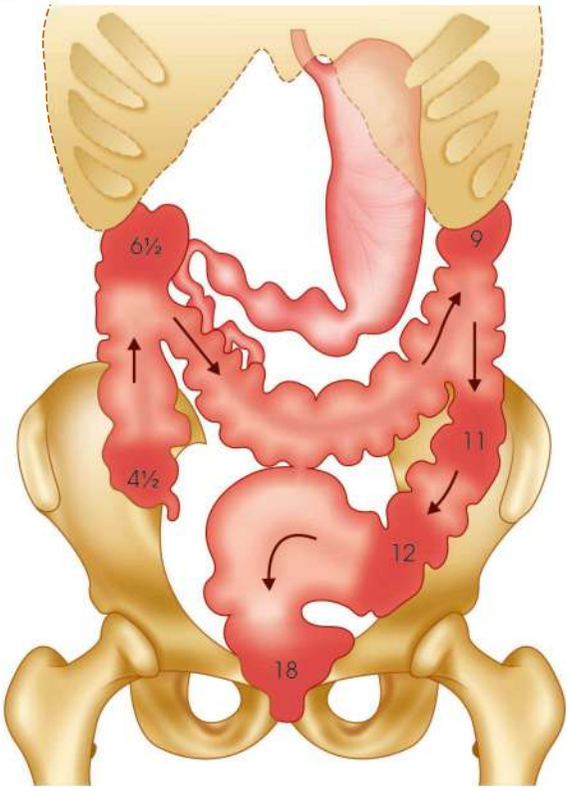

Fig. 59.10: Rate of progress of barium meal

Diarrhoea: Diarrhoea due to acute bacterial infection or cholera toxins, viral infection (Norovirus or Rotavirus) or giardiasis (Giardia intestinal parasite) increases gastro-intestinal secretion and motility produce loose watery stool. The patient may present with complains of weakness, abdominal pain or discomfort and increased frequency of defaecation with loose watery stools.

Defaecation

Defaecation is an act of emptying of entire distal colon from the splenic flexure through the anal orifice into the exterior which is a reflex process. The reflex is initiated by the rise of intraluminal pressure of about 20–25 cm of H_2O in the rectum containing.

Pressure receptor which not only detects increase of pressure but also differentiates whether the increase in pressure is due to gas, liquid or solid. The reflex centres have been located in the hypothalamus, in the lower lumbar and upper sacral segments of the spinal cord and in the ganglionic plexus of the gut. This is a reflex which is under some degree of voluntary control.

Mechanism

The rectum is normally empty. The faecal matter is stored in the sigmoid and pelvic colon and not in the rectum. As soon as some faecal matter enters the rectum due to mass movement there is a desire for defaecation along with voluntary effort, e.g. assumption of appropriate posture, voluntary relaxation of external anal sphincter, abdominal compression in the adult, etc. which may further augment visceral reflexes. These reflexes result mass contraction of entire length of colon and relaxation of internal anal sphincter. Thus, the colon contents pass into the pelvic colon, rectum, anal canal and finally removed from the body. Defaecation reflex can be initiated earlier or inhibited voluntarily.

EXAM-ORIENTED QUESTIONS

Essay

1. Describe the movements of alimentary tract.
2. Describe the stages of swallowing.
3. Describe the movements of small intestine and large intestine.

Short Notes

1. Gastric emptying
2. Gastric function test
3. Vomiting
4. Defaecation
5. Irritable bowel syndrome
6. Movements of villi
7. Blind loop syndrome
8. Hirschsprung's disease

Chapter

60

Digestion and Absorption of Foodstuffs

INTRODUCTION

The term digestion may be defined as the process of biochemical transformation of complex and larger food particles in the gut enzymatically into a simple form suitable for absorption and assimilation, the complex and higher molecules being unsuitable for absorption.

If a brief survey is made of the whole process of digestion the following broad facts are seen: There are four digestive juices: Saliva, gastric juice, pancreatic juice and succus entericus. Bile may be taken as the fifth one.

Digestion is carried out by enzymes. Various other factors help the enzymes to carry out their functions. Excepting the milk-coagulating enzyme, all the other digestive enzymes perform their functions by a process of hydrolysis. One digestive juice does not possess all the enzymes necessary for the digestion of all the varieties of foodstuffs completely. For this reason, digestion is carried out in 'relays'. One digestive juice digests up to a certain stage, further digestion is carried out by the next one and in this way the process goes on to completion. It should be further noted that simultaneous absorption along with digestion is an essential factor for complete digestion, because, if not removed by absorption, the end products of digestion will accumulate and set up a reversible enzyme action. Thus, complete digestion will not be possible.

DIGESTION OF CARBOHYDRATES

Introduction

Although dietary carbohydrate is entirely dispensable but the diet of most of the countries includes 60–80% carbohydrate and they are eaten mostly as oligosaccharides or polysaccharides and to lesser extent monosaccharides. Monosaccharides are usually absorbed into the portal vein by the intestinal mucosa so polysaccharides or oligosaccharides should be digested. Daily intake of carbohydrate ranges from 300 to 800 gm per day in adults which generally supplies 1000 to 2500 calories.

Different forms of Carbohydrates

The different forms of carbohydrates which are generally included in diet are as follows:

1. ***Polysaccharides:*** Starch, dextrin, glycogen and cellulose.
2. ***Oligosaccharides (disaccharides):*** Lactose, maltose, sucrose.
3. ***Monosaccharides:*** Glucose and fructose.

The digestion of carbohydrates includes the digestion of polysaccharides and oligosaccharides. Digestion of polysaccharides and oligosaccharides starts in the saliva and is completed in the succus entericus. Digestion of oligosaccharides (disaccharides) chiefly takes place in the succus entericus, but may occur to a slight extent by other digestive juices.

The brief details of the digestion of starch and disaccharides are as follows.

Digestion in the Saliva

Saliva contains (a) chiefly salivary amylase or ptyalin, (b) traces of maltase. Salivary amylase, whose origin in the saliva, acts on starch (which is mostly amylopectin type) and hydrolyses them to maltose and other glucose polymers.

Ptyalin

Peculiarities of ptyalin action.

1. Ptyalin acts on boiled starch only. It cannot penetrate the intact cellulose covering of the unboiled starch particle.
2. Optimum reaction is slightly acid (pH 6.8), but it can also act in neutral or slightly alkaline medium.
3. Strong acid (such as HCl of gastric juice) destroys ptyalin.
4. Optimum temperature is about 45°C and at 60°C it is destroyed.
5. Effects of salts (such as chlorides) are necessary for ptyalin action.
6. Ptyalin digests starch up to the maltose stage only.

As food remains in stomach for a very short period in mouth only 5% of starch is hydrolysed before food gets swallowed. The action of salivary amylase continues in stomach.

Digestion in the Gastric Juice

Gastric juice does not possess any carbohydrate splitting enzyme, but gastric HCl can carry on some hydrolysis of sucrose. The acid gastric juices inhibit action of salivary amylase but prior to this nearly 30–40% of starch is hydrolysed.

Digestion in the Pancreatic Juice

Pancreatic juice contains various enzymes that act on carbohydrates:

Pancreatic juice contains enzymes such as trypsinogen, chymotrypsinogen, elastase, carboxypeptidase, pancreatic lipase, nucleases and amylase.

Pancreatic Amylase

Conditions of action of pancreatic amylase are as follows:

1. It can act on both boiled and unboiled starch.
2. Its action is much more rapid than ptyalin. Most of the starch is converted into maltose within a few minutes.
3. Optimum reaction ranges from pH 6.7 to 7, i.e. slightly acid or neutral. It can also act in slightly alkaline medium.
4. Optimum temperature is about 45°C.
5. Amylase is not present in the pancreatic juice of infants up to the age of about 6 months. Hence, during this period the baby should not be given any starchy food.

Stages of action of pancreatic amylase: The pancreatic amylase digests starch into maltose within 20–30 minutes as chyme enters duodenum.

Role of Intestinal Gland in Digestion

The enterocytes in the small intestinal mucosa contain digestive enzymes peptidase, sucrase, maltase, lactase and intestinal lipase. Succus entericus contains oligo-1, 6-glucosidase which splits α1-, 6-glucosidic linkages of dextrin formed by the action of α-amylase thus providing scope of further activity of α-amylase and of isomaltose converting it to glucose. It also contains maltase which hydrolyses maltase to glucose. So, the starch is completely hydrolysed to glucose by joint action of α-1, 4-amylase present in saliva and pancreatic juice and α-1, 6-glucosidase and maltase in the succus entericus. The other disaccharides taken in food are hydrolysed by lactase and sucrase (invertase) present in this juice. The enzymes, and their substrates upon which they act, and the respective end products are given below.

1. *Sucrase (invertase):* Acts on sucrose producing one, molecule of fructose and one molecule of glucose.
2. *Lactase:* Acts on lactose giving one molecule of glucose and one molecule of galactose.

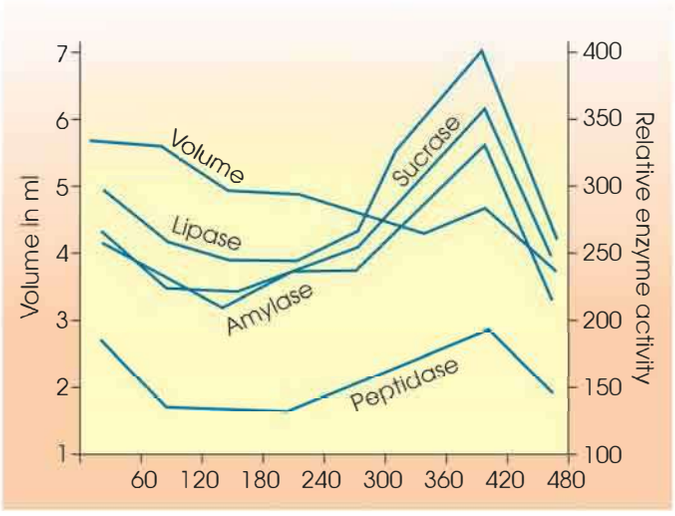

Fig. 60.1: Graphical tracing representing average secretion of succus entericus (intestinal juice) from isolated jejunal loop

3. *Oligo-1, 6-glucosidase:* Converts isomaltose into glucose and splits α-1, 6-linkages of dextrin.
4. *Maltase:* Acts on maltose giving two glucose molecules.
5. *Amylase:* Traces of this enzyme may be present in the succus entericus. The presence of this enzyme at a low concentration has been established. It is supposed to act on that little quantity of starch and dextrin which might have escaped pancreatic digestion.

The graphical representation with average secretion of succus entericus from isolated jejunal loop is depicted in big 60%.

DIGESTION OF PROTEINS

Introduction

Dietary proteins are indispensable so the adult require 0.5–0.8 gm of protein per kg of body weight per day to remain in nitrogen balance and growing children require more, i.e. 4 gm per kg of body weight per day. Proteins are absorbed in the form of amino acids. So, the proteins are digested to amino acid from prior to their absorption. The protein digestion which takes place in the intestine includes the (a) protein contained in the digestive juices (30 gm) and (b) protein derived from desquamated cells (25 gm) along with ingested protein (Fig. 60.2).

Different Forms of Protein

The different forms in which proteins are taken in diet are as follows:

1. Various types of albumins and globulins. This is the chief form of food protein
2. Nucleoprotein
3. Caseinogen (of milk)
4. Collagen and gelatin
5. Mucin
6. Elastin

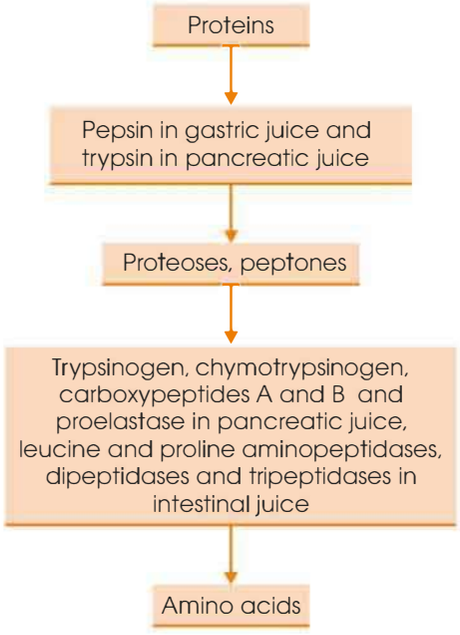

Fig. 60.2: Protein digestion

Of these proteins, elastin cannot be digested. All the other varieties are broken down up to the stage of amino acids by the endopeptidases, e.g. pepsin, trypsin and chymotrypsin and by the exopeptidases in the lower part of the intestinal tract. Albumin and globulin being the chief proteins of diet whose digestion will be described in detail. The differential peculiarities of the other forms of proteins will be mentioned separately. Protein digestion starts in gastric juice and is finished in the succus entericus due to the absence of proteolytic enzyme in saliva. Brief details are as follows.

Digestion in the Gastric Juice

Pepsin is the proteolytic enzyme of gastric juice. It acts with the help of HCl and converts all digestible proteins up to the peptone stage. Proteolytic enzymes other than pepsin (e.g. cathepsin, parapepsins, gastricsin) with the pH optima from 1.8 to 4.5 are present in gastric juice. Although gastric digestion of protein is dispensable (since atrophy of gastric mucosa does not interfere nitrogen equilibrium) but in normal functioning of stomach at best 10–15% of protein is converted to proteoses, peptones and a few amino acids by pepsin.

Digestion in the Small Intestine

1. The proteolytic enzymes of pancreas involved in digestion of proteins are trypsin, chymotrypsin, elastase and carboxypeptidase.
2. The endopeptidases trypsin, chymotrypsin and elastin break the internal peptide bond of protein and convert them into smaller peptides.
3. The endopeptidases are converted from there inactive form into active form in intestinal lumen by enterokinase enzyme.
4. The inactive pancreatic juice contains two zymogens:
   a. Trypsinogen
   b. Chymotrypsinogen.

   Enterokinase converts trypsinogen into trypsin and trypsin activates chymotrypsinogen into chymotrypsin. So that when enterokinase is added to inactive pancreatic juice both the zymogens become activated. Trypsin itself can similarly activate trypsinogen into trypsin as a process of autocatalysis. Chymotrypsin coagulates milk like rennin. It also hydrolyses casein and gelatin, but its mode of action is different from trypsin. Both trypsin and chymotrypsin are endopeptidases.
5. Further breakdown of proteins is carried out by the exopeptidases, e.g. (a) aminopeptidase, (b) carboxypeptidase, (c) tripeptidase and (d) dipeptidase. These enzymes act upon different fractions of the digested protein molecule. Aminopeptidase splits off those amino acids from the polypeptide molecule which possess a free amino group.
6. Similarly, carboxypeptidase takes away those ones which possess a free carboxyl group. Tripeptidase and dipeptidase split off tri- and dipeptides to amino acids. Pancreatic juice also contains two other protein-splitting enzymes, namely elastase and collagenase.

Digestion in the Succus Entericus

1. Digestion of protein in the intestinal juice depends upon the tryptic activity as the proteolytic enzyme present in the intestinal juice is unable to hydrolyse the protein as such.
2. The peptidases present on the brush border of enterocytes of intestinal lumen splits the larger polypeptides into amino acids, dipeptides and tripeptides and then these products are transferred into enterocytes via the microvilli.
3. The dipeptides and tripeptides are broken down into amino acids in the enterocytes.
4. The amino acids are absorbed into the blood.

Digestion of Nucleoprotein

The proteolytic enzymes, pepsin and trypsin, hydrolyses nucleoproteins into their protein and prosthetic part nucleic acid components, in the first step. The former is digested as outlined in protein digestion. The nucleic acids are depolymerised by the hydrolytic action of pancreatic ribonuclease and deoxyribonuclease with the production of oligonucleotides and certain pyrimidine mononucleotides. The former are converted to mononucleotides by intestinal phosphodiesterases. The mononucleotides are hydrolysed by non-specific phosphatases yielding nucleosides and inorganic phosphate.

Digestion in the intestine takes place partly in the lumen by succus entericus, containing enzyme derived from desquamated cells and mainly in the epithelium

of the gut, because, the enzymes are concentrated in the epithelial cells.

Digestion of Casein

1. The chief protein of milk is casein, a complex phosphoprotein. The fourth stomach of ruminants in young mammals contains a protein-splitting enzyme rennin which causes clotting of milk.
2. Rennin causes coagulation of milk, and is important in the digestive processes of infants because it prevents the rapid passage of milk from the stomach. It liberates para-casein from casein which is precipitated as calcium para-caseinate which is later acted over by pepsin.
3. Rennin, which is also known as chymosin, is a proteolytic enzyme synthesized by chief cells in the stomach.
4. Chymosin is distinct from pepsin and absent in adults.
5. Other proteases present in the stomach can convert casein to para-casein. Tryptic and chymotryptic digestions convert it to phosphopeptones containing phosphoserine.

Digestion of Milk

Three constituents of milk require digestion:

1. **Protein**
   - *Caseinogen:* Its digestion has been described above.
   - *Lactalbumin and laciglobulin:* They are digested in the same way as protein—successively by pepsin, trypsin and erepsin.
2. **Fats:** They undergo the same process of digestion as other fats.

   In infant, fat in mother's milk is the major source of nutrition. The lingual lipase is the main enzyme which catalyzes the hydrolysis of dietary fat in infant fed breast milk which serves as a complement to the poorly developed pancreatic lipase activity.
3. **Lactose:** Digested by lactase to glucose and galactose.

Digestion of Collagen and Gelatin

Collagen is present in white fibrous tissue, tendons, bones, etc. Gelatin is prepared from it by boiling with acid. Its digestion proceeds as follows: In the gastric juice: Collagen → gelatin → gelatoses → gelatin peptone. Pancreatic juice digests this peptone up to polypeptide which in its turn is digested by succus entericus up to amino acids.

DIGESTION OF MUCIN

It is a glycoprotein. Gastric juice breaks it into glucosamine and another peptone-like substance. The latter is further digested as other peptones.

Digestion of Lipids

Different Forms of Lipids

Different forms of lipids taken in diet are (a) neutral fats, (b) phospholipids, (c) cholesterides, (d) free cholesterol, (e) fatty acids, and glycerol (Fig. 60.3). The last two groups do not require any digestion, because they are absorbed as such. The first three groups are digested in the alimentary canal.

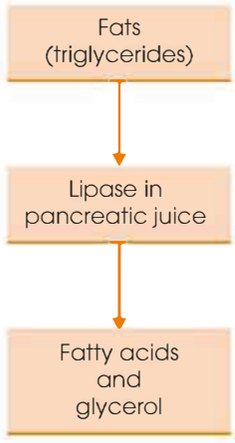

Fig. 60.3: Showing fat digestion by lipase, facilitated by emulsion by bile (simplified)

Neutral Fats

Neutral fats are digested into fatty acid and glycerol. Hydrolysis of fats takes place in stages. In the first stage there is formation of one molecule of fatty acid and a diglyceride. The diglyceride is broken down into another molecule of fatty acid and a monoglyceride. The monoglyceride is finally hydrolyzed into another molecule of fatty acid and glycerol. So, the fat molecule after complete hydrolysis gives rise to three molecules of fatty acids and one molecule of glycerol. All other fats are digested in similar manner but their end products are different.

Digestion in the Mouth

Saliva does contain small quantity of lingual lipase. The total amount of fat digested by lingual lipase is less than ten percent. Digestion starts in the stomach and ends in the succus entericus.

Digestion in the Gastric Juice

1. It breaks neutral fat into one molecule of glycerol and 3 molecules of fatty acids.
2. It acts best in slightly acid medium, but is destroyed by 0.02% HCl in 15 minutes. Hence, its action takes place only in the initial stages of gastric secretion when the acidity is not high.
3. Optimum temperature for its action is about 40°C.
4. It acts best on emulsified fat, i.e. egg-yolk, milk, etc.

Due to these characteristics gastric lipase is not of much importance. Hydrolysis of fat in stomach is not significant, since most of the ingested fat is found in the duodenum and presence of lipase in the stomach is by way of regurgitation from the small intestine.

Digestion in the Pancreatic Juice

Fat digestion is mainly carried out in the duodenum by pancreatic lipase or sometimes called as steapsin. Pancreatic lipase, also known as pancreatic triacylglycerol lipase, is an enzyme which hydrolyses (breaks down) dietary fat molecules converting triglyceride substrates of food to monoglycerides and free fatty acids.

Since fat is insoluble, pancreatic lipase acts on triglycerides in emulsified form in the lumen by bile salts into smaller droplets from larger fat droplets thereby increasing the overall surface area of the fat, and this break apart the fat more effectively. Emulsification of fat takes place by bile salts, fatty acids, cholesterol, monoglycerides, lysolecithins and proteins. Fatty acids and monoglycerides pass into micelle phase with bile salts and they in this way are made water-soluble. The formed monomers moves by way of peristalsis along the small intestine and get absorbed into the lymphatic system by a specialized vessel called a lacteal.

Fat digestion is nearly completed by the pancreatic juice. This is due to the fact that not only pancreatic lipase, but several other factors also help in the process.

The factors that help lipase in its action are as follows:

1. Bile salts help fat digestion in a number of ways (as discussed earlier).
2. Presence of antiperistalsis in the duodenum is of considerable importance in this respect. It helps thorough admixture and keeps fatty food for a longer time in the duodenum, to ensure maximum digestion.
3. Pancreatic lipase acts best in a slightly alkaline medium (pH 8). The alkalinity of the pancreatic juice and bile helps to maintain a suitable reaction and facilitates lipase action.
4. Presence of fats in the duodenum liberates enterogastrone which inhibits gastric secretion and motility. Thus, emptying of stomach is delayed. Fats also stimulate bile secretion as well as bile expulsion. Thus, fatty food adapts the environment in such a way that its own digestion is facilitated.
5. Amino acids like arginine, histidine and lysine also help in the hydrolysis of fat.
6. Phospholipases, cholesterol esterases: These are other enzymes of pancreatic juice acting on other lipids for their digestion. The former removes one fatty acid from lecithin and cephalins yielding substances called lysolecithins and lysocephalins. These are acted upon by phospholipases A_2, which removes the second fatty acid yielding glycerol—phosphorylcholine, and other similar compounds. Glycerol is split off by phosphodiesterase, and the base choline and sphingosine, etc. are subsequently freed by the action of phosphatases. Cholesterol is freed from cholesterides by cholesterol esterase.
7. The digestion of lipid is facilitated by colipase a protein secreted by pancreatic juice.

ABSORPTION

Definition

Absorption is the process by which the end products of digestion pass through the intestinal epithelium and enter the blood stream.

Passive and active transport: In the passive transport mechanism, special physical force is not required. For instance, if any particular food substance remains in higher concentration in the lumen of the intestine the energy for movement is derived from the higher concentration of the food substance.

In the active transport mechanism, there is a carrier mechanism which helps in movement of the food substance against the electrical forces and the chemical gradient, ATP supplies energy to this carrier system.

ABSORPTION OF CARBOHYDRATES

1. End products of carbohydrate digestion are all monosaccharides, such as glucose, laevulose, galactose, xylose, mannose, arabinose, etc. It is in this form that carbohydrates are absorbed. All carbohydrates in food are absorbed in form of monosaccharides and a small portion as disaccharides.
2. Duodenum and jejunum are the site of maximum carbohydrate absorption.
3. *Transport of glucose:* The glucose absorption occurs in a co-transport mode and is accompanied with active transport of sodium. The sodium dependent glucose transport (SGLT) is responsible for the co-transport. The active transport of sodium ions from the basolateral membrane of the intestinal cells into the blood occurs first. The low concentration of sodium inside the cell causes movement of sodium from lumen into the cell through the brush borders along concentration gradient by secondary active transport. Sodium drags along with it glucose inside the cell. As it reaches the epithelium cell other transport proteins GLUT-2 cause transport of glucose via interstitium into the blood.
4. *Transport of galactose:* Galactose is transported by similar mechanism as glucose.
5. Fructose is transported via facilitated diffusion through intestinal epithelium and this is aided by GLUT-4 and GLUT-5.

ABSORPTION OF PROTEINS

1. The digested proteins are absorbed through luminal membrane of the intestinal epithelial cells. The products that are absorbed are dipeptides, tripeptides and a few free amino acids.
2. The amino acids and peptides are transported by seven types of transport systems. The sodium ions are involved in the co-transport in five of these systems while two requires chloride ion.

3. The energy for this transport is mediated via sodium co-transport mechanism, e.g. sodium-amino acid co-transport. The amino acids or other peptides bind with a specific transport protein which requires binding of sodium first to the transport protein and then along with the sodium the peptides and amino acids are carried to inside of the cell along the electrochemical gradient by secondary active co-transport.
4. The tripeptides and dipeptides are transported with aid of H^+ into the enterocytes by peptide transporter 1.
5. Some amino acids are transported by facilitated diffusion with the aid of special membrane transport protein. The absorption occurs mainly in duodenum and jejunum.
6. Amino acids are further transported into blood by facilitated diffusion.

Fats Absorption

Process of absorption

1. Most dietary fat of either vegetable or animal origin comprises triglycerides in which glycerol is combined in low-energy ester linkages with three fatty acids and the fatty acids are of even number of carbon atoms. Fatty acids are both saturated and unsaturated which are almost entirely palmitic and stearic in case of former and in case of the latter oleic and linoleic acids. These are long-chain fatty acids. Milk fat contains 3–10% (C4–14 acids) contributing shorter-chain fatty acids.
2. Since fats are insoluble in water and immiscible in chyme, so fat neither is absorbed as such nor is digested by lipase (due to lack of contact with lipase) to fatty acid and glycerol for absorption.
3. Emulsification of fat by different emulsifying agent is required for preparing it suitable for both digestion and absorption and this process (emulsification) possible in small intestine where bile salt and other agents are present.
4. Bile salts themselves are relatively weaker than the mixture of bile salts and a polar body—lecithin, lysolecithin or monoglycerides as emulsifying agent.

The latter two are produced by the action of pancreatic lipase on lecithin or triglycerides. Thus, the enzymic action tends to stabilize the emulsion.

Aspects of Fat Absorption

It has been observed that

1. The lipids are absorbed in form of monoglycerides and free fatty acids. The fatty acid is absorbed more readily than any other components, i.e. triglycerides, 1-monoglycerides, 2-monoglycerides, diglycerides and free fatty acids.
2. The monoglycerides and free fatty acids are transported by diffusion into the microvilli of the intestinal cells in small intestine.
3. In the intestinal cells the larger fatty acids are re-esterified to triglycerides while small fatty acids are directly carried to the portal circulation.
4. The triglycerides are coated with proteins, phospholipids and cholesterol to form chylomicron.
5. The droplet of fat which is chylomicron is formed (by aggregation of fat molecules) before the delivery of resynthesized fat into lymph. The droplets are enclosed in a membrane, composed of small amount of protein, free cholesterol and saturated triglycerides in a monolayer of phospholipid. The fat in the chylomicron reflects the composition of ingested fat (dietary) partly since long-chain fatty acids of the dietary fat are added to mucosal cells for resynthesis whereas most of short and medium-chain fatty acids and some glycerol are shunted to portal blood. Chylomicron cannot enter the fenestrae of the capillaries due to its particle size. It enters the lacteals through open channels existing between interstitial spaces and the lymphatic lumen.
6. The small amount of long-chain fatty acid are absorbed in ileum while the major portion is absorbed in upper small intestine.
7. The colonic bacteria by its action on starch and carbohydrates produce short-chain fatty acids in colon.

These are reabsorbed into colon by the specific transporter of colons epithelial cell.

Water Absorption

1. Very little water is absorbed from the stomach. Water introduced into the stomach almost immediately passes into the small intestine which is the chief seat of water absorption.
2. The intestinal contents near the ileocaecal valve contain the same proportion of water as the upper part of jejunum. But their absolute amount is much smaller. The large intestine absorbs all the residual water and forms solid stool.
3. The main physico-chemical forces which help water absorption are hydrostatic pressure, endosmosis and osmotic pressure. Although the hydrostatic pressure and endosmosis do not play any significant part but the osmotic pressure plays an important role in the absorption process.
4. Water and salts are absorbed passively and actively. During passive absorption, water and small water-soluble substances traverse the intestinal mucosa along osmotic or electrochemical gradient, the energy moving them being derived from whatever processes established the gradients in the first place. Besides this, water and sodium can also be absorbed against osmotic and electrochemical gradient (active transport).

ABSORPTION OF ELECTROLYTES

1. The sodium is absorbed by secondary active transport and facilitated diffusion all along the intestine.

2. Calcium is absorbed in presence of vitamin D, protein and lactose. It is actively absorbed from the duodenum.
3. Iron is absorbed in combination with a special protein called ferritin. Absorption is promoted by ascorbic acid which keeps it reduced. The duodenum and upper jejunum are the sites of iron absorption. Iron absorption is regulated by the amount of iron stored in the body.
4. Phosphate ions are absorbed by all segments of the small intestine but most effectively from the ileum.
5. *Chloride ions:* It is secreted via gastric, intestinal and pancreatic secretion into lumen of intestine. The chloride is chiefly absorbed in jejunum. In colon the presence of chloride channels in enterocytes aids its absorption. In ileum chloride is absorbed in exchange of bicarbonates.
6. Bicarbonates are mainly absorbed in jejunum. It is secreted in the pancreatic secretion.
7. *Potassium:* It is absorbed from jejunum and ileum by passive diffusion. It is secreted and re-absorbed in the large intestine.

Faeces

The chyme derived from the first food intake requires 36 hours for its solidification to be converted into solid residual matter known as faeces. Roughly about 150 gm of solid stool is passed in 24 hours. If vegetable, coarse cereals and cellulose be excluded from the diet, the faeces show a fairly constant composition as follows:

1. **Water:** 65%
2. **Solid:** 35%
3. **Ash:** 15% (mainly calcium, phosphates, iron and magnesium).
4. **Ether-soluble substances (fats):** 15% (neutral fats, fatty acids, lecithin, cholic acid and coprosterol).
5. **Nitrogen:** 5% (derived from purine bases, about 0.11 gm per day).
6. **Desquamated epithelial cells,** bacteria, mucus, undigested and unabsorbed food.

Contents and Characteristics

1. The reaction of stool is generally neutral or acid, but may be slightly alkaline. It is composed of: (a) Food residues, (b) intestinal secretions, (c) bile, (d) leucocytes, (e) bacteria, (f) epithelial cells, and (g) substances excreted through the large intestine. Since, under normal healthy conditions very little food residue is left, faeces are not derived from any of the ingested food.
2. On analysis no soluble carbohydrates, proteins, or their derivatives are found in the faeces. Moreover, animals, in state of complete starvation, from faeces which shows the same composition as that formed after food. This shows that in a healthy man living upon well-balanced diet, stool is independent of food.
3. Bacteria usually constitute about 9% of the total solids, but it may be as high as 50% of the total weight of the faeces.
4. The colour of the faeces is due to the presence of stercobilin derived from the bile pigments—biliverdin and bilirubin and also to bilifuscins. Biliverdin and bilirubin are reduced to urobilinogen in the large intestine. A certain amount of urobilinogen is absorbed through the intestinal wall into the blood where it passes via portal vein to liver. The liver excretes these pigments as a major constituent of bile, a part of which carried to the kidney through general circulation for their excretion through urine. Unabsorbed urobilinogen in the large intestine is converted to stercobilin. Interference in the flow of bile by obstruction in the bile duct or by a tumour formation results pale appearance of faeces which conclusively indicates that the normal fate of bile pigments is the formation of stercobilin and its excretion through faeces giving its characteristic in yellow colour.
5. The colour also varies somewhat with the character of the diet, being paler on a high milk diet, black after Fe, etc.
6. The odour of the stool is due chiefly to aromatic substances like indole, skatole, etc. and also gases like H_2S.
7. Indole and skatole result specifically from the action of bacteria on amino acid tryptophan.
8. Under normal conditions about 500 ml of gas is passed out per day.
9. If coarse cereals and vegetables be taken, the composition of the faeces does not remain constant. Because, the cellulose, being indigestible, increases the amount of stool.
10. Also various other substances, which remain inside a cellulose covering, are also found in the faeces, under such conditions.

In this way the composition varies. However, cellulose serves the important purpose of increasing the

Summary: Absorption of lipids

The digested products of fats are the monoglycerides and free fatty acids are dissolved in the lipid portion of the bile micelle. These micelles are 3 to 6 nanometer in diameter and are soluble in chyme. The dissolved monoglycerides and free fatty acids in micelle are carried to microvilli surface of the intestinal cell. The monoglycerides and free fatty acids diffuse out of micelle into the epithelial cells as they are also soluble in lipid soluble portion of membrane of epithelial cell. The bile micelles which are in chyme further help in absorption of more monoglycerides and free fatty acids. Nearly 97% of fat is absorbed by ferrying action. In the epithelial cell the monoglycerides and free fatty acids in the smooth endoplasmic reticulum forms triglycerides and they are further released as chylomicrons at the base border of epithelial cell to move into the general circulation via lymphatics.

bulk of stool and thus stimulating movement of the large intestine.

EXAM-ORIENTED QUESTIONS

Essay

1. Describe the mechanism of absorption, secretion and digestion of carbohydrates along the gastro-intestinal tract.
2. Describe the mechanism of absorption, secretion and digestion of lipids along the gastro-intestinal tract.
3. Describe the mechanism of absorption, secretion and digestion of proteins along the gastro-intestinal tract.

Short Notes

1. Water absorption
2. Absorption of salt
3. Chylomicron
4. Digestion of lipids
5. Absorption of lipids
6. Peculiarities of ptyalin action

Chapter

61

Gastro-intestinal Hormones

INTRODUCTION

From a localised area of the gastro-intestinal tract, certain hormones are secreted. These are generally known as **gastro-intestinal hormones** (Fig. 61.1). Some of the hormones, of course, are not exclusively secreted from the gastro-intestinal areas but also from other areas too. These hormones regulate the motor and secretory activities of the digestive organs. The hormones are polypeptide in nature, a structural resemblance between the chemical nature of these hormones themselves, and also other polypeptide hormones like glucagon and caerulein (amphibian skin secretion) has been noted. Their secretions are mostly conditioned by the presence or absence of specific food materials in the lumen of the gastro-intestinal tract, rather than by other glandular products in the circulation. The specific cells responsible for the synthesis of these hormones are not known with certainty. By appropriate stimuli, these hormones are secreted quickly and also destroyed rapidly following withdrawal of the appropriate stimuli.

Gastrin

It stimulates the secretion of HCl and pepsin by the stomach. It is secreted from G cells present in the pyloric gland. It is a polypeptide hormones found in forms such

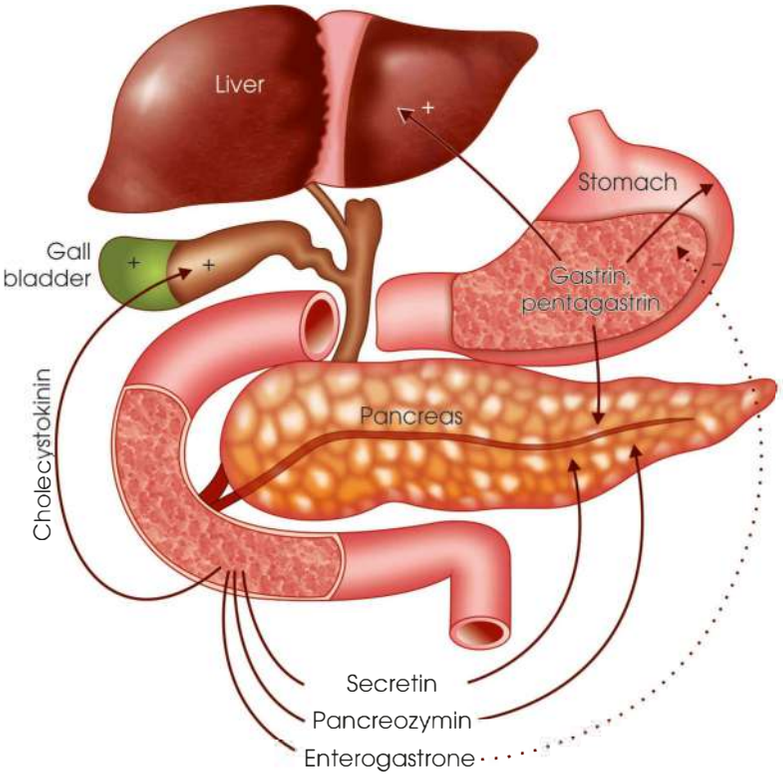

Fig. 61.1: Source and action of the gastrointestinal principles

as G34, G17 and G14, G17. They have half-life of around three minutes. The inactivation of gastrin occurs in small intestine and kidney.

Functions of Gastrin

It stimulates gastric motility.

- It also stimulates chief and oxyntic glandular cells to secrete pepsinogen and acid (HCl), pancreas to secrete bicarbonate containing fluid and enzymes, duodenal mucosa for the release of secretin.
- It also stimulates insulin secretion after protein intake.
- It also produces contraction of gastro-oesophageal sphincter.

Regulation of Gastrin Secretion

1. Vagal stimulation, acetylcholine, digested protein products, and bombesin stimulate gastrin secretion.
2. Somatostatin, acidity in stomach, secretin, glucagon, VIP and GIP inhibit gastrin secretion.

Cholecystokinin (CCK) or Pancreozymin

Cholecystokinin was previously called *pancreozymin*. It is synthesized and secreted by enteroendocrine cells in the duodenum, the first segment of the small intestine. But it is now clear that it is a single hormone and is secreted from the mucosa of the upper small intestine when foodstuffs (especially dilute acids, lipids, fatty acids, peptones, etc.) come in contact with it. Cholecystokinin depending on post-translational modification of the 150-amino acid precursor, preprocholecystokinin consists of varied numbers of amino acids. CCK peptide hormone exists in several forms and is labelled as CCK58, CCK33, CCK22 and CCK8. CCK12 depending on number of amino acids it contains.

Cholecystokinin hormone has got action on the contraction of the gall bladder; it also releases stored bile into the intestine, and stimulates the secretion of pancreatic juice by secreting enzyme-rich pancreatic juice. It inhibits gastric acid secretion and gastric emptying. It increases the secretion of enterokinase. It increases the action of secretin in producing alkaline juices. It stimulates the secretion of calcitonin and glucagon. It also enhances the motility of colon and small intestine.

Regulation of Secretion of Cholecystokinin or Pancreozymin (CCK)

Products of digestion, peptides, amino acids and fats increases cholecystokinin secretion.

Secretin

It is secreted by the duodenal mucosa due to presence of chyme from stomach; the release of pancreatic juice but not enzymes is stimulated. Bayliss and Starling (1902) first reported the presence of a blood-borne factor which is responsible for the secretion of pancreatic juice. In addition to hydrochloric acid, fatty acids, hydrolyzed protein, etc. even water are effective stimulant. Removal of the vagus does not affect release of secretion, but hypophysectomy lowers it. Secretion is ineffective by mouth and is destroyed in the body due to the action of an enzyme "secretinase". The jejunum cells are responsible for its synthesis. It contains 27-amino acid residues and is a polypeptide hormone.

It increases the secretion of bicarbonate by duct cells of pancreas and biliary duct. It also potentiates the secretion of CCK to produce enzyme rich pancreatic secretion. Secretin mainly increases the secretion of bicarbonate by the biliary duct and duct cells of pancreas.

Regulation of Secretin Secretion

The decreased gastric acid secretion may cause contraction of pyloric sphincter. Increased acid in upper small intestine and products of protein digestion increases secretin secretion. Bile salts in intestine stimulates secretin production.

Glucagon-like Peptide-1

Glucagon-like peptide-1 (GLP-1): It is a neuropeptide and an incretin derived from the proglucagon gene. The major source of secretion of GLP-1 is the intestinal L cell. The GLP-1 is secreted by the ileal L cells and is dependent on the presence of dietary carbohydrate, protein and lipid in the small intestine. It increases secretion of insulin from the pancreas and is glucose dependent. It increases beta cell mass and insulin expression and secretion (Fig. 61.2).

GASTRIC INHIBITORY POLYPEPTIDE (GIP)

GIP is produced by cells in the mucosa of the duodenum and jejunum. It is released in response to presence of glucose and fat in the gut. It consists of 42 amino acids.

It stimulates insulin secretion and in high doses inhibits gastric juice secretion and its motility.

VASOACTIVE INTESTINAL PEPTIDE (VIP)

VIP is released from jejunum in response to fatty meals. It contains 28 amino acids.

It increases secretions of electrolytes and water from intestine, inhibits secretion of gastric juices, dilates peripheral blood vessels and brings over relaxation of intestinal smooth muscle including the sphincters.

Urogastrone

This is a polypeptide and has been isolated from urine of men and dogs. It can inhibit the gastric secretion in response to cholinergic drugs.

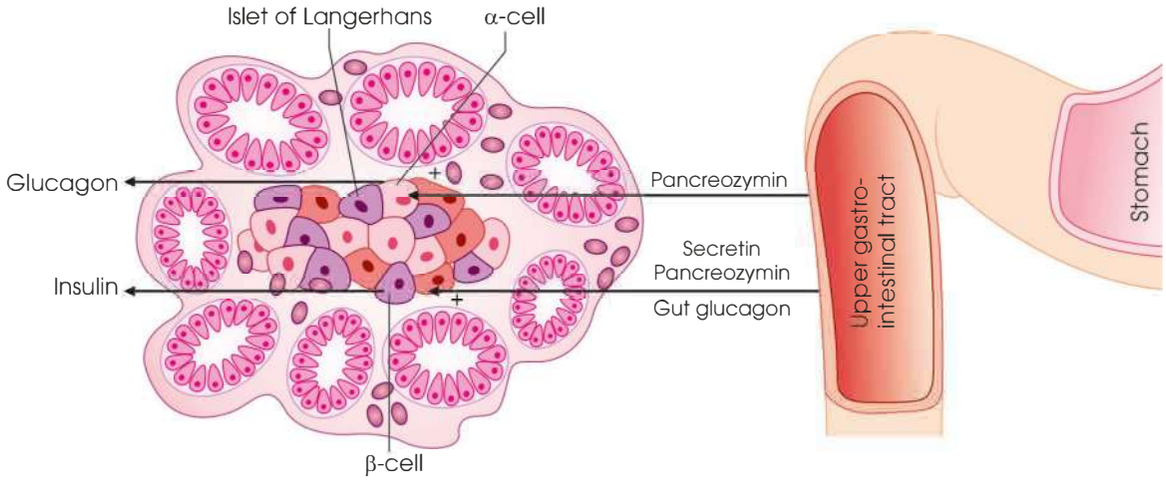

Fig. 61.2: Diagram shows gastro-intestinal hormones which stimulate glucagon and insulin release

Villikinin

This hormone is believed to be secreted from the mucosa of the upper small intestine by chyme. It is known to stimulate movement of villi in the small intestine.

Enterocrinin

This hormone is secreted in the intestinal lumen. It is known to increase the secretion of intestinal juice.

MOTILIN

It is secreted by cells in the stomach, duodenal and colon mucosa; some of these cells are enterochromaffin cells. It contains 28 amino acids. It regulates intestinal motility in inter-digestive phase. It prepares the intestine for next meal. It also regulates the migrating motor complexes.

NEUROTENSIN

It is secreted by mucosal cells of the ileum in presence of fatty acids. Neurotensin increases ileal blood flow and inhibits gastric motility.

SOMATOSTATIN

Somatostatin is also known as growth hormone inhibiting hormone (GHIH). It is released from cells in the pancreatic islets and GIT mucosa. It inhibits gastric acid secretion and motility, inhibits gall bladder contraction, and also inhibits the absorption of glucose, amino acids and triglycerides. It also inhibits the secretion of gastrin, secretin, vaso-active intestinal peptides, gastro-intestinal peptides and motilin.

GASTRIN RELEASING PEPTIDE

It is present at vagal nerve endings and when released cause increases in gastrin secretion.

GHRELIN

It is secreted by the cells in stomach. It regulates intake of food. Ghrelin secretion is increased on fasting and decreased after ingestion of food. It also stimulates growth hormones secretion.

PEPTIDE YY

It is secreted by the jejunum in response to fatty acid in diet. It inhibits gastric acid juice secretion and gastric motility.

SUBSTANCE P

It is released by the endocrine cells and nerve cells in the GIT and is responsible for increasing motility of the small intestine.

BOMBESIN

It is 14 amino acid peptides. It stimulates release of cholecystokinin (CCK) and gastrin. Bombesin is a tumour marker for small cell carcinoma of lung, neuroblastoma, gastric cancer and pancreatic cancer.

61

EXAM-ORIENTED QUESTIONS

1. Discuss the physiological functions of various hormones of GIT.

Short Notes

1. Gastrin
2. Cholecystokinin (CCK) or pancreozymin
3. Secretin
4. Motilin
5. Ghrelin
6. Substance P
7. Vaso-active intestinal peptides

CLINICAL CASE SCENARIO

Gastrointestinal Tract

Q1. A 22-year-old male having dental caries, complained of dry mouth, difficulty in swallowing and slurred speech with no salivary secretion. What is the likely diagnosis? What is the cause for the same?

Ans. The patient is suffering from aptyalism. The salivary duct obstruction due to calculus is the most common cause.

Q2. A 30-year-old male complained of recurrent heart burn, belching and oesophageal discomfort. What are the likely diagnoses for the symptoms?

Ans. The patient is suffering from gastro-oesophageal reflux disease (GERD), there is reflux of acid gastric content into the oesophagus leading to heartburn and oesophageal discomfort.

Q3. A patient was diagnosed as a case of peptic ulcer. He was prescribed ranitidine (H_2 receptor antagonist) initially and later the drug was changed to a (proton pump blocker). Describe the mechanism of action of both the drugs.

Ans. Ranitidine blocks the H_2 receptor thereby preventing the release of histamine as histamine is known to stimulate release of HCl from parietal cells. Omeprazole inhibits the H^+/K^+ pump, thereby preventing HCl secretion.

Q4. A 40-year-old male complained of acute pain in abdomen, tachycardia and vomiting. His blood investigation revealed increased plasma level of serum amylase. What is the diagnosis? Enlist any four causes for the clinical condition.

Ans. The patient is suffering from acute pancreatitis. The common causes are chronic alcoholism, increased triglyceride levels, blunt injury to abdomen and obstruction of sphincter of Oddi by gall stones.

Q5. A 42-year-old obese fertile female presented with complaints of spasmodic abdominal pain. Her ultrasound revealed stone in the biliary tract. What is the diagnosis? Enlist any two causes for developing the condition.

Ans. This clinical condition is known as cholelithiasis. It is due to formation of gall stone in the gall bladder. The decreased flow of bile (bile stasis) and bile supersaturated with cholesterol leads to gall stone formation.

Q6. A patient having history of loose motion producing water diarrhoea was identified as a case of cholera. What is the cause for watery diarrhoea?

Ans. There is active secretion of chlorides and bicarbonates into the intestinal lumen due to increased cyclic AMP concentration produced by the cholera toxins leading to watery stools.

Q7. What is the advantage of initiating oral rehydration therapy in patients of diarrhoea?

Ans. The patients of diarrhoea have history of loose motions which leads to loss of water and electrolytes in the stool. The oral rehydration solution supplements the body with sodium and glucose which reinstate water and electrolyte imbalance.

Recent Advances: Treatment of Peptic Ulcer

Barry J Marshall
1951

J Robin Warren
1937

Barry J Marshall and J Robin Warren were awarded the Nobel Prize in Physiology or Medicine in 2005 "for their discovery of the bacterium *Helicobacter pylori* and its role in gastritis and peptic ulcer disease". *Helicobacter pylori* are also linked to the development of gastric carcinoma and duodenum ulcer. *Helicobacter pylorus* is a gram-negative, microaerophilic bacterium colonising generally in the stomach. The standard first-line therapy is a one-week "triple therapy" consisting of any one of the proton pump inhibitors such as omeprazole/pantoprazole/rabeprazole and the antibiotics clarithromycin and amoxicillin (or metronidazole for people who are allergic to penicillin). This revolutionary therapy has helped in cure of peptic ulcers.

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RECENT ADVANCES: CAPSULE ENDOSCOPY

An endoscopy is a simple procedure by which inside of the human bodies can be visualised using an endoscope. The key hole surgery is performed by the cutting tool attached to the end of the endoscope. Advancement in endoscopy technology brought over capsule endoscopy. Capsule endoscopy is a way to record images of the digestive tract for clinical diagnostic and therapeutic purpose. The capsule used for the procedure has the size and shape of a pill and encompass a tiny camera. The patient is asked to swallows the capsule. It takes pictures of the inner

Capsule endoscope

structures of the gastrointestinal tract. The most important use of capsule endoscopy is to examine areas of the small intestine which cannot be observed by colonoscopy or esophagogastro-duodenoscopy (EGD).

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Section

VII

Renal Physiology

Chapter

62

Functional Anatomy of Excretory System

INTRODUCTION

Kidneys, skin, lungs, gastro-intestinal tract, salivary glands and liver are the main channels through which excretion takes place. There seems to be a general rule regarding the channels of excretion of different substances. For instance, the soluble, non-irritant solid substances and water are mainly excreted through the kidneys and to a lesser extent through the skin; volatile substances, such as CO_2, ammonia, alcohol, ketone bodies, aromatic oils, water vapour, etc. are excreted through the lungs. Heavy metals are excreted through the gastro-intestinal tract, especially through the large intestine and slightly through the liver and saliva; fats and fat derivatives are passed out of the body through the skin, as sebum, and through the liver along with bile.

These are the broad principles on which excretion from the body takes place. To understand the way in which the kidney carries out these functions, it is essential to understand first the way in which it is supplied with blood. About 25% of left ventricle's total output of blood in each min is distributed through the renal arteries to the kidneys for filtration at rest.

Among the main channels of excretion, kidneys are the chief. The kidney performs excretory, homeostatic and endocrine functions. The urinary system consists of those organs which produce urine and eliminate it from the body. These organs comprise two kidneys, two ureters, one bladder and one urethra (Flowchart 62.1).

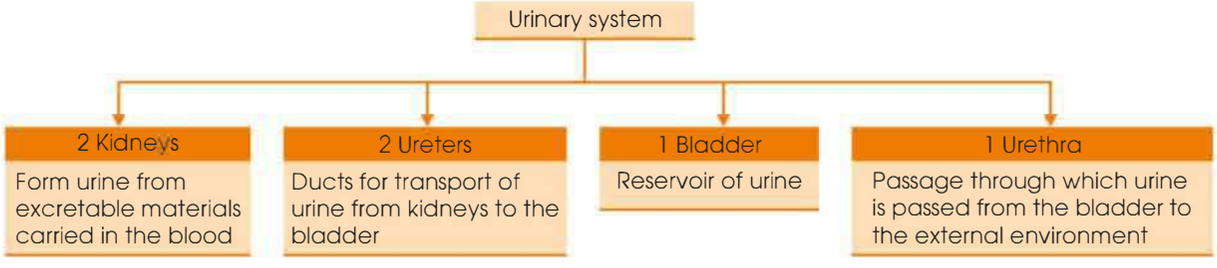
The secretion of urine and its elimination from the body are vital functions because they together constitute one of the most important mechanisms for maintaining the bodily homeostasis. Smith has phrased thus. "The composition of the blood (and internal environment) is determined not by what the mouth ingests but by what the kidney keeps".

KIDNEYS

Characteristic Features

1. The human kidneys are bean-shaped paired organs situated just behind the vertebral column in the abdomen at the twelfth thoracic to third lumbar segment (Fig. 62.1).
2. An average-sized kidney measures 10 to 12 cm in length, 5 to 6 cm in width and 3 to 4 cm in thickness, each weighing about 150 gm in adult male and about 135 gm in adult female. Usually the right kidney is slightly smaller than the left one.
3. A deep notch or concavity is present at the medial border—the hilus (hilum), and it is through this region the blood vessels—renal artery and vein, ureter and nerves pass.
4. If the kidney is sectioned along its long axis and examined with the naked eye, a dark reddish-brown peripheral region (opposite the hilus), the cortex, and the rest lighter area—the medulla are distinguishable.
5. The medulla is again divided into 10 to 15 conical areas, the renal pyramids having their broad base

Flowchart 62.1: Organs of urinary system



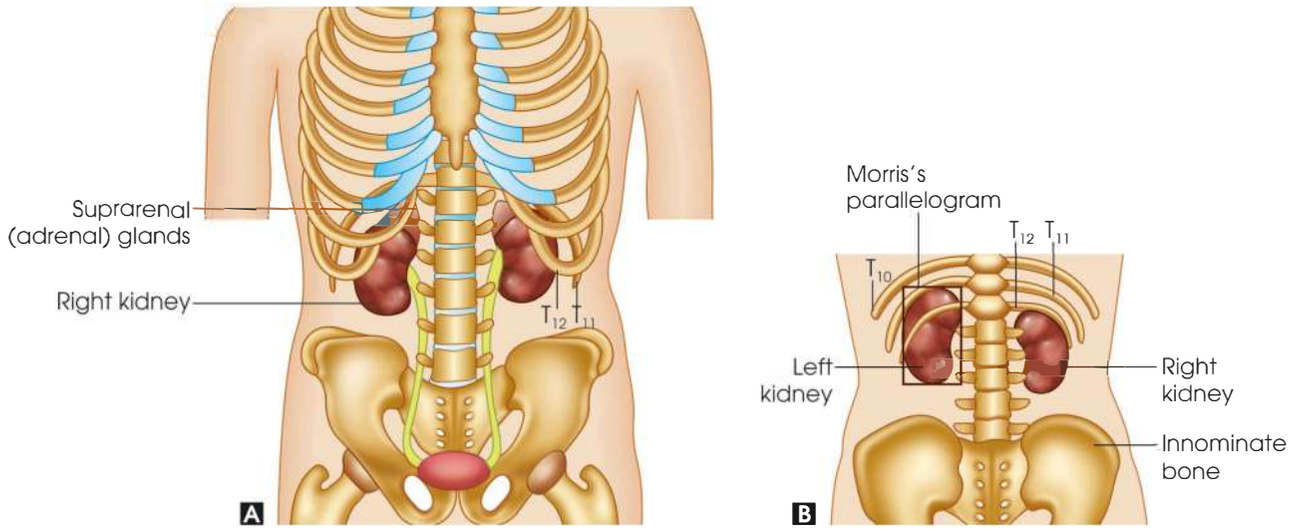


Fig. 62.1A and B: Diagrammatic representation of the normal position of urinary organs. (A) Ventral view; (B) Dorsal view

towards the cortex and the vertex, apex or papilla projected into the lumen of the minor calyx.

6. The lateral boundaries of the pyramid are formed by the projections of the darker cortex which are named as renal columns of Bertin. The substance of the pyramid is radially striated by brownish lines which converge towards the apex which is due to the straight uriniferous tubules and the blood vessels parallel to them. The apex or papilla (the minor calyx) is known as the area cribrosa, which is perforated by a number of openings of the collecting tubules to the minor calyx.
7. The renal medulla is known to be a site of origin for PGA_2, PGE_2 and PGF_2—the prostaglandins concerned with blood pressure regulation.

NEPHRON

The two kidneys are the chief excretory organs of the body. They are made up of numerous functional microscopic units called nephron. They perform excretory and homeostatic functions. There are about one million nephrons in each human kidney. The nephrons ultimately drain into the pelvis of the ureter (Fig. 62.2). From here urine passes down the ureters and collected in the urinary bladder.

Uriniferous Tubules

They consist of:

1. Secretory portion or nephron
2. Non-secretory portion or collecting portion or duct system.

Types of Nephrons

Histologically there are two types of nephrons, according to their relative position in the cortex: The superficial nephrons (superficial glomeruli) occupy the outer two-thirds of the cortex and make up about 85% of the total number. The juxtamedullary nephrons (juxtamedullary glomeruli) occupy the inner third of the cortex and constitute about 15% of the total number. The superficial nephrons are smaller in size and are fully functional under normal conditions. The juxtamedullary nephrons are large and work only in conditions of stress.

The nephrons consist of the following parts:

1. Renal or malpighian corpuscle
2. Proximal convoluted tubule
3. Descending limb of loop of Henle
4. Ascending limb of loop of Henle: The ascending limb of loop of Henle comprises two segments: Thin ascending limb of loop of Henle and thick ascending limb of loop of Henle.
5. Distal convoluted tubule.

The proximal convoluted and distal convoluted tubules are in the cortex and in close proximity to the renal corpuscle. The loop of Henle extends from the cortex to the variable distance in the medulla according to the position of the corpuscles in the cortex.

Malpighian corpuscles (malpighian body or renal corpuscle): Found only in the cortex of the kidney. It measures about 200 microns in diameter. The corpuscle consists of two parts:

1. Glomerulus
2. Bowman's capsule (Fig. 62.3).

1. Glomerulus: It is the capillary tuft which invaginates Bowman's capsule. The afferent arteriole breaks up into about fifty capillary loops and forms the glomerular tuft which lies within Bowman's capsule, a double-walled epithelial sac. Each nephron starts with a tuft of 6 to 8 renal blood capillaries invaginated into the end of a tubule. This structure is named as glomerulus. Although these glomerular capillaries are not homogeneous loops, but they make up freely

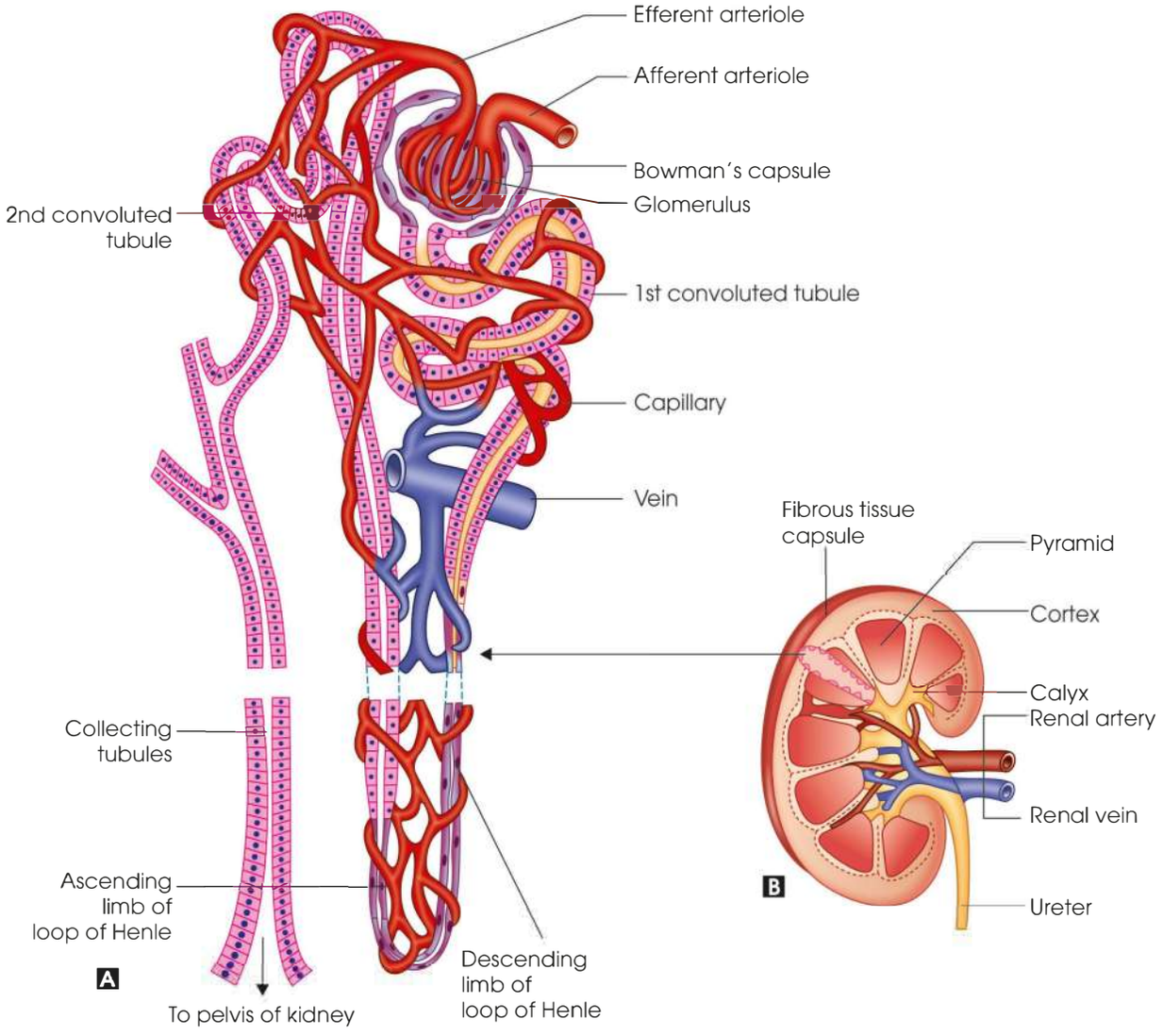


Fig. 62.2A and B: (A) Diagram show renal tubules (B) Longitudinal section of the right kidney on the right side

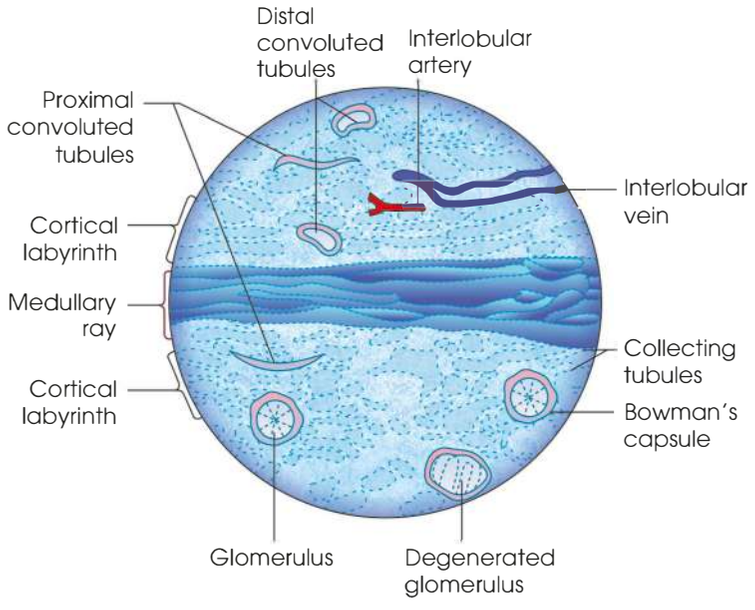


Fig. 62.3: Diagrammatic representation of the cortex of human kidney showing its different structures

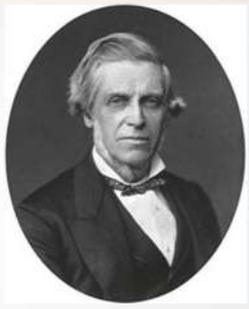
Marcello Malpighi is famous for his discovery of the pulmonary capillaries and alveoli. He also studied and revealed the structural details of the renal glomeruli, urinary tubules, dermal papillae, taste buds, and the glandular components of the liver. Renal pyramids (Malpighi's pyramids) are named after Marcello Malpighi.

Marcello Malpighi
1628–1694

branching anastomotic network. There are about 20 to 50 capillary loops in all. Just before entering the glomerulus, the media of the afferent arteriole is found to contain a thick cuff of large modified muscle cells—the juxtaglomerular cells. The capillary tuft re-unites and forms the efferent arteriole which passes out of the glomerulus. The afferent vessel is short and wide whereas the efferent vessel is narrow and long (Figs 62.4 to 62.6). This arrangement makes the glomerular blood pressure much higher (70 mm Hg) than the capillary bed elsewhere and facilitates filtration. The total surface area of all the glomerular capillaries in two kidneys (i.e. the total filter bed) is about 1.73 square metres.

4. ***Bowman's capsule:*** It is the dilated blind end of the nephron, invaginated by the glomerular tuft—the vascular end or pole. It consists of two layers—parietal and visceral. The parietal layer is made up of typical squamous epithelium of flat polygonal cells. It gradually becomes continuous with the tubular epithelium at the tubular end of Bowman's capsule.

Bowman's capsule is a cup-like sac at the beginning of the tubular component of a nephron named after Sir William Bowman, who identified it in 1842.



Sir William Bowman
1816–1892

5. The filtering membrane of the malpighian corpuscle (Fig. 62.6) consists of the following layers: (a) the endothelial cell layer of the capillaries, (b) the basement membrane, (c) the epithelial cells of the visceral layer of Bowman's capsule.

The cells of the visceral layer during development undergo extensive modification and are known as podocytes (glomerular epithelial cells). These give rise to a number of radiating tentacle-like cytoplasmic processes which in turn give up a large number of small branches—the pedicles or end feet. The pedicles are actually attached to the basal lamina (Fig. 62.7). It is seen with electron micrograph that the cell body along with the nucleus of the podocyte remains about 1.0 or 2.0 µm away from a single, continuous basal lamina. The space between the basement membrane and the podocyte is known as the subpodocytic space. The adjacent foot processes are not in intimate contact with one another but a narrow gap or slit of 25 nm wide is present in between them and is known as the filtration slit or slit pore. In high resolution of electron micrograph, a thin dark line of about 6 nm in thickness (the slit membrane) is observed to bridge the slit pore. The nucleus of podocyte has got infolding and is of a complex form.

The cells of the endothelial lining of glomerular capillaries are flat and fenestrated having circular pores of 50 to 100 nm in diameter (Fig. 62.8). These are different from that of the capillaries of any other place, because the fenestrae (pores) do not have the thin diaphragm and are larger in size and more in number.

The nucleus remains towards the lumen of the capillaries. The endothelium of the glomerulus also contains between endothelium and basal lamina another type of cells known as deep cells or mesangial (inter-capillary or stalk) cells (Fig. 62.6) branched stellate cells having similar character to pericytes of capillaries of any other place. The functions of these cells may become proliferative and phagocytic in certain pathological conditions. In adult kidneys, the endothelium of the glomerulus and the epithelium of the visceral layer are separated by the common single basement membrane of 0.1 to 0.5 µm in thickness. The basement membrane is made up of a felt work of very fine filaments embedded in a homogeneous matrix. This membrane is the only continuous barrier between the capillary lumen and capsular space.

The modern studies with horse reddish peroxidase (mol wt 40,000) and myeloperoxidase (mol wt 160,000) as protein tracer, it is evidenced that the smaller peroxidase (horse reddish) can pass easily through filtering membrane but the larger one (myeloperoxidase) can pass up to the basement membrane but held up at the epithelial slits. From this finding it is interpreted that the epithelial slit pores are actually responsible for the differential permeability of the renal corpuscle.

Renal Tubules (Fig. 62.2)

The renal tubule comprises the following structure:

1. *Proximal convoluted tubule:* It is lined by simple cuboidal epithelium comprising brush borders.

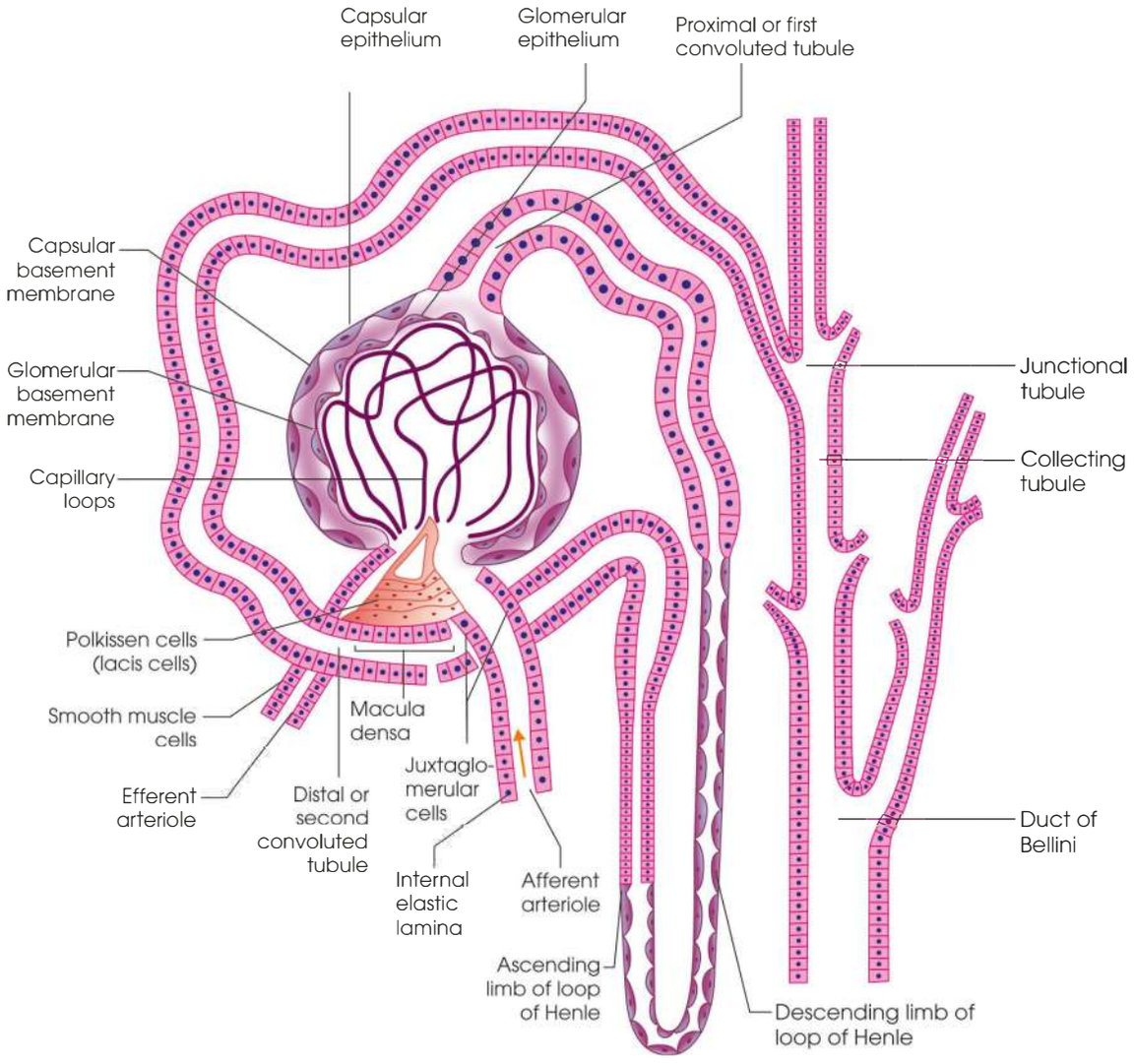


Fig. 62.4: Diagrammatic representation of the juxtaglomerular apparatus showing the respective positions of the juxtaglomerular cells, Polkissen or 'Lacis' cells and macula densa of the same glomerulus and nephron

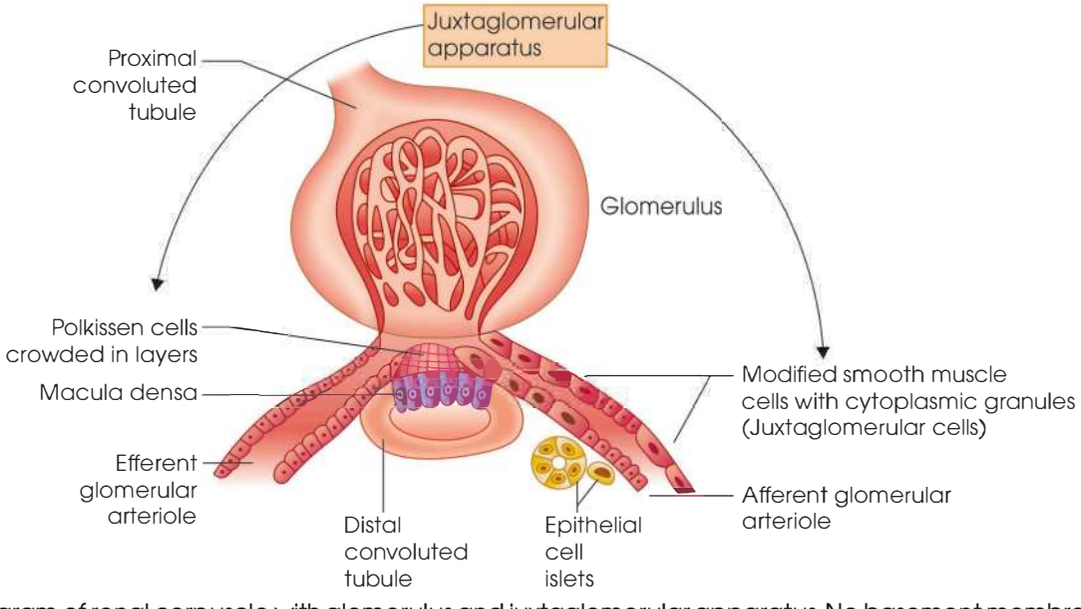


Fig. 62.5: Diagram of renal corpuscle with glomerulus and juxtaglomerular apparatus. No basement membrane separates the juxtaglomerular from the macula densa cells

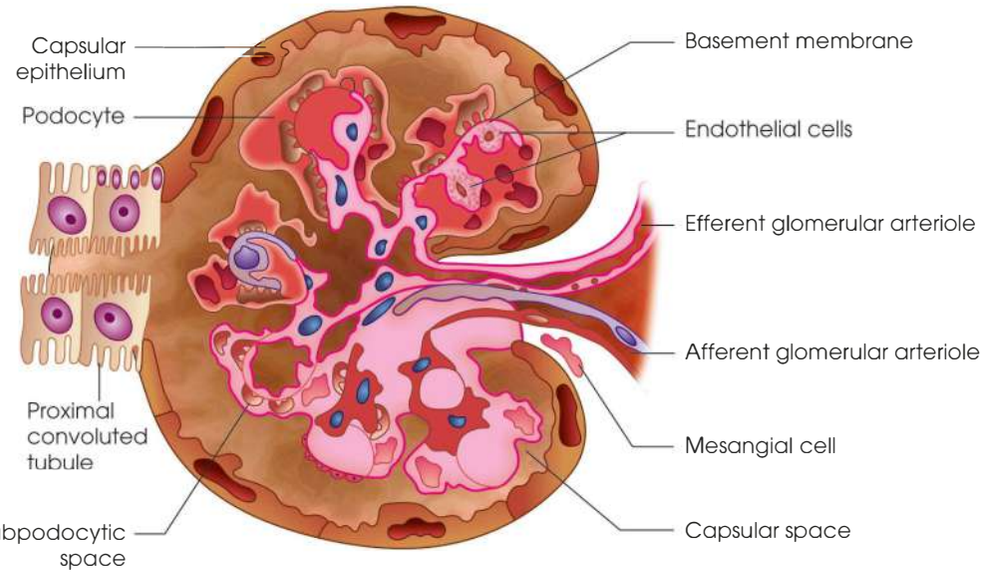


Fig. 62.6: Diagram of renal corpuscle. Note the presence of mesangial cells in the connective tissue skeleton of the tuft

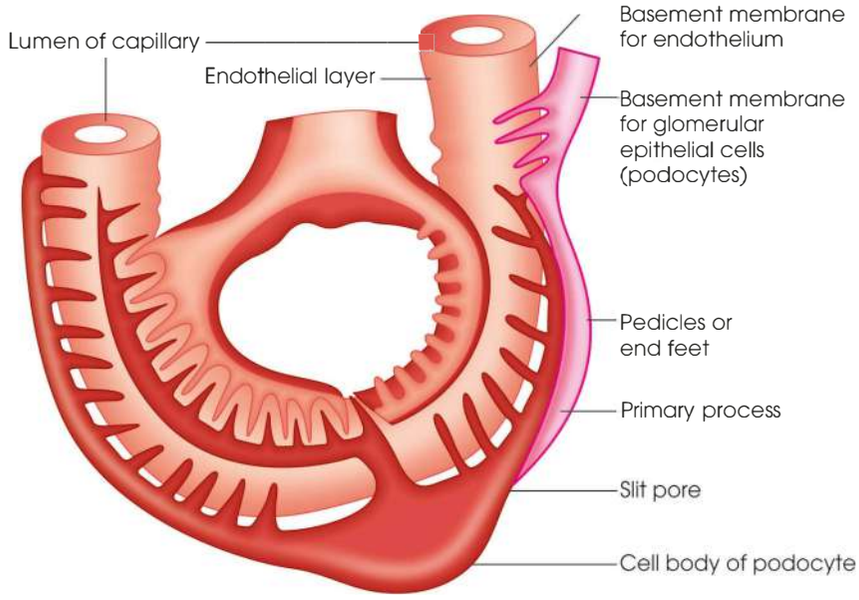


Fig. 62.7: Podocytes of glomerular epithelial cells lining the visceral layer of Bowman's capsule

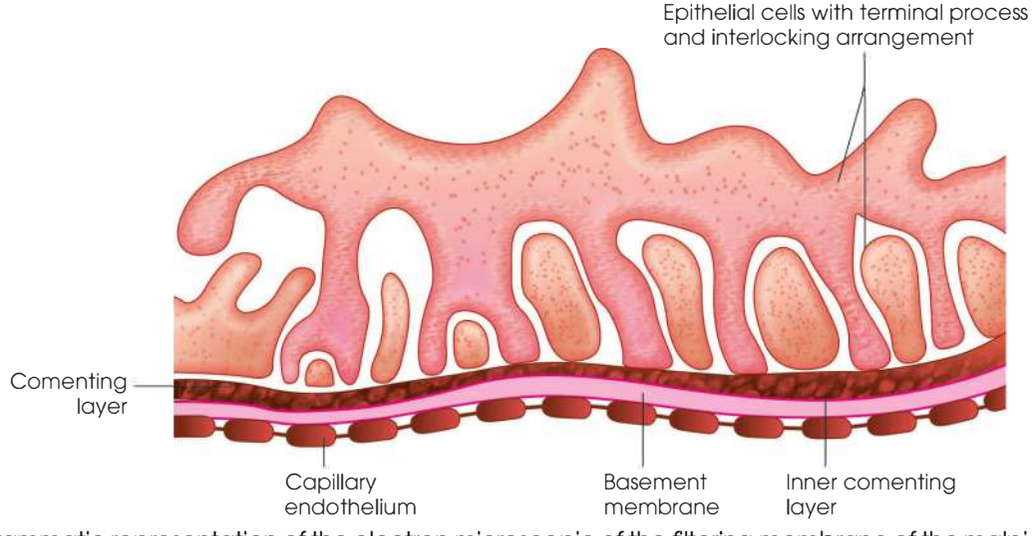


Fig. 62.8: Diagrammatic representation of the electron microscopic of the filtering membrane of the malpighian corpuscle

62

2. *Loop of Henle* (hair-pin like, i.e. U-shaped and lies in medulla)
   a. Descending limb of loop of Henle
   b. Ascending limb of loop of Henle: The ascending limb of loop of Henle comprises two segments: Thin ascending limb of loop of Henle and thick ascending limb of loop of Henle (the thick ascending limb of loop of Henle enters cortex and becomes distal convoluted tubule—DCT).
3. *Distal convoluted tubule:* The human renal tubule is about 3 cm long, 20–60 microns wide, and since there are about 2 millions of nephrons, the total length of the tubules is about 65 km or 40 miles. Just below the glomerulus, the tubule has a very short constricted portion—the neck. After the neck, the tubules consist of the following serial parts.

Proximal Convoluted Tubule (PCT or Pars Convoluta)

It is about 14 mm long, external diameter 60 µm and inside diameter 15–25 µm and extremely tortuous. It is lined by a single layer of cubical cells, the free borders (at the lumen) of which are striated or brush bordered. Electron microscopy has revealed that the brush border in composed of processes or microvilli (prolongations of the cell cytoplasm) which enormously increase the surface area of the cells for absorption in the lumen of the proximal tubule.

There are numerous tubular invaginations arising at the clefts between the microvilli, extending downwards into the apical cytoplasm and are known as apical canaliculi. In the apical region, clear vacuoles of various sizes are also noted, which along with the apical canaliculi are involved in the cellular mechanism of absorption of protein from the filtrate. Protein enzymes are also demonstrated at the brush border (microvilli) and the canaliculi. Inside the cytoplasm there are rod-like structures of mitochondria at the basal half of the cell. The mitochondria are oriented perpendicular to the cell membrane. There are also a number of lateral processes at the base of the cells and are extended under the neighbouring cells to occupy deep recesses in their base (Fig. 62.9). The cells of proximal convoluted tubule are very active metabolically and contain a large number of enzymes. These cells reabsorb about two-thirds of the water of the glomerular filtrate and all the glucose and part of sodium chloride and phosphate.

Henle's Loop (Pars Recta)

It is a U-shaped loop, dipping for a variable length into the medulla. Anatomically the loop of Henle can be divided into (i) descending limb (ii) thin-walled ascending limb, and (iii) thick-walled ascending limb. The proximal four-fifths of the descending limb have the same diameter (proximal straight tubule) and epithelium as above but the cells have very few and

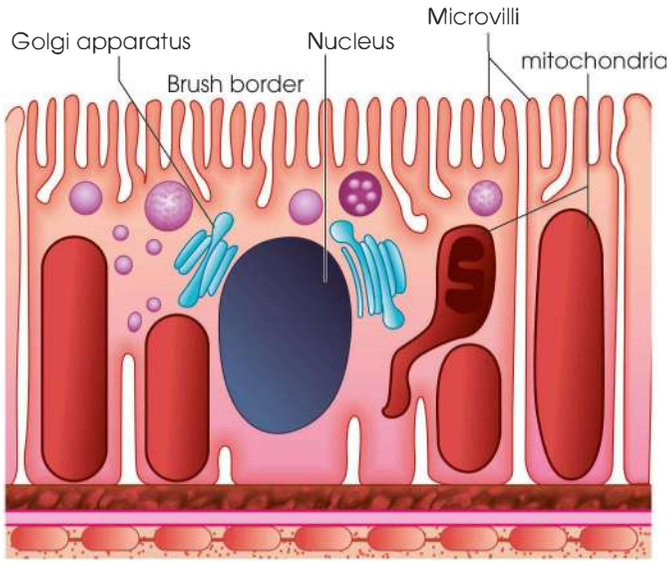


Fig. 62.9: Diagrammatic representation of the electron microscopic structure of a cell lining the proximal or first convoluted tubule. Rod-shaped mitochondria lying across the cell

small mircovilli and have got no striated appearance (Fig. 62.10). The distal one-fifth of this limb (in superficial nephrons) and also a variable part of the ascending limb (only in juxtamedullary nephrons), are lined by flat epithelial cells. The cells of the distal portion of the ascending limb are of cuboidal shape with distinct vertical striation due to presence of mitochondria (Fig. 62.11), but they are not brush bordered, i.e. no microvilli. In the superficial nephrons the loop is very short and dips only slightly into the medulla, whereas in juxtamedullary nephrons the loop is of considerable length and passes deep into the medulla. The average length of the loop is about 20 mm.

In the thin-walled descending limb being devoid of obvious intracellular structures isosmotic fluid delivered from the PCT becomes increasingly hypertonic as it progresses from the renal cortex towards the renal papilla until it reaches the maximum

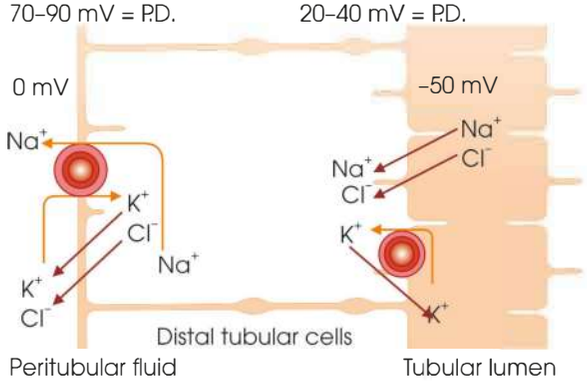


Fig. 62.10: Diagrammatic representation of electron microscopic structure of a cell living the descending limb of loop of Henle

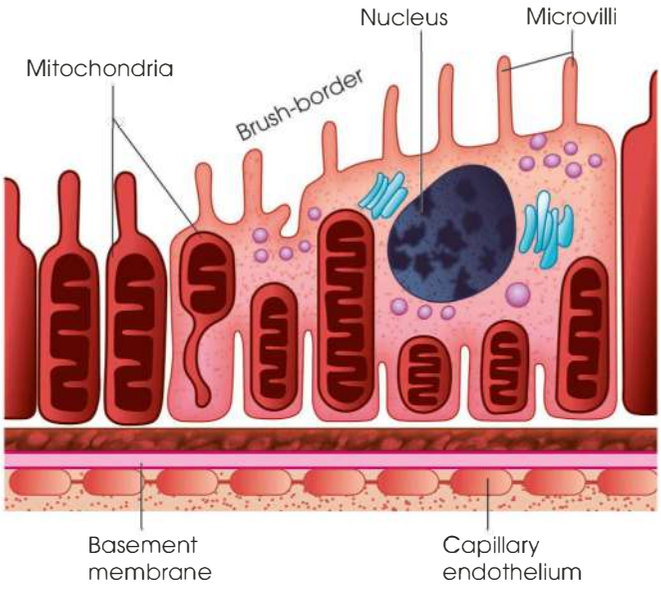


Fig. 62.11: Diagram shows the electron microscopic structure of a cell lining the ascending limb of Henle's loop

concentration and the membrane of this portion is more permeable to H_2O than solute urea, NaCl and in order to effect equilibrium, H_2O is removed. In the thin segment of the ascending limb the tubular fluid becomes progressively less concentrated due to its backflow towards the renal cortex as a consequence of loss of NaCl from the renal tubule. The thin ascending limb is impermeable to water and ions, except sodium and chloride which moves across the tubules in this part by diffusion. The thin ascending limb lies in the medulla of the kidney. The thick ascending limb occupied the medulla as well as cortex. The ascending limb is much thicker than the descending limb.

Thick ascending limb: The thick ascending limb in medulla portion remains impermeable to water. The transport of sodium, potassium and chloride from the tubule to the interstitium in the thick ascending limb takes place with the aid of sodium/potassium/2-chloride co-transporter. The descending limb is permeable to water but not solutes while the thick ascending loop is not permeable to water but solutes are pumped out. The thick ascending limb is the site of action of loop diuretics. The loop diuretics furosemide blocks the $K^+/Na^+/2Cl^-$ co-transporter.

Applied Physiology

Bartter's syndrome: Mutation of gene for Barttin's an integral protein, which is required for chloride channel functioning leads to defective transport of solute in thick ascending limb of loop of Henle.

Fanconi's syndrome: It occurs due to mutation of a carrier or transport proteins. It is inherited as an autosomal or X-linked disease. This leads to defective reabsorption of glucose, phosphate, amino acid, protein, potassium and bicarbonate in proximal convoluted tubules. The patient may present with glycosuria, hypokalemia, hypouricaemia, hypophosphataemia, aminoaciduria and proximal renal tubular acidosis.

Distal Convoluted Tubule (DCT)

This portion of the nephron is situated in the cortex having an average length of 4.6 to 5.2 mm and diameter 20–50 µm and lined by cubical epithelium which is rodded but without any true brush border. There is extensive infolding of the cell membrane. It has a much smaller number of microvilli (Fig. 62.12). Electron micrograph reveals that the basal invagination is more extensive than that of the proximal tubules. The diameter of the lumen of distal convoluted tubule is greater than that of proximal one and the tubules contain a larger number of cells.

The proximal part of the distal tubule actually comes in contact with the juxtaglomerular cells of the corresponding afferent vessel. The cells of this region have got closely packed nuclei and form the macula densa (Fig. 62.4). Macula densa cells, nearby juxtaglomerular cells and 'lacis' cells form juxtaglomerular apparatus.

The reabsorptive activity of the distal convoluted tubule and collecting duct is under the influence of ADH, vasopressin, which is one determinant of the volume of urinary output. Distal tubular cells are able to reabsorb small quantities of water and electrolytes due to a small number of microvilli in addition to determining urinary acid–base balance (Fig. 62.13).

Collecting Tubule

The renal collecting duct system comprises series of tubules and ducts that connect the nephrons to the

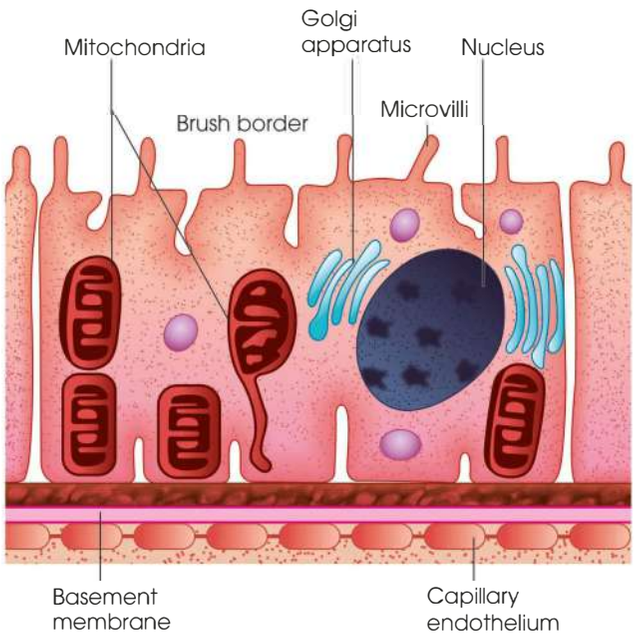


Fig. 62.12: Diagram shows the electron microscopic structure of a cell lining the distal convoluted tubule

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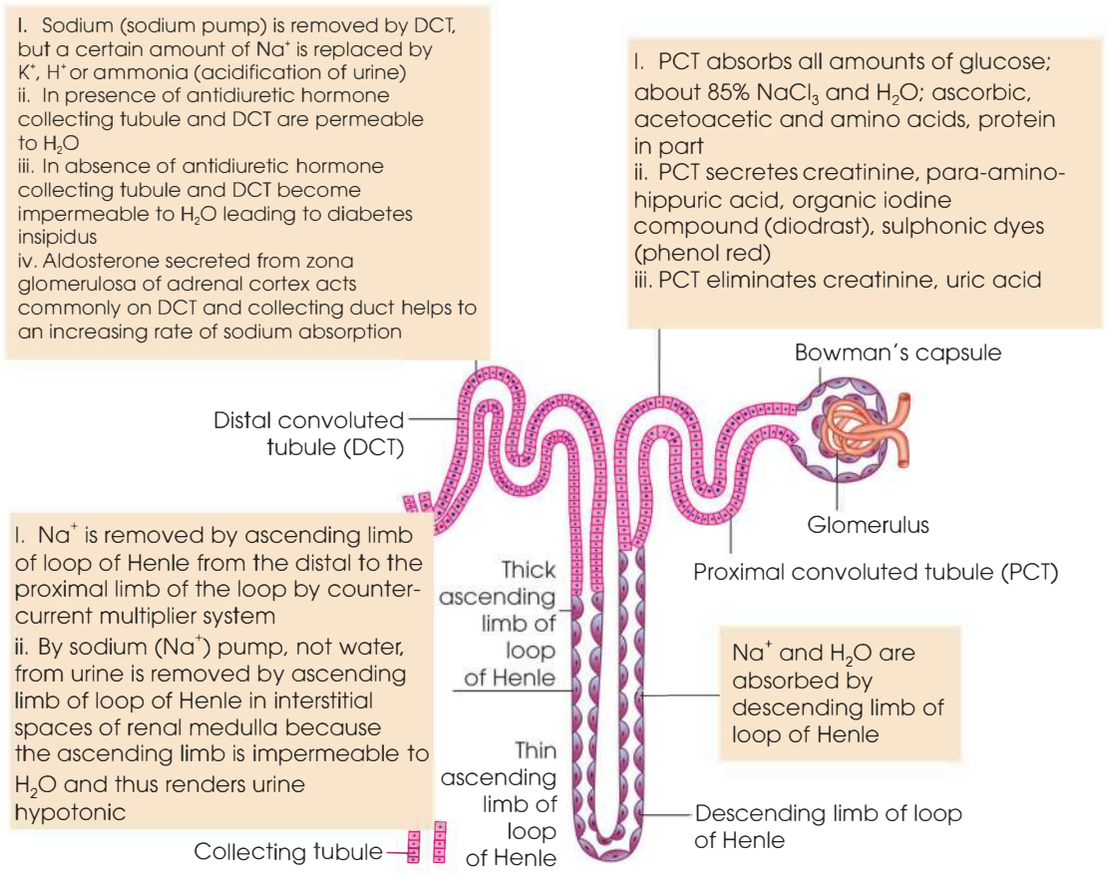


Fig. 62.13: Simplified diagram represents functions of different parts of the nephron

ureter. The collecting duct system includes the connecting tubules, cortical collecting ducts, and medullary collecting ducts. The connecting tubules from numerous neighbouring nephrons join to form cortical collecting tubules, and these unite to form cortical collecting ducts. The medullary collecting ducts divide into outer and inner segments. The inner segment reaches more interior into the medulla and is the part of the collecting duct system between the outer segment and the papillary ducts. The terminal portions of the medullary collecting ducts are the papillary ducts. The papillary duct ends at the renal papilla and further enjoins the minor calyx. The collecting duct accounts for 4–5% of the kidney's reabsorption of sodium and nearly 5% of the kidney's reabsorption of water. The outer medullary and cortical collecting ducts are impermeable to water without the presence of antidiuretic hormone.

JUXTAGLOMERULAR APPARATUS

Goormaghtigh suggested the term juxtaglomerular apparatus (Figs 62.4, 62.5 and 62.14) which included the (i) granular juxtaglomerular cells in the afferent arteriole, (ii) macula densa of the distal convoluted tubule, and (iii) agranular Polkissen or 'lacis' cells situated in the angle created by the entrance and exit of the afferent and efferent arterioles of each glomerulus. But some have preferred to use the term juxtaglomerular complex, instead of juxtaglomerular apparatus, only because of unequivocal functional relationship existing between the juxtaglomerular cells and macula densa, and the fundamental structural differences between them. The smooth muscle cells of the afferent and efferent arterioles and also the cells of the 'lacis' are continuous with the mesangium. Juxtaglomerular cells are granular epithelioid cells situated in the media of the pre-glomerular portion of the afferent and occasionally of the efferent arterioles and also in the adjacent connective tissue.

Functional Key Features of JGA

- The components of the juxtaglomerular apparatus have been related in some way to the control of blood pressure, renal blood flow, salt balance and erythropoiesis. Renal hypertension is claimed to be the cause of secretion of renin from the juxtaglomerular apparatus thus helping in the formation of angiotensin II (Fig. 62.15).
- Granulation in the juxtaglomerular cells is related with the presence of secretable renin. Also it is observed that fluorescent antirenin antibodies localise in the juxtaglomerular cells. These two are

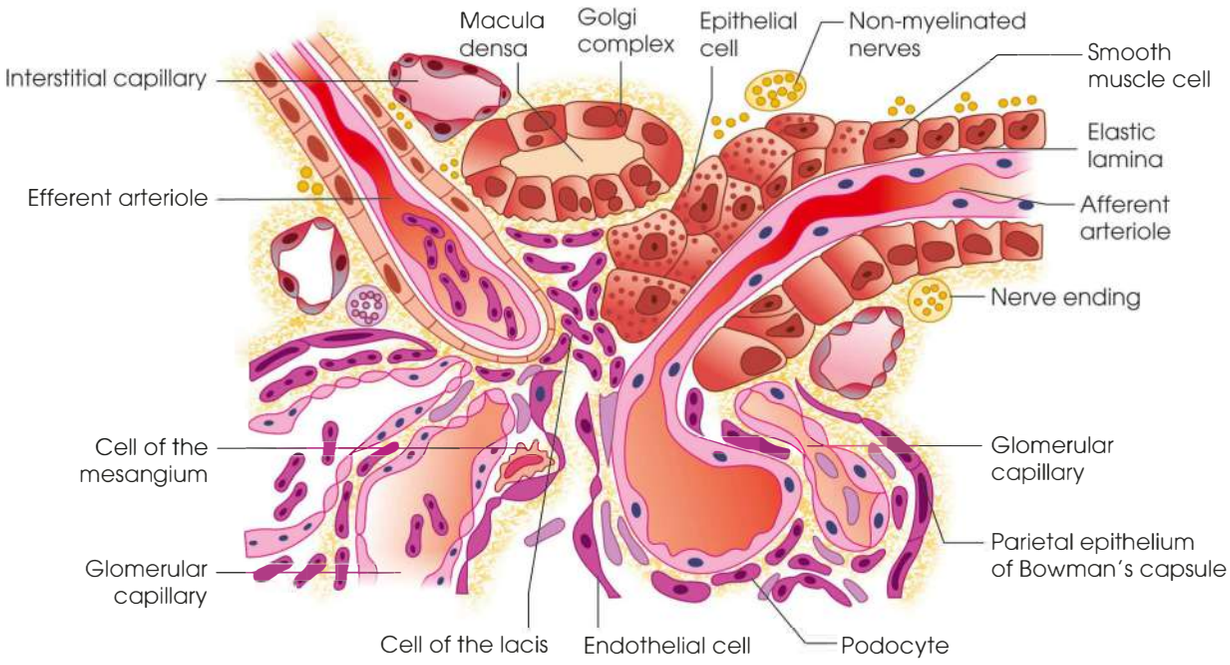


Fig. 62.14: Diagrammatic representation of the juxtaglomerular apparatus

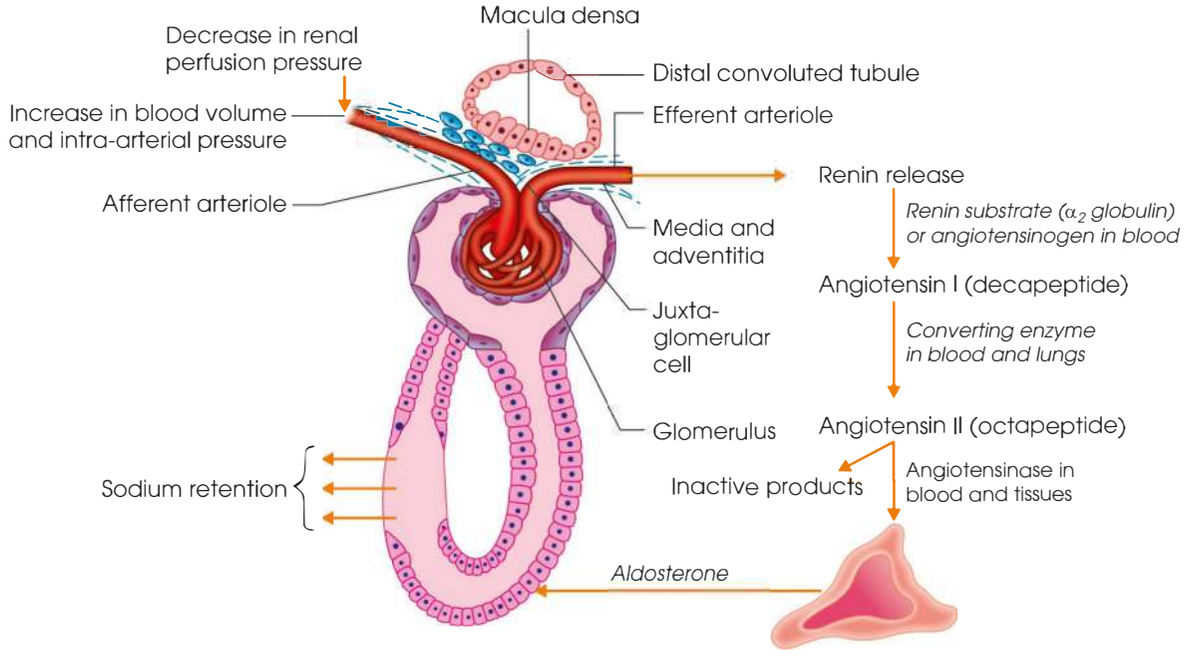


Fig. 62.15: Regulation of aldosterone secretion (renin-angiotensin system)

evidences to suggest that the juxtaglomerular cells are the source of renin. Renin secretion is determined by the degree of stretch of the afferent glomerulus and also by the Na concentration of the macula densa cells.

- The REF or erythrogenin is known to be the secretion of juxtaglomerular cells. Its production is increased by hypoxia, cobalt salts and androgens. Cobalt salt stimulates the production of erythropoietin by causing degranulation of the epithelioid cells. The erythropoietin which is known as a hormone and having molecular weight of about 25,000–40,000 causes the certain stem cells (erythropoietin-sensitive stem cells) in the bone marrow to be converted to proerythroblast. It is claimed that the action of erythropoietin is apparently mediated through the stimulation of mRNA synthesis. The blood level of erythropoietin thus plays an important part in the control of erythropoiesis (Fig. 62.16).

Macula densa is a modified epithelial cell in the portion of the distal convoluted tubule lying in contact with the afferent glomerular vessels of the same

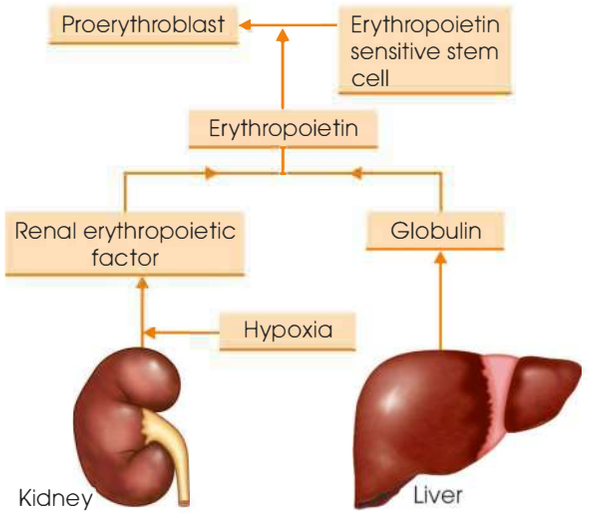


Fig. 62.16: Schematic representation of the role of kidney in erythropoiesis

nephron. Macula densa cell does not possess the basement membrane (Fig. 62.5) and possess Golgi apparatus which lies between the nucleus of the epithelial cell and outer border of the cell. Towards the glomerular side of the tubule, the cells are taller and thinner (Fig. 62.4). 'Lacis cell' is also known as polkissen (polar cushion) cell, Goormaghtigh's cell, pseudo-meissnerian cell. It is a cell mass formed mainly by the agranular or occasional granular cells. These cells lie in close contact with the macula densa cells and also within the vascular pole formed by the afferent and efferent glomerular vessels.

RENAL CIRCULATION

Peculiarities

1. The renal arteries arise nearly at right angle from the sides of the abdominal aorta. Upon or just before entering the hilus of the kidney the renal artery (70%) on each side divides into an anterior and a posterior division. The primary branches of the division supply the five renal vascular segments (anterior, apical, upper middle, lower middle, lower and posterior). These primary branches are called segmental arteries. Usually these segmental arteries are end arteries. Each segmental artery divides into lobar branches, one for each renal pyramid (Fig. 62.17). Those branches which pass between renal pyramids are known as interlobar (straight) arteries (Fig. 62.18). Each interlobar artery gives off branches which run across the bases of the pyramids and are called arcuate (arciform) arteries. There is no communication between these arcuate arteries (Fig. 62.19).

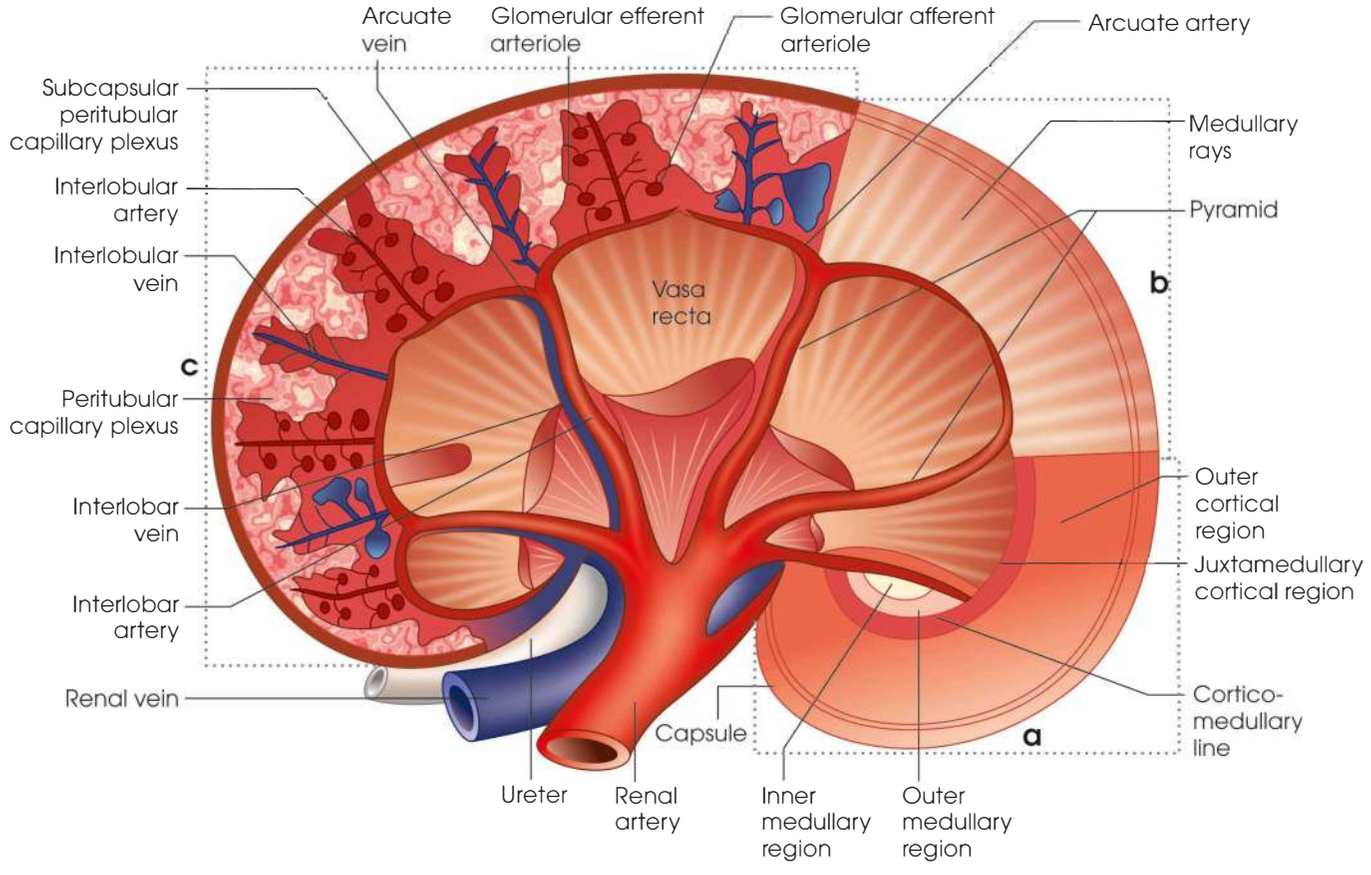


Fig. 62.17: Composite diagram showing the kidney circulatory pattern (c), medullary rays and pyramid (b), and different zones of the kidney (a)

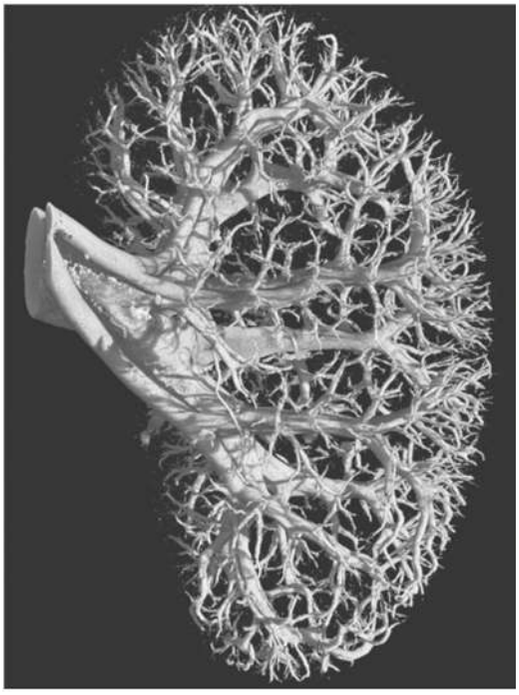


Fig. 62.18: Corrosion specimen of kidney showing branching arrangement of the renal artery

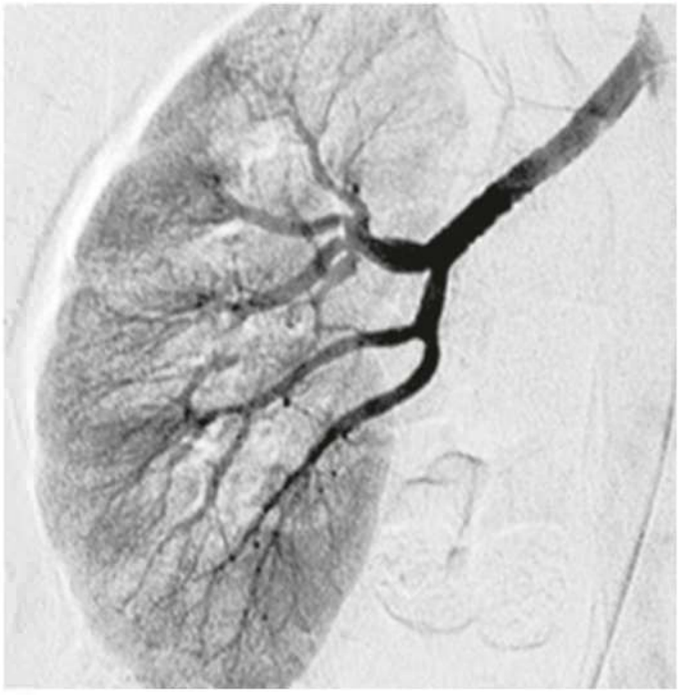


Fig. 62.19: Arteriogram on coronal section of kidney showing absence of arcuate arteries

These arteries again give off small branches called small interlobular arteries which run radially through the cortex towards the renal surface.

2. In the case of superficial cortical glomeruli, the interlobular artery breaks up into a number of arterioles, most of which one forms an afferent vessel to the glomerulus.
3. Just before entering the glomerulus, the afferent arterioles are found to contain numerous juxtaglomerular cells. Each afferent arteriole divides into about 50 capillaries which stay close together to form the glomerulus which lies within the cortex of the kidney. This capillary tuft reunites and forms the efferent arteriole. This vessel again breaks up into a second set of capillary network which twines round the different parts of the corresponding renal tubule (peritubular network) (Fig. 62.17).
4. The afferent vessel is short and wide, while the efferent vessel is thin and long. This arrangement makes the blood pressure in the glomeruli relatively higher-making it more suitable for filtration.
5. Moreover, the afferent vessel arises directly from the interlobular artery at an angle advantageous for the free flow of blood. Since superficial cortical glomeruli constitute 85% of the total number, the major part of renal circulation normally passes through them.

In the case of juxtamedullary glomeruli, circulatory peculiarities (Fig. 62.20) are as follows:

1. There is no disparity between afferent and efferent vessels; even the efferent arteriole may be larger.
2. The afferent vessel though large yet arises at an angle not suitable for free flow of the blood stream. Hence, normally they have a little work to do. Very little blood passes through them. But in time of stress a large quantity of blood may be shunted through them.
3. The efferent vessel breaks up into a number of straight vessels—the vasa recta, which for the most parts do not join the peritubular network but descends closely applied to the descending and ascending loops of Henle and to the collecting ducts and drain almost directly into the capillary venous plexus (Trueta shunt). These are known as descending vasa recta. Thus, the medulla gets blood supply mostly from the vasa recta originating from the efferent arterioles of the juxtamedullary glomeruli.
4. Blood from the capillary venous plexus in the medulla drain into the arcuate or interlobular veins via ascending vasa recta. In the outer medulla both ascending and descending vasa recta are grouped into vascular bundles within which they are closely appose to each other.
5. This close relationship of ascending and descending vasa recta with each other and with the surrounding duct system provides the structural basis for diffusion exchanges between outflowing and inflowing blood (counter-current exchange and multiplier system).
6. The venous return of the kidneys starts in a venous plexus in the cortex where all the capillaries drain. From this plexus blood passes

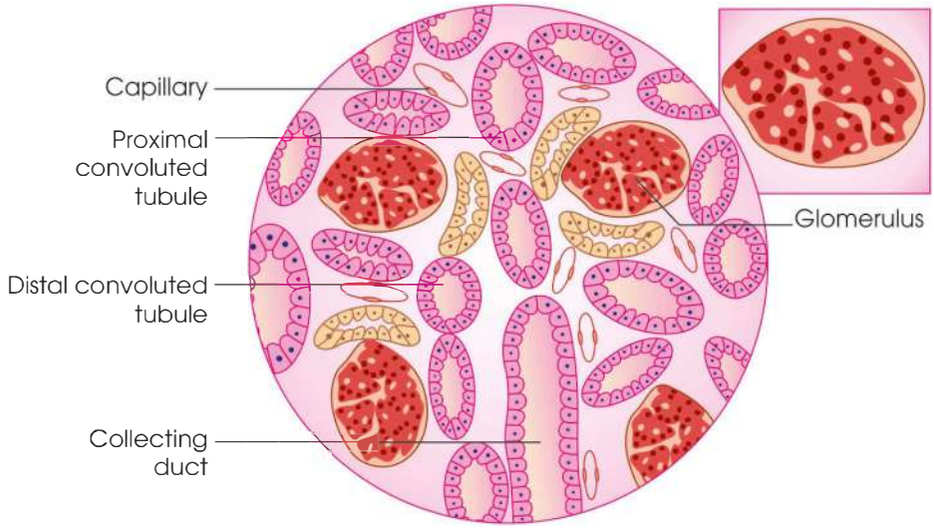


Fig. 62.20: Histological appearance of glomerulus

through the interlobular veins which accompany the corresponding arteries. The interlobular veins unite to form the large arcuate (arciform) veins. In the medulla straight radially arranged veins known as venulae rectae directly open into the arcuate veins. The arcuate veins fuse to form interlobar veins which ultimately join the renal vein. Some venous blood passes through the venae stellatae under the capsule and joins the perirenal veins.

PECULIARITIES OF RENAL CIRCULATION

From the above, the peculiarities of renal circulation (Fig. 62.21) may be summarised as follows:

1. Almost all blood that passes through the kidney has to pass through the glomerular tuft. Renal circulation is a portal system. The blood has to pass through double capillary network—at first through the glomerular capillaries and then through the peritubular capillaries. So, almost all blood that reaches the tubule has to cross the glomerular tuft. These two capillary systems serve two different functions: The glomerular tuft filters and the tubular tuft reabsorbs.
2. Renal blood pressure is comparatively high. This is due to two reasons:
   - The renal artery is short and wide, and arises directly from the aorta. It divides into a small number of wide branches. Owing to this arrangement, blood enters the kidneys at a comparatively high pressure.
   - The afferent glomerular vessel is wider and shorter than the efferent vessel. Due to this, glomerular pressure remains fairly high and is about three to four times more than the capillary pressure elsewhere. It is about 75 mm Hg (same as the diastolic pressure of the subject). This high glomerular pressure is very suitable for filtration.
3. Rate of blood supply to the kidney is comparatively high. About 1,200–1,300 ml of blood (about 25–30% of cardiac output) pass through the two kidneys per minute in a man weighing about 70 kg at rest and the plasma flow is 600 ml per minute as is determined by para-aminohippuric clearance test. The total quantity of blood in the human body may circulate through the kidneys in four to five minutes.
4. There are two circulations in the kidney—the greater and the lesser. The greater circulation carries 85% of blood and first passes through the superficial cortical glomeruli, then through the peritubular network and finally joins the renal vein. The lesser circulation carries only 15% of blood normally and passes through the juxtamedullary glomeruli. The efferent glomerular vessels, after a short straight course, vasa recta, join (mostly) the renal vein directly and join partly the peritubular network. Under abnormal conditions it may act as a shunt—Trueta shunt.

In man and animal under basal condition, renal blood flow is not altered in denervated or innervated kidney. This shows that there is no neurogenic vascular tone in kidney under basal state.

Lymphatic supply: An abundant lymphatic supply of the kidney drains via the thoracic duct into the venous circulation in the thorax.

Nerve supply: The kidneys are supplied mainly by the sympathetic but there are some para-sympathetic fibres also. The sympathetic come mainly from the 10th to 12th thoracic segments and the para-sympathetic from the vagus. The sympathetic carries vasoconstrictor and afferent fibres. The autonomic fibres also supply the tubular cells and reabsorption of sodium is probably influenced by these nerves.

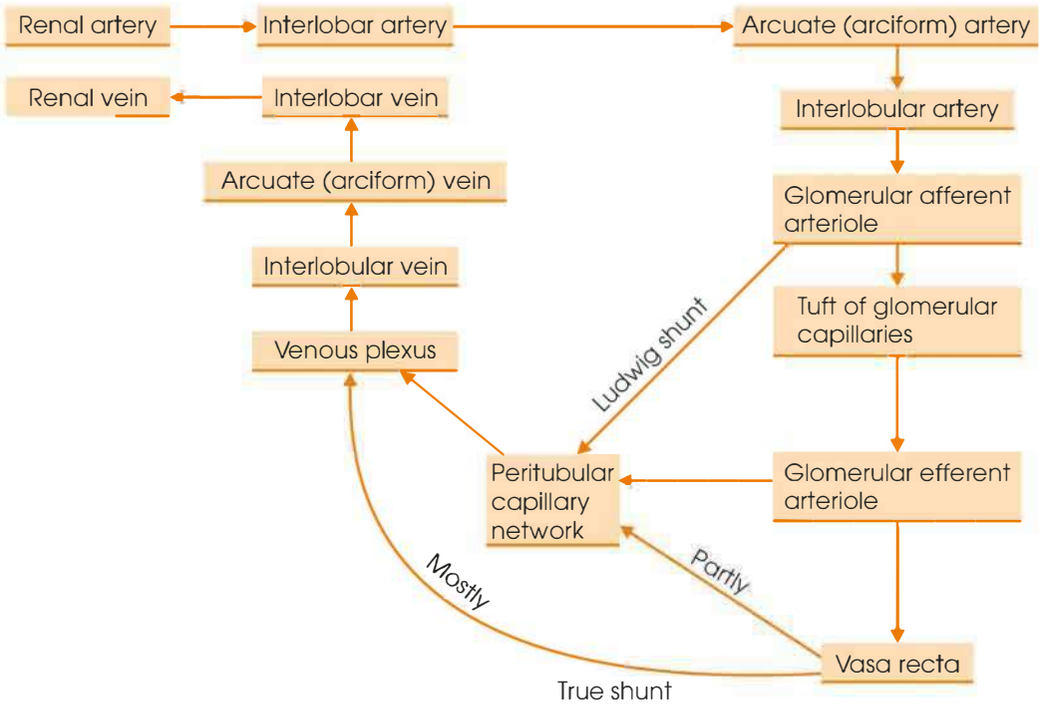


Fig. 62.21: Schematic representation of the anatomical organisation of the renal circulation

EXAM-ORIENTED QUESTIONS

Essay

1. Describe the functional anatomy of excretory system.

Short Notes

1. Functions of kidney
2. Malpighian corpuscles
3. Peculiarities of renal circulation
4. Distal convoluted tubules
5. Loop of Henle
6. Collecting duct
7. Juxtaglomerular apparatus
8. Functional key features of JGA
9. Renal circulation

Chapter

63

Renal Blood Flow

INTRODUCTION

Normal renal blood flow: The blood flow is much greater in the renal cortex then in the renal medulla. In a resting state and with a normal arterial blood pressure about 1,200–1,300 ml of blood or 25–30% of total cardiac output passes through both human kidneys per minute. Blood flow per gram of kidney tissue has been observed to be 4 ml per minute, but the distribution pattern of blood to the different parts of the kidneys is not similar and varies from area to area. In the kidneys there are two capillary beds united in series. These are the glomerular capillaries and the peritubular capillaries. These capillary beds are separated by vasa efferentia. The vasa afferentia (pre-glomerular) and vasa efferentia (post-glomerular) behave functionally and anatomically like arterioles. Main pressure drops in the kidneys are observed in these vascular beds. Pressure gradients in the renal vascular beds have been observed in Fig. 63.1.

Blood flow rate in the cortex is higher than that in the medulla. Blood flow values obtained in the unanaesthetised dog is 4.5–5 ml/gm/minute in the cortex, 1.25–1.50 ml/gm/minute in the outer medulla and 0.15–0.2 ml/gm/minute in the inner medulla.

This lower blood flow rate in the medulla is possibly due to (i) unusual length of the vasa recta and (ii) increase of blood viscosity in the medulla caused by the transmembranous shunting of water from the descending limb to the ascending limb of the vasa recta.

Autoregulation of Renal Blood Flow

Within the physiological limit kidney has the peculiarity of maintaining the normal blood flow under any condition. This self-controlling mechanism of blood flow is known as autoregulation. Mostly all the vascular beds follow the haemodynamic principle with the exception of renal bed, which behaves indifferently. In the renal bed if the pressure is increased then the flow is also increased in response to pressure up to 90 mm Hg and further increase of pressure up to 250 mm Hg will have a little effect in renal plasma flow (Fig. 63.2). But if the pressure is increased further above 250 mm Hg, then regulation may fail.

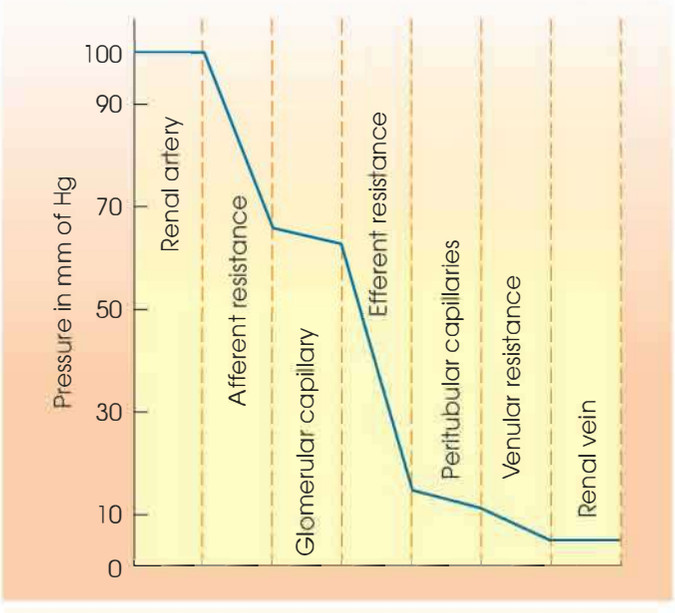


Fig. 63.1: Graphical representation of pressure

Glomerular filtration rate (GFR) varies less than the renal plasma flow (RPF). In an average sized man the GFR is about 125 ml per minute. But fall of systemic blood pressure below 90 mm Hg drops the GFR very sharply (Fig. 63.3). This autoregulation is only present in the cortex. Extrinsic reflex mechanism is non-existent and it is observed in isolated as well as in denervated kidneys.

Mechanism of autoregulation: The main mechanisms involved in autoregulation of renal plasma flow are:

1. *Myogenic theory:* According to this theory, the smooth muscles which are present in the pre-glomerular blood vessels are capable of reacting to distension by shortening during elevation of perfusion pressure. Thus, the pre-glomerular vascular tone is increased due to this myogenic response to increased arterial

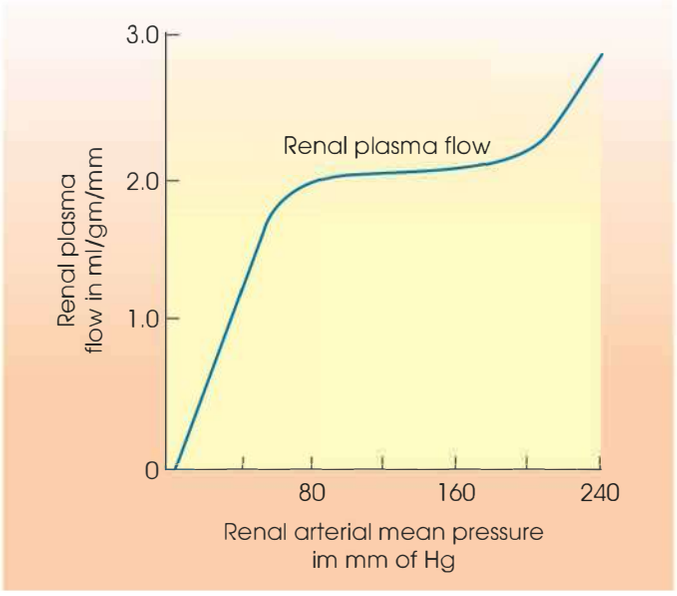


Fig. 63.2: Graphical tracing shows the autoregulation of RPF

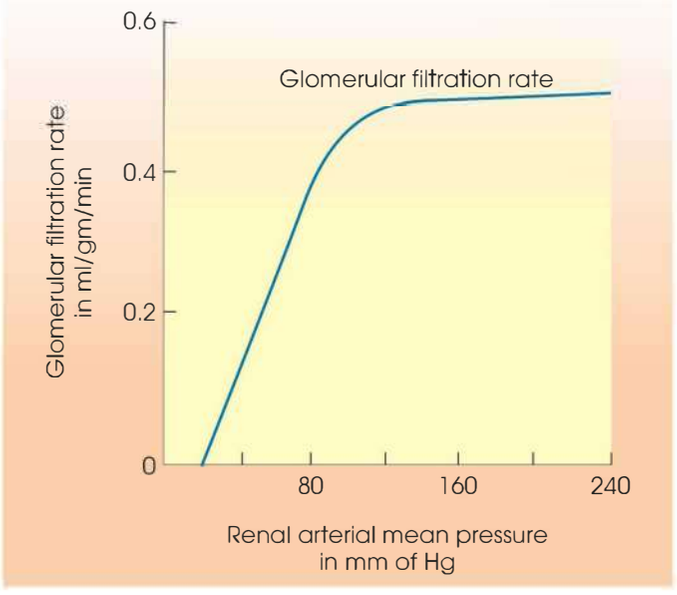


Fig. 63.3: Graphical tracing shows the autoregulation of GFR

blood pressure. This reactivity is abolished after paralysing the smooth muscles by potassium cyanide, papaverine and by high concentration of procaine.

2. *Glomerular tubular feedback mechanism:* Intrarenal Na feedback theory: In this theory, importance has been given to the macula densa cells in the regulation of renin secretion from the glomerular afferent arterioles. If it is supposed that there is no autoregulation in the kidney, then with the increase of blood pressure, renal blood flow as well as the glomerular filtration rate (GFR), will be increased. Due to increased glomerular filtration rate, distal convoluted tubule will have greater Na load which stimulates the liberation of renin from the juxtaglomerular cells. The renal blood flow as well as the glomerular filtration rate will be decreased due to renin-induced cortical pre-glomerular vasoconstriction. So, the Na load in the distal convoluted tubule at the juxtaglomerular region may be the determinant of renal autoregulation. The intrarenal Na feedback theory can be described schematically in Fig. 63.4.

Nervous Control

Though the kidney has got both sympathetic (via splanchnic nerves originating from T10 and L2 intermediolateral grey segment of spinal cord) and para-sympathetic nerve supply (via vagus). There is tonic sympathetic discharge in renal nerves in resting condition and is mediated by cerebral cortex and the vasomotor centre. The sympathetic nerve supply is to afferent and efferent arteriole, DCT, PCT, thick ascending limb of loop of Henle and its fibres ends around JG cells and renal tubular cells.

Other Factors

Other factors which may modify the renal circulatory haemodynamics are as follows.

1. Exercise, Posture and Central Blood Volume

Exercise: Renal blood flow is decreased during exercise. The increase sympathetic discharges via angiotensin II and NE reduce renal blood flow.

Posture: It has been observed that during upright posture central blood volume is reduced and this via baroreceptor reflex reflexly produce vasoconstriction in the renal vascular bed.

On the contrary, if the central blood volume is increased by negative pressure breathing, instead of renal vasodilatation, diuresis occurs. This diuretic effect has been claimed to be the reflex inhibition of secretion of antidiuretic hormone (ADH) through the stimulation of stretch receptors situated at the left atrial wall by increased central blood volume.

2. Hypoxia, Hypercapnia and Acidosis

With mild hypoxia, there may be a slight renal vasodilatation due to reactive hyperaemia but if the hypoxia is severe and prolonged then there may be profound vasoconstriction. This vasoconstrictor response during severe hypoxia has been claimed to be reflex chemoreceptor effect from the carotid body and aortic body because denervation either of the chemoreceptor or of the kidney fails to alter the renal haemodynamics.

Hypercapnia and acidosis also reduce renal blood flow. This effect has been described to be centrally mediated neurogenic action rather than local action, because this response is abolished following denervation of the kidney.

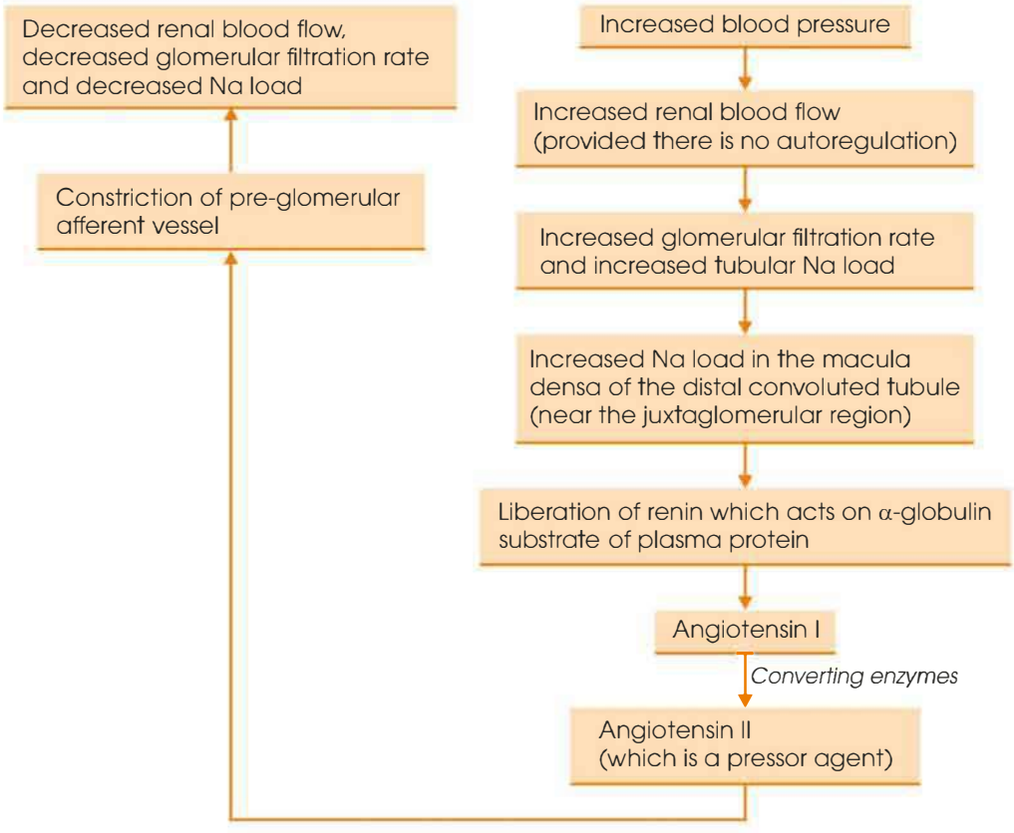


Fig. 63.4: Schematic representation showing the importance of macula densa in the autoregulation of renal blood flow through the Na feedback theory

3. Hormones

- Catecholamines constrict renal blood vessels.
- Adrenaline (epinephrine) and noradrenaline (norepinephrine) in small doses have a less effect on the afferent than the efferent arterioles. They have got constrictor effect on the renal blood vessels either given intravenously or directly into the renal artery.
- Pitressin in a physiological dose with anti-diuretic effect has got no effect on renal blood flow. But in higher doses it may produce vasoconstriction.
- Serotonin (5-hydroxytryptamine) has got peculiar effect on renal circulation. If it is infused directly into the renal artery in a dosage of 10–100 µg per minute then it increases vascular resistance through vasoconstriction. This effect is observed in both denervated and innervated kidneys. On the other hand, if the serotonin is administered intravenously then it produces renal hyperaemia with a striking antidiuretic effect. This renal hyperaemia is reflex in origin.

4. Other Pharmacological Agents

- Ephedrine, phenylpropanolamine, hydroxyamphetamine when injected intravenously have got no constrictor effect but if administered directly into the renal artery, then they produce vasoconstriction.
- Dopamine, nitric oxide and bradykinin increase renal blood flow as these increase capillary pressure in glomerulus.
- Xanthine group of drugs like caffeine and theophylline have got effect on renal circulation. It increases the glomerular filtration rate.

5. Protein Diet High

Protein diet increases renal blood flow. Bacterial pyrogen increases renal blood flow.

6. Anaesthetic Agents

It has been pointed out by many that anaesthetic agents have got effects on renal plasma flow, glomerular filtration rate, etc. During light anaesthesia, there may be a redistribution of blood from splanchnic beds to the skin and muscles of the extremities. So, under such state the kidney is also affected. But during deep and sustained anaesthesia, renal blood flow is decreased all throughout.

Hormones influencing Renal Functions

1. Renin is a proteolytic enzyme and interacts with a normal plasma α_2-globulin (angiotensinogen or

hypertensinogen) and forms angiotensin I. Angiotensin I (decapeptide) is an inactive compound. Plasma enzyme (converting enzyme) interacts with angiotensin I and forms angiotensin II (octapeptide). Angiotensin II (formerly known as hypertensin or angiotensin) constricts blood vessels and raises systemic blood pressure. It also stimulates the secretion of aldosterone from the adrenal cortex. It has been suggested that the secretion of aldosterone through angiotensin II is primarily dependent upon adrenocorticotrophic hormone (ACTH) level in blood. Angiotensinase or hypertensinase, present in many tissues, breaks down angiotensin II into inactive angiotensin.

2. REF or erythrogenin and erythropoietin: In hypoxia, renal erythropoietin factor (REF) is secreted from glomerular cells and it is related to the formation of erythropoietin. This erythropoietin is concerned with the regulation of normal erythropoiesis.
3. Prostaglandins: In the kidneys, primary prostaglandins cause an increase in renal blood flow and vasodilatation.
4. Kininogen: It is produced by the kidneys, has an anti-hypertensive effect and regulates blood pressure.

Measurement of Renal Blood Flow

It can be measured in animals (1) by venous shunt technique or (2) by Fick principle.

Venous Shunt Technique

A cannula is inserted in the left ovarian vein (which joins the left renal vein), the renal vein is occluded for a brief period, and venous blood from left kidney is shunted along the ovarian vein and is directly measured by a volume recorder. This method can be adopted for measuring the venous flow in any part of the body where two veins join to form a common vein.

Fick Principle

Let *P* and *V* be the concentrations in mg% of a particular constituent (C) in the plasma of renal arterial and renal venous blood respectively. Let *Um* be the output in mg of the same substance (C) in the urine per minute. So that 100 ml of arterial plasma enter kidney with *P*, leave with V and loss *P–V*.

P–V is, therefore, excreted from 100 ml of renal plasma flow.

Therefore, *Um* will be excreted from $\frac{100}{P-V} \times Um =$ ml of renal plasma flow per minute.

Thus, renal plasma flow per minute is known. From the haematocrit reading, the total blood flow can be calculated.

In man, renal vein puncture is not practicable. This principle, therefore, can only be used if the degree of 'extraction' is constant and is known. The term extraction indicates the ratio $(P–V)/P$ and represents that fraction of the arterial plasma concentration (of a constituent) which is excreted by the kidney. If the extraction is complete and none of the constituent (C) escapes in the renal vein, V becomes 0. Consequently, the formula for renal plasma flow becomes $(100/P) \times Um$. That is, only arterial blood and urine need to be analysed.

Para-aminohippurate (PAH) clearance is used to measure renal plasma flow. This reflects the renal functional status. The method employs technique to evaluate concentration of PAH in arterial blood (P_{PAH}) and concentration of PAH in urine sample (U_{PAH}). The urine flow (V) is also evaluated. The renal perfusion flow is then calculated by:

$$RPF = \frac{U_{PAH}}{P_{PAH}} \times V$$

The calculation estimates the effective renal plasma flow (eRPF). As the renal extraction ratio of PAH is nearly equal to 1, therefore eRPF almost equals RPF. The estimation of RPF by this method underestimates RPF by approximately 10%. This margin of error is acceptable taking into consideration the ease with which eRPF is measured.

With this method the human renal blood flow comes to about 900–1,300 ml (plasma 450–700 ml) per minute.

FUNCTIONS OF KIDNEY AND GLOMERULUS

Functions of Kidney

1. It excretes waste products, especially the nitrogenous and sulphur-containing end products of protein metabolism.
2. It helps to maintain the normal hydrogen–ion concentration of body fluids and electrolytes. It helps to maintain water balance of the body and thereby plasma volume.
3. It helps to maintain the optimum concentration of certain constituents of blood (by the process of selective reabsorption). It eliminates drugs and various toxic substances from the body.
4. It manufactures certain new substances—ammonia, hippuric acid and inorganic phosphates. Ammonia helps in preserving acid–base equilibrium.
5. It helps in maintaining the osmotic pressure in blood and tissues.
6. It helps in the regulation of blood pressure during hypoxia in condition of emergency through the liberation of renin from the juxtaglomerular apparatus.
7. It helps in the regulation of erythropoiesis through the formation of erythropoietin by REF secreted from the glomerular cells.
8. It plays an important role in vitamin D metabolism.

EXAM-ORIENTED QUESTIONS

Essay

1. Describe the mechanism of autoregulation of renal blood flow.
2. Describe the other factors which may modify the renal circulatory haemodynamics.
3. Describe the hormones influencing renal functions.

Short Notes

1. Normal renal blood flow
2. Measurement of renal blood flow
3. Functions of kidney
4. Function of glomerulus

Chapter

64

Glomerular Filtration Rate

INTRODUCTION

The protein-free plasma filters through the glomerular capillaries into Bowman's capsule. Approximately about 20% of the plasma that enters the glomerulus gets filtered. This process is known as glomerular filtration which is the first step in urine formation. The rate at which glomerular filtrate is formed is termed as glomerular filtration rate.

GFR is determined by the balance of hydrostatic and colloid osmotic forces acting across the capillary membrane and the capillary filtration coefficient (K_f). The glomerular capillaries have a high filtration rate than most other capillaries because of high glomerular hydrostatic pressure and a large K_f. In the average adult human the GFR is about 125 ml/min or 180 L/day. The fraction of the renal plasma flow that is filtered (the filtration fraction) averages about 0.2. Filtration fraction = GFR/renal plasma flow

$$GFR = K_f \times \text{Net filtration pressure}$$

Filtration pressure: It is the force that drives the fluid and its dissolved substances through the glomerular filter.

The net filtration pressure—NFP (or net hydrostatic pressure—NHP) is the difference between three pressures:

1. Glomerular (blood) hydrostatic pressure (GHP) or GBHP
2. Capsular hydrostatic pressure (CHP)
3. Blood colloid osmotic pressure (BCOP)

The relationship can be expressed by

$$NPF = GBHP - (CHP + BCOP)$$

Forces favouring filtration

- Glomerular hydrostatic pressure 60 mm Hg
- Bowman's capsule colloid osmotic pressure 0 mm Hg

Forces opposing filtration

- Bowman's capsule hydrostatic pressure 18 mm Hg
- Glomerular capillary colloid osmotic pressure 32 mm Hg

Thus, NPF = 60 mm Hg – (32 mm Hg – 18 mm Hg)
NFP = 10 mm Hg

Net filtration pressure/effective filtration pressure: The glomerular hydrostatic (capillary) pressure averages about 60 mm Hg. The colloidal osmotic pressure of plasma proteins averages only about 32 mm Hg. The hydrostatic pressure in Bowman's capsule (intracapsular hydrostatic pressure) is about 18 mm Hg (interstitial pressure, 10 mm Hg and intra-tubular fluid pressure, 10 mm Hg). Of these three factors, blood pressure is the motive force for filtration. The other two oppose the process of filtration. So, the net or effective filtration pressure is about 10 mm Hg and this is the force upon which filtration depends it has been proved by the experiment in which the pressure in the capsular space is artificially raised to the same height as the effective filtration pressure, when formation of filtrate is found to cease. It is also observed by the fact that a rise of glomerular pressure increases, whereas a fall of pressure diminishes the amount of capsular fluid formed. On the other hand, when the concentration of plasma proteins is lowered (such as, by intravenous saline injections), the colloidal osmotic pressure is reduced increasing the effective filtration pressure and thereby increasing the formation of capsular filtrate.

Calculation of GFR

$$GFR = K_f \times \text{Net filtration pressure}$$

The K_f is a measure of the product of the hydraulic conductivity and surface area of the glomerular capillaries averages around 12.5.

Hence GFR = 12.5 × 10 = 125 ml/min

Mechanism involved in regulation of GFR

1. *Myogenic response:* The stretching of afferent arteriole walls due to increased blood pressure leads to contraction of smooth muscles in afferent arteriole wall thereby decreases GFR by constricting the lumen. The decreased glomerular blood pressure dilates the afferent arteriole and

constrict the efferent arteriole of glomerular capillaries thereby increasing GFR.

2. *Tubuloglomerular feedback:* The kidneys govern a feedback mechanism by which macula densa cells sense changes in sodium chloride concentration and thereby control the renal arteriolar resistance. The decreased GFR slows the flow rate in the loop of Henle causing increased reabsorption of sodium and chloride ions and reducing the concentration of sodium chloride at the macula densa cells. This reduction in sodium chloride concentration initiates a signal response from the macula densa that has two effects: (1) It decreases resistance to blood flow in the afferent arterioles which increase the glomerular hydrostatic pressure and return GFR toward normal. (2) It increases renin release from the juxtaglomerular cells of the afferent and efferent arterioles. Renin via angiotensin II constricts the efferent arterioles, increasing glomerular hydrostatic pressure and helping to return GFR toward normal.
3. *Sympathetic nervous system* activation decreases GFR and the renal blood flow due to constriction of the renal arterioles.
4. *Hormones*
   - Norepinephrine, epinephrine, and endothelin constrict renal blood vessels and decrease GFR.
   - Angiotensin II preferentially constricts efferent arterioles. Constriction of both afferent and efferent arterioles decreases GFR.
   - Atrial natriuretic peptides: Stretching of the atrial muscle due to increased blood volume causes relaxation of the mesangial cells increasing filtration surface and thus increasing GFR.
   - Anti-diuretic hormone stimulates insertion of aquaporin-2 (water channels) in apical membrane or principal cells. Increases water reabsorption and increases blood volume to return GFR to normal.
   - Aldosterone increases reabsorption of Na^+ and water by principal cells of the DCT collecting duct and thus increases blood volume to return GFR to normal.

Presence of filtering pores in the filtering bed of the glomerulus: Filtering pores are actually present in the capillary endothelium and capsular epithelium. Their diameters can be measured by injecting substances with known molecular weight and size, and watching their appearance in the urine. If the injected substance be found in the urine, obviously it has passed through the filter bed. So, the size of the filtering pores is bigger than the size of the molecule. With such experiments, it is seen that serum albumin

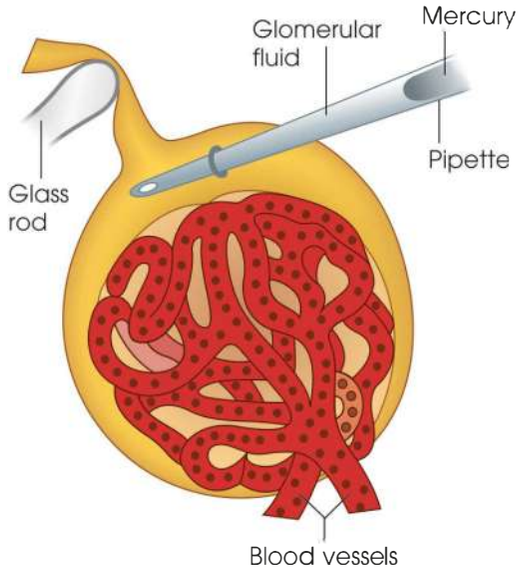


Fig. 64.1: Illustrating Richards method of obtaining a sample of the fluid from Bowman's capsule

(molecular weight 70,000, size 6–7 milli microns) cannot pass. Serum globulin (mol wt 170,000) and fibrinogen having still bigger molecule also fail to pass. But haemoglobin (mol wt 68,000) can freely pass out. Gelatin, Bence-Jones proteins, egg albumin (mol wt 35,000 and size about 4 milli microns), being smaller than haemoglobin, easily pass through.

This proves that the size of the filtering pores is such as will allow the molecules with less than 70,000 mol wt to pass out, but not the bigger ones. Serum albumin thus lies on the borderline.

- Normally a very small amount of serum protein (protein percent less than 0.03) is present in the glomerular fluid and is reabsorbed in the renal tubules.
- Electron micrograph has revealed that the diameter of the filtering pores of the capillary endothelium and capsular epithelium are about 50–100 nm and 25 nm respectively. In anoxia, heart failure, and other pathological conditions, where the membrane becomes damaged, the filtering pores enlarge in size, so that serum albumin passes out in the largest amounts and appears in the urine. This is one of the causes of albuminuria.

All the above evidences go to prove that glomeruli act as ultra-filters.

Functions of Glomerulus

A fine filter (millipore filter) can separate the red cells from the plasma (blood), yet the process is a simple filtration. Still finer process is ultra-filtration by which colloidal materials, e.g. proteins and fats can be removed from solution. The glomeruli act as ultrafilters. The

process of filtration in the glomerulus is a passive process and never an active one. They filter all the constituents of plasma excepting the colloids (i.e. proteins and fats). The facts upon which this conclusion is based are briefly summarised below:

1. The effective filtration pressure of the glomerular capillaries.
2. The amount of fluid formed must be directly proportional to the quantity of blood flowing through the kidneys per unit time.
3. The capsular fluid must have the same composition as the plasma, excepting its colloids.
4. Actual filtering pores must be present in the filtering membrane.

Each one of these conditions is found to be satisfied by the glomerulus.

EXAM-ORIENTED QUESTIONS

Essay

1. Define GFR. Describe the mechanism involved in regulation of GFR.

Short Note

1. Glomerular filtration rate

Chapter

65 Physiology of Renal Tubules: Water and Electrolytes Balance and Counter-current Mechanism

INTRODUCTION

It is known that the capsular filtrate has the same composition as plasma excepting the colloids. When this fluid is compared to the fully formed urine, certain remarkable differences are noticed. The differences are briefly summarised below:

1. The total volume of urine in 24 hours is much less than the amount of glomerular filtrate formed during the same time. It is known that about 170 litres of filtrate are formed by the glomeruli in 24 hours, whereas only 1–5 litres of urine are excreted per day.
2. Some substances are present in the glomerular filtrate but are practically absent in the urine, for instance, glucose and bicarbonates.
3. The solid constituents are much more concentrated in urine than in the glomerular fluid (i.e. in plasma).
4. All the solids do not undergo the same degree of concentration. Each one of them has a different concentration in the urine.
5. The total quantity of some solid substances in the urine is much more than that of glomerular filtrate.
6. Glomerular filtrate is alkaline in reaction (same as plasma) but the urine is acid.
7. Some new substances are present in the urine which is not present in the renal artery. It is obvious that these changes must have taken place in the glomerular filtrate when the latter passes down the tubules.

In other words, the function of the tubules is to bring about these changes actively in the glomerular filtrate and thus convert the latter into fully formed urine.

The modern conception about the functions of the renal tubules is that it performs the following three functions:

1. *Selective reabsorption:* The renal tubules reabsorb water and other dissolved substances from the lumen of the tubule neck into the blood stream. The reabsorption is differential and purposefully selective. Each substance is reabsorbed to a particular degree. The more useful substances are reabsorbed to a higher degree than the less useful ones.
2. *Tubular secretion:* Tubules not only reabsorb but can also actively transfer certain substances from the blood stream into the lumen of the tubules.
3. *Formation of some new substances:* The tubular epithelium can also form certain new substances such as ammonia, inorganic phosphate, etc.

Methods of Study of Tubular Functions

The tubular function has been studied by (1) analysis of the composition of the fluid withdrawn from the tubules with micro-pipettes by the micro-puncture technique (Fig. 65.1) and (2) stop-flow method.

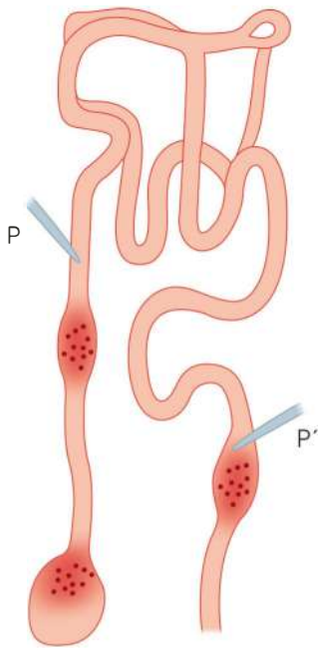


Fig. 65.1: Showing Richards method of collecting samples of fluid from different parts of the tubule by insertion of micropipettes. P and P′: Micro-pipettes

Selective Reabsorption

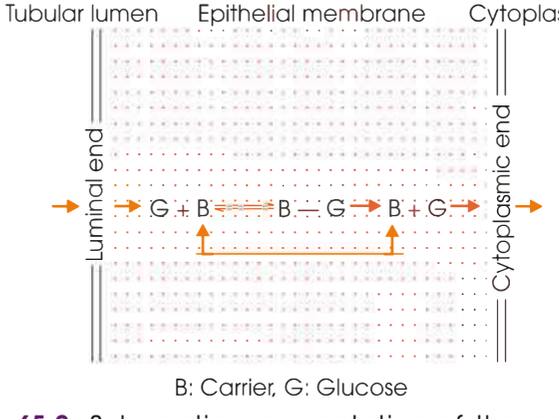
1. **Protein:** Normally a small amount of protein that is filtered into glomerular filtrate is reabsorbed through the brush border of the proximal tubular epithelium by the process of pinocytosis.
2. **Glucose:** Ninety-eight percent of glucose is absorbed in the proximal convoluted tubles. Two percent of glucose is absorbed from beyond the distal convoluted tubule. The glucose in the proximal tubule is co-transported with sodium ions into the proximal convoluted tubule walls via the SGLT2 co-transporter. Once in the tubule wall, the glucose and amino acids pass by diffusion directly into the blood capillaries along a concentration gradient.

   The carrier substance is present at the luminal wall of the tubular epithelium at the proximal end. The amount of these carrier substances is fixed and limited. At the luminal surface of the apical membrane, the carrier (B) combines with glucose (G) of the tubular fluid to form a reversible carrier-glucose (G-B) complex which then migrates towards the cytoplasmic end of the membrane and splits up. The free glucose is then delivered to the cytoplasm. This free carrier again goes back to its original position to combine with another molecule of glucose (Fig. 65.2).

   The reabsorption rate of glucose depends on the availability of the carrier. This limitation of the capacity of tubular cells to reabsorb glucose is termed as Tm for glucose or transfer maximum glucose (TmG) in short. So, the glucose reabsorptive mechanism is a Tm limited one. The average value for man is 375 mg/minute/1.73 square metre of body surface area and for women are 300 mg/minute/1.73 square metre of body surface area. If the glucose concentration of the filtrate is lower than this value, the total amount of glucose is reabsorbed. Under hyperglycaemic state and also in higher glucose concentration in glomerular filtrate (GF) than the TmG, the capacity of the carrier system fails to reabsorb glucose completely, and so the excess glucose appears in the urine (glycosuria) (Fig. 65.3). This is often observed when the venous blood glucose level exceeds the threshold value [(180 mg%) which corresponds to an arterial level of 200 mg%]. It is suggested that not all of the 2 million nephrons in the kidneys have exactly same transfer maximum glucose (TmG) or filtration rate; in some, TmG is exceeded at low levels of plasma glucose (PG). Or some glucose escapes reabsorption when the amount filtered is below the TmG, because the reactions involved in glucose transport are not completely irreversible. This deviation is called splay.
3. **Water:** Out of 170 litres of water filtered by the glomeruli per day, 168.5 litres are reabsorbed in the renal tubules. Only 1.5 litres are excreted as urine. The reabsorption of water occurs in the proximal, distal and collecting tubules. The normal osmolality of plasma is about 280 mOsm/L and is maintained by the kidney's ability to excrete water and sodium. The osmolality of urine is maintained by the kidney (Fig. 65.4).

Key Points

1. The proximal tubule has got high water permeability. About two-thirds to seven-eighths of water of the glomerular filtrate are reabsorbed in the proximal tubule. Active reabsorption of sodium along with passive reabsorption of water takes place in the proximal tubule. Here water and electrolytes are reabsorbed as an isosmotic solution and do not play any part in the concentration of dilution process. This is called obligatory water reabsorption. In the proximal portion of renal tubule, the sodium as well as potassium is reabsorbed actively but chloride reabsorption is a passive process (Fig. 65.5). Water is reabsorbed passively at osmotically equivalent rates. So, the tubular fluid leaving the proximal tubule is isotonic.



B: Carrier, G: Glucose

Fig. 65.2: Schematic representation of the possible mechanism of reabsorption of glucose in the renal tubule

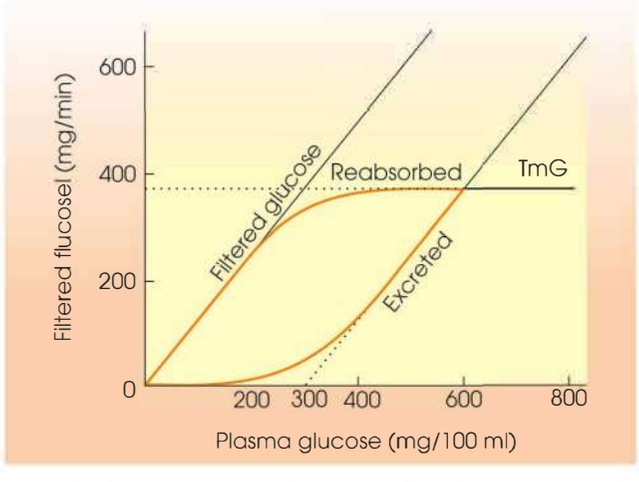


Fig. 65.3: Relative rates of glucose filtration, reabsorption and excretion in human kidney (diagrammatic)

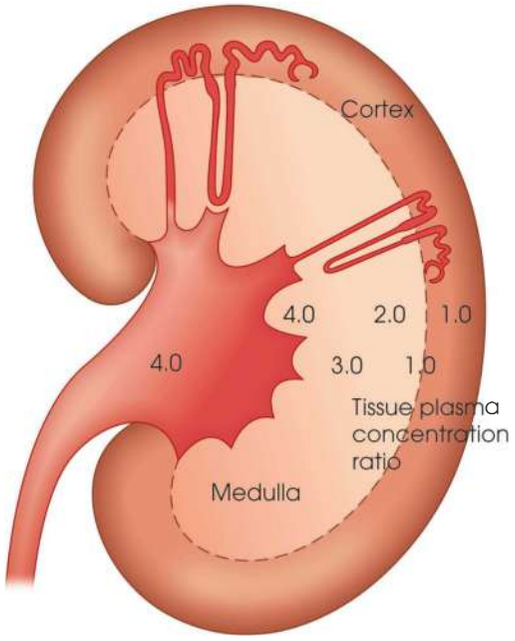


Fig. 65.4: The mechanism of osmolar concentration of urine at a depth of the kidney. (*Courtesy:* After Janaieson and Kay's Textbook of Surgical Physiology)

2. The fluid in the descending limb of loop of Henle maintains the same osmotic pressure as the interstitial fluid, and H_2O and Na pass by diffusion into and from the interstitium respectively.
3. The ascending limb of loop of Henle is impermeable to H_2O and it is there that sodium is pumped out actively into the interstitium so raising the osmolality and providing a hyperosmolar medium which allows the loop to act as a counter-current multiplier system. There is active reabsorption of sodium and as a result the tubular fluid becomes dilute and hypotonic.
4. The distal portion of the renal tubule does the function of reabsorption of water and ions in the following manners: The epithelial cells of this part can establish a relatively high ion concentration gradient between the blood and tubular fluid which is altered with the change of the blood content of ions and controlled by mineralocorticoids of the adrenal cortex. This portion can also establish relatively high osmolar concentration gradient between blood plasma and tubular fluid which is altered with the percentage of body fluid (water) content.

Water permeability through the epithelium of the distal portion of the renal tubule is controlled by the circulating anti-diuretic hormone (ADH) level secreted by the posterior pituitary gland. The secretion of this hormone (ADH) is controlled by water content of the body. In addition, the ADH may stimulate the pumping of sodium in the loop of Henle.

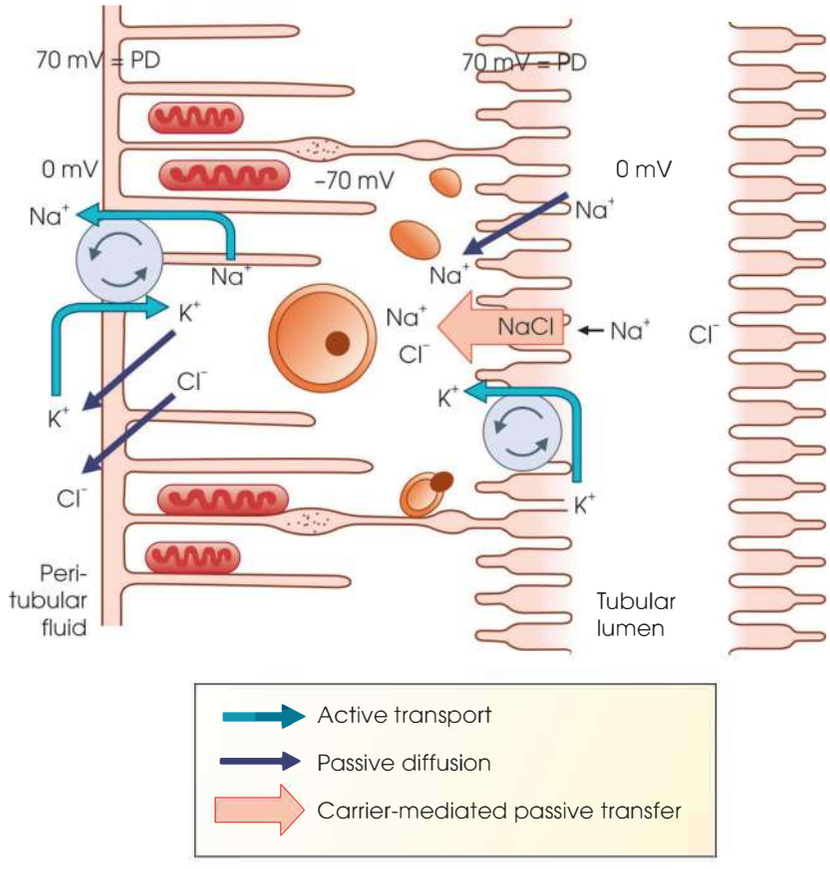


Fig. 65.5: Schematic representation of ion exchange in the first convoluted tubular cells (diagrammatic)

However, in summary it may be stated that the reabsorption of water at proximal tubule, loop of Henle and distal tubule, is a passive process which is determined by the osmotic forces created by the reabsorption of ions.

5. When the fluid passes into the collecting ducts; which are permeable to H_2O; this passes through the duct walls with the osmotic gradient into the medullary interstitium. It is here the ADH has its effect in enhancing the movement of H_2O so producing a hyperosmolar urine. Under conditions of hydropenia, water is re-absorbed quickly across the tubular epithelium, i.e. the tubular epithelium becomes highly permeable to water.
6. The transport of water across the epithelial cells depends on presence of aquaporins. There are four types of aquaporins found in humans; these are aquaporin 1, aquaporin 2, aquaporin 5 and aquaporin 9. The aquaporin 1 is found in proximal tubule and loop of Henle. The aquaporin 2 is found in collecting duct. The aquaporin helps in water reabsorption in the kidney.

Concentration of Urine

The urine is not concentrated in the distal tubule but in the collecting ducts. In the distal tubule facultative water reabsorption occurs and is regulated (Fig. 65.6) by the anti-diuretic hormone (ADH). ADH increases the permeability of the distal tubule and collecting duct cells in the kidney to water. There is reabsorption of sodium and chloride in the distal tubule and the tubular fluid becomes isotonic. The isotonic fluid enters the collecting tubule and is more concentrated (hyperosmotic) here due to further reabsorption of water.

So, the process of reabsorption of water may be summed up in the following ways:

1. In the proximal tubule two-thirds to seven-eighths of the water are re-absorbed. This is called

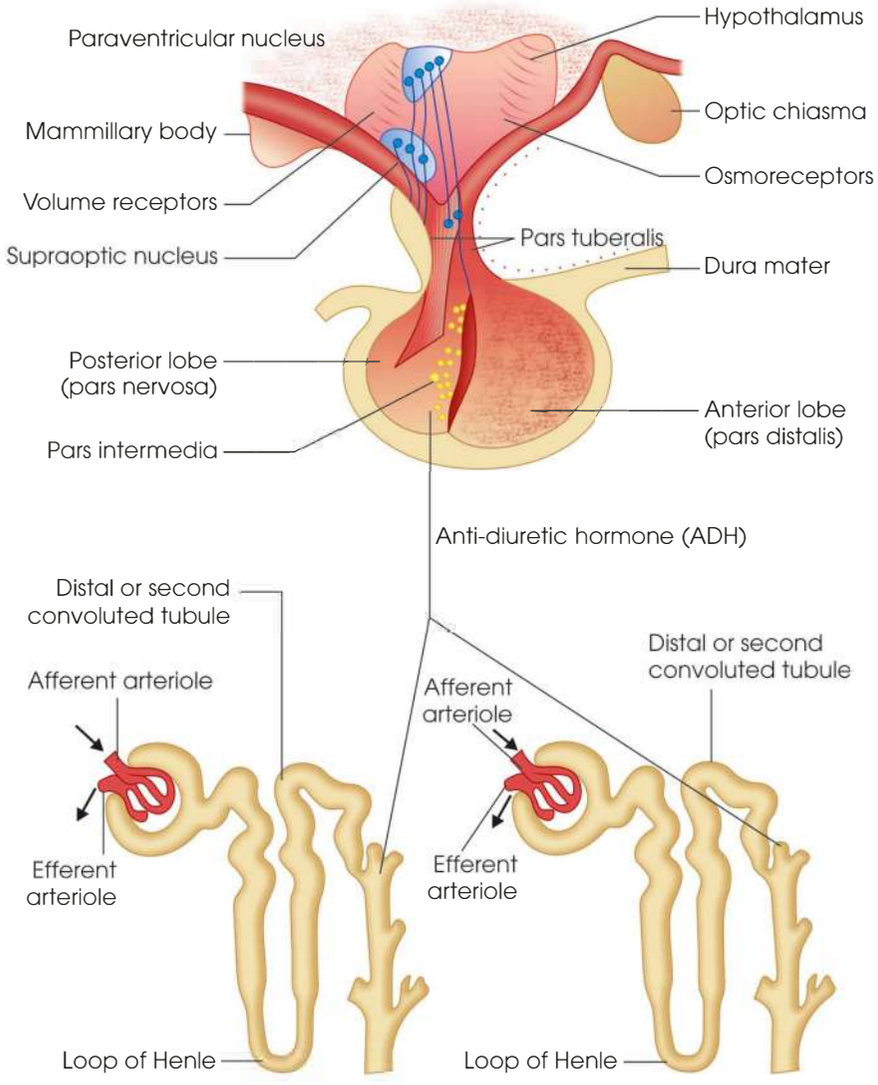


Fig. 65.6: Diagrammatic representation showing the regulation of secretion of anti-diuretic hormone and its action on renal tubules

'obligatory water reabsorption'—a passive process.

2. In the distal tubule water is reabsorbed with the help of ADH and the tubular fluid that leaves the distal tubule is isotonic.
3. Distal tubular reabsorption is called 'facultative water reabsorption' as it is regulated (Fig. 65.6) by the anti-diuretic hormone (ADH).
4. In the collecting ducts, the reabsorption of hyperosmotic fluid occurs. The water reabsorption in collecting duct is under influence of anti-diuretic hormone (ADH).

In healthy individual the fluid passing the ascending loop of Henle and early part of distal tubule is always dilute irrespective of ADH level and further as fluid passes into late distal tubules and collecting duct, the urine is further diluted as this portion of tubule is also impermeable to water and moreover addition reabsorption of sodium chloride occurs here. In presence of anti-diuretic hormone (ADH), its permeability to water increases and concentrated urine is formed.

Since reabsorption has to work against the gradually rising osmotic pressure, it is obvious that a point will come where these two opposite forces will exactly equalise and further reabsorption will cease. In other words, there is a maximum limit beyond which the urine cannot be concentrated. The solid constituents of urine, therefore, require a certain minimum amount of water for excretion. This is called the minimum volume or obligatory volume.

Anti-diuretic hormone (ADH) is an octapeptide and is produced in the supra-optic and para-ventricular nuclei of the hypothalamus from the neuro-hypophysis, where the ADH is stored. The secretion of ADH from the posterior lobe of the pituitary gland is controlled by the hypothalamus. Hypothalamus receives impulses from the osmoreceptor cells supplied by internal carotid artery and from volume receptors in the thorax. An osmoreceptor is a sensory receptor primarily found in the hypothalamus. Osmoreceptors are found in various locations such as organum vasculosum of the lamina terminalis (OVLT) and the sub-fornical organ (SFO). Increased osmotic pressure in the blood or extra-cellular fluid stimulates the osmoreceptors, which in its turn increase the intensity of impulse to the hypothalamus which increases or decreases vasopressin (ADH) secretion from the posterior pituitary. This impulse leads to increased secretion of ADH. Thus, they contribute to fluid balance in the body.

Increase in blood volume through the volume receptors produces opposite effect, i.e. inhibits ADH secretion (Fig. 65.6). In a disease called diabetes insipidus there is elimination of large quantity of water due to lack of secretion of ADH.

Summary: Role of ADH

1. It increases the water permeability of distal convoluted tubule and collecting duct cells in the kidney. The increased water reabsorption is mediated via the increased transcription and insertion of water channels (aquaporin-2) into the apical membrane of distal convoluted tubule and collecting duct epithelial cells.
2. ADH increases permeability of the inner medullary portion of the collecting duct to urea and urea is reabsorbed into the medullary interstitium as it moves along the concentration gradient created by removal of water from the cortical and outer medullary collecting duct.
3. The sodium absorption across the ascending loop of Henle is influenced by ADH.

Counter-current multiplication theory of urine concentration.

Key Points

1. The different phases through which urine become by Wirz and his associates (1951) hypertonic have been studied and later by Bray (1960). It is a complex process and has got close relation with the anatomical distribution of the tubules along with the Na concentration and different depths from the cortex towards the medulla of the kidney (Fig. 65.7).
2. The loop of Henle along with blood vessels passes from the corticomedullary junction to the medulla and again returns towards the cortex and thus simulates a hairpin counter-current arrangement.

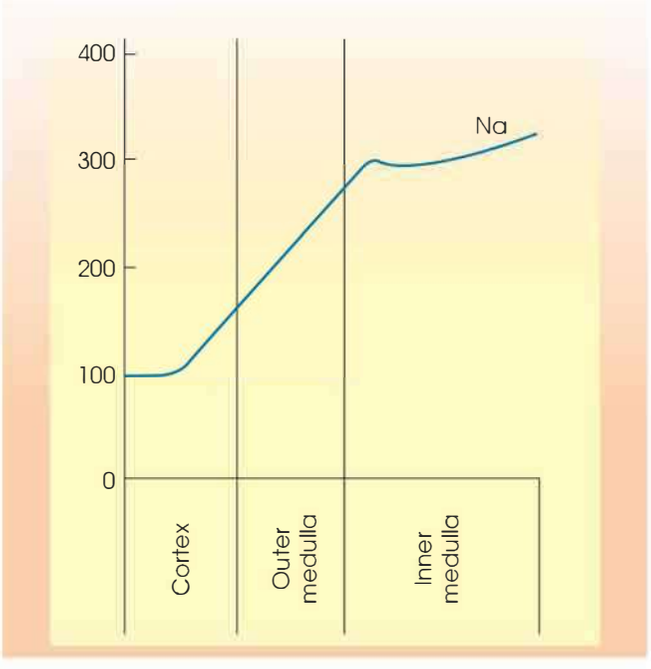


Fig. 65.7: Graphical representation of concentration of sodium in slices of the cortex and of the outer and inner region of the medulla in the hydropenic animal

3. The glomerular filtrate has a concentration same as blood plasma and although about 80% of the filtrate is re-absorbed in the proximal tubule, yet the remaining 20% enters into the descending loop of Henle in an isotonic state. So, as the isotonic fluid passes through the descending limb, there is passive diffusion of sodium into the tubule from the surrounding hypertonic tissue fluid and becomes hypertonic.
4. Now this hypertonic fluid enters into the ascending limb and gradually becomes hypotonic due to active transport of sodium from the tubule to the surrounding tissue and also due to impermeability of this portion of the tubule to water. So, this hypotonic fluid is entering into the distal convoluted tubule where the anti-diuretic hormone (ADH) increases the permeability to water of the distal convoluted tubular epithelium and the hypotonic fluid again attains isotonic state.
5. Vasopressin regulates the body's retention of water by acting to increase water reabsorption in the kidney's collecting duct. Now, this isotonic fluid passing through the collecting tubule becomes hypertonic due to the ionic and water exchange in between the tubular fluid and the hypertonic medullary tissue fluid. And thus the hypertonic urine is formed and passes through the ureter (Figs 65.8B and 65.9).

Counter-current exchange theory for the vasa recta (Berliner and his associates, 1958): It is a passive process and depends on the diffusion of solutes and water in both the directions across the permeable walls of the vasa recta. As the descending limb of the vasa recta gradually enters deep into the medulla, water diffuses out from, and osmotically active particles diffuse into the blood vessel. In the ascending loop, the diffusion process is just in opposite direction and thus isotonic blood is leaving the medulla (Fig. 65.9). The counter-current exchange reduces the rate of dissipation and hence reduces the rate at which the counter-current multiplier must pump Na^+ to maintain any given gradient.

Sodium and Chloride

Sodium is absorbed along with chloride. There is active transport of Cl^- in the thick-walled ascending limb of Henle's loop. Out of 560 gm of Na^+ filtered by the glomeruli per day; 490 gm are reabsorbed in the proximal tubule, 65 gm are reabsorbed in the distal tubule and 5 gm are excreted in the urine. Reabsorption of Na^+ in the tubules is an active process and is due to the activity of the cells of the proximal tubule, the ascending limb of loop of Henle and the distal tubule (Figs 65.5, 65.9 and 65.10). But in the descending limb of Henle's loop the sodium movement is just in the reverse direction, i.e. sodium enters into the tubular lumen passively through the process of diffusion. Reabsorption of water follows passively due to active reabsorption of sodium in the tubule. The reabsorption is influenced by aldosterone, the adrenal cortical hormone. Aldosterone release is controlled by blood volume, a decrease of blood volume causes an increase in hormone secretion and thus increased retention of sodium occurs. Renin secreted from juxtaglomerular

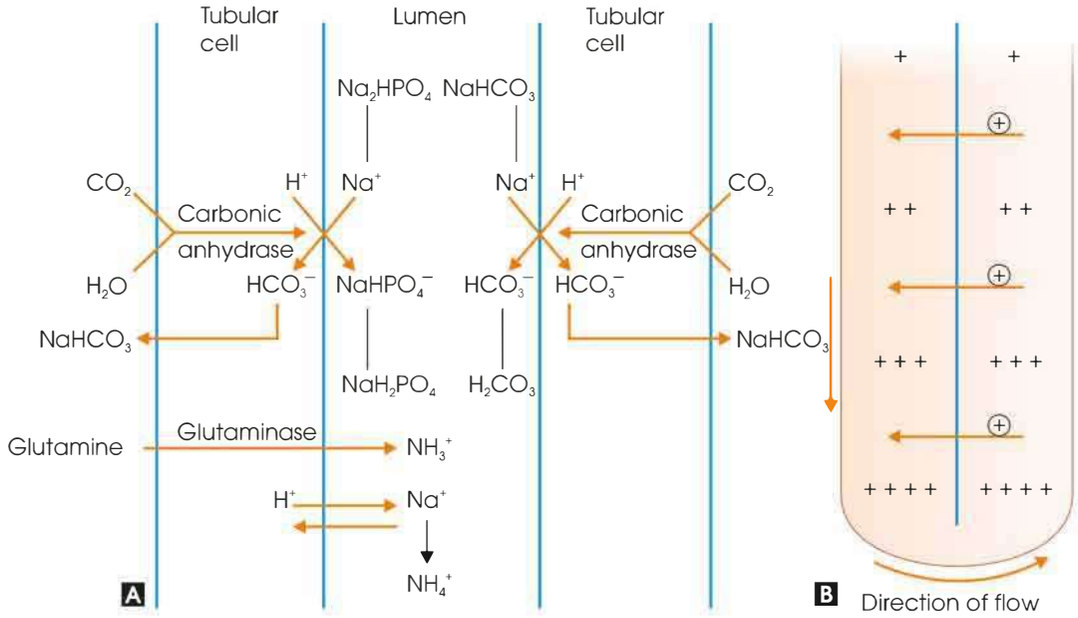


Fig. 65.8A and B: Diagram A illustrating the ionic movements across the tubular cell concerned in the excretion of H^+ and conservation of cation. Diagram B illustrates mechanism of a counter-current multiplier. The transfer of a single ion at each side indicated results in gain of three ions at the HP of loop, although the osmotic gradient through which ions has to pass is only unity

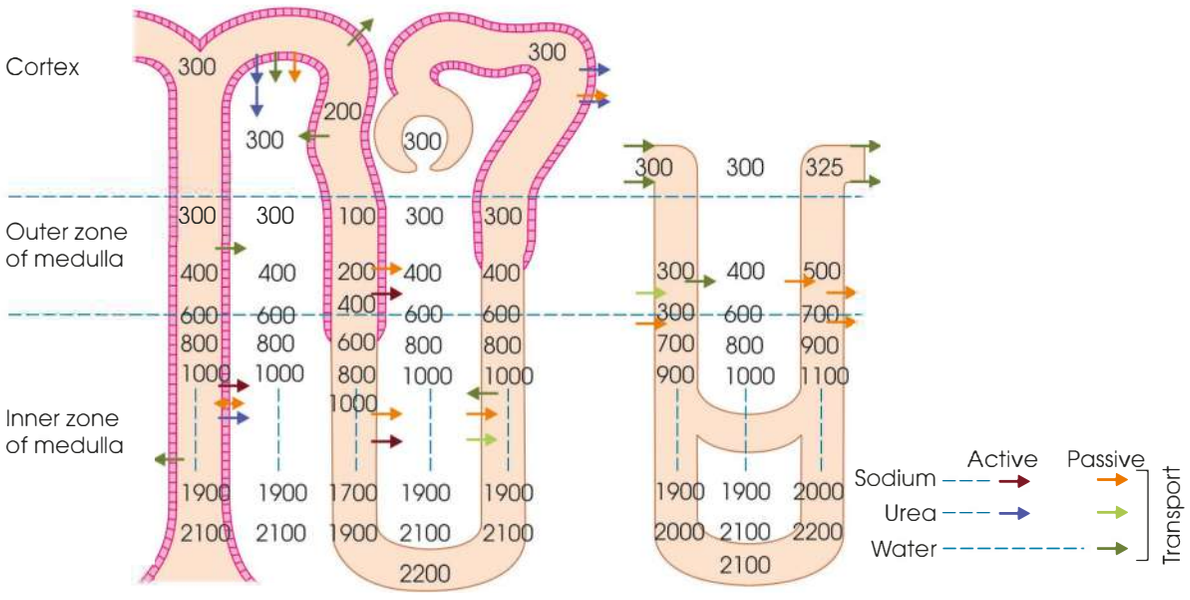


Fig. 65.9: Diagram illustrating the counter-current mechanism as it is believed to operate in a nephron with a long loop and in the vasa recta (represent on the right). The numbers represent hypothetic osmolality values. No quantitative significance is to be attached to the number of arrows and only net movements are indicated

apparatus causes increased formation of angiotensin II, which controls aldosterone secretion. As sodium is actively absorbed by tubular cells, it can be inhibited by chemical agents like mercury. The reabsorption of chloride in the renal tubules is also a passive process which works with the electrochemical gradient (Figs 65.5 and 65.10).

Potassium

About 80–95% of the potassium filtered is re-absorbed in the proximal tubule and a specific active transport process is involved in it. The secretion of potassium takes place in the second convoluted tubule. Reabsorption of sodium ions and secretion of potassium ions

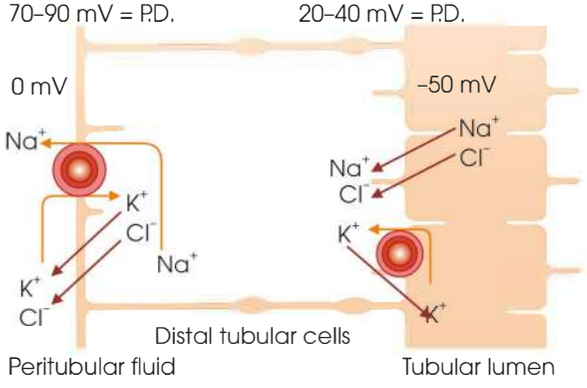


Fig. 65.10: Schematic representation of ion transport in the second convoluted tubular cells (diagrammatic)

are simultaneous processes. About 75% of urinary potassium is accounting from the process of secretion. The secretion of potassium is augmented during alkalosis.

Bicarbonate

Bicarbonate is completely reabsorbed in the renal tubule. pH of the fluid collected from the proximal tubule is alkaline in reaction. In the lumen of the tubule HCO_3, combines with H which diffuses from the tubular epithelial cells in exchange of reabsorption of Na^+ *. H_2CO_3, is formed which yields CO_2, and H_2O. CO_2,

It diffuses into the tubular cells and with the help of carbonic anhydrase (CA) forms H_2CO_3. The cell wall at the luminal side is not permeable to HCO_3 ion. Na which is separately absorbed is transferred to the blood capillaries together with HCO_3 from the tubular cells. During alkalosis, less amount of H ion is present for exchange with sodium and $NaHCO_3$ is excreted in urine. During acidosis, on the other hand, more CO_2 is present and the amount of H_2CO_3 formed inside the tubular cells under the influence of carbonic anhydrase is increased (Figs 65.11 and 65.12).

The renal threshold of bicarbonate is 28 mM/L in man. This value is influenced by the following four factors:

1. CO_2 tension in arterial blood
2. Potassium store in the body

*Acidification of urine. The tubular cells synthesise and secrete H ions in exchange of Na ions which diffuse into the tubular cells. H ions are responsible for making the urine acid.

3. Chloride level in the blood
4. Amount of secretion of adrenal cortical hormones.

The bicarbonate reabsorption increases with the increment of CO_2 in the blood and depletion of potassium or chloride and *vice versa*. The bicarbonate reabsorptive mechanism is certainly affected by the secretion of adrenal cortical hormones though the mechanism of action is not known. Hyper-secretion of mineralocorticoids and glucocorticoids cause metabolic acidosis along with increased bicarbonate reabsorption.

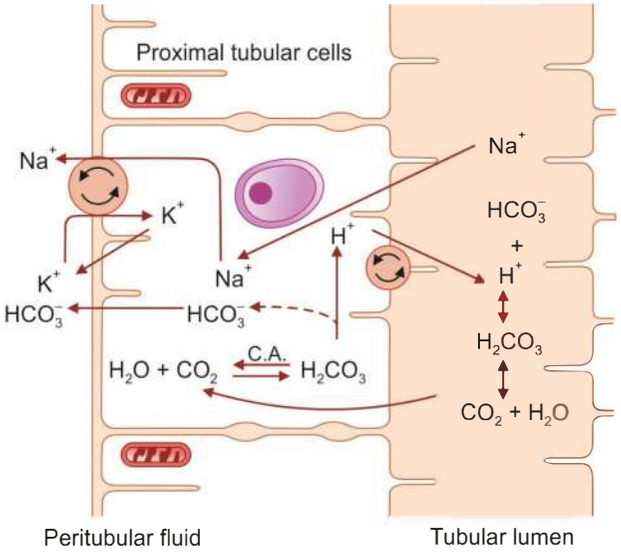


Fig. 65.11: Diagrammatic representation of bicarbonate reabsorption in the first convoluted tubular cells

Phosphate

Reabsorption of phosphate takes place in the proximal tubule. Phosphate reabsorption is an active process. The transport mechanism is influenced by a number of factors. Parathyroid hormone depresses the transport mechanism.

Acidosis increases excretion of phosphate. The mechanism of reabsorption of the inorganic phosphates resembles in many aspects with that of glucose.

The differences are:

1. The kidney regulates the plasma phosphate concentration as slight alteration of plasma phosphate content alters the rate of phosphate excretion.
2. The absorptive capacity of the tubular epithelium is much more variable and influenced by body store of ions and also by adrenal cortical and parathyroid hormones but in case of glucose, the absorptive capacity is remarkably stable.

Reabsorption of other Substances

Like sulphate, uric acid, ascorbic acid, β-hydroxybutyric acid, ketone bodies, aceto-acetic acid, creatinine and some amino acids also occur in the tubules. The mechanism of reabsorption of sulphate in the renal tubule is in resemblance with that of phosphate and is also an active process. The reabsorption of amino acids is more or less complete in renal tubules and is controlled by the plasma concentration of the same and ultimately negligible quantities are excreted. Creatine is reabsorbed completely from the renal filtrate. But reabsorption of creatine is completely blocked by

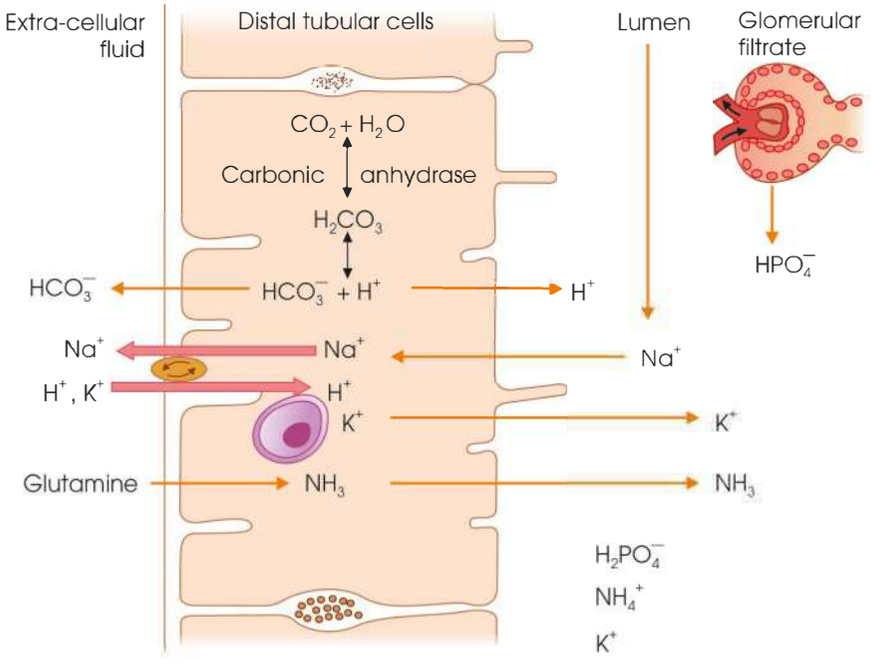


Fig. 65.12: Diagrammatic representation of transport of bicarbonate and sodium by the second convoluted tubular cells

saturation of the reabsorptive mechanism by glycine and alanine which indicates that these three substances may be reabsorbed through a single mechanism.

Tubular Secretion

Although selective reabsorption is the main function of the renal tubules but the tubules also secrete some substances. Tubular secretion is an active process carried out by the tubular cells. Various ethereal sulphates, steroid and other glucuronides, and 5α-hydroxyindoleacetic acid which are produced normally in the body are secreted by the tubules. In some fishes there is no glomerulus in the kidneys but the urine contains chloride, creatinine, uric acid, water, creatine, magnesium, potassium, sulphate, urea, etc. As there is no filtration due to lack of glomeruli, these substances must be the secretory products of the tubules. In man, certain substances, e.g. phenol red, diodrast (diodone), penicillin, para-aminohippuric acid (PAH), etc. are actively transferred by the tubules from the blood stream into the lumen of the tubules. Potassium, creatinine and hydrogen ion are also secreted by the tubules. Normally reabsorption of potassium is complete in the proximal tubules. Studies with radioactive potassium show that potassium present in the urine is the secretory product of the tubules. When Na^+ ions is reabsorbed in the tubules, potassium and hydrogen ion are at the same time secreted by the tubules. When reabsorption of Na^+ is increased by the hormone aldosterone, K^+ is secreted in larger amounts. On the other hand, when the secretion of H^+ ion increases, that of potassium is decreased. EDTA (ethylenediaminetetra-acetic acid) is also secreted. PAH reduces the secretion of 18- and 3-glucuronide derivatives of aldosterone. Up to a range of 6 mg PAH/100 ml of plasma, the rate of filtration, secretion and excretion of PAH changes proportionately. But about 10 mg/100 ml, the secretion of PAH is maintaining a constant value, i.e. independent of plasma concentration. The Tm value for PAH secretion is about 80 mg/min/1.73 square metre of body surface area under normal condition.

Determination of Tubular Secretory or Excretory Power

Like selective reabsorption, tubular secretion or excretion also depends upon the vital activity of the epithelium. The maximum power of the tubules to excrete substances (e.g. PAH) can be determined and is called Tm or tubular maximum. The term PAH Tm means the maximum power of the tubule to excrete it. This may be determined by injecting PAH and thereby raising its concentration in the plasma well above that level, up to which complete extraction is possible; urinary PAH excretion (Um) is noted; the amount of PAH filtered (F) is calculated from the plasma PAH concentration (Fig. 65.13) and the volume of glomerular filtrate formed (inulin clearance value), viz. Um – F = Tm. The Tm value is related to the number of functioning tubules and their excretory efficiency. The decrease in the Tm represents decrease in the number of tubules or their efficiency or both.

Formation of Some New Substances

Formation of ammonia: The cells of the renal tubules contain deaminases (amino acid oxidases) which can produce ammonia by the deamination of amino acids. This is proved by the fact that (1) renal vein contains greater amount of ammonia than renal artery and (2) experiments with 15N-labelled amino acids also produce the same isotopic ammonium salts. About 60% of ammonia is formed from glutamine (Fig. 65.14). The rest is formed from glycine, alanine, leucine and aspartic acid.

It is said that urinary ammonia is mainly produced in the kidney.

The ammonia, thus formed is passed out into the lumen of the tubules where it combines with the acid

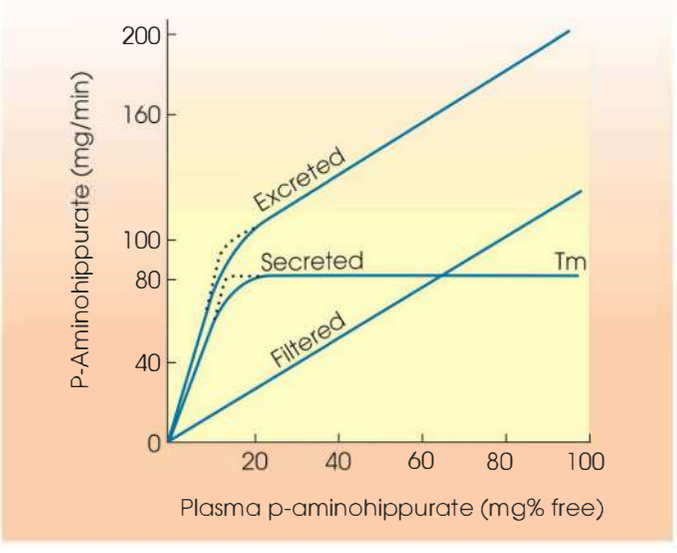


Fig. 65.13: Graphical representation of relative filtration, secretion and excretion of PAH by the kidney as functions of plasma concentration

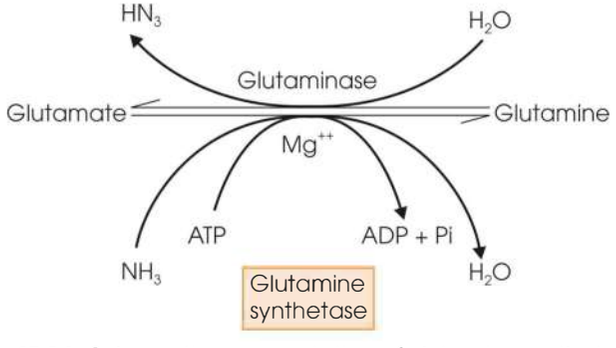


Fig. 65.14: Schematic representation of glutamine synthetase reaction

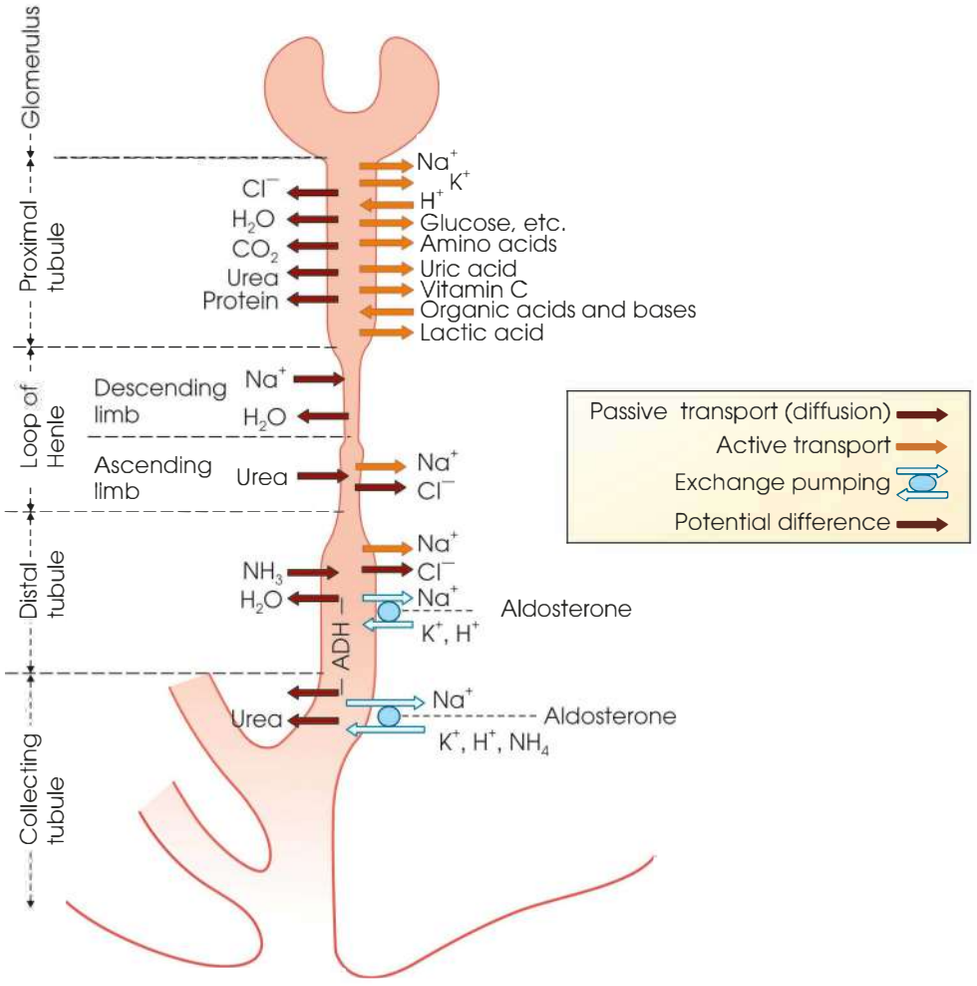


Fig. 65.15: Diagrammatic representation of the tubular reabsorption and secretion on the course of urine formation

radicals and liberates the fixed bases (e.g. Na^+, K^+, etc.). These bases are reabsorbed to maintain the alkali reserve. The amount of ammonia formation markedly increases during acidosis. This is an important way by which kidneys help to maintain a constant blood reaction

The tubules can synthesize hippuric acid by combining benzoic acid with glycine.

Formation of inorganic phosphates: Part of the inorganic phosphates eliminated by the kidneys is manufactured in the tubules. The enzyme phosphatase hydrolyses organic phosphates (hexose phosphate) and liberates inorganic phosphate. Role of renal tubules in the formation of urine has been summarised diagrammatically in Fig. 65.15.

EXAM-ORIENTED QUESTIONS

Essay

1. Describe the methods of studies of tubular functions.
2. Describe the counter-current multiplier mechanism and counter current exchanger mechanism.

Short Notes

1. Concentration of urine
2. Dilution of urine
3. Counter-current multipliers
4. Counter-current exchangers

Chapter

66

Renal Function Test, Urine, Urinary Characteristic and its Mechanism of Formation

INTRODUCTION

These tests depend upon blood analysis only

1. Estimation of nitrogenous constituents, to find nitrogen retention if any, e.g. urea, blood urea nitrogen (BUN), non-protein nitrogen (NPN), uric acid, creatinine, etc.
2. Estimation of cholesterol and albumin/globulin ratio (nephrosis).

Tests depending upon urine analysis only

1. Volume, reaction, ammonia coefficient, concentration of normal constituents (e.g. urea, etc.).
2. Specific gravity test.
3. Presence of abnormal constituents (e.g. albumin, casts, blood cells, etc.).

Tests depending upon the elimination of some substances

1. *Water elimination test:* 568 ml (a pint) of water is given to a fasting patient and the urine is collected and measured at hourly intervals. In a normal subject 568 ml should be excreted in three hours.
2. *Urea concentration test:* Bladder is emptied and 15 gm of urea, in about 142 gm of water, is given to the subject. Urine is collected after one and two hours. The second sample should have a urea concentration of 2% or more
3. *Indigo carmine test:* 0.1 gm of the dye is injected intramuscularly. It should appear in the urine in 6–8 minutes. Any delay means renal inefficiency. Excretion is usually complete by 12–24 hours. This test may indicate which particular kidney is wrong if ureteric catheter is used.
4. *Iodoxyl test (intravenous pyelography):* This compound is rapidly excreted by the kidney and is opaque to X-ray. About 15 gm of the substance in 20 ml of a 10% solution of invert sugar, is given intravenously, and a radiograph is taken. In normal persons, a distinct pyelogram will be obtained in 2–l0 minutes. In cases of renal diseases it may be delayed for hours. This method is useful in demonstrating the size and shape of kidney, renal pelvis, calyces, ureters, presence of stone, etc.

Tests depending upon both blood and urine analysis (clearance tests): The best tests for renal function are the renal clearance tests.

Renal clearance can be estimated by calculating the urine concentration of the substance [mmol/L], urine flow (volume/time) and plasma concentration [mmol/L] of the substance.

Assuming

K is the clearance [ml/min], C_U is the urine concentration [mmol/L or mg/ml], Q is the urine flow (volume/time) [ml/min or often ml/24 hr] and C_B is the plasma concentration [mmol/L often mg/ml]

$K = C_U$ is the urine concentration × Q is the urine flow (volume/time)/C_B is the plasma concentration.

Three types of clearance values can be obtained according to the nature of the substance used:

1. When the substance used is filtered by the glomeruli and is neither reabsorbed nor excreted by the tubules, its clearance value is equal to the volume of glomerular filtrate. This applies for inulin. Actually, glomerular filtrate can be measured by inulin clearance test (125 ml).
2. When the substance is filtered by the glomeruli but is reabsorbed by the tubules, its clearance value is less than the volume of glomerular filtrate, i.e. inulin clearance. This is true for urea clearance (maximum clearance—Cm 75 ml, standard clearance—Cs 54 ml). The more is the degree of reabsorption, the less will be the clearance value.
3. When the substance is filtered by the glomeruli, as well as excreted by the tubules, its clearance is more than the glomerular filtrate. This is true for creatinine (170 ml). If a substance is completely cleared from the plasma, its clearance value is

equal to the renal plasma flow. This is true for diodrast (iodopyracetate) or PAH.

The following clearance tests are commonly used.

INULIN CLEARANCE (A MEASURE OF GLOMERULAR FILTRATION RATE)

This inulin clearance test is performed to estimate the glomerular filtration rate. Any substance may be regarded suitable for estimating glomerular filtration rate if it possesses the following four physiological properties:

1. It is freely filterable through the renal corpuscular filtrating membrane.
2. It is biologically inert, neither reabsorbed nor secreted by the tubules.
3. It is non-toxic and does not alter renal function.
4. It is easily and accurately estimable. The clearance of such substance will be equal to the glomerular filtration rate.

Procedure: The principle and procedure is as follows: 10 gm of inulin, dissolved in 100 ml of normal saline, is given intravenously at the rate of 10 ml per minute. Since inulin is filtered by glomeruli and is neither reabsorbed nor excreted by the tubules, clearance value will measure the rate of glomerular filtration.

Calculation: Inulin clearance can be calculated from the formula:

$$C_{In} = \frac{U_{In} \times V}{P_{In}} \text{ ml/minute}$$

Where U_{In} = inulin concentration in mg per 1 ml of urine; V = volume of urine in ml per minute; and P_{In} = concentration of inulin in mg per 1 ml of plasma; C_{In} = inulin clearance in ml per minute.

Normally, inulin clearance is 125 ml per minute.

UREA CLEARANCE TEST (VAN SLYKE)

Procedure: Two one-hourly samples of urine are collected. Blood is collected at the end of first hour and its urea content (mg%) is estimated. The urine samples are measured and their urea concentrations separately estimated (mg%). The minute output of urine is calculated from these data.

Calculation

Maximum clearance: If the minute output be 2 ml or more, the result is called maximum clearance (Cm). The formula for it is as follows:

$$= \frac{U_u \times V}{P_u}$$

where U_u = urine urea in mg per ml; V = volume of urea in ml per minute; P_u = urea in mg per ml of plasma.

In normal human adults, the value is relatively constant and the average is 15 ml per minute. The result is usually expressed as percentage of the clearance, as follows: For Cm 75 ml is taken as the 100% efficiency.

Cm × 100/75, i.e. Cm × 1.33% will give the percentage efficiency for maximum clearance. For instance, if the Cm of a normal subject is found to be 50 ml, the renal efficiency will be 50 × 1.33, i.e. 66.5%

Standard clearance: If the minute output of urine be less than 2 ml, the value is named as standard clearance (Cs). For it, the following formula is used:

$$= \frac{U_u \times V}{P_u}$$

The normal range of Cs is 40–65 ml (average 54 ml). To express as percentage of normal, Cs is to be multiplied by 100/54, i.e. 1.85. For example, if the Cs of a subject is found to be 50, the renal efficiency will be 50 × 18.5, i.e. 92.5%.

This test, according to Van Slyke, is a reliable method for estimating renal efficiency. In a person with high protein diet or who injected urea, the plasma concentration of urea (P_u) increases and the same time the rate of excretion of urea ($U_u \times V$) also increases in direct proportion. So, the ratio $\frac{U_u \times V}{P_u}$ remains unaltered. Thus, the urea clearance value is independent of plasma urea.

Concentration and the rate of urea concentration: The urea clearance value varies proportionately with the filtration rate. In renal disease, the urea clearance value is reduced. In severe congestive failure, dehydration, shock and cirrhosis with ascites, the urea clearance value is reduced.

CREATININE CLEARANCE TEST

Glomerular filtration rate (GFR) is most commonly estimated by creatinine clearance. The measurement of serum creatinine, urine creatinine, and 24-hour urine volume helps to calculate the creatinine clearance.

The advantage of use of creatinine clearance test for GFR estimation is:

Creatinine is freely filtered across the glomerulus. It is neither reabsorbed nor metabolized by kidney. As the proximal tubules secrete approximately 15% of urinary creatinine in healthy individuals having normal GFR; there over-estimation of the GFR. The proximal

Table 66.1: Plasma clearances of substances used for determination of renal functions

Substance	*Plasma clearance per minute*	*Used as a measure of*
Inulin	125	Glomerular filtration
Diodrast	560	Plasma flow and blood flow through the kidneys
PAH	585	Do
Urea	75	Overall renal efficiency
Creatinine	140	GFR

tubular secretion is neglected as all of the filtered creatinine (the GFR product × serum creatinine concentration) is excreted (equal to product of the urine creatinine concentration [U_{Cr}] × urine flow rate or volume [V]).

Estimation of Creatinine Clearance

Assuming creatinine clearance (C_{Cr}), creatinine concentration in urine sample (U_{Cr}), urine flow rate (Vdt), the plasma concentration (P_{Cr}), and the creatinine clearance is found to be:

$$C_{Cr} = \frac{U_{Cr} \times V}{P_{Cr}}$$

This can be expressed as follows:

GFR × serum creatinine concentration = U_{Cr} × V and GFR = [U_{Cr} × V]/serum creatinine concentration.

The decrease in creatinine clearance indicates decreased GFR and impaired renal function. The normal range, however, is between 97 to 137 ml/min for males, and 88 to 128 ml/min for females.

Peter Agre discovered aquaporin the water-channel proteins. Aquaporins are water-channel proteins that move water molecules through the cell membrane.

Peter Agre
Born 1949

URINE, URINARY CHARACTERISTIC AND ITS MECHANISM OF FORMATION

INTRODUCTION

Volume of urine: Quantity of urine formed in 24 hours in an adult normal individual varies from 600 to 2500 ml. Normally it depends upon (a) water intake, (b) diet, (c) environmental temperature, (d) mental state, and (e) physical conditions of the subject. About half as much urine is formed during sleep as during activity. There is a special relationship in between the skin and the kidneys where excretion of water is appreciably made by these two organs. Thus, perspiration has got inverse relation with the formation of urine.

Substances that stimulate the formation of urine are termed as diuretics. Tea, coffee, alcoholic beverages and also nitrogenous end products have got diuretic effects. Low threshold substances like urea, sulphate and phosphate resulting from protein metabolism retain water in the tubules to keep those materials in solution. For this reason after high protein diet, urine volume is increased. High threshold substances like glucose, amino acids, etc. also produce diuresis when present in the tubule still in excess after reabsorption, because these substances require water for keeping themselves in solution. For the same reason, diabetes is always accompanied with diuresis.

Table 66.2: Mechanism of action of some diuretics

Drugs	*Mechanism of action*
Mannitol and glucose	Produces osmotic diuresis
Organic salts of mercury	Inhibit Na^+ and Cl^- reabsorption beyond the proximal tubule; inhibit K^+ secretion
Carbonic anhydrase inhibitors: Acetazolamide (Diamox)	Decrease H^+ secretion with resultant increase in excretion of K^+ and Na^+
Thiazides	Inhibit Na^+ reabsorption in the distal portion of loop of Henle and proximal portion of the distal tubule
Furosemide ethacrynic acid	Inhibit Na^+ reabsorption in the loop of Henle

Table 66.2 summarises mechanism of action of some of the drugs commonly used in medical practice as diuretics.

OTHER CHARACTERISTICS

Colour

Normal colour of the urine is pale-yellow or amber and it is mainly dependent upon the presence of pigment, urochrome. This colour also varies with the quantity and concentration of the urine voided. In fever, urine becomes dark-yellow or brownish. If vitamin riboflavin is taken then it is excreted through the urine causing its colour yellow. In liver disease, bile pigment may give the urine green, brown or deep-yellow. Blood and haemoglobin colours the urine smoky to red. Homogentisic acid and methaemoglobin may give urine a dark-brown colour.

Reaction

Normal freshly voided urine is usually clear and acidic in reaction with a pH value as low as 4.5 and as high as 8.2. The mean pH of the normal mixed 24-hour urine is about 6.

Specific Gravity

In normal urine specific gravity varies in between 1.010 and 1.050; and may be subject to wide fluctuation. As a result of excessive water intake, specific gravity may fall to 1.003 and on the other hand, may rise to 1.040 or higher because of haemoconcentration due to excessive perspiration. The specific gravity of urine is directly proportional to the solute content and varies inversely with the volume.

Turbidity

Usually freshly voided urine is transparent but alkaline urine on standing may become cloudy due to precipitation of calcium phosphate. In pathological condition the urine may be turbid due to presence of mucoid, nucleoprotein, epithelial cells, pus cells, etc.

Odour

The colour of normal urine is faintly aromatic and is due to presence of number of volatile organic substances. Sometimes, the odour of urine may be attributed to a neutral ill-smelling substance-urinod (C_6H_8O).

Osmotic Pressure

Osmotic pressure of the urine is much above that of blood plasma.

COMPOSITION OF URINE

Normal Constituents of Urine

Organic Constituents

1. **Urea:** It constitutes about half of the total urinary solid and is the principal end product of protein metabolism. In human beings, it represents about 80–90% of the total urinary nitrogen. About 25–30 gm of urea is excreted per 24-hour.
2. **Ammonia:** It is the second and most important nitrogenous constituent. Ordinarily there is very little ammonia in the freshly voided urine. It is about 2.5 to 4.5% of the total urinary nitrogen and in average 0.7 gm is excreted per day.
3. **Uric acid:** It is the end product of the purine metabolism in the body. About 0.7 gm uric acid is excreted through the urine per 24-hour.
4. **Creatinine and creatine:** Creatinine is the product of the breakdown of creatine. The amount of creatinine excreted through the urine by an adult individual is about 1.2 to 1.7 gm per 24-hour. Excretion of urine per 24-hour is practically constant in the subject with creatinine-free diet. Creatinine coefficient may be defined as the ratio between the amounts excreted in 24 hours and the body weight in kilogram. It is commonly 20 to 26 mg per kg per 24-hour in normal man and 14 to 22 mg per kg in normal woman per 24-hour. Creatine is present in the urine of children and in much smaller amounts in normal adult man. It is observed that normal males excrete about 6% of the total creatinine output of creatine (60–150 mg/day). In females, this amount is higher than that of the males.
5. **Oxalate:** Normal urine contains about 10–30 mg of oxalate per 24-hour. Though some quantity of oxalic acid comes from the metabolism of glycine and ascorbic acid, major portion of oxalic acid comes from the diet such as asparagus, spinach.
6. **Amino acids:** In adults, about 150–200 mg of amino acids are excreted through the urine in 24 hours.
7. **Hippuric acid:** It is chemically benzoyl glycine. It is the detoxication products of benzoic acid with glycine. The quantity of hippuric acid excreted through the urine is about 0.7 gm (ranges about 0.1 to 1 gm).
8. **Allantoin:** It is derived from partial oxidation of uric acid and present almost in all mammals. In human beings, it is present in very small amount.
9. **Vitamins, hormones and enzymes** are also excreted in normal urine. In adults, 15–50 mg of ascorbic acids are excreted per 24-hour. Metabolic degradation, products of adrenocortical hormones are excreted through the normal urine of both males and females.

Inorganic Constituents

1. **Chloride:** Next to urea, it is the chief solid constituent of urine. Generally, 6–9 gm per 24-hour as chloride and 10–15 gm as sodium chloride are excreted through the urine.
2. **Phosphate:** The amount of phosphate excreted through the urine varies widely and it depends largely upon the diet. It is normally excreted 0.8–1.3 gm of phosphate per day. Urine phosphates are combination of Na^+ and K^+ phosphates as well as calcium and magnesium phosphates.
3. **Sulphates:** Urinary sulphates are derived mainly from the metabolic degradation of sulphur containing amino acids—methionine, cystine and cysteine. The quantity of excretion of sulphate depends upon the protein consumption and the breakdown of tissue protein.
4. **Minerals:** The four cations—Na^+, K^+, Ca^{++} and Mg^{++} are present in the urine. The quantity of sodium is excreted through the urine normally ranges from 4 to 5 gm per 24-hour. Urinary potassium is about 2.5 to 3.0 gm per day and rises when the intake is increased or in presence of excessive tissue catabolism. Potassium and sodium ratio is about 3:5. Calcium and magnesium are practically excreted through the faeces and very little amount is excreted through the urine. Daily excretion of calcium is about 0.1 to 0.3 gm and of magnesium is about 0.1 to 0.2 gm. The excretions of these two ions are dependent upon the nature of diet. Iodine, arsenic and lead are also excreted (Table 66.3).

ABNORMAL CONSTITUENT OF URINE

1. Protein

The normal urine contains <150 mg/day of protein. Normally only very trace or micro-amount of albumin (<20 mg/dl) are excreted through the urine. The most of the normal protein in the urine is Tamm-Horsfall

Table 66.3: Normal and abnormal constituents of urine

Normal constituents of urine		*Abnormal constituents of urine*
A. *Organic constituents*		• Protein
Nitrogen (total)	25–35 gm	• Albumin
Urea	25–30 gm	• Globulin
Creatine	60–150 gm (approx)	• Bence Jones proteins
Creatinine	1.4 gm (1.2–1.7 gm)	• Sugar
Ammonia	0.7 gm (0.3–1.0 gm)	• Glucose
Uric acid	0.7 gm (0.5–0.8 gm)	• Fructose
Hippuric acid	0.1–1.0 gm	• Galactose
Oxalic acid	10–30 mg	• Lactose
Amino acids (amino acid nitrogen)	150–200 mg	• Pentose
Allantoin	Small quantity	• Ketone bodies
Vitamins, hormones and enzymes	Small quantity	• Indican
B. *Inorganic constituents (per 24-hr)*		• Blood
Chloride	6–9 gm	• Pigments
Chloride as NaCl	10–15 gm	• Bile pigments
Phosphate as P	0.8–1.3 gm	• Urochromogen
Sulphate (total sulphur)	0.8–1.4 (average 1 gm)	• Porphyrin
Potassium	2.5–3 gm	• Melanin
Sodium	4–5 gm	• Casts
Calcium	0.1–0.3 gm	• Calculi
Magnesium	0.1–0.2 gm	• Pus
Iodine	50–250 µg	• Hormones
Arsenic	50 µg	
Lead	50 µg	

mucoprotein, which is produced by the thick ascending limb of loop of Henle.

Proteinuria (albuminuria) is the condition where albumin and globulin are frequently present in abnormally high concentration in the urine. Besides these, nucleoprotein, fibrin, myoglobin, haemoglobin, proteoses, peptones and Bence Jones proteins are present in the urine. Proteinuria is of two types: (i) Physiological and (ii) pathological (accidental). In physiological proteinuria, less than 0.5% protein is present and this may occur due to severe exercise, high protein meal, pregnancy, etc. Pathological proteinuria is of three types: (a) Pre-renal in which primary causes are the factors operating before the kidney is reached, (b) renal, where kidneys are involved primarily (i) causing filtration of increased amount of protein through the leaky glomeruli (glomerular proteinuria) and (ii) failure of proximal tubular function of reabsorption of α_2- and β_2-globulins and hence excretion through the urine (tubular proteinuria) and (c) post-renal, where destructive lesions occur in lower urinary tract. In pathological proteinuria along with protein, casts are found in the urine.

Casts are always accompanied by proteinuria. Casts are definite evidence of renal lesions and these are: (i) Epithelial casts shed from the tubules, (ii) coarse granular casts and (iii) waxy casts. Casts are generally composed of (i) albuminous material, (ii) degenerated tubule cells, (iii) red blood cells, and (iv) pus cells.

- Nephritis is the condition which represents a diffuse inflammation of the glomeruli and is always associated with urinary albumin, casts, haemoglobin, etc. In glomerulonephritis, glomeruli are characterised by inflammation along with secondary changes in the tubule.
- Nephrosis or degenerative nephritis is always characterised by degenerative changes in the tubules and also in the glomeruli both. Nephrosis is always associated with albuminuria, haematuria (blood in the urine), oedema, hypertension and anuria.
- Nephrosclerosis is the condition where there are pathological changes in the glomeruli as well as marked arteriosclerosis of the arteries and veins of the kidney.

Bence Jones protein: It is considered as a low-molecular weight globulin and may occur in the urine of patients with multiple myeloma, leukaemia (rarely), Hodgkin's disease and also in lymphosarcoma. The Bence Jones proteins have got a peculiar solubility. These proteins are precipitated out if the urine is heated at 50–60°C but redissolve again almost completely if the urine is heated further at 100°C. The precipitation reappears on cooling.

2. Glucose

In normal urine very negligible amount of glucose is present and it is clinically insignificant. About 140 mg per 24-hour is excreted through the urine. Abnormal excretion of glucose through the urine is called glycosuria. In diabetes mellitus, excessive glucose is excreted through the urine. About 10% or more glucose is excreted through the urine. This is associated with the increased blood sugar levels exceeding the threshold level.

Other sugars

- *Fructose/Fructosuria* is a condition when excess fructose is excreted through the urine. It is observed in severe cases of diabetes mellitus in which fructose is excreted along with glucose.
- *Galactose/Galactosuria* is a condition in which galactose is excreted through the urine of nursing infants.
- *Lactose:* It is frequently present in urine (lactosuria) of lactating mother as because the lactose may frequently come from the mammary gland in the general circulation. Since lactose as such is not utilised by the tissue, it is excreted.
- *Pentose/Pentosuria* is the condition in which transient excretion of pentose is observed through the urine. Congenital pentosuria is a benign genetic defect which is characterised by inability to metabolise L-xylulose.

3. Ketone Bodies

In normal individual 3–15 mg of ketone bodies are excreted through the urine per 24-hour. In diabetes, starvation, pregnancy and ether anaesthesia, excessive ketone bodies are excreted. The ketone bodies come from the excessive oxidation of fatty acids.

4. Indican

It is indoxyl potassium sulphate and is formed from indole. Indole is formed in the large intestine due to putrefaction of protein food. Indole is absorbed in the blood and being toxic to the body, is detoxified in the liver with the formation of indican, the less toxic and excretable substances. In normal urine very little amount of indican is formed but in abnormal condition when excessive putrefaction of proteins occurs, excess indican is excreted through the urine.

5. Blood

In cases of acute inflammation of the kidney, blood may be present in the urine (haematuria) which may be the result of a lesion in the kidney or urinary tract (e.g. after trauma in the urinary tract). In tuberculosis, cancer and renal stone, haematuria is frequently observed.

6. Pigments

- A number of pigments is found in the urine. These are urochromogen, bilirubin, porphyrin and melanin. Urochrome gives a normal colour of the urine. This urochrome comes from haemoglobin through series of reactions. In tuberculosis, urochromogen is excreted through the urine instead of urochrome.
- Bilirubin is excreted through the urine in obstructive jaundice when bile is re-absorbed from the biliary tract into the blood stream. Bile pigment makes the urine a greenish-yellow or golden-brown colour. In chronic malaria, wasting diseases, melanin is excreted through the urine.

7. Calculi and Casts

Mineral salts may precipitate and form calculi or stones. These stones may be formed in any part of the kidney tubules. These calculi or stones may be present in the urine.

In some abnormal conditions, kidney tubules become lined with substances that harden or form cast inside the tube. These casts are excreted through the urine. Nature of casts has already been discussed.

8. Pus

Pus cells often are formed in the urine in suppurative condition of the urinary system.

9. Hormones

Urinary excretions of different adrenal steroid hormones and gonadal hormones are altered considerably in different physiological and pathological conditions. Urinary neutral 17-ketosteroids are the reflection of the androgenic function of the subject. In adrenocortical carcinoma, hyperplasia of the cortex and testicular tumours, excretion of urinary 17-ketosteroids are tremendously increased. Excretion of 17-ketosteroids is decreased greatly in Addison's disease, pituitary dwarfism, Simmond's disease, etc. Pituitary gonadotrophins and placental gonadotrophins are also excreted through the urine in different physio-pathological conditions of the organ. Excretion of human chorionic gonadotrophin (hCG) is increased greatly in certain stages of pregnancy and in chorion epitheliomas and hydatidiform moles.

FACTORS AFFECTING FORMATION OF URINE

1. **Water intake:** When large quantities of water (1–2 litres) are taken, diuresis starts after a latent period of about 15–30 minutes. The flow becomes maximum in the second hour, when the output may be as high as 1,300 ml per hour (resting 50 ml per hour). Then it declines and comes back to normal in about three hours time. Even 5 litres of water drunk during two hours may

be completely excreted by the kidney in 4–5 hours. Rise of blood volume and the consequent dilution of plasma and the lowering of the osmotic pressure are very slight. Because, water passes out into the tissues almost as rapidly as it is absorbed from the intestine. As diuresis proceeds the water is slowly drawn in. As the quantity of urine rises, its specific gravity falls and may go down to 1.003.

Consequently, this diuresis is due to decreased reabsorption of water by the renal tubules. It is believed that dilution of blood, however small, reduces the secretion of anti-diuretic hormone of the posterior pituitary (through hypothalamus) and thereby reduces reabsorption of water.

2. **Effects of salts**
   - *Increased salt intake:* Experimentally, it has been shown that after ingestion of 28 gm of NaCl, flow of urine was increased to 120 ml per hour. The rate of salt excretion is comparatively slower. Consequently, after intake of salt, considerable amount of water has to be taken to dilute the retained salt and thus to maintain optimum osmotic pressure. The excess fluid and salt are slowly excreted in the course of a few days.
   - *Deprivation of salt:* This can be produced in man by giving a salt-free direct or by increasing the salt loss by inducing profuse sweating. In such cases, it is found that at first both plasma chloride level and chloride excretion in the urine progressively fall. Later on chloride excretion practically ceases due to complete reabsorption. At this stage the level of plasma chloride rises to some extent and remains only slightly below normal.

     Under such conditions, it is found that kidney function is seriously disturbed although blood pressure remains unaltered. Glomerular filtration is decreased by 30%, urea clearance is decreased from 40 to 80% of normal, blood urea increases and a state of uraemia develops. The decreased rate of glomerular filtration may be one cause in producing this uraemia. Water intake does not induce the normal diuresis in such cases.
3. **Effect of water deprivation**
   - *In adults:* The renal circulation remains unchanged. There is no decrease in plasma volume and no haemoconcentration. Because, fluid is drawn from the tissue spaces. Body weight decreases by about 3–5 kg. The volume of glomerular filtrate is reduced by 20% and the volume of urine per hour is reduced to 30–40 ml. At this rate of output (30 ml per hour), urea, creatinine, phosphate and other nitrogenous and non-nitrogenous solids in urine become concentrated to the maximum. It means that unless the urine volume is increased, larger amounts of these constituents cannot be eliminated. On the other hand, if urine volume is further reduced, total solid excretion diminishes, so that they accumulate in the blood leading to uraemic symptoms.
   - *In children:* Effects of water deprivation are more disastrous for the children. Because, at birth the kidney is not functionally developed, as is proved by the fact that infant urine is hypotonic (and not hypertonic as in adults). Because, the kidneys cannot concentrate the urine. Hence, more water is required to excrete a given amount of solid. Comparatively, a small quantity of fluid is available in the body to be out as urine, as with diarrhoea or vomiting, and consequently there is retention of urea and other nitrogenous constituents producing uraemia, rapidly. Hence in children, suffering from diarrhoea, vomiting, etc. the fluid intake should be kept high.
4. **Effects of exercise:** It always reduces urine volume. The initial effects are not due to adrenaline secretion or renal vasoconstriction. Emotional states also produce similar results. It is suggested that both exercise and emotion, acting upon the hypothalamo-pituitary mechanism, increase the secretion of anti-diuretic hormone and thus urine output is reduced. After severe exercise the urine volume is further reduced and becomes more acid in reaction.

REACTION OF URINE

The reaction of urine is generally acid throughout the greater part of the day. The normal range of pH of urine is 7.5–5.0, the average being 5.3. After a meal the human urine may be alkaline. This phenomenon is known as alkaline tide. It is believed to be due to relative increase of alkaline radicals in blood caused by the passage of large amount of HCl in the gastric juice. Increased respiration due to loss of CO_2 causes alkaline urine. Urinary acidity also occurs during exercise due to increased production of lactic acid and in diabetic ketosis.

Total amount of acids eliminated per day varies from 600–700 ml of N/10 acid, of which about 400 ml is excreted as ammonium salts (not titratable) and the rest 300 ml is in the form of titratable acidy. The pH of the urine may be determined by indicators or by titration or by hydrogen electrode. Owing to the buffering action of phosphates, the titratable acidity of urine is always higher than is suggested by its H^+ ion concentration.

For all practical purposes any alteration of the reaction of urine reflects faithfully the tendency of blood reaction. In acidosis, it becomes more acidic, in alkalosis, it becomes alkaline. The explicit purpose is to maintain the body reaction constant.

The mechanisms by which kidney regulates the blood reactions and consequently alter the reaction of urine are described as follows.

MECHANISM OF REGULATION OF BLOOD REACTION BY THE KIDNEYS

Maintenance of blood reaction to neutrality is an essential condition for life. Various factors take part in this regulation in which kidneys have the main role. During acidosis, the acidity of urine might be 1,000 times more than plasma. Kidneys regulate the blood reaction in the following five ways:

BICARBONATE MECHANISM

1. The epithelial cells of kidney tubules (proximal, distal and collecting one) secrete hydrogen ion into tubular fluids and change the reaction of the urine accordingly. Bicarbonate usually in combination with Na^+ is present in glomerular filtrate, but it is absent from the normal urine due to complete reabsorption. Na^+ is absorbed by the tubular cells in exchange of H^+ which is secreted by them.
2. The entry of Na^+ ions into the proximal tubular epithelium is a passive process as the movement is only diffusion down a concentration gradient. But on the contrary, the hydrogen ion moves in the reverse direction with an active secretory pump system.
3. The hydrogen ion combines with bicarbonate ion to form carbonic acid. The carbonic acid breaks up into CO_2 and H_2O, the CO_2 diffuses into the tubular cells. Inside the cell, this CO_2 unites with water to form carbonic acid again; the reaction is catalysed by the enzyme, carbonic anhydrase. The carbonic acid dissociates into bicarbonate ion and hydrogen ion, the latter is secreted into the tubular lumen. If enough hydrogen ions are present, almost all the bicarbonate filtered by the glomerulus is re-absorbed.
4. The net result of this mechanism is re-absorption of Na^+ and bicarbonate of the tubular fluid into blood capillaries in exchange of H^+ ion.

The factors affecting bicarbonate transport are as follows:

a. *Changes in the carbon dioxide tension (pCO_2) of the arterial blood:* Increased pCO_2 in the arterial blood causes significant increment in bicarbonate reabsorption.

b. *Variations in the body store of potassium:* The intracellular concentration of the potassium and not of the plasma is the factor that controls the bicarbonate reabsorption. Cooke, Darrow and others have shown that when excess potassium was infused, potassium rapidly enters into the cell in the exchange of H^+ ion. The H^+ ions leaving the cells are buffered by bicarbonate of extracellular fluid and the plasma bicarbonate content is reduced. So, in the renal tubular epithelium gives up less H^+ ion and thereby less bicarbonate is re-absorbed.

c. *Variations in plasma level of chloride:* Increase or decrease of chloride concentration in plasma causes decrease or increase of bicarbonate concentration respectively. When the chloride excess is excreted or the chloride deficit is replenished, the plasma level of bicarbonate rapidly returns to normal.

d. *Variation in the secretion of the adrenal cortical hormones:* The hormones of the adrenal cortex have some role in bicarbonate reabsorption but the actual mechanism is not known.

PHOSPHATE MECHANISM

1. Both disodium hydrogen phosphate and monosodium dihydrogen phosphate are present in the plasma. The pH of the urine is determined by the ratio of disodium hydrogen phosphate to the monosodium dihydrogen phosphate.
2. In plasma, the concentration of the disodium hydrogen phosphate (alkaline phosphate) exceeds that of the monosodium dihydrogen phosphate (acid phosphate) and the ratio of 4:1. But in the urine the concentration of the monosodium dihydrogen phosphate exceeds that of the disodium hydrogen phosphate and the ratio becomes 9:1.
3. The kidneys naturally eliminate more acid phosphate and retain the basic phosphate as far as possible. This is mainly done by altering the excretion of alkaline and acid phosphates.
4. In acidosis, more acid phosphates are eliminated. In alkalosis, alkaline phosphates are excreted in excess. In this way the constant ratio between the two phosphates in blood is maintained and the blood reaction is kept constant. The possible theories for phosphate reabsorption along with excretion are shown in Figs 66.1 to 66.3.

During excretion of large amounts of acid the tubular cells also manufacture ammonia from the glutamine

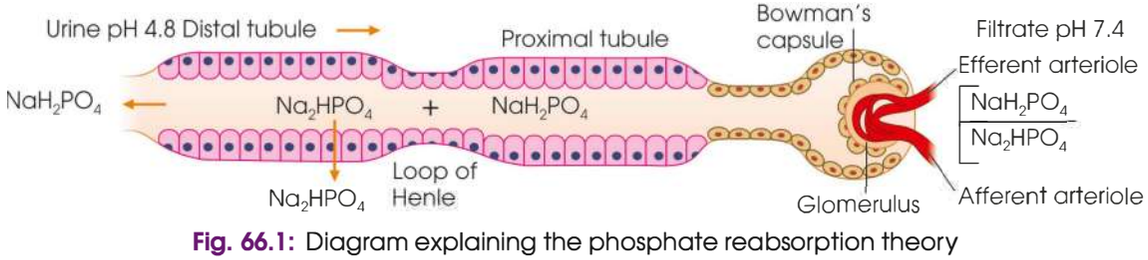


Fig. 66.1: Diagram explaining the phosphate reabsorption theory

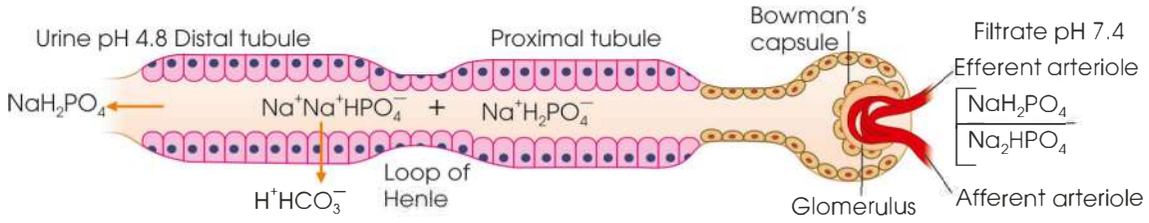


Fig. 66.2: Diagram explaining the tubular ion exchange theory

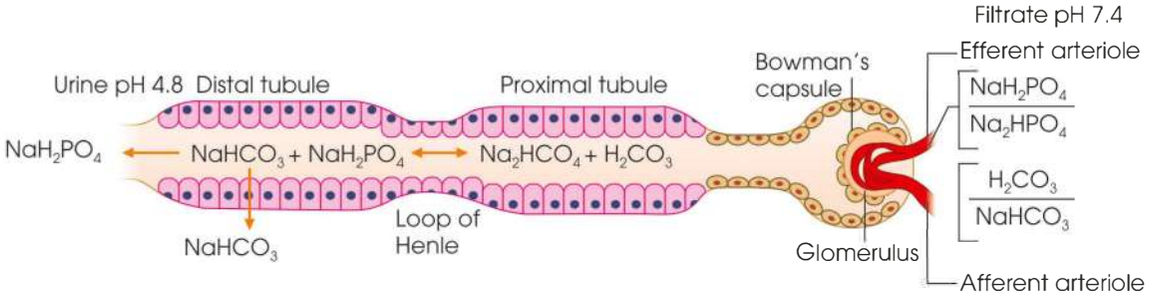


Fig. 66.3: Diagram explaining the carbonic acid filtration theory

and other amino acids, and ammonium salts are formed and excreted.

AMMONIA MECHANISM

1. It has been discussed earlier that the cells of the renal tubules contain deaminases (amino acid oxidases) which can produce ammonia by the deamination of amino acids. The ammonia, formed with the action of glutaminate on glutamine (60%) and also with other reactions is passed out into the lumen of the tubules where ammonia combines with the acid radicals and thus spares the fixed bases (e.g. Na^+, K^+, etc.). These bases are reabsorbed to maintain the alkali reserve.
2. During acidosis the amount of ammonia formation is markedly increased. The effect might be due to an increase in the renal enzyme, glutaminase or amino acid oxidase. Adrenal cortical hormone has some role in this adaptive mechanism also. This is an important way by which kidneys help to maintain a constant blood reaction.

ELIMINATION OF ACIDS

There are evidences that the kidney tubules can secrete H^+ ion to maintain the blood reaction. For the mechanism of secretion of H^+ ion, two theories have been suggested. Either molecular acid combines with titratable acids after secreted by the renal tubules, or the H^+ ion is exchanged against the cations across the tubular epithelium. The second theory has been supported by many evidences and is now mostly accepted one (Fig. 66.4). There are three major factors that affect the rate of formation of titratable acids:

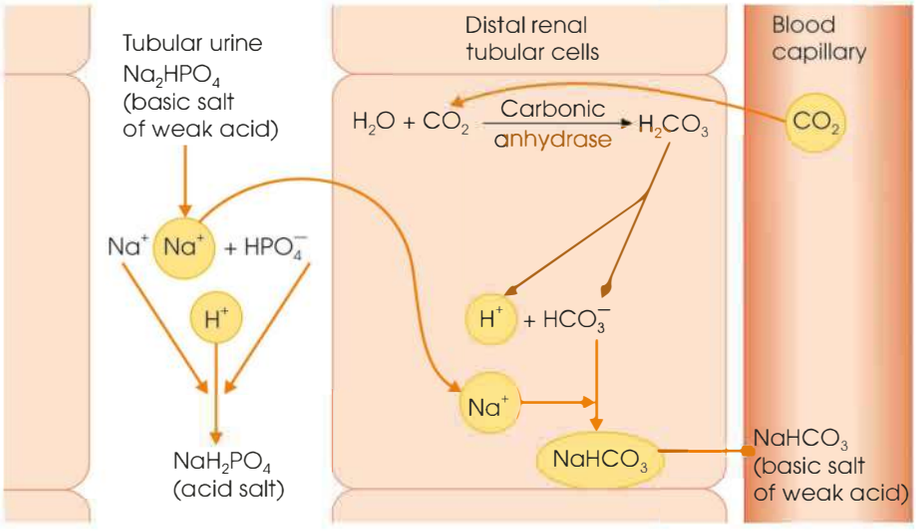


Fig. 66.4: Schematic representation of mechanism of formation of titratable acid by ion exchange in the distal renal tubular cells

(i) Rate of excretion of the buffer, (ii) the pK' of the buffer and (iii) the degree of acidosis.

Kidneys excrete directly some acids, such as uric acid, hippuric acid, the abnormal acids like aceto-acetic acid, β-hydroxybutyric acid, glucuronic acid, homogentisic acid and so on. Although their acid value is not of much practical importance, yet it is a distinct attempt on the part of the kidneys to maintain blood reaction.

ELIMINATION OF ALKALIS

1. Sodium bicarbonate, being a high threshold substance, is completely reabsorbed by the renal tubules.
2. But in alkalosis or when excess sodium bicarbonate is administered, it may be directly eliminated in the urine. Under such conditions, both bicarbonates and alkaline phosphates are found in the urine. One curious fact is the presence of large amounts of chloride in the diet; which diminishes the excretion of bicarbonates through the kidneys and therefore hampers the function of kidneys to combat alkalosis.
3. Although it is customary to say that urine reaction reflects the tendency of blood reaction, yet it seems that preservation of an optimum saline concentration and crystalloid osmotic pressure is of greater importance than the maintenance of a constant H ion concentration. In order to attain this end, the normal pH level might have to be sacrificed. In excessive vomiting and diarrhoea, increased loss of chlorides occurs, leading to a fall of plasma chlorides and consequently the osmotic pressure. The base left behind by the loss of Cl^-, combines with HCO_3^- to form more bicarbonate. These excess bicarbonates help to maintain the osmotic pressure of blood but, at the same time, cause alkalaemia (Fig. 66.5).

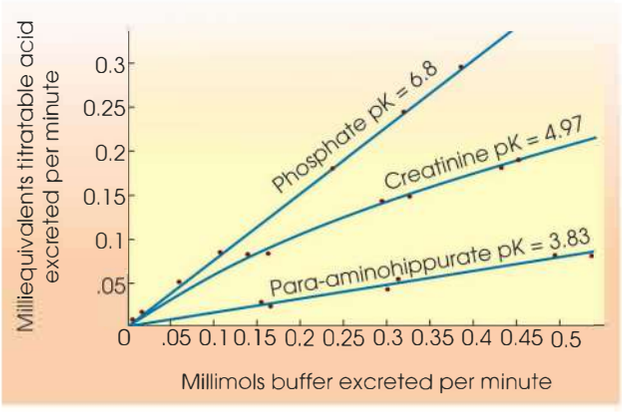


Fig. 66.5: Graphical representation of relationships of the rate of excretion of titratable acids to the rate of excretion of buffer and to pK' of the buffer in an acidotic subjects: Neutral sodium phosphate (having the highest pK), creatinine and sodium p -aminohippuric acid are infused at such rates as to cause equimolar increases in rates of excretion

In such cases, acid urine may be passed in spite of alkalosis. This shows that probably electrolyte balance and osmotic pressure are of more importance than a constant pH. Whenever, there comes a clash between these two factors, kidneys will try to maintain the more essential, sacrificing the lesser one.

4. In addition to carbonic acid, which is eliminated by the respiratory organs as CO_2, other acids which are not volatile are produced by metabolic processes. These include lactic and pyruvic acids and the most inorganic acids—hydrochloric, phosphoric and sulphuric. About 50–150 mEq of these inorganic acids are eliminated by the kidneys in a 24-hour period. It is of course necessary that these acids be practically buffered with cation largely sodium, but in the distal tubules of the kidneys some of the cation is reabsorbed (actually exchanged for hydrogen ion) and the pH of the urine is allowed to fall. This acidification of the urine in the distal tubule is a valuable function of the kidney in conserving the reserves of cation in the body.
5. Another device used by the kidney to buffer acids and thus to conserve fixed base (cation) is the production of ammonia from amino acids. The ammonia is substituted for alkaline. Cations are the amounts of ammonia mobilised for this purpose may be markedly increased when the production of acids within the body is in leisure (e.g. as in metabolic acidosis such as occurs as a reason of the ketosis of uncontrolled diabetes).

When alkali is in excess, the kidney excretes alkaline urine to correct this imbalance (the details of the renal regulation of acid–base equilibrium are discussed elsewhere).

In kidney disease, glomerular and tubular damage results in considerable impairment of these important renal mechanisms for the regulation of acid–base balance.

GLYCOSURIA

Glycosuria is a condition in which glucose is present in the urine in such quantities as will reduce Benedict's or Fehling's reagent.*

It is obvious that sugar may be present in the urine under two general conditions:

1. When the blood sugar level goes above the renal threshold value of sugar, i.e. 0.18%.
2. When the degree of tubular reabsorption of sugar is deficient, i.e. where the renal threshold is low.

All the different types of glycosuria can be put under these two groups: The first group is always attended with hyperglycaemia. In the second group, the blood sugar may be normal or even low. By the term

*Urine may also contain other reducing substances such as uric acid, oxalates, glucuronic acid lactose (in advances pregnancy or in lactating mothers) and these conditions should be carefully eleminated for the diagnosis of a case of glycosuric

hyperglycaemia is meant a condition, in which blood sugar rises above the maximum normal range, i.e. 0.12%. When this level goes beyond 0.18%, glycosuria takes place. So that, hyperglycaemia between 0.12% and 0.18% is not attended with glycosuria. Thus, glycosuria may be either due to hyperglycaemia or due to less tubular reabsorption or diminished renal threshold. These two groups are described below.

GLYCOSURIA DUE TO HYPERGLYCAEMIA

The following conditions give rise to hyperglycaemia which may lead to glycosuria:

Alimentary glycosuria: When a large carbohydrate diet is taken, blood sugar may rise and cause glycosuria. This condition does not seem to be a normal process because hormonal control of carbohydrate metabolism is so efficient that a large amount of it can be taken as food without any appreciable variation in blood sugar level. Alimentary glycosuria therefore is only possible in those subjects in whom the power of sugar utilisation is deficient and subjects should be considered as early cases of diabetes.

Nervous glycosuria: Stimulation of the sympathetic nerves to the liver or of the splanchnic nerves breaks down liver glycogen and produces hyperglycaemia, which may lead to glycosuria. Nervous stimulation mentioned above causes glycogenolysis directly, and is also responsible for increased liberation of adrenaline, which also activates glycogenolysis. Thus anything that stimulates sympathetic system, such as excitement, stress, etc. may produce glycosuria.

Endocrine glycosuria: Deranged function of a number of endocrine glands produces hyperglycaemia which may result in glycosuria. The important examples are as follows:

- **Pancreas (diabetes mellitus or pancreatic diabetes):** This has been fully dealt within the chapters of Metabolism of Carbohydrates and Endocrines. In this condition the β-cells of the islets of Langerhans fail to secrete adequate amount of insulin. Lack of insulin produces hyperglycaemia.
  - Urine may also contain other reducing substances; such as uric acid, oxalates, glucuronic acid, lactose (in advanced pregnancy or in lactating mothers), and these conditions should be carefully eliminated for the diagnosis of a case of glycosuria.
- **Hyperthyroidism:** Hyperactivity of the thyroid is always attended with low sugar tolerance, hyperglycaemia and may be, glycosuria.
- **Adrenaline (epinephrine):** Increased secretion of adrenaline stimulates synthesis of cyclic adenylic acid which activates inactive phosphorylase in hepatic and extra-hepatic tissues. The active phosphorylase then causes breakdown of glycogen to glucose phosphate and then to glucose. The resultant effect is hyperglycaemia and ultimately glycosuria. Prolonged administration of adrenaline through subcutaneous route may increase the amount of liver glycogen by converting muscle glycogen into lactic acid and then lactic acid to liver glycogen.
- **Anterior pituitary:** Hyperactivity of the anterior pituitary is also attended with hyperglycaemia and glycosuria (20–30% cases). This is supposed to be due to increased secretion of the growth or somatotrophic and adrenocorticotrophic hormones.
- **Adrenal cortex:** Hyperactivity of the adrenal cortex is believed to cause hyperglycaemia which may lead to glycosuria. Adrenal corticoid, e.g. cortisol, stimulates neoglucogenesis and produces hyperglycaemia and glycosuria and increased resistance to insulin.
- **Glucagon:** It is secreted by the δ-cells of the islets of Langerhans. It also activates the inactive phosphorylase (only in liver) like that of adrenaline and thus stimulates glycogenolysis, producing hyperglycaemia and glycosuria.
- In many clinical conditions, glycosuria is found to take place. For instance: Asphyxia, exited conditions, acute infective conditions, administration of anaesthetics, acute painful conditions, coma, etc. usually cause hyperglycaemia and often glycosuria. In all such conditions liver glycogen is found to be depleted. All these conditions are believed to act by causing sympathetic stimulation and thereby adrenaline secretion. There may also be an active inhibition of the β-cells of the islets of Langerhans.

GLYCOSURIA DUE TO LESS TUBULAR REABSORPTION

In the following conditions, renal reabsorption of sugar is defective. Blood sugar may be normal or low but still there is sugar in the urine.

- **Renal glycosuria:** This is usually a developmental defect. The renal glycosuria is congenital and often demonstrated as familial. In one type the tubular maximum for tubular reabsorption (TmG) is reduced in the same line with the renal threshold. But in the other type there is no such relationship or any definite system. The cause for this irregularity in the second type may either be due to excessive glomerulotubular imbalance or to altered kinetics of the carrier system. It is probable that the receptor carrier mechanism, which is responsible for the reabsorption of sugar by the renal tubule, fails to develop properly in this condition. This type of glycosuria is not attended with ketosis or the other ill effects generally found in diabetes mellitus. The man is otherwise completely healthy.
- **Phloridzin glycosuria:** Phloridzin is a glucoside, found in the root of apple tree. When hydrolysed it gives glucose and phloretin. When phloridzin is

administered subcutaneously it gives rise to intense glycosuria (the dose given to dogs is 1 gm per day, in oil, subcultaneously). Certain other glucosides, such as arbutin have similar effects. Phloridzin binds up strongly with membrane carrier preventing the glucose to attach with the membrane carrier and thus inhibits glucose reabsorption.

- **Diseases of the renal tubules:** In some cases of kidney disease, the tubules may be damaged, thus failing to reabsorb sugar. Consequently, sugar may be present in the urine.
- **Due to heavy metal poisoning:** The heavy metals like lead, cadmium, mercury, etc. produce damage to the kidney cells thereby causing renal type of glycosuria.
- **In pregnancy:** There is glycosuria due to lowering of renal threshold with the advancement of pregnancy.

From the above, it will be evident that glycosuria may take place under a variety of conditions. Investigation of a case of glycosuria is of immense importance for clinical purposes. Sugar tolerance test is of considerable value in this connection.

FACTORS CONTROLLING VOLUME OF URINE

The average volume of urine per day is 1,500 ml. But this volume is not a fixed quantity. It may increase or diminish considerably. There are two principles on which urine volume will depend. One is the rate of glomerular filtration and the other is the degree of tubular reabsorption. Any factor, having influence on urine volume, will have to work on one or both of these two principles.

The factors that affect urine volume may be briefly summarised as follows:

1. *Water intake:* Urine volume is directly proportional to the amount of water taken.
2. *Elimination of water by other channels:* Urine volume is inversely proportional to the amount of water lost through other channels. For instance, diarrhoea, dysentery, cholera, excessive vomiting, etc. will all reduce the output of urine.
3. *Rate of renal circulation:* Urine volume is directly proportional to the degree of renal circulation. During sleep urine output is decreased. This is partly due to reduced renal circulation. Anything that improves renal circulation will increase urinary volume.
4. *Available filtration pressure:* Available filtration pressure is the resultant of many opposing forces. Raised general blood pressure (as also increased blood volume—intravenous saline, etc.) increases filtration, and thereby urine volume. On the other hand, with fall of general blood pressure (as in shock, haemorrhage, heart failure, vasomotor failure, etc.), urine volume is reduced. Glomerular pressure may vary independent of general blood pressure. For instance, constriction of afferent arteriole (e.g. by adrenaline) will reduce glomerular pressure and reduce urine volume. On the other hand, dilatation of the afferent vessel (e.g. by caffeine) or constriction of the efferent vessel increases glomerular pressure and raises the amount of urine. Pressure in the ureter exerts an opposite effect. Rise of ureteric pressure, such as by a stone, reduces filtration and diminishes urine flow.
5. Colloidal osmotic pressure of plasma also affects urine volume. Reduction of colloidal osmotic pressure (such as by intravenous fluid administration or after ingestion of a large amount of water) increases filtration and thereby the volume of urine. On the other hand, increased concentration of plasma proteins (which may occur by excessive fluid loss through other channels) reduces urine volume.
6. *Number of active glomeruli:* It is obvious that, other factors remaining constant, amount of filtration will depend upon the number of active glomeruli. It is possible that during increased activity of kidney, a proportion of the inactive glomeruli will open up and help to increase the flow of urine.
7. *Permeability of the filter bed:* Urine volume directly depends upon the permeability of the glomerular membrane. Anything that reduces the permeability reduces the urine volume.
8. *Degree of tubular reabsorption:* Normally, the renal tubules reabsorb water to about 90% from the capsular filtrate. The degree of reabsorption depends upon the anti-diuretic hormone secreted by the posterior pituitary. This hormone increases the degree of reabsorption. In any condition, where the blood is more diluted (increased water intake, intravenous saline and so on) the secretion of this hormone is depressed, and therefore, more water is allowed to pass out. In conditions of haemoconcentration more of this hormone is secreted, better tubular reabsorption takes place, resulting in less volume of urine. There is a pathological condition in which the secretion of this hormone is lacking (disease of the posterior pituitary of hypothalamus) and this may produce very large amount of dilute urine. This condition is known as diabetes insipidus where excretion of very large amounts of pale urine of low specific gravity and extreme thirst are caused.
9. Certain drugs such as caffeine, mercurial diuretics, etc. reduce the power of absorption and increase the urine flow. In some types of kidney

disease, in which the tubules degenerate, amount of urine increases due to failure of reabsorption.

10. *Amount of solids to be excreted by the kidney:* The osmotic pressure of the tubular contents work against reabsorption. In any condition, therefore, where urinary solids increase in amount, the osmotic pressure will rise, reabsorption will be retarded and as such the volume of urine will rise. Some such conditions are as follows:
    - Excess intake of salts.
    - Excess intake of sulphates, urea, etc.
    - Excess protein diet produces more urea, hence, more urine flow.

    Thyroxine stimulates metabolism, produces more metabolites and, therefore, increases the amount of urine.

- Hypo-function of adrenal cortex increases urine volume. It is partly due to associated salt loss and probably also due to reduced reabsorption of water by tubules.
- In diabetes mellitus a large amount of sugar is present in the urine. This naturally increases the osmotic pressure and reduces reabsorption of water. It is due to this, that in diabetes, urine volume increases.

Various other factors may be mentioned which affect urine volume. But in all cases they will be found to work either by altering the rate of filtration or the rate of tubular reabsorption or both. On the whole it should be remembered that one function of kidney is to maintain the water balance of the body. In any condition where the water content of the body increases, urine volume will also rise. When the water content falls, volume of urine will be reduced. It must also be remembered that to eliminate the solid waste products a minimum amount of water must pass out.

Thus on the whole, the volume of urine represents either the inevitable minimum or the unnecessary excess. The regulating machinery is extremely efficient.

Abnormal Volume of Urine

1. **Nocturia**, early stage of kidney disease indicated by passage of more urine at night.
2. **Oliguria**, marked reduction of urine volume due to impairment of kidney function.
3. **Anuria**, complete suppression of urine flow due to severe kidney disease.

Applied Physiology

Renal Failure

The decreased output of urine due to loss of nephron functional capacity may progressively lead to acute and chronic renal failure.

Acute renal failure: Progressive and reversible loss of renal functions with decreasing urine output may manifest as acute renal failure. The causes for acute renal failure may be due to renal causes (glomerular disease, interstitial kidney disease, acute tubular necrosis, etc.; *post-renal causes* (prostate enlargement, renal tumour, renal carcinoma, renal stones, etc.; *pre-renal causes*: Systemic heart failure, hypovolemic shock conditions, etc. The patient presents with oliguria (urine output is less than 400 ml/day, anorexia, nausea, vomiting, confusion, hyperkalaemia, anaemia, etc. The management includes treatment for underlying causes, e.g. correction of metabolic acidosis, hyperkalaemia, etc. IV fluids for electrolyte balance and renal dialysis if needed.

Chronic renal failure: The irreversible changes and severe deterioration in renal function leads to chronic renal failure. The common causes are hypertension, diabetes mellitus, glomerular disease, polycystic kidney, systemic inflammatory disease, renal artery stenosis, etc. Fluid replacement, renal dialysis and kidney transplant are the only way of its management.

Joseph Edward Murray who performed the first successful human kidney transplant. Murray shared the Nobel Prize in Physiology or Medicine in 1990 with E. Donnall Thomas for their discoveries concerning "organ and cell transplantation" in the treatment of human disease.

Joseph Edward Murray
1919–2012

EXAM-ORIENTED QUESTIONS

Essay

1. Discuss regarding the renal clearance test.
2. Describe the abnormal constituents of urine. Discuss the cause for the physiological variation.
3. Discuss the factors affecting formation of urine.
4. Describe the factors that affect urine volume.
5. Describe the mechanism of regulation of blood reaction by the kidneys.

Short Notes

1. Inulin clearance test
2. Creatine clearance test
3. Glycosuria
4. Alimentary glycosuria
5. Nervous glycosuria
6. Renal failure

Chapter

67

Micturition

INTRODUCTION

The micturition is the process of accumulation of formed urine in the urinary bladder and evacuation of the same from the bladder from time to time, which are controlled by the nervous mechanism.

STRUCTURE OF URINARY BLADDER

Bladder serves as a distensible reservoir for urine and to evacuate its contents at suitable intervals. The urinary bladder consists of two main parts: (1) The body and (2) the trigone (Fig. 67.1). The body is a hollow sac of detrusor muscle; the interior is lined by layer of transitional epithelium which rests on lamina propria. The transitional epithelium of the innermost layer has got corrugations—rugae which are gradually flattened during filling of the bladder. During filling, the body of the bladder expands and the muscles contract during micturition reflex to evacuate it. The trigone is a small triangular region formed by connecting the openings of two ureters and the urethra. The smooth muscles surrounding the opening of the urethra form the internal sphincter of bladder. The muscles are tonically contracted till the pressure inside the bladder is raised high enough to open it. Distal to this the striated muscle of urogenital diaphragm forms the external sphincter of bladder. This muscle is also tonically contracted all the time excepting during micturition when it relaxes under reflex or voluntary control. The nerve supply of bladder and urethra in man are given in Fig. 67.2 (Tables 67.1 and 67.2).

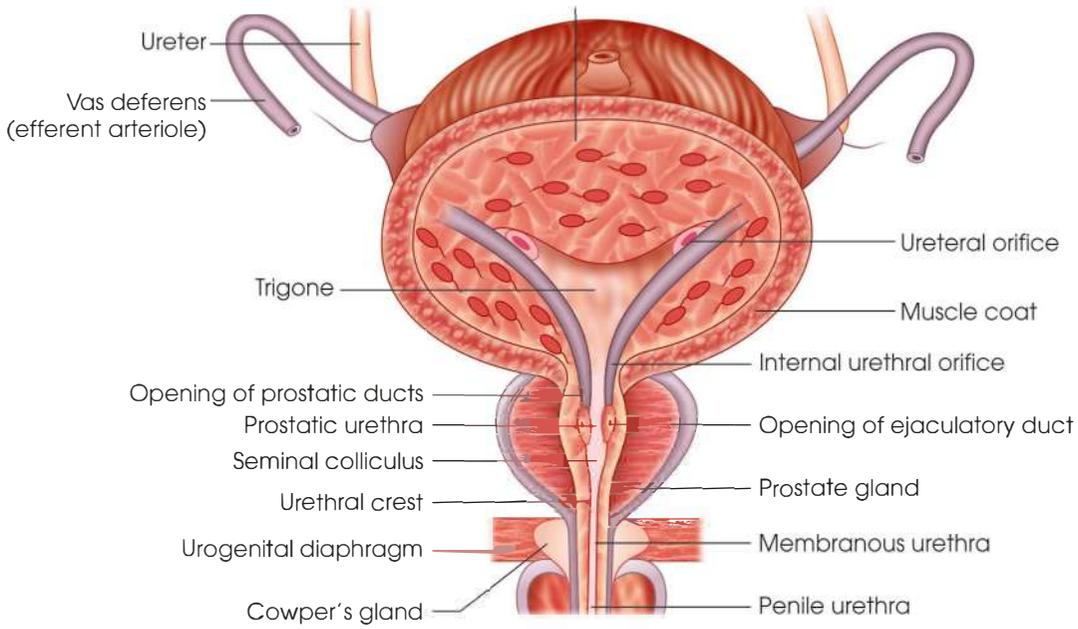


Fig. 67.1: Diagrammatic representation of the bladder to show the bladder wall, orifices of the ureters, urethra and related structures. The orifices of the urethra and ureters form the trigone. Also note that the ureters enter the bladder wall from the posterior and run vertically downwards

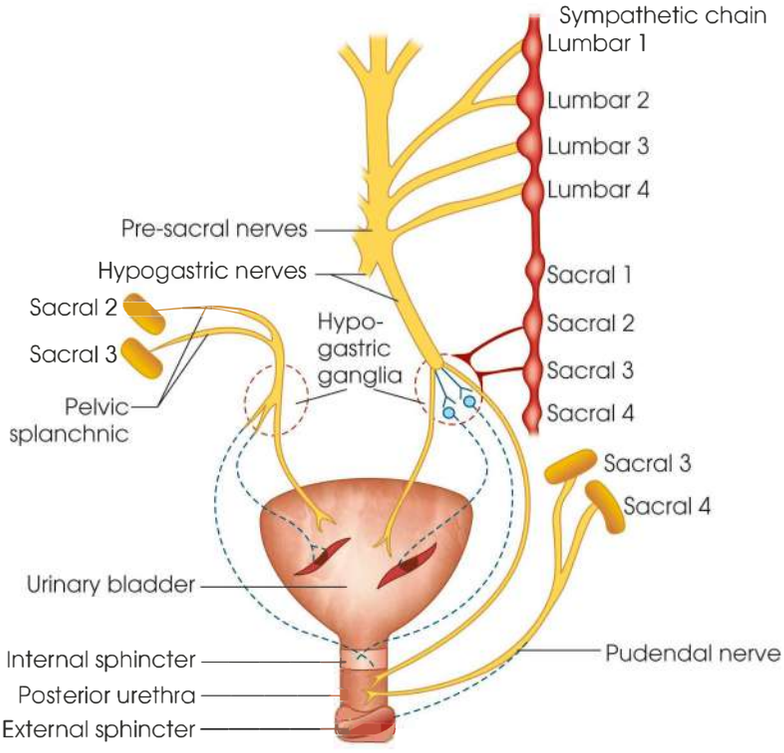


Fig. 67.2: Diagrammatic representation of the innervations of the bladder and the urethra in man

Table 67.1: Nerve supply of bladder

Efferent fibres (motor)	*Nerve roots*	*Peripheral nerves*	*Structures innervated*	*Functions*
Somatic	S_3 and S_4	Pudendal nerve	External sphincter and distal urethra	Control micturition (voluntary)
Sympathetic	T_{11} and T_{12} and L_3–L_4	Hypogastric nerves	Bladder and internal sphincter	Relax the bladder wall and constrict the internal sphincter
Parasympathetic	S_2–S_4	Nervi erigentes (pelvic nerves)	Muscles of bladder (detrusor) and internal sphincter	Contraction of the bladder and relaxation of the internal sphincter

Table 67.2: Nerve supply of urethra

Afferent (sensory) fibres (concerned with pain and conscious awareness of distension) from the organ/structure	*Peripheral nerves*
Bladder cavity	Hypogastric nerves
Muscles of the bladder (detrusor)	Pelvic nerves
Urethra	Pudendal nerve

MECHANISM OF FILLING OF BLADDER

Normally, bladder becomes filled up with urine coming from the two ureters. Peristaltic waves pass down the ureters from the renal pelvis to the bladder. With each such wave a jet of urine enters the bladder. The waves travel at a speed of 20–25 mm per second and with a frequency of 1–5 per minute. With each wave, urine enters the bladder and the intra-vesical pressure rises, which is followed by an immediate fall of pressure (nearly to the initial level) due to relaxation of the detrusor muscle. In this way, the bladder volume increases due to gradual relaxation of the detrusor muscle with the increased volume of urine accumulated in it, but without any appreciable rise of pressure (Laplace's law). This process continues until the urine volume (i.e. bladder volume) is about 400 ml beyond which the bladder wall fails to relax further and so the pressure rises up without any appreciable fall (Fig. 67.3). In this way, the bladder gradually fills up. When about 350 to 400 ml of urine is collected the normal desire for micturition is felt. By voluntary effort, the onset of micturition can be delayed till a maximum of about 700–800 ml of urine accumulates in the bladder when it becomes urgent and painful. No further inhibition is possible beyond this stage and micturition will automatically begin. Under normal conditions, both the internal and external sphincters remain tonically contracted.

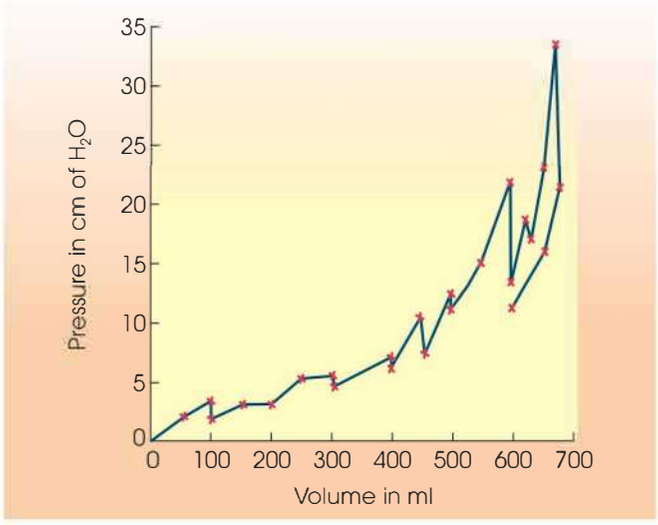


Fig. 67.3: Graphical representation of the pressure changes in the bladder during filling and emptying

MECHANISM OF MICTURITION

Key Features

1. The act of micturition involves the co-ordinated contraction of the smooth muscle of the bladder wall (detrusor muscle), abdominal wall and muscles of the pelvic floor, fixation of the chest wall and diaphragm, relaxation of the internal and external urethral sphincters. Accordingly, there are involvements of autonomic and voluntary activities.
2. The process of micturition can be studied by inserting catheter in the bladder, connecting it with a suitable manometer and studying the process under various conditions (cystometrogram). When the normal process of micturition is compared with the behaviour of a denervated bladder, the following facts are seen: In the denervated bladder the power of tone adaptation is lost and micturition starts much earlier than in a normal bladder. Although micturition starts yet the act is not complete. Only partial emptying of bladder takes place and some residual urine is always left behind. Whereas with normal bladder the power of tone adaptation is perfect, micturition starts much later (i.e. greater filling is allowed), and the act of micturition is a complete process without any urine being left behind.

   This remarkable difference in the behaviour of normal and denervated bladders indicates that, under normal conditions, bladder does not behave simply as a hollow muscular organ, but that, nerves are necessary for its normal activity. In other words, the normal act of micturition depends on the integrity of the nerves of bladder. These considerations show that micturition depends on nervous reflexes.
3. In the denervated bladder, which acts simply as a muscular bag, only moderate filling raises the pressure to the adequate level causing contraction of the muscles and thereby starting micturition. When some quantity of urine is passed out, intra-vesical pressure falls below the critical level and further contraction ceases, till the requisite amount of urine accumulates again. In this way, always residual urine is left behind. But in normal bladder when micturition starts, intra-vesical pressure does not fall although fluid passes out. Because during the process, many sensory impulses arise from the bladder and the urethra and reflexly stimulate further contraction of the bladder until the act is complete.
4. Voluntary effort has enormous control over the process of micturition. Experimentally it has been shown that micturition can be inhibited for a long time or started much earlier by volitional effort. Volition can exert its influence both upon the sympathetic and parasympathetic supply of bladder and alter their activity. It is believed that in the normal micturition there is at first a voluntary removal of the constant inhibition upon the bladder, so that the lower centres are released. In this way micturition begins. When the bladder is not sufficiently filled, contraction of the bladder can be brought about by stimulating the pelvic visceral nerves by volitional efforts.
5. Moreover, raised intra-abdominal pressure caused by voluntary contraction of the abdominal muscle and that of the diaphragm, exerts pressure upon the bladder and mobilises micturition reflexes. In infants, due to non-myelination of the pyramidal tracts, the act of micturition is not under voluntary control. But since, the other centres are working properly the act although involuntary, yet becomes complete.

Micturition Reflex

As the bladder radius progressively increases with accumulation of urine coming from the ureters, the tension on the bladder wall increases. This tension on the vesical wall stimulates the proprioceptive end organs situated in the bladder wall which sends afferent impulses in the spinal reflex centre for micturition (S_2–S_4), in the reflex centre for micturition in the brain stem (pons) and in the voluntary centre for micturition in the paracentral lobule in the cerebral cortex.

Normally the spinal reflex centre for micturition is inhibited by impulses from the brain stem centre even though the bladder is full. When an opportunity to empty the bladder is found, the inhibition of the higher centre to the spinal reflex centre is lifted and the micturition reflex begins. Parasympathetic efferent impulses from the spinal reflex centre to the detrusor muscle of the bladder wall. Contraction of the detrusor widens and shortens the posterior urethra resulting in passage of a bolus of urine into the posterior urethra. Urine in the posterior urethra stimulates the spinal

centre which reflexly relaxes the external sphincter of the urethra and urine flows through the urethra.

Micturition reflex is a 'self-regenerative process' which means initial contraction of the bladder further stimulates the receptors to cause further increase in reflex contraction of the bladder. Thus, a cycle starts which repeats again and again until the bladder reaches a state of strong contraction. After some time the reflex begins to fatigue and the self-regenerative cycle ceased.

Centres of Micturition

The central control of micturition lies at four levels: (1) Cortical, (2) hypothalamic, (3) brain stem, and (4) spinal. The micturition is under the joint control of all these centres.

Cortical centre: The centre for control of the bladder is supposed to be present in the motor area of the cortex as well as in the upper part of the post-central gyrus. The motor fibres arising from the cortex lie near the pyramidal tracts in the cord being mixed up with spinocerebellar fibres. Electrical stimulation of the pre-motor area raises the intra-vesical pressure which is followed by micturition. A bilateral lesion of the cortical areas (motor areas, 4 and 6) causes loss of voluntary control over the act of micturition. In other words, the voluntary holding up of urine is lost.

Hypothalamic centre: It was observed that the tone of the detrusor muscle increases after electrical stimulation of the anterior nuclei of the hypothalamus and the diminution of tone after stimulation of the posterior nuclei of the hypothalamus.

Brain stem centre: Section below the superior colliculus makes the bladder so excitable that micturition starts when only a few millilitre of urine is collected. Section below the inferior colliculus makes the bladder function reduced to the spinal state. The dominant centres for Barrington's first and second reflexes lie in the hind-brain (Table 67.3).

Spinal centres: These centres remain in the second, third and fourth sacral segments. When these spinal centres are injured, the act becomes involuntary and incomplete. These spinal centres can be excited reflexly by various types of sensory stimulation. The spinal centres are under inhibitory control of the cerebral cortex and midbrain. Irritation of the pelvic organs, as caused by intestinal worms in children, may give rise to reflex involuntary micturition. This is one of the commonest causes of nocturia in children. Voluntary micturition is abolished after lesion of the spinal cord above the sacral segments. The tone of the muscles of the bladder wall (destrusor) and of the sphincter is lost for a certain period. Reflex contraction of the detrusor muscle and relaxation of the sphincter muscle do not occur. But after some time the detrusor muscle regains some tone and minor contractions take place which is not sufficient to overcome the sphincter tone which has recovered earlier. One or two weeks later, the urethral resistance diminishes. Urine is collected in the bladder which is gradually over-stretched causing a rise in the intra-vesical pressure. When this raised pressure overcomes the sphincter tone, a small quantity of urine dribbles out, leaving more urine in the bladder. This condition is called retention with overflow.

From the above, it will be seen that the phenomenon of micturition involves a number of complex reflexes controlled by many centres. It is also interesting to find that both somatic and autonomic nervous systems are involved in the process. It is one of the few processes in the body which is almost equally under the dual control of somatic and autonomic nervous system.

EFFECT OF LESION OF AUTONOMIC NERVES

Effects of lesion of the afferent sympathetic and para-sympathetic nerves: There is loss of perception of pain and stretch sensibility of the bladder. The subject cannot feel the distension of the bladder, and as a result urine accumulates, and when the intra-vesical pressure becomes high, the resistance of the sphincter is overcome and dribbling occurs. In tabes dorsalis there is lesion of the posterior nerve roots in the lumbosacral region and similar changes are noticed.

Effects of lesion of the efferent sympathetic nerves: The internal sphincter, the trigone and the ureteric orifices will be relaxed. There may be rise in frequency of micturition at the initial stage. Later the internal sphincter regains its tone. Gradually normal function of the bladder is re-established.

Table 67.3: Summary of Barrington's reflexes

Name of reflex	*Stimulus*	*Afferent nerve*	*Efferent nerve*	*Centre of reflex*	*Response*
First	Distension of bladder	Pelvic	Pelvic	Hindbrain	Contraction of detrusor
Second	Fluid through urethra	Pudendal	Pelvic	Hindbrain	Contraction of detrusor
Third	Distension of posterior urethra	Hypogastric	Hypogastric	Spinal cord	Contraction of detrusor
Fourth	Fluid through urethra	Pudendal	Pudendal	Spinal cord	Relaxation of urethra
Fifth	Distension of bladder	Pelvic	Pudendal	Spinal cord	Relaxation of urethra
Sixth	Distension of bladder	Pelvic	Pelvic	Spinal cord	Relaxation of posterior third of the urethra

Effects of lesion of the efferent para-sympathetic (sacral autonomic) nerves: Voluntary micturition is almost abolished. The detrusor muscle becomes atonic. The bladder becomes flaccid neurogenic (Fig. 67.4). The tone of the internal sphincter rises. There is retention of urine. The intra-vesical pressure rises with volume and when it overcomes the resistance of the sphincter, the dribbling of urine occurs. But soon this condition disappears and the bladder empties itself automatically through local nervous reflex. It is called an isolated bladder. The detrusor muscle contracts as soon as the intra-vesical pressure rises, but the contractions of the detrusor muscle are not strong and some residual urine always remains.

Effects of complete transection of the spinal cord: The bladder becomes complete spastic neurogenic (Fig. 67.5). The bladder capacity is diminished. There is increment in intra-vesical pressure, spasm of the external sphincter and involuntary contraction of detrusor muscle. Cerebral control is lacking. The bladder works in conjunction with its sacral reflex arc.

Effects of incomplete lesions of the cerebral cortex or the pyramidal tracts: The cerebral restraint becomes either weakened or abolished due to incomplete lesions of the cortex or the pyramidal (motor) tracts (Fig. 67.6). There may have frequent micturition, nocturia and uncontrollable urgency. These facts are also present in normal infants.

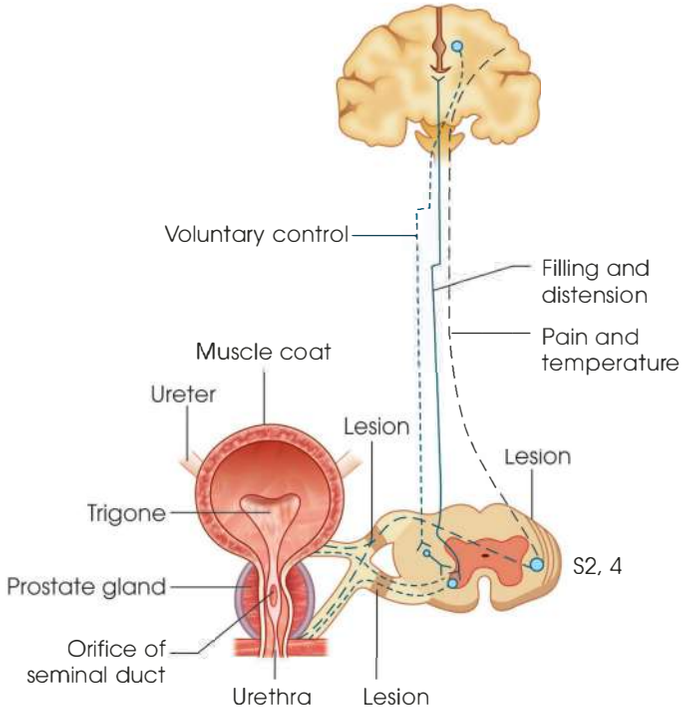


Fig. 67.4: Diagrammatic representation of a flaccid neurogenic bladder caused by a lesion of the sacral portion of the spinal cord or of the cauda equina

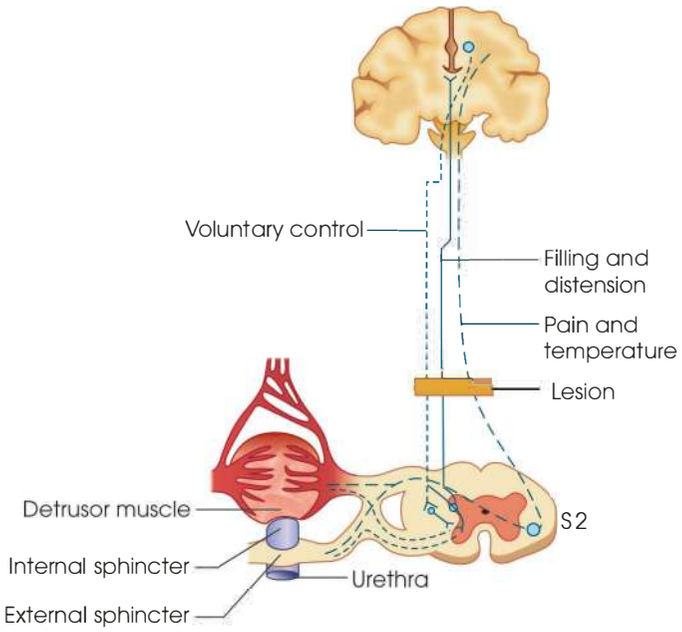


Fig. 67.5: Diagrammatic representation of a complete spastic neurogenic bladder caused by a more or less complete transection of the sacral spinal cord above S2

ARTIFICIAL KIDNEY

If the kidney fails to maintain its functions adequately then improvised device can be adapted so as to eliminate the incompatible substances of the body. This improvised artificial device is the artificial kidney. It is based on the principle of dialysing out the incompatible

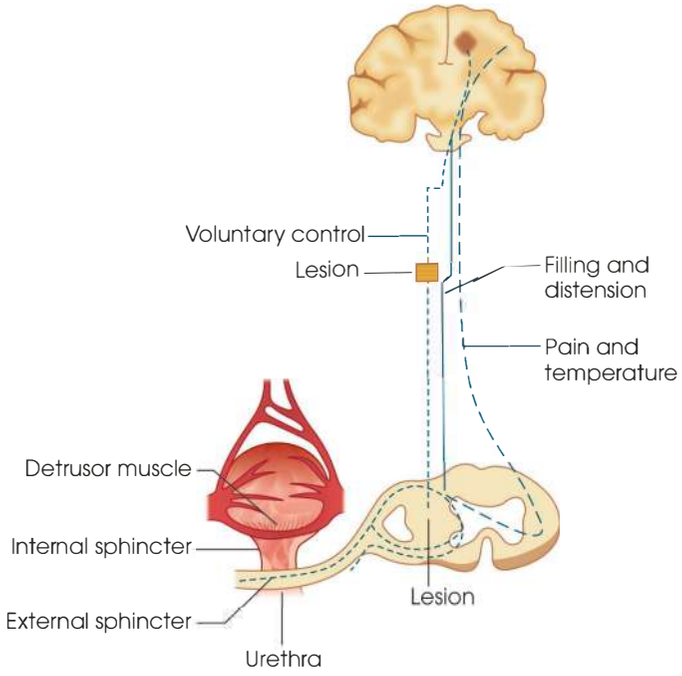


Fig. 67.6: Diagrammatic representation of an uninhibited neurogenic bladder caused by a lesion of the inhibitory centres of the cerebral cortex or pyramidal tracts

substances from the blood. The artificial kidney consists of a membrane through which incompatible substances are diffused out of the blood. This membrane allows all the constituents except the protein. The substance to be removed from the body is diffused out by adjusting the concentration gradient of dialysing fluid. For the operation of the artificial kidney, the arterial blood is passed through artificial kidney and the blood, after being filtered, is returned back to the body through the vein. For prevention of coagulation, heparin is always used.

EXAM-ORIENTED QUESTIONS

Essay

1. Describe the mechanism of micturition and micturition reflex.
2. Describe the effects of lesion of the afferent sympathetic and para-sympathetic nerves of urinary bladder in emptying of bladder.

Short Notes

1. Mechanism of filling of bladder
2. Artificial kidney

Chapter

68

Skin, Body Temperature and its Regulation

INTRODUCTION

Skin acts as a protective covering for the body minimising loss of water from the body tissues. Various sensory nerve endings on the skin help to protect the body from injury by invoking appropriate response to noxious stimuli. The skin also plays an important role in temperature regulation.

Structure

Two Main Layers

1. Epidermis
2. Dermis (Fig. 68.1)

Epidermis

Epidermis is the most superficial layer and is composed of stratified squamous type of epithelium. From outside inward stratified epithelium may be divided into 5 layers:

1. ***Stratum corneum*** is most superficially placed. The cells are keratinised. The cell outlines are indistinct and the nuclei are absent. This layer is thickest at the sole and the palm and thinnest at the lip. Hairs, loops, nails, feathers, scales, etc. are special outgrowth of this layer.
2. ***Stratum lucidum:*** This is a thin more or less transparent layer 3 to 5 cells deep placed below the stratum corneum. The cell outlines are indistinct and the nuclei are absent. The cells contain droplets of 'eleidin' which is precursor of keratin.
3. ***Stratum granulosum*** is situated below the stratum lucidum and consists of 3 to 5 layers of flattened polyhedral cells filled with keratohyalin granules which take a deep stain with haematoxylin.
4. ***Stratum spinosum:*** This is a broad layer of variable thickness and is made up of polyhedral cells. The surface of these cells is apparently covered with minute spines, which interdigitate with similar spines of adjacent cells. These are consequently known as "prickle cells". As the cells move towards the surface, keratin is synthesized within them. Microscopic studies indicate that the prickle cells are in fact cytoplasmic protrusions and the branches from two cells actually do not have cytoplasmic continuity, but attached by well-developed cytoplasmic nodes called desmosomes. These cells are basophilic and are supported by a network of cytoplasmic fibrils. Scattered irregularly throughout this layer are branched star-shaped cells known as Langerhans cells demonstrable by appropriate technique. The functions of these cells are not known and they are capable of active synthesis of DNA.
5. ***Stratum germinativum (stratum malpighi):*** This growing layer is composed of a single layer of columnar epithelium which has got transverse, thin, short cytoplasmic processes on its basal lamina by means of which they anchor the epithelium to the underlying dermis. These cuboidal to columnar cells with oblong nuclei, placed perpendicularly on the basement membrane, produce new cells to replace those of the above layers by the process of mitosis. Mitotic activity of the epidermis occurs in rhythmic diurnal cycles, the greatest activity of the epidermis occurs in rhythmic diurnal cycles, the greatest activity in humans occurring during the hours of sleep. At the junction of the epidermis with the dermis there occur number of melanin-containing branched cells, known as melanocytes*. They send processes among the malpighian cells and the melanin present in the deeper cells of the stratum. Malpighi is produced by these melanocytes. Cytocrine secretion transfers the fully-formed melanin granules from melanocytes to the malpighian cells. The ratio of melanocytes to basal epidermal cells varies between 1 to 4 and 1 to 10.

Epidermis of the Body in General

Above characteristic features of the epidermis are mostly present in the palm and the sole. Epidermis in general, i.e. of other than soles and palms is thin and

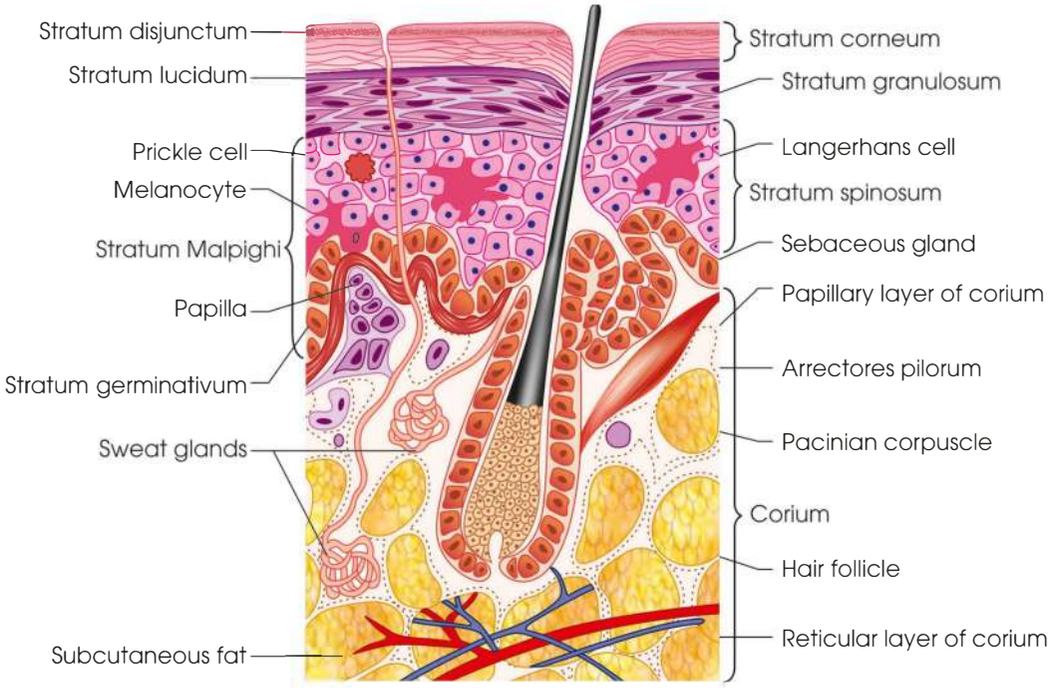


Fig. 68.1: Diagram shows section of skin

stratum Malpighi and stratum corneum are always present. Stratum corneum is also thin and just identifiable. Definite stratum lucidum is not seen.

Dermis (Cutis Vera or True Skin)

The true skin is made up of connective tissue and lies below the epidermal layer which it supports and binds to the underlying tissues. It is made up chiefly of collagenous and elastic fibres which provide it with a tensile strength equal to that of a skin steel wire. This layer is utilised for the production of leather after chemical processing. From the structural point of view the superficial part of the dermis is compact and forms the papillary layer because it sends innumerable finger-like projections (papillae) into the prickle cells layer of epidermis. The deeper part of the dermis is composed of rather loose connective tissue and is infiltrated with fat. This layer constitutes what is called reticular layer of dermis. The reticular layer of the dermis merges imperceptibly into the subcutaneous layer of fat.

Cells of the Dermis

These are fibroblasts from which the fibrous tissue of the dermis develops.

These cells are belonging to the reticulo-endothelial system which protects the body from invading bacteria. Some of these cells are loaded with melanin pigments and are known as *melanophores*. Besides the above two types of cells—dermis also contains other types of cells which are found in areolar tissue of other regions of the body.

Blood Vessels of the Dermis

Though the epidermis is devoid of blood vessels, the dermis has got a luxuriant supply of capillary blood vessels which form a network from which "hairpin" loops supply the tip of the dermal papillae. The blood vessels play an important role in temperature regulation and have got rich vasomotor innervations.

There is also a well-organised lymphatic system in the dermis.

Nerves of the Dermis

Besides the vasomotor nerves mentioned above the dermis is provided with sensory nerve endings of various types, which keep the individual informed about the surroundings.

Glands of the Dermis

The lower ends of the sweat glands reach deep part of the dermis and act as islands for re-growth of epidermis in case of injury or burns involving loss of epidermis.

Sebaceous glands located in the dermis open into the hair follicles and secrete oily material called sebum.

Note

The ceruminous glands of the external auditory meatus and mammary glands are modified sweat glands.

Muscles of the Dermis (Corium)

1. Arrectores pilorum: A small bundle of involuntary muscles attached to the hair in such a way that they cause the hair to stand on end.

2. Tunica dartos and other involuntary muscles of the skin of the penis, nipple and areola are located deep in the dermis.

Nowadays, melanocytes are used instead of melanoblast. Melanoblast is now used for such cells in embryonic life.

Pigmentation of the Skin

The colour of the human skin is derived from a variety of chemical and physical properties associated with the skin structure. Five pigments are known to influence skin colour: All individuals except albinos have some melanin pigment in their skin. Melanin is a yellow to black pigment which is found mainly in the stratum Malpighi, and is formed on a specific cell particle, the melanosome within the melanocytes (Fig. 68.2). In man, melanosomes are uniformly elongated except red-haired individuals where they are spherical. During pregnancy, the pigmentation of the areola of the nipples increases. Melanin contributes colour quality to the skin and protects the organism from the ultra-violet rays. The stability of melanosomes after their incorporation into the cytoplasm of malpighian cells along with their absolute increase in numbers is the main cause of the marked difference in the skin colour between black and white individuals.

Melanoid is supposed to be a degradation product of melanin and is diffused through the epidermis. Melanoid has a different absorption band of visible light.

Carotene is a yellow-orange pigment and present in lipid-rich areas (i.e. the stratum corneum and the fat of the corium and subcutaneous tissue). Carotene adds the strong yellow component to skin colour of the women in greater concentration than that of men.

Oxyhaemoglobin imparts a reddish hue to the skin colour and is evident to areas where there is rich arterial supply (i.e. face, neck, palms, soles and nipples).

Reduced haemoglobin contributes a bluish or purple character to skin colour and is more evident in lower areas of the trunk. Melanin concentration and skin thickness have a tendency to suppress the haemoglobin pigment colour component effects.

The distribution pattern and concentration of these pigments also influence the skin colour. Variations in the thickness of the skin may modify the skin colour. Subjects with thin epidermis have a ruddier colour complexion and with a thicker epidermis, look yellower. The thick epidermis is less transparent than the thin and the thin epidermis allows the colour of blood pigments to express their colour characteristics more readily. The light-scattering qualities of the skin may also act as a factor modifying skin colour. As the transparent stratum corneum scatters light slightly, the deeper layer appears bluer because of the scattering effect of light in the overlying coarser tissue layer where dark-pigment granules are commonly found. Pigmentation of the skin is supposed to protect the underline tissue against the potentially harmful effects of solar radiation.

FUNCTIONS OF SKIN

1. **Protection:** Stratum corneum which is the outermost layer is horny and formed by the keratinised stratified epithelial cells resist the action of external agencies. It protects the internal individual injury and bacterial invasion. The nails (hoops, horns, etc. in animals) are also defensive appendages of the skin.
2. **Regulation of body temperature:** Cutaneous vasoconstriction diverts the blood to the interior of

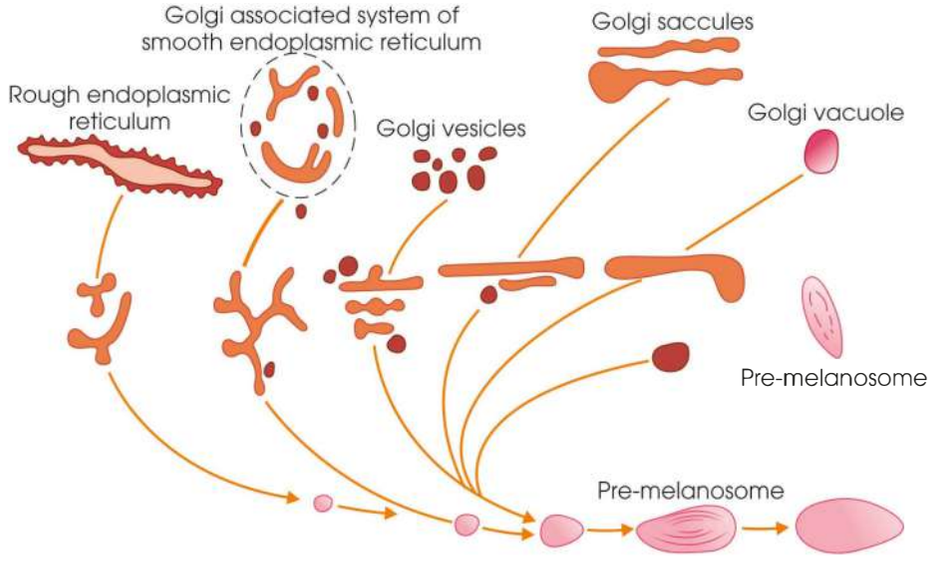


Fig. 68.2: Composite diagram shows the region in a melanocyte

the body and so diminishes heat loss. This is an important mechanism of protection against cold environment. Vasodilatation of the skin helps in elimination of heat from the body. Sweating—evaporation of sweat cools the body, the latent heat of vaporisation being 0.56 cal/gm of H_2O vaporised. Hairs of the skin—in lower animals entangle a layer of air in its meshes. This layer of stagnant air being poor conductor of heat intervenes between the warm air in contact with the skin and odd atmospheric air and helps to preserve body temperature.

3. **General sensation:** The skin serves as the medium for receiving the general sensation. Touch, pain, temperature, etc. are sub-served by the respective nerve endings present in the skin. The hair roots are richly supplied with nerves. Consequently, slight movement of the hair, such as by a blast of wind arouses sensation. In this way, hairs help the sensory functions of the skin.
4. **Excretion:** Through sweat and insensible perspiration, salts and metabolites are excreted to some extent. Synthetic function: Vitamin D is synthesized by ultra-violet rays of the sun acting upon the ergosterole present in the skin and subcutaneous tissue. For this reason exposure to the sun ensures supply of vitamin D.
5. **Secretion:** *Sebum:* The sebaceous glands secrete a fatty substance which is rich in cholesterol, called sebum. It helps to keep the skin greasy and prevents drying. In cold climates the secretion is depressed and the skin becomes dry and scaly. Sweat (described in detail below) which is the active secretion of sweat glands of skin plays a vital role in keeping the body cool in hot environment. Milk, the secretion of the mammary glands, which are modified sweat glands. [In the toads, certain poisonous glands are present in the skin. They secrete a highly irritant fluid and in this way act as a defensive weapon for the animal.]
6. **Absorption:** Waxy layer hinders water absorption through the skin. But the skin is not completely waterproof and on prolonged exposure to water, there is water absorption causing swelling of the stratum corneum. Lipids are easily permeable through the skin. Lipid-soluble substances like vitamins are easily absorbed through the skin.
7. **Water balance:** Formation and evaporation of sweat is an important factor in the regulation of water balance of the body.
8. **Acid–base equilibrium:** Sweat being acid in reaction, a good amount of acid is excreted through it. In acidosis it becomes more acidic and in this way helps to maintain a constant reaction in the body fluids. *Storage functions:* The dermis as well as the subcutaneous tissue can store (a) fats, (b) water, (c) salts, and (d) glucose and such other substances. It is found that when blood sugar level suddenly rises, considerable amount of sugar may be temporarily stored in the subcutaneous tissue and in the skin. As sugar is gradually used up, the skin sugar is slowly drawn in. The areolar tissue of the skin and subcutaneous tissue have great affinity for water and any excess of water in the body is stored mainly inside the skin and subcutaneous tissue. After haemorrhage, the lost blood volume is quickly replenished by drawing fluid from the tissue and half of it comes from the skin. Similarly, a large quantity of NaCl may remain stored in the skin. After a chloride-rich diet or after an intravenous saline injection, the heaviest load of NaCl is found in the skin and subcutaneous tissue. Whereas during salt deprivation; blood chlorides are maintained by drawing upon the chlorides stored in the skin. The sub-papillary plexuses of the dermis can store up to about 1,000 ml of blood, which supplies muscles and other organs in times of emergency.
9. **Gaseous exchange:** Absorption of oxygen and excretion of CO_2 may go onto a considerable extent through the skin in those animals whose skin is thin and moist, e.g. frogs. It is said that it can be carried to such an extent that these animals may live even after the extirpation of the lungs or in the hibernating period when the lungs do not function. In man, this effect is negligible. No oxygen is absorbed but a small amount of CO_2 is eliminated through the skin (probably it passes out being dissolved in the sweat). Skin also exhibits signs of vitamin deficiency, malnutrition and advancement of aging (appearance of wrinkles on the skin).

Glands in the Skin

Sweat Glands

Modified smooth muscle cells, known as myoepithelial cells invest the base of the glands and wind round their ducts in such a way that by their contraction they help in elimination of sweat. There are about 3 million of active sweat glands in the body. They are divided into two groups: Eccrine and apocrine (Fig. 68.3)

Eccrine sweat gland: The eccrine glands constitute the majority and are generally found throughout the surface. They are most numerous on the palms and soles, than on the head, but much less on the trunk and the extremities. In lower mammals they are relatively sparse being found mainly on hairless areas such as foot pads. They reach their peak of development in man where they may be 200–400/sq cm of the skin surface. They are innervated by cholinergic sympathetic fibres whose discharge is altered primarily by changes in deep body temperature. The glands on palms and soles do not respond to temperature but secrete at time of

emotional stress. They secrete dilute sweat-containing NaCl, urea, lactates, creatinine, uric acid, ammonia, amino acids, glucose, water-soluble vitamins B and C. NaCl content is variable. The sweat is not a sample of an active process though the composition depends on the blood constituents.

Apocrine sweat gland*: The apocrine glands are larger sweat glands. They are derived from the hair follicles and include the ceruminal glands of the ear, those of the eyelid, and the mammary glands. The distribution of these large glands varies very much from individual to individual and from race to race and are found only in special regions, such as axilla, areola of the nipples, mons pubis, labia majora, etc. they are said to be, in many instances, of sexual significance and to become less so as we ascend the evolutionary scale they respond to circulating adrenaline. They do not become active until puberty their secretion is viscid, milky and odourless at first. After puberty their secretions vary in composition and possess a characteristic odour.

The odour is due to indoxyl, volatile fatty acids, hydroxy acids, ammonia, etc. Bacterial activity in the presence of the secretions contributes to the intensity and quality of the odour. The mammary glands which eject milk are form of the apocrine sweat glands and an interesting study in evolution.

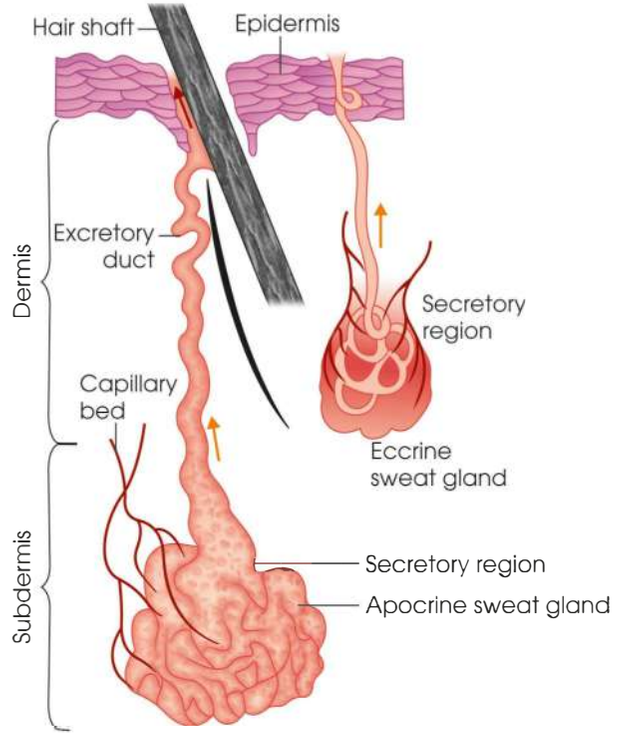


Fig. 68.3: Diagram shows the excretory duct, the tightly coiled secretory region, and the vascular bed of eccrine and apocrine sweat glands

Mechanism of Secretion of Sweat

Though the eccrine glands are supplied by the sympathetic fibres, yet adrenaline (epinephrine) has got no action on them. Pilocarpine which stimulates the para-sympathetic fibres increases the flow of sweat and atropine which paralyses the para-sympathetic endings, abolishes sweating. From these observations and from similar others, it is believed that acetylcholine is liberated at the nerve endings. Hence, although the fibres are automatically sympathetic, yet they are functionally cholinergic. Hilton has shown that the sweat secreted from the eccrine glands contains bradykinin-forming enzyme. This enzyme diffuses into the surrounding tissue and helps in forming bradykinin (Fig. 68.4). Bradykinin is a polypeptide and causes dilatation of the cutaneous blood vessels and blood vessels supplying the sweat glands. Injection has only very slight effect on this vasodilatation.

Different Types of Sweating

1. Insensible perspiration which occurs even in cold climate amounts to 600 to 800 ml daily.
2. Thermal sweating occurs in hot environmental temperature, the threshold being 28°C for man and 31°C for woman. As the environmental temperature rises sweating increases and may amount to 11 litre/day under extreme conditions. It is to be emphasized that when the ambient temperature is higher than body temperature sweating is the only method of keeping the body temperature normal, provided the humidity is low so that the sweat can evaporate.
3. Psychic sweating
4. *Emotional (mental) sweating:* In the emotional conditions sweating occurs chiefly in the palms, soles and axilla, but it is also present at the head and neck and elsewhere. In the extreme emotional conditions it may be more generalised (hyperhidrosis). Emotional (cold) sweating is due to impulses discharged from the higher centres, affecting the sweat centres directly (Fig. 68.5). The sweating that occurs under the emotional condition is probably under the control of the pre-motor area of the cerebral cortex.
5. In muscular exercise, the sweating both thermal and mental. Sweating is reduced by cold, which at the same time reduces cutaneous circulation. It is also reduced by dehydration, whether the result of deprivation of fluid intake or by sweating itself. The taking of a glass of cold water which dilutes the blood produces a profuse sweating.
6. In hot climates, eating of spicy foods (e.g. capsicum or curry) stimulates sweating (gustatory sweating), because pain nerve endings in the mouth are stimulated. Hence, reflex sweating occurs in the head and neck.

*According to Jenkinson's view, apocrine should be dropped to describe the sweat gland, because the original concept of formation of apocrine sweat by shedding the outer portion of secretory cells is now known to be incorrect.

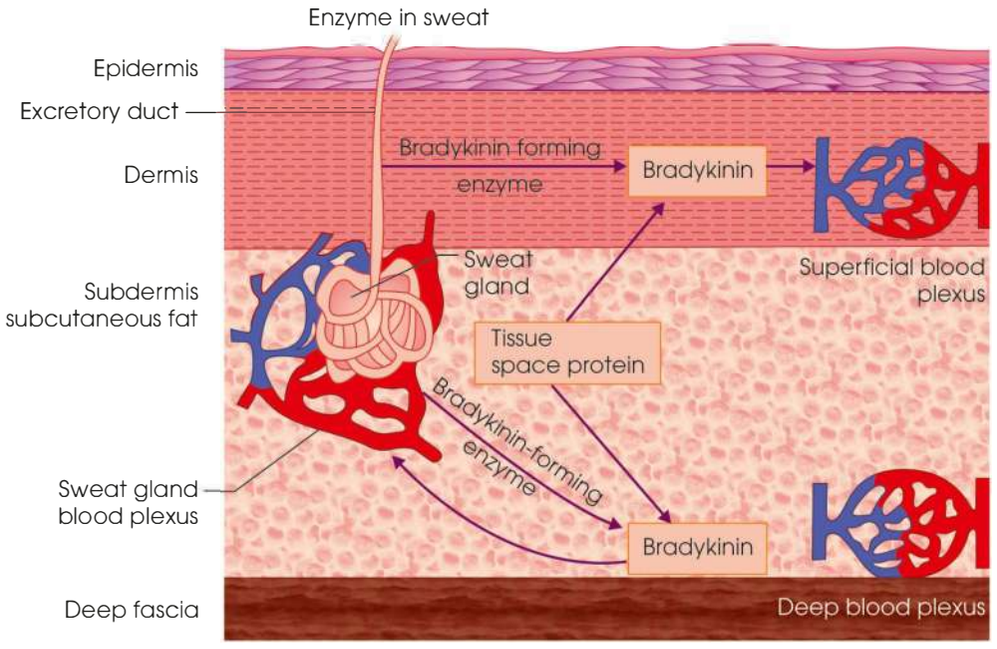


Fig. 68.4: Diagram showing the formation of bradykinin in human skin during body warming

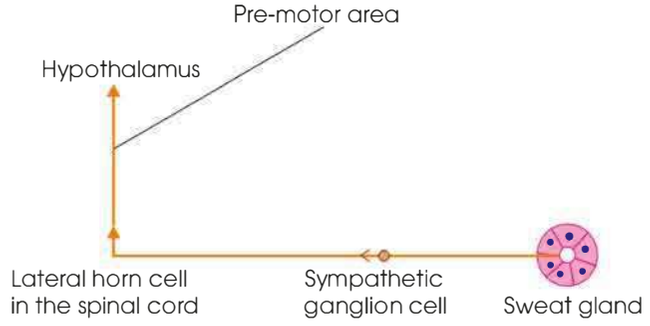


Fig. 68.5: Diagram shows the nervous path for regulation of sweat secretion

7. Owing to sympathetic activity, nausea and vomiting, fainting, hypoglycaemia and asphyxia cause secretion of sweat.

COMPOSITION OF SWEAT

Sweat mainly consists of secretions of the eccrine glands. It is the most dilute of all animal fluids. When freshly collected, it contains epithelial cells and some sebum. When filtered, it forms a clear colourless fluid. The human sweat has specific gravity of about 1.001–1.006, pH 3.8 to 6.5 and the average composition is as follows (Table 68.1).

Aldosterone, the secretion of adrenal cortex, reduces both the sodium and chloride ions in sweat and thus decreases their loss. Excessive sweating may cause increased secretion of aldosterone, and prevents depletion of salt reserves of the body (accommodation of sweat glands). Urea has about the same concentration as in blood, but lactates seem to be either concentrated of actually formed by the sweat glands.

Sebaceous Glands

Sebum is the oily secretion of the sebaceous glands (Fig. 68.6). These glands are pear-shaped bodies opening into the root of the hair follicles. But where they are independent of hairs, their ducts open directly to the free surface of the skin (e.g. in gland penis, basal glands of the eyelids, etc.). Nicoll and Cortese (1972) have reviewed the current concepts on the ultrastructural anatomy and on the physiology of sebaceous gland cells. They have described the life cycle of sebaceous cells into three distinct cell types. These are:

1. The peripheral cells which contain ribosomes and are formed against the basement membrane that surrounds the acinus.
2. The partially differentiated cells, which are actively synthesizing and storing sebum droplets within the cells.
3. The fully differentiated cells, which contain a multitude of tightly packed sebum vacuoles ready to be released upon rupture of the cell.

Cellular maturation and sebum lipogenesis represent a dynamic process. In the early stage of lipogenesis, glycogen, smooth endoplasmic reticulum and ribosomes predominate in the cytoplasm of cells. For the cellular maturation, numerous mitochondria along with smooth surface vesicles, ribosomes, glycogen and Golgi membranes fill cytoplasmic substance of the cells. As maturation of cells is completed, numerous lipid vacuoles, smooth membranes, and a minimal number of ribosomes and mitochondria appear within the cells. The smooth-surfaced endoplasmic reticulum is primarily involved in lipogenesis and mitochondria may play a role in producing energy. The sebum is thus formed in the cells of the gland and thereby the cells become impregnated with fat. These matured cells

Table 68.1: Composition of sweat

Water	99.221–97.742 gm/100 ml
Solids	1.174–1.587 gm/100 ml
Ash	0.144–0.566 gm/100 ml
Creatinine	0.1–1.3 mg/100 ml
Urea	12–57 mg/100 ml
Lactic acid	285–336 mg/100 ml
Carbolic acid	2–8 gm/100 ml
Sugar (as glucose)	1–3 mg/100 ml
Uric acid	0.07–0.25 mg/100 ml
Ascorbic acid (as dehydroascorbic acid)	70.5 µg/100 ml
Total nitrogen	33.2 mg/100 ml
Non-protein nitrogen	27–64 mg/100 ml
Amino acid N	1.1–10.2 mg/100 ml
Ammonia N	5–9 mg/100 ml
Urea N	5–36 mg/100 ml
Calcium	1–8 mg/100 ml
Iodine	0.5–1.2 µg/100 ml
Iron	0.022–0.045 mg/100 ml
Chloride	36–468 mg/100 ml
Na^+	24–312 mg/100 ml
K^+	21–126 mg/100 ml
Sulphur	0.7–7.4 mg/100 ml
Copper	0.006 mg/100 ml
Amino acids (total)	43.62 mg/100 ml

It is to be noted that the only conspicuous constituent is NaCl, which varies between 0.2 and 0.5%. Muscular exercise increases the salt concentration of sweat. Sweat secreted by clothed skin has a higher salt concentration than that of naked skin. When sweating is profuse the total elimination of NPN per hour may be as much as 0.5–1.0 gm. Regarding salts a man may lose as much as 10 gm of chlorides by profuse sweating in 3 hours.

finally degenerate and break apart releasing the sebum to the excretory duct. The residue ultimately is coming out the skin surface through the hair root canal (Fig. 68.6). They are totally absent at the palms and soles. The sebaceous glands are very active during adolescence. When the secretion is not properly discharged, it lodges in the ducts as whitehead. The outer portion of the substance may be blackened by oxidation and then constitutes a blackhead. Formation of sebum is determined by alteration of vascularity of the skin.

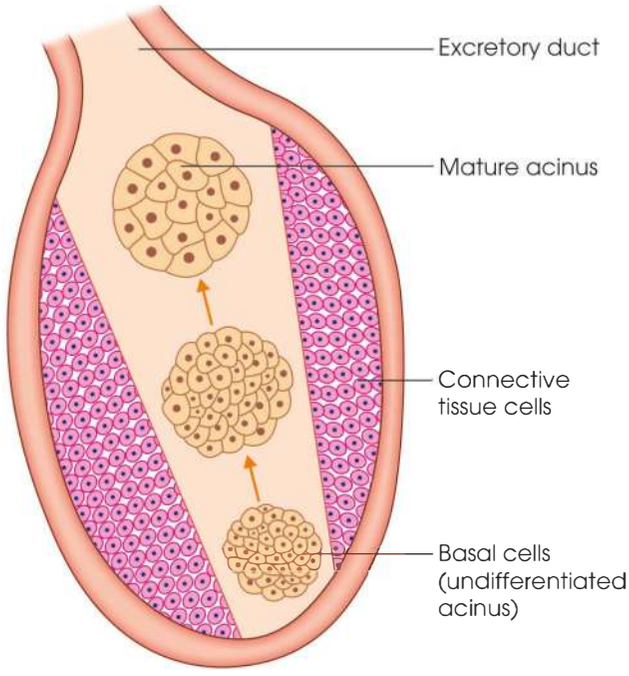


Fig. 68.6: Diagram showing the development of a sebaceous acinus

Composition of Sebum

Composition of sebum is not fully known. Sebum has a characteristic colour. Sebum is rich in fatty acids, stored cholesterol, cholesterol esters, triglycerides, wax esters and other aliphatic components. This cholesterol has two additional advantages over ordinary fat. First, it can absorb about 100% of water and thus helps to keep the surface moist. Secondly, it is not attacked by bacteria and, therefore, does not become rancid. Irradiation of sebum gives rise to vitamin D.

Control

There is no nervous control for the secretion of sebum. Hormonal regulation of sebaceous glands is well established. Experimental evidence suggests that excess administration of progesterone causes pronounced enlargement of sebaceous glands. This has given rise to the belief that in the female, acne is incited by

progesterone formed by the corpus luteum of the ovary. But it is not ascertained whether the progesterone affects the sebaceous glands in physiological doses. Oestrogen is known to decrease the size of the sebaceous glands as well as to inhibit the formation of sebum in human. Current evidence suggests that sebum production is stimulated primarily by androgens secreted either from adrenal glands, testes or ovaries. In human males, testosterone is considered as the major cause of pubertal acne. Contraction of arrectores pilorum helps in the expulsion of sebum. These muscles contract by the application of cold, during excitement and in response to adrenaline.

Functions

Sebum has dual functions. It acts as a bacteriostatic agent and as a lubricant. It has also the property preventing damage of the epidermis during hot season and conservation of heat during cold.

Cerumen is a yellow, pasty substance resembling wax which is secreted by the modified sweat glands (ceruminal glands) located in the external auditory canal. An accumulation of cerumen deep in the auditory canal may interfere with hearing.

SPECIAL STRUCTURES OF SKIN

Nails (Ungues)

The nails form the protective covering on the dorsal surface of the terminal phalanges of the fingers and toes (Fig. 68.7) and are made up of horny plates. With continuous proliferation and differentiation of cells of the lower part of the matrix (stratum germinativum), the forming nail plate (modified stratum corneum) is pushed out of the groove and slowly advances over the dorsal surface of the digits towards the distal end. Component cells contain numerous cytoplasmic fibrils which are lost at the later stage as the cells become homogeneous, cornified and join the nail plate. The keratin of the nail is turned hard. The epidermis immediately beneath the nail plate constitutes the nail-bed (matrix). The nail plate is contained within the nail groove and flanked by a skin-fold, the nail wall (eponychium-modified stratum corneum). The nailbed is made up of the deeper layers of the epidermis and lacks sweat glands and hair follicles.

Functions

Protection, defence, in animals the nail helps in capturing the prey.

Hair (Pili)

These are elastic keratinised threads which develop from the epidermis and extend downward into the subcutaneous tissue. They vary in length from 1 to 1,500 mm and in thickness 0.05 to 0.5 mm. The hairs are present all over the skin except for palms, soles and urogenital apertures. Each hair has a free shaft projected beyond the surface of the skin and a root embedded in the tubular hair follicle in the skin. The hair (Fig. 68.8) is composed of two parts: Epidermal (epithelial) and dermal (connective tissue). The expanded lower part of the follicle is named as the hair bulb which is indented at the basal end by a connective tissue papilla. Hairs have no blood vessels but receive nourishment from the blood vessels of the papilla. The follicle is associated into one or more sebaceous glands and a bundle of smooth muscle—arrector pili, contraction of which causes erection of the hair which normally remains in an obtuse angle to the skin surface. Hairs grow from the papilla by multiplications of matrix cells which become elongated to form the fibres of fibrous

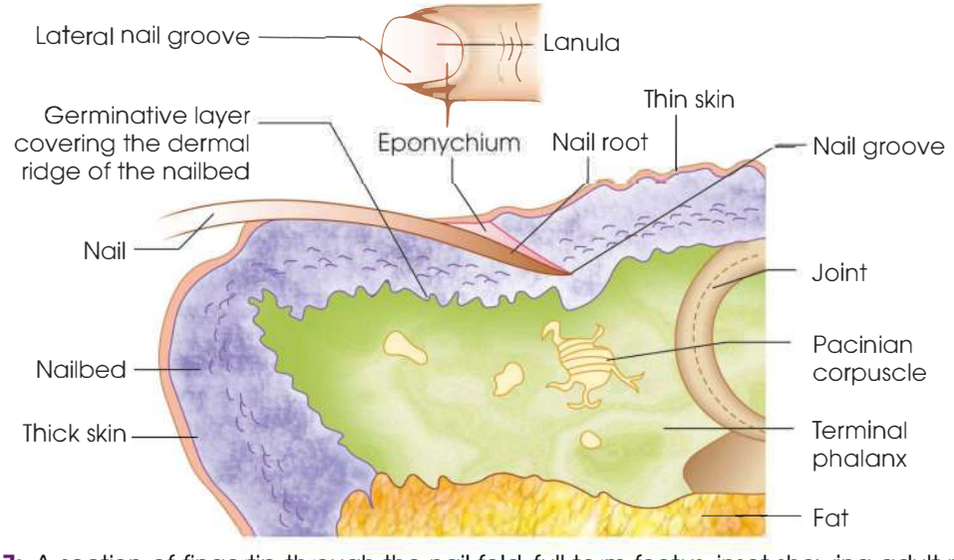


Fig. 68.7: A section of fingertip through the nail fold, full term foetus, inset showing adult nail fold

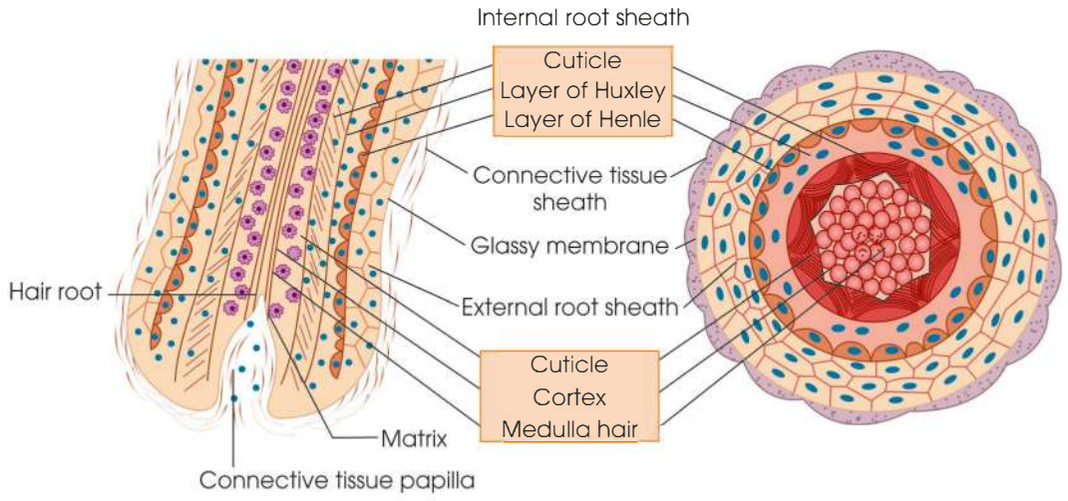


Fig. 68.8: Diagrammatic representation of a hair follicle in longitudinal section (left) and in transverse section (right)

part and when they are pushed to the surface, they become flattened and form the cuticle. Shedding of hairs is seasonal in some animals but not in man. The newly developed hair is longitudinal section (left) and in transverse section (right), present on the side of the shedding hair. Failure of replacement results to baldness, uncommon in the females. An acute inflammation of a hair follicle and its sebaceous gland gives rise to a boil due the entrance of staphylococci through the follicle.

Functions of Skin

1. Presence of hair protects the body from the extreme temperature of the environment by acting as an insulator, especially in animals of cold countries.
2. Scalp hairs protect the head from mechanical injury as well as from heat, rays, etc. The hair also does the tactile receptor function.
3. Hair serves a sexual function in promoting the evaporation of the apocrine sweat and the accompanying characteristic odour that goes along with it provides a sexual attraction for the lower animals.

BODY TEMPERATURE AND ITS REGULATION

INTRODUCTION

The animal kingdom can be broadly classified into two groups, depending upon their body temperature. Those who can maintain their body temperature relatively constant in the face of wide variations of environmental temperature are known as warm-blooded animals or homeotherms, whereas those whose temperature fluctuates with fluctuations of the environmental temperature are termed cold-blooded animals or poikilotherms. In the course of evolution, from poikilotherms to homoiotherms, there exist another group who are known as hibernants, going into hibernation in winter, otherwise behaving like the warm-blooded animals in the remaining period. Hibernating mammals do not require an external source of heat to raise their body temperature to normal and can rouse themselves probably by activating their large stores of brown fat. Even in homeotherms, who are capable of maintaining constant body temperature, it has been found that different parts of the body, even different organs, have different temperatures, and this is a normal phenomenon. However, the average temperature as measured has been found to lie within a constant range, hence justifying the statement of having a constant body temperature.

Normal temperature: Oral 97°–99°F or 36.11°–37.22°C (average 98.4°F or 36.89°C). Axillary (or groin) temperature is 1°F or 0.55°C less, while rectal and oesophageal temperatures are 1°F or 0.55°C more. The body is hypothetically divided into core and shell (Fig. 68.9). The core temperature, i.e. temperature of intra-abdominal, intra-thoracic and intra-cranial content is maintained at a constant temperature. Rectal and oesophageal temperatures represent core temperature.

Oesophageal temperature taken at heart level is a good index of rapid changes of cardiac and aortic blood temperatures (as during induction of hypothermia, rewarming from hypothermia), whereas rectal temperature gives a poor reflection of rapid change of blood temperature and may be misleading. Shell temperature, i.e. temperature of the limbs and the surface layer of the trunk exhibits wide variation of the temperature. Although rectal and oesophageal temperatures are most reliable, yet, for practical advantage, oral temperature is taken for routine clinical purposes.

In some special circumstances axillary temperature may have to be taken. Clinical thermometer is the commonest instrument used. It should be kept under the tongue for 2 to 5 minutes for precise measurement. [Thermocouple is a more sensitive instrument and is

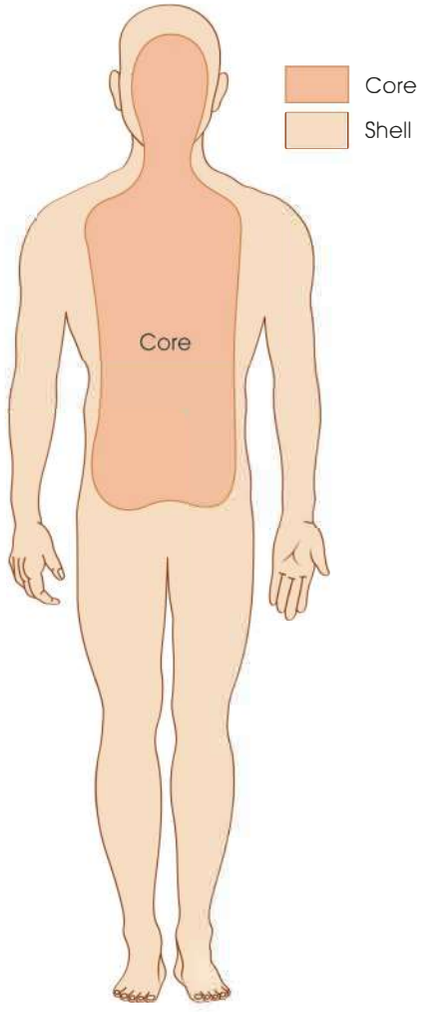


Fig. 68.9: Division of the body into hypothetical areas of 'core' and 'shell'

routinely used during and after heart surgery, neurosurgery and in intensive care units in clinical practice.

Besides these, the instrument is also used for experimental purposes. The temperature recorded by a thermocouple on the tympanic membrane has been found to reflect accurately changes in arterial temperature.]

FACTORS AFFECTING BODY TEMPERATURE

1. **Diurnal variation:** It is highest in the evening (after the day's labour—between 5 and 7 pm) and lowest in the early hours of the morning (after the night's rest). In the night-workers, the rhythm is reversed. The average range of variation is 1°F (0.55°C) to 1.5°F (0.83°C). This diurnal variation is related to exercise and specific dynamic action (SDA) of food. Fasting and absolute bed rest abolishes this variation.
2. **Age:** In infants regulation is imperfect. Hence, range of variation is wider. A fit of crying may raise and a cold bath may lower the body temperature. In old age, the body temperature may be subnormal due to low BMR.
3. **Size:** Heat production and heat loss depend upon the ratio of mass to body surface area. In a mouse, heat production is 452 large calories per kilogram body weight per 24 hours; whereas in a horse it is only 14.5 large calories.
4. **Sex:** In females the body temperature may be a little lower. This is due to relatively low BMR and thick layer of subcutaneous fat (non-conductor). During menstruation, temperature slightly falls (0.3°F or 0.17°C). Then it gradually rises and becomes maximum 24 to 48 hours after the ovulation. This rise is due to progesterone level of blood which is secreted by the corpus luteum. Regular record of oral temperature in the early morning is sometimes used to detect the exact date of ovulation in a woman, in clinical practice.
5. **Food:** Protein food, due to high SDA may raise body temperature. The act of ingestion of food may also raise body temperature.
6. **Exercise:** Increases temperature (only 25% of muscular energy is converted into mechanical work, the rest comes out as heat).
7. **Atmospheric conditions:** Temperature, humidity and movement of air are directly concerned with the amount of heat loss from the surface and thus affect body temperature.
8. **Cold and warm baths:** These have a far greater influence than air at the same temperature, but since the duration of exposure to these baths are short, they have a little effect on the normal body temperature. However, body temperature may remain elevated for a considerable time after a prolonged hot water bath.
9. **Sleep:** Because of muscular inactivity, sleep results in a slight fall of body temperature.
10. **Emotion:** Body temperature may rise due to emotional disturbances. The rise of temperature may be as high as 2°C.
11. **General anaesthetics or chlorpromazine** reduce the body temperature by depressing the activity of the ascending reticular system.
    - Tubocurarine paralyses the skeletal muscle, and reduces heat production and results in fall of temperature.
    - Antipyretic drugs, e.g. sodium salicylate and acetylsalicylic acid (aspirin) antagonise the action of pyrogen on the hypothalamus, induce cutaneous vasodilatation and sweating and thereby help in reduction of temperature in febrile states.
12. **Posture, piloerection and clothing** are also important factors which affect the body temperature. All animals and even man may conserve heat or may prevent heat loss by curling them up during exposure to cold.

REGULATION OF BODY TEMPERATURE

Although a large amount of heat is produced and lost from the body constantly, yet the body temperature remains fixed within a limited range. This shows that there is strong machinery which keeps an exact balance between gain and loss of heat and thereby maintains a constant body temperature. The physiologic process of heat production in the body is known as thermogenesis. Dissipation of bodily heat by means of radiation, evaporation, etc. is called thermolysis. The mechanism by which body temperature is normally adjusted is known as the thermotaxis (Fig. 68.10). The controlling mechanism consists of the following is given below.

Mechanisms of Heat Production (Thermogenesis)

Heat production takes place through physiological oxidation of food materials in the body—by combustion of carbohydrates, proteins and fats. Cold climate stimulates appetite. Subjects take more food and proportionately higher amounts of fat. Higher fat intake increases heat production.

Although heat produced by skeletal muscles is variable and depends upon physical activity, yet the heat produced by skeletal muscles is by far the more important. As these constitute about one-half of the active structures of the body, they furnish the largest amount of heat produced among the body tissues. Increasing the activity of the muscles whether voluntarily or involuntarily thus automatically increases the heat production. Shivering increase heat production although not as high as voluntary muscular exercise. Ingestion of hot foods or drinks contributes to heat production in negligible amounts. Of the organs, the liver contributes the highest amount. Heat produced by liver and heart is relatively constant. The action of some internal secretion and enzymes, e.g. thyroxine and epinephrine (possibly) also helps to heat production. During digestion the peristaltic action of intestines and the activity of various digestive glands produce heat. Increased heat production which occurs by increasing the BMR and metabolic activity is termed chemical thermogenesis, while the heat production by increased muscular activity is termed physical thermogenesis.

Mechanisms of Heat Loss (Thermolysis)

Heat is lost from the body by three channels, the (1) skin, (2) lungs and (3) excretion, mainly through the processes of radiation, conduction, convection and evaporation. The bodily changes that regulate the exchange of heat between the body and the environment are referred to as physical heat regulation.

From the skin (proportional to the total surface area):

1. *Radiation:* Due to the difference of temperature existing between the body and the cooler environment heat is lost from the body by radiation (loss by electromagnetic waves). The body however does not radiate to the surrounding air, but through the air to the solid objects in the vicinity. When a number of people are present in a room, they radiate towards one another as well as to the surrounding objects. Amount of heat lost by this process is about 55% of total heat lost. The amount of radiation from an object is determined by several factors. It is proportional to the surface area of the body, to its emissive power, and to the difference in temperature between the radiating body and the surrounding objects (actually to the difference between the fourth power of the absolute temperatures of each respectively). The color of clothing's may play a part, white and pastel shades being suitable for tropical climate.

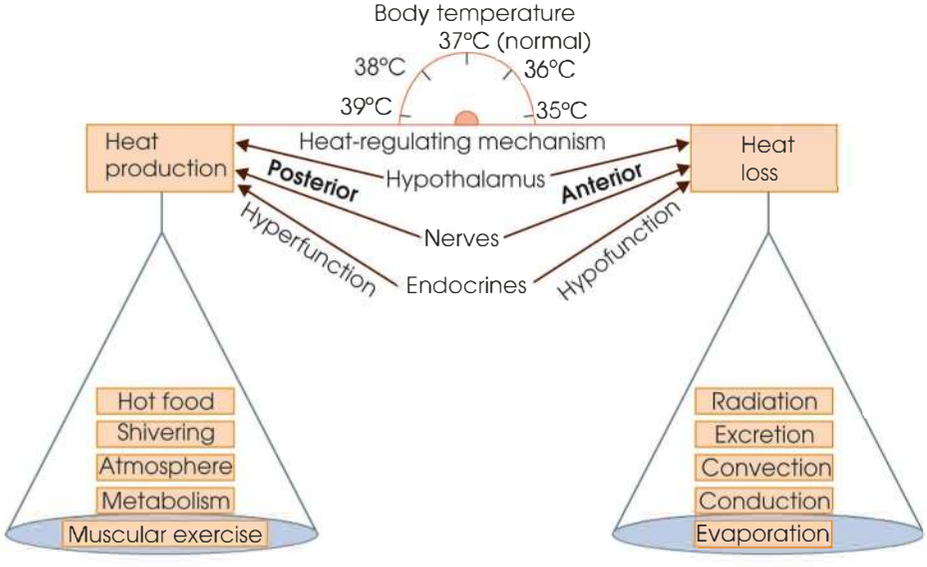


Fig. 68.10: Diagrammatic representation of the mechanism of heat production and heat loss in the body

However, the colour of human skin has no effect upon the degree of radiation; both white and black skin is a 97% perfect black body. [A body absorbing 100% of the radiant energy falling on its surface is a perfect black body.]

2. *Conduction and convection:* About 20% of heat is lost from the body through conduction and convection. The heat loss through these processes depends upon the temperature of the surrounding atmosphere. When the temperature of the surrounding atmosphere is low, heat is lost from the skin to the surrounding air. The molecules of the air gradually get warmed and move away from the skin. Another layer of cooler air takes its place. Heat loss through convection depends upon the relative density and temperature of air and wind velocity but not on relative humidity of the air. Besides these factors, adjustment of the blood vascular system plays an important role. Not only variation of blood flow and calibre of the cutaneous blood vessels alter the skin temperature but arteriovenous anastomoses and alignment of veins and arteries—venae comitantes also help to regulate temperature. Vasoconstriction of the cutaneous vessels reduces blood flow and thereby less heat is lost from the body, whereas vasodilatation produces opposite effect. Wearing woollen clothes, which are bad conductors of heat, also decrease the heat loss through conduction. Heat loss is prevented due to the entrapment of air between clothes and body surface. 1–4 units of comfortable at low temperature (CLO) can keep a man comfortable at 12°C. [One CLO is defined as the amount of clothing necessary to keep a standard man comfortable in low temperatures.]
3. *Evaporation:* About 25% of heat is lost by evaporation from the body including lungs.

From skin: Insensible perspiration occurs due to continuous diffusion of fluid from the capillaries of the deeper layer of skin to the dry surface of skin. The sweat is vapourised from the surface of the skin, which decreases its temperature, because it is found that 1 gm of water vapourised from the surface produces the loss of about 580 kcal. Evaporation decreases to a great extent if the humidity of the atmosphere is high. For this reason a person can better tolerate high but dry atmospheric temperature than high humid one.

From lungs

- Evaporation of water in expired air is the main pathway through which heat is lost in dogs and sheep.
- Heat lost for warming the inspired air is about 2% in man.

By excreta: Urine, faeces, etc. about 2%. Protrusion of the tongue facilitates heat loss through salivation in dogs.

The simultaneous influences of convection, evaporation and radiation in relation to metabolism and heat change can be determined by the technique of partitional calorimetry. When equilibrium exists, i.e. when heat production is balanced by heat loss, the exchange of heat between body and environment can be expressed by the equation $M \pm C \pm R - E = \pm S$ where M (metabolic heat production) is always positive, E (heat lost by evaporation) always negative, while C (heat lost/gained by convection and conduction) and R (heat lost/gained by radiation) may be either positive or negative. In some conditions, equilibrium is not maintained and heat may be either stored or lost, which is denoted by S. Thus, in vigorous exercise, heat produced is not lost as rapidly and thus body gains heat. On the other hand, on exposure to cold, heat loss is great, and the body temporarily is in negative heat balance.

Nervous System and Thermotaxis

Nervous system controls both heat production and heat loss in the following ways:

1. **Role of cerebrum:** Removal of cerebrum makes very little change. The regulating capacity only becomes slightly restricted. The animal responds normally to external heat or cold but the body temperature falls if kept in the cold room for a long time.
2. **Role of hypothalamus:** The heat-regulating centre lies in the hypothalamus. Section below the hypothalamus (midbrain preparation) destroys the mechanism and makes the animal cold-blooded. These findings show that the hypothalamus is the main centre (Fig. 68.11). Stimulation of the cephalic or anterior part of the hypothalamus causes vasodilatation, sweating, etc. and helps in heat loss. Lesion (disease) of the anterior part of the hypothalamus abolishes these reactions and leads to a loss of power to withstand high temperature. The response to reduced temperature is controlled by the posterior part of the hypothalamus. Lesion of the posterior part of the hypothalamus leads to subnormal body temperature. Thus, it may be concluded that the anterior part controls the rate of heat loss and thereby prevents overheating and the posterior part governs heat production and thereby prevents chilling of the body. Shivering centre is also situated in the posterior part of the hypothalamus. Hypothalamus exerts its effects by controlling autonomic nervous system and by controlling the ductless glands.
3. **Role of autonomic nervous system:** Only a few thermal responses are mediated by the para-sympathetic division, e.g. salivary secretion, secretion of glands of the pharynx and respiratory tract, and local vasodilatation followed by activity. Greater part of the generalised thermal responses in visceral effectors is due to sympathetic control, e.g. constriction of peripheral vessels, erection of hair and feathers, liberation of epinephrine and norepinephrine, sweating and cutaneous vasodilatation. It has been

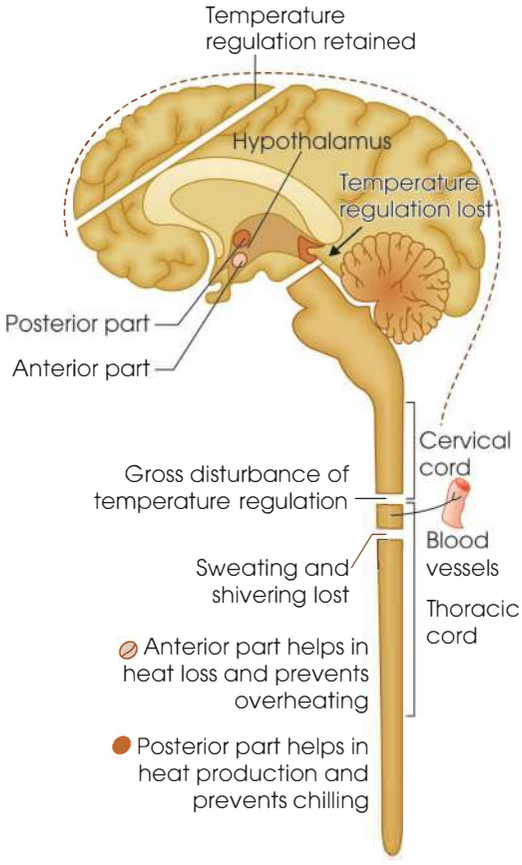


Fig. 68.11: Diagrammatic representation of the role of different parts of central nervous system in thermotaxis

definitely established that adrenal medulla is an integral part of the sympathetic system.

4. **Role of spinal cord in heat regulation:** Spinal cord is the connecting path between the heat-regulating centres in the hypothalamus, peripheral thermoreceptors and effector organs (muscles). The cervical segment of the spinal cord transmits greater part of the sympathetic outflow, which regulates peripheral circulation and hence heat regulation. Spinothalamic tracts of the spinal cord carry the efferent impulse for shivering from higher centres. Effect of section through spinal cord on thermoregulation depends upon the level. When the section of the cord is made above or through the level of sympathetic outflow (cervical segments), gross disturbance of temperature regulation occurs. Transection of the spinal cord from the level of upper thoracic segments downwards abolishes sweating and shivering below the level of transection, i.e. in the paralysed parts (Fig. 68.11).
5. **Role of motor fibres of the cerebrospinal system in heat regulation:** Muscle tone alone (even without locomotion and exercise) is a continuous source of heat production. Central nervous system maintains the muscle tone (thermal muscle tone) by continuous discharge of impulses to the muscles via the motor fibres. Exaggerated 'thermal muscle tone' to the extent of tremor is described as shivering. Shivering impulses from the shivering centre are not transmitted via the sympathetic system but via the motor fibres of cerebrospinal system.

Interaction of Central and Peripheral Factors

Central and Reflex Control

The normal stimulus which mobilises nervous influence arises in two ways:

1. Variations of external temperature affect the skin thermal receptors and reflexly regulate the heat-regulating centres of the hypothalamus. Recent evidences indicate that there are temperature sensitive cells in the hypothalamus (thermodetectors) that respond to high temperature.
2. Temperature of blood, directly bathing the heat-regulating centres, adjusts its activities. For instance, warming the blood in the carotid artery causes those changes which increase heat loss, viz. sweating, cutaneous vasodilatation, increased respiration, etc. Cooling the carotid blood causes opposite changes. The relative roles played by peripheral and central thermoreceptors in the regulation of body temperature are not easily determined. There are species differences as well as differences among individuals of the same species as to the mode of interaction. The skin thermal receptors are responsible for bringing about early thermoregulatory changes in response to environmental temperature changes. Hypothalamic thermodetector cells are more important in that, final thermoregulatory adjustments are brought about by them.

Role of Endocrines

Certain endocrine glands also take part in heat production and heat loss. For instance:

Thyroid: Thyroxine stimulates BMR. Cold stimulates and heat reduces thyroid secretion. In cold, excess thyroid-stimulating hormone (TSH) is liberated from the anterior pituitary and thereby excess thyroid hormones are secreted from the thyroid gland in controlling low body temperature. In cretinism and myxoedema body temperature is subnormal. Thyroidectomised animals cannot maintain the normal body temperature.

Anterior pituitary: Thyrotrophic hormone stimulates secretion of thyroxine and helps in the maintenance of body temperature. Adrenocorticotrophic hormone (ACTH) is secreted under increased or decreased body temperature (cold stress or heat stress respectively).

Adrenal medulla: It helps in both ways. Cold reflexly stimulates adrenaline secretion, which increases heat production by stimulating metabolism. There is increased tissue oxidation and also accelerated

conversion of liver glycogen to blood glucose. The former is of special importance in temperature regulation. It also reduces heat loss by peripheral vasoconstriction.

Adrenal cortex: Adrenal corticoid secretion is stimulated by the increase or decrease of environmental temperature. Usually a low body temperature has been noted in Addison's disease (hypofunction of adrenal cortex).

The hypothalamus contains high concentration of 5-hydroxytryptamine (serotonin) and noradrenaline (norepinephrine). The effect of these two amines, so far the temperature regulation is concerned, varies in different animals. It is presumed that these hypothalamic amines play some part in the regulation of body temperature in normal and in pathological states.

TEMPERATURE REGULATION IN THE NEWBORN INFANT

Body temperature of neonates is generally unstable. They cannot control heat loss by controlling of sweat glands and cutaneous circulation. Infants rarely shiver but can raise metabolic rate by 2–3 times. This occurs principally in two ways: (a) By utilising brown fat and (b) by increasing O_2 consumption. All newborn mammals possess 'brown fat' which has rich blood and sympathetic adrenergic nerve supply. When the infants are exposed to cold, the blood supply to the fat increases and there occurs depletion of intracellular fat droplets.

EFFECTS OF EXPOSURE TO HIGH AND LOW ATMOSPHERIC TEMPERATURE

Physiological Alterations of Body Mechanisms on Exposure to Hot Atmosphere

1. Excessive sweating: When the atmospheric temperature is higher than that of body temperature, heat loss occurs mainly from evaporation of sweat. If the humidity is high, it will prevent rapid evaporation of sweat and reduce heat loss.
2. Rise of pulse rate, increase of cardiac output initially, followed by fall of cardiac output, fall of diastolic blood pressure due to diminished peripheral resistance resulting from vasodilatation.
3. Normal postural adjustment of vascular tone does not occur. Prolonged erect posture may result in fainting attack.
4. Hyperpnoea and respiratory alkalosis develops.
5. Urine volume diminishes; reaction of urine becomes alkaline, NH_3 formation by the kidney decreases.
6. In heat exhaustion syndrome besides hyperpyrexia, sweating is profuse, there is profound loss of Na^+, Cl^- and water. Due to anhydraemia and salt loss there are changes in the muscular activity, e.g. fatigue and heat cramp. There is tachycardia and fall of blood pressure. There is another syndrome known as heat stroke. This also occurs after exposure to excessive heat. There is also hyperpyrexia. The rectal temperature is high. The skin remains dry. The patient may suddenly become unconscious. The heat-regulating mechanisms completely fail. If severe, the nervous system may be permanently damaged.

Life in Deserts

Despite excessive temperature, many animals adapt desert life. Mechanism of survivals depends largely on the size of the animal. Small animals like kangaroo rats burrow hole and hide themselves under the ground in the daytime. A camel sits down early in the morning in a cool patch of sand and tucks its legs underneath exposing as little as possible of its surface to the sun in the daytime.

Sweating is minimal in or absent in desert animals. Thereby they conserve water. They pass dry faeces. Their urine is highly concentrated. Salt content of the urine of kangaroo rats and camel is higher than sea-water. This is probably due to their relatively long renal tubules particularly the loops of Henle. Their long and tortuous upper respiratory tract helps in minimising loss of water.

They can withstand a greater amount of water loss (up to 30% of body weight). Drinking capacity of a camel in one sitting is more than 150 litres. Camel's body temperature remains steady if it has plentiful of water. However, a camel can tolerate a 10°C swing of body temperature. A camel has a body coat of several centimetres thick, which gives it a good protection against heat gain by radiation.

Man has no special adaptations for desert life. Provided there is liberal supply of water and salt he can keep his body temperature within normal range. An explosive heat rise takes place before 15% of the original body water has been lost. In deserts long loose all—enveloping clothes are preferable to shorts.

Physiological Alterations of Body Mechanisms on Exposure to Cold Atmosphere

When the atmospheric temperature is lower, the body temperature is maintained in two ways:

1. By increasing heat production
2. By reducing heat loss.

Heat production is increased by:

1. Stimulating thyroxine and adrenaline secretion (reflexly) this increases the metabolic activities.
2. Increased intake of food (proteins, due to high SDA, are of additional value).
3. Causing reflex shivering, if the cold is too severe.

Heat loss is reduced by:

1. Reflex vasoconstriction of the skin (less blood to the surface, hence less heat loss).

2. Depression of sweating (less heat loss due to less evaporation).
3. Thick layer of subcutaneous fat, clothing.

Extreme or protracted exposure of the limbs to cold may cause vascular changers of the limb, which depend upon the duration of exposure, wetness and chilling. After prolonged exposure, on re-warming the limb shows colour changes of varying degrees, pain and swelling. In its extreme form, the limb may show complete or patchy gangrene (death of tissues). The condition has been designated by various names according to the cause, e.g. frost bite, 'immersion foot', 'trench foot', etc.

Causes of this cold injury are many of which the most important ones are:

1. Formation of ice crystals within the tissues
2. Alteration of local blood supply
3. Alteration of local electrolyte concentration
4. Alteration of tissue pH.

Effect of Sudden Change of Atmospheric Temperature

When a person enters into a warm room after having been out in the cold or from an air-conditioned room, he may feel much colder or even may start shivering. Sudden vasodilatation produced by the warmth leads to sudden drop in blood temperature due to initial return of cold blood from the skin.

PYREXIA

Body temperature, between 99°F (37.22°C) and 105°F (45.57°C) and onwards, is called pyrexia, while rise of body temperature above 107°F (41.66°C) is called hyperpyrexia. Body temperature rises due to derangement of the heat-regulating mechanism. Toxins (pyrogens) act on WBC and produce endogenous pyrogen. This acts directly on the anterior hypothalamus and the body temperature is elevated. Fever occurs due to any of the causes such as infections (e.g. pneumonia, typhoid fever, etc.), injury to nervous centres, dehydration, tissue destruction, administration of some drugs, etc. In some fever, e.g. malaria, shivering or rigor occurs.

Physiological Responses due to Pyrexia (Fever)

1. Metabolism increases.
2. Blood pressure, pulse rate and cardiac output increase. Rate of respiration increases.
3. There occurs negative nitrogen balance.
4. There occurs dehydration and fall of plasma chloride level.

HYPOTHERMIA

Deliberate reduction of body temperature in order to facilitate some surgical operations, e.g. operation on the heart and great blood vessels, and on brain, have been practised with great success. This deliberate lowering of body temperature is known as hypothermia. There are three grades of hypothermia according to the temperature (Table 68.2).

Table 68.2: Grades of hypothermia

1. Mild hypothermia	35°C–31°C
2. Moderate hypothermia	30°C–25°C
3. Deep hypothermia	Below 25°C

The **cooling** may be achieved by various means, e.g.

1. **Surface cooling,** i.e. by exposing the body surface to ice packs, or cold water.
2. **Blood stream cooling,** i.e. blood is collected from major veins (superior vena cava and inferior vena cava or the femoral vein), passed through a system outside the body (heat exchanger) and again returned to an artery with the help of a pump (Fig. 68.12).

Physiological Responses to (Deliberate) Hypothermia

When hypothermia is induced deliberately as an adjunct to surgery, some of the normal physiological responses to cold exposure, e.g. shivering, vasoconstriction, etc. are also stopped by giving muscle relaxants (Curari) and vasodilators (Largactil). By preventing shivering and vasoconstriction, the cooling is accelerated and deleterious effects of unintentional hypothermia or accidental hypothermia as happens in ship-wrecked mariners may be averted. The physiological responses to hypothermia when shivering is stopped are different from unintentional hypothermia with shivering and are of great practical value.

1. **Metabolism:** In absence of shivering, the oxygen consumption falls as the body temperature is reduced. The whole body oxygen consumption may be reduced to half when the mean body temperature is lowered by 10°C. The glucose metabolism is impaired when the body temperature is lowered to 30°C due to inhibition of hexokinase activity. Glucose given intravenously is redistributed into extracellular fluid and injection of insulin does not help in the utilization of sugar.
2. **Heart:** With the reduction of temperature heart rate is also reduced being halved at 25°C, due to the effect of cooling on the pacemaker. In absence of shivering, cardiac output falls with diminishing temperature. Though oxygen uptake by the heart diminishes under hypothermia, yet it remains relatively higher, as compared to most other tissues. When the temperature is lowered below 25°C, ventricular fibrillation starts.
3. **Respiratory system:** There is an increase in the anatomical and physiological dead spaces under hypothermia, probably due to bronchodilatation. A combined respiratory and metabolic acidosis develops.

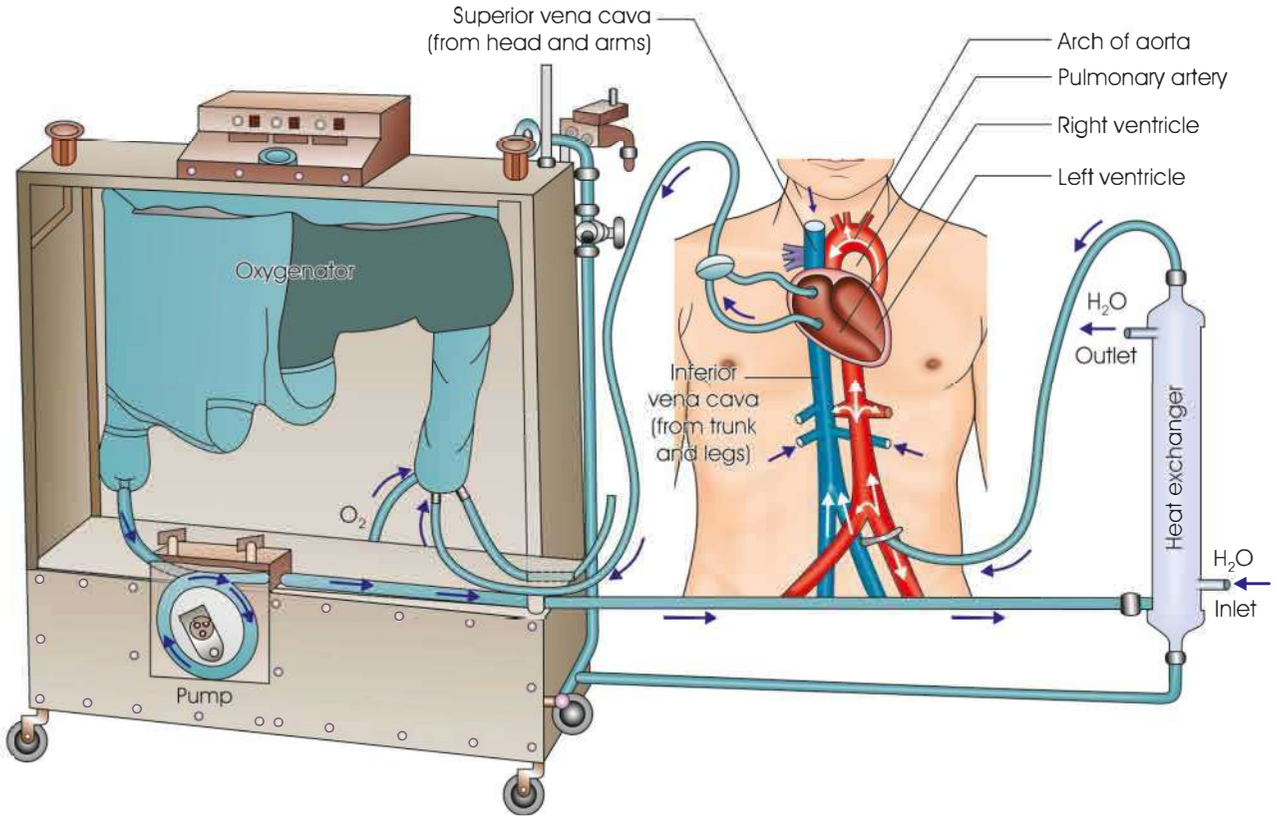


Fig. 68.12: Diagrammatic representation of the method of inducing hypothermia with the help of a heat exchanger incorporated in an extracorporeal circuit (heart-lung machine) consisting of a pump (means heart) and a bubble oxygenator (means lung)

4. **Circulatory changes:** With the reduction of body temperature, blood flow through brain, coronary arteries, kidneys and splanchnic areas is reduced.
5. **Brain:** At normothermia, brain cannot withstand oxygen lack for more than 3 minutes. But when the body temperature is lowered to 25°C, brain circulation can be kept arrested for more than 10 minutes. This advantage of hypothermia is taken in undertaking brain and cardiac operations by stopping circulation through the heart. In absence of shivering cerebrospinal fluid pressure falls under hypothermia.
6. **Kidney:** At 25°C body temperature there is 30% reduction in the glomerular filtration rate and blood flow through the kidney.
7. **Endocrine function:** Deliberate hypothermia under anaesthesia and in absence of shivering, causes of all ductless glands to remain markedly depressed. But if shivering is there, most of the endocrine glands show marked activity.

EXAM-ORIENTED QUESTIONS

Essay

1. What is the normal body temperature? What are the factors affecting body temperature?
2. What is the normal body temperature? Describe the mechanism involved in regulation of body temperature.

Short Notes

1. Functions of skin
2. Mechanism of formation of sweat
3. Composition of sweat
4. Functions of sweat
5. Sweat glands
6. Secretion of sweat
7. Types of sweating
8. Pyrexia
9. Hyperthermia
10. Hypothermia
11. Physiological alterations of body mechanisms on exposure to cold atmosphere

Chapter

69 Regulation of Reaction of Blood and Disturbances in Acid–Base Regulation

INTRODUCTION

Although, large quantities of acids and alkalis derived from food and metabolites, are constantly entering the blood stream, yet the reaction of blood remains rigidly constant with limits of pH varying from 7.36 to 7.44, the H^+ concentration ranging between 44 nmol and 36 nmol per litre. This constant blood reaction is one of the essential requisites of life and any material variation on either side, seriously disturbs the vital processes and may lead to death.

This constant blood reaction is the result of a dynamic process by which equilibrium between the production and excretion of hydrogen ions is maintained. Major sources of hydrogen ion production in the human body may be conveniently and arbitrarily divided into two categories, viz.

1. Respiratory H^+

The process of respiration delivers oxygen to the tissue and carbon dioxide which is generated combines with water molecules forms carbonic acid which finally dissociate to form hydrogen ions and bicarbonate.

Source: $CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$

In alveolar hypoventilation CO_2 is retained and so $H_2CO_3^-$ and H^+ are increased in the blood (respiratory acidosis). The reaction moves to the right. In hyperventilation the reaction shifts to the left and H^+ is eliminated from the blood.

2. Metabolic H^+

The process of metabolism generates formation of H_2SO_4, phosphoric acids, keto acids and lactic acids.

Channels of elimination of H^+ ion from the body are two, viz.

1. *Lungs:* 15,000 mmol of CO_2 are eliminated daily by this route.
2. *End product of metabolism:* The source of hydrogen ion is carbon dioxide production as end product of metabolism. 20–40 mEq of metabolic H^+ is formed daily by this method and 50 mEq of H^+ are eliminated daily by the kidney.

Mechanism of Regulation of Blood Reaction

Factors responsible for maintaining blood reaction within narrow limits of H^+ concentration are mainly through the buffer system.

Buffers of the blood: These are discussed below in greater details.

Buffers

These are substances which fix up the H^+ or OH^- as soon as they are formed, till they are disposed off by the lung or the kidney. They are formed by a weak acid and its salt with a strong base.

Example: $H^+ + HCO_3^- \rightleftharpoons H_2CO_3 \rightleftharpoons H_2O + CO_2$ (eliminated)

$H^+ + Na_2HPO_4 \rightleftharpoons Na + NaH_2PO_4$ (excreted by kidney)

These buffers (Fig. 69.1) may be classified as follows:

1. **Bicarbonate buffers** [(HCO_3/H_2CO_3) = (salt/acid)].
   a. This consists of carbonic acid and its salt with strong base, sodium bicarbonate. Normal [sodium bicarbonate/carbonic acid] ratio in plasma is 20:1.
   b. They are the chief buffers of blood and constitute the so-called "alkali reserve" of plasma.

They help in the following ways: Lactic acid + $NaHCO_3$ = Na lactate + H_2CO_3. The H_2CO_3, thus formed is eliminated through diffusion of CO_2 into the alveolar air in the lungs. Thus, the bicarbonate buffer system is directly linked up with respiration.

$$H^+ + HCO_3^- \rightleftharpoons H_2CO_3 \rightleftharpoons H_2O + CO_2$$

Similarly if a strong base NaOH is added, the reaction is

$$NaOH + H_2CO_3 \rightleftharpoons NaHCO_3 + H_2O$$

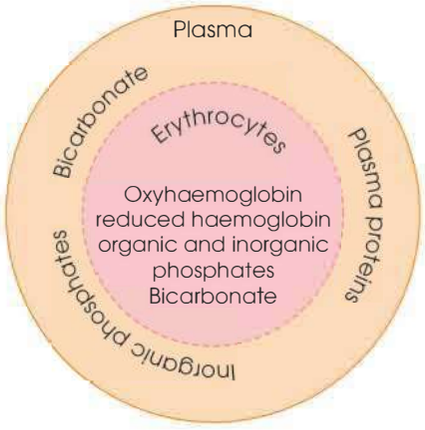


Fig. 69.1: In whole blood, four principal buffer systems are present. In the plasma: Bicarbonate, plasma proteins and phosphates. In the erythrocytes: Haemoglobin, phosphates and bicarbonate

The bicarbonate concentration in glomerular filtrate is 24 mEq/L. Most of the secreted hydrogen ions in the proximal convoluted tubule react with bicarbonate to form carbonic acid and this is useful in bicarbonate reabsorption. The carbon dioxide formed from secreted hydrogen ions returns back to the tubular cells. Thus, there is no net hydrogen ion secretion. While the hydrogen ions which get excreted in excess to one required for absorption of bicarbonate are excreted in urine as titratable acids. Physiologically as most of the secreted hydrogen ions are removed from tubules they do not get excreted in urine.

The kidney maintains the [HCO_3^-] at 24 mEq/L. Ventilation normally maintains the pCO_2 at 40 mm Hg. Thus, the resulting pH is 7.40.

2. **Phosphate buffers:** The efficacy of dibasic phosphate as buffer is a function of the urinary pH in relation to the pK of HPO_4^{2-}. The pK of $HPO_4^{2-}/H_2PO_4^-$ is 6.8, and nearly 90% of the buffering by HPO_4^{2-} occurs above a pH of 5.8. The 30–40 mEq of dibasic phosphate that is filtered each day accounts for the excretion of approximately one half of the daily fixed H^+ excretion. The secreted hydrogen ions react with phosphates rather than bicarbonates. Approximately 10–30 mEq of dibasic phosphate are buffered with H^+. The exchange of hydrogen ions for sodium converts the sodium dihydrogen phosphate into acidic sodium dihydrogen phosphate which is excreted in urine as titratable acids.

The kidneys naturally eliminate more acid phosphate and retain the basic phosphate as far as possible. This is mainly done by altering the excretion of alkaline and acid phosphates. Thus to conclude the hydrogen ion excretion depends on the quantity of dibasic phosphate which is filtered (*refer* diagram 69.3).

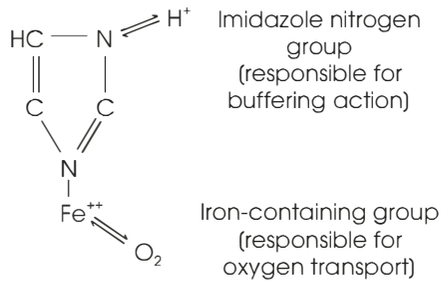


Fig. 69.2: Structure of haemoglobin

3. **Haemoglobin:** Haemoglobin is one of the most important buffers of the blood. Each molecule of haemoglobin contains 38 molecules of histidine. Histidine in haemoglobin molecule contains imidazole, which contains two groups: iron-containing group concerned with carriage of oxygen and imidazole nitrogen group, which is responsible for (Fig. 69.2) buffering property of haemoglobin since it remains dissociated in acid and conjugate base forms.

   1 mmol of reduced Hb can accept 0.7 mmol of H^+ without any change of blood reaction. Carbamino-compounds formed by direct union of CO_2 with NH_2 part of protein molecule, confers upon the proteins the ability to act as buffers, since the reaction is reversible.
4. **Plasma proteins** (Na^+ proteinate/HPr) = (salt/acid): Buffering capacity of the plasma proteins is weaker than haemoglobin. Proteins contain a large number of acidic and basic groups due to presence of COOH and NH_2 groups respectively in their structure. In acid solution they act as a buffer in that the basic amino group takes up excess H^+ ions forming (NH_3^+), whereas in basic solutions, the acidic COOH groups give up hydrogen ion forming COO^-. Carbamino-compounds formed by direct union of CO_2 with NH_2 part of protein molecule, confers upon the proteins the ability to act as buffers, since the reaction is reversible.

Role of Respiration in Acid–Base Balance

Under normal conditions the alveolar ventilation is adjusted so as to maintain an arterial pCO_2 of 40 mm Hg and blood pH 7.4. Respiratory regulation of blood pH is effected by rapid alteration of the volume of alveolar ventilation according to increase or decrease of [H^+] of blood. Thus, as a compensatory response to acidaemia—the respiratory centre is stimulated and the reaction:

$$H^+ + HCO_3^- \rightleftharpoons H_2CO_3^- \rightleftharpoons H_2O + CO_2^-$$

$H_2O + CO_2$ moves to the right while the CO_2 is eliminated and H^+ is converted to H_2O. Likewise, in alkalosis the reaction moves in the opposite direction

and CO_2 is retained in blood when the concentration of H_2CO_3 increases, as a compensatory measure.

Role of Kidneys in Acid–Base Balance

Kidneys play an important role in maintaining a constant blood reaction by the mechanisms described below.

1. **Absorption of filtered $NaHCO_3$:** Under normal conditions urine is almost free of bicarbonate. This helps in maintaining the 'alkali reserve' of the blood. In other words it helps to preserve the HCO_3 buffers of the blood. The obligatory reabsorption of bicarbonate in the kidney occurs in proximal convoluted tubules. DCT and CT are the sites where controlled reabsorption of bicarbonate occurs.
2. Manufacturing of H^+ by the renal tubular epithelium from H_2CO_3 and utilisation of the H^+ for formation of titratable acid (24 to 28 mmol/L)
   $Na_2HPO_4 = Na^+ + Na^+ + HPO_4^- + H^+$
   NaH_2PO_4 (excreted) + Na^+ (reabsorbed as $NaHCO_3$)
   Under conditions of acidosis some of the H^+ manufactured may combine directly with the acid radical Na lactate + H^+
   Na lactate + $H^+ \leftrightarrow$ lactic acid + Na^+ (reabsorbed as $NaHCO_3$)
3. 50–100 mEq of metabolically produced non-carbonic acid is excreted by the kidney daily.
4. The buffers which are active in pH control in kidney are bicarbonate system, dibasic phosphate system and ammonia system. The bicarbonate system, dibasic phosphate system have already been discussed earlier.
5. Role of ammonium buffer (Fig 69.4): Under physiological condition 30–40 mEq of fixed acid is excreted in kidney per day in form of ammonium. Whenever there is increased acid burden the titratable acid is excreted as ammonium up to 300 mEq/day. It (NH_4) is produced mostly within the proximal tubular cells. The predominant source is from glutamine which enters the cell from the peritubular capillaries (80%) and the filtrate (20%). It is produced from glutamine by the action of the glutaminase. Further ammonium is produced when the glutamate is metabolised to produce α-ketoglutarate. It is primarily synthesized in the proximal tubular cells by the deamination of glutamine to glutamate and ammonium cation. It is transported into the interstitium in the thick ascending limb substituting for K^+ on the Na^+-K^+-$2Cl^-$ carrier. It then dissociates to ammonia in the medullary interstitium under the influence of a relatively higher pH. Ammonia thereafter diffuses into the medullary collecting duct and is trapped in acidic urine as NH_4. Thus, 30–40 mEq of fixed acid is excreted in kidney per day as ammonium.

Note

Mechanism of formation of H^+ by kidney.

Note

a. The ammonia system has pK value about 9 and due to this all the NH_3 entering the distal convoluted tubules at pH value of 6 combines with hydrogen ions to form NH_4. Ammonium system eventually conserves sodium and bicarbonates of the body.
b. The transport mechanism can secrete hydrogen ions with the maximal H^+ gradient at pH 4.4. This is the limiting pH of urine that is the lowest pH attainable in urine.

DISTURBANCES IN ACID–BASE REGULATION

69

Alteration of hydrogen ion concentration of blood may lead to two main patho-chemical conditions:

1. Acidosis
2. Alkalosis.

Acidosis is defined as a patho-chemical condition with lowering of blood pH below 7.36 resulting from primary accumulation of acid or primary loss of base from the body or from some specific fluid compartment of the body.

Alkalosis is a patho-chemical condition with increase of blood pH above 7.44 resulting from primary accumulation of base or primary loss of acid from the body or some specific fluid compartment of the body.

RESPIRATORY ACIDOSIS

Definition

It is a clinical condition caused due to decreased alveolar ventilation (especially respiratory diseases or other associate causes) which leads to reduced arterial pH, increased carbon dioxide tension and increased plasma bicarbonate.

Pathophysiology

There is decreased alveolar ventilation leading to increased pCO_2 which increases H_2CO_3 (carbonic acid) and thus increases H^+ concentration and decreases the pH and increases the bicarbonate concentration in the arterial plasma.

Causes

The decreased alveolar ventilation may be due to emphysema, chronic obstructive pulmonary disease, overdose of respiratory depressant, breathing 7% CO_2, bulbar poliomyelitis, chest injuries (tension pneumothorax), sleep-causing depressed respiration, congenital heart diseases, etc.

$$H_2O + CO_2 \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

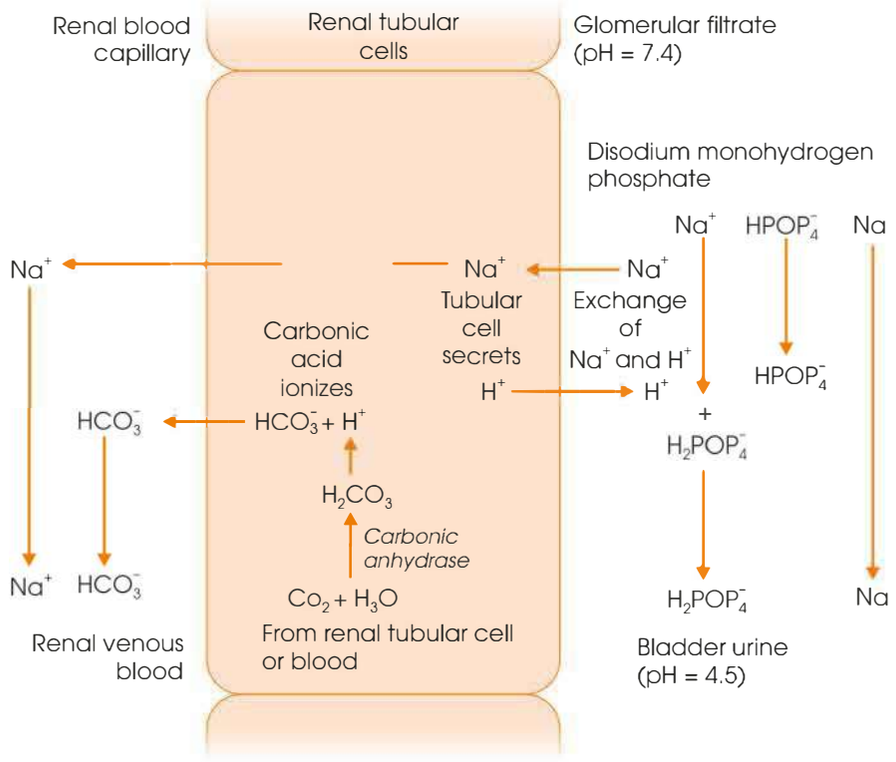


Fig. 69.3: Schematic representation of acid-base equilibrium by the kidneys by the disodium phosphate buffer

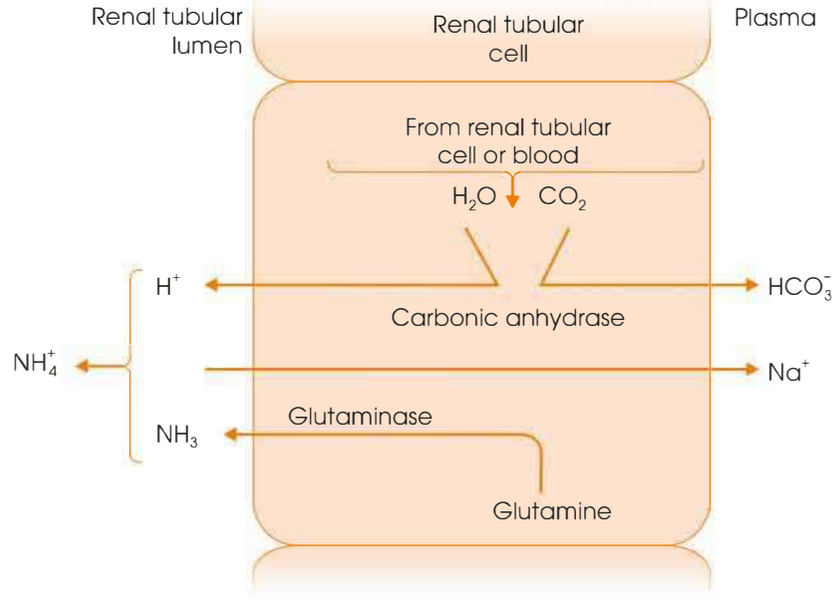


Fig. 69.4: Schematic representation of acid-base equilibrium by the kidneys by the ammonia mechanism

Compensatory Mechanism

Renal Mechanism

1. The renal tubular secretion of hydrogen ions increases thereby pH increases and pH returns to normal physiological level.
2. There is increased bicarbonate absorption accompanied by renal tubular secretion of hydrogen ions (although plasma bicarbonate level is high) further increases plasma bicarbonate and promotes chloride excretion in urine in exchange of bicarbonate reabsorption.

METABOLIC ALKALOSIS

Definition

It is a clinical disorder characterised by increased arterial pH and increased plasma bicarbonate.

Causes of Metabolic Alkalosis

It may occur under the two general conditions:

Accumulation of bicarbonate content of plasma or primary loss of acids occurs in the following conditions:

1. Excess of alkali intake—as in the treatment of peptic ulcer (milk-alkali syndrome)
2. Vomiting specially in the early stages (in the late stages of vomiting acidosis and ketosis occur due to starvation), viz. (i) in pyloric stenosis, (ii) in high intestinal obstruction.
3. Overzealous treatment of acidosis without repeated assessment for the acid–base status.
4. Potassium deficiency leads to intracellular migration of H^+ ion in exchange of potassium lost from the cell. Unlike other types of alkalosis, there occur aciduria in alkalosis following potassium deficiency (paradoxical aciduria).
5. Fall of pCO_2 of blood occurs in the following conditions:
   - Voluntary hyperpnoea.
   - High external temperature with moist air, causing increased pulmonary ventilation to help heat loss.
   - Excess CO_2 is also lost, hence, alkalosis.
   - Anoxia.
   - High altitude, etc.

Patients with alkalosis suffer from lethargy, muscular weakness and mental confusion. Acidosis and alkalosis are classified as follows:

Henderson-Hasselbalch equation states that:

$$pH = pK + \log \frac{[HCO_3^-] = [\text{Bicarbonate}]}{[H_2CO_3] = [\text{Carbonic acid}]}$$

$$= 6.1 + \log \frac{[HCO_3^-]}{0.0301 \times \text{partial pressure of } CO_2\ (pCO_2)}$$

It is evident from the above equation that pH of a sample of blood depends on the $[HCO_3]/[pCO_2]$ ratio. When this ratio is increased, pH will be elevated resulting in alkalosis. The lowering of pH will occur when $[HCO_3]/[pCO_3]$ ratio is decreased. The decrease of the ratio may be due to the decrease of the numerator (bicarbonate) or due to the increase of the denominator (partial pressure of CO_2). In case, acidosis or alkalosis, when the alteration of the pH is primarily due to bicarbonate concentration of blood, the condition is designated as metabolic or non-respiratory. Similarly, the alteration of pH resulting in acidosis or alkalosis primarily due to carbon dioxide tension of the blood, is designated as respiratory or gaseous.

Respiratory Compensation

Alkalosis at once depresses respiration leading to rise in pCO_2 and H_2CO_3 in blood (respiratory compensation)

$$H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

Some of the HCO_3^- are buffered by non-bicarbonate buffers:

$$HCO_3^- + H\,Buf^- \rightleftharpoons H_2CO_3 + Buf^-$$

Renal Compensation

There thus occurs increase in pCO_2 along with rise in total buffer base as well as bicarbonate in the blood. It is slow to develop and consists mainly in excretion of excess of alkali from the body in the form of alkaline urine. It may be noted that the high pCO_2 favours the formation of H^+ by the kidney and excretion of titratable acid and (NH_4) salts. In this respect the respiratory compensatory effect counteracts to some extent the renal compensatory effects. But nevertheless, if adequate treatment is available—the continued excretion of alkaline urine by the kidney establishes the normal blood pH within a reasonable period.

METABOLIC ACIDOSIS

Definition

It is a clinical disorder characterised by low arterial pH or decreased plasma bicarbonate concentration.

Causes

1. Ingestion of excessive alcohol, salicylate or ammonium chloride.
2. Loss of bicarbonate as seen in clinical condition such as fistula or severe diarrhoea.
3. Diabetic ketoacidosis.
4. Excessive accumulation of lactic acid in case of prolonged severe exercises.

Compensation

1. **Respiratory compensation:** Partial compensation is effected by hyperventilation caused by acidosis. This results in fall in pCO_2 and the amount of H_2CO_3 in the blood.
2. Renal compensation—takes longer time to be established but are more effective.
   a. The kidney continues excreting all bicarbonate which is filtered and hydrogen in form of titratable acid and NH_3 salts. If metabolic defects are rectified in mean time by, e.g. in diabetic ketoacidosis the bicarbonate concentration of the

blood increases (due to continued reabsorption by the kidney) and the blood buffer is restored to normal:

$$H\ Buf^- + HCO_3^- \rightleftharpoons Buf^- + H_2CO_3$$

b. The renal tubular cell secretes hydrogen ions in exchange for sodium and with secretion of each hydrogen ion; one sodium and one bicarbonate ions are added to the blood thus restoring bicarbonate levels.

RESPIRATORY ALKALOSIS

Definition

It is a clinical disorder characterised by variable decrease in plasma bicarbonate, low carbon dioxide tension and increased arterial pH.

Causes

1. Anoxia leading to hyperventilation
2. Voluntary hyperventilation
3. High altitude hyperventilation
4. Any condition which leads to hyperventilation such as anxiety or hysteria will drive out CO_2 from the body and the concentration of H_2CO_3 in the blood will fall with relative excess of HCO_3, which is responsible for alkalosis.

   The excess HCO_3 is buffered as follows:

$$HCO_3^- + H\ Buf \rightleftharpoons Buf^- + H_2O + CO_2\ (expired)$$

This tends to diminish HCO_3^- with corresponding increase in Buf^- so that the total BB and base excess remains unaltered.

In a typical case the biochemical data may be as follows: pH = 7.52, pCO_2 = 22 mm Hg (due to hyperventilation). Plasma bicarbonates are 16 mEq/L (due to buffers as noted above). BE = 0.

Renal Compensation

a. The amount of HCO_3^- filtered by the kidneys is not reabsorbed by the renal tubule and is excreted in the urine. H^+ secreted by the tubule is pCO_2 dependent and since pCO_2 is low, H^+ secreted by the renal tubule is low. Therefore, the titratable acidity and the NH_3 is coefficient of the urine is both decreased and the kidney secretes an alkaline urine to correct the alkalosis.

b. The renal tubular secretion of H^+ decreases leading to retention of H^+. And thus the pH is decreased.

Assessment of the Acid–Base Status

Precise evaluation of acid–base abnormalities is necessary for instituting proper treatment in many clinical conditions, to indicate the type and severity of the acid–base disturbance, determination of (1) pH, (2) arterial blood gas test.

pH

Nowadays pH of whole blood is universally determined electrometrically using reliable pH metres, equipped with thermostated glass electrode at a temperature of 37°C.

Arterial Blood Gas Test

An arterial blood gas (ABG) test measures the blood gas tension values of the arterial partial pressure of oxygen, and the arterial partial pressure of carbon dioxide, and the blood's pH.

EXAM-ORIENTED QUESTIONS

Essay

1. Describe the role of kidney in acid–base balance.
2. Describe the mechanism of action of various buffers involved in acid–base balance.

Short Notes

1. Metabolic acidosis
2. Metabolic alkalosis
3. Respiratory acidosis
4. Respiratory alkalosis

CLINICAL CASE SCENARIO

Kidney

Q1. A 55-year-old male who was a known patient of anxiety neurosis complained of headache, nervousness and irritability due to over work in the office. His heart rate variability report revealed sympathetic dominance; blood pressure was 140/90 and urine glucose report revealed glycosuria. Correlate the cause of nervous glycosuria.

Ans. Nervous stimulation is responsible for increase liberation of adrenaline which activates glycogenolysis. The chronic nervous conditions increase blood glucose over a prolonged period of time and also produce glycosuria. The stimulation of sympathetic nerves to the liver or of the splanchnic nerves breaks down liver glycogen and thus causes glycosuria. Thus, factors that stimulate sympathetic system such as excitement and stress can produce glycosuria.

Q2. Two friends 18-year-old planned a trip to desert in summer season. Discuses the preventive measures they would plan to prevent heat stroke and heat exhaustion.

Ans. Human does not have any special adaptations for desert life hence they should carry with them adequate water and salt (electrolyte) supplementing drinks so that liberal supply of water and salt will keep their body temperature with normal range. An explosive rise in body heat will take place when nearly 15% of original body water has been lost. In deserts, long loose clothes are preferable to shorts.

Q3. A 32-year-old male was diagnosed as case of frost bite as result of cold injury. What are the causes of cold injury?

Ans. The causes of cold injury are:

a. Formation of ice crystal within the tissue.
b. Alteration of local blood supply.
c. Alteration of local electrolyte to solution
d. Alteration of tissue pH.

Q4. A patient of diarrhoea landed up in acidosis. Explain the cause of acidosis in diarrhoea.

Ans. Diarrhoea leads to dehydration, impaired renal circulation and thereby impairs kidney function. The anoxia from impaired circulation leads to lactic acid accumulation in the tissue and further the loss of bases in the faeces leads to acidosis and ketosis (due to starvation, as person avoids intake of food due to discomfort and feeling of nausea and vomiting).

Q5. A patient reported to casualty with complains of vomiting since one week. He was a known case of acid peptic disease and was on daily intake of antacids. On basis of his investigation of acid blood gas analysis he was diagnosed as a case of metabolic alkalosis. What is the cause for the same?

Ans. The patients past history revealed prolonged treatment with antacid for acid peptic disease. This high intake of antacid over time with the loss of hydrochloric acid in vomiting history of one week might have lead to metabolic alkalosis.

Q6. A patient of tabes dorsalis developed automatic bladder. Explain the disease and cause for development of automatic bladder.

Ans. The injury of the afferent nerves of spinal cord produces lesion in dorsal lumbo-sacral nerve root in patent of syphilis. The patient can micturate voluntarily but cannot sense the fullness or distended state of bladder leading, to incomplete evacuation of bladder. This produces distension of bladder and it becomes thin leading to a state of automatic bladder in which emptying is due to stretch induced contraction of detrusor muscle of bladder.

Q7. A 50-year-old was admitted to casualty and diagnosed as a case of congestive cardiac failure. Explain the classical signs seen in patients of congestive cardiac failure.

Ans. The congestive cardiac failure may manifest as right heart failure or left heart failure. The vital signs seen in right ventricular failure are distended neck veins, increased jugular venous pressure, and oedema in lower limb and sacral region and hepatosplenomegaly. The left ventricular failure patient may present with froth sputum, dyspnoea, paroxysmal nocturnal dyspnoea and orthopnoea (dyspnoea in lying down position).

Applied Physiology

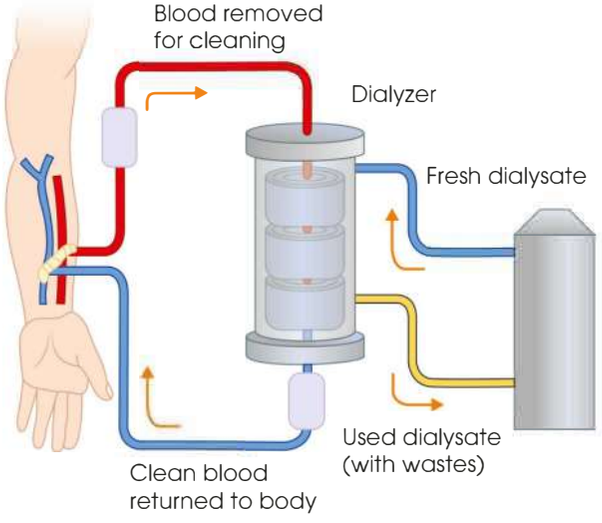
Dialysis Techniques

Dialysis is a process for removing accumulating metabolic waste and excess water from the blood and is used primarily as a renal replacement therapy in patients of renal failure. The two common methods used in dialysis are haemodialysis and peritoneal dialysis.

Haemodialysis: The patient's blood is passed through the dialyzer and pumped through the dialyzers blood compartment, thereby exposing the blood to a partially permeable membrane. The dialyzer composes of thousands of tiny hollow synthetic fibres which form the fibre wall. The fibre wall acts as the semipermeable membrane. As the blood flows through the fibres, it is exposed to the dialysis solution which flows around the outside of the fibres, and water and wastes move between blood and the dialysis solution. The cleansed blood is then returned via the circuit back to the body. Ultra-filtration occurs by increasing the hydrostatic pressure across the dialyzer membrane.

Peritoneal Dialysis

A sterile solution containing glucose (dialysate) is run via a tube into the peritoneal cavity. The peritoneal membrane acts as a partially permeable membrane. The



Haemodialysis in progress

Note

Paediatric dialysis: Newer generation modified biocompatible synthetic membranes, small size material dialyzers and newer extra-corporeal volume tubing have been designed for infant dialysis. The arterial and venous tubing length are made of minimum length and diameter (<80 to <110 ml volume tubing) is designed for paediatric patients. In paediatric patient the pump speed should be kept at low side depending on patient's blood output capacity as high flux dialysis is not recommended in paediatric cases.

peritoneal membrane is a layer of tissue containing blood vessels that line and surround the peritoneal, or abdominal cavity and the internal abdominal organs. The process of osmosis and diffusion drive excess fluid and waste product through the peritoneum into the dialysate until the dialysate achieves equilibrium with the body's fluids. Dialysate is thereafter drained, discarded, and replaced with fresh dialysate.

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Recent Updates: Transplanting the Islet Cells from the Pancreas

The patient in renal failure with diabetic complications may require kidney and transplantation of the islet cells from the pancreas. Transplanting just the islet cells from the pancreas is still in the experimental stage and needs further exploration. The deceased donor pancreas is received, it is carefully dissected over and the islet cells that make insulin are extracted. These cells are injected through a catheter into the recipient and are lodged in the liver. The recipient is administered immunosuppressant drugs to avoid rejection, but it does not require any surgery.

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Appendices

Appendix

1

Some Physico-chemical Laws Applied to Physiology (Biophysics)

Physiological processes are dependent upon certain physico-chemical principles which are described in this chapter.

UNITS OF CONCENTRATION OF SOLUTIONS

Percentage: Weight in grams of solute per 100 gm of solution is percentage by weight and the same amount dissolved in 100 ml is percent by volume.

Molar solution: Molecular weight in grams of a solute dissolved in 1000 ml is a molar solution, e.g. mol wt of H_2SO_4 is 98.016, so a molar solution is 98.016 gm per 1000 ml.

Molal solution: Molecular weight in gram of a solute dissolved in 1000 gm of solvent. A mol (M) or gram-molecular weight is the amount of substance in grams equal to the molecular weight of the substance. One millimol (mM) is equal to one-thousandth of a mol (M) and 1 micromol (μM), to one-thousandth of a millimol.

Normal solution: A normal solution is one which contains per litre, the amount equal to gram-molecular weight of the substance divided by equivalent weight or number of replaceable hydrogen atoms.

Example: Molecular weight of H_2SO_4 is 98.016 where $H^+ + H^+$ and SO_4 ions are present. As number of replaceable hydrogen atoms is 2, then normal solution of H_2SO_4 will contain 98.016 divided by 2, i.e. 49.008 gm in a litre.

Gram equivalent is the molecular weight in grams divided by the number of valence of ions present. One milliequivalent (mEq) is one-thousandth of such gram equivalent.

IONS, ELECTROLYTES AND NON-ELECTROLYTES

Ions

On an electric current being passed through solution of a compound in water, some elements migrate at the positive pole or anode and some at the negative pole or cathode. The charged particles are called ions. Negative ions (anions) move towards the anode and positive ions (cations) to cathode due to attraction of unlike charges.

Electrolytes

Electrolytes are compounds which can be dissociated into anions and cations on an electric current being passed. e.g. acid, bases, salts, etc.

Non-electrolytes

Compounds which cannot be dissociated are called non-electrolytes.

FILTRATION

Filtration is the process by which undissolved particles are separated from a liquid through a membrane as a result of a mechanical force (filtering force). It is done through a porous substance, such as a piece of linen or filter paper. This filtering force is either gravity or hydrostatic pressure—positive or negative.

Physiological Importance

Whenever there is a difference of hydrostatic pressure between the two sides of a membrane, filtration will occur.

The important examples are: (a) Absorption from the small intestine, (b) passage of water, salts, foodstuffs, etc. from the blood stream to the tissue fluid—hydrostatic pressure in the capillaries being higher than in the latter.

DIFFUSION

Molecules of a substance are continuously in motion. This motion is least in the solids, maximum in the gases and intermediate in the liquids. When two such substances (for instance—a solid and a liquid, or two miscible liquids, or a liquid and a gas or two gases) are kept in contact, the molecules of the two substances

will pass into each other, until an uniform admixture is obtained. If a layer of water be carefully added upon a layer of concentrated sugar solution, the two liquids will gradually run into each other and ultimately the sugar concentration will be same everywhere. This spontaneous admixture of the molecules of the two substances in contact (due to inherent molecular movement) is called diffusion (Fig. A1.1).

Anything that alters the molecular movement also alters the rate of diffusion proportionally, viz. heat increases and cold depresses. The diffusibility of different substances is not same. Other factors remaining constant, diffusion depends upon the weight and size of the molecules.

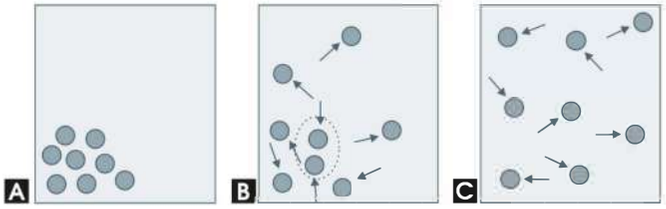


Fig. A1.1A to C: Diffusion (schematic representation): (A) Molecules are introduced into one corner; (B) Molecules are in random movement due to their kinetic energy. They can collide with one another and with the walls of the container; (C) Molecules are commencing to be evenly distributed throughout

Physiological Importance

When two substances, as mentioned above, come in contact, directly or through a permeable membrane, diffusion will take place, when the molecular concentration of a substance in solution is higher in one part of a liquid than in another and when any absolute barrier does not intervene (Fig. A1.2). Some of the important examples are:

a. Admixture of foodstuffs with digestive juices
b. Absorption from the intestine
c. Exchange between plasma and red cells
d. Exchange in the capillary bed, e.g. foodstuffs, oxygen, etc. pass out from the blood stream (highest concentration) to the tissue fluid (lower concentration) and then to the tissue cells (lowest concentration), where they are used up.
e. The metabolites including CO_2, on the other hand, come out of the cells (highest concentration) and enters into the capillary and carried out through circulatory system and thereafter while CO_2 from venous blood (higher concentration) diffuses out into the air (lower concentration).

OSMOSIS

The diffusion of water through a semipermeable membrane is called osmosis. If a layer of water be separated from a sugar solution by a semipermeable

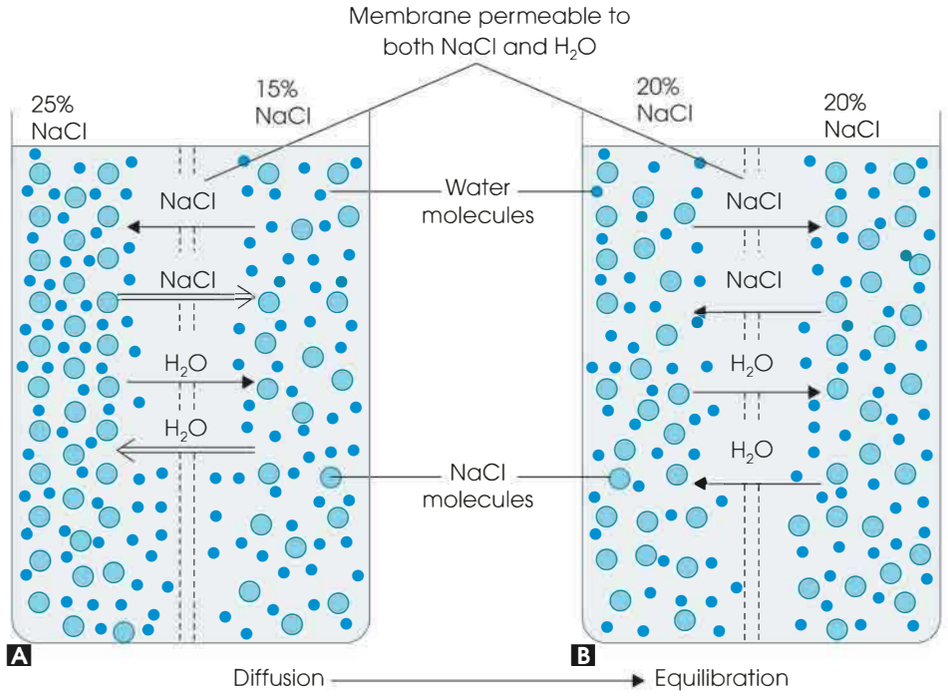


Fig. A1.2A and B: Mechanism of diffusion of two solutions of unequal strength. (A) Contains NaCl, compartment (a) contains 25% NaCl and (b) has 15%. NaCl solution diffuses rapidly from (a to b) But H_2O from (a) diffuses slowly to (b) and diffuses rapidly from (b to a). But NaCl diffuses very slowly from (b to a). (B) Equilibration of two solutions after diffusion through membrane are produced, to the tissue fluid (lower concentration), and then to the blood stream (lowest concentration). (e) Exchange in the lung capillaries, e.g. O_2 from air (higher concentration) enters the venous blood (lower concentration), while CO_2 from venous blood (higher concentration) diffuses out into the air (lower concentration). (f) Admixture of gases in the lungs. There are many other examples

membrane (which will allow only the water molecules to pass but not the sugar molecules); it will be seen that the sugar solution gradually increases in volume for some time and then there will be no further change (Fig. A1.3). What happens is that, the sugar molecules being impermeable, more water molecules will pass from the water layer into the sugar solution than will pass from the latter to the former (Fig. A1.3A and B). Due to this, the volume and therefore the level of sugar solution will rise. This will raise the hydrostatic pressure of the sugar solution and this increased pressure will force more and more water molecules to pass out of the sugar solution. Thus, a time will come when the movement of water molecules on either side will be same, so that no further alteration of volume will take place. At this stage (Fig. A1.3C), the hydrostatic pressure of sugar solution S exactly neutralises the attractive force of the solution for water molecules. This attractive force is the osmotic pressure. The amount of pressure which has to be applied on solution S to prevent the movement of water molecules is defined as the osmotic pressure of the particular concentration of the substance. If the semipermeable membrane is placed between two sugar solutions of different strengths, water molecules will continuously pass from one side to the other till the concentrations on two sides are equal. Stronger solution will always draw more water from the weaker solution. Therefore, the force under which a solvent moves from a solution of lower solute concentration to a solution of higher solute concentration when a selectively permeable membrane separates these solutions is called osmotic (oncotic) pressure (Fig. A1.3). In true sense, it is the pressure which must be put upon a solution to keep it in an equilibrium with the pure solvent when the two are separated by a semipermeable membrane. For this reason osmotic pressure does not depend upon the size of the molecules but upon the total number of discrete particles per unit volume. If the solute be ionisable, the osmotic pressure (OP) will be proportionally more. If more pressure (weight) is applied on piston (Fig. A1.4) water will be forced out from the stronger solution S to the weaker solution W. This passage of water against concentration gradient is known as ultra-filtration. If two solutions, separated by a membrane, have the same OP, they are called isotonic. One having lesser OP is called hypotonic, while that having higher OP, hypertonic. 0.9% sodium chloride solution is isotonic with blood plasma, commonly known as normal saline or physiological saline. A 5% solution of glucose has also similar osmotic pressure. Either of them can be injected intravenously in human patient if properly sterilised. Two solutions having same number of particles per unit volume are called isosmotic. But since, their permeability through a membrane may vary, they are not necessarily isotonic.

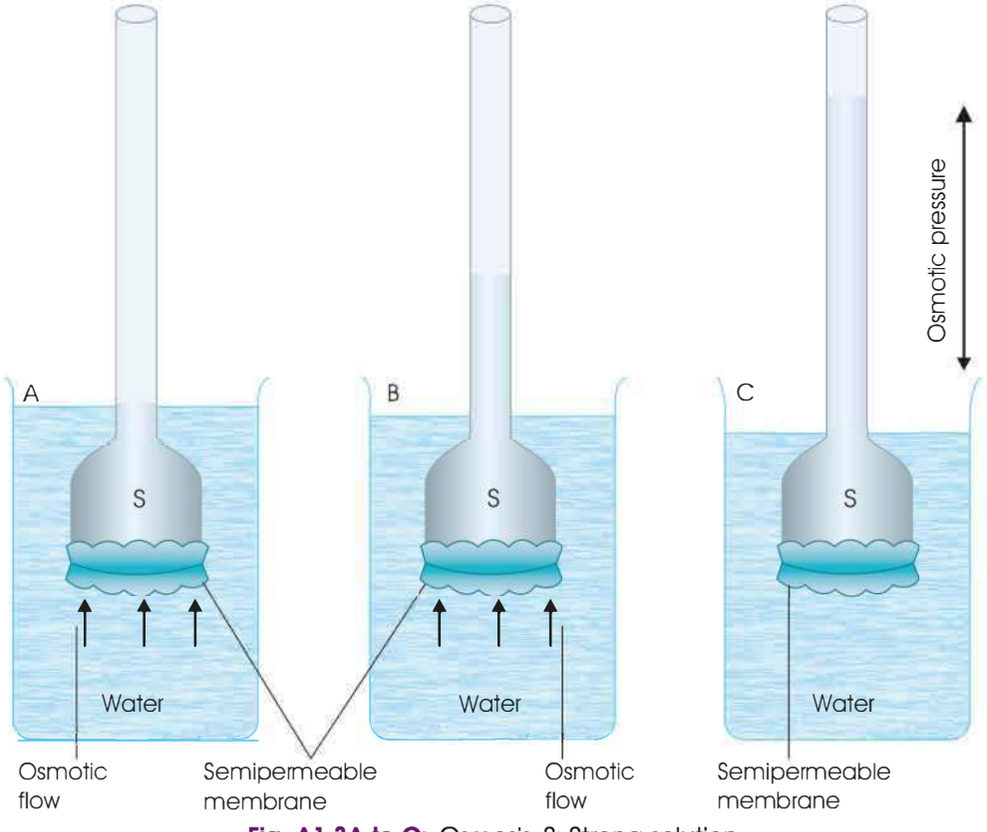


Fig. A1.3A to C: Osmosis. S: Strong solution

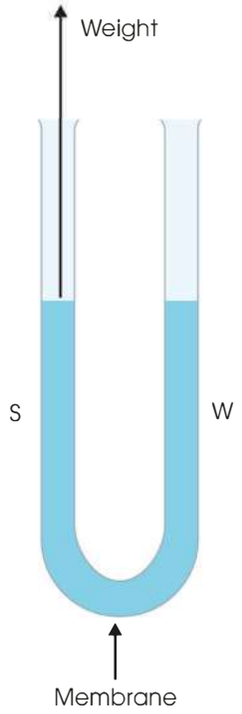


Fig. A1.4: Measuring OP by weight

Physiological Importance

Osmotic pressure plays a great role in physiology. A few examples are as follows:

1. Absorption from the intestine
2. Exchange in the capillary bed. Continuous osmotic exchange is going on between blood, tissue fluid, tissue cells and lymph.
3. Regulation of urine formation.
4. Reabsorption of cerebrospinal fluid.
5. Continuous osmotic exchange between plasma and red cells.

Clinical use: Injections of isotonic, hypotonic and hypertonic saline and other solutions are given in suitable cases by way of treatment. Saline purgatives (magnesium sulphate, etc.) and saline diuretics also work on osmotic principle.

Laboratory use: Sodium chloride and other solutions of different osmotic pressures are used in the laboratory for various experiments.

Methods of Measurement

OP can be measured in various ways which are described below.

Mechanical Methods

By putting weights: The simplest way is to apply adequate pressure (i.e. weight) upon the stronger solution to prevent any rise of volume. That pressure which is just needed to stop the increase of volume of a particular solution is the measure of its OP (Fig. A1.4).

By a manometer: The same thing can be done by connecting the apparatus with a suitable manometer in which the pressure will gradually rise till it equalises with the OP of the solution, at which point further rise will stop (Peffer's method, Fig. A1.5).

Biological Methods

Hamburger's red corpuscle method: Red cells are kept in the unknown solution for some time after which the cell volume is noted. If the cell volume be reduced, the solution is hypertonic than plasma (hence, water has been drawn out), if the cells swell up, the solution is hypotonic (so that water has entered), if no change—the solution is isotonic, if sufficiently hypotonic the red cells will gradually swell up and ultimately burst (haemolysis).

De Vris' plant cell method: The same principle is followed as above, only plant cells are used instead of red cells and the comparison is made with the cell sap inside. In hypertonic solutions, the cells will shrink, in isotonic—there will be no change, while in hypotonic solutions the cells will swell up and may burst (plasmolysis).

Physical Methods

By noting depression of freezing point: Higher the concentration, lower will be the freezing point, and therefore higher will be the OP.

By Noting the Vapour Tension

Higher the concentration, lower will be the rate of evaporation from the solution and higher the OP.

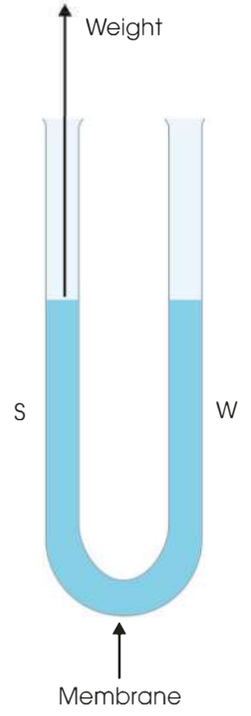


Fig. A1.5: Measuring OP with manometer

Hill's method: Using thermopile. Higher the rate of evaporation more will be the fall of temperature and less will be the OP. Comparison is made with a solution of known OP.

Barger's capillary glass tube method: Alternate drops of known and unknown solutions separated by air bubbles are drawn in a standard capillary glass tube and after some time the edges of the solutions noted. The edges will shift according to the rate of evaporation. From these data OP can be calculated.

ULTRA-FILTRATION

Ultra-filtration is a kind of filtration through a jelly filter or any ultra-filter which serves to separate colloid solutions from crystalloids and to separate particles of different size in a colloid mixture. Due to the opposite of osmosis ultra-filtration results from the exertion of a pressure on a solution. This pressure forces the solution through a membrane impermeable to one or more of the solutes.

Physiological Importance

The blood plasma is placed in a vessel of which one end is a collodion membrane. The collodion membrane was first utilized by Fick's in 1855 to study diffusion. Later flexible collodion membranes were used. Now, if a pressure is exerted on the blood plasma an ultra-filtrate will be separated. This separation will take out from the solution all the constituents of the plasma except the protein which has been contained in the gross plasma. This occurrence results in due to small pores of the collodion membrane. The extracellular fluid (ECF) is also an ultra-filtrate into the plasma through the porous capillary membrane. If the counter pressure is not exerted on the plasma, the ECF shall pass back into the plasma.

DIALYSIS

Dialysis is a process by which the more diffusible materials can be separated from non-diffusible material. In Fig. A1.6A, water solution of egg albumin and sugar has taken in the upper smaller container whose open bottom is covered with a semipermeable membrane. The semipermeable membrane has got selective permeability to water and sugar molecules but not to macromolecules—the egg albumin. This container is suspended (partially) in the water of a large container. Due to selective permeability, the sugar molecules will ultimately go into the water leaving behind only the albumin and a little water. As the albumin is impermeable to this membrane, this will rebound from the membrane during the process of dialysis (Fig. A1.6).

Physiological Importance

Whenever the bigger particles are held back and only the smaller particles are allowed to pass through a

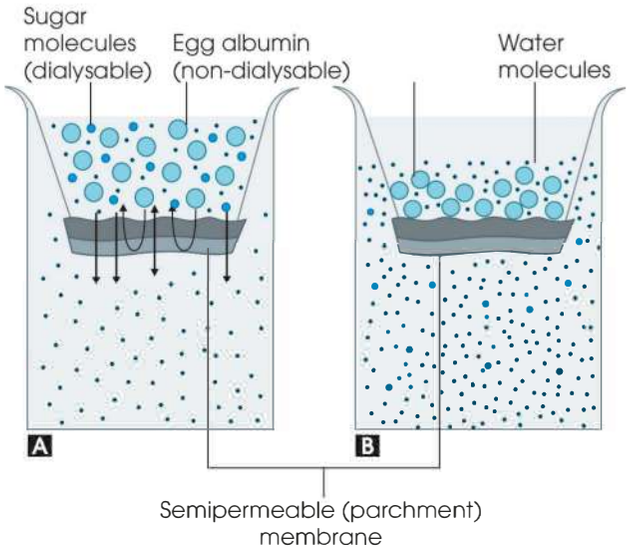


Fig. A1.6A and B: The process of dialysis. (A) Onset of the process; (B) After completion of the process

membrane depending upon osmotic pressure (i.e. diffusion), dialysis comes into action. For instance: (a) During absorption from the intestine bigger food particles are held back, (b) in the capillary area the bigger albumin, globulin, etc. particles are not ordinarily allowed to pass into the tissue fluid. It is to be noted that diffusion, osmosis and dialysis are the manifestations of the same principle (inherent molecular movement) and usually go on simultaneously.

SURFACE TENSION

Surface tension is the manifestation of attracting forces in between atoms or molecules. As elsewhere, so also in a liquid, the molecules attract each other. Within the depth of the liquid, each molecule is attracted equally from all directions (Fig. A1.7). Hence, the molecule can move freely in all directions. But a molecule at the liquid-air surface is attracted only by the molecules within the depth of the liquid and there are relatively few molecules in the gas above the water surface to exert any upward force. Consequently, it tends to be pulled inwards and its freedom of movement is restricted. Thus, at the surface of a liquid, a layer is

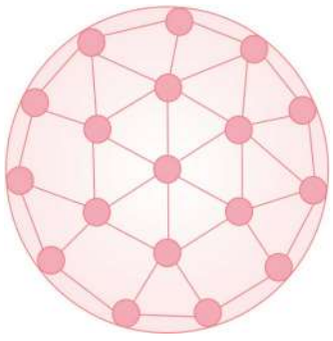


Fig. A1.7: The surface tension of water molecules in a beaker as seen from above

formed in which the molecules are arranged more densely. For the same reason the surface of the liquid tries to pull itself together and shrinks in order to occupy the least possible area. This energy with which the surface molecules closely adhere together is called surface tension. The solutes influence surface tension. Inorganic salts generally raise while organic substances reduce surface tension of water. Of the latter—bile salts, proteins, phospholipids, oils, soaps, etc. are important examples. (Hay's sulphur test, for the detection of bile salts in urine, depends upon this principle.)

Physiological Importance

(a) The globular shape of an oil drop in water, of the fat particles in milk, etc. is due to surface tension. (b) Bile salts reduce the surface tension of fat converting it into an emulsion in the intestine. This helps digestion and absorption of fat. (c) The formation of cell membrane is at least partly due to surface tension of the cell cytoplasm. There are innumerable such examples.

ADSORPTION

Adsorption is a peculiar form of combination in which substances adhere together on their surfaces. It is a sort of union by surface contact. It is not a true chemical reaction, because no definite quantitative relation is found. The possible mechanisms of adsorption are as follows:

- **Surface tension:** Adsorption is probably a manifestation of surface tension developed from the attraction of dissimilar molecules of two substances.
- **Residual valence:** The chemical nature of the particles and their residual valences may be such as to make such a loose combination possible.
- **Electrical state:** The electrical charge of the particles may be of opposite nature; so that they attract each other and thus make a sort of contact combination. Failure of adsorption means that the electrical charges are similar, so that they repel each other. During the process of adsorption much kinetic energy is lost and this kinetic energy appears as heat of adsorption.

Physiological Importance

In physiology adsorption plays a great role. Some of them are as follows: (a) Enzyme action—both the enzyme and the substrate are colloidal in nature. By process of adsorption they come in closer contact and the interaction is hastened. (b) The combination between toxin and antitoxin, by which they neutralise each other, is another adsorption phenomenon. (c) Various adsorption compounds are formed in the body, such as lecithin with protein (found in the brain) and such others. The blue compound formed by adding iodine with starch is an adsorption compound.

HYDROTROPY

Certain substances have the property of making water-insoluble substance soluble in water. This is called hydrotropy or hydrotropic action. How this action is brought about is not definitely known? It is said that the hydrotropic substances form loose compounds with the insoluble substances and thereby make them soluble and diffusible through membranes. This view is supported by the fact that a quantitative relation is often found between them. For instance, when glycocholic acid forms hydrotropic compound with oleic acid they have a molecular ratio of 3:1. Hydrotropic substances of physiological importance are:

1. Bile salts and such other compounds of cholic acid (vide under 'Bile Salts'). This is possibly the chief member.
2. Lecithin, soaps of higher fatty acids, phenylacetic acid, benzoic acid, hippuric acid, etc.
3. Hydrotropic substances of unknown nature are also found in the intestinal juice, in the intestinal mucosa, in the blood plasma and possibly in other tissues and body fluids.
4. The insoluble substances which are made soluble in this way are—fats, certain phospholipids, sterols specially cholesterol, insoluble soaps, uric acid and inorganic salts of Ca, Mg, and possibly of Fe, Cu, Mn, etc.

Physiological Importance

This is of immense physiological value. There are many chemicals in the body which are kept in solution with the help of hydrotropic action. Some examples are given below: (a) In the bile (in the liver, gall bladder, common bile duct), cholesterol and certain other compounds are kept in solution with the help of bile salts. (b) In the intestine the insoluble soaps, sterols, fatty acids, inorganic salts, etc. make hydrotropic compounds with bile slats and other hydrotropic substances present in the intestinal juice and mucosa and are thus easily absorbed. (c) In the blood plasma and other body fluids (and possible in the tissues) there are a number of hydrotropic substances which keep many otherwise insoluble substances in solution, for instance, sterol, Ca and other inorganic salts, uric acid, etc.

DONAN EQUILIBRIUM

When a saline solution and distilled water are separated by a permeable membrane final equilibrium will be reached when the concentration of salt on both sides will be same. But if there be any non-diffusible ion on one side, a different phenomenon will be seen. Suppose NaP is an ionisable compound of which P is non-diffusible. If this compound is kept on one side and NaCl on the other, Na and Cl ions will freely pass but not P. When the final equilibrium will be reached, it

will be seen that the product of Na and Cl on one side is equal to the product of the same two ions on the other side. For example:

Initial state		*Final state*
Na:Na		Na:Na
P:Cl	⟶	Cl:Cl
:		P:

In the final state Na + Cl (left) = Na + Cl (right). It is obvious that total Na of the left side is greater than total Na of the right side and the total chloride of the right side is greater than total chloride of the left side.

Similarly, if NaP be on the left side and H_2O on the right side, in the final state the reaction on the right side will be alkaline due to Na and OH ions. If a compound ClP be on the left and H_2O on the right, in the final state the reaction on the right side will be acid due to H and Cl ions. This type of equilibrium in which the products of the same pair of ions on two sides of the permeable membrane become same, is called Donnan equilibrium. It is obvious that in the final state there will be a great difference in the nature and quantity of diffusible ions on two sides of the membrane which will lead to a difference of electric potential and chemical reaction on the two sides. Since, in our body there are many compounds of the NaP type, Donnan equilibrium is of great physiological importance.

Physiological Importance

It explains how difference of electric potential can be established on two sides of a membrane, how stomach can secrete a strongly acid juice and pancreas can secrete an alkaline juice. The phenomenon of chloride shift can also be explained from this standpoint.

COLLOID

When sugar, urea, NaCl, etc. be dissolved in water the result is a clear permanent true solution. But if proteins, starch, glycogen, etc. be placed in water, they will make a thick opalescent unstable solution. Graham (1861) called the first type as the crystalloidal solution, because solute particles form crystals and pass through parchment membrane, and called the second group, colloidal solution, for the solute particles in this case show reverse properties. Modern idea indicates that the difference between true solution and colloidal solution depends on the size of the solute (the dispersed phase) molecules in the solvent (the dispersion medium). If the size is greater than 200 mμ they remain as suspensions and if less than 1 mμ as true solution. The size of the molecules in colloidal solutions varies from 1 to 200 mμ.

To understand the difference between a crystalloid and a colloidal solution, one should remember that the forces which keep the solute particles in solution are roughly as follows: (a) Inherent movement of the solute particles, i.e. their diffusibility. (b) Inherent movement of the solvent molecules which continuously dash against the solute particles and thus help to keep them in solution. (c) Electric charge—positive or negative—carried by the particles, which by constant attraction and repulsion also help uniform solution. (d) Hydration (carrying water molecules) of the solute particles, and so on. If the solute particles be very small (below 1 mμ—a micron) all these forces will be acting to their maximum and the result will be a permanent true solution. Now, if the solute particles be gradually made larger and larger (1–200 mμ—submicrons), their own movement (diffusibility) will gradually be reduced and ultimately will be almost nil. The first factor, mentioned above, will therefore, be completely out of the field. The other forces, such as the dashing by the solvent molecules, electric charge, etc. will then try to keep the large solute particles somehow in solution and will be able to do so up to a certain extent. Such a solution will be unstable and heterogeneous—a sort of pseudo solution. This is a colloid. If then, the solute particles be made still larger (over 200 mμ—microns) all the forces will completely fail and the solute particles will not go into solution at all. Hence, if the solute–solvent relations be studied as a series of phenomena, it will be found that, at one extreme there is complete solubility (true solution), at the other extreme—complete insolubility, while in the intermediate stages there will be a phenomenon of semi-solubility. This is colloidal solution. Thus, other factors remaining constant, the real difference between a true solution and colloidal solution lies in the size of the solute particles, and not upon their chemical nature.

Thus, a colloid may be defined as a substance (e.g. gelatin or cell cytoplasm), which by reason of the size of its molecules, is slowly diffusible rather than soluble in water (its hydrates being gelatinous in consistence), and is incapable of passing through an animal membrane (a semipermeable membrane). In this substance the solute particles are proportionally larger than the solvent molecules.

Emulsoid and Suspensoid

Colloids fall into two classes—emulsoid (emulsion) or lyophilic colloids and suspensoid (suspension) or lyophobic colloids. Difference between the two is shown in Table A1.1.

Emulsoids being more stable, can impart their own stability to suspensoids. In other words, when suspensions of solid particles are made in a solvent which is already an emulsion, the resulting suspensoid

Table A1.1: Difference between emulsoid and suspensoid

Colloid	
Emulsoid/lyophilic	*Suspensoid/lyophobic*
1. Solutions have a great affinity for the solvent	1. No affinity between solute and solvent
2. Electrolyte required in large quantity for precipitation	2. Very small amount of electrolyte required
3. Precipitate formed is reversible *Example:* Polysaccharides, proteins, etc.	3. Precipitate formed is irreversible *Example:* Inorganic salts, metals

will be relatively more stable*. This is called the protective action of emulsoids. For instance, blood is a suspension of red cells (7.2 μm) in plasma, which in itself is an emulsion of proteins in water. This makes blood, a relatively stable suspensoid.

Sol and Gel

A colloid may remain in two states—as a liquid or as a solid. The former is called sol, the latter, gel. These, two states are reversible. Sol can be transformed into gel by altering temperature, H ion concentration, salt concentration, concentration of the solute, etc. During this process, a relatively small amount of solute particles runs together and sets into a semi-solid mass, entangling in its meshes a fairly large amount of the solvent in the form of isolated droplets. The explanation is as follows. Being heterogeneous, colloidal solution has two distinct components—called phases. The solvent, being continuous, is called the continuous phase. The solute, being discontinuous or dispersed, is known as dispersed phase. The two phases are interchangeable. When the solute is more or less solid and the solvent (continuous phase) is liquid—it becomes a sol (if the liquid is water—a hydro sol). If, on the other hand, the solvent (continuous phase) is more or less solid and the solute (dispersed phase) be liquid—it makes a gel (if the latter is water—a hydro gel). For instance, a hot solution of agar-agar is a liquid—a sol. On cooling, it sets into a semisolid jelly—a gel. Milk is a sol, butter is a gel (Fig. A1.8).

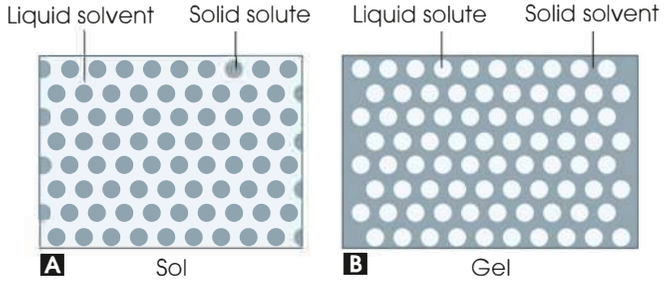


Fig. A1.8A and B: Reversal of phases of a colloid. (A) Sol; (B) Gel

PROPERTIES OF COLLOIDS

Brownian movement: Due to the impact of the solvent molecules, the colloid particles are continuously moving about.

Faraday-Tyndall phenomenon (optical phenomenon): When a beam of light is passed through a colloidal solution and observed at right angles, the track of light, which is invisible in a true solution, may be visible here as a white line. This is caused by the dispersal of light rays by the colloid particles. If the particles be sufficiently small, the light, when viewed through a Nicol's prism, will be found to be polarised at right angles to the beam.

Electrical phenomenon

1. Colloid particles carry electric charge—which may be positive or negative (colloidal ions).
2. *Isoelectric pH*: By addition of salt, weak acid, weak alkalis, etc. the hydrogen ion concentration of the medium can be adjusted to an isoelectric pH where the electric charge of the colloidal ions will be completely neutralised. At this pH the colloid particles become less soluble and may be precipitated. Almost every colloid, particularly proteins, has its characteristic isoelectric pH.
3. *Electrophoresis*

Precipitation: The colloid particles can be precipitated in various ways. Isoelectric precipitation has been mentioned above. In precipitation, there is no intramolecular change and it can be made to go back into solution again.

Coagulation: This also is a property of the colloid. Here, the intramolecular change takes place. The coagulum cannot be redissolved.

Osmotic pressure (oncotic pressure): Colloids exert some osmotic pressure. But the particles being large, it is proportionally much smaller. However, it is of great physiological importance.

Imbibitions of water: Emulsoid particles can imbibe a good amount of water.

Adsorption: This also is a colloidal phenomenon.

Separation of Colloids

May be done by:

- ***Dialysis***
- ***Isoelectric precipitation*** (*vide* above).
- ***Salting out:*** By adding suitable amounts of various salts. It is a kind of precipitation of a protein from its solution by saturation or partial saturation with such neutral salts as sodium, chloride, magnesium sulphate, or ammonium sulphate.

Electrophoresis: If an electric current be passed through a colloidal solution, the positively charged colloid ions

*Many pharmacopoeial emulsions, such as of bismuth salts are made upon this principle, by adding to the mixture—gum acacia and such other substances which with water easily makes an emulsion.

will accumulate around the negative pole, while the negatively charged particles will accumulate around the positive pole. In this way they can be separated. Even their rate of movement can be measured by noting the concentration of a particular colloid at different points in the electric field.

Adsorption: By suitable methods, colloids can be isolated by adsorption.

Ultra-centrifugalisation: If centrifugalize at high speed colloid particles collect at the bottom of the centrifuge tubes at different rates, the heavier ones settle out faster.

Ultra-filtration: By this process the colloidal (larger) and crystalloid (smaller) dissolved molecules can be separated from each other. Colloid of different particle size can be separated from one another by ultra-filtration through membranes of different pore sizes.

Physiological Importance

Colloids are of immense physiological value. Some examples are as follows:

1. ***Cell protoplasm:*** In every cell exists in a colloidal state—mostly as emulsoid. 90% of organic matter of the body remains as colloid.
2. ***Milk, plasma and lymph:*** They are all emulsoids. Blood is a suspension of red cells in plasma.
3. ***Interfacial reaction:*** Being dispersed in the form of minute particles, colloids afford a very large surface area for various reactions to occur, such as adsorption, surface tension, enzyme action, etc. For instance: (a) The emulsification of fats during digestion, provide a large surface of contact for the enzymes to act and thus quickens the process of digestion. (b) The total surface area of red cells being very large, facilitates rapid exchange of gases, ions, etc. in the blood stream.
4. ***Imbibition of water:*** Since emulsoids readily imbibe water, a good deal of water remains stored in the body in this way.
5. ***Adsorption:*** It is of great physiological importance-is a colloidal phenomenon.
6. ***Blood clotting:*** It is essentially a colloidal process, in which a sol (plasma) is converted into a gel (clot).
7. ***Colloid osmotic pressure:*** When human red blood corpuscles are suspended in 0.5% sodium chloride solution (hypotonic), water and sodium chloride pass into the cells due to lower osmotic pressure inside the cells. The red cells are swollen and burst. If the red cells are suspended in more than 1.0% sodium chloride solution (hypertonic) opposite change occurs, i.e. water and salt pass out of the red cells. The red cells are shrunken and become crenated. If the red cells are suspended in normal saline solution (0.9%) there is practically no change in the cells. Plasma contains both diffusible and non-diffusible large colloidal particles. The total osmotic pressure of plasma is about 6.5 atmospheres. This is due to crystalloids and colloids. The osmotic pressure of the plasma proteins is 25 mm of Hg. The osmotic pressure of the plasma proteins depends on serum albumin and serum globulin. Serum albumin exerts a greater osmotic pressure than serum globulin. By virtue of the osmotic pressure of the plasma proteins, the fluid in the blood capillaries is retained and the plasma volume is maintained. The capillary blood pressure is a filtering force tending to drive protein-free fluid into the interstitial spaces, whereas the osmotic pressure of the plasma protein exerts an opposite effect. The osmotic pressure of plasma proteins also plays a great role in glomerular filtration.

THE ATOMIC STRUCTURE

The atom of all elements (103 known) is composed of the smallest and elementary particles. Neutrons, protons and electrons are the elementary particles. The neutron and proton occur in the centre of an atom where they form an atomic nucleus. The electron orbits the atomic nucleus at some distance from its centre where it is virtually weightless and carries negative electrical charge. Each neutron and proton (nucleons) has one unit of atomic weight or mass and is about 1800 times heavier than the electron. A proton's electrical charge is positive whereas a neutron is electrically neutral.

Hence, the numbers of positive and negative charges are exactly balanced and so an atom is electrically neutral. The structure of atoms of some elements as shown in Fig. A1.9 and indicate each element has a distinctive number of protons and electrons.

The chemical properties of the elements however depend upon the number and configuration of electrons. The number of such planetary electrons in the neutral atom of any element is equal to the number of protons in the nucleus of that atom. An element is characterised by a fixed and definite atomic number of protons in the nuclei of its atom balanced by an equal number of planetary electrons. With the exception of the nucleus of the ordinary hydrogen all atomic nuclei contain one or more neutrons in addition to protons.

Isotopes

The numbers of neutrons in the nuclei of the atoms of an element are not fixed, but vary within certain limits. The atoms of most elements contain nuclei with different numbers of neutrons and consequently of different mass numbers. Each such atomic variety with a specific mass of an element constitutes isotopes. Therefore, isotopes are atoms of the same elements whose nuclei contain different numbers of neutrons but

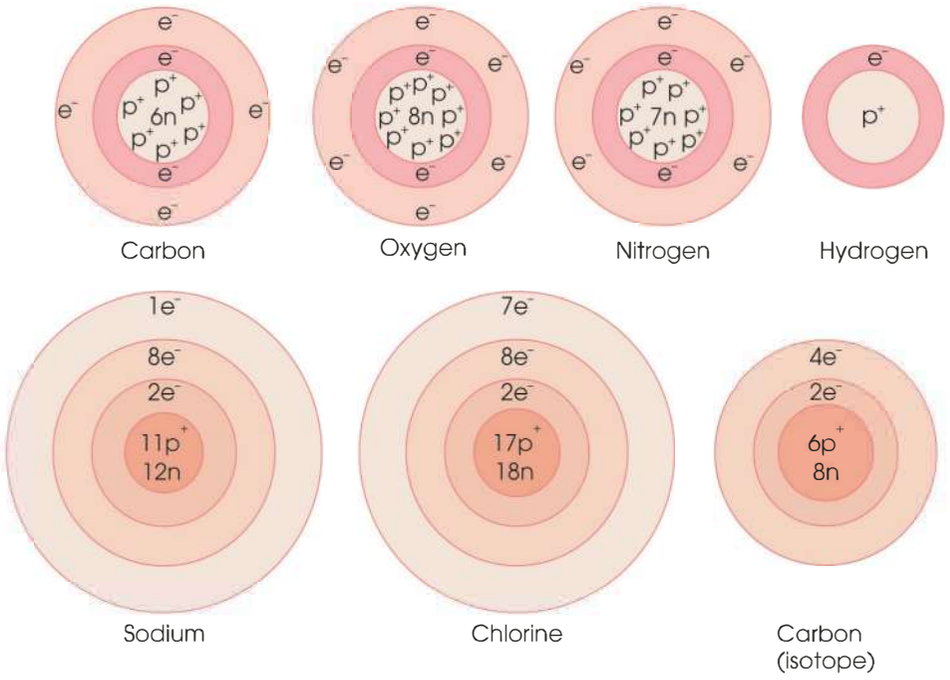


Fig. A1.9: The electron shells of some atoms. The circle represents the atomic nucleus of the atom containing protons (p^+) and neutron (n) and electrons (e^-). The distance of electron from the atomic nucleus denotes various energy levels of atoms. The isotope of carbon varies only in number of neutrons contained in the atomic nucleus

the same number of electrons and protons. Isotopic atoms (nuclides) can be detected and distinguished physically but not chemically.

Natural Isotopes

They belong to the first class and remain as mixtures in all natural sources and can be isolated by careful fractionation. The isotopes of physiological importance are those of carbon, hydrogen, nitrogen, oxygen, sulphur, chlorine, etc. For instance, carbon having the familiar atomic weight 12 has isotopes with atomic wt. 11, 12, 13 and 14 (^{11}C, ^{12}C, ^{13}C, ^{14}C or written as C^{11}, C^{12}, C^{13}, C^{14}). Hydrogen has the usual atomic weight 1, but isotopic heavy hydrogen has an atomic weight 2 (^2H). Oxygen with atomic wt. 16, has isotopes with atomic weight 17 and 18 (^{17}O, ^{18}O). Similarly, sulphur (atomic weight 32) has isotopes with weight 33 and 34 (^{33}S, ^{34}S) and chlorine with the usual atomic weight of 35 may have an isotopic variety with 37 (^{37}Cl).

Of special interest are the hydrogen isotopes (radio hydrogen) (^2H and H^b) and ^2H which have been given special names—deuterium and tritium, respectively. The deuterium is symbolised by D (Urey, 1932). D_2O (heavy water) is present to some extent in all natural samples of water (tap water contains 1 part in 9,000 parts) and traces of D_2O (deuterium oxide) and THO (radioactive tritium oxide) are present throughout the body fluids. By determining D_2O and THO spaces, the total body fluid would be obtained. Electrolysis of water preferentially dissociates H_2O but leaves D_2O untouched. Consequently after electrolysis of water fairly concentrated solution of D_2O can be obtained and can be subsequently isolated by fractionation. Although in chemical properties it is identical with H_2O, D_2O has different mass, recognised by mass spectrometer.

Radioactive Isotopes

Some isotopes are stable, but some are unstable due to their relative number of protons and neutrons in the nuclei. These are radioactive isotopes which break down constantly into more stable atoms. Elements like uranium and radium are radioactive, they emit α-, β- and γ-rays. Although majority of the naturally occurring elements are not radioactive, radioactive isotopes of all of them can be prepared artificially by bombardment with a cyclotron or by uranium pile. The emitting α-radiation from the disintegrating atom is not easily detected. β-radiation and γ-radiation are usually measured with Geiger-Müller counter. In some special cases either Windowless or Scintillation counter is used. The stability of a radioactive isotope is measured by its half-life. The half-life of a radioactive isotope is the time taken by the radiation to come down to half the original strength. The half-life of radioactive elements varies from minutes to years. Those applied for physiological studies must obviously have sufficiently long-life to enable an elaborate study of their life-history in the body.

Half-line of some radioactive isotopes: ^{42}K, 12.4 h.; ^{24}Na, 15 h.; ^{131}I, 8 d.; ^{59}Fe, 45 d.; ^{32}P, 14.3 d., ^{35}S, 87.1 d.; ^{45}Ca, 152 d.; ^{60}Co, 5.3 y.; ^{14}C, 5700 y.; ^3H, 12.5 y.; etc. (h denotes hours, d, days and y, years).

Physiological Application of Isotopes

The isotopes, either natural or radioactive, have identical chemical properties with the parent element and so they are treated similarly in the living system as their normal homologue. A very low concentration of them can however be detected by physical method and so they can be used to 'label' molecules and are used extensively in different physiological and clinical studies in recent times.

The latest advancement in the study of metabolic processes is the application of natural and radioactive isotopes. Compounds labelled with such isotopes are administered. The changes undergone in such 'labelled' compounds, their migration from place to place and finally their excretion from the body, are traced and observed with the help of suitable instruments and of other physico-chemical properties. Hence, such elements are referred to as tracer elements.

Some physiological studies based on application of radioactive isotopes are listed below:

1. *By dilution of radioactivity added:* (a) Measurement of red cell volume with ^{51}Cr labelled red blood corpuscles. (b) Determination of plasma volume with ^{131}I labelled serum albumin. (c) Determination of sodium space with either ^{22}Na or ^{24}Na. (d) Estimation of total body water with iodo-antipyrine labelled with ^{131}I.
2. *Membrane-transfer measurement:* (a) Absorption studies of iron (^{59}Fe), vitamin B_{12} (^{60}Co), fatty acids (^{14}C oleic acid), etc. (b) Transport of Na and K across the intestinal wall. (c) Exchange of ions in the kidney tubules.
3. *Studies based on distribution of a particular isotope:* (a) Uptake of ^{131}I by thyroid gland in thyroid function test. (b) Uptake of ^{32}P by malignant tissue in its tracing in the body. (c) Distribution of different drugs.
4. *Metabolic studies:* This is one of the widest applications of radioactive isotope. Studies on synthesis, degradation and isolation of intermediate products in almost all metabolic pathways in normal physiological conditions and its variations in different diseases have been attempted. They include carbohydrate, protein and nucleic acids, fatty acids and steroids and mineral metabolism. Synthesis and degradation of various hormones have also been studied.
5. *As a radiation source in medicine:* (a) ^{131}I in the treatment of hyperthyroidism and thyroid cancer. (b) ^{32}P in the treatment of leukaemia and polycythemia vera. (c) Clinically for destruction of tumour.

Autoradiography

The capacity of radioactive isotope to blacken photographic emulsion is termed the technique of autoradiography. Mixtures of substances are first of all separated by paper chromatography and then the paper is kept in contact with X-ray film or similar photographic emulsion. The grade of black hue gives the amount of radioactivity of the particular fraction. If applied on histological section; this technique can detect the presence of radioactivity inside the different cellular compartments (nuclei, mitochondria, etc.).

ACID–BASE, HYDROGEN ION CONCENTRATION AND pH

Acid and Base

An acid may be defined as a substance which, when dissolved in water, produces hydrogen ions [H^+] or in other words, it is the compound of electronegative element with ionisable hydrogen [H^+]. Similarly, a base or alkali is a substance which produces hydroxyl ions [OH^-] when dissolved in water. That is electropositive elements with ionisable hydroxyl groups [OH^-] are the bases. A normal solution of any acid will exactly neutralise an equal volume of a normal solution of any base, no matter, how strong or weak, may be characteristics of two reactants. During neutralisation equal numbers of hydrogen ions of acid and hydroxyl ions of base unite to form water.

1. *Free acidity:* Amount of acidity present in a solution in free state and not combined with any other substance.
2. *Actual acidity:* Amount of [H^+] ion concentration in a solution.
3. *Total acidity or titratable acidity:* Amount of free acid plus that present in combination. This can be titrated against a base.

The equilibrium governing the ionisation of water is as follows:

$$H_2O \rightleftharpoons [H^+] + [OH]$$

At a particular temperature the product of a number of hydrogen ions multiplied by a number of hydroxyl ions is a constant. Thus, at 23°C

$$[H^+] \times [OH^-] = 10^{-14}$$

Concentration of [H^+] and [OH^-] ions in aqueous solution is such that their product is always equal to 10^{-14} gram-molecular weight per litre. This concentration product or dissociation constant of water can be denoted as Kw*. So the equation will be

$$[H^+] \times [OH^-] = Kw = 0.00000000000001 = 1 \times 10^{-14}.$$

In pure water the concentration of [H^+] ions is equal to the concentration of [OH^-].

So $[H^+] \times [OH^-] = 1 \times 10^{-14}$ and $[H^+] = 1 \times 10^{-7}$, $[OH^-] = 1 \times 10^{-7}$.

*By law of mass action $[H^+][OH^-]/[HO] = K$, where K is constant. So, $[H^+]\,[OH^-] = Kw$.

Therefore, pH of the pure water will be pH = log (1/[H^+]) = log (1/[1 × 10^{-7}]) = 7. So, the pure water having equal number of [H^+] and [OH^-] ions is neutral at pH 7.

Hydrogen Ion Concentration

It is a measure of extent of acidity or alkalinity of a solution. As the product of hydrogen and hydroxyl ion concentrations in a solution is constant, if the former is known, the latter can be calculated. Thus, the hydrogen ion concentration of neutral water is 10^{-7} and of normal sodium hydroxide is 10^{-14}.

pH

The pH of a solution is defined as the negative logarithm (base 10) of the hydrogen ion concentration, i.e. pH = –log [H^+] or pH = log (1/[H^+]).

Calculation of pH

For calculation of pH, [H^+] ion concentration must be ascertained. If the [H^+] ion concentration of a solution is known then the pH of a solution can be calculated. When the [H^+] ion concentration of a solution is 2.86 × 10^{-4} then the pH can be calculated as follows:

[H^+] = 2.86 × 10^{-4}

pH = log (1/[H^+])

= log (1/[2.86 × 10^{-4}])

= log (1/[100.456 × 10^{-4}])

= log (1/$10^{-3.544}$) = 3.544

Or pH = 3.544.

Calculation of [H^+] Ion Concentration

For calculation of [H^+] ion concentration from a known pH value, the calculation will be reversed. Supposing the pH value of a solution is 3.544 then the [H^+] ion concentration will be: pH 3.544 = log 103.544

= log (1/$10^{-3.544}$)

= log (1/[100.456 × 10^{-4}])

= log (1/[2.86 × 10^{-4}]) (anti-log of 0.456 = 2.86)

[H^+] ion concentration will be 2.86 × 10^{-4}.

Greater the value of pH, lower will be the value of acidity and conversely the lower value of pH will indicate the higher value of acidity.

Hydrogen ion concentration (cH) can be expressed in three ways (Table A1.2).

The pH Scale

Sorensen introduced the simple pH scale. The degree of acidity can be measured on a scale of pH units. Pure water is neutral, i.e. neither acid not alkaline, has a pH of 7. Acid substances produce more [H^+] ions than those are found in pure water. They possess pH values of less than 7. Alkaline (basic) substances combine with [H^+] ions leaving fewer than those are found in pure water. They have pH values of more than 7 (Fig. A1.10). A change of one unit on the pH scale means a ten-fold change in hydrogen ion concentration. A two-fold change in hydrogen ion concentration is expressed by a movement of 0.3 on the pH scale.

Indicators: For standardization of solution, an indicator is generally used to determine the end point. Indicator

Table A1.2: Hydrogen ion concentration (cH)

	pH (–log)	*Power of Ten*	*Decimal*	
	pH 0	1 N	1.0 N	
	pH 1	10^{-1} N	0.01 N	
	pH 2	10^{-2} N	0.001 N	
Acid	pH 3	10^{-3} N	0.0001 N	Acid
	pH 4	10^{-4} N	0.00001 N	
	pH 5	10^{-5} N	0.000001 N	
	pH 6	10^{-6} N	0.0000001 N	
Neutral	pH 7	10^{-7} N	0.00000001 N	Neutral
	pH 8	10^{-8} N	0.000000001 N	
	pH 9	10^{-9} N	0.000000001 N	
	pH 10	10^{-10} N	0.0000000001 N	
Alkaline	pH 11	10^{-11} N	0.00000000001 N	Alkaline
	pH 12	10^{-12} N	0.000000000001 N	
	pH 13	10^{-13} N	0.0000000000001 N	
	pH 14	10^{-14} N	0.00000000000001 N	

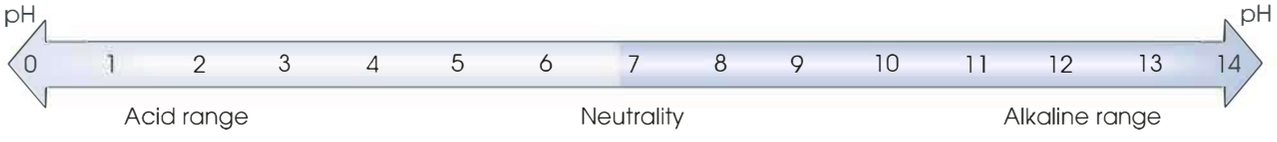


Fig. A1.10: The scale of pH

can be defined as the very weak organic acid or base that undergoes change of structure with consequent change of colour in the presence of certain concentration of hydrogen and hydroxyl ion. Some common indicators along with their range of colour over a range of concentration have been presented in Table A1.3.

Table A1.3: Common indicators

Some common indicators	*Ranges of pH*	*Changes in colour*
Alizarin red	5.0 to 6.8	Yellow to red
Bromocresol green	4.0 to 5.6	Yellow to blue
Bromocresol purple	5.4 to 7.0	Yellow to purple
Congo red	3.0 to 5.0	Blue to red
Litmus	4.5 to 8.3	Red to blue
Methyl orange	3.1 to 4.4	Orange-red to yellow
Methyl red	4.2 to 6.3	Red to yellow
Phenolphthalein	8.3 to 10.0	Colourless to red
Phenol red	6.6 to 8.2	Yellow to red
Töpfer's reagent	2.9 to 4.0	Red to yellow

Physiological Importance of Maintenance of pH

A large number of living cells is extremely sensitive to changes in the pH of their extracellular fluids. For the extracellular fluid (ECF), an useful requirement is that it would not be either too acid or too alkaline (basic). The pH of human blood plasma is usually maintained between 7.36 and 7.45. If these limits change, i.e. above pH 7.8 or below 6.8, fatal illness and perhaps death would ensue. Luckily our blood plasma equalises this limit with substances called buffers which act to prevent any sudden shift in the pH.

Buffers are usually a mixture of either weak acids with their salts of strong bases or strong acids with their salts of weak bases. The buffer system of the body has the capability of resisting the change in pH by accepting either the $[H^+]$ or $[OH^-]$ ions. Buffering action is very important in the biological system in maintenance of normal acid–base balance of the body. It is most important in the action of enzymes, both *in vivo* and *in vitro*.

Henderson-Hasselbalch equation can be used for determination of pH of a buffer solution or for determination of relative concentration of the salt and acid required for achieving the normal pH.

The pH of the buffer solution prepared by mixing 35 ml of (N/10) acetic acid with 15 ml of (N/10) NaOH can be determined by the Henderson-Hasselbalch equation:

pH = pKa + log (salt/acid), where pKa is log (1/K a). Ka* is the dissociation constant of the acid. When these two solutions are mixed up 15 ml of (N/10) NaOH will neutralise 15 ml of (N/10) acetic acid so as to form 15 ml of the salt, Na acetate. So, 20 ml of acetic acid will remain unneutralised.

So, in the buffer solution, the salt and unneutralised acid ratio will be (15/20). The dissociation constant (Ka) of acetic acid is (1.86×10^{-5}) and so the pKa will be log (1/Ka), that is 4.73.

So, in Henderson-Hasselbalch equation

$$\begin{aligned} pH &= 4.73 + \log (15/20) \\ &= 4.73 + \log 0.75 \\ &= 4.73 + 1.8751 \\ &= 4.6. \end{aligned}$$

If the ratio of salt and unneutralised acid ratio becomes 1:1, then the pH value of the buffer mixture will be equal to pKa of the acid.

As for example, if 30 ml of (N/10) acetic acid and 15 ml of (N/10) NaOH are mixed up then 15 ml of salt.

Na acetate will be formed and 15 ml of acetic acid will remain as unneutralised acid. So, the salt and acid ratio will be (15/15) = 1. So, interpolating the value in the Henderson-Hasselbalch equation the result will be:

$$\begin{aligned} pH &= pKa + \log (salt/acid) \\ &= 4.73 + \log (15/15) \\ &= 4.73 + \log 1 \\ &= 4.73 + 0 \\ &= 4.73. \end{aligned}$$

So, the buffering action in this case is at its maximum and can react either as acid or as base.

*Consider the equilibrium holding in a pure solution of a week acid is

$$HA \rightleftharpoons [H^+] + [A^-]$$

By law of mass action $[H^+]\,[A^-]/[HA] = Ka$ or $[H^+] = Ka\,[HA]/[A^-]$

So $\log [H^+] = \log Ka + \log [HA] - \log [A^-]$

or $-\log [H^-] = -\log Ka - \log [HA] + \log [A^-]$

Accoding to Sørensen $-\log [H^+] = pH$, by similar reasoning $-\log Ka = pKa$

Therefore, $pH = pKa + \log [A^-]/[HA]$

Appendix

2

Normal Balanced Diet

INTRODUCTION

In general terms, adequate diet is one which permits normal growth, maintenance and reproduction. The feeding and satiety centre in hypothalamus (Fig. A2.1) which are influenced by state of fullness of stomach, emotional factors (sight and smell of food) and other factors (Fig. A2.2). The following points should be taken into consideration while estimating adequate diet for an individual:

Essential Constituents of Diet

Normal diet must contain the following six items: (1) proteins (2) fats, (3) carbohydrates, (4) vitamins, (5) minerals, (6) water. The first three are of energy production, growth and mechanism, i.e. for the utilisation of energy, synthesis of various necessary metabolites, viz. enzymes, hormones, etc.

Quantity of Food

Energy for physiological processes is provided by the combination of carbohydrates, fats and proteins. The daily energy requirement or the daily calorific need is the sum of the basal energy demands plus that required for the additional work of the day. The energy is quantitatively expressed as units of heat which in this case is the kilocalorie. It is the amount of heat required to raise the temperature of a litre of water by 1°C at a range around 15° to 16°C. However, recent convention, has been agreed to have the joule as the unit of energy and to convert 1 kcal = 4.10 kJ (or 1 kcal is equal to 4184 or 4188.5 joules).

The quantity of food will be proportional to the total energy requirement of the individual. The total energy requirement can be calculated from the following data:

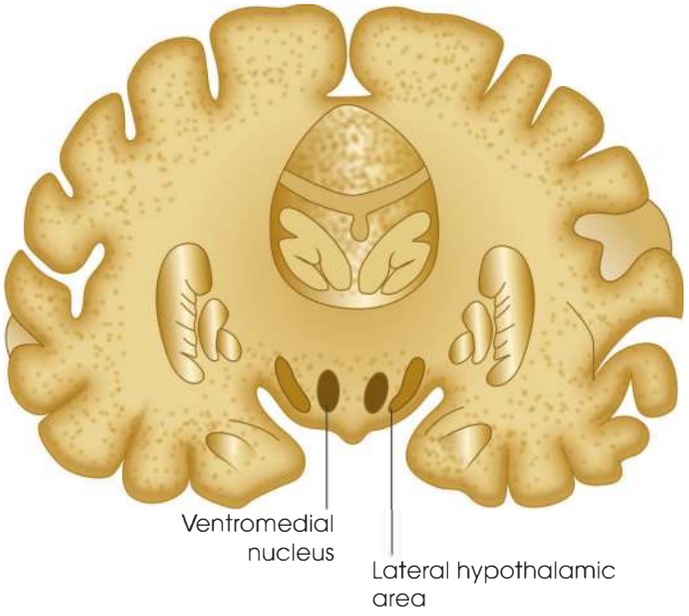


Fig. A2.1: The anatomical positions of ventromedial nuclei and lateral areas of the hypothalamus in which satiety centre and hunger centre are situates respectively (diagrammatic representation)

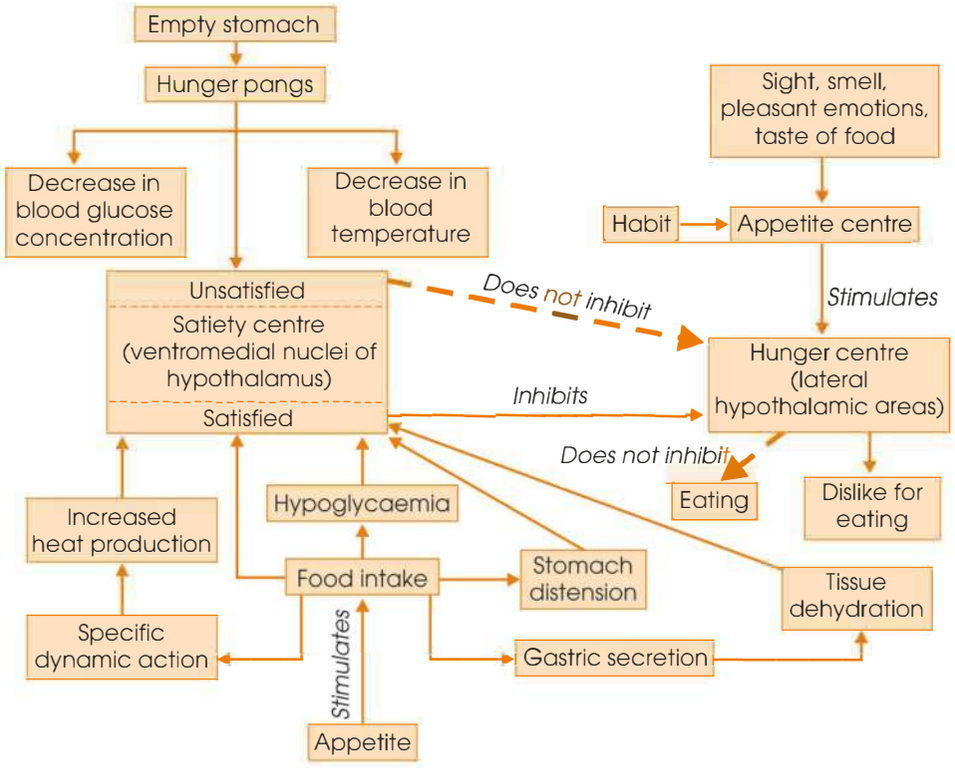


Fig. A2.2: Factors influencing the hunger and appetite centres present in the hypothalamus (schematic representation)

BMR: BMR can be determined from the surface area on the whole, the average adult male has a surface area of 1.8 sq-m and a BMR of about 72% per hour or 40°C per sq-m of body surface. The Nomogram for estimating surface area of infants and young children is shown in Fig. A2.3 while that in older children and adult in Fig. A2.4.

Nature of work done by the individual: Work involves expenditure of energy, over and above the basal metabolic rate. The following is a rough estimate:

Sedentary work: without any muscular effort (such as the brain workers)—20–25% (about 400°C)

1. Light work: 30–40% (700°C)
2. Moderate exercise: 50–60% (1,000°C)
3. Heavy work: 100% or more (2000°C).

The total energy requirement will be BMR plus these figures.

Allowance for growth: Infants, growing children, pregnant women, mothers, athletes and convalescent patients require at least 50% more food over and above their actual BMR. This additional amount is necessary to provide for active growth.

During waking hours: Ingestion of food stimulates metabolism by 5–10%.

Calorie

The basal calorie requirement of an individual is 40 calorie per sq-m per hour. Thus, in an average adult male, having a surface area of 1.8 sq-m, the total energy requirement during 24-hour period may be obtained by calculating as follows:

8 hours' sleep = (40 × 1.8) × 8 = 576°C

8 hours' awake = basal + 30% (i.e. 10% for stimulating action of food + 20% for minor activities, etc.) = 576 + 174 = 750°C

8 hours' moderate work = basal (576) + 1,000 = 1,576°C

Total = (576 + 750 + 1,576) = 2,902°C (roughly about 3,000°C).

To provide for the 10% loss in cooking and faulty absorption, the purchased value of the food should have 300 calories more, total 3,300°C.

The average calorific requirement of a man doing light work is 3,000°C net or 3,300°C as purchased. The average housewife needs about 10% less (2,700°C net) a lady doing more active work has the same requirement as the average male. Men doing hard work should receive up to 4,000°C. The requirements of the children are suggested to be as follows:

Table A2.1: Calorie requirement in children

Age in years	*Calories*
1–2	1,000
2–3	1,250
3–6	1,550
6–8	1,850
8–10	2,150
10–12	2,550
12–14	2,900

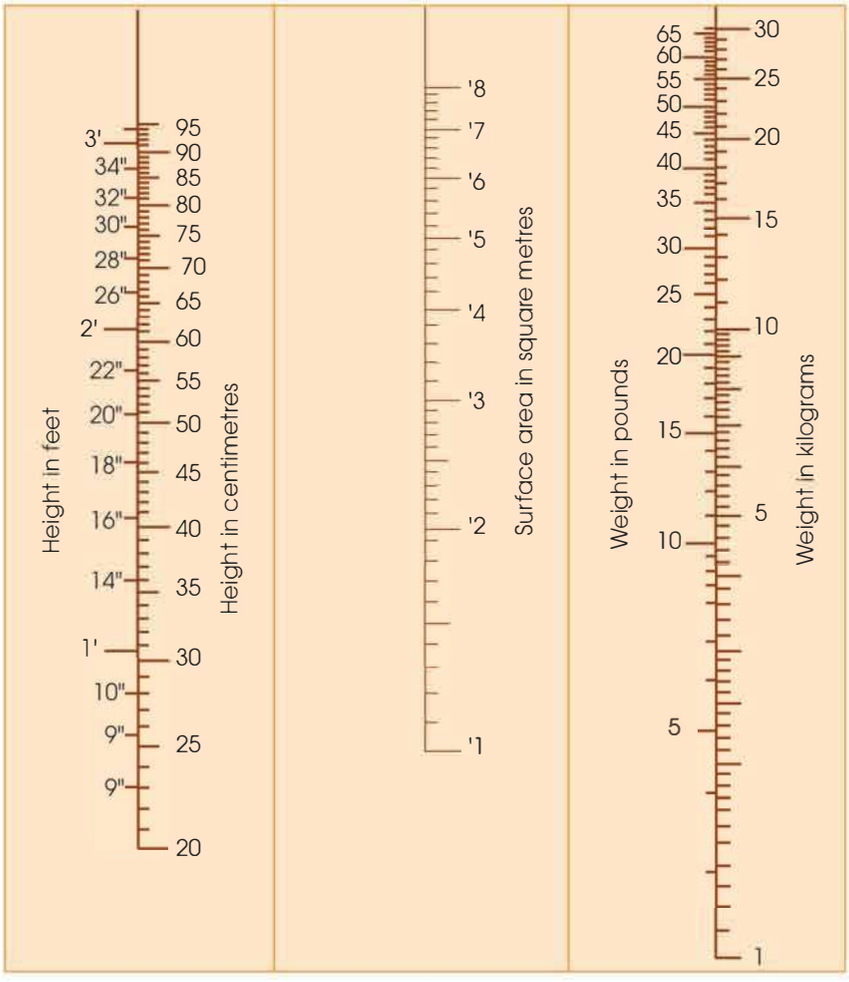


Fig. A2.3: Nomogram for estimating surface area of infants and young children. To determine the surface area of the individual it can be drawn a straight line between the point representing his height on the left-hand vertical scale to the point representing his weight on the right-hand vertical scale. The point which this line intersects the middle vertical scale represents the individual's surface area in square metres

It is to be noted that children of 12 and over require as much food as an adult. Girls between 14 and 18 should have 2,800°–3,000°C, and boys of the same age should consume 3,000°–3,400°C. (A little over the requirements of an adult light worker.) According to Lusk, the following are the energy requirements for different age groups in the young people [1.0 represents 3,000°C or 1 adult unit (called man value)] Age 0–6. = 0.5 (i.e. 1,500°C). Age 6–10 = 0.7 (i.e. 2,100°C). Age 10–14 = 0.83 (i.e. 2,500°C). Boys 14–20 = 1 (i.e. 3,000°C). Girls 14–20 = 0.83 (i.e. 2,500°C).

Distribution of Calories in the Diet

Protein requirement: The protein requirement must be carefully maintained. A minimal amount of protein is indispensable in the diet to provide for the replacement of tissue proteins which undergo wear and tear. If the protein content of diet is in excess, the remainder is utilised to produce energy. The requirement of protein is not only quantitative but also qualitative, since metabolic aspect of protein is intimately connected with its amino acid composition. The essential amino acids must be supplied in the dietary protein which is the basis of the qualitative aspect of protein material. First class proteins contain more essential amino acids than others. For adults, the adequate maintenance dose is 1 gm of protein per kg of body weight per day. To provide enough margin of safety, 100 gm per day is advocated. One-half of it must be given in the form of 'first class' proteins or proteins of 'high biological value'. For infants and growing children 3–4 grams of proteins per kg of body weight per day; for school boys and girls, pregnancy, lactation, etc. 2–3 gm per kg of body weight per day.

To provide for the specific dynamic action of proteins on the average the SDA is approximately 5–10% of BMR. This is to be provided for by adding proportional amount of carbohydrates and fats.

Fat requirement: Fat produces high energy and serves as a vehicle for the fat-soluble vitamins A, D, E and K. It also contains essential fatty acids, i.e. linoleic, linolenic

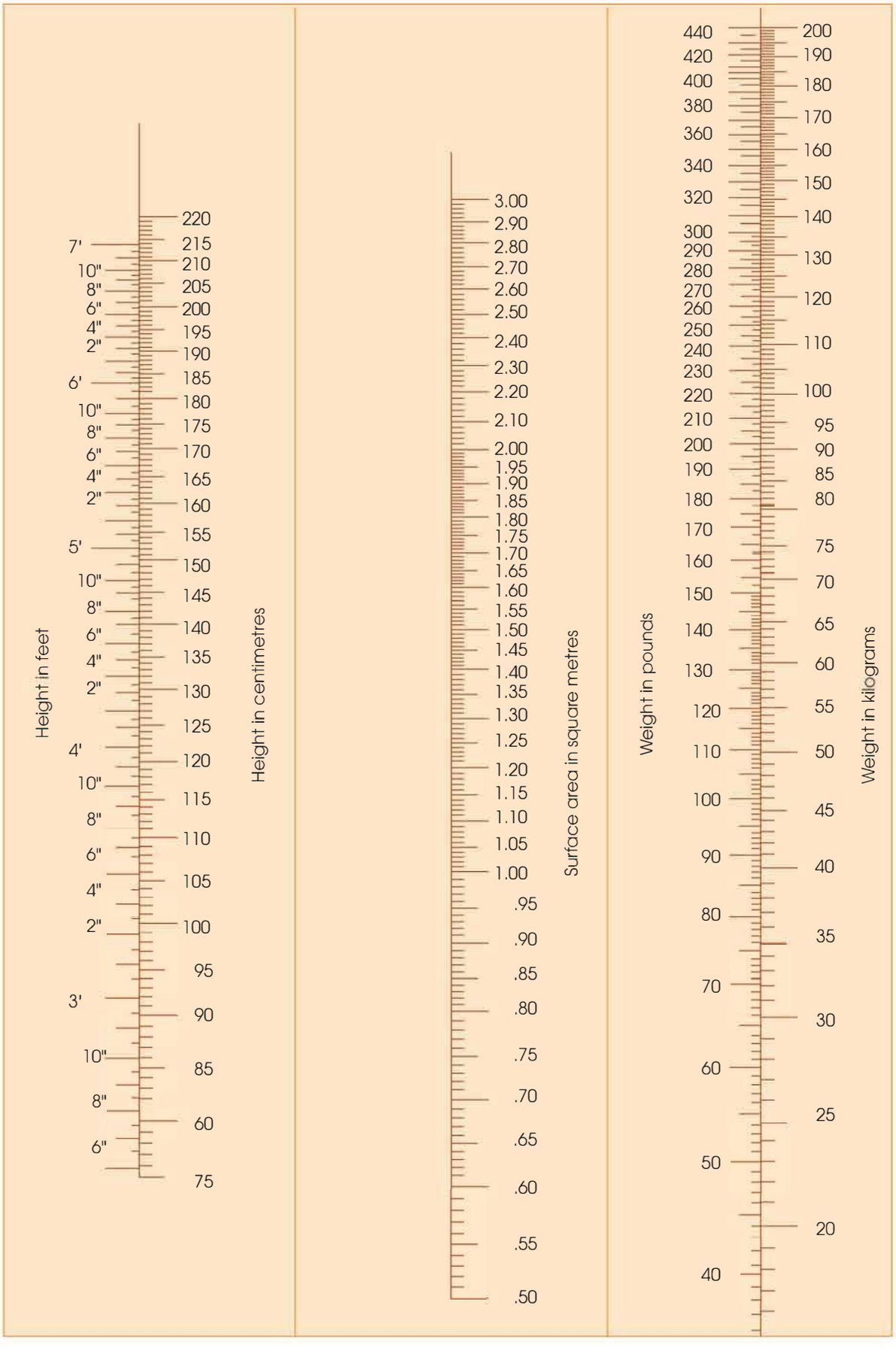


Fig. A2.4: Nomogram for estimating surface area of older children and adults. The description is same as in Fig. A2.3

and arachidonic acids. Out of the total energy requirement, 20–30% should come from fats of the diet. The daily intake of fat should be between 50 and 100 gm per day.

Carbohydrate requirement: There should be sufficient carbohydrate in the diet to prevent ketosis. Out of the total energy requirement, 60–70% should come from carbohydrates.

Loss during digestion, absorption and cooking: The full calorific value of the purchased food is not physiologically available. During preparation and cooking about 5% of the energy of the food is lost. Another 5% loss takes place due to incomplete absorption of food. The total loss is 10%. So that, in order to obtain 1,000°C, the diet intake should be equivalent to 1,100°C approximately as purchased from the market.

The Proportion of Fat, Protein and Carbohydrate

A correct proportion of these three constituents is essential to avoid ketosis. This can be done in two ways:

1. The first method is that of the ratio between protein, fat and carbohydrate should be approximately 1:1:4, i.e. if 100 gm of protein be given, fat should also be 100 gm and carbohydrate 400 gm. But considerable variations, especially of carbohydrates, may be done without any harm. In India, fat consumption is much less.
2. The second method is that of the total energy requirement, 10–15% should come from proteins, 20–30% from fats and 60–70% from carbohydrates. This principle has got more in favour than the other one. In this way the three constituents should be distributed.

Mineral Requirement

All salts are adequately supplied in normal diet. In lactating and expectant mothers as well as in infants additional amounts of iron, phosphorus and calcium should be given. In people who undergo a lot of sweating extra-quantity of salts should be given.

Choice of Foodstuff

The articles of food should be selected keeping in view the following facts: (a) Digestibility: Certain articles cannot be digested by human beings. For instance, elastin and cellulose. Hence, they should not be taken into account for calculating the calorific value of that particular food, but the presence of cellulose in the diet to be considered as they give bulk and provide adequate roughage for prevention of constipation. (b) Age: An infant before six months does not develop starch-splitting enzymes specially the pancreatic amylase. Hence, starch should be given in moderation before this age. (c) Habit: Articles should be selected in such a way that the subject is generally accustomed to it. Unaccustomed diet cannot be easily digested. (d) Religious and social factor: Certain classes of people and certain religions have certain restrictions on animal proteins. (e) Cost: It is needless to emphasise that the articles should be selected in such a way that the subject can easily afford to buy.

Value of Cooking

Cooking has certain advantages as well as certain disadvantages. The advantages are that (a) the food is sterilised, (b) the food is partly digested (as a result of hydrolysis) and (c) it is made palatable (which stimulates gastric secretion and thereby digestion). Addition of spices and condiments also serve this purpose. The disadvantages are: (a) Some quantity of vitamin is lost, (b) a small part of energy of the food (about 5%) is lost. Hence, to avoid this loss of vitamin by cooking, certain amount of uncooked fresh food should be taken (fruits, green vegetables and milk).

Conclusions

From the above principles, the diet of any individual, viz. adult male or female, lactating or expectant mother, children, infants, etc. may be calculated. It should be specially remembered that in infants before six months age, starch should be given in moderation and milk should be the chief diet, with added amounts of minerals and vitamins. As a matter of fact it is not definitely known how much food is just adequate for infants and children. A little excess over the calculated value is always desirable. The following few actual examples may be helpful:

A man weighing 70 kg and with energy requirement of 3,000°C should have the following diet: Protein: 70 gm (or to provide for a margin of safety: 100 gm). Thus, will yield 100 × 4.1 = 410°C. Fats—same amount as proteins, i.e. 100 gm. This will yield 100 × 9.3 = 930°C. Total of these two is 1,340°C. The remaining, i.e. 3000 – 1340 = 1660°C should come from 1,660/4 = 415 gm of carbohydrates. However, for practical purposes, it is usually presumed that 1 gm of carbohydrate yields 4°C, 1 gm of protein 4°C and 1 gm of fat 9°C, and necessary adjustments are made in the calculation of calorific requirement in the diet.

A man in nitrogen equilibrium excretes 12 gm of nitrogen per day. His total calorific requirement is 3,000°C. His diet chart should be as follows: Protein requirement = 12 × 6.25 = 75 gm. This is his actual protein requirement. This will yield 75 × 4.1 = about 308°C. Fats approximately same quantity, i.e. 75 gm. This will yield 75 × 9.3 = about 698°C. These two make up 1,006°C. The remaining 3,000 – 1,006 = 1,994°C should be supplied by 1,994/4 = about 500 gm of carbohydrates.

It has been observed that an average adult can maintain his nitrogen equilibrium by taking as low as

35–45 gm of protein daily. In economically backward countries the total calorie obtained from a combination of protein and fat is usually as low as 15–25%.

Diet of school student: For school student (age 10–15), the calorific requirement is same as that of an adult. The protein requirement is high and is about 2.5 gm per kg of body weight per day. Rest of the calculations should be done in the above method.

Diet of an infant: For infants before six months, the total calorific supply should be aimed at between 700° and 1,000°C. [May be approximately calculated from the above mentioned principles.] It should receive small amounts of polysaccharide. Whole of his diet should preferably be given in the form of milk till the fourth month, and after that it can be supplemented with some form of semi-solid food. The amount of milk can be calculated from the percentage composition of cow's milk, from which its calorific value per ounce can be known. The protein requirement should not be less than 4 gm per kg of body weight per day. It is to be noted that the fat content of this milk diet is in large excess than the accepted standard for adults. This is very helpful in infants whose BMR is very high as it supplies enough energy in comparatively small volume.

Diet for the aged: The calorific requirement should be reduced as the age advances according to the following:

- 3% reduction between 30 and 50 years
- 7.5% reduction between 50 and 70 years
- 10% reduction between 70 and 80 years

Diet for pregnant or lactating mothers: Additional allowance over the normal requirements: During pregnancy 500 extra-calorie and 20 gm extra-protein, during lactation 1,000°C and 40 gm protein. Extra-amount of iron, calcium and different vitamins should also be provided.

Lacto-vegetarian diet: Protein should be given from milk and pulses. Other same as above.

PERCENTAGE COMPOSITION OF THE ORDINARY ARTICLES OF DIET

For prescribing diet charts where actual articles of food have to be mentioned, the following facts are helpful.

According to some nutritionists there are in all seven basic food groups: (1) bread and cereals, (2) dairy foods, (3) meats, fish, eggs, (4) butter, oils, (5) green-leafy and yellow vegetables, (6) citrus fruits, (7) potatoes and other vegetables, while others suggest four main groups, such as (i) breads and cereals, (ii) milk food, (iii) meats, fish, eggs, (iv) vegetables and fruits. However, whatever the grouping, the diet should be balanced, containing all the items of food in adequate proportions which will supply the required energy in the body as well as maintain proper growth, normal cytoarchitecture and functioning of the body.

For proteins, the following articles should be selected: Fish, meat, milk, eggs ('first class' proteins or proteins of 'high biological value'), and pulses ('second class' proteins or proteins of 'low biological value'). The protein contents of the other varieties of foodstuffs are so low that for practical purposes they can be neglected. The protein content of fresh fish is roughly 20% (fat

Table A2.2: Qualitative and quantitative composition of diet with approximate calorific value

Qualitative composition of diet	*Corresponding quantitative values*	*Corresponding calorific value*
Protein	70 gm	2,500 calories
Fat	50 gm	
Carbohydrate	440 gm	
Calcium	0.8 gm	
Phosphorus	1.4 gm	
Iron	40 mg	
Vitamin A	7.300 IU	
Thiamine (vitamin B)	1.8 mg	
Vitamin C	200 mg	

Table A2.3: Balanced diets for adult man

Dietary articles	*Sedentary work*		*Moderate work*		*Heavy work*	
	Vegetarian (gm)	*Non-vegetarian (gm)*	*Vegetarian (gm)*	*Non-vegetarian (gm)*	*Vegetarian (gm)*	*Non-vegetarian (gm)*
Cereals	400	400	475	475	650	650
Pulses	70	55	80	65	80	65
Green-leafy vegetables	100	100	125	125	125	125
Other vegetables	75	75	75	75	100	100
Roots and tubers	75	75	100	100	100	100
Fruits	30	30	30	30	30	30
Milk	300	100	300	100	300	100
Fats and oils	40	35	45	40	50	45
Meat and fish	—	30	—	50	—	60
Egg	—	30	—	30	—	30
Sugar and jaggery	30	30	40	40	55	55
Groundnuts	—	—	—	—	50+	50+

Table A2.4: Balanced diets for adult woman

Dietary articles	*Pre-school children*				*School children*			
	1–3 years		*4–6 years*		*7–9 years*		*10–12 years*	
	Vegetarian (gm)	*Non-vegetarian (gm)*	*Vegetarian (gm)*	*Non-vegetarian (gm)*	*Vegetarian (gm)*	*Non-vegetarian (gm)*	*Vegetarian (gm)*	*Non-vegetarian (gm)*
Cereals	300	300	350	350	475	475	50	100
Pulses	60	45	70	55	70	55	—	10
Green-leafy vegetables	125	125	125	125	125	125	25	25
Other vegetables	75	75	75	75	100	100	—	—
Roots and tubers	50	50	75	75	100	100	—	—
Fruits	30	30	30	30	30	30	—	—
Milk	200	100	200	100	200	100	125	125
Fats and oils	35	30	40	35	50	45	—	15
Meat and fish	—	30	—	30	—	50	—	—
Egg	—	30	—	40	—	30	—	—
Sugar and jaggery	30	30	30	30	40	40	10	20
Groundnuts	—	—	—	—	40*	40*	—	—

*An additional 25 gm of fats and oils can be included in the diet in place of groundnuts.
Adapted from tables on balanced diests as given the report of the Nutrition Expert Group, Indian Council of Medical Research (1968).

Table A2.5: Balanced diets for children

Dietary articles	*Pre-school children*				*School children*			
	1–3 years		*4–6 years*		*7–9 years*		*10–12 years*	
	Vegetarian (gm)	*Non-vegetarian (gm)*	*Vegetarian (gm)*	*Non-vegetarian (gm)*	*Vegetarian (gm)*	*Non-vegetarian (gm)*	*Vegetarian (gm)*	*Non-vegetarian (gm)*
Cereals	150	150	200	200	250	250	320	320
Pulses	50	40	60	50	70	60	70	60
Green-leafy vegetables	50	50	75	75	75	75	100	100
Other vegetables, roots and tubers	30	30	50	50	50	50	75	75
Fruits	50	50	50	50	60	60	60	60
Milk	500	200	400	200	500	200	400	200
Fats and oils	20	20	25	25	30	30	35	35
Meat and fish, egg	—	30	—	30	—	40	—	50
Sugar and jaggery	30	30	40	40	50	50	50	50

Table A2.6: Balanced diets for adolescent boys and girls

Dietary articles	*Boys*				*Girls*	
	13–15 years		*16–18 years*		*13–18 years*	
	Vegetarian (gm)	*Non-vegetarian (gm)*	*Vegetarian (gm)*	*Non-vegetarian (gm)*	*Vegetarian (gm)*	*Non-vegetarian (gm)*
Cereals	430	430	450	450	350	350
Pulses	70	50	70	50	70	50
Green-leafy vegetables	100	100	100	100	150	150
Other vegetables	75	75	75	75	75	75
Roots and tubers	75	75	100	100	75	75
Fruits	30	30	30	30	30	30
Milk	500	200	400	150	400	150
Fats and oils	35	40	45	50	35	40
Meat and fish	—	60	—	70	—	80
Egg	—	30	—	30	—	30
Sugar and jaggery	30	30	40	40	30	30
Groundnuts	—	—	50+	50+	—	—

Note: +An additional 30 gm of fat and oil can be included in the diet in place of groundnut

1.5%); that of meat is roughly 25% (fats about 15%); that of pulses are approximately 15–20%. Whole egg contains 12.8% protein (fat 11.3%) and yolk only has 15.5% (fat 33.3%) of it. Rice and wheat contain 7–8% and 10–11% protein respectively on the average. Although rice contains less protein than that of pulses, its biological value is higher.

For fats: Vegetable oils, ghee, butter (all of which are pure fat) and milk (fat approximately 4%) should be selected.

For carbohydrates: Cereals (mainly rice and wheat) and root vegetables (e.g. potatoes) contribute the major portion of dietary carbohydrate. All these contain approximately 70–80% carbohydrates (and 6–10% proteins).

REGULATION OF FOOD INTAKE

A good number of factors operates together as a complex mechanism for regulating food intake

Food intake regulation: Since eating is a voluntary act so conscious sensation (hunger and appetite). Indicating whether food is needed or not, is a prerequisite for the regulation of food intake (eating). Hunger is aroused by the physical need of the food whereas appetite is the emotional (psychic) desire to which may or may not be associated with the need of food.

Hunger, i.e. desire for taking food is controlled by a centre in the hypothalamus, i.e. lateral hypothalamic area. There is another centre, i.e. ventromedial hypothalamic nuclei (Fig. A2.1) in tuber cinerium called satiety centre, which inhibits the hunger centre when it is satisfied with the amount of food appropriate to calorific requirement resulting loss of desire for taking food. Reversely when the satiety centre is not satisfied with the amount of food appropriate to calorific requirement, then this centre is not satisfied to inhibit the centre so the hunger centre is not inhibited resulting the desire for food.

Appetite, on the other hand, is conditional and not dependent to calorific need.

The mode of activation of these centres has not been established as yet. Several theories have been put forward suggesting the regulatory activity of the appetite centre or appestat.

They are as follows:

1. *Concentration of blood glucose:* Low glucose concentration stimulates the lateral hypothalamic nucleus producing increased hunger and food intake. When glucose concentration increases after intake of food, the ventromedial centre of the hypothalamus is satisfied and hunger centre is inhibited, thus the animal stops eating.
2. *Temperature variation of blood:* Low temperature from normal stimulates food intake and *vice versa*.
3. The proportionate amount of active fat in fat cells to passive fat also acts in a regulatory manner.
4. The concentration of serum amino acids also regulates the hunger centre.

Other factors also play some part. The hypothalamic hunger centre receives projection from the cortex. So, it is under voluntary control. Emotional stress, such as worry, tension, etc. also play a major part (Fig. A2.2).

The satisfaction achieved by food intake and its regulatory role are partly dependent also on the digestive tract. The feeling of fullness after a heavy meal may depend on the distension of the stomach, which may be brought about by proprioception. Other nervous projections may also play a part.

Appendix

3

Energy Metabolism

INTRODUCTION

There is a continual exchange of energy between a living organism and its environment as in accordance with the principles of thermodynamics applied to non-living physical and chemical systems. There is a fundamental difference between plants and animals, in the way of their utilization of energy for their life processes. Plants unlike animals, can convert the energy of solar photons* into the energy of chemical bonds. Plants are, therefore, interposed between the sun and all animals in the energy-flow relationship in nature. It follows therefore that the energy required for our bodily activities is supplied by food which is stored with energy and the original source of energy is the sun.

The Body's Input and Output of Energy

The energy exchange is based on the first law of thermodynamics as derived by Mayer, Joule and Helmholtz and later proved applicable in case of the animal body also by Voit, Pettenkofer and Rubner, which states that energy is neither gained nor lost when it is converted from one form to the other—mechanical, thermal, electrical, chemical, etc. The unit of energy is mainly expressed as heat unit or energy equivalent of foodstuffs—the calorie (cal), also known as small calorie, standard calorie or gram-calorie. This is defined as the amount of heat energy which can raise the temperature of 1 gm of water from 15 to 16°C. The measurement of heat is known as calorimetry—the apparatus for measurement being known as the calorimeter. In the biological system, i.e. in physiology and medicine, the unit used is the large calorie or kilocalorie (cal or k cal) which is equal to 1000 small calories. It is defined as the amount of heat required to raise the temperature of 1 kg of water from 15 to 16°C.

The total energy obtained from food sources are utilized as:

1. For the synthesis of different types of protoplasmic constituents characteristic of different cells and tissues, e.g. phospholipid, proteins, RNA, DNA, enzyme, co-enzyme, other complex substances, etc.
2. For the operation of different organs and as a whole organism, e.g. heart, lung, gland, kidney, etc.
3. For maintenance of body temperature
4. For generation of electrical potentials and current, viz. in central nervous system, heart, etc.
5. For the transport of substances against the concentration gradient
6. For growth and maintenance

Joule versus calorie: Amounts of heat may be expressed as equivalent amounts of energy, with the joule as the unit. According to the pamphlet circulated to the Nutrition Society by the Royal Society (1968) the amount of heat must be expressed in terms of energy and the relationship between the two units is 1 kcal equal to 4184 or 4188.5 or 4186.8 joules or about 4.19 kJ.

Methods for Determination of Energy Output

Energy output can be determined by measuring the heat production of an individual over a measured amount of time.

Direct calorimetry: This is done by measuring the heat output of the subject for a given period, by putting him inside a specially prepared heat proof chamber (Atwater-Benedict's respiration calorimeter). Heat produced is measured by changes in temperature of circulating water. This method, although very accurate, requires much elaborate apparatus and can hardly be used for ready clinical purposes.

Indirect calorimetry: Due to complications involved in direct calorimetry, heat output is calculated indirectly from O_2 consumption and CO_2 output.

*Photons = unit of energy (i.e. 1 absolute (k) cal = 4.184 × 107 ergs), carbohydrate containing energy which is released during its breakdown] and store them in their leaves, stems, fruits, etc. which serves as food for the animals.

Closed circuit method (clinical type): Various apparatus may be used for this purpose, such as Benedict-Roth apparatus (Fig. A3.1) and other apparatus of similar type. Benedict-Roth apparatus is very useful for clinical purposes as the heat production can be calculated in this type of apparatus by the oxygen consumption only without determination of CO_2 elimination. The subject is allowed to breathe from O_2 reservoir through a mouthpiece, the nose being clipped. The CO_2 eliminated in expiration is absorbed by soda lime to keep the O_2 reservoir pure. The fall in the level of O_2 during the experiment is recorded which gives the value of O_2 consumption at the specified time. In this method, respiratory quotient (RQ) of the subject is not determined and the average RQ is taken as 0.82. 4.825 cal of heat is liberated at this RQ when one litre of O_2 consumed (Table A3.1). The energy output during the experiment is calculated by multiplying litres of O_2 consumed at that time with 4.825.

(a) ***Open circuit method*** (Haldane type). The subject inspires atmospheric air. The expired air is collected in a special air-tight bag known as Douglas bag. The total volume of expired air collected in the bag at the end of experiment is measured and the samples are analysed for CO_2 and O_2 in Haldane gas analysis apparatus or in Scholander's micro-gas analysis apparatus. The amount of oxygen consumed and carbon dioxide given off is calculated from the difference of percentage of O_2 and CO_2 between the atmospheric and expiratory air. The RQ and the calorific value of O_2 can then be determined. The Douglas bag method can conveniently be used to measure the energy output during different types of activities. A respirometer devised at the Max-Planck Institute at Dortmund is also recently used for this purpose, and is much less cumbersome and easier to manipulate than the Douglas bag.

Table A3.1: The amount of heat produced per litre of O_2 consumed, at different RQ

RQ	*Calories evolved per litre of O_2 consumed*
0.71	4.795
0.75	4.829
0.80	4.875
0.85	4.921
0.90	4.967
0.95	5.012
1.0	5.058

RESPIRATORY QUOTIENT (RQ)

Definition: It is the ratio of the volume of CO_2 produced by the volume of O_2 consumed (i.e. CO_2/O_2) during a given time. It should be noted that RQ is simply a ratio. It gives no idea as to the absolute quantity of gaseous interchange. Proportional increase or diminution of CO_2 produced and O_2 utilised, will keep the ratio unchanged. But any disproportionate variation will be reflected by a corresponding change in the RQ.

Normal RQ: In a healthy adult it is 0.85 for a mixed diet.

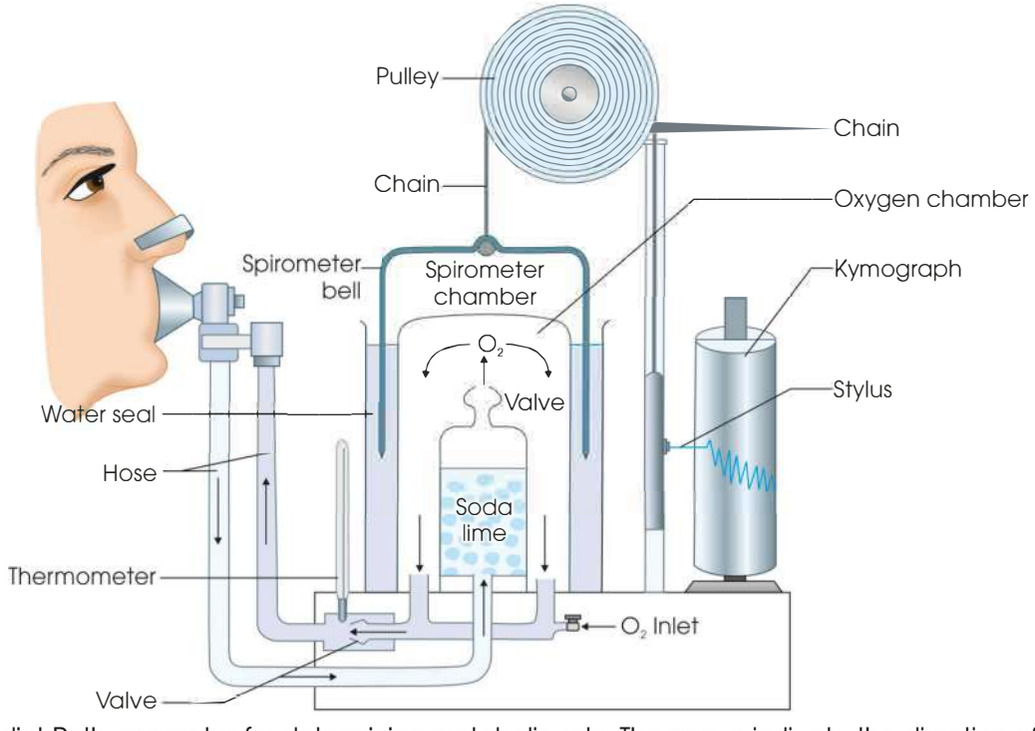


Fig. A3.1: Benedict-Roth apparatus for determining metabolic rate. The arrows indicate the direction of air flow during respiration

Method of determination: This is done by measuring the volume of O_2 consumed and CO_2 produced during a given time with the help of Douglas bag and other similar instruments.

Factors Affecting Respiratory Quotient

Role of diet: In case of carbohydrate diet the RQ is unity. Because in carbohydrate diet the volume of CO_2 produced is same as the volume of oxygen consumed. This is due to the fact that, in the carbohydrate molecule, the amount of O_2 present is just sufficient to oxidise the H present in the same molecule. Hence, external oxygen is necessary only to convert the C of the molecule into CO_2. So that, the volume of O_2 consumed and the volume of CO_2 produced will be same. This is represented in the following equation:

$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O$. $RQ = 6CO_2/6O_2 = 1.0$

In case of fats the RQ will be lowest and is about 0.7; because fat is an oxygen-poor compound. The oxygen present in it cannot fully oxidise the H of the molecule. So that, oxygen consumed from outside, is used for two purposes: First, for oxidising C and producing CO_2 and secondly, for oxidising H giving H_2O. Consequently, the volume of CO_2 produced will be less than the volume of O_2 utilised. Hence,

RQ will fall and will be about 0.7. This is shown in the following equations:

$$2C_3H_5(C18H_35O_2)_3 + 163O_2$$
(Tristearin)
$$= 114CO_2 + 110H_2O$$

In case of proteins the RQ is about 0.8.

$2C_3H_7O_2N + 6O_2 = (NH_2)_2CO + 5CO_2 + 5H_2O$. $RQ = 114CO_2/163O_2 = 0.7$ (Alanine)

$$RQ = 5CO_2/6O_2 = 0.8$$

In any condition where fats are burnt chiefly (starvation, advanced diabetes, etc.); the RQ will be about 0.7.Whereas with predominant carbohydrate combustion the RQ will approach 1.

Effect of inter-conversion in the body: When carbohydrates are converted into fats in the body, RQ will rise. Because in the process an oxygen-rich substance is converted into an oxygen-poor compound. So that some amount of O_2, liberated from carbohydrate, will be utilized for purposes of oxidation. Consequently, less oxygen will be needed from outside. Hence, the amount of CO_2 produced will be more than the amount of O_2 consumed. So that, RQ will rise. When fat is converted into carbohydrate just the opposite effects will be produced and RQ will fall. It is, therefore, evident that RQ value will indicate the following: (a) The type of foodstuff burning in the body and (b) the nature of conversion of one foodstuff into another in the body.

Acidosis: During acidosis CO_2 output is greater than O_2 consumption so the RQ rises. The RQ falls due to the reverse condition of acidosis, i.e. CO_2 output is lesser than O_2 consumption.

Alkalosis: Here the RQ will fall, because respiration is depressed and CO_2 will be retained in the body (i.e. less CO_2 is produced).

Rise of body temperature: It may increase RQ as in acidosis. [Rise of body temperature, such as in fever, will cause increased breathing and thereby will wash out more CO_2.]

Diabetes mellitus: In advanced cases of diabetes, when a little carbohydrate is burning, energy is supplied mainly by oxidation of fats. Hence, RQ will fall. In such cases, if insulin is injected, carbohydrates will start burning and RQ will rise.

Starvation: Here the subject has to live on its own body tissues. In the first stages (1–2 days) energy is derived mainly from the stored glycogen, so that the RQ, although it falls below normal (0.85), is proportionately high—0.78. But later on, when energy is derived chiefly from the combustion of fats, RQ will fall still further and will be about 0.7.

Voluntary hyperpnoea: By this process excess CO_2 is washed out without a corresponding increase of O_2 utilised, so that RQ will be above unity.

Muscular Exercise

1. With moderate exercise (with a normal mixed diet) the RQ remains almost unaltered. Because in exercise the body uses different foodstuffs in the same proportion as at rest.
2. With violent exercise lactic acid enters blood and produces acidosis. Pulmonary ventilation will be raised washing out more CO_2. Consequently, RQ rises and may go above 2 even.
3. During recovery from violent exercise RQ falls, because less CO_2 is evolved. Gradually it goes back to normal.

Value of Determining Respiratory Quotient

1. RQ acts as a guide as to the type of food is burning or the nature of synthesis taking place in the whole body as well as in a particular organ.
2. RQ is very helpful in determining metabolic rate.
3. Non-protein RQ helps in finding out the proportion of the three foodstuffs that are being utilised in the body.
4. Determination of RQ helps in the diagnosis of various pathological conditions, such as acidosis, alkalosis, etc.

The RQ of Various Organs and Tissues

The RQ of the individual organs, such as liver, spleen, heart, brain, etc. can be measured by noting (a) the total blood flow in a given time through the organ and (b) the oxygen consumed and CO_2 produced by the organ at same time. This can be found out by determining

the arteriovenous difference of these gases and then multiplying the figure by the total volume of blood flow through the organs. In this way the RQ of various organs can be worked out. The total gaseous interchange of an organ will show its metabolic rate and RQ of the organ will indicate the nature of foodstuff burning in it.

Basal Metabolism

Energy, in terms of heat, produced as a by-product of total cellular metabolism is essential for the maintenance of life of the organism. Although the amount of energy required for any individual varies directly with the degree of activity and environmental condition, but the rate of energy production in an individual by its overall cellular metabolism is more or less constant under some standard conditions known as basal metabolism and the rate of its energy production at basal condition per hour and per sq-m of body surface is known as basal metabolic rate (BMR). The basal conditions are as follows:

1. The person should be in awake but at complete rest, both physical and mental.
2. The person should remain in normal condition of environment, i.e. at normal temperature, pressure and humidity.
3. The person should be without food at least for 12–18 hours, i.e. in the post-absorptive state.

Thus, the BMR may be defined as the amount of heat given out by a subject who, though awake, is lying in a state of maximum physical and mental rest under comfortable conditions of temperature, pressure and humidity, 12–18 hours (post-absorptive)* after meal. It is also called resting metabolic rate (RMR).

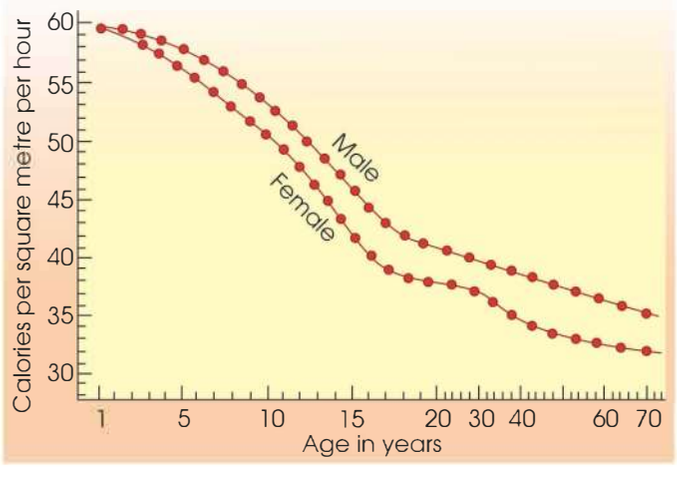


Fig. A3.2: Graphical tracing of normal BMR at different ages (diagrammatic representation)

Basal Metabolic Rate (BMR) (Fig. A3.2)

It is usually expressed as; the heat production per square metre of body surface area per hour. In adult male, normal BMR is about 40 calories per sq-m of body surface per hour and in the adult female about 37 calories. The surface area of an average adult is about 1.8 sq-m, and can be calculated from the following formula by DuBois.

$$S = 0.0071 \times W0.425 \times H0.725$$

When W = body weight in kg, H = height in cm, and S = surface area in square metres.

The rate of metabolism at basal conditions has been formed to vary in different individuals and, therefore, the BMR varies with different factors.

Factors Affecting Basal Metabolic Rate (Fig. A3.3)

1. **Age:** The BMR of children is much higher than the adults. Roughly speaking it is inversely proportional to the age.** In other words, with advancing age BMR gradually falls. This is due to the fact that children possess a greater surface area in proportion to their body weight. [But in newly born baby it is low—about 25 calories per sq m of body surface per hour. In pre-mature infants it is still lower.]
2. **Sex:** The BMR of the males is slightly higher than the females (Fig. A3.3). The figures are mentioned above.
3. **Surface area:** The BMR is directly proportional to the surface area of the subject. Larger the surface area greater will be the heat loss, and equally higher will be the heat production (i.e. metabolic rate). Otherwise temperature balance will be upset. For this reason BMR depends upon the surface area.
4. **Climate:** In colder climates the BMR is high and in tropical climates the BMR is proportionally low. Habit: Trained athletes and manual workers have a slightly higher BMR than persons leading a sedentary life.
5. **Diet:** Prolonged undernutrition lowers the metabolic rate.
6. **Hormones:** Circulating levels of hormones secreted by adrenal medulla, adrenal cortex, thyroid and the anterior pituitary increase BMR. One milligram of thyroxine increases BMR by about 1,000 calories. In thyrotoxicosis, BMR may increase by 50–100% above normal, but RQ remains unaltered since both O_2 consumption and CO_2 production increase proportionately in such cases. In myxoedema, BMR

*This period is allowed to pass for avoiding the effects of digestion and absorption, the effects of specific dynamic action (SDA) of foodstuffs and also to prevent any chance of starvation.

**At the age six it is 57.5 calories; at twelve 50.4 calories; between twenty and thirty 40 calories; between forty and seventy it is 38.5 calories.

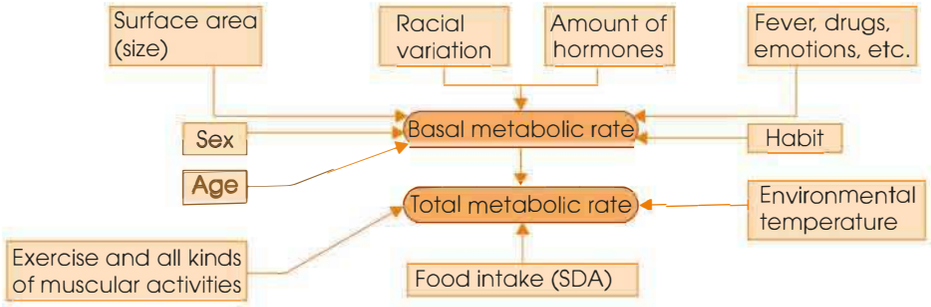


Fig. A3.3: Factors affecting basal metabolic rate

is diminished to 30% or even 45% below normal. Anterior pituitary through its thyroid-stimulating hormone (TSH) affects BMR. Growth hormone (STH) secreted by this gland also causes BMR about 20% rise. Adrenaline (epinephrine) and noradrenaline (norepinephrine) increase BMR by about 20% of the resting value. Male sex hormones cause a 10% increase in BMR, female sex hormones increase it insignificantly.

7. **Barometric pressure:** Moderate reduction of atmospheric pressure does not affect the BMR; but a fall of pressure to half an atmosphere (viz. O_2 tension—75 mm of Hg)—as occurs in mountain climbing—increases BMR, but increased pressure of oxygen does not raise BMR.
8. **Pregnancy:** The BMR of the pregnant mother, after six months of gestation, rises. It is seen that the BMR of the mother is the sumtotal of her own metabolism as in her non-pregnant state and combined with that of the foetus. Hence, pregnancy exerts no specific effect upon BMR.
9. **Body temperature:** The BMR increases by about 12% with the rise of 1°C (such as in fever). This is due to the fact that increased temperature stimulates the chemical processes of the body and thereby increases BMR.
10. **Drugs:** Some drugs like caffeine, benzedrine, etc. increase the basal metabolic rate. The reverse is observed by anaesthetics.
11. **Racial variation:** Natives of Yucatan were found to have their higher metabolic rate than the Americans, whereas South-Eastern Asians were found to have much lower rate. Thus, there may be significant deviations in the metabolism according to race.

In addition to the above factors, the metabolic rate of an individual may be influenced by the following:

(a) Muscular exercise—light exercise, as sitting, standing, etc. increases metabolism by 30–40%. Moderate exercise, as walking, by 50–60%, and severe exercise (hard work), by 100% of the BMR. (b) Mental exercise causes a slight rise: Solving mathematical problems increases BMR by 3–4% only. But it should be remembered that the basal oxygen consumption of brain is high and amounts to about 10% of the total O_2 requirement of entire body. (c) Strong emotions increase metabolism by 5–10%. (d) Sleep reduces it by 10–13%.

Disease Causing Variation of BMR

Changes of BMR are found in the following pathological conditions:

1. *Conditions increasing BMR*: (a) Hyperthyroidism (may rise up to 100%), (b) fever, (c) cardio renal disease with dyspnoea (25–50%), (d) leukaemia (21–80%), (e) polycythaemia (10–40%), etc.
2. *Conditions reducing BMR*: (a) Starvation and under nutrition, (b) hypothyroidism, (c) Addison's disease, (d) lipid nephrosis, etc.

Measurement of BMR

The apparatus most commonly employed clinically for the determination of BMR is Benedict-Roth apparatus, although Tissot method and Douglas bag method are also sometimes employed.

Read's formula: This formula gives a rough estimate of BMR and is often and at the bedside. It is as follows:

BMR = 0.75 (PR + 0.74 × PP) – 72 where PR = pulse rate, PP = pulse pressure.

The result comes out as the percentage of the normal and is correct within a range of ± 10%, viz. if above 10% the BMR is higher, if below 10% it is lower than normal.

Importance of Noting BMR

1. For prescribing a diet of adequate calorific value.
2. For the diagnosis of various pathological conditions specially in hypothyroidism and hyperthyroidism. To note the effect of different types of food and drug on basal metabolic rate.

Mechanical Work and Heat

Energy: It is a physical concept which cannot be realised but measured since it is equivalent by its capacity due to the equality of the physical dimensions in both

energy and work. Although there are different forms of energy, viz. chemical, electrical, mechanical, surface, radiant, etc. which are interconvertible, i.e. when one form is produced, an equivalent amount of other form disappears, but the total quantity in the universe remains same (law of conservation of energy). The ultimate source of energy is the radiant energy coming from the sun which is converted to chemical energy and then to different forms of work in biological system, i.e. biosynthesis, osmotic, mechanical and transport work.

Of the total energy released from the breakdown of the chemical substances in the biological system, only a fraction of it (known as free energy) is utilised for the work (second law of the thermodynamics). Energy of a system unavailable to do work is termed as entropy. The energy which is consumed or released is termed as enthalpy.

When mechanical work is completely transformed into heat, or heat is completely converted into mechanical work, the amount of mechanical work is equivalent to quantity and unit of heat which is called mechanical equivalent of heat.

Joule established the relation between the mechanical work and heat mathematically, with the help of the following formula: If W, unit of work, is converted into H, unit of heat or vice versa, we have,

$W = f \times H$ or $W/H = 7$, where f is a constant, assuming that all the work is spent in the production of heat and no part of it is wasted by friction, radiation, etc. The constant is known as the mechanical equivalent of heat, and the accepted value of f is 4.184 × 107 ergs per calorie.

1 kg-m = 7.23 foot-pounds
= 0.002343 calorie
= 0.81 × 107 ergs.

Work may also be defined as the product of force and the distance through which the force acts. When a man lifts a 10 kg weight at a height of two metres from the ground, the work done is 10 kg × 2 m = 20 kg-m. But time factor need also to be considered. If a man has lifted 10 kg weight at a height of two metres from the ground in one second, the work done is 20 kg-m/sec. If he performs the same work at same rate for 60 sec, the total work performed is 20 kg-m × 60 seconds or 1,200 kg-m per minute. The mechanical work done can be measured by different instruments like bicycle ergometer, treadmill, etc.

Bicycle Ergometer

It is a modified bicycle (Fig. A3.4) specially constructed for carrying out standardised work (exercise) whose wheel is rotated by pedalling without the actual progress of the bicycle in the ground. A brake-band is applied to the front wheel. The work done depends on the rate of pedalling and is determined by the revolution counter attached to the front wheel. The work done is calculated from the tension difference between the two sides of the bands and the linear velocity of the circumference of the wheel.

A metre, calibrated in kilogram-metre per minute, is kept above the front of the stationary bicycle for noting the constant work output by the subject.

With the help of the bicycle ergometer various physiological changes from a 'resting level' to a 'working level' can be noted. Heart rate, blood pressure, cardiac output, pulmonary ventilation, oxygen consumption, CO_2 output, body temperature, etc. change during physical activity. By measuring these variables during activity it is possible to determine in what degree the 'working level' differs from the 'resting level'. In the bicycle ergometer, mechanical work is imposed on the body. Even after the stoppage of mechanical work, physiological work continues above the 'resting rate' till recovery is not complete. A complete 'work cycle' indicates the 'physiological cost of work' and 'physiological cost of recovery'.

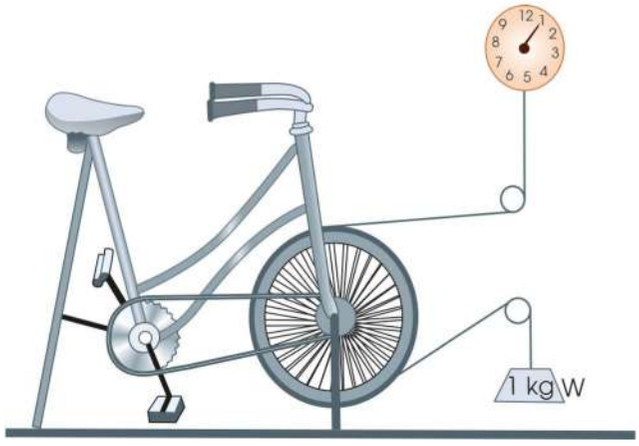


Fig. A3.4: Sketch of bicycle ergometer: Weight (W) is put with the help of string against the bicycle wheel and a recording metre (M) records the weight. Bicycle wheel is to be rotated 60 times a minute by pedalling. The friction of the string against the bicycle wheel will produce work according to load W. In the figure the load W is given as 1 kg

Appendix

4

Ageing

INTRODUCTION

The pattern of life changes that occurs as one grows older in age is ageing. Worldwide researchers are investigating methods to prevent or in fact slowing ageing process. Gerontology is the collective study of ageing processes in an individual. The biological age is the relative age or condition of a person's organs and body systems while the legal age is determine by the chronological years of existence of life.

The factors which influence ageing process are discussed along with theories of ageing.

Theories of Ageing

Ageing is much influenced by genetic factors and is also mainly hereditary based. Ageing or senescence is not inevitable in humans and can be delayed. The process of ageing is complex, and many different mechanisms varied reasons attribute towards occurrence of ageing.

The various proposed theories of ageing are:

1. *Membrane theory of ageing:* This theory suggests that with ageing the cells ability to transfer chemicals, heat and electrical processes in physiological functioning of body declines. The lipid and water solubility across the cell membrane decreases along with the decline in receptor response sensitivity. This impedes cellular functioning and there is a toxic accumulation of products in the cells.
2. *Mitochondria decline theory:* The mitochondria are the power house of the cell and produces adenosine triphosphate (ATP). Nutrients such as NADH, acetyl-l-carnitine, CoQ10 (Idebenone), few B complex vitamins, etc. which are required during oxidative phosphorylation and so also in other metabolic cycles in mitochondria. Age related decline in mitochondria functioning brings over agility. Adequate numbers of mitochondria and there effective functioning are vital for preventing and slowing ageing. Supplementation of above mention nutrients aids mitochondria in its functioning and delays ageing.
3. *Programmed senescence theory:* Ageing is under control of the biological clocks. The biological clock control depends on gene expression and their variations and this affects the mechanism involved in maintenance, repair, and defence responses. Ageing occurs due to sequential switching on and off of different genes, and age-associated deficits in functioning of body cells and tissues manifest with diseases and agility.
4. *Cell replication theory:* The replicating cells in our body example blood cells or gastro-intestinal tract cells or those replicating during injury such as endothelial cells, and fibroblasts replicate up to a limit beyond that the replication fails to occur. Similarly, the telomeres shorten on each event of cell division. As they get too short, they can no longer divide leading to various age associated changes. Telomeres are specialised DNA sequences at the end of chromosomes. The shortening of telomeres occurs with each cell division. When the telomeres become too short, the cell enters the senescence stage.
5. *The neuroendocrine theory:* Hypothalamus regulates the vital activities of human body such as growth, autonomic, metabolic, reproduction and so also influences the functioning of various target glands such as thyroid, parathyroid, adrenals, pancreas, etc. The hypothalamus regulatory ability declines with ageing and the receptors which are involved in uptake individual hormones become less sensitive to them. This leads to decreased secretion of many hormones declines and their effectiveness especially due to down-grading of the receptors.
6. *Free radical theory:* Accumulation of free radicals creates oxidative stress which damages the cell membrane, decreasing its efficiency. The body

produces antioxidants that scavenge the free radicals. The intake of anti-oxidants prevents occurrence of cardiovascular diseases, metabolic disorders and ageing. It combats and prevents occurrence of tissue damage due to free radical.

7. *Wear and tear theory:* The lipofuscin, a yellow brown pigment is seen in most of the tissues of an agile person. It is formed from the breakdown of mitochondria or endoplasmic reticulum in the lysosomes. The concentration of lipofuscin is directly proportionate to the age of an individual. The lipofuscin granules interrupt with the normal functioning of cell leading to cell death.

Cross-linking theory: It is also known as the glycosylation theory of ageing. The accumulation of cross-linked proteins damages cells and tissue, slowing down bodily processes. The binding of glucose with protein renders the protein impaired in performing its functions efficiently. The known cross-linking clinical disorders include in senile cataract, loss of flexibility of connective tissue, microvascular changes in arteries, etc.

Effect of Ageing on Body Systems

1. **Sensory hearing, vision, smell and taste**
   Hearing: Hearing threshold increases with ageing and it may also be due to damage caused to hair cells of inner ear as a result of varied exposures to noise sound levels over the period of life. There is loss in ability to hear and may this may lead to social isolation and can be resolved by using hearing aids.
   Vision: Healthy vision status having no impaired vision may be observed in some old individual. But by and large all ageing people have cataract. The near vision defect called presbyopia appears by 40 years of age; and focusing becomes less accurate, sensitivity to glare increases and the night vision are not as acute as in childhood and adult age.
   Taste and smell: Due to decline efficacy of receptor cells for smell and taste with ageing there is some loss in taste and smell.
2. **Cardiovascular system:** The yellowish brown granules called lipofuscin accumulates in the myocardium with ageing. Atherosclerotic changes with narrowing of coronary arteries occur with ageing; especially in obese, smokers and alcoholics. The capillary density in cardiovascular musculature decreases with ageing. The regulatory control sensitivity to the baroreceptors and chemoreceptors declines with ageing and this result in increased blood pressure with ageing. The altered fatty diet with sedentary lifestyle makes the older persons more prone for cardiovascular disease.
3. **Respiratory system:** Ageing related changes in lungs: The decreasing number of cilia and reduced ciliary activity, decrease in number of functional alveoli, thinning of alveolar walls, decreased elasticity of the lungs which may be due to collagen cross-linking and also decreased value of lung volumes and capacities are some of the major physiological alteration in lung with ageing. The respiratory muscles lose strength and endurance and this leads to decreased lung compliance. The pulmonary artery becomes less elastic, thickens and enlarges thereby increasing resistance to blood flow in lungs and may lead to increased pulmonary arterial pressure.
4. **Central nervous system:** Cognitive functions decline with ageing. There is a gradual reduction in the weight and volume of the brain as a person agile. This decline is about 2% per decade. There is neuronal loss in the brain with ageing. All superficial and deep reflexes become sluggish in old age. There is development of senile dementia and Alzheimer's disease in seventies. The degeneration of dopaminergic neurons leads to parkinsonism with ageing.
5. **Peripheral nervous system:** There is slowed conduction velocity in peripheral nervous system.
6. **Kidney:** With ageing the renal blood flow decreases from about 600 ml/min (age of 40) to about 300 ml/min (age of 80). The kidney size decreases by 20–30% by age of 90. These occur loss of the glomerular cells in cortex (glomerular cells decrease by 30–40% by age of 80). There is decreased GFR. There is a decline in the number of renal tubular cells, and thickening of the tubular walls occurs with ageing. The ability to dilute and concentrate urine decreases with ageing. The bladder control is declining along with ageing especially in late seventies and eighties. The overall kidney function, however, remains normal.
7. **Musculoskeletal system:** There is decreased muscle mass and contractile force with ageing. There is gradual loss of bone mass, decreased water content in cartilage, and decreased water in the cartilage of the inter-vertebral discs. This affects in compressibility and flexibility. Similarly, there is some decrease in water content of tendons and ligaments and this affects the mobility and movements.
8. **GI tract:** The basal and maximal stomach acid production diminishes with ageing. The gastric cells decrease in numbers and thereby there is decreased production of HCl. All secretions in GIT including that of pancreas, small intestine and large intestine decrease with ageing. The decreased tone in stomach and intestines result in decreased peristaltic movements producing constipation.
9. **Autonomic nervous system:** The temperature regulating mechanism is altered with ageing. Many

old individual develop postural hypotension due to partial failure of baroreceptors mechanism.

10. **Haematological changes:** Iron deficiency anaemia is commonly seen in the elderly due to nutritional deficiency and poor absorption of iron and vitamin B_{12} in the gut. There is decreased resistance to infections due to declined immune functions. T-lymphocyte and B-lymphocyte functions reduce with ageing hence agile persons are more prone to infections.
11. **Endocrines:** There is some atrophy and decreased secretion from endocrine glands with ageing. There is poor glycaemic control, altered metabolism, decreased sexual function, declining autonomic control including lower levels of thyroid and other adrenal hormones in circulation.
12. **Reproductive system:** *Males:* The testosterone level decreases with ageing in males. The erectile firmness of penis decreases. The sperm count and their motility decrease but still the sperm are viable and can produce progeny even at any age in males. The prostate gland enlarges; resulting in compression of the urethra which may inhibit the flow of urine. *Females:* There is gradual decrease of estrogen and progesterone secretion during transition from perimenopause to menopause. The pubic hairs thin down and there is greying. The ovaries and uterus decrease in size and weight. The breast sag over as there is loss of glandular tissue. The other physical changes may include hot flashes, sweating, irritability, depression, headaches, and myalgias. Sexual desire is variable.
13. **Immune system:** The non-specific defences become less effective and the ability of the body to produce antibodies declines with ageing. The incidence of auto-immune disorders may occur in old age and there are evidences of findings of positive rheumatoid factor, anti-nuclear antibody, and false-positive syphilis screens in healthy older individuals. The thymus gland (which produces hormones that activate T cells) undergo atrophy with ageing.
14. **Hair changes:** The male's hairline recedes around 16 years of age and male pattern baldness may occur in their thirties. The females may experience a receding hairline; hair becomes thinner and increased hair growth about chin and around lips. There is loss of body hairs in males and females.
15. **Skin changes—dermis:** The number of epidermal cells decreases by 8–10% every decade and the cells replication slows down making the skin less able to repair itself fast. The skin wrinkle and sag, the dermal layer thins down, and elastin fibres wear out. The decreased function of sebaceous and sweat glands contribute to dry skin.

Healthy Ageing

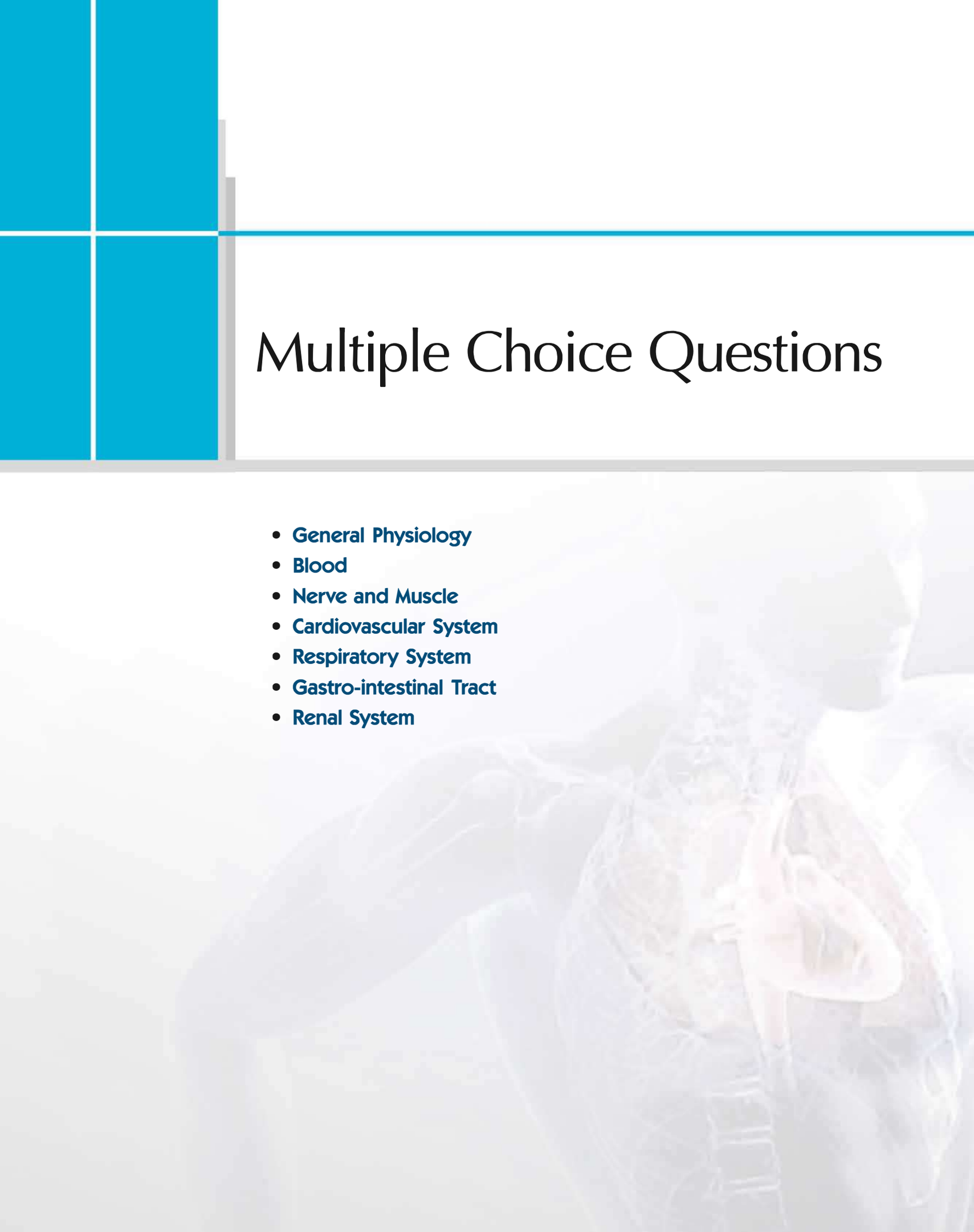
Balanced diet, regular exercises, regular intake of supplementary vitamins and anti-oxidants and health lifestyle prevents early ageing.

Nutrition: Balanced diet intake provides adequate calories and nutrients so that body can defend infections and especially prevent chronic diseases, maintain digestive health, functional ability of all aspects of human body such as bones, liver, kidney, heart, GIT, oral health, vision, etc.

Exercise: Regular exercise habits prevent cognitive decline in later life and individuals have better cognitive function. All adults, including older people should exercise at least 30 minutes (five days a week) and these exercises should be of moderate intensity. Exercise will improve bone and muscular strength and balance of body. It will prevent diseases such as osteoporosis, anaemia, diabetes, heart disease, stroke, constipation and other gastrointestinal disorders. It will increase longevity of life.

Multiple Choice Questions

- General Physiology
- Blood
- Nerve and Muscle
- Cardiovascular System
- Respiratory System
- Gastro-intestinal Tract
- Renal System



GENERAL PHYSIOLOGY

1. **The respiratory enzyme in mitochondria are:**
   a. Flavoprotein and cytochrome
   b. Albumin and calcium
   c. Renin and angiotensin
   d. Cobalt and zinc
2. **The digestive apparatus of cell is:**
   a. Nucleus b. Lysosomes
   c. Endoplasmic reticulum d. Centrosome
3. **The chromatin are classified on genetic bases as:**
   a. Euchromatin and heterochromatin
   b. Neochramatin and paleochromatin
   c. Heteropyknotic and hyopyknotic
   d. Somatochromatin and cytochromatin
4. **The enzyme which activates apoptosis is:**
   a. Caspases b. Ribonucleases
   c. Carboxylase d. Transaminase
5. **Potassium ion channels are:**
   a. Dimmers b. Monomers
   c. Tetramers d. None of the above
6. **The osmolarity of normal human plasma is:**
   a. 490 mOsm/L b. 190 mOsm/L
   c. 390 mOsm/L d. 290 mOsm/L
7. **Match the following:**

a. Rhodopsin	1. Secondary hyperparathyroidism
b. Nicotinic acid receptor	2. Colour blindness
c. Ca^{2+} receptor	3. Retinitis pigmentosa
d. Cone opsin	4. Myasthenia gravis

8. **In Gibbs done non-membrane equilibrium:**
   a. Diffusible anion (Cl^-) concentration is lesser on side without the non-diffusible anion
   b. Diffusible anion (Cl^-) concentration is equal on either side
   c. Diffusible anion (Cl^-) concentration is greater on side without the non-diffusible anion
   d. None of the above
9. **Electrotonic potentials are:**
   a. Catelectrotonic potential
   b. Anelectrotonic potential
   c. Neutral potential
   d. Both catelectrotonic potential and anelectrotonic potential
10. **Resting membrane potential in pacemaker average around:**
    a. – 90 mV b. – 35 mV
    c. – 55 mV d. – 70 mV
11. **The total amount of plasma in human body is:**
    a. 5% of body weight b. 10% of body weight
    c. 15% of body weight d. 20% of body weight
12. **Transcellular water amounts to ..... % of extracellular fluid.**
    a. 8% b. 4%
    c. 2.5% d. 12%
13. **Interstitial fluid and lymph amounts to ..... % of extracellular fluid.**
    a. 7.5% b. 20%
    c. 30% d. 40%
14. **The rate of low of lymph along the thoracic duct is:**
    a. 10–15 ml per minute
    b. 1.0–1.5 ml per minute
    c. 5 –10 ml per minute
    d. 50–60 ml per minute
15. **The pressure of the lymph tissue and thoracic duct is:**
    a. Tissue (8 to 10 mm Hg) and thoracic duct (0 to 4 mm Hg)
    b. Tissue (20 to 30 mm Hg) and thoracic duct (6 to 8 mm Hg)
    c. Tissue (10 to 15 mm Hg) and thoracic duct (8 to 10 mm Hg)
    d. None of the above
16. **Microglia are found in:**
    a. Liver
    b. Bone marrow
    c. Spleen
    d. Central nervous system

ANSWERS

1. (a) 2. (b) 3. (a) 4. (a) 5. (c) 6. (d) 7. (a-3, b-4, c-1, d-2) 8. (c) 9. (d)
10. (c) 11. (a) 12. (c) 13. (b) 14. (b) 15. (a) 16. (d)

BLOOD

1. **The specific gravity of whole blood varies from:**
   a. 1.025 to 1.030 b. 1.005 to 1.010
   c. 1.055 to 1.060 d. 1.015 to 1.020
2. **The approximate percentage of total amount of protein in human blood is:**
   a. 15.5% b. 10.5%
   c. 7.5% d. 20.5%
3. **The albumin–globulin ratio ordinarily is:**
   a. 2.5 : 0.5
   b. 1.5 : 1
   c. 4 : 3
   d. 5: 2.5
4. **The total amount of serum albumin is:**
   a. 4.7 to 5.7% b. 1.3 to 2.5%
   c. 0.2 to 0.4% d. 0.1 to 0.2%

5. **The substance prepared from leucocytes, which are necessary for tissue cell growth in culture, are:**
   a. Globulin b. Albumin
   c. Seromucoid d. Trephines
6. **The pH of blood varies between:**
   a. 7.36 and 7.45 b. 7.26 and 7.36
   c. 7.45 and 7.60 d. 7.15 and 7.25
7. **The normal clotting time in siliconised tube is:**
   a. 9 to 15 minutes b. 5 to 10 minutes
   c. 15 to 20 minutes d. 19 to 60 minutes
8. **An approximate prothrombin time is:**
   a. 5 to 10 seconds b. 11 to 16 seconds
   c. 1 to 2 seconds d. 2 to 6 seconds
9. **Vitamin K deficiency leads to:**
   a. Decrease synthesis of factor Xl and factor XII
   b. Decrease synthesis of factor I and factor II
   c. Decrease synthesis of factor VII and prothrombin
   d. None of the above
10. **The ratio of WBC : RBC is**
    a. 1 : 17 b. 1 : 70
    c. 1: 700 d. 1 : 7000
11. **The mean corpuscular thickness is:**
    a. 0.5 to 1 µm b. 2.1 to 2.2 µm
    c. 3 to 4 µm d. 4 to 5 µm
12. **The normal range of saturation index is:**
    a. 0.1 to 0 3 b. 0.2 to 0.5
    c. 0.7 to 0.8 d. 0.9 to 1.1
13. **In platelet serotonin is secreted from:**
    a. Alpha granules b. Syndesmosomes
    c. Very dense granules d. Glycogen granules
14. **The paternity test for confirmation which is prominently used in medicolegal cases are:**
    a. M and N factors b. ABO blood group
    c. Rh blood group system d. None of the above
15. **Routine blood storage is ..... for stored packed red blood cells.**
    a. 42 days b. 4 days
    c. 20 days d. 2 days
16. **Enormous exposure to X-ray or γ-ray can lead to:**
    a. Iron deficiency anaemia
    b. Aplastic anaemia
    c. Megaloblastic anaemia
    d. Thalassaemia
17. **The osmotic fragility of red blood cells in healthy individual:**
    a. Starts at 0.48%, ends at 0.33%
    b. Starts at 0.65%, ends at 0.48%
    c. Starts at 0.35%, ends at 0.20%
    d. Starts at 0.10%, ends at 0.15%
18. **Microcytic hypochromic anaemia is seen in:**
    a. Iron deficiency anaemia
    b. Pernicious anaemia
    c. Megaloblastic anaemia
    d. All of the above
19. **The deficiency of factors V, VII and leads to:**
    a. Haemophilia B b. Haemophilia C
    c. Haemophilia A d. Pseudohaemophilia
20. **Erythrocyte in newborn is:**
    a. 5–6 mm per hour b. 3–4 mm per hour
    c. 0–2.0 mm per hour d. 2–3 mm per hour

ANSWERS

1. (c)	2. (c)	3. (b)	4. (a)	5. (d)	6. (a)	7. (d)	8. (b)	9. (c)	10. (c)
11. (a)	12. (d)	13. (c)	14. (a)	15. (a)	16. (b)	17. (a)	18. (a)	19. (d)	20. (c)

NERVE AND MUSCLE

1. **The water content of skeletal muscle is:**
   a. 20% b. 40%
   c. 60% d. 75%
2. **The muscle haemoglobin is:**
   a. Oxyhaemoglobin
   b. Carboxyhaemoglobin
   c. Myohaemoglobin
   d. Sulphur-methyl-haemoglobin
3. **The lactic acid percentage in resting muscle is:**
   a. 0.02%
   b. 0.08%
   c. 0.10%
   d. 0.2%
4. **The striation in smooth muscles is:**
   a. Longitudinal and transverse
   b. Transverse
   c. Longitudinal
   d. All of the above
5. **The T system is pre-eminent and present at Z-line in:**
   a. Skeletal muscle b. Smooth muscle
   c. Cardiac muscle d. All of the above
6. **The whole contraction period is absolute refractory in:**
   a. Smooth muscle b. Skeletal muscle
   c. Cardiac muscle d. All of the above
7. **The maximum protein is present in the muscle type:**
   a. Skeletal b. Cardiac
   c. Smooth d. Mixed type
8. **Tetanus is not possible in cardiac muscle due to:**
   a. Long refractory period
   b. Prolong relaxation phase
   c. Short refractory period
   d. All of the above
9. **The muscle having highest blood supply is:**
   a. Skeletal muscle b. Cardiac muscle
   c. Smooth muscle d. All of the above

10. **The minimum galvanic current when allowed to flow indefinitely will exit a tissue is:**
    a. Chronaxie b. Barthomocity
    c. Rheobase d. Excitability
11. **The maximum conduction velocity in unmyelinated:**
    a. 10 mt/sec b. 5 mt/sec
    c. 1 mt/sec d. 4 mt/sec
12. **As per Sunderland grading of injury interruption of endoneurial injury is:**
    a. Second degree injury b. First degree injury
    c. Fourth degree injury d. Third degree injury
13. **The competitive blockers are:**
    a. Tubocurarine and gallamine
    b. Suxamethonium
    c. Dexamethonium
    d. Salbutamol
14. **The disease associated with antibodies against calcium channel in nerve ending of neuro- muscular junction manifests as syndrome is:**
    a. Lambert-Eaton syndrome
    b. Myasthenia gravis
    c. Auto-immune thyroditis
    d. Systemic lupus erythematosus
15. **Red muscle fibres are resistant to fatigue as:**
    a. They are large in size
    b. They contain numerous nucleoli
    c. They are highly vascular and have abundant mitochondrion
    d. They contain endoplasmic reticulum
16. **In isotonic muscle contraction the amount of energy expanded in doing work is:**
    a. Approximately 20%
    b. Approximately 10%
    c. Approximately 25%
    d. Approximately 50%
17. **Voltage-gated channels are highly concentrated up to 2000–12000% at:**
    a. Node of Ranvier b. Initial segment of axon
    c. Dendrites d. Soma
18. **The nerve growth factor involved in maintenance of cutaneous mechanoreceptors is:**
    a. Nerve growth factor
    b. Neurotrophins 3, 4
    c. Brain derived neurotrophic factor (BDNF)
    d. None of the above
19. **When axon gets damage or cut the part distal to it degenerates and the process in known as:**
    a. Wallerian degeneration
    b. Whipple's degeneration
    c. Watson's degeneration
    d. White and Lee degeneration
20. **Sliding filament theory was proposed by:**
    a. AF Huxley and HE Huxley
    b. Watson and Bricks
    c. Robertson Hooke
    d. Sunderland
21. **Goldman-Hodgkin-Katz equation states that the membrane potential will be mainly influenced by:**
    a. Equilibrium potential for ion to which membrane is least permeable
    b. Equilibrium potential for ion to which membrane is most permeable
    c. Equilibrium potential for all ions to which they are partially permeable
    d. All of the above
22. **The duration of action potential in cardiac muscle is:**
    a. 20–30 millisecond b. 1–2 millisecond
    c. 200–300 millisecond d. 400–500 millisecond
23. **Resting membrane potential in neurons is:**
    a. – 70 mV b. – 90 mV
    c. +35 mV d. + 10 mV
24. **Absolute refractory period is:**
    a. Period from the firing level is reached the time repolarization is 1/3rd complete
    b. Period from the firing level is reached till time repolarization is half complete
    c. Period from the firing level is reached till time repolarization is 2/3rd complete
    d. None of the above

ANSWERS

1. (d)	2. (c)	3. (a)	4. (c)	5. (c)	6. (c)	7. (a)	8. (a)	9. (b)	10. (c)
11. (c)	12. (d)	13. (a)	14. (a)	15. (c)	16. (c)	17. (d)	18. (c)	19. (a)	20. (a)
21. (b)	22. (c)	23. (a)	24. (a)						

CARDIOVASCULAR SYSTEM

1. **The time required for one cardiac cycle is:**
    a. 0.12 sec b. 0.35 sec
    c. 0.5 sec d. 0.8 sec
2. **The total ventricular filling time is:**
    a. 0.38 sec b. 0.25 sec
    c. 0.32 sec d. 0.22 sec
3. **First heart sound is due to:**
    a. Sudden closure of the AV valve
    b. Sudden closure of semi-lunar valve
    c. Contraction of atria
    d. Sudden rush of arterial blood into ventricle
4. **The duration of second heart sound is:**
    a. 0.08–0.10 sec
    b. 0.05–0.10 sec
    c. 0.1–0.14 sec
    d. 0.01–0.06 sec

Multiple Choice Questions

5. **The slow repolarisation of the intra-ventricular septum produces:**
   a. P wave b. U wave
   c. Q wave d. S wave
6. **The conduction time from SA node to ventricle is denoted by:**
   a. PR interval b. TP interval
   c. QT interval d. PP interval
7. **The average cardiac index is:**
   a. 5.2 litres/min/m^2 b. 4.8 litres/min/m^2
   c. 3.3 litres/min/m^2 d. 2.6 litres/min/m^2
8. **The increase of pulse during inspiration and fall during expiration occurs in:**
   a. Pulsus paradoxus b. Pulsus alternans
   c. Sinus arrhythmia d. Pulsus bisferiens
9. **The total oxygen consumption of brain is:**
   a. 200 ml/min/100 gm b. 300 ml/min/100 gm
   c. 150 ml/min/100 gm d. 50 ml/min/100 gm
10. **The capillary pressure in pulmonary artery is:**
    a. 60 mm Hg b. 25 mm Hg
    c. 100 mm Hg d. 80 mm Hg
11. **The average blood flow through myocardium is:**
    a. 40 ml/100 gm/min b. 80 ml/100 gm/min
    c. 220 ml/100 gm/min d. 250 ml/100 gm/min
12. **Capacitance vessels are:**
    a. Arteries b. Metarterioles
    c. Veins d. Capillaries
13. **Total ventricular systolic time is denoted by:**
    a. PP interval b. RR interval
    c. QT interval d. PR interval
14. **The peripheral resistance indicate:**
    a. Diastolic blood pressure
    b. Systolic blood pressure
    c. Pulse pressure
    d. None of the above
15. **The resting blood flow of muscle which is 7–9/ml/100 gm tissue is increased to during exercise:**
    a. About 20 ml/100 gm tissue
    b. About 50 ml/100 gm tissue
    c. About 60 ml/100 gm tissue
    d. More than 100 ml/100 gm tissue
16. **Respiratory quotient during moderate exercise more or less same as resting state is:**
    a. 0.69 to 0.79 b. 0.85 to 0.89
    c. 0.55 to 0.65 d. 0.25 to 0.35
17. **During severs exercise the lactate content of blood goes up to (resting state is 10–20 mg/%):**
    a. 20.40 mg% b. 40 to 50 mg%
    c. 50 to 70 mg% d. 100–200 mg%
18. **Average venous pressure of human being in recumbent position is about:**
    a. 0–5 cm H_2O b. 5–20 cm H_2O
    c. 60–120 cm H_2O d. 180–350 cm H_2O
19. **Rate of conduction (metre) in SA node is:**
    a. 0.05 per sec b. 0.1 per sec
    c. 1.0 per sec d. 0.4 per sec
20. **The normal direction of mean cardiac vector ranges from:**
    a. –60 to –30 degree b. 60 to 120 degree
    c. 110 to 18 degree d. –30 to 110 degree
21. **Baroreceptor regulate blood pressure in pressure range of:**
    a. 60 to 200 mm Hg b. 50 to 200 mm Hg
    c. 80 to 120 mm Hg d. 60 to 100 mm Hg
22. **Increase distension of ventricle due to excess ventricular filling leads to:**
    a. Tachycardias, vasodilatation, hypertension
    b. Bradycardia and vasoconstriction
    c. Bradycardia, vasodilatation and hypotension
    d. All of the above
23. **Endothelium derive relaxing factor produces:**
    a. Contraction of vascular smooth muscle and reduces blood pressure
    b. Relaxation of vascular smooth muscle and increases blood pressure
    c. Relaxation of vascular smooth muscle and decreased blood pressure
    d. It has no effect on smooth on used
24. **High output failure occurs in all conditions, *except*:**
    a. Beriberi b. Paget's disease
    c. Tuberculosis d. Arteriovenous fistula
25. **Endotoxin released by bacteria producing venodilatation may lead to:**
    a. Neurogenic shock b. Septic shock
    c. Obstructive shock d. Endotoxic shock

ANSWERS

1. (d)	2. (a)	3. (a)	4. (c)	5. (b)	6. (a)	7. (c)	8. (c)	9. (d)	10. (b)
11. (b)	12. (c)	13. (c)	14. (a)	15. (d)	16. (b)	17. (d)	18. (c)	19. (a)	20. (d)
21. (a)	22. (c)	23. (c)	24. (c)	25. (d)					

RESPIRATORY SYSTEM

1. **The normal intra-pleural pressure is:**
   a. –2 mm Hg b. –1 mm Hg
   c. –0.5 mm Hg d. –4 mm Hg
2. **The average residual volume in lung is:**
   a. 1.2 litres b. 1.8 liters
   c. 2.2 liters d. 3.2 liters
3. **The respiratory minute volume of lung is:**
   a. 2 litres/minute b. 3 litters/minute
   c. 4 litres/minute d. 6 litres/minute
4. **The normal tidal volume of lung is:**
   a. 1000 ml b. 700 ml
   c. 500 ml d. 300 ml

5. **The accessory muscle of respiration is:**
   a. Scalene b. Sternocleidomastoid
   c. Platysma d. All of the above
6. **The oxygen volume % in atmospheric air:**
   a. 20.94 b. 14
   c. 10 d. 12
7. **The pulmonary diffusion capacity of oxygen at rest is:**
   a. 10 to 15 ml/min/mm Hg
   b. 15 to 20 ml/min/mm Hg
   c. 0 to 5 ml/min/mm Hg
   d. 20 to 25 ml/min/mm Hg
8. **The oxygen content in ml/dl in mixed venous blood is:**
   a. 10.8 b. 19.3
   c. 14.2 d. 18.3
9. **1 gm of haemoglobin when fully saturated combines with ml of oxygen:**
   a. 2.65 ml b. 3.55 ml
   c. 4.22 ml d. 1.34 ml
10. **More than 80% of CO_2 is carried as:**
    a. In solution
    b. As carbaminocompound
    c. Bicarbonate
    d. Sulphate
11. **Central pattern generator:**
    a. Pre-Botzinger complex
    b. Nucleus of tractus solitarius
    c. Nucleus ambiguus
    d. Retrofacialis
12. **The chemosensitive type I cell in carotid body is:**
    a. Sustentacular cells b. Glomus cells
    c. Reticular cells d. Vestibular cells
13. **Hering-Breuer reflex inflation protects lung from:**
    a. Circulating toxins
    b. Circulating phagocytes
    c. Overinflation
    d. Under ventilation
14. **Diphtherial toxins inhibit synthesis of cytochrome and may lead:**
    a. Anaemic hypoxia b. Stagnant hypoxia
    c. Hypoxic hypoxia d. Histotoxic hypoxia
15. **The oxygen solubility in plasma is:**
    a. 0.6 ml/100 ml/mm Hg
    b. 2 ml/100 ml/mm Hg
    c. 0.03 ml/100 ml/mm Hg
    d. 3.2 ml/100 ml/mm Hg
16. **The conditions that increase pulmonary diffusing capacity are:**
    a. Exercise b. Emphysema
    c. Collagen disease d. Interstitial oedema
17. **The normal pulmonary vascular resistance is:**
    a. 50–150 dynes/seconds/cm^{-5}
    b. 150–200 dynes/seconds/cm^{-5}
    c. 200–250 dynes/seconds/cm^{-5}
    d. 0–50 dynes/seconds/cm^{-5}
18. **The type of respiration seen in diabetic ketoacidosis is:**
    a. Cheyne-Stroke breathing
    b. Biot's respiration
    c. Hysteric breathing
    d. Kussmaul breathing
19. **Monges disease is:**
    a. Acute bronchitis
    b. Chronic bronchitis
    c. Emphysema
    d. Chronic mountain sickness
20. **The hormone stimulating surfactant secretions are:**
    a. Insulin b. Glucocorticoids
    c. Thyroxine d. All of the above

ANSWERS

1. (a)	2. (a)	3. (d)	4. (c)	5. (d)	6. (a)	7. (b)	8. (c)	9. (d)	10. (c)
11. (a)	12. (b)	13. (c)	14. (d)	15. (c)	16. (a)	17. (a)	18. (d)	19. (d)	20. (b)

GASTRO-INTESTINAL TRACT

1. **The total amount of saliva produced in 24-hour is:**
   a. 200–400 ml
   b. 1200–1500 ml
   c. 500–1000 ml
   d. 100–200 ml
2. **Saliva excretes:**
   a. Thiocyanates b. Iodide
   c. Penicillin d. All of the above
3. **pH of gastric juice is:**
   a. 0.1–1.5 b. 2–4
   c. 4–5 d. 5–6
4. **Total quantity of juices produced by succus entericus in 24 hours is:**
   a. 2–3 litres b. 1–2 litres
   c. 3–4 lites d. 5 liters
5. **The pigments formed by oxidation of biliverdin is, *except*:**
   a. Bilicyanin b. Bilifuscin
   c. Biliprasin d. Choletelin
6. **Lipase which is tributyrase and acts on butter tributyrin:**
   a. Intestinal lipase b. Salivary lipase
   c. Gastric lipase d. Pancreatic lipase
7. **The movements especially occur in stomach are:**
   a. Peristaltic waves
   b. Systolic contraction of the terminal nature
   c. Diminution in size of fundus and body
   d. All of the above
8. **Ludwig pendulum movement is seen in:**
   a. Oesophagus b. Stomach
   c. Small intestine d. Ilium

9. The movements of large intestine include all, *except*:
a. Haustral contraction
b. Segmenting contraction
c. Kneading movements
d. Rhythmic segmentation

10. The following facts are true about trypsin:
a. Trypsin is protein in nature
b. Trypsin is secreted as inactive trypsinogen
c. It acts on native protein and products of protein digestion
d. All of the above

11. The water content of faeces averages around:
a. 35% b. 25%
c. 65% d. 40%

12. Hyper-salivation is seen in all, except:
a. Aptyalism
b. Neoplasm of mouth, stomach, etc.
c. Pregnancy
d. Ulceration of oesophagus and stomach

13. The drugs which stimulates the gall bladder are all, *except*:
a. Adrenaline b. Atropine
c. Histamine d. Pitressin

14. The hormone which stimulates the movement villi is:
a. Bradykinin b. Villikinin
c. Gastrin d. Ptyalin

15. The proton pump inhibitor used in treatment of peptic ulcer is:
a. Omeprazole b. Cimetidine
c. Sucralfate d. Ranitidine

16. The following are true about, *except*:
a. It contains 31 amino acid
b. It is also produced by neurons
c. It inhibits GI motility
d. It decreases ileal blood flow

17. The strong orexigenic agent which increases food intake is:
a. Ghrelin b. Gianylin
c. Motilin d. Peptide

18. Tumour of VIP secreting cell is known as:
a. Gastrinoma b. VIPoma
c. Hepatoma d. None of the above

19. The drug which binds to motilin receptor facilitates intestinal motility is:
a. Penicillin b. Cephalexin
c. Sulphonamides d. Erythromycin

20. The enzyme that damages pancreatic tissue and leads to necrotic sign of surrounding fat in acute pancreatitis is:
a. Carboxylase b. Bradykinin
c. Lysolecithin d. Villikinin

ANSWERS

1. (b)	2. (d)	3. (a)	4. (b)	5. (c)	6. (c)	7. (d)	8. (c)	9. (d)	10. (d)
11. (c)	12. (a)	13. (d)	14. (b)	15. (a)	16. (d)	17. (a)	18. (b)	19. (d)	20. (c)

RENAL SYSTEM

For each of the following numbered items in the left-hand column, choose the most correct lettered item from the right-hand column that best applies:

1. Juxtaglomerular cells
a. Afferent vessels in the corticomedullary joining directly the peritubular capillaries without forming glomerular tuft

2. Renal columns of Bertin
b. Branched satellate cells between the endothelium and basal lamina of the glomerulus having proliferative and phagocytic properties

3. Mesangial cells
c. Modified epithelial cells of the distal convoluted tubules lying in contact with the afferent glomerular vessels of the same nephron

4. Macula densa
d. Agranular cell mass lying within the vascular pole formed by the afferent and efferent golmerular vessels

5. 'Lacis' cells
e. Projections of renal cortex in between the two pyramids forming its lateral boundaries

6. Ludwig shunt
f. Marked reduction of urine volume

7. Trueta shunt
g. Thick cuff of modified muscle cells in the media of afferent arteriole just before it enters the glomerulus

8. Anuria
h. Efferent vessels of juxtamedulllary glomeruli draining directly into the venous plexus without joining the peritubular network

9. Oliguria
i. Complete suppression of urine volume

10. In resting state flowing blood through a kidney per minute is approximately:
a. 200–300 ml per min
b. 400–500 ml per min
c. 12000–13000 ml per min
d. 5000–6000 ml per min
e. None of these

11. Glomerular filtration pressure is:
a. 15 mm of Hg b. 25 mm of Hg
c. 35 mm of Hg d. 65 mm of Hg
e. 100 mm of Hg

12. The normal glomerular filtration rate is approximately:
a. 20 ml per min b. 50 ml per min
c. 120–125 ml per min d. 300 ml per min
e. None of these

13. Over 99% of the glomerular filtrate are reabsorbed and are approximately:
a. 184 litres b. 90 litres
c. 25 litres d. 2 litres
e. None of these

14. Tuft of capillary vessels which is surrounded by the Bowman's capsule is known as:
a. Glomerulus
b. Malpighian corpuscle
c. Loop of Henle
d. Juxtaglomerular apparatus
e. None of these

15. Area cribrosa is the apex of the renal papilla. It opens directly into the:
a. Major calyx b. Minor calyx
c. Bowman's capsule d. Ureter
e. Urinary bladder

16. Human renal tubule is:
a. 100 mm in length b. 60 mm in length
c. 30 mm in length d. 15 mm in length
e. None of these

17. Blood flow per gram of kidney tissue per minute is:
a. 0.05–1 ml b. 3–4 ml
c. 10–12 ml d. 20–25 ml
e. None of these

18. Intrarenal sodium-feedback theory is related with:
a. Autoregulation of renal blood flow
b. Regulation of acid–base balance by the kidney
c. Mechanism of micturition
d. All of these
e. None of these

19. Release of rennin probably depends on:
a. Glomerular filtration rate (GFR)
b. Sympathetic nerve activity
c. Parasympathetic nerve activity
d. Both (a) and (b)
e. Both (a) and (c)

20. Glucose reabsorption takes place in the:
a. Bowman's capsule
b. First-half of the proximal tubule
c. Distal tubule
d. Loop of Henle
e. None of these

21. The interior of urinary bladder is lined by:
a. Columnar epithelium b. Squamous epithelium
c. Transitional epithelium d. Goblet cells
e. None of these

22. Normal desire for micturition is felt when the amount of urine collected in the bladder is:
a. 25–50 ml b. 50–100 ml
c. 100–200 ml d. 300–400 ml
e. 1000–1500 ml

23. The antidiuretic hormone (ADH) is secreted by:
a. Renal cortex b. Renal medulla
c. Anterior pituitary d. Posterior pituitary
e. None of these

24. Administration of the antidiuretic hormone (ADH):
a. Decreases water loss by the lungs
b. Increases perspiration
c. Decreases the rate of the active reabsorption of water by the kidney
d. Increases active reabsorption of water by the distal tubule of the kidney
e. None of these

25. A substance generally used to measure the glomerular filtration rate is:
a. Inulin
b. Insulin
c. Diodrast
d. Para-aminohippuric (PAH) acid
e. None of these

26. A substance generally used for measuring renal plasma flow:
a. Ethyl alcohol
b. Insulin
c. Para-aminohippuric acid
d. Glucose
e. Diodrast

From the following directions given below, select answers for questions 27–30:
a. If (i) is greater than (ii)
b. If (ii) is greater than (i)
c. If (i) and (ii) are equal or very nearly equal

27. i. Blood flow rate in the cortex of the kidney
ii. Blood flow rate in the medulla of the kidney

28. i. Size of the right kidney
ii. Size of the left kidney

29. i. Total number of superfrcial nephrons
ii. Total number of juxtamedullary nephrons

30. i. Length of afferent vessels of glomerulus
ii. Length of efferent vessels of glomerulus

From the following directions given below, select answers for questions 31–37:
a. If statements (i), (ii) and (iii) are all correct
b. If statements (i) and (iii) are correct
c. If statements (ii) and (iv) are correct
d. If the statement (iv) only is correct

31. i. Renal artery divides into anterior and posterior divisions
ii. Branches of the anterior division anastomose with one other
iii. Branches from the posterior division also anastomose with one other
iv. Arcuate arteries which are branches from the interlobar arteries freely communicate with one other

32. i. In the kidney, the blood usually passes through double capillary networks—the glomerular tuft and the peritubular tuft
ii. The function of glomerular tuft is to reabsorb and the peritubular tuft is to filter
iii. Glomerular blood pressure is 3–4 times more than the capillary kidney
iv. Under basal condition, renal blood flow is altered in denervated kidney

33. i. Administration of high dose of epinephrine increases blood pressure but profoundly decreases the renal blood flow
ii. Exercise in recumbent position profoundly decrases renal blood flow

iii. Exercise in upright position produces renal vasoconstriction and decreases in renal blood flow
iv. Hypercapnia and acidosis increase renal blood flow

34. i. Absorption of glucose by the renal tube is an active process
ii. The reabsorption rate of glucose depends on the availability of the carrier substance present in the tubular epithelium
iii. Phloridzin binds strongly to the carrier substance and prevents combination of glucose with the carrier substance
iv. Insulin inhibits glucose reabsorption

35. i. In the proximal tubule water and chloride reabsorptions are active process
ii. Water reabsorption from the proximal tubule is known as facultative water reabsorption
iii. Disal tubular water reabsorption is a passive process and is known as obligatory water reabsorption
iv. There is a maximum limit beyond which the urine cannot be concentrated

36. A nephrone is the functional unit of the kidney:
a. There are about 1000 nephrons in each human kidney
b. In man, it consists of Bowman's capsule and renal ubules
c. These two parts of the nephron have same function
d. In certain fishes the nephron possesses only the tubule

37. In clearance tests both blood and urine are analysed:
a. Urea clearance test is quantity of urea that is cleared from plasma
b. Urea clearance test is the volume of plasma completely cleared of urea
c. In renal diseases urea clearance value is increased
d. In normal human adults maximum urea clearance averages 70 + 15 ml/minute

From the following directions, select answers for questions 38–45:

a. If the statement is correct and the reason is also correct
b. If the statement is correct but the reason is wrong
c. If the statement is wrong but the reason is correct
d. If the statement is wrong and the reason is also wrong

38. The glomerular blood pressure is much higher than the capillary bed elsewhere, because the afferent vessels are long and narrow, whereas the efferent vessels are short and wide.

39. Major part of the renal circulation normally passes through the juxtamedullar glomeruli, because the afferent vessels of juxtamedullary glomeruli arise at an angle most suitable for free flow of blood stream.

40. Ischaemic kidney produces hypertension, because such kidney liberates a substance—renin.

41. Glucose is not reabsorbed through phosphorylation, because dinitrophenol which uncouples oxidation and phosphorylation does not block reabsorption of glucose.

42. After high protein diet urine volume is reduced, because urea, sulphate and phosphate resulting from protein metabolism retain water in the tubules to keep those materials in solution.

43. In fever, diabetes or in excessive adrenocortical activity urea excretion in the urine is increased, because in the above conditions there is increased protein catabolism.

44. In nephritis, excretion of urea is increased, because ability of the kidney to excrete urea is severely impaired in nephritis.

45. Hypogastric nerves (sympathetic) are called the nerves of emptying, because sympathetic stimulation causes strong contraction of the bladder and relaxation of internal sphincter.

For each of the following numbered items in the left-hand column, choose the most correct lettered items from the right-hand column that best applies:

46. Homoiotherms
a. Behaves like warm-blooded animals except in winter

47. Poikilotherms
b. Deliberate reduction of body temperature.

48. Hibernants
c. Who can maintain their body temperature constant in the face of wide variations of the environmental temperature.

49. Core temperature
d. Body temperature between 99°F and 105°F and onwards

50. Shell temperature
e. Temperature of intra-abdominal, intrathoracic and intracranial contents

51. Pyrexia
f. Temperature of limbs and surface layer of the trunk

52. Hypothermia
g. Whose temperatures fluctuates with the fluctuation of the environmental temperature

53. Among the organs largest amount of heat is produced by:
a. Brain
b. Liver
c. Intestine
d. Heart
e. None of these

54. Physiological response due to pyrexia is:
a. Decreased metabolism.
b. Fall of blood pressure, pulse rate and cardiac output.
c. Positive nitrogen balance.
d. Rise of plasma chloride level.
e. None of these.

55. Among the body tissues largest amount of heat is produced by:
a. Subcutaneous tissues
b. Adipose tissues
c. Skeletal muscle
d. Blood
e. None of these

56. After a prolonged hot bath:
a. Temperature falls rapidly below normal
b. No effect on body temperature is observed
c. Body temperature remains elevated for a considerable time
d. Vasoconstriction occurs
e. None of these

From the following directions given below, select answers for questions 57–60:

a. If statements (i), (ii), (iii) are all correct
b. If statements (i) and (iii) are correct
c. If statements (ii) and (iv) are correct
d. If statements (iv) only is correct
e. If all the statement are correct

57. Nervous system controls thermotaxis:
i. Heat-regulating centre lies in the medulla
ii. Greater part of the generalized thermal response in the visceral effectors is due to sympathetic control
iii. Cooling of the carotid artery blood causes sweating, cutaneous vasodilation and increased respiration
iv. Shivering centre is situated in the posterior part of the hypothalamus

58. Many factors affect the body temperature:
i. Protein foods raise body temperature
ii. Body temperature is above normal in old age
iii. 25% of muscular energy is converted into mechanical work and the rest comes out as heat
iv. The colour of human skin has profound effect upon the degree of heat loss by radiation

59. These are four principal mechanisms for physical heat regulation:
i. 55% of body heat is lost by radiation
ii. 20% of body heat is lost by conduction and convection
iii. 25% of heat is lost by evaporation
iv. 15% of heat is lost for warming the inspired air

60. For practical advantage, mouth temperature is taken as clinical purposes:
i. Mouth temperature at 5 pm is higher than at 5 am in day workers.
ii. Mouth temperature is higher than rectal temperature.
iii. Axillary temperature is 1°F. less than mouth temperature.
iv. Emotional disturbance has no influence on body temperature.

From the following directions given below, select answers for questions 61–66:

a. If the statement is correct and the reason is also correct
b. If the statement is correct but the reason is wrong
c. If the statement is wrong but the reason is correct

61. Woollen clothes decrease heat loss through conduction, because woolen clothes are bad conductors of heat.

62. High but dry atmospheric temperature is better tolerated by a person than high humid temperature, because evaporation decreases to a great extent if the humidity of the atmosphere is high.

63. Stimulation of the anterior part of the hypothalamus helps in heat production, because the anterior part of hypothalamus contains the centre which governs the heat production.

64. Lesions of the posterior part of the hypothalamus lead to subnormal body temperature, because response to reduced temperature is controlled by the anterior part of the hypothalamus.

65. On entering a warm room after having been in the cold, a person may start shivering because of inexplicable phenomenon.

66. In a woman body temperature gradually rises and becomes maximum during menstruation because of high progesterone level of blood during menstruation.

For each of the following numbered items on the left-hand column, choose the most correct lettered items from the right-hand column that best applies:

67. Bicarbonate buffers
a. Sodium bicarbonate concentration of blood not combined with non-volatile acid (NVA).

68. Phosphate buffers
b. Pathochemical condition resulting from primary loss of base

69. Alkali reserve
c. Directly linked up with respiration

70. Acidosis
d. Work in co-operation with the kidneys

71. Alkalosis
e. Retention of large amount of fluid in the body

72. Hyperchloraemic acidosis
f. Pathochemical condition resulting from primary loss of acid

73. Dilution acidosis
g. Ureterosigmoidostomy

74. Metabolic acidosis may be brought about by:
a. Loss of CO_2 by increased ventilation
b. Retention of CO_2 brought about by respiratory obstruction
c. Pyloric stenosis
d. Methyl alcohol poisoning
e. None of these

75. Respiratory acidosis may be brought about by:
a. Emphysema b. Barbiturate overdose
c. Respiratory obstruction d. Chest injuries
e. All of these

76. Metabolic alkalosis may be brought about by:
a. Prolonged hypotension leading to poor tissue perfusion
b. High altitude
c. High intestinal obstruction
d. Diabetes mellitus
e. None of these

77. Respiratory alkalosis may be brought about by:
a. Diarrhoea
b. Cardiac arrest
c. Excess of alkali intake
d. Renal failure
e. Voluntary hyperventilation

78. Main buffers of blood are:
a. Potassium and sodium
b. Bicarbonates, plasma proteins, haemoglobin and phosphates
c. Bicarbonates only
d. γ-globulin, carbohydrates, fats
e. None of these

79. As blood passes through systemic capillaries:
a. Its pH rises
b. HCO_3 passes from the red cells to the plasma
c. Concentration of chloride ions in the red cells falls
d. Oxygen dissociation curve shifts to the left
e. K^+ ions come out of the cells

80. Buffer is a:
a. Mixture of weak acid and its salt with strong base
b. Mixture of a strong acid and its salt with strong base
c. Mixture of a strong acid and a weak acid
d. Mixture of a strong base and a weak base
e. None of these

81. Sodium bicarbonate/carbonic acid ratio in plasma is:
a. 20 : 1 b. 4 : 1
c. 50 : 1 d. 1 : 20
e. 1 : 100

82. Respiration acts as the chief process for maintaining the pH of the body fluid constant by:
a. Elimination of ammonia
b. Elimination of phosphates
c. Changing dissolved CO_2 content of plasma
d. Elimination of non-volatile acid (NVA)
e. None of these

83. In respiratory alkalosis:
a. pCO_2 falls below 35 mm of Hg
b. pCO_2 rises above 35 mm of Hg
c. pCO_2 remains the normal

84. Carbonic anhydrase is present in high concentration in:
a. WBC
b. Renal tubular cells
c. Plasma

85. In compensated metabolic acidosis:
a. pCO_2 rises above 35 mm of Hg.
b. pCO_2 falls below 35 mm of Hg.
c. pCO_2 remains the same normal.

86. pH of urine depends upon:
a. The ratio of sodium blcarbonate to carbonic acid
b. The ratio of sodium chloride to ammonium chloride
c. The ration of Na_2HPO_4 to NaH_2PO_4
d. All of these
e. None of these

From the following directions given below, select answers for questions 87–89:
a. If (i) is greater than (ii)
b. If (ii) is greater than (i)
c. If (i) and (ii) are equal or very nearly equal

87. i. pCO_2 in uncompensated metabolic acidosis
ii. pCO_2 in compensated metabolic acidosis

88. i. Buffering capacity of plasma proteins
ii. Buffering capacity of haemoglobin

89. i. pCO_2 in respiratory acidosis
ii. pCO_2 in normal individual

From the following directions given below, select answers for questions 90–92:
a. If the statement and the reason are correct
b. If the statement is correct but the reason is wrong
c. If the statement is wrong but the reason is correct
d. If both the statement and the reason are incorrect

90. Oxyhaemoglobin is more acidic than reduced haemoglobin, because 1 mMol of reduced haemoglobin can accept 0.7 mMol of hydrogen without change of blood reaction.

91. In metabolic acidosis the respiration will be depressed, because in metabolic acidosis the respiratory centre is depressed.

92. Methyl alcohol even in small amount may cause profound metabolic acidosis, because methyl alcohol is converted into acetic acid.

ANSWERS

1. (g)	2. (e)	3. (b)	4. (c)	5. (d)	6. (h)	7. (a)	8. (i)	9. (f)	10. (b)
11. (a)	12. (c)	13. (c)	14. (b)	15. (b)	16. (c)	17. (e)	18. (a)	19. (d)	20. (b)
21. (c)	22. (d)	23. (d)	24. (d)	25. (a)	26. (c)	27. (a)	28. (c)	29. (a)	30. (b)
31. (b)	32. (b)	33. (b)	34. (a)	35. (d)	36. (c)	37. (c)	38. (b)	39. (d)	40. (a)
41. (a)	42. (c)	43. (c)	44. (c)	45. (d)	46. (c)	47. (g)	48. (a)	49. (e)	50. (f)
51. (d)	52. (b)	53. (b)	54. (e)	55. (c)	56. (b)	57. (c)	58. (b)	59. (d)	60. (b)
61. (a)	62. (a)	63. (d)	64. (b)	65. (b)	66. (d)	67. (c)	68. (d)	69. (a)	70. (b)
71. (f)	72. (g)	73. (e)	74. (d)	75. (e)	76. (c)	77. (e)	78. (b)	79. (b)	80. (a)
81. (a)	82. (c)	83. (a)	84. (b)	85. (b)	86. (b)	87. (a)	88. (b)	89. (a)	90. (a)
91. (d)	92. (b)								

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