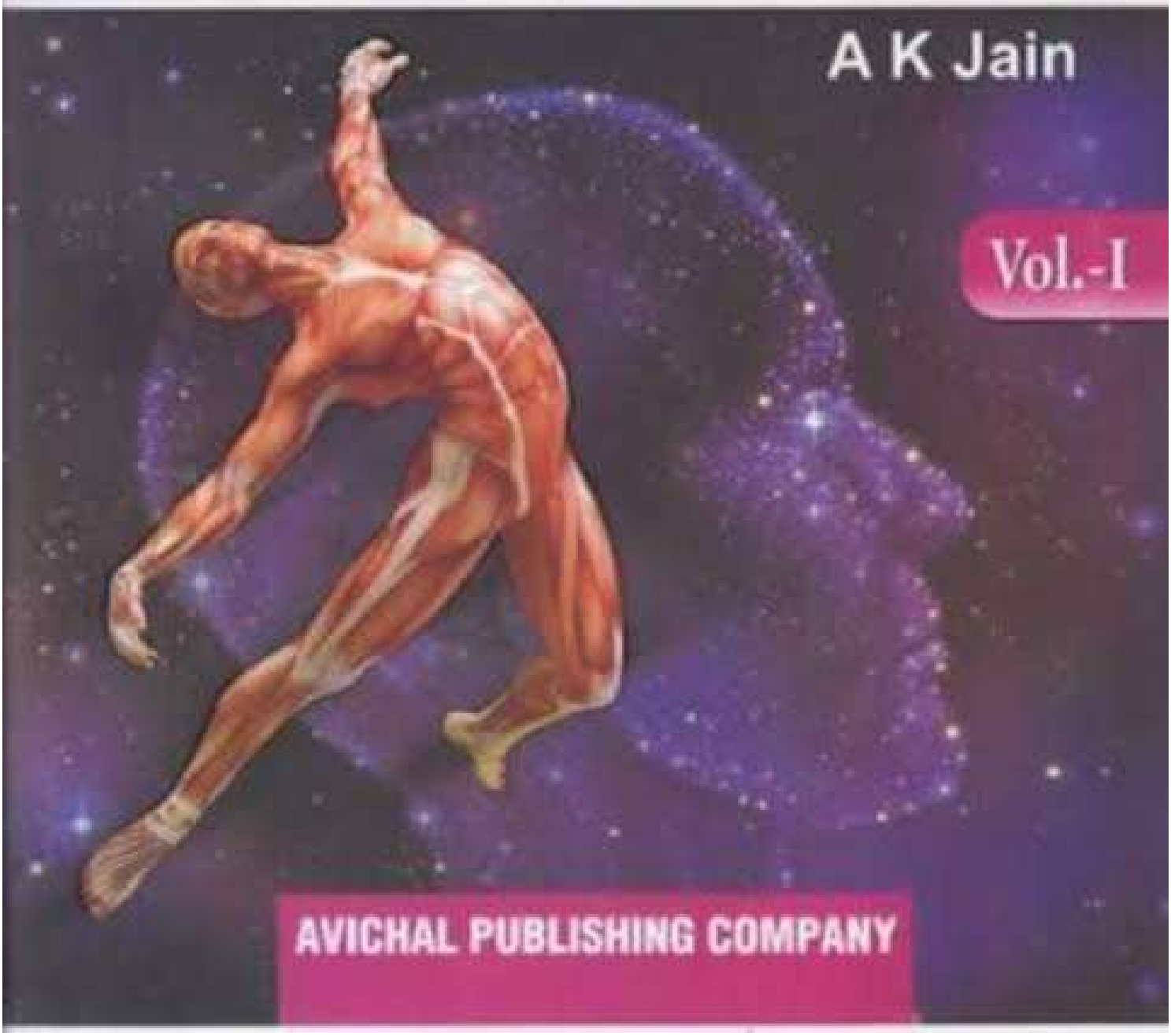
APC

SIXTH EDITION

TEXTBOOK OF PHYSIOLOGY

A K Jain

Vol.-I



AVICHAL PUBLISHING COMPANY

Preface to the First Edition

In my 20 years as a teacher, I have always relished teaching Physiology. It was because of my love for the subject and constant inspiration from my students that I could come up with Textbook of Physiology, which hopefully would lack the inadequacies the students face in other textbooks. In this age when the basic sciences are being vastly updated, this book attempts to summarize the current state of knowledge about the functional organization of the human body, taking care to make learning of the subject an interesting and enriching experience for the students.

Some of the salient features of the book are:

1. A rapid preview has been presented to help the readers to have a bird's eye view of the chapter's content.
2. Well labelled diagrams, flow charts and summarizing tables have been incorporated to make learning easier.
3. Highlighting of important terms has been done by using italics, bold letters or pin-pointing important notes.
4. New concepts and latest developments have been included.
5. The text has been so presented that the student would find it easy to attempt any questions in the form of objective type, multiple choice or essay type after going through the book.
6. Various systemic functions tests have been discussed in detail.
7. Applied aspects of clinically related topics have been discussed.
8. The book has been presented in two volumes for students convenience.

For whom is the book intended? The book is geared to students in health related professions like medicine, dentistry, nursing, occupational therapy, physiotherapy and medical technology. Because of its scope, the text is useful for students in biological sciences.

It is impossible task to come out with a balanced textbook of Physiology, the first time round. Still, I have tried my very best by putting forth my life time's experiences as a teacher into this book. I am aware that I may be having shortcomings in this first effort. Suggestions and new ideas for further improvement of this book shall always be welcomed and widely appreciated.

I could not have even conceived this book, had it not been for the help and encouragement put forth by the undergraduate and postgraduate students of Physiology. Special appreciation is hereby expressed with gratitude for the most assured cooperation provided by all the members of the Department of Physiology, Maulana Azad Medical College, New Delhi. I acknowledge with thanks the valuable time devoted by Dr (Mrs) Urvashi Gupta for correcting the vast manuscript. Dr S. Suresh, an intern, deserves special thanks for his timely valuable contributions.

I extend my heartfelt thanks and sincere regards to Shri V.K. Manchanda and Shri Rajiv Manchanda of M/s. Laser Tech Prints, New Delhi, for giving their valued views for better presentation of this book. I am grateful to Shri Sunil Dutt, Artist, who has given form to my ideas by presenting beautiful diagrams throughout the book.

I am immensely grateful for the support my family members have given me throughout my endeavour. Indebtness is acknowledged and appreciation is expressed to my wife, Smt. Shailesh Jain, nephew Manish and sons Ashish and Avnish, for providing all facilities and keeping me free from all day to day activities to enable me to complete this book. I owe a great deal of my achievements to my respected mother Shrimati Lajwanti Jain and Tauji Shri Man Singh. Without their blessings this book would not have seen the light of the day.

It will be unfair on my part if I fail to pay my gratitude to Dr Vipin Gupta, a unique unmatched personality, who constantly and repeatedly inspired me to start the work on this project.

Finally, I must thank my publishers, M/s. Avichal Publishing Company. Without their sincere efforts my dreams would not have materialized.

Dr A K Jain

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Unit IX : The Endocrine System

Unit X : The Reproductive System

Unit XI : The Nervous System

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Unit I

GENERAL PHYSIOLOGY

Chapter 1: The Structure and Function of a Cell

What is Physiology?

Physiological systems

Homeostatic regulation

Structure and function of a cell

Junctional Complexes: Cell junctions

Apoptosis-programmed cell death

Chapter 2: Transport Across Cell Membranes

Passive transport processes: Diffusion, simple, facilitated

Osmosis: Osmotic pressure, tonicity

Active transport processes: Primary (Na^+–K^+ pump), Secondary, Carrier type (Uniporters, Symporters, Antiporters)

Vesicular transport processes: Endocytosis (phagocytosis), Pinocytosis Exocytosis

Intercellular communication (chemical messengers): Protein Kinases, Role of Ca^{2+} as second messenger, Receptor and G-protein diseases

Chapter 3: Body Water and Body Fluids

Introduction

Distribution of total body water (TBW)

Measurement of body fluid volumes and with ionic composition

Units for measuring concentration of solutes: Moles, Equivalents, Osmoles, Concept of pH and H^+ concentration, Concept of buffer system

Chapter 4: The Membrane Potentials

Ionic Composition of body fluids

Gibbs-Donnan membrane equilibrium

Resting membrane potential: definition; genesis; equilibrium potential; variations in membrane potential

Action potential: origin; phases; ionic basis; properties; electrotonic potentials (graded potentials); extracellular (surface) recording - biphasic and monophasic; injury (demarcation) potential

Chapter 1

The Structure and Function of a Cell

I. Introduction – What is physiology?
II. Physiological Systems
III. Homeostatic Regulation
IV. The Structure and Function of a Cell
V. Junctional Complexes Cell Junctions
VI. Apoptosis-programmed cell death

INTRODUCTION —WHAT IS PHYSIOLOGY?

The term ***physiology*** was originally derived from a Greek root with Latin equivalent **Physiologia**, which denoted *natural knowledge*. It now denotes a study of the *functions of the living organism* as a whole or its constituent parts.

1. A study of ***mammalian physiology***, which is a study of the dynamic inter-relationship among different tissues and organs, is mostly carried out at the organism level. The knowledge of physiology is important to appreciate the role of mechanism that control bodily functions.
2. ***Clinical Physiology*** is study of physiological responses or compensatory mechanisms that occur in normal systems when other parts of the body are diseased, for example, the study of changes in the lungs, liver or kidneys when the heart goes into failure.
3. ***Applied Physiology*** is study of underlying mechanisms that control body functions with aging, during exercise, the effects of low or high barometric pressures, oxygen lack, yoga, meditation etc.

Physiology is, therefore, the discipline that deals with the bodily functions and their control. It is however, only concerned with the normal.

PHYSIOLOGICAL SYSTEMS

Fig. 1.1 shows the different **levels of organization** of living organisms. At a fundamental level, **atoms** of elements link together to form mocules. The smallest unit of structure capable of carrying out all life processes is the **cell**. Simple organisms are composed of only one cell, but complex organisms have many cells with different structural and functional speciliazations. Collection of cells that carry out related function are known as *tissues*. Tissues form structural and functional units known as *organs*, and group of organs integrate their functions to create *organ system* (Fig. 1.2) and (Table 1.1).

HOMEOSTATIC REGULATION

In the nineteenth century, *Claude Bernard* (a French Physiologist) was first to recognize the importance of maintaining a stable *internal environment*. The cells, tissues, organs and organ systems of the body are interconnected and live together in a shared (internal) environment. Blood forms internal environment of the cell *i.e.* ***Millieu Interieur*** in terms of *volume, (water) composition, ion concentrations, pH and temperature*. This is regulated to normal (narrow) physiological limits with respect to minor changes in the

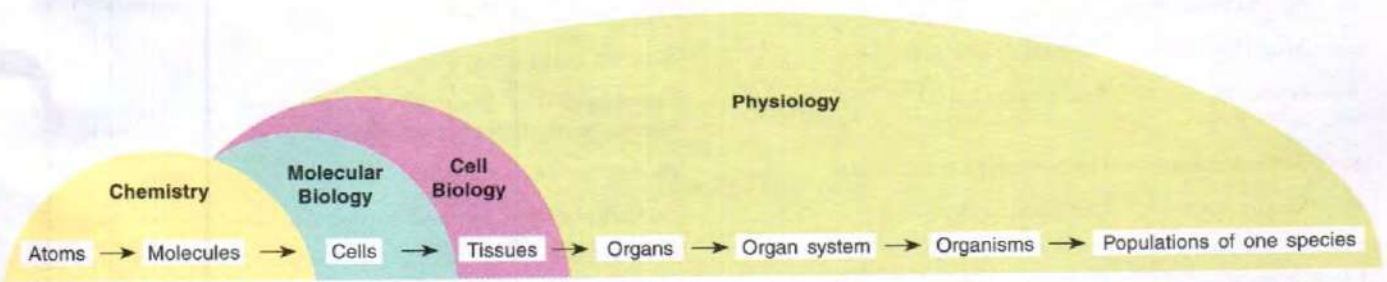


Fig. 1.1 Levels of organization of living organisms

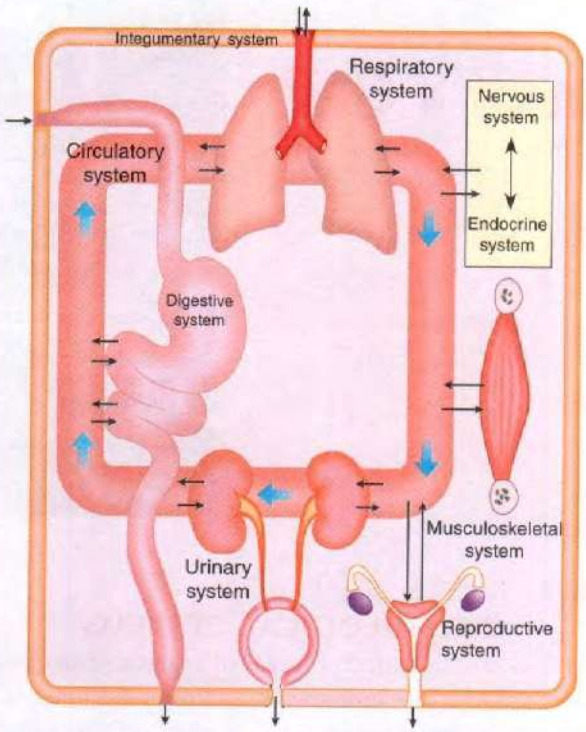


Fig. 1.2 Physiological organ systems in the human body

body. A variety of physiological mechanisms which act to stabilize the internal environment, are called ***Homeostasis Mechanisms*** (A term coined by an American physiologist *W. B. Cannon* in the twentieth century). The adjustments in physiological systems that are responsible for the preservation of homeostasis are referred to as *Homeostatic Regulation*.

Homeostatic regulation usually involves a *receptor*, sensitive to a particular *stimulus* and an *effector* whose activity affects the same stimulus.

(Also refer to pages 507, 557).

A. NEGATIVE FEEDBACK MECHANISMS

Most homeostatic mechanisms involve ***Negative feedback*** *i.e.* a corrective mechanism involving an action that directly opposes a variation from normal limits. Therefore, an increase or decrease in the variable being regulated brings about responses that tend to push the variable in the direction opposite (*negative*) the direction of the original change. For example,

Rise in blood pressure (BP) (*a stimulus*) → via negative feedback mechanism → Decrease in B.P. to normal limits (*response*)

Note

Here the initial stimulus produces a response that depresses the stimulus *i.e.* stimulus and response are opposite to each other.

In general, the *nervous system* performs corrective management by directing rapid, short-term and very specific response. On the other hand, the *endocrine* system releases chemical messengers (*hormones*) that affect tissues and organs throughout the body. The response may be slow to begin with but often persists for days or weeks. However, both systems are usually controlled by negative feedback mechanisms.

Tablel 1.1: Organ Systems of the Human Body

	System Name	Organs (or tissues)	Function(s)
1.	Circulatory	Heart, blood vessels, blood	Transport of materials between all cells of the body
2.	Digestive	Stomach, intestines, liver, pancreas	Conversion of food into particles that can be transported into the body; elimination of wastes
3.	Endocrine	Thyroid gland, adrenal gland etc.	Coordination of body function through synthesis and release of regulatory molecules
4.	Immune	Thymus, spleen, lymph nodes	Defence against foreign invaders
5.	Integumentary	Skin	Protection from external environment
6.	Musculoskeletal	Skeletal muscles, bones	Support and movement
7.	Nervous	Brain, spinal cord	Coordination of body function through electrical signals and release of regulatory molecules
8.	Reproductive	Ovaries and uterus, testes	Production of the species
9.	Respiratory	Lungs, airways	Exchange of oxygen and carbon dioxide between the internal and external environments
10.	Urinary	Kidneys, bladder	Maintenance of water and solutes in the internal environment; waste removal

B. POSITIVE FEEDBACK MECHANISMS

In few instances homeostatic regulation involves ***Positive feedback mechanisms***. *i.e.* an initial disturbance in a system sets off a chain of events that increases the disturbance even further. Therefore, it does not usually favour stability and often abruptly displaces a system away from its steady state operating point.

Example Marked fall in B.P. (*stimulus*)
↓
Decreased blood supply to the heart
↓
Decrease in myocardial contraction
↓
Further fall in the B.P. (*response*)

Note

Here, the initial stimulus produces response that reinforces (exaggerates) the original stimulus.

Positive feedback mechanism can sometimes be useful:

Example 1. Injury to blood vessels
↓
Initiates clotting processes
↓
Clotting proceeds
↓
Release of chemicals
↓
Enhances clotting processes
↓
Seals break in blood vessel wall

Example 2. Refer to page 805

(Also refer to page 656)

THE STRUCTURE AND FUNCTION OF A CELL

The fundamental unit of life is a *cell*, since virtually all tissues and any organised activity can be equated to the cellular level. Though no ***typical*** or ***generalised cell*** exists, it is convenient to create one to serve as a conceptual model within which most cell functions can be incorporated. (Fig. 1.3)

Note

Most cells in a human being have diameters of 10-20 μm (range 2-120 μm).

The three principal constituents of a cell are:
(A) Cell membrane

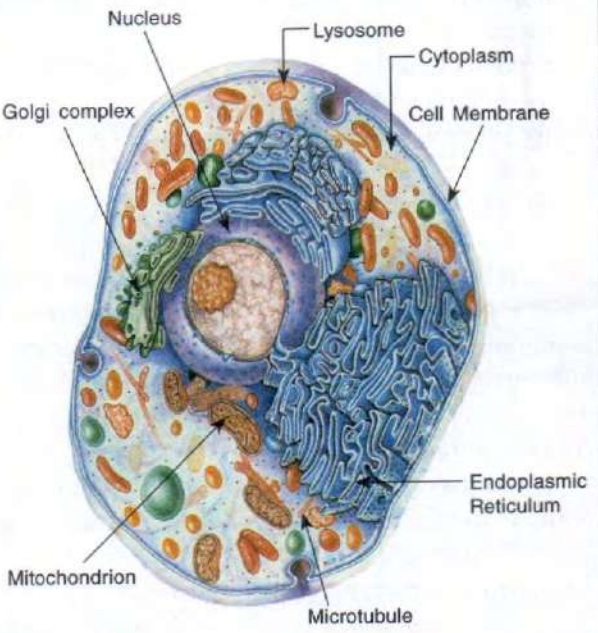


Fig. 1.3 Structure of a Generalised Cell

(B) Nucleus and its chromosomes
(C) Cytoplasm and its organelles

Note

The clear fluid portion of the cytoplasm in which the particles are dispersed is called *cytosol*.

A. CELL MEMBRANE or PLASMA MEMBRANE or UNIT MEMBRANE

Thickness

70-100 Angstrom (Å) or 7-10 nanometer (nm) (1 nm = 10^{-9} mts; 1Å = 10^{-10} mts).

Structure (Fluid Mosaic Model) (Fig. 1.4)

1. All membranes consist of a double layer of lipid molecules in which proteins are embedded. The lipids

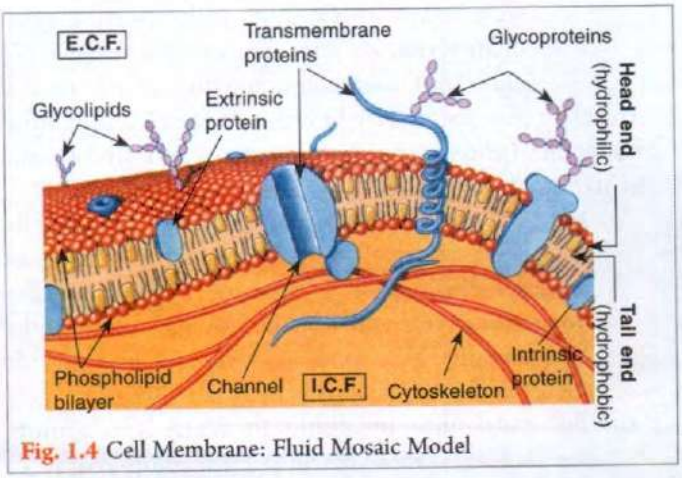


Fig. 1.4 Cell Membrane: Fluid Mosaic Model

normally constitute 20-40% of the dry weight of the membrane.

2. Proteins make upto 60-70% of the dry weight of the membrane and are of 2 types:
   (i) *Lipoproteins* (proteins containing lipids): function as enzymes and ion channels.
   (ii) *Glycoproteins* (proteins containing carbohydrates constituting 1-5% of the dry weight): function as receptors for hormones and neurotransmitters.

Some proteins are located in the inner surface of the membrane (***intrinsic proteins***); some are located in the outer surface of the membranes (***extrinsic*** and ***peripheral proteins***); while some extend through the membrane (***transmembrane proteins***):

(i) Intrinsic proteins serve mainly as 'enzymes'.
(ii) Extrinsic proteins contribute to the *cytoskeleton* (framework of the cell).
(iii) Transmembrane proteins serve as:
   (a) ***Channels***, through which ions or small water soluble substances can diffuse (pages 15 and 521).
   (b) ***Carriers***, which passively or actively transport materials across the lipid layer (pages 20 and 520).
   (c) ***Pumps***, which actively transport ions across the lipid layer (pages 18 and 520).
   (d) ***Receptors***, which when activated initiate intracellular reactions. The number of receptors in a cell are not constant but their number increases and decreases in response to various stimuli, and their properties change with change in physiological condition. For example, when a hormone or neurotransmitter is present in excess, the number of active receptors decreases (called *down regulation*); whereas during their deficiency, the number of active receptors increases (called *up regulation*) (Also refer to page 651). These effects on receptors are of physiological significance in explaining the phenomenon of ***denervation hypersensitivity*** (pages 171 and 189) and tolerance to certain drugs.

3. The clear area formed by bimolecular thickness of lipid molecules (phospholipids, cholesterol and glycolipids) is arranged as follows: **(Fig. 1.4)**
   (i) *Head end:* contains phosphate portion, is positively charged and quite soluble in water (*i.e.* **polar** or **hydrophilic**). Polar groups of lipid molecules have affinity for water (water loving) and face the aqueous phase *i.e.* exterior of the cell on one side (ECF) and cytoplasm on the other (ICF).
   (ii) *Tail end:* quite insoluble in water (no affinity for water/water fearing) (*i.e.* **non-polar** or **hydrophobic**), contains two fatty acid chains. The hydrophobic ends facing each other meet in the water-poor interior of the membrane.

Important Note

The bimolecular lipid layer in the membrane has the characteristics of a fluid due to presence of cholesterol. This fluidity makes the membrane quite flexible, thus allows cells to undergo considerable changes in shape without disruption of their structural integrity.

Functions

1. ***Protective*** – it forms outermost boundary of the cell organelles.
2. ***Digestive*** – takes in food and excretes waste products.
3. ***Property of selective permeability:***
   (i) *Non-polar molecules* (gases like O_2, CO_2 and N_2, lipids, steroid hormones, alcohol) can dissolve in the non-polar regions of the membrane and thus move rapidly across the membrane. *Polar molecules* (water soluble substances: ions, glucose, urea etc.) have much lower solubility, therefore, penetrate the membrane much more slowly.
   (iii) Chemical and physical characteristics of the membrane control the free passage of ions and molecules into and out of the cell.

   This property of selective permeability of the cell membrane helps in maintaining the difference of composition between ECF and ICF (page 29).
4. ***Insulating properties:*** It act as the ***dielectric material*** (such as rubber) of a charged condenser, thus the cell membrane has a very high insulating value.
5. It provides a framework for the arrangement of an ordered sequence of protein molecules (enzymes, pumps, receptors, ions, channels, Co-factors, carriers) in a functionally meaningful pattern.
6. It links adjacent cells together by junctional complexes to form tissues (page 10).

Summary: Cell membrane components and Functions **(Fig. 1.5)**

B. NUCLEUS AND ITS CHROMOSOMES

Structure

1. It is a spherical structure (10 µm diameter) surrounded by a relatively permeable membrane called ***nuclear membrane*** (or envelope). This is composed of two unit membranes and shows large pores of 1000 Å diameter which are closed by thin homogenous membrane. Therefore, passage of macromolecules like RNA can take place through these pores. The space between the two folds is 300 Å and is called ***perinuclear cistern***. **(Fig. 1.6)**

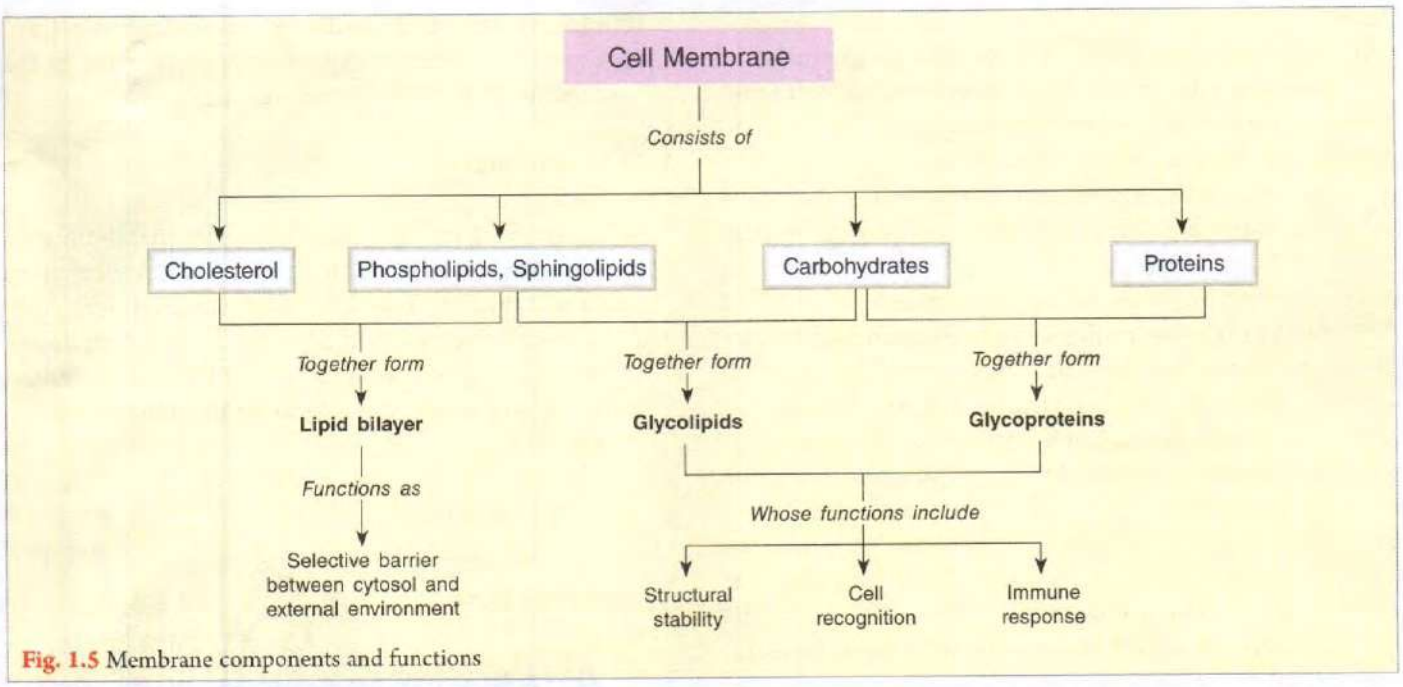


Fig. 1.5 Membrane components and functions

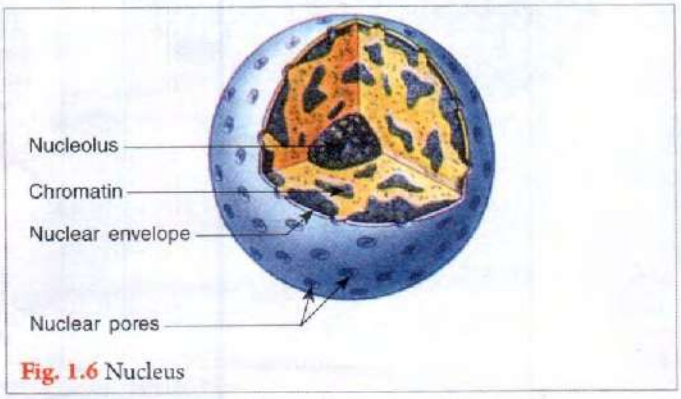


Fig. 1.6 Nucleus

2. It is made up of ***chromosomes*** (each chromosome is made up of supporting protein plus giant molecule of Deoxyribonucleic Acid-DNA), on which genes are present. ***Gene*** is a portion of DNA molecule which carries a complete blue print for all the heritable species and individual characteristics of an animal. During cell division, the pairs of chromosomes become visible, but between cell divisions the irregular clumps of dark material called ***chromatin*** are the only evidence of their presence.
3. It contains a ***nucleolus*** which is densest of all the nuclear material *i.e.* a patch work of granules rich in *Ribonucleic Acid* (RNA). Nucleoli are most prominent and numerous in growing cells. They synthesize the RNA for the ribosomes.

Functions

1. DNA in nucleus serves as a 'template' (block) for synthesis of RNA, which then moves to the cytoplasm where it regulates the synthesis of proteins by the cells. The information coded into the DNA molecules is conveyed from the nucleus to the cytoplasm by messenger RNA where actually the synthetic work of the cell takes place.

Note

80% of the dry weight of nucleus is protein, the remainder is made up by 18% DNA and 2% RNA.

2. Genes are units of hereditary characteristics.
3. It is concerned with cellular reproduction and multiplication; the development of chromosomal threads form the network, being the first step towards cell division.

C. CYTOPLASM AND ITS ORGANELLES

1. Endoplasmic Reticulum (ER)

It is a complex series of tubules whose walls are made up of unit membrane. Through this network of tubules, substances may be delivered from the outer membrane of cell proper to the membrane of the nucleus or to other inclusion bodies of the cells *e.g.* mitochondria **(Fig. 1.7)**.

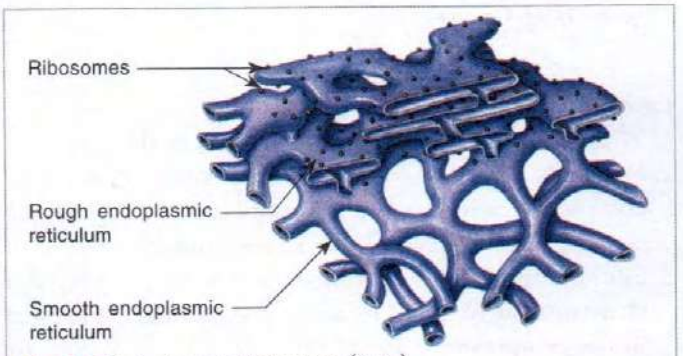


Fig. 1.7 Endoplasmic Reticulum (E.R.)

Types

(i) *Agranular* or *Smooth ER*: Contains no granules.
   (a) It is site of steroid (Adrenocortical hormone) synthesis in steroid secreting cells and the site of detoxification processes in other cells.
   (b) As the *sarcoplasmic reticulum*, it plays important role in skeletal and cardiac muscle (page 160).

(ii) *Granular* or *Rough ER* or *Ergastoplasm*.
   (a) Contains granules called ***ribosomes*** (diameter 15 nm; contains 65% RNA and 35% protein: *Ribonucleoprotein*) which are attached to the cytoplasmic side of the membrane. 3-5 ribosomes clump together to form ***polyribosomes*** or polysomes.
   (b) It is the site of protein synthesis *e.g.*, hormones that are secreted by the cell; and proteins that are found in enzymes.
   (c) Free ribosomes are also found in the cytoplasm, they synthesize cytoplasmic protein *e.g.*, *Haemoglobin*.

2. Golgi Complex (or Golgi Bodies)

It is a collection of membranous tubules and vesicles found always in the neighbourhood of the nucleus, prominent in actively secreting gland cells. **(Fig. 1.8)**

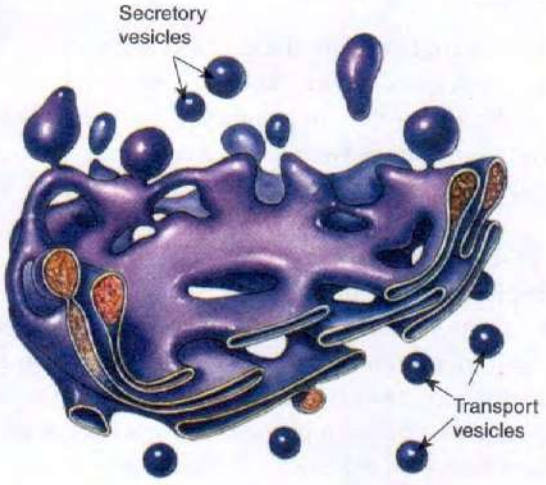


Fig. 1.8 Golgi Complex

Functions

1. Wrapping and packaging department of the cell.
2. Produces *secretion granules i.e.* membrane enclosed complexes, which store hormones and enzymes in protein secreting cells; it packages proteins.
3. Site of formation of *lysosomes i.e.* large irregular structures surrounded by membrane which are present in the cytoplasm.
4. It adds certain carbohydrates to proteins to form glycoproteins, which play an important role in the association of the cells to form tissues.

3. Mitochondrion

Structure

(i) Length 5–12 μm; diameter 0.5–1 μm; filamentous or globular in shape; occur in variable numbers from a few hundred to few thousands in different cells.
(ii) Made up of outer membrane and inner membrane. Inner membrane folded to form *cristae* (shelves) which project into the interior of the mitochondrion. **(Fig. 1.9)**

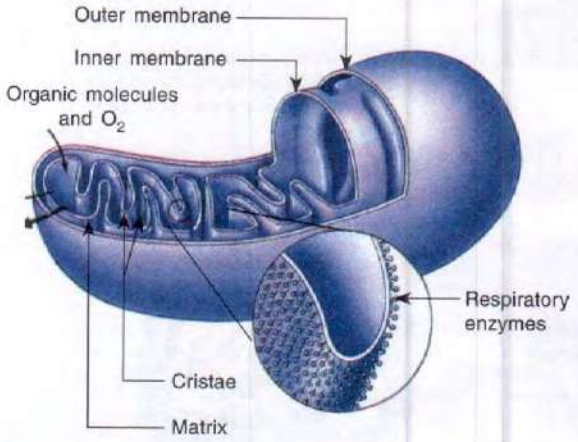


Fig. 1.9 Mitochondrion

(iii) **Outer membrane**: Studded with the enzymes concerned with *biological oxidation* (oxidation being catalyzed by enzymes).
(iv) *Interior (matrix)* of mitochondrion contains enzymes concerned with *'citric acid cycle'* (page 604) and 'respiratory chain oxidation' (page 598).
(v) **Inner membrane** contains adenosine triphosphatase (ATPase) and other enzymes concerned with synthesis and metabolism of ATP.

Functions

(i) Mitochondria are *power generating* units of the cells and are plentiful and best developed in parts of cells where energy requiring processes take place *e.g.* rapidly contracting skeletal muscles where they comprise 40-50% of the cell volume.
(ii) Also contain DNA and can synthesize proteins.

4. Lysosomes

Structure

1. These are large irregular structures surrounded by unit membrane and are found in the cytoplasm;

250-750 nm in diameter. A typical cell may contain several hundred lysosomes.

2. It is filled with large number of small granules, 5-8 nm in diameter which contain variety of enzymes, called ***lysozymes*** **(Table 1.2)**.

Table 1.2: Lysosomal enzymes (lysozymes) and the substrates on which they act

	Enzymes	Substrate
(i)	Ribonuclease	RNA
(ii)	Deoxyribo nuclease	DNA
(iii)	Phosphatase	Phosphate esters
(iv)	Glycosidase	Complex carbohydrates, glycosides and polysaccharides
(v)	Arylsulphatases	Sulphate esters
(vi)	Collagenase	Collagen proteins
(vii)	Cathepsins	Proteins

3. The interior is kept acidic (near pH 5.0) by the action of *proton pump* or H^+ or *ATPase*. Lysozymes are all *acid hydrolases* as they function best at the acidic pH.

Functions

(i) Acts as a form of digestive (lytic) system for the cell, because enzymes present in it can digest essentially all macromolecules.
(ii) Engulf worn out components of the cells in which they are located.
(iii) Engulf exogenous substances *e.g.* bacteria etc. and degrade them.
(iv) When a cell dies, lysosomal enzymes cause autolysis of the remnant *i.e.* why lysosomes are called as *suicidal bags*.

5. Peroxisomes

(i) Its structure is similar to that of lysosomes but with a different chemical composition. It contain *oxidases* (enzymes that produce H_2O_2) rather than hydrolases.
(ii) They consume oxygen in small amounts that is not used in the chemical reactions associated with ATP formation.
(iii) They destroy certain products formed from oxygen, especially hydrogen peroxide, that can be toxic to the cells, hence the name peroxisomes.

Note

The alcohol, a person drinks is mainly detoxified by the peroxisomes of the liver cells.

6. Centrioles or Centrosomes

Structure

(i) These are two short cylinders called *'centrioles'* visible only during cell division.
(ii) They are located at each pole near the nucleus and are so arranged such that they are at right angles to each other.
(iii) Tubules in group of three (triplets) run longitudinally in the walls of the centrioles. There are nine of these triplets spaced at regular intervals around the circumference.

Function

They are concerned with the movement of the chromosomes during cell division.

7. Microtubules and Microfilaments

Microtubules are long hollow structures approx. 25 nm in diameter; make up structures or tracts on which chromosomes, mitochondria and secretion granules move from one part of the cell to another **(Fig. 1.10** and **1.11)**.

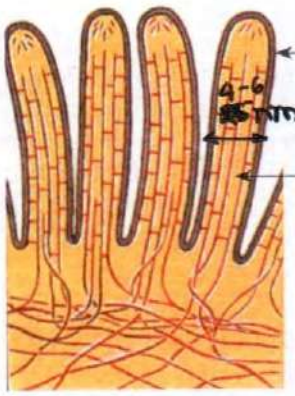


Fig. 1.10 Microtubule

Microfilaments are long solid fibers 4-6 nm in diameter. They comprise the contractile protein actin and are responsible for the cell motion. **(Fig. 1.11)**

Functions

These are involved in the:

(i) movements of the chromosomes;
(ii) cell movement;
(iii) processes that move secretion granules in the cell; and
(iv) movement of proteins within the cell membrane.

8. Secretion Granules: Page 8.

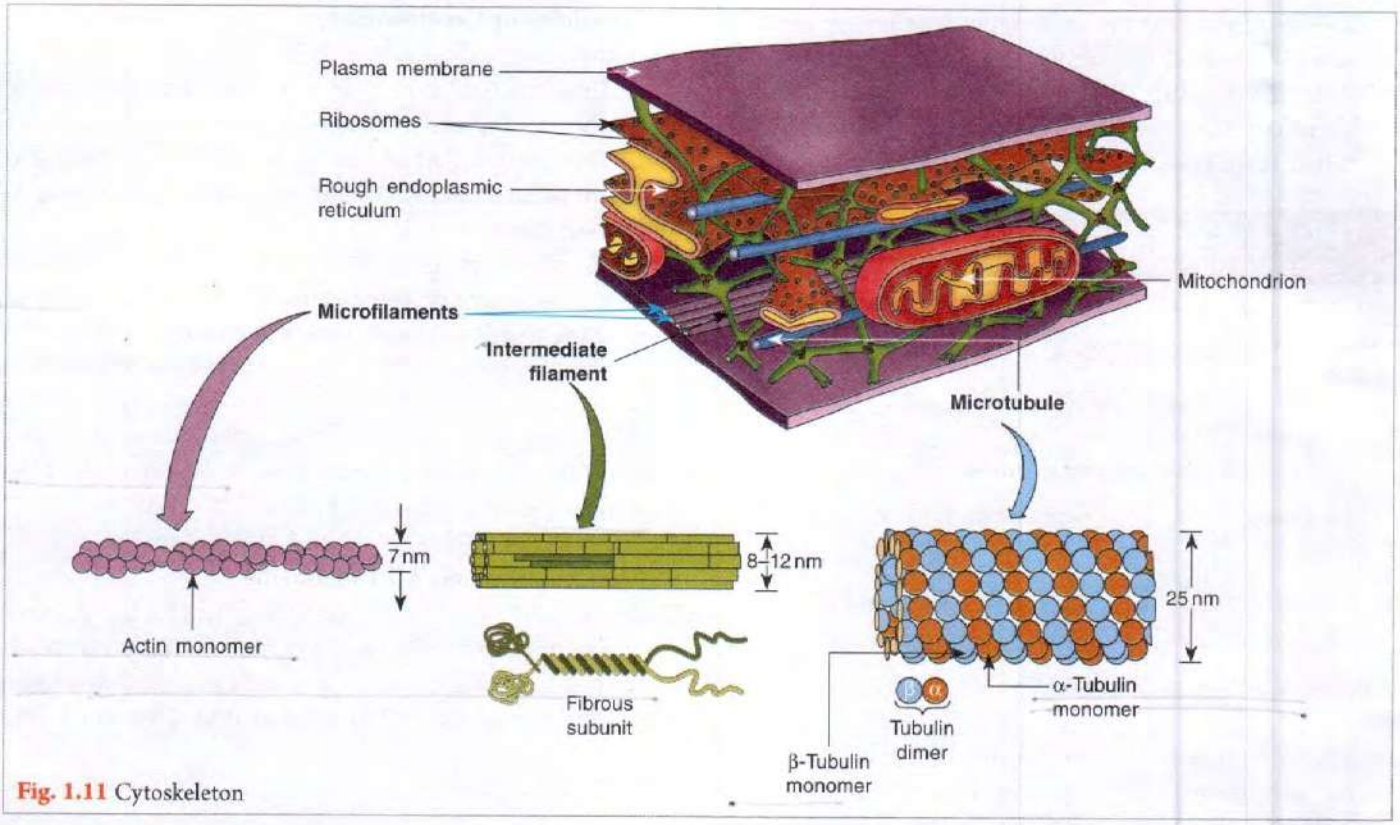


Fig. 1.11 Cytoskeleton

Important Note

All cells have a system of fibers called *cytoskeleton* that maintains the structure of the cell. It allows a cell to change shape and also permits its movement. The cytoskeleton comprises of microtubules and microfilaments, along with proteins that bind them together (Fig. 1.11).

JUNCTIONAL COMPLEXES: CELL JUNCTIONS

The cells are associated into tissues by various means (Fig. 1.12):

1. *Tight Junction:* In this, membranes of two cells become opposed and outer layers of the membranes fuse strongly, thus obliterating the space between the cells. This type of junction is characteristically seen along the apical margins of cells in epithelium such as the intestinal mucosa, the walls of the renal tubules, and the choroid plexus. Tight junction forms a barrier to the movement of ions and other solutes from one side of the epithelium to the other.
2. *Desmosomes or Adherens Junction:* Here two membranes are separated by a 150-350 Å (15-20 nm) space. There is dense accumulation of proteins on both the surfaces of the membrane with fibers extending from the cytoplasmic surface of each membrane into the cell. This holds adjacent cells firmly together in areas that are subjected to stretching, such as the skin.
3. *Gap Junction or Nexus:* There is 2 nm to 20 nm space between the opposing membranes. This gap is filled with densely packed particles through each of which there appears to be a channel that connects the two cells. The diameter of each channel is regulated by intracellular Ca^{2+}, pH and voltage.

   Other advantages of gap junction:

   (i) It permits rapid propagation of electrical potential changes from one cell to another, *e.g.* cardiac and smooth muscle cells (page 175).

   (ii) It permits the direct transfer of ions and other small molecules upto MW 1000 (*e.g.* sugars, amino acids) between the cells without traversing the extracellular space.

Important Note

Cells are attached to each other by *cell adhesion molecules (CAMs).* They also transmit signals into and out of the cell. These adhesion proteins (viz. laminin, intergin, IgG, cadherin, selectin) play important role in:

(i) embryonic development
(ii) formation of the nervous system
(iii) holding tissues together
(iv) inflammation and wound healing, and
(v) metastasis of tumours.

Cells with abnormal CAMs have a higher rate of apoptosis (see below).

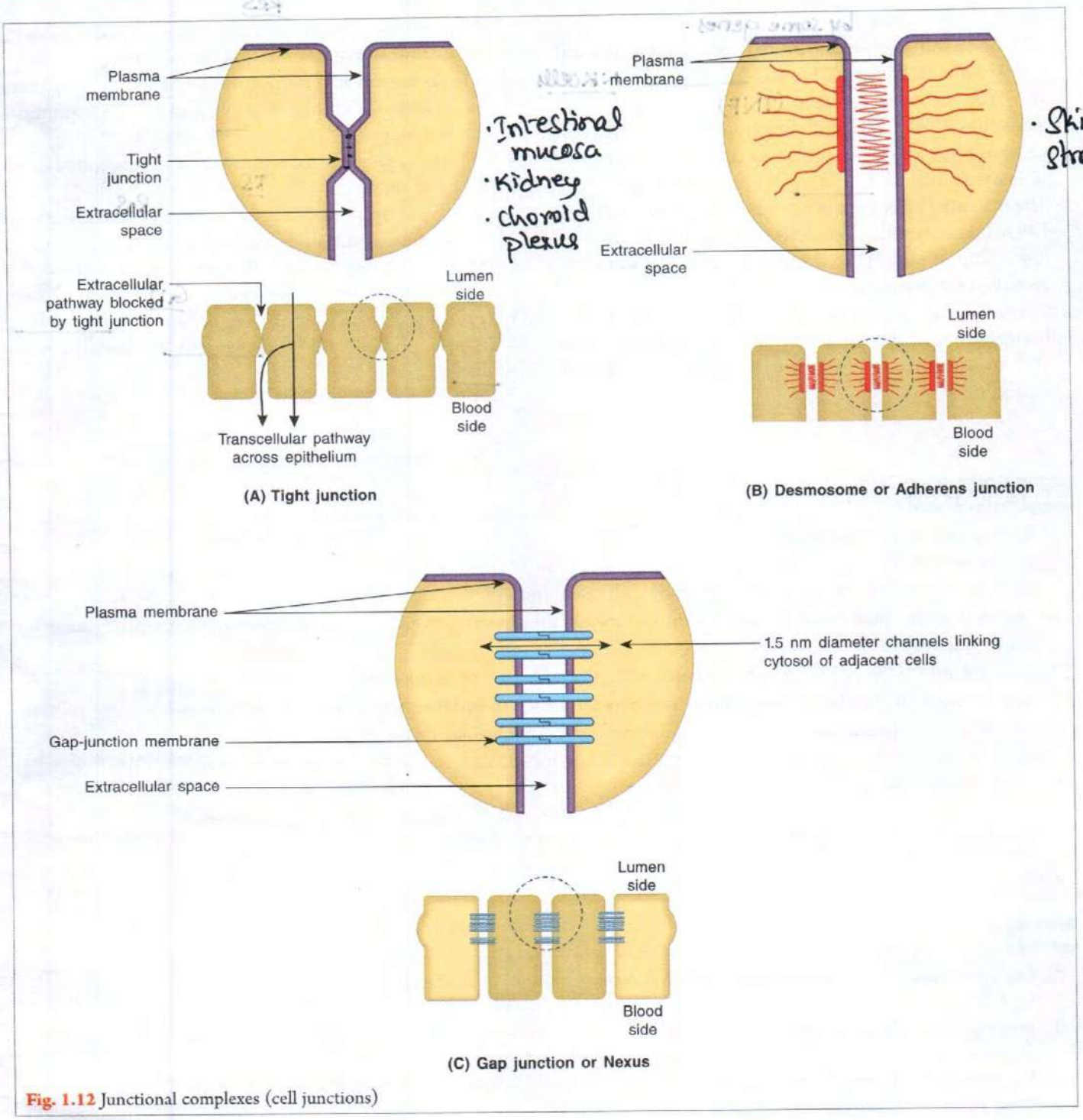


Fig. 1.12 Junctional complexes (cell junctions)

APOPTOSIS: PROGRAMMED CELL DEATH

It is a Greek word which means loosening or falling. (*Apo* means 'away' and *Ptosis* means 'fall')

1. Apoptosis is a process of programmed cell death in which body cells die and get absorbed (phagocytosed) under genetic control. Here cell's own gene plays an active role on its death, therefore, also called as *cell suicide*.

Important Note

Cell necrosis or *cell murder* is a process in which neighbouring healthy cells are destroyed by a disease such as inflammation. However apoptosis is an orderly cell death in which neighbouring cells usually remain healthy.

Mechanism. Apoptosis may be initiated by:

(i) environmental processes such as inflammation

(ii) internal stimuli
(iii) *Fas*, a transmembrane protein produced by natural killer cells (page 122) and T-lymphocyes
(iv) Tumour necrosis factor.

The ultimate pathway initiating apoptosis is activation of group of cysteine proteases inactivate enzymes (together called as, *Caspases*) within the mitochondria. The activated apoptotic gene causes the cell to undergo DNA fragmentation, condensation of cytoplasm and chromatin; finally the cell break up and remnants are removed by phagocytes.

2. ***Physiological significance.*** Apoptosis plays an important role during embryonal development and also in adulthood. It removes un-needed cells. For example,
   (i) it is responsible for regression of duct system during sex differentiation in the foetus;
   (ii) it is responsible for degeneration and regeneration of neurons within the CNS and for the formation of synapse;
   (iii) it is responsible for removal of inappropriate clones of immune cells;
   (iv) it is responsible for cyclical shedding of endometrium at the time of menstruation; and
   (v) it is responsible for cell shed from the tip of the villi in the small intestine.
3. ***Applied.*** Abnormal apoptosis occurs in autoimmune diseases (page 128), degenerative diseases and cancers.

Study Questions

1. **Give physiological significance of:**
   (i) cellular cytoskeleton (ii) Millieu interieur (iii) homeostatic regulation (iv) Junctional complexes
2. **Give the electron microscopic structure of the cell membrane.**
3. **Justify the term 'fluid mosaic model' for the cell membrane structure. Which cell membrane component is responsible for its fluidity?**
4. **Give the role of the cell membrane in maintaining the difference of composition between ECF and ICF.**
5. **List the prominent cell organelles. Briefly describe the structure and functions of any one of them.**
6. **Describe the structure and function of the different types of junctions found between cells.**
7. **Give an account of programmed cell death. How is it initiated? Give its physio-clinical significance.**
8. **Write short notes on:**
   (i) Peroxisomes (ii) Lysosomes (iii) Cell adhesion molecules
   (iv) Negative versus positive feedback mechanisms. (v) Caspases

MCQs

1. **On weight basis, the cell membrane contains protein and lipid in the ratio of:**
   (a) 1:2 (b) 1:1 (c) 2:1 (d) 4:1
2. **One major function of the cell membrane is:**
   (a) Protective (b) Digestive
   (c) Property of selective permeability (d) Links adjacent cells together to form tissues
3. **Main function of nucleus is:**
   (a) To control chemical and physical characteristics of the cell
   (b) To bring about cellular reproduction and multiplication
   (c) To synthesize protein for the cell
   (d) To help in cellular movement
4. **Endoplasmic reticulum is associated with all of the followings *except*:**
   (a) Enzymatic secretion (b) Lipid secretion
   (c) Glycogen synthesis (d) Glycogenolysis
5. **Mitochondria are plentiful and best developed in parts of cells where:**
   (a) Active protein synthesis takes place (b) Energy requiring processes take place
   (c) Active detoxification process is going on (d) Active secretion occurs

6. **Peroxisomes:**
   (a) Their structure and chemical composition is similar to that of lysosomes
   (b) They destroy products formed from oxygen, especially hydrogen peroxide
   (c) They engulf exogenous substances and degrade them
   (d) They consume oxygen in large amounts, hence the name peroxisomes

7. **Cytoskeleton comprises:**
   (a) Microtubules and microfilaments
   (b) Cell membrane
   (c) Golgi complex
   (d) Cell junctions

8. **All are true for gap junction, *except*:**
   (a) It permits rapid propagation of electrical potential changes from one cell to another
   (b) It permits direct transfer of ions between the cells
   (c) It is traversed by a channel that connects the two cells
   (d) It is plentiful in skeletal muscle cells

9. **Which of the following is *false* about apoptosis?**
   (a) It is a process of programmed cell death
   (b) It is also called as cell suicide
   (c) It plays an important role during embryonal development
   (d) It occurs as a natural process in autoimmune diseases

10. **Which of the following moves rapidly across the cell membrane?**
    (a) CO_2 (b) Water (c) Glucose (d) Urea

11. **The bimolecular lipid layer in the cell membrane has the characteristics of a fluid due to presence of:**
    (a) Phospholipids (b) Cholesterol (c) Glycolipids (d) Glycoproteins

Answers

1. (d) 2. (c) 3. (b) 4. (c) 5. (b) 6. (b) 7. (a) 8. (d) 9. (d) 10. (a) 11. (b)

Chapter 2

Transport Across Cell Membranes

I. Passive Transport Processes:
   (A) Diffusion: simple, facilitated (B) Osmosis: osmotic pressure, tonicity
II. Active transport processes:
   (A) Primary (Na^+–K^+ pump)
   (B) Secondary
   (C) Carrier type (uniporters, symporters, antiporters)
   (D) Vesicular transport processes: endocytosis (phagocytosis), pinocytosis, exocytosis
III. Intercellular communication: chemical messengers

Substances move through the cell membrane by two major processes: passive and active. *Passive transport* requires **no** energy; *active transport* on the other hand does consume energy.

PASSIVE TRANSPORT PROCESSES

Here substances move across the cell membrane without any energy expenditure by the cell. It includes: *Diffusion* and *Osmosis*.

A. DIFFUSION

Diffusion is a ***passive process*** (*i.e.* **no** external source of energy is required) by which molecules move from areas of high concentration to areas of low concentration (*down their 'chemical gradient'*); and cations (positively charged molecules) move to negatively charged areas whereas anions move to the positively charged areas (*down their 'electrical gradient'*). It is of two types:
(1) *simple diffusion, and*
(2) *facilitated diffusion.*

1. Simple Diffusion

Characteristic features

(i) It occurs because the heat content of the solution keeps the solvent and the solute particles of the solution in constant motion.
(ii) Net movement stops when the concentration of the molecules is equal everywhere within the solution (***diffusional equilibrium***).
(iii) Although random movements of the molecules continue after diffusional equilibrium is achieved, the concentration of the molecules throughout the solution remains the same.
(iv) It is the only form of transport that is *not* carrier mediated.
(v) *Factors affecting diffusion.* The 'rate' at which a material diffuses through a membrane (flux) is given by *Fick's law of diffusion i.e.*

Net rate of diffusion (flux) =

$$-\frac{\text{Diffusion coefficient (D)} \times \text{Area of the membrane (A)}}{\text{Thickness of membrane (or diffusion distance) (T)}} \times (C_{in} - C_{out})$$

C_{in} and C_{out} = Concentration of the material inside and outside of the membrane. The negative sign indicates that the material is moving down its concentration gradient.

(Also see to page 355)

(a) *Distance:* The greater the distance, the longer the time required. In the human body, diffusion distances are usually small as diffusion of substances occurs across the cell membranes of uniform thickness (10 nm).
(b) *Size of the gradient:* The larger the concentration gradient, faster the diffusion proceeds.
(c) *Temperature:* The higher the temperature, faster the diffusion rate. At normal body temperature of 37°C diffusion is optimal (maximum).
(d) *Molecular size:* The permeability of cell membrane to a substance falls rapidly with increase in molecular weight in the range between 10,000 to 60,000. This is why glucose diffuses faster than large proteins.
(e) *Lipid solubility:*
– *Lipid soluble molecules* (O_2, CO_2, N_2 and alcohols) diffuse rapidly with ease through the lipid layer of the membrane.

– *Water soluble molecules* (ions, glucose, urea) can cross the cell membrane slowly as they diffuse through the aqueous channels formed by transmembrane proteins.

Ion channels → PORE without gate.
'Ions' also utilize *ionic channels* to cross the cell membrane. Some channels are continuously open, whereas others are 'gated' **(Fig. 2.1)**.

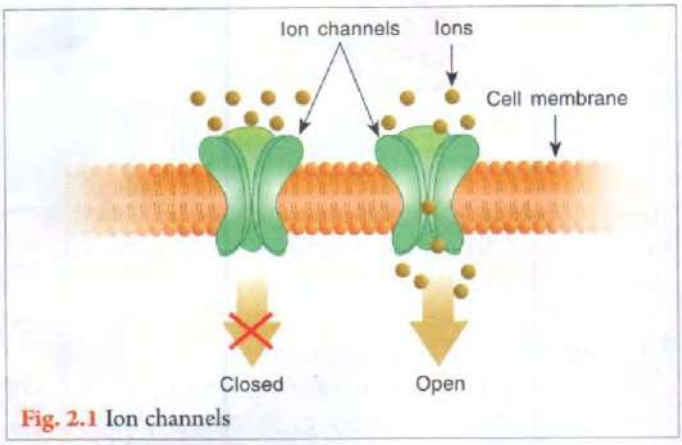


Fig. 2.1 Ion channels

There are ion channels *specific* (for Na^+, K^+, Ca^{2+} and cl^-) and *non-specific* (for cations or anions). Each type of channel exists in multiple forms with different properties. Most are made up of identical or very similar protein subunits. For example: NON-Gated ion channels

1. K^+ channels are *tetramers* with four similar protein subunits through which K^+ pass. Similarly aquaporin water channels (page 530) are tetramers with an intracellular channel in each protein subunit.

2. Ligand gated cations or anion channels (see below) have five identical protein subunits.

3. Several types of Cl^- channels are *dimers* with an intracellular pore in each subunit.

Applied: Ion channel mutations cause a variety of ***channelopathies*** - diseases that mostly affect muscle and brain tissue and produce: periodic paralysis, myotonia, myasthenia or convulsions.

Gated channels have gates that open or close either:
(i) by alteration in membrane potential (***voltage gated***) *e.g.* Na^+ and Ca^{2+} channels (page 156); or
(ii) when they bind a ligand *i.e.* either an ion or a specific molecule (***ligand gated***). The *ligand* is either *external* (*e.g.* neurotransmitter - page 156 or a hormone) or *internal* (*e.g.* intracellular Ca^{2+}, cAMP, or G protein produced in the cells - page 654) **(Fig. 2.2)**.
Some channels are also opened by mechanical stretch (***mechanosensitive channel*** - pages 190, 867), which play an important role in cell movement.

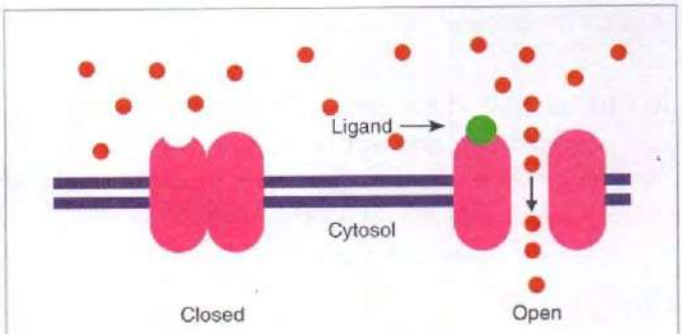


Fig. 2.2 Ligand gated channels

Important Notes

1. The variation in the membrane permeability in different cell membranes reflects differences in the number of ion channels in the membranes.
2. The extracellular ligands are called *first messengers* and the intracellular mediators are called *second messengers*. The second messengers generally activate protein kinases (page 654).

2. Facilitated Diffusion

Characteristic features

(i) It is a *carrier-mediated process* that enables molecules that are too large to flow through membrane *channels* by simple diffusion **(Fig. 2.3)**. For example,
  (a) glucose transport by the *glucose transporter (GLUT)* across intestinal epithelium (page 260), and
  (b) the transport of glucose into RBCs, muscles and adipose tissue in the presence of insulin.
(ii) It is more rapid than simple diffusion.
(iii) The carrier protein undergoes repetitive spontaneous configurational changes during which the binding site for the substance is alternately exposed to the ICF and ECF.

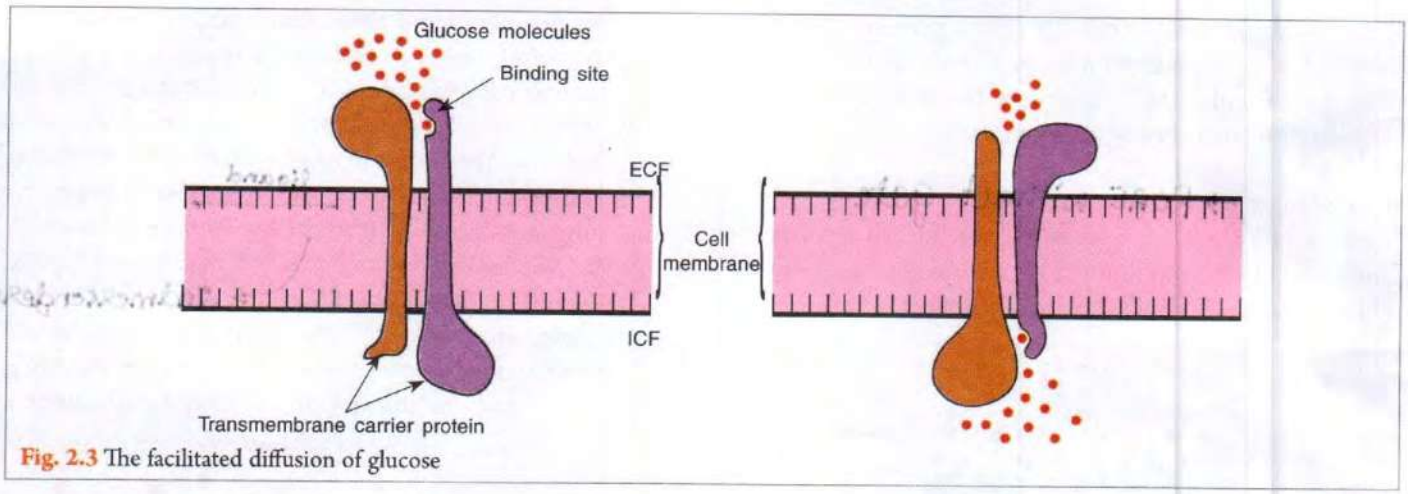


Fig. 2.3 The facilitated diffusion of glucose

(iv) Its rate of diffusion increases with increase in concentration gradient to reach a plateau when all the binding sites on the carrier proteins are filled **(Fig. 2.4)**. This is called 'saturation'.

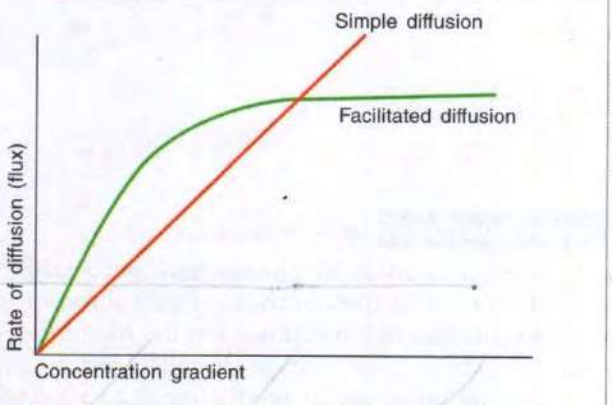


Fig. 2.4 The diffusion rate (simple and facilitated) compared

(v) There are many types of carrier proteins in membranes, each type having binding sites that are specific for a particular substance.

Important Notes

1. **Diffusion is a major force affecting the distribution of water and solutes in different body compartments.**
2. **In diabetes mellitus, glucose uptake by muscle and fat cells is impaired because the carrier for facilitated diffusion of glucose require insulin.**

Some substances (weak acids and weak bases) that are quite soluble in cell membranes in the undissociated form, cross the membrane with difficulty in the ionic form. However, if an undissociated substance diffuses from one side of the membrane to the other end and then dissociates, there occurs net movement of the undissociated substance from one side of the membrane to another, called *Non-ionic diffusion*. This phenomenon is seen in the GIT and kidneys.

B. OSMOSIS

Definition: Osmosis is the ***passive*** flow of the solvent *e.g.* water across a selectively permeable membrane (*i.e.* membrane permeable to solvent but not to the solute), into a region where there is a higher concentration of a solute to which the membrane is impermeable.

The Osmotic Pressure

The tendency for movement of solvent molecules to pass across a membrane from a low concentration of solute to a region of greater solute concentration can be prevented by applying pressure to the more concentrated solution. The amount of pressure exactly required to prevent solvent migration (*i.e. osmosis*) is called the *osmotic pressure* of the solution **(Fig. 2.5)**.

Osmotic pressure of a solution is related to the:

(1) Number of particles (molecules or ions) dissolved in the solution rather than their size, type, molecular weight or chemical composition.

(2) Temperature and volume in the same way as the pressure (P) of a gas *i.e.*

$$P = \frac{nRT}{V}$$

where n = number of particles; R = gas constant; T = absolute temperature and V = volume of solution.

If 'T' is kept constant then osmotic pressure is proportionate to the number of particles in a solution per unit volume of solution.

Since the body fluids are not ideal solutions, the number of particles free to exert an osmotic effect is reduced due to interaction among the ions. Thus, it is actually the effective

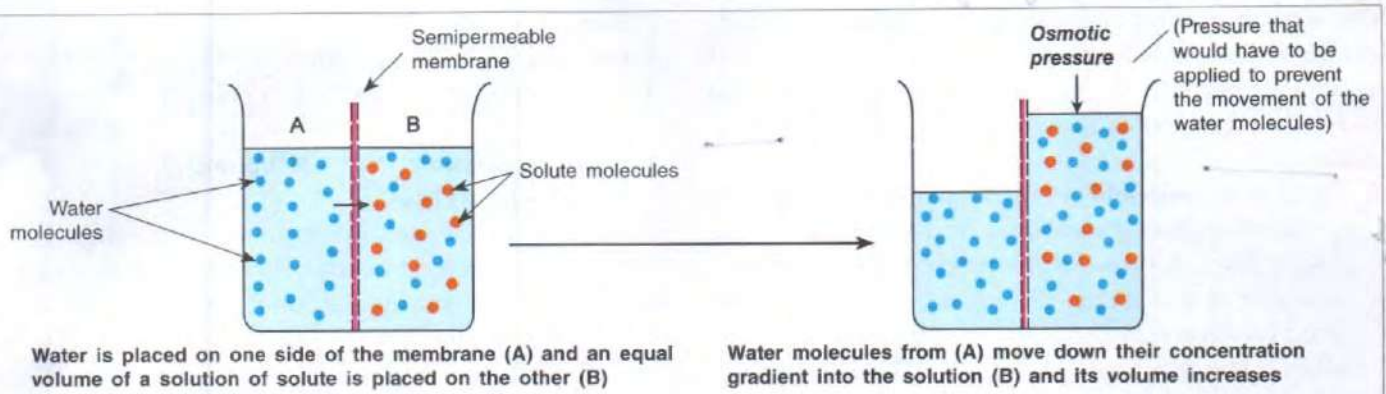


Fig. 2.5 Diagrammatic representation of osmosis

electrolyte's concentration in the body fluids rather than the number of equivalents of an electrolyte in solution that determines its osmotic effect.

Note

The colloidal osmotic pressure due to the plasma colloids is called ONCOTIC PRESSURE (refer to page 55).

The Osmotic Pressure Gradient

The *osmotic pressure gradient* is created by the presence of different concentrations of solutes in the solutions on either side of the membrane. Thus a homogeneous solution containing osmotically active particles can exert osmotic pressure only when it is in contact with another solution across a membrane permeable to the solvent and not to the solute.

Osmolal Concentration of Plasma: Tonicity

The term concentration refers to the total concentration of solute particles in a solution, irrespective of their chemical composition. The concentration of osmotically active particles is expressed in *osmoles* (osm) or *'milliosmoles'* (1/1000 of 1 osm) (page 30).

The ***osmolarity*** is the number of osmoles per litre of solution *e.g.* plasma; whereas the ***osmolality*** is the number of osmoles per kilogram of the solvent. Therefore, osmolarity is affected by the volume of the various solutes in the solution and the temperature, while the osmolality is not. Osmotically active substances in the body are dissolved in water, and as the density of water is 1, so osmolal concentration is expressed as osm/L of water.

The osmolality of normal human plasma is 290 mosm/L. The osmolality of a solution relative to plasma is called ***tonicity***. Solutions that have the same osmolality as plasma are said to be ***isotonic*** (*e.g.* 0.9% sodium chloride solution or 5% glucose solution); those with greater osmolality are ***hypertonic***; and those with lesser osmolality are ***hypotonic***.

Important Note

In contrast to isotonic, hypertonic and hypotonic, another set of terms *isosmotic*, *hyperosmotic* and *hyposmotic* denote simply the osmolarity (*i.e.* total solute concentration) of a solution relative to another solution regardless of its composition. The two sets of terms are therefore not synonymous.

Relative contribution of the various plasma components to the total osmolal concentration of plasma:

1. Approximately 270 of the 290 mosm/L of normal plasma are contributed by Na^+ and its accompanying anions, mainly Cl^- and HCO_3^-, because there is no net movement of these osmotically active particles into the cells and these particles are not metabolised.
2. Although the concentration of the plasma proteins is large (average 6.4–8.3 gm / dL), they normally contribute less than 2 mosm/ L because of their very high molecular weights.
3. The major non-electrolytes of plasma, glucose and urea, which are in equilibrium with the cells (i.e. easily crosses most cell membranes) contribute about 5 mosm/L. But this can be quite large in hyperglycemia or uremia.

Note

Clinically, a rough estimation of plasma osmolality can be made by using the formula:

Osmolality (mosm/L) =

$$2[Na^+] + 0.055\,[\text{glucose}] + 0.36\,[\text{Blood urea nitrogen}]$$
$$(\text{mEq/L}) \qquad (\text{mg/dL}) \qquad (\text{mg/dL})$$

Clinical significance

The total plasma osmolality is important in assessing

dehydration, overhydration, and other fluid and electrolyte abnormalities. For example:

1. Hyperosmolality can cause coma (***Hyperosmolar coma***) by causing water to flow out of the cells *i.e.* cellular dehydration
2. The flow of water into or out of the capillaries depends on whether the *colloidal osmotic pressure* or *oncotic pressure* (osmotic pressure produced by plasma proteins) is greater or lesser than the *hydrostatic pressure* of the blood (produced by systemic arterial blood pressure). When water flows into or out of capillaries, it carries dissolved particles with it. This force is called *solvent drag*. Its effects are very small in the body.

Note

At normal body temperature (37°C), a concentration of 1 mosm/L will cause 19.3 mm Hg osmotic pressure in the solution.

ACTIVE TRANSPORT PROCESSES

Here, substances are transported against their chemical and electrical gradient. This form of transport requires energy and is called *active transport*. It includes:

(A) Primary active transport processes,
(B) Secondary active transport processes,
(C) Carrier type processes, and
(D) Vesicular transport processes

Note

Because active transport processes require energy, they are often referred to as *pumps*.

A. PRIMARY ACTIVE TRANSPORT PROCESSES

They directly use the energy obtained from the hydrolysis of adenosine triphosphate (ATP). The primary active transport system consists of:

1. Sodium-potassium ($Na^+ - K^+$) pump,
2. Calcium (Ca^{2+}) pump, and
3. Potassium hydrogen ($K^+ - H^+$) pump.

1. Sodium-potassium ($Na^+ - K^+$) pump or $Na^+ - K^+$ ATPase

It is the most common pump found in all parts of the body. It uses the membrane-bound ATPase as a carrier molecule *i.e.* an enzyme that catalyses the hydrolysis of ATP. One of these ATPase is sodium-potassium activated ATPase ($Na^+ - K^+$ ATPase).

Structure

ATPase is composed of 6 subunits, three α (α_1, α_2 and α_3) and three β (β_1, β_2 and β_3), all extend through the membrane of most cells; however they have specialized function in certain tissues. Sodium and potassium transport occurs through α subunit. α-subunit has:

(i) *ATPase enzymatic activity i.e.* the ability to convert ATP to adenosine diphosphate (ADP), thereby releasing energy; and
(ii) *binding sites* on its intracellular and extracellular faces. The former contains binding sites for *three* Na^+ ions and an ATP molecule whereas the latter contains binding sites for *two* K^+ ions.

Mechanism of operation

The operation of the $Na^+ - K^+$ pump consists of two steps **(Fig. 2.6)**:

Step 1: Binding of $3Na^+$ ions and ATP to a carrier protein inside the cell transfers high energy phosphate group from ATP to aspartic acid residue of α subunit of ATPase (*phosphorylation*). This causes change in configuration of protein resulting in $3Na^+$ ions to move out of the cell.

Step 2: When $2K^+$ ions bind to the carrier protein on the outside of the cell, the aspartic acid-phosphate bond is hydrolysed (*dephosphorylation*). This causes second change

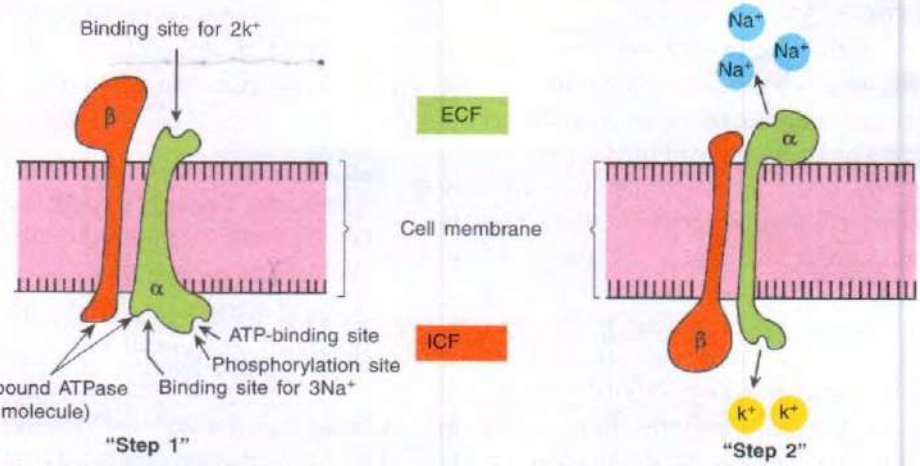


Fig. 2.6 Mechanism of operation of $Na^+ - K^+$ pump

in configuration of protein resulting in $2K^+$ ions to move into the cell.

Functions

1. $Na^+ - K^+$ pump is responsible for maintaining the high K^+ and low Na^+ concentrations in the cell. How? $Na^+ - K^+$ pump catalyzes the hydrolysis of ATP to ADP and uses the energy to force out $3Na^+$ ions from the cell and take $2K^+$ ions into the cell for each mole of ATP hydrolyzed. Thus it is an ***electrogenic pump*** with a ***coupling ratio*** of 3/2 and produces net movement of positive charge out of the cell. This electrical potential is a basic requirement in nerve and muscle fibers for transmitting electrical signals.
2. *Active transport of Na^+ ions and K^+ ions is one of the major energy-using processes in the body. It accounts for a large part of the basal metabolism.*
3. It helps in regulation of normal cell volume and pressure (page 35).

Inhibition of the pump

1. The pump requires binding by Na^+, K^+ and ATP for its operation. Therefore, if the concentration of any of these substances is too low, the pump does not function.
2. When temperature is reduced.
3. During oxygen lack.
4. Metabolic poisons *e.g.* 2,4 dinitrophenol (DNP) that prevents the formation of ATP.

Note

Na^+–K^+ pump activity is increased by: thyroid hormones, insulin, aldosterone and G-actin, whereas dopamine inhibits its activity.

Clinical Significance

Digitalis, a drug used for the treatment of heart failure, increases myocardial contractility by binding to the extracellular face of the α-subunit and interfering with the dephosphorylation step of the transport process. This inhibits the pump thereby increasing intracellular Ca^{2+} concentration and ultimately leads to increased myocardial contractility (also see to page 342).

2. Calcium (Ca^{2+}) pump or Ca^{2+} ATPase

(i) It is one of the ATPase other than $Na^+ - K^+$ ATPase. It is present in the sarcoplasmic reticulum of muscle cells, which maintains the intracellular ionic Ca^{2+} concentration below 0.1 mmol/L.

(ii) It is also located in the cell membrane and in many cell organelle membranes. In the cell membrane, the direction of Ca^{2+} transport is from cytoplasm to ECF. In cell organelle membranes it is from cytoplasm into the organelle lumen. This is why the cytoplasm of most cells has a very low Ca^{2+} concentration.

3. Potassium-hydrogen ($K^+ - H^+$) pump or $K^+ - H^+$ ATPase (or $H^+ - K^+$ ATPase)

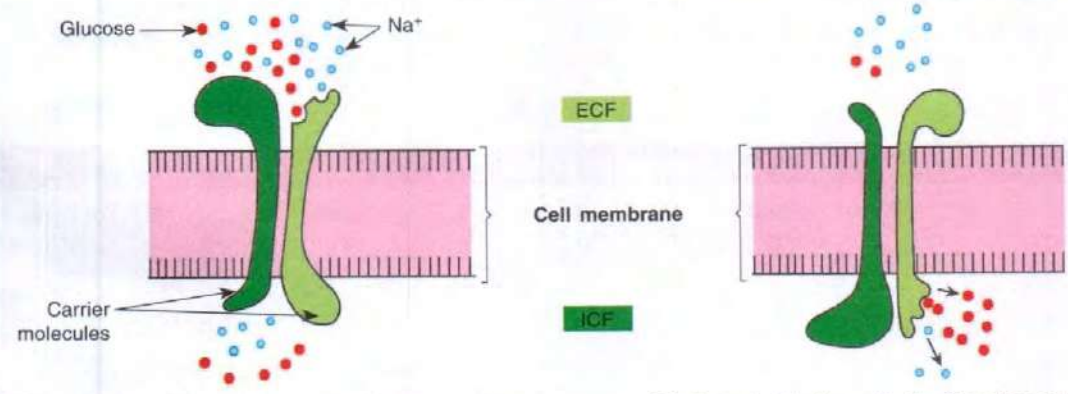
It is present in the cells of the gastric mucosa (page 218) and renal tubules (page 527) where it causes the secretion of H^+.

4. Proton ATPase

It is present in many cell organelles such as golgi complex and lysosomes (page 8) where it help to keep the interior acidic.

B. SECONDARY ACTIVE TRANSPORT PROCESSES

In some tissues, the active transport of Na^+ is coupled (linked) to the transport of other substances *i.e.* the transport of many ions and nutrients along their electrochemical energy gradients is accomplished by Na^+-dependent secondary active transport (Fig. 2.7). For example:



(A) The affinity of the protein carrier for glucose is increased in the presence of Na^+. The glucose and Na^+ pass through the membrane together.

(B) Once inside the cell, the Na^+ dissociates from the carrier, reducing the carrier's affinity for glucose and causing the glucose to dissociate from the carrier.

Fig. 2.7 Secondary active transport of glucose (*i.e.* active transport that uses an indirect energy source)

(1) Glucose and amino acids are reabsorbed from the proximal renal tubules (page 521) or absorbed from the intestinal lumen (page 260) only if Na^+ binds to the protein and is transported down its electrochemical gradient at the same time.
(2) Calcium is exchanged from the cytoplasm of cardiac and other muscle cells for extracellular Na^+, called ***$Na^+ - Ca^{2+}$ exchanger***. This causes muscle relaxation.
(3) Iodide pump (page 681).

Mechanism of secondary active transport

When Na^+ binds to the carrier molecule, the carrier molecule increases its affinity for the substances to be transported. When both Na^+ and the substance are bound to the carrier molecule, the carrier undergoes a configurational change during which both molecules are transported across the membrane.

In some cases, both Na^+ and the substance are transported in the same direction, whereas in others they are transported in opposite directions.

C. CARRIER TYPE PROCESSES

Carriers are transport proteins that binds ions and other molecules and then change their configuration, moving the bound molecules from one side of the cell membrane to the other. **(Fig. 2.8)**

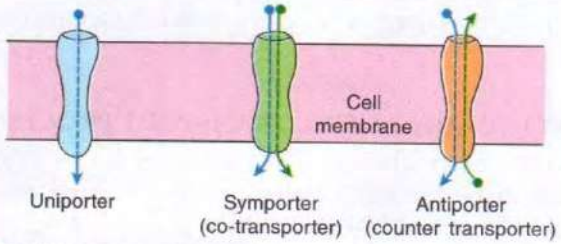


Fig. 2.8 Carrier type processes

Types

1. ***Uniporters*** are carriers that transport a single particle in one direction, such as the facilitated diffusion of glucose.
2. ***Symporters*** *(co-transporters)* transport two particles together in the same direction, such as the secondary active transport of glucose (see above).
3. ***Antiporters*** (*counter transporters*) transport molecules in opposite directions *i.e.* they exchange one substance for another. For example:
   (i) $Na^+ - K^+$ pump which moves $3Na^+$ out of the cell in exchange for $2K^+$ that moves into the cell.
   (ii) $Na^+ - Ca^{2+}$ exchangers in the muscle cells.
   (iii) $Na^+ - H^+$ exchangers in the renal tubules.

Important Note

Carrier type processes can be active or passive, depending on the location.

D. VESICULAR TRANSPORT PROCESSES or TRANSCYTOSIS

Many substances are transported across the cell membrane by *endocytosis* and *exocytosis*.

1. **Endocytosis** – 2 types:
   (i) ***Phagocytosis*** (cell eating). It is the process by which extracellular substances (bacteria, dead tissue, foreign particles etc.) are engulfed by the cells. The substance makes contact with the cell membrane, which then invaginates. The endocytic vesicle pinches off from the cell membrane and fuses with another intracellular vesicle *e.g.* lysosome, from which the ingested substance is released into ICF. **(Fig. 2.9)**

Note

If the substance ingested is in solution form, the process is called *pinocytosis (cell-drinking)*.

   (ii) ***Receptor-mediated endocytosis*** – the material to be transported first binds to a receptor, and then the receptor-substance complex is ingested by endocytosis. For example, transport of iron and cholesterol into the cells.

Summary: Characteristics of different types of transport

Type	Electro-chemical gradient	Carrier mediated	Metabolic energy required	Effect of inhibition $Na^+ - K^+$ pump
1. Simple diffusion	Downhill	No	No	Nil
2. Facilitated diffusion	Downhill	Yes	No	Nil
3. Primary active transport	Uphill	Yes	Yes	Inhibited
4. Cotransport	Uphill	Yes	Yes (indirectly)	Inhibited
5. Counter transport	Uphill	Yes	Yes (indirectly)	Inhibited

Downhill or Uphill means along or against the electrochemical gradient respectively

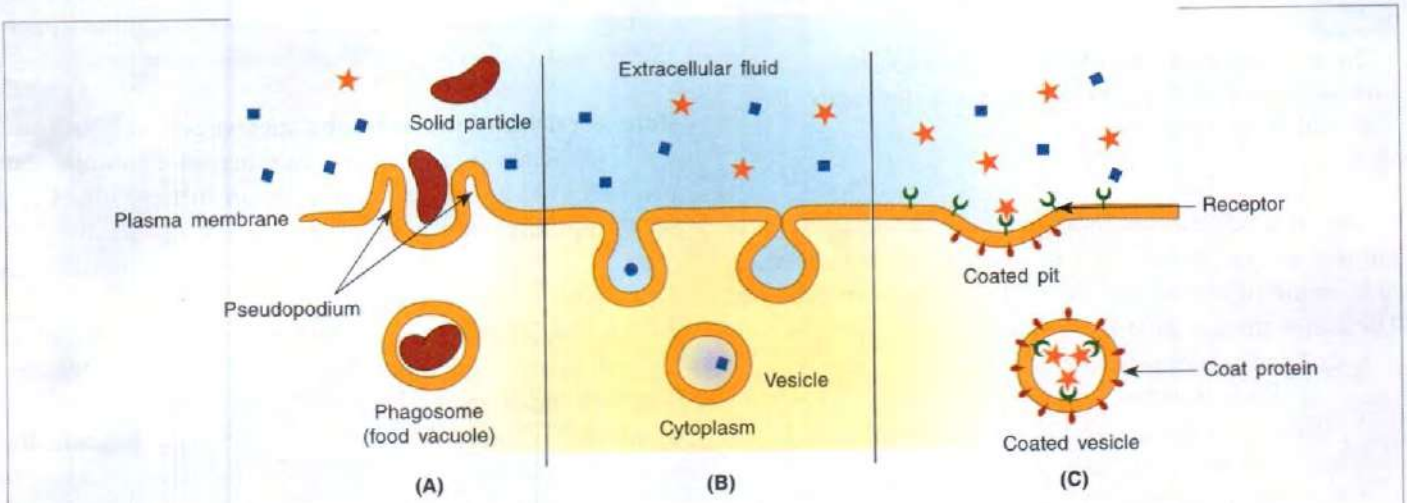


Fig. 2.9 Sequence of events in endocytosis (A), Pinocytosis (B) and Receptor mediated endocytosis (C)

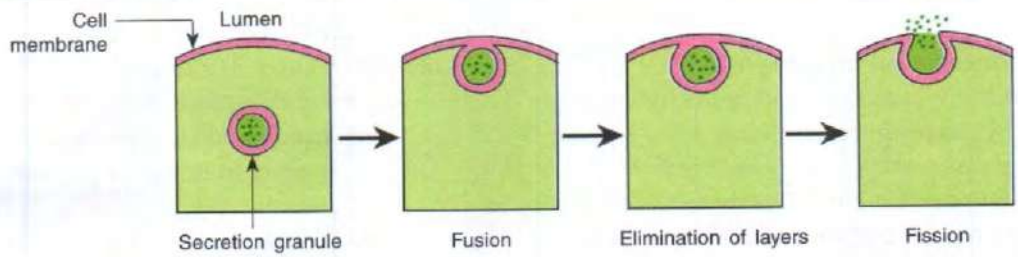


Fig. 2.10 Sequence of events in exocytosis

2. **Exocytosis** – Here substances secreted by the cell are trapped within vesicles or granules which fuse with the cell membrane and release their contents to the ECF. This leaves the contents of the vesicles or granules outside the cell and the cell membrane intact. It requires Ca^{2+} and energy **(Fig. 2.10)**. Hormones, digestive enzymes and synaptic transmitters are examples of substances transported out of the cell by this process.

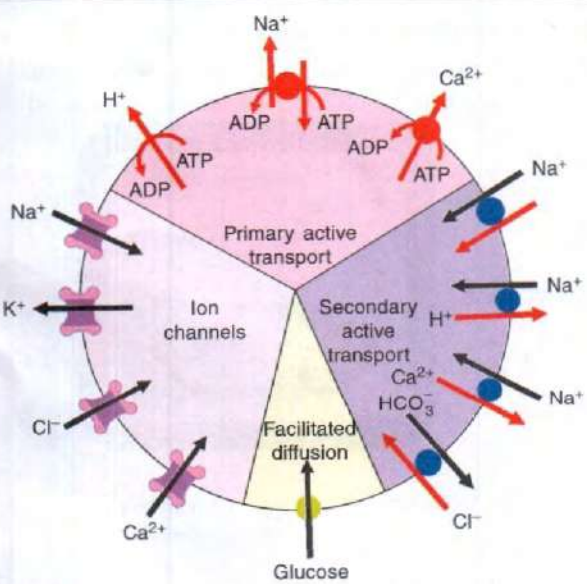


Fig. 2.11 Summary: Movements of substances across cell membranes.

Note

Vesicular transport processes of any kind require metabolic energy in the form of ATP.

INTERCELLULAR COMMUNICATION: CHEMICAL MESSENGERS

Cells communicate with each other via chemical messengers that include amines, amino acids, steroids, polypeptides, lipids, nucleotides etc. Some messengers move from cell to cell via *gap junction* without entering the ECF (page 10), and some messengers are secreted into the ECF to affect the functions of neighbouring cells. The intercellular communication mediated by messengers in the ECF are of *three* types:

1. *Neural communication i.e.* communication via synaptic junction (page 155).
2. *Paracrine communication.* Here products of cells diffuse in the ECF to affect the neighbouring cells. In addition, if product of cells bind to receptors on the same cell, it is called **autocrine communication**.
3. *Endocrine communication* in which hormones reach cells via the circulating blood (page 655).

Note

The extracellular ligands (page 15) are called ***First Messengers*** and the intracellular mediators are called ***Second Messengers.***

The second messengers generally activate ***protein kinases*** *i.e.* enzymes that catalyze the phosphorylation of tyrosine or serine and threonine residues in proteins. The major protein kinases are:

1. Calmodulin dependent *e.g.*
   (i) Phosphorylase kinase
   (ii) Ca^{2+}/calmodulin kinase I, II and III
   (iii) Myosin light-chain kinase
2. Protein kinase C
3. cAMP dependent kinase-protein kinase A
4. cGMP dependent kinase

Mode of action of chemical messengers

The mechanisms by which chemical messengers in the ECF bring about changes in cell function are:

(A). *By opening or closing ion channels in the cell membrane.* Some ligands (page 15) bind directly to ion channels in the cell membrane and then alter the permeability of membrane to the ion. For example, action of A-ch on motor end plate (page 155).

(B) *By stimulation of transcription.* The chemical messenger acts via cytoplasmic or nuclear receptors to increase transcription of mRNA. Thyroid and steroid hormones exert their effects in this fashion (page 654).

(C) *By activating phospholipase C.* Binding of ligand to its receptor activates phospholipase C on the inner surface of the membrane. Phospholipase C catalyzes the hydrolysis of *phosphotidylinositol diphosphate (PIP_2)* to form *inositol triphosphate (IP_3)* and *diacylglycerol (DAG)* both of which act as ***second messenger.***

(i) The IP_3 diffuses to the endoplasmic reticulum (ER), where it triggers the release of Ca^{2+} into the cell to produce physiological effects.

(ii) DAG stays in the cell membrane, where it activates protein kinase C to produce physiological effects **(Fig. 2.12).**

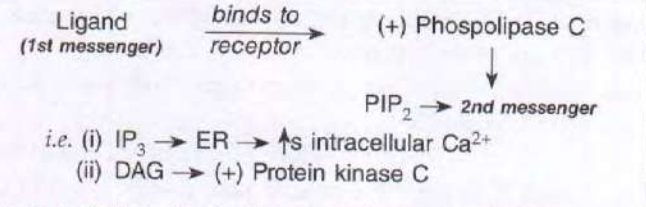


Fig. 2.12 Pathway for the formation of inositol triphosphate (IP_3) and diacylglycerol (DAG) from phosphotidylinositol diphosphate (PIP_2). (↑*s: increases; (+): activates; ER: endoplasmic* reticulum)

Angiotensin II (page 506) and catecholamines (page 735) and ADH (page 672) act in this fashion.

Role of calcium ions as second messenger

Ca^{2+} regulates a very large number of physiological processes (page 699), so regulation of intracellular Ca^{2+} is of great importance.

Regulation of intracellular Ca^{2+} **(Fig. 2.13)**

1. The free $[Ca^{2+}]$ in the cytoplasm at rest is 100 nmol/L and in the interstitial fluid is 1,200,000 nmol/L *i.e.* 12,000 times the cytoplasmic concentration.
2. Much of the intracellular Ca^{2+} is bound by the endoplasmic reticulum, mitochondria and ***calcium-binding proteins.*** The major Ca^{2+} binding protiens include:
   (i) *Troponin:* Ca^{2+} binding protein involved in contraction of skeletal muscle.
   (ii) *Calmodulin:* When it binds Ca^{2+}, it activates five different *calmodulin-dependent kinases.*
      (a) *myosin light-chain kinase,* which phosphorylates myosin to bring about contraction in smooth muscle.
      (b) *Phosphorylase kinase,* which activates phosphorylase
      (c) & (d) ***Ca^{2+}/calmodulin kinase I and II,*** are concerned with synaptic function; and
      (d) *Ca^{2+}/calmodulin kinase III,* concerned with protein synthesis.
   (iii) *Calcineurin* that inactivates Ca^{2+} channels by dephosphorylating them and also plays a role in activating T-cells.
3. Ca^{2+} enters the cells along its concentration and electrical gradient and cause opening of ***store-operated Ca^{2+} channel*** (***SOCC***) in the cell membrane to increase the concentration of free Ca^{2+} in the cytoplasm. This

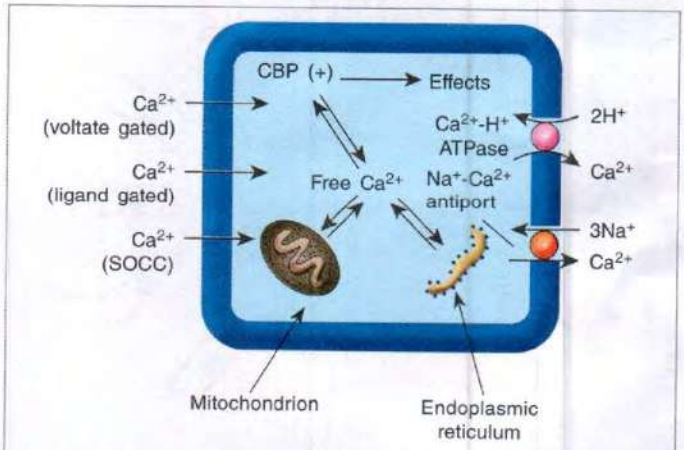


Fig. 2.13 Regulation of intracellular Ca^{2+}.
SOCC: Store-operated Ca^{2+} channel in cell membrane
CBP: Calcium binding proteins; (+): Stimulation

Summary: Mode of action of chemical messengers (See text for details)	
Mechanism	**Examples**
A. By opening or closing of ion channels in the cell membrane	(i) Action of A-ch on motor end plate (page 155) (ii) Action of catecholamine on ion channel on the heart (page 177)
B. By stimulation of transcription of mRNA	Action of steroid and thyroid hormones
C. By activating phosphorylase C with intracellular production of IP_3 and DAG.	(i) Angiotensin II (page 506) (ii) Catecholamines (page 736) (iii) ADH (page 671)
D. By altering activity of adenylate cyclase	Activation of phosphorylase kinase in the liver by epinephrine (page 601)
E. By increasing cGMP in cell	(i) Vasodilator action of nitric oxide on blood vessels (page 322) (ii) Phototransduction in rods and cones (page 1108)
F. By activation of G-protein	Actions of catecholamines (page 736); A-ch opioids (page 900); Angiotensin II, TSH; FSH, light (page 1108) etc.

in turn binds to and activates Ca^{2+}-binding protein and eventually activate a number of protein-kinases.

4. Ca^{2+} is pumped out of the cell by:
   (i) Ca^{2+}-H^+ pump (Ca^{2+}-H^+ ATPase) in exchange for two H^+; and
   (ii) by an antiport driven by the Na^+ gradient that exchanges $3Na^+$ for each Ca^{2+} *(Na^+-Ca^{2+} antiport)*
5. Many second messengers (specially IP_3) act by increasing the free cytoplasmic [Ca^{2+}] by releasing Ca^{2+} from intracellular stores - primarily the endoplasmic reticulum.

Mechanism of variable Ca^{2+} actions as second messenger

Ca^{2+} have different effects at low and at high intracellular concentrations. For example:

(1) Ca^{2+} may be in high concentration at the site of its release from an organelle or calcium bindng protein or a channel (***Ca^{2+} sparks***); and

(2) Ca^{2+} may be in low concentration after it diffuses throughout the cell.

Note

Ca^{2+} sparks plays an important role in the control of vascular tone. In addition, once released intracellular [Ca^{2+}] fluctuate at regular intervals and the amplitude of such fluctuations codes information for effector mechanisms.

(D) *By altering activity of adenylate cyclase* causing increased or decreased intracellular production of cAMP (page 653). A typical example is the activation of phosphorylase kinase in the liver by epinephrine via cAMP (page 601).

(E) *By increasing cyclic guanosine monophosphate (cGMP) in cell.* Increase in intracellular cGMP activates cGMP dependent kinase producing physiological effects. The enzyme guanylyl cyclase catalyzes the formation of cGMP. Nitric oxide produces vasodilation via this mechanism (page 322). cGMP also play important role in vision (page 1108).

(F) *G-proteins linked receptor activation* **(Fig. 2.14)**

(i) G-proteins or GTP (Guanosine triphosphate: An analog of ATP) binding proteins are membrane bound nucleotide regulatory proteins having 3 sub-units: α, β and γ. When the largest of 3 sub-units (*i.e.* α-sub-unit) binds GDP (Guanosine diphosphate), the 3 sub-units associate together.

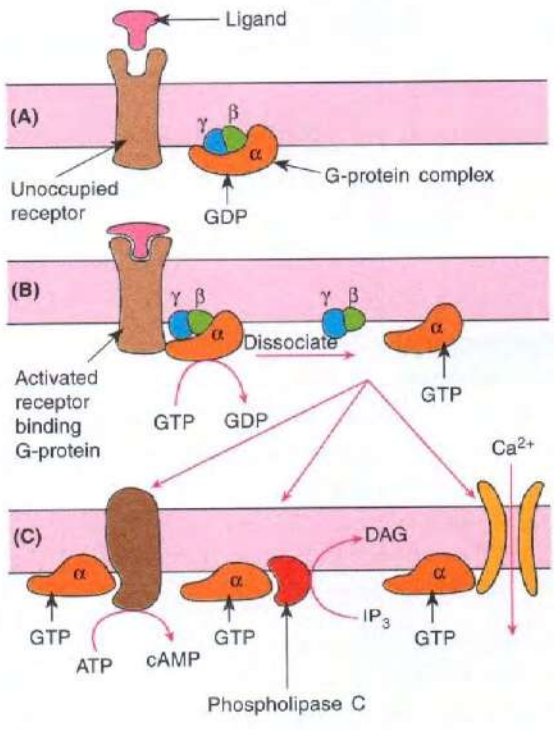


Fig. 2.14 G-protein linked receptor activation. (A) Receptor G-protein interaction; (B) Activation of G-protein linked receptor; (C) Effects of free α-sub-unit of G-protein on enzymes and channels.

(ii) G-protein gets activated when a receptor binds to its specific ligand. When this occurs, α-sub-unit exchanges *bound* GDP for GTP and dissociates from the β and γ sub-units. G-proteins, therefore, dissociate into 2 parts: the α-subunit and the βγ-subunit complex.

(iii) The free α-subunit then migrates in the cell membrane to modulate the activity of target cell in 3 ways:
  (a) interaction with adenylyl cyclase; or
  (b) interaction with phospholipase C; or
  (c) directly open an ion-channel.

(iv) Examples of some of the ligands for receptors that act via G-proteins are: Catecholamines (page 736), A-ch, opioids (page 900), angiotensin II, TSH, FSH, light (page 1108) etc.

Note

All G-protein coupled receptors tranverse the cell membrane seven times (called *serpentine receptors*).

Applied Aspects

Abnormalities caused by alteration in the function of G-protein receptors are given in Table 2.1.

Table 2.1: Receptor and G-protein diseases

Receptor Involved	Disease
I. Diseases due to mutation of the genes for receptors	
A. Due to loss of function of receptors	
1. Cone opsins	Colour blindness (page 1113)
2. Rhodopsin	Retinitis pigmentosa (page 1089) and night blindness (page 1108)
3. V_2-vasopressin (page 696)	Nephrogenic diabetes insipidus (page 674)
4. Endothelin and receptors	Hirschsprung's disease (page 253)
B. Due to gain of function of receptors	
1. Ca^{2+} receptors	Familial hypercalciuric hypocalcemia and secondary hyperparathyroidism (page 707)
2. G-protein	Acidophilic cell tumour of anterior pituitary with acromegaly (page 665)
II. Diseases due to production of antibodies against receptors	
1. Thyroid stimulating hormone (TSH)	Grave's disease (page 691)
2. Nicotinic A-ch receptors	Myasthenia gravis (page 158)

Study Questions

1. **Give physio-clinical significance of: diffusion, osmosis, non-ionic diffusion, solvent drag, hyperosmolar coma; chemical messengers; Fick's law of diffusion and factors affecting it; Receptor mediated endocytosis.**
2. **What determine distribution of water and solutes in different body compartments.**
3. **Differentiate between:**
   (i) active and passive transport processes
   (ii) simple and facilitated diffusion
   (iii) osmotic and oncotic pressure
   (iv) osmolarity and osmolality
   (v) endocytosis and exocytosis
   (vi) phagocytosis and pinocytosis
   (vii) isotonic and isosmotic solutions
   (viii) primary and secondary active transport processes.
   (ix) first and second messenger
4. **Depict diagrammatically**
   (i) mechanism of operation of $Na^+ - K^+$ pump.
   (ii) Phenomenon of Osmosis
   (iii) Secondary active transport
   (iv) Sequence of events in encocytosis and exocytosis
5. **What are the requirements for operation of $Na^+ - K^+$ pump. What will happen if the pump does not function?**

6. Give the normal value of human plasma osmolality. Enumerate the plasma components that contribute to it. Add a note on its clinical significance.
7. List the major processes that brings about the transport of substances across the cell membranes.
8. Write briefly about:
   (i) Protein kinases
   (ii) Role of calcium ion as second messenger
   (iii) Calcium binding proteins
   (iv) G-protein linked receptor activation
   (v) Ion channels
   (vi) Mechanism of diversity of calcium action.
   (vii) Solvent drag
9. Describe briefly principal ways that a chemical messengers in the extracellular fluid produce changes inside the cells.
10. Explain how does intercellular communication affect cellular physiology?
11. Briefly describe abnormalities caused by alteration in functions of G-protein receptors.

MCQs

1. Rate of diffusion of a substance from a region of its higher concentration to a region of lower concentration is directly proportional to:
   (a) Molecular size of the substance
   (b) Temperature
   (c) Thickness of the membrane
   (d) Water solubility of the substance
2. The concentration of sodium on the first side of a membrane is two times as great as on the second side of the membrane. This concentration is now increased to five times as great on the first side as on the second side. How many times does the net rate of sodium diffusion through the membrane increase?
   (a) Five times (b) Four times (c) 2.5 times (d) Two times
3. Gated ionic channels open or close by:
   (a) Alteration in membrane potential
   (b) Only when they bind to an internal ligand
   (c) Only when they bind to an external ligand
   (d) Depending on the lipid solubility of an ion
4. A major force affecting the distribution of water and solutes in different body compartments is:
   (a) Diffusion
   (b) Osmosis
   (c) Active transport processes
   (d) Sodium-potassium pump
5. Osmotic pressure of a solution is related to the:
   (a) Number of particles dissolved in the solution
   (b) Size and type of the particles
   (c) Chemical composition of the solution
   (d) Number of equivalents of an electrolyte in the solution
6. Approx. 90% osmolality of plasma is contributed by:
   (a) Plasma proteins
   (b) Glucose
   (c) Sodium and its accompanying anions
   (d) Urea
7. Active transport processes:
   (a) Are often referred as pumps
   (b) Are most used processes to move substances through the cell membrane
   (c) Always use the energy obtained from transport of other substances
   (d) Help transport of substances across cell membrane along the electro-chemical gradient
8. Sodium-potassium pump is characterised by all of the following *except*:
   (a) Requires binding by only Na^+ and K^+ for its activation
   (b) Uses membrane bound ATPase as a carrier molecule
   (c) Is responsible for maintaining high K^+ and low Na^+ concentration in the cell
   (d) Accounts for a large part of the basal metabolism
9. A large part of the basal metabolism is due to:
   (a) Operation of sodium-potassium pump
   (b) Flow of large molecules through membrane channels by facilitated diffusion
   (c) Phenomenon of osmosis
   (d) Sodium dependent secondary active transport
10. Sodium potassium pump can be inhibited by all, *except:*
    (a) If concentration of Na^+, K^+ and ATP is too low
    (b) When temperature is reduced
    (c) If plasma Ca^{2+} concentration decreases
    (d) Oxygen lack

11. **The exocytosis (or reverse pinocytosis) requires which ion?**
(a) Na^+ (b) K^+ (c) Ca^{2+} (d) Mg^{2+}

12. **Which is *not true* about second messengers?**
(a) Mediate intracellular responses to many different hormones and neurotransmitters
(b) Generally activate protein kinases
(c) are substances that interact with first messenger outside cells
(d) are intracellular mediators.

13. **The mechanism by which epinephrine activates the phosphorylase kinase in liver is mediated by:**
(a) opening or closing of ion channels in the cell membrane
(b) activation of transcription of mRNA
(c) Activation of G-protein
(d) Activation of adenylate cyclase.

14. **All are the major calcium binding proteins *except***
(a) Calmodulin (b) Actinin (c) Troponin (d) Calcineurin

15. **Simple diffusion *differs* from facilitated diffusion through a membrane in that simple diffusion:**
(a) Does not follow saturation kinetics
(b) Is a carrier mediated process
(c) Is an active process
(d) Occurs only across a selectively permeable membrane

16. **Oncotic pressure is:**
(a) Same as osmotic pressure
(b) Osmotic pressure across the capillary wall
(c) Pressure due to the plasma colloids
(d) 37 mmHg at capillary arteriolar end

17. **Hyperosmolar coma is associated with:**
(a) Cellular overhydration
(b) Increased colloidal osmotic pressure of the interstitial fluid
(c) Cellular dehydration
(d) Increased hydrostatic pressure of the plasma

Answers

1. (b)	2. (b)	3. (a)	4. (a)	5. (a)	6. (c)	7. (a)	8. (a)	9. (a)	10. (c)
11. (c)	12. (c)	13. (d)	14. (b)	15. (a)	16. (c)	17. (c)			

Chapter 3

Body Water and Body Fluids

I. Introduction
II. Distribution of total body water (TBW)
III. Measurement of body fluid volumes
IV. Ionic composition of body fluids
V. Units for measuring concentration of solutes
(A) Moles (B) Equivalents (C) Osmoles
(D) Concept of pH and H^+ concentration (E) Concept of buffer system

INTRODUCTION

1. In an average young adult male (Fig. 3.1):
   (i) 7% of body weight is mineral
   (ii) 15% is fat
   (iii) 18% is protein and related substances, and
   (iv) 60% is water, called ***total body water (TBW)***.
2. TBW is about 10% lower in young females due to relatively greater amount of adipose tissue (subcutaneous fat)
3. In *infants* TBW is 65-75% of body weight.

DISTRIBUTION OF TOTAL BODY WATER (TBW)

The distribution of the total water in the body is as given in Table 3.1.

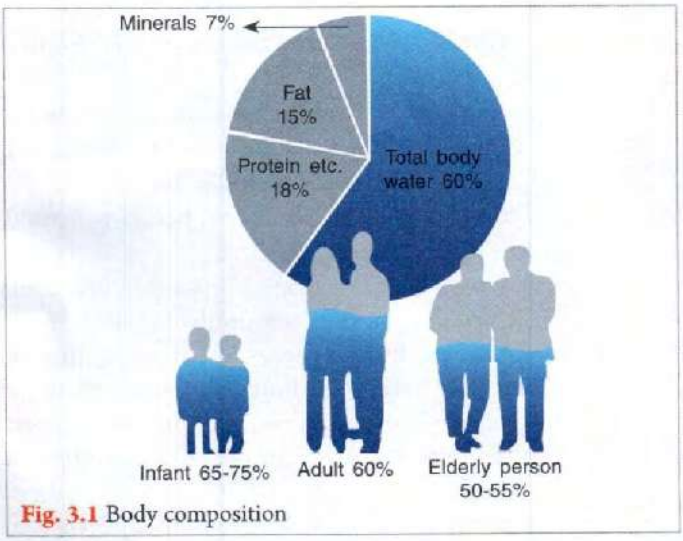


Fig. 3.1 Body composition

Table 3.1: Distribution of total body water (TBW) in a normal adult 70 kg person

Compartment	Volume (L)	Percent	
		Body weight	Body water
Total Body Water (TBW)	42	60	100
A. Extra Cellular Fluid (ECF)	14	20	33 (1/3rd TBW)
B. Intra Cellular Fluid (ICF)	28	40	67 (2/3rd TBW)

A. EXTRA CELLULAR FLUID (ECF) COMPARTMENT

This is fluid contained in the spaces outside the cell. ECF compartment includes: *Plasma, interstitial fluid* and *transcellular fluid*. (Table 3.2)

Table 3.2: Distribution of extra cellular fluid (ECF) in a normal adult 70 kg person

Compartment	Volume (L)	Percent	
		Body weight	Body water
Plasma (25% of ECF)	3.5	4-5	8
Interstitial plus Transcellular Fluid (75% of ECF)	10.5	15	25

1. ***Plasma*** is the fluid portion of the blood (page 49). It represents 25% of the ECF. Its volume can be calculated from blood volume and PCV (packed cell volume) as under:

$$\text{Plasma Volume (L)} = \text{Blood volume (L)} \times \frac{100 - \text{Hematocrit (PCV)}}{100}$$

Blood Volume *i.e.* plasma and blood cells which fill the vascular system. It is approx. 80 mL/kg of body weight or 8% of the total body weight.

2. ***Interstitial Fluid:*** It is that part of ECF that is outside the vascular system. It surrounds all cells except blood cells and includes ***lymph***. It is in constant motion throughout the body and is transported rapidly in the circulating blood. Lymph constitutes 2–3% of the total body weight.
3. ***Transcellular fluid:*** It represents fluid in the lumen of structures lined by epithelium. It includes: digestive secretions; sweat; cerebrospinal fluid (CSF); pleural, peritoneal, synovial, intraocular (aqueous and vitreous humours) and pericardial fluids, bile and luminal fluids of the gut, thyroid and cochlea. Transcellular fluid volume is relatively small, about 1L ≡ 15 mL/kg of body weight (1.5% of body weight).

Important Note

The normal cell function depends upon the constancy of the fluid that forms the actual immediate environment of the cells. For this reason blood is called the internal environment of the body or *Millieu Interieur* (a term coined by *Claude Bernard*, French physiologist). (Also refer to page 3)

B. INTRA CELLULAR FLUID (ICF) COMPARTMENT

This is the fluid contained within the body cells. This is larger of the two major fluid compartments. The volume of the ICF compartment varies.

MEASUREMENT OF BODY FLUID VOLUMES

The volume of water in each fluid compartment can be measured by the ***indicator dilution principle***. This principle is based on the relationship between:

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

The equation for the relationship is:

$$C = \frac{A}{V} \quad i.e. \quad V = \frac{A}{C}$$

Characteristics of the Indicator (marker) used

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

The indicator may leave the compartment by excretion or metabolism during the time allowed for mixing. Then

$$\text{Volume of distribution (V)} = \frac{\text{(A) administered minus (A) removed}}{C}$$

A. EXTRACELLULAR FLUID VOLUME (ECFV) MEASUREMENT

The ECFV is difficult to measure, because:

(i) limits of this space are ill-defined;
(ii) few substances mix rapidly in all parts of the space while remaining exclusively extracellular.

Methods

1. Most accurate method to measure ECFV is by using ***inulin*** (polysaccharide, MW 5200). Radioactive (RA) inulin is prepared by substituting ^{14}C for one of the carbon atoms of the molecule. RA inulin levels are easily determined by counting the samples with suitable radiation detectors.
2. As Cl^- is largely extracellular, RA isotopes of Cl^- ($^{36}Cl^-$ and $^{38}Cl^-$) have been used for determining ECFV. However, some Cl^- is intracellular, therefore, ECFV determined using Cl^- are greater than actual ECFV.
3. Like Cl^-, other anions that have been used to measure ECFV includes: ^{82}Br, sulphate, thiosulphate, thiocyanate and ferrocyanide. These ions interchange with Cl^- in the body and determine greater values for ECFV.
4. Mannitol and sucrose have also been used to measure ECFV.
   (1) ***Plasma Volume*** is measured using either of the two dilution methods.
      (i) The first method employs substances that neither leave the vascular system nor penetrate the RBCs; such substances include:
         (a) *Evans Blue Dye* (T-1824) that becomes bound to plasma proteins.
         (b) *Radio-iodinated human serum albumin* (RISA) *i.e.* serum albumin labelled with RA iodine. It slowly leaks out of the circulation into the interstitial fluid. Suitable sample of injected solution and plasma samples obtained after injection are counted in a scintillation counter.
         (c) *Radio-iodinated gamma globulin and fibrinogen.* These substances generally do not leak out of the blood stream.

(ii) The second method is based on the fact that the radio-isotopes of phosphorus (^{32}P), iron ($^{55,59}Fe$) and chromium (^{51}Cr) penetrate and bind to RBCs. Therefore, the ***Red Cell Volume*** *i.e.* volume occupied by all the circulating RBCs in the body can be measured by injecting tagged RBCs intravenously (I.V.) and after mixing has occurred, measuring the fraction of the RBCs that is tagged. Commonly used tag is *^{51}Cr (RA isotope of chromium)* that is attached to the cells by incubating them in a suitable 'Cr' solution. Then

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

(practically PCV = RBC volume)

(2) ***Interstitial Fluid Volume*** cannot be measured directly because:

(i) difficult to sample it;

(ii) no substance is distributed exclusively in this compartment.

Substances which librate here also equilibrate in plasma. Therefore, the interstitial fluid volume is determined as the difference between ECFV and plasma volume (*i.e.* ECFV minus plasma volume).

B. INTRACELLULAR FLUID VOLUME (ICFV) MEASUREMENT

ICFV cannot be measured directly by dilution, because no substance is confined exclusively to this compartment after I.V. administration. It is determined indirectly as:
ICFV = TBW – ECFV.

TBW can be measured by indicator dilution principle (page 28). *Deuterium Oxide* (D_2O, heavy water) has properties that are slightly different from H_2O, but in equilibration experiments for measuring TBW, it gives accurate results. *Tritium Oxide* and *aminopyrine* have also been used for this purpose.

Important Note

The ECFV/ICFV ratio is larger in infants and children than it is in adults, but the absolute volume of ECF in children is smaller than it is in adults. Therefore, dehydration develops more rapidly and is frequently more severe in children than in adults.

IONIC COMPOSITION OF BODY FLUIDS

Characteristic features

1. Ions constitute approx. 95% of the solutes in the body fluids (Table 3.3).
2. The distribution of electrolytes in various body compartments differs markedly. *However, the sum of the concentrations of the cations equals the sum of the concentrations of the anions in each respective compartment,* making the fluid in each compartment electrically neutral.
3. Na^+, Ca^{2+}, Cl^- and HCO_3^- are largely extracellular; whereas, K^+, Mg^{2+}, organic phosphates (PO_4^{3-}), and proteins ($prot^-$) are predominantly present in the ICF.

Table 3.3: Ionic distribution in the various body fluids (concentration in mEq/L of H_2O)

	Ion	Plasma	Interstitial Fluid	I.C.F.
Cations				
	Na^+	153	145	12
	K^+	5.4	5	155
	Mg^{2+}	1.9	2	15
	Others	2.7	2	2
	Total Cations	**163**	**154**	**184**
Anions				
	Cl^-	111	110	8
	HCO^{3-}	26.2	27	8
	Phosphate (PO_4^{3-})	1	2	90
	Proteins ($Prot^-$)	17.2	15	60
	Others	7.6	–	18
	Total Anions	**163**	**154**	**184**
Total Ions		**326**	**308**	**368**

Note

Ca^{2+} concentration gradient on outside to inside the cell is 12,000:1, see pages 22 and 36.

4. Essentially all of the body K^+ is in the exchangeable pool, whereas only 65%–70% of the body Na^+ is exchangeable. *Only the exchangeable solutes are osmotically active.*
5. Almost all of the body Ca^{2+} (in bone) and most of the body Mg^{2+} (in bone and cells) are non-exchangeable.

UNITS FOR MEASURING CONCENTRATION OF SOLUTES

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

A. MOLES

The mole is the standard unit for expressing the amount of substances in the SI unit system. A ***mole*** is the gram-molecular weight of a substance, *i.e.*, the molecular weight of the substance in grams. Each mole (mol) consists of approx. 6×10^{23} molecules. Thus, 1 mole of KCl = 39 + 35.5 gm = 74.5 gm (*i.e.* sum of atomic masses of all the atoms in the molecule).

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

1. The concentrations of two different substances on the basis of number of grams per litre of solution does not indicate how many molecules of each compound are present. Therefore, concentrations in units of grams per litre are often used when the chemical structure of the solute is unknown.
2. When the structure of a molecule is known, concentrations are expressed as moles per litre. This provides a unit of concentration based upon the number of molecules of the solute in solution. Thus a solution containing 74.5 gm of KCl in 1 litre of solution is said to be ***one-molar (1M or 1 mol/L)*** solution of KCl.
3. Since 1 mole of any molecule will have the same number of molecules (6×10^{23}), therefore, 1M solution of KCl contains the same number of solute molecules per litre as a 1M solution of glucose or any other substance.

Note

The concentrations of many solutes dissolved in the body fluids are much less than 1M, being in the range of millimoles per litre (1 mM = 0.001M).

B. EQUIVALENTS

1. The *equivalent* is the standard unit for expressing the solutes in the body which are in the form of charged particles. One ***equivalent (Eq)*** is 1 mole of an ionized substance divided by its valency. One mole of KCl dissociates into 1 Eq of K^+ and 1 Eq of Cl^-. One Eq of K^+ = 39 gm/1 = 39 gm; but 1 Eq of Ca^{2+} = 40gm/2 = 20 gm. The milliequivalent (mEq) is 1/1000 of 1 Eq.
2. The ***normality*** (N) of a solution is the number of gram equivalents in 1 litre. Therefore, 1 N solution of hydrochloric acid contains 1 + 35.5 gm/L = 36.5 gm/L.

C. OSMOLES

1. The amount of concentrations of osmotically active particles are usually expressed in *osmoles (osm)*. One ***osmole*** equals the gram molecular weight (*i.e.* one mole) of the substance divided by the number of freely moving particles each molecule liberates in solution.

The ***milliosmole (mosm)*** is 1/1000 of 1 osm.

$$\text{As 1 osmole} = \frac{\text{Gram molecular weight (\textit{i.e.} one mole) of a substance}}{\text{Number of freely movable particles, each molecule liberates in solution}}$$

therefore

(i) if a solute is a non-ionizing compound (*e.g.* glucose), then one osmole is equal to 1 mole of solute particle. Thus, 1-molar solution of glucose has a concentration of 1 osm (1 osmole per litre);

(ii) if the solute ionizes and forms an ideal solution, each ion is an osmotically active particle, thus in a 1-molar solution of NaCl, NaCl would dissociate into Na^+ and Cl^- ions, so that each mole in solution would supply 2 osmoles of solute per litre of solution. Similarly, one mole of $CaCl_2$ would dissociate into Ca^{2+}, Cl^- and Cl^- supplying 3 osmoles.

2. The number of osmoles per litre of solution is called ***Osmolarity***, *e.g.* plasma; whereas the number of osmoles per kilogram of solvent is ***Osmolality***.

Note

***Osmolarity* is affected by the volume of the various solutes in the solution and the temperature, while the osmolality is not.**

3. Osmotically active substances in the body are dissolved in water; as the density of water is 1, therefore, *osmolal* concentration is expressed in osmoles per litre (osm/L) of water.

D. CONCEPT OF pH AND H^+ CONCENTRATION

1. H^+ concentration of various body fluids is expressed in two different ways, either directly as $[H^+]$ or indirectly as pH (pH stands for power of hydrogen). **(Table 3.4)** pH refers to the negative logarithm of the $[H^+]$.
   The relation between $[H^+]$ and pH can be expressed as:
   (i) $pH = \log_{10} 1/[H^+]$
   (ii) $pH = -\log_{10} [H^+]$

Note

For decrease in each pH unit (*e.g.* from 7.0 to 6.0), the $[H^+]$ is increased 10 fold; for each pH unit increase (*e.g.* 7 to 8), it is decreased 10 fold.

2. ***pH and $[H^+]$ are inversely related.*** Another advantage of the pH concept is that when the pK of a buffer system is known, it is immediately possible to determine the effective pH range of the buffer, where K = the ionization or dissociation constant. Therefore,

Table 3.4: pH and $[H^+]$ of various body fluids

pH		$[H^+]$ nmol/L or nEq/L = 10^{-9} mol/L
8.00	(maximum alkaline urine)	10
7.70	of plasma (severe alkalosis)	20
7.41	(arterial blood)	
7.40	(plasma)	40 How? plasma at pH 7.4 *i.e.* $[H^+]$ of 40×10^{-9} mol/L
7.38	(mixed venous blood)	
7.30	(CSF)	50
7.20	(RBC)	
7.10	(ICF)	80
7.00	(pure water) 'neutral'	100; pH of water at 25°C, in which H^+ and OH^- are present in equal numbers, $[H^+] = [OH^-] = 10^{-7}$ mol/L
6.9	of plasma (severe acidosis)	126
6.0	(normal urine)	1000
4.5	(urine maximum acidity)	31600
1.0	(pure gastric juice)	10^8

pK = negative log of K (–log K) and is equal to the pH at which half of the acid molecules are dissociated and half are undissociated.

3. Blood pH always refers to plasma pH (7.4), the range of $[H^+]$ that is *compatible with life* is 20-126 mEq/L ≡ pH of 7.7 to 6.9.

Optimal pH range for blood at which human body functions properly is 7.35 to 7.45 (7.4 ± 0.05). Clinically, blood pH < 7.35 is referred as ***Acidosis****, and blood pH > 7.45, as* ***Alkalosis*** **(Fig. 3.2).**

E. CONCEPT OF BUFFER SYSTEM

A buffer is a substance that has the ability to bind or release H^+ in solution.

A buffer in a solution consists of a weak acid and its conjugate base, thus keeping the pH of the solution relatively constant despite the addition of considerable quantities of acid or base. Buffering is the primary means by which large changes in $[H^+]$ are minimized (also refer to page 55).

Dynamics of Buffering: The Henderson-Hasselbalch Equation

The general equation for a buffer system is

$$HA \rightleftharpoons H^+ + A^-$$

where A^- represents any anion and HA the *undissociated acid*.

If an acid stronger than HA is added to a solution containing this buffer system, the equilibrium is shifted to the left. H^+ are 'tied up' in the formation of more undissociated HA, so the increase in H^+ concentration is much less than it would otherwise be. Conversely, if a base is added to the solution, H^+ and OH^- react to form H_2O; but more HA dissociates, limiting the decrease in H^+ concentration. By the *law of mass action*, the product of the concentrations of the products in a chemical reaction divided by the product of the concentration of the reactants at equilibrium is a constant:

$$\frac{[H^+]\,[A^-]}{[HA]} = K$$

If this equation is solved for H^+ and put in pH notation (pH is the negative log of $[H^+]$), the resulting equation is that originally derived by Henderson and Hasselbalch to describe the pH changes resulting from addition of H^+ or OH^- to any buffer system *(Henderson-Hasselbalch equation)*:

$$pH = pK + \log \frac{[A^-]}{[HA]}$$

It is apparent from these equations that the buffering capacity of a system is greatest when the amount of free anion is equal to the amount of undissociated HA *i.e.* when $[A^-]/[HA] = 1$, so that $\log [A^-]/[HA] = 0$ and pH = pK. *This is why the most effective buffers in the body would be expected to be those with pKs close to the pH in which they operate.* The pH of the blood is normally 7.4; that of the cells is probably about 7.2; and that of urine varies from 4.5 to 8.0.

The pK of the bicarbonate system is 6.1
The pK of the dibasic system is 6.8
The pK of the Ammonia system is 9.0
(For details refer to pages 551 and 559)

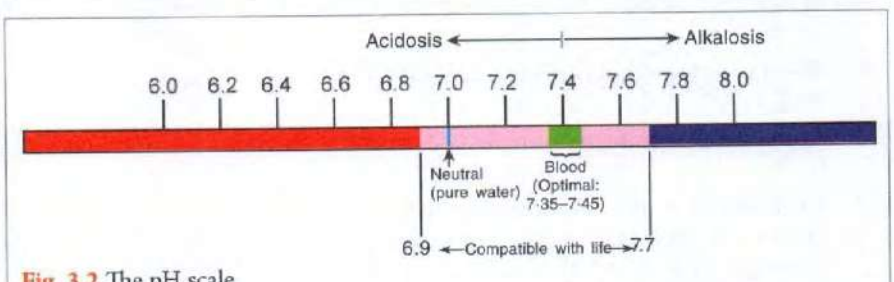


Fig. 3.2 The pH scale

Study Questions

1. **Give physiological significance of:**
   (i) transcellular fluid
   (ii) moles, osmoles and equivalent
   (iii) pH and buffering
   (iv) total body water
2. **Name the different body fluid compartments in the human body. Describe the ways in which their volumes can be measured.**
3. **Explain, why dehydration develops rapidly and is often more severe in children than in adults.**
4. **Give the range of normal pH of blood. What blood pH is compatible with life and why?**
5. **Give distribution of ions in various body fluids compartments. Explain its physiologic basis.**
6. **Depict diagramatically: The pH scale.**

MCQs

1. **Total blood volume is approximately:**
   (a) 8% (or 80 mL/kg) of body weight
   (b) 15% (or 150 mL/kg) of body weight
   (c) 25% (or 250 mL/kg) of body weight
   (d) 30% (or 300 mL/kg) of body weight
2. **Synovial, intraocular and pericardial fluids are examples of:**
   (a) Lymph
   (b) Interstitial fluid
   (c) Transcellular fluid
   (d) Intracellular fluid
3. **If plasma volume is 3 litres and PCV is 40%, then total blood volume is:**
   (a) 4 litres
   (b) 4.5 litres
   (c) 5 litres
   (d) 5.5 litres
4. **Dehydration develops more rapidly and is frequently more severe in children than adults because in children:**
   (a) ECFV/ICFV ratio is smaller
   (b) ECFV/ICFV ratio is larger
   (c) Total body water is larger
   (d) Total ECFV is smaller
5. **Which of the following ion is almost non-exchangeable in the body?**
   (a) Sodium
   (b) Potassium
   (c) Chloride
   (d) Calcium
6. **Major anion in intracellular fluid is:**
   (a) Protein
   (b) Phosphate
   (c) Chloride
   (d) Bicarbonate
7. **A mole of albumin with molecular weight 69,000 exerts an osmotic effect identical to:**
   (a) 69 mmHg
   (b) Cannot exert an osmotic effect because of very high molecular weight
   (c) Na^+ which has an atomic weight of 23
   (d) Oncotic pressure
8. **One mole is:**
   (a) Sum of atomic masses of all the atoms in the molecule
   (b) Mole of an ionized substance divided by its valency
   (c) Number of gram equivalents in one litre
   (d) Gram molecular weight of the substance divided by the number of freely moving particles each molecule liberate in solution
9. **In intracellular fluid, the single substance with the greatest osmotic activity is:**
   (a) Protein (b) Potassium (c) Urea (d) Phosphate
10. **Blood pH range that is compatible with life is:**
    (a) 7.4 ± 0.5 (b) 6.9 – 7.7 (c) 6.7 – 7.9 (d) 4.5 – 6.00
11. **In an average young adult male, ...% is fat:**
    (a) 7 (b) 15 (c) 18 (d) 25
12. **E.C.F. differs from I.C.F. in man in that:**
    (a) It forms major portion of total body water
    (b) It has lower tonicity
    (c) Its principal anions are organic
    (d) It has higher $Na^+ : K^+$ molar ratio

13. **The fluid in each body compartment is electrically neutral because:**
    (a) Ions constitute approx. 95% of the solutes in the body fluids
    (b) Na^+ and Cl^- are largely extracellular, whereas K^+ and PO_4^{3-} are predominantly intracellular
    (c) Essentially all of the body K^+ and Na^+ is in the exchangeable pool
    (d) Sum of concentration of cations equals the sum of concentration of anions in each respective compartment

14. **For bicarbonate buffer system the Henderson-Hasselbach equation is:**
    (a) $pH = pK + HCO_3^-/H_2CO_3$
    (b) $pH = pK + H_2CO_3/HCO_3^-$
    (c) $pH = pK + \log HCO_3^-/H_2CO_3$
    (d) $pH = pK + \log H_2CO_3/HCO_3^-$

Answers

1. (a) 2. (c) 3. (c) 4. (d) 5. (d) 6. (b) 7. (c) 8. (a) 9. (d) 10. (b)
11. (b) 12. (d) 13. (d) 14. (c)

Chapter 4

The Membrane Potentials

I. Introduction: Ionic composition of body fluids
Gibbs-Donnan membrane equilibrium

II. Resting membrane potential:
Genesis, equilibrium potential; variation in membrane potential.

III. Action potential:
Definition; Origin; phases; ionic basis; properties; electrotonic potentials (graded potentials); extracellular (surface) recording – biphasic and monophasic; injury (demarcation) potential.

INTRODUCTION

1. The cell membrane separates the intracellular fluid (ICF) from extracellular fluid (ECF) *i.e.* separating the two compartments having widely different ionic compositions (**Table 3.3**, page 29).
2. *What causes differences in the ionic composition of ICF and ECF?*
   (i) Resting cell membrane is effectively impermeable (or moderately permeable) to Na^+.
   (ii) Resting cell membrane is freely permeable to K^+ and Cl^- (K^+ permeability 50-100 times greater than Na^+ permeability).
   (iii) Cell membrane is practically impermeable to most of the intracellular anions such as proteins and organic phosphate ions.
   (iv) Presence of *carriers* for certain ions and other molecules on the cell membrane, *e.g.* glucose etc.
3. *Why are there differences in the permeability of cell membrane to various small ions?*
   The ions in the body are *hydrated i.e.* ion with its full complement of water. Thus, although the atomic weight of potassium (39) is greater than the atomic weight of sodium (23), the hydrated Na^+ is larger than the hydrated K^+; that is why Na^+ moves through the cell membrane with greater difficulty than K^+. **(Table 4.1)**
4. ***Gibbs-Donnan membrane equilibrium***
   Inspite of marked concentration gradient across the cell membrane, this uneven distribution of ions is constantly maintained under resting conditions. *How?*
   Consider two ionized solutions 'a' and 'b' filling compartments of constant volume and separated by a membrane permeable to cations as well as anions. At equilibrium two things occur:

Table 4.1: Size of hydrated ions and other substances

Substance	Atomic or Molecular weight	Radius (nm)
K^+	39	0.12
Cl^-	35.5	0.12
H_2O	18	0.12
Ca^{2+}	40	0.15
Na^+	23	0.18
Urea	60	0.23
Glucose	180	0.38
Albumin	69,000	7.50

solution 'a'	solution 'b'

(i) Each solution will be electrically neutral *i.e.* total charges on cations will be equal to those on anions. Therefore,
$(Cations)_a = (Anions)_a$ and
$(Cations)_b = (Anions)_b$ ... (1)

(ii) The product of *diffusible* (penetrating) ions on one side of the membrane will be equal to product of *diffusible* ions on the other. Therefore,
$(Diffusible\ cations)_a \times (Diffusible\ anions)_a$
$= (Diffusible\ cations)_b \times (Diffusible\ anions)_b$ ... (2)

$$i.e.\ \frac{(diffusible\ cations)_a}{(diffusible\ anions)_b} = \frac{(diffusible\ cations)_b}{(diffusible\ anions)_a}$$

But if one or more indiffusible (non-penetrating) ions are also present, the ionic distribution of the diffusible ions

at equilibrium will be asymmetrical. Consider that KCl is present in solution 'a' and 'b', but only 'a' contains a salt K^+X^-, where X^- is an non-diffusible anion unable to cross the membrane (*e.g.* protein or organic phosphate anion).

solution 'a'	solution 'b'
K^+	K^+
Cl^-	Cl^-
X^-	

Therefore, the penetrating ions K^+ and Cl^- diffuse until equilibrium is attained, the two criteria (1) and (2) established above will hold *i.e.*

$$\left.\begin{aligned}(K^+)_a &= (Cl^-)_a + (X^-)_a \\ (K^+)_b &= (Cl^-)_b\end{aligned}\right\} \text{(electrically neutral)}$$

and $$\left.\begin{aligned}(K^+)_a \times (Cl^-)_a &= (K^+)_b \times (Cl^-)_b \\ \frac{(K^+)_a}{(K^+)_b} &= \frac{(Cl^-)_b}{(Cl^-)_a}\end{aligned}\right\} \text{(products and ratios of diffusible ions)}$$

From the relationship it follows that:

$$(K^+)_a > (Cl^-)_a$$

and, therefore, that

$$(K^+)_a > (K^+)_b \text{ and } (Cl^-)_a < (Cl^-)_b$$

and $$(K^+)_a + (Cl^-)_a > (K^+)_b + (Cl^-)_b$$

Hence at equilibrium two things will occur:

(i) *the diffusible cation (K^+) concentration on the side of the membrane containing the non-penetrating anion X^- is greater than the diffusible cation concentration on the other side;*

(ii) *the diffusible anion (Cl^-) concentration will be greater on the side without the non-diffusible anion. This is known as the* ***Gibbs Donnan Membrane Equilibrium.***

5. ***Gibbs-Donnan effect***

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

(i) There will be an electrical difference across the cell membrane whose magnitude can be determined by ***Nernst equation*** (page 36).

(ii) Because of protein anions ($prot^-$) and other organic molecules in the cells, there are more osmotically active particles in the cells than the interstitial fluid. This would make them swell and eventually rupture; but prevented by operation of $Na^+ - K^+$ pump which produces net movement of positive charge out of the cell and keeps the inside and outside of the cell in osmotic equilibrium. Thus normal cell volume and pressure depend on $Na^+ - K^+$ pump. (Page 18)

Important Note

Ischaemia decreases $Na^+ - K^+$ pump activity causing swelling and damage of body cells, that is why cerebral ischaemia, if prolonged, is always fatal.

RESTING MEMBRANE POTENTIAL or STEADY POTENTIAL or TRANSMEMBRANE POTENTIAL

When two electrodes are placed on the surface of a cell and are connected through a suitable amplifier to a *cathode ray oscilloscope* (CRO *i.e.* voltage amplifier recorder system), no potential difference is observed. However, if one electrode is inserted into the interior of the cell, a constant (or *steady*) potential difference is observed between the inside and the outside of the cell at rest, called as the ***Resting Membrane Potential*** **(Fig. 4.1)**. This is also termed as *steady potential* or *transmembrane potential* and indicates the resting state *i.e.* ***state of polarisation*** of the cell membrane. It is written with a *minus sign*, signifying that inside is negative relative to the exterior. Its *magnitude* varies considerably from tissue to tissue *ranging from –10mV to –100mV.*

GENESIS OF RESTING MEMBRANE POTENTIAL (RMP)

Why negativity inside and positivity outside the membrane?

(i) It is due to distribution of ions across the cell membrane. Some K^+ diffuses out of the cell along its concentration gradient while non-diffusible anions (*e.g.* proteins etc.) stay in the cell, creating a potential difference across the membrane. Therefore, there is a slight excess of cations outside the membrane and slight excess of anions inside. (It is worth noting that the actual number of ions responsible for the membrane potential is a minute fraction of the total number present across the cell membrane in each respective compartment.)

(ii) $Na^+ - K^+$ pump is ***electrogenic*** at rest with a coupling ratio of 3/2 (page 19). It does not generate the membrane potential when the coupling ratio is 1. However, it maintains the concentration gradient on which the existence of the membrane potential depends.

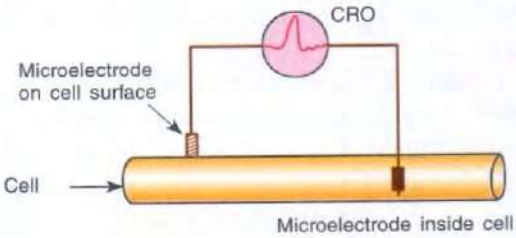


Fig. 4.1 Arrangement to record resting membrane potential (R.M.P.)

(iii) Na^+ influx does not compensate for K^+ efflux, because membrane at rest is much less permeable to Na^+ than K^+.

(iv) Cl^- diffuses inwards down its concentration gradient but its movement is balanced by the electrical gradient.

THE EQUILIBRIUM POTENTIAL:

Forces acting on ions

The forces acting on ions across the cell membrane produces the variations in membrane potential. In the above mentioned system, the magnitude of forces acting across the cell membrane on each ion can be analysed by ***Nernst equation*** as follows:

$$E_{ion} = -\frac{RT}{ZF} \ln \frac{C_{in}}{C_{out}}$$

where,

E_{ion} = equilibrium potential
R = the natural gas constant (8.216 joules/degree)
T = absolute temperature
Z = the valency of ion (for both Na^+ and $K^+ = 1$; for $Ca^{2+} = +2$; for $Cl^- = -1$)
F = The Faraday constant (No. of coulombs/mole of charge = 96,500 coulombs/mol)
ln = Symbol for natural logarithm
C_{in} and C_{out} = concentration of the ion inside and outside.

At normal body temperature of 37°C, the Nernst equation can be simplified by substituting for the constants (R, T and F) and converting to common logarithms, then

$$E_{ion} = -61.5 \log \frac{C_{in}}{C_{out}}$$

A. Forces on Cl^-

(i) Cl^- being mainly extracellular, tends to diffuse into the cell along its 'Concentration Gradient'.

(ii) Inside of the cell being negative with reference to outside, therefore, Cl^- is pushed out of the cell along this 'Electrical Gradient'.

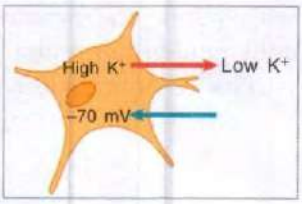
An equilibrium is reached at which Cl^- influx and Cl^- efflux are equal, therefore the membrane potential at which the electric force is equal in magnitude but opposite in direction to the concentration force is called ***Equilibrium Potential*** *for that ion.* **(Table 4.2)**

E_{Cl^-} of a neuron calculated from the Nernst equation is –70mV, and the resting membrane potential of a nerve fiber is also –70mV. Therefore, forces applied on Cl^- to explain the distribution of Cl^- across the membrane are no forces other than those represented by the concentration and electrical gradient *i.e.* Cl^- moves into and out of the cell membrane *passively.*

Table 4.2: Equilibrium potential (E) for important ions in a neuron

E_{Na^+}	+60 mV
E_{K^+}	–90mV
$E_{Ca^{2+}}$	+130mV
E_{H^+}	–25mV
E_{Cl^-}	–70mV
$E_{HCO_3^-}$	–25mV

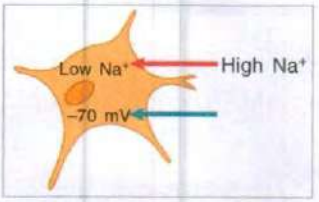
B. Forces on K^+



In this case, concentration gradient is outwards and electrical gradient is inwards.

E_{K^+} of a neuron is –90mV. Since the resting membrane potential is –70mV, there is something else than passive forces (*i.e.* concentration and electrical gradient) acting on K^+ which is responsible for active influx of K^+. If only passive forces are acting across the membrane then cell will lose K^+.

C. Forces on Na^+



Here, concentration gradient is inwards and electrical gradient is also inwards.

E_{Na^+} of a neuron is +60mV, since resting membrane potential is –70mV and membrane is moderately permeable to Na^+; therefore, forces other than passive forces are acting on Na^+ which are responsible for active efflux of Na^+. If only passive forces are acting across the membrane then cell will gain Na^+.

However, the intracellular concentration of Na^+ and K^+ remains constant. Since $Na^+ - K^+$ pump actively transports $3Na^+$ out of the cell against its electrical and concentration gradient and this transport is coupled to active transport of $2K^+$ into the cell.

D. Forces on Ca^{2+}

The ionized Ca^{2+} concentration in ECF is 1.2 mmol/L and in ICF is much lower, 10^{-4} mmol/L, (*i.e.* Ca^{2+} concentration ECF : ICF :: 12,000:1), therefore, both electrical and chemical gradients are directed inwards. The Ca^{2+} permeability of the nerve cell membrane is approx. 100 times less than the Na^+ permeability, but some Ca^{2+} does enter. The intracellular Ca^{2+} is kept low by active transport of Ca^{2+} out of the cell.

The situation in muscles and various other types of cells is similar. In muscles e.g. the resting membrane potential is approx. –90 mV; E_{Cl^-} is –86mV; E_{K^+} is –100 mV and E_{Na^+} is +55 mV.

VARIATIONS IN MEMBRANE POTENTIAL

The magnitude of the membrane potential at any given time depends upon the distribution of Na^+, K^+ and Cl^- and the permeability of the membrane to each of these ions. An equation that describes this relationship with considerable accuracy is the ***Goldman-Hodgkin-Katz (GHK) equation***. It is derived from the laws of diffusion and, therefore, uses permeability as a measure of the ease with which an ion passes through the membrane.

$$V = -\frac{RT}{F} \ln \frac{P_K [K^+]_{in} + P_{Na} [Na^+]_{in} + P_{Cl} [Cl^-]_{out}}{P_K [K^+]_{out} + P_{Na} [Na^+]_{out} + P_{Cl} [Cl^-]_{in}}$$

where,

V = membrane potential

R = the natural gas constant (8.216 joules/degree)

T = absolute temperature

F = the Faraday constant (No. of coulombs/mole of charge = 96,500 coulombs/mol)

ln = symbol for natural logarithm

P_{Cl} P_K and P_{Na} = permeability of the membrane to Cl^-, K^+ and Na^+ respectively

$[\ ]_{in}$ and $[\ ]_{out}$ = signify concentration of ions inside and outside the cell.

Since P_{Na} is low relative to P_K in the resting cells, Na^+ contributes little to the value of membrane potential and *changes in extracellular* $[Na^+]$ *produce little change in the resting membrane potential.* (However, size of the action potential decreases).

(i) If the permeability of Na^+ is zero, the GHK equation reduces to the Nernst equation for K^+.

(ii) Because the permeability of K^+ is so much higher than the permeability for Na^+, the membrane potential is primarily dependent on the concentration gradient for K^+.

(a) *Increase in extracellular* K^+, which makes the equilibrium potential for K^+ more positive, cause the membrane potential to decrease (***depolarize***) *i.e.* become more positive. Thus the neuron becomes more excitable.

(b) *Decrease in extracellular* K^+, which makes the equilibrium potential for K^+ more negative, cause the membrane potential to increase (***hyperpolarize***) *i.e.* become more negative (less excitable).

(iii) Effect of change in ECF $[Ca^{2+}]$: Refer to page 143

(iv) The GHK equation predicts that the membrane potential will be most influenced by the equilibrium potential for the ion to which the membrane is most permeable (or to which it has the highest *conductance*, a measure of permeability).

Important Note

Measurement of permeability changes show that the permeability to Na^+ is altered whenever the membrane potential changes; depolarization of membrane causes an increase in sodium permeability, whereas hyperpolarization causes a decrease (page 38).

ACTION POTENTIAL

A. DEFINITION

The brief sequence of changes which occur in the membrane potential following excitation is called ***action potential***.

B. ORIGIN

Excitable cells *e.g.* nerve and muscle cells, generate action potentials when they are stimulated by a change in membrane potential. It is due to the disturbance in the ionic equilibrium across the receptive zone of the cell membrane *i.e.* changes in the conduction of ions across the cell membrane that are produced by alterations in the ion channels.

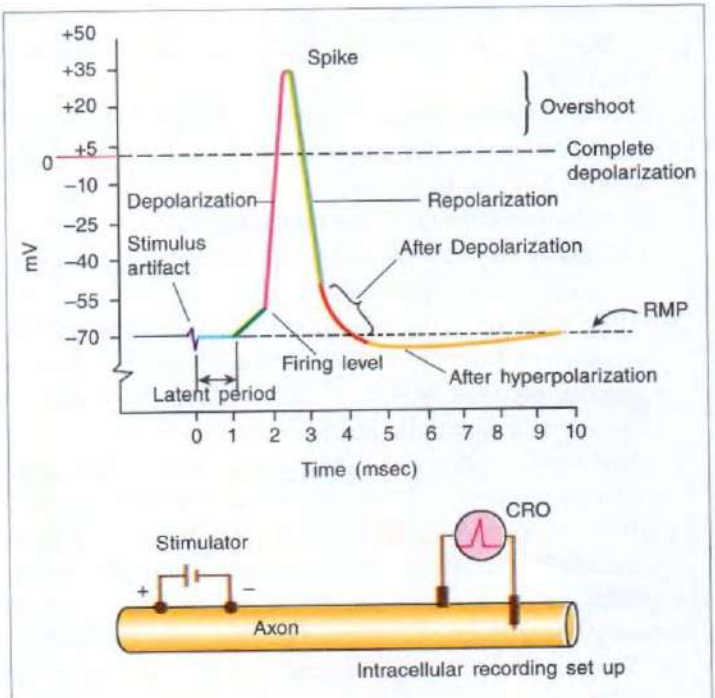


Fig. 4.2 Action potential in a neuron (intracellular recording)

C. PHASES

The *phases* of action potential produced by various cell types differ slightly but their origin is the same. *The phases of the nerve cell action potential are described here* **(Fig. 4.2)**:

1. *During resting (polarised) state,* the inside of nerve is negative and the outside of nerve is positive and the resting membrane potential in most of neurons is –70 mV.
2. When a stimulus is applied there is a brief irregular deflection of the base line, the ***Stimulus Artifact***. This is due to a current leakage from the stimulating electrode to the recording electrode. It marks the point of stimulus. The stimulus artifact is followed by an isopotential interval or ***latent period*** which ends with the next potential change. It corresponds to the time it takes the impulse to travel along the axon from the site of stimulation to the recording electrode. Its duration is proportionate to the distance between the stimulating and recording electrodes; and inversely proportional to the speed of conduction of the axon.
3. The first manifestation of the approaching impulse is a beginning of ***Depolarization*** or *Reverse Polarization* of membrane. Depolarization means reduction in the membrane potential from its negative value towards zero.
4. After an initial 15 mV of depolarization the rate of depolarization increases; the point at which this change in rate occurs is called the ***firing level*** (or *threshold excitation*).
5. The tracing rises rapidly, then reaches and overshoots the zero potential line to approx. +35 mV.
6. It then reverses and falls rapidly towards the resting level, called ***Repolarization***.
7. When repolarization is about 70% completed, the rate of repolarization decreases and tracing approaches the resting level more slowly.
   The sharp rise and the rapid fall are the ***Spike Potential*** of the axon, and the slower fall at the end of the process is the *After Depolarization*.
8. After reaching the previous resting level, the tracing becomes slightly more negative than its resting value for quite some time. This small but prolonged increase in membrane potential is called ***After hyperpolarization***.

After depolarization lasts for approx. 4 msec. while *after hyperpolarization* is 1-2 mV in amplitude and lasts for approx. 35-40 msec. These potentials represent recovery processes in the neurons. If the nerve has been conducting repetitively for a long time, the *after hyperpolarization* is usually quite large. *'Depolarization' decreases the stability of the membrane, while 'hyperpolarization' increases the stability.*

During the local response and *after-depolarization*, the threshold is lowered but during the rising and falling phases of *spike potential*, and *after-hyperpolarization*, threshold is increased.

Note

The excitability is the reciprocal of threshold.

The size and duration of action potential varies from tissue to tissue. Duration of action potential in a mammalian axon is not more than a few msec. but that in cardiac muscle fiber is approx. 200-300 msec.

D. IONIC BASIS (MECHANISM OF DEVELOPMENT) OF ACTION POTENTIAL (Fig. 4.3)

1. Resting (polarised) state: R.M.P.

At rest, inside of the membrane is negative and outside is positive. Why? (Page 35). Since K^+ permeability is greater than Na^+ permeability, therefore, K^+ channels maintain the RMP (which is close to the equilibrium potential for K^+).

2. Depolarization

(*i.e.* during activation of the membrane)

At the point of stimulation, a slight decrease in the RMP due to passive redistribution of ions, leads to increased K^+ and Cl^- influx, restoring the RMP. However, when depolarization exceeds 7 mV, "Na^+ channel activation" occurs through 'm' gates (*i.e.* the voltage-gated Na^+ channels start to open at an increased rate); and when firing level is reached, influx of Na^+ along its concentration and electrical gradient is so great (18-20 times) that it overcomes the repolarizing forces and run away depolarization results.

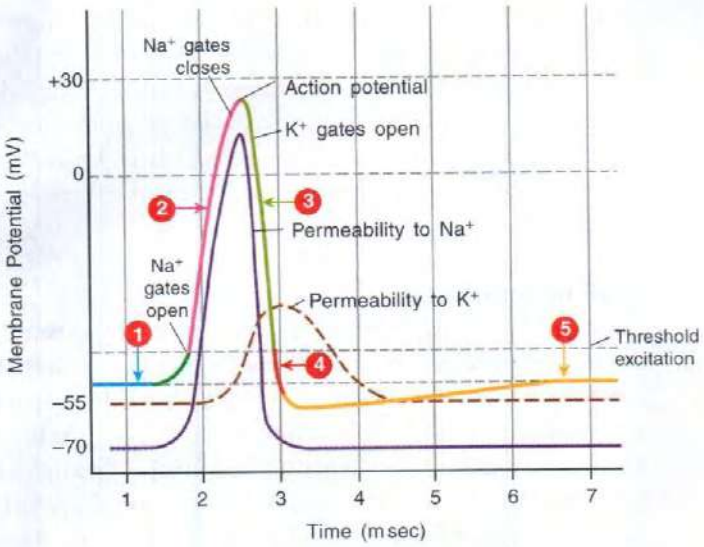
The membrane potential fails to reach +60 mV (*i.e.* value of E_{Na+}) during the action potential because increase in Na^+ permeability is short-lived.

Factors which limit Na^+ influx are:

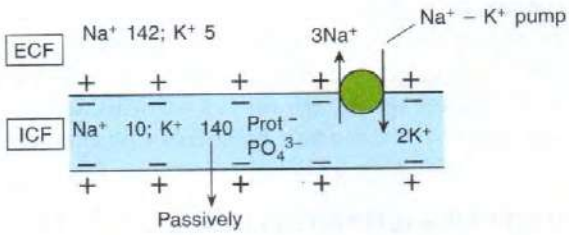
(i) inactivation of Na^+ channels through 'h' gates (Na^+ channels rapidly enter a closed state)
(ii) the direction of electrical gradient for Na^+ is reversed during 'overshoot' because membrane potential is reversed
(iii) opening of voltage-gated K^+ channels ('n' gates), therefore, within 0.3 msec of the threshold excitation, K^+ leaves the cell along its concentration gradient.

Note

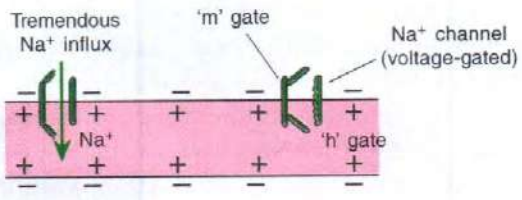
Decreasing the ECF [Na^+] reduces the size of the action potential but has little effect on the RMP.



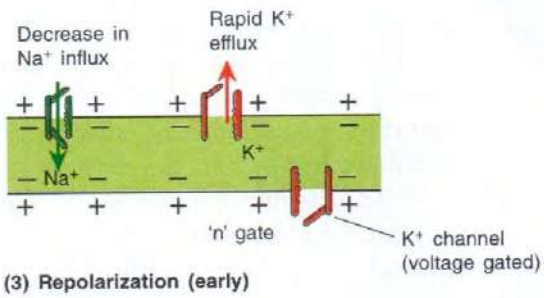
Change in ion permeability during the action potential



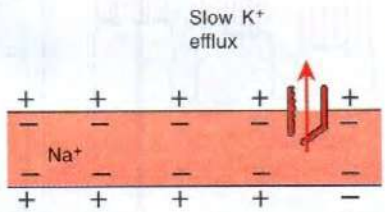
(1) Resting Membrane Potential



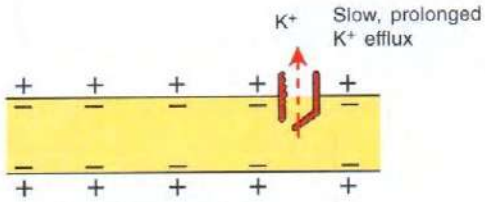
(2) Depolarization (reversal of polarity)



(3) Repolarization (early)



(4) After-depolarization (late slow repolarization)



(5) After-hyperpolarization

Fig. 4.3 Events during action potential: ionic basis (values in mEq/L)

3. Repolarization

Repolarization starts with K^+ efflux due to opening of voltage gated K^+ channels and decreases in further Na^+ influx. The opening of voltage gated K^+ channels is slower and more prolonged than the opening of Na^+ channels.

K^+ efflux cause net transfer of positive charge out of the cell that serve to complete repolarization.

4. After depolarization

At the termination of spike potential K^+ conduction is slowed down and thus a few milliseconds are delayed in restoring the membrane potential. This last phase of slow K^+ efflux is called *after depolarization*.

5. After hyperpolarization

With the disappearance of the after depolarization, though the resting membrane potential is achieved yet the resting ionic status (composition) is not established. It is achieved by slow return of the K^+ channels to the closed state (although the membrane permeability to Na^+ has returned to baseline levels) **(Fig. 4.3)**.

Important Notes

1. Generation of action potential is prevented by *local anaesthetics* such as xylocaine as it blocks opening of the sodium channels thereby reducing membrane excitability.
2. Decreasing extracellular Na^+ concentration decreases the size of the action potential but has little effect on the RMP. (Also refer to page 38).
3. Decrease in extracellular $[Ca^{2+}]$ causes activation (opening) of Na^+ channels. This increases the excitability of nerve by decreasing the amount of depolarization necessary to initiate the action potential. Conversely, an increase in extracellular $[Ca^{2+}]$ decreases membrane permeability to Na^+ and stabilizes the membrane by decreasing excitability.

E. PROPERTIES (CHARACTERISTIC FEATURES) OF ACTION POTENTIAL

1. Threshold Stimulus

Excitable cells undergo rapid depolarization if the membrane potential is reduced to critical level, called the *threshold potential*. It is possible to determine the minimal intensity of stimulating current (*threshold intensity*) that, acting for a given duration, will just produce an action potential. The relationship between the strength of the stimulating current and the duration it must be applied to produce a response is called ***strength-duration curve***.

Stimuli of extremely short duration will not excite the tissue, no matter how intense they may be. With stimuli of longer duration, *threshold intensity* is inversely related to the duration of stimulus. Furthermore, with weak stimuli, a point is reached where no response occurs no matter how long the stimulus is applied **(Fig. 4.4)**.

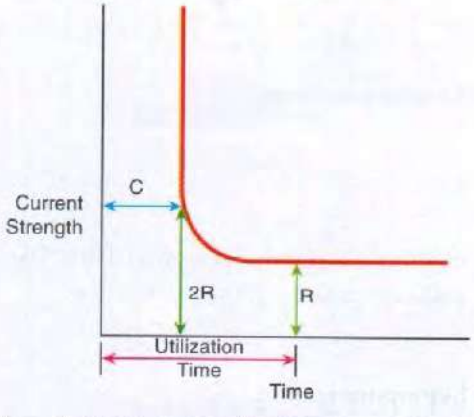


Fig. 4.4 Strength-duration curve (R: Rheobase; C-chronaxie)

(i) The *weakest current* strength which can excite a tissue, if allowed to flow through it for an adequate time, is called ***Rheobase***. The time for which it must be applied is the ***utilization time***. A still weak current fails to stimulate the tissue no matter how long the duration of current flow is.

(ii) The *length of time* for which a current of twice 'rheobase' intensity must be applied to produce a response is called ***chronaxie***. Within limits, the *chronaxie* of any given excitable tissue is constant for that tissue. Therefore, *chronaxie values as a* ***unit of excitability*** *have been used to compare excitability of various tissues.*

2. All or none response

The action potential is an *all or none response* to a stimulus *i.e.* if the stimulus is *subthreshold* (not adequate), no action potential is produced. Once *threshold* intensity is reached, a full-fledged action potential is produced. Further increase in the intensity of a stimulus produces no increment in the height of the action potential provided the other experimental conditions remains the same. However, *once threshold voltage is reached, the stronger the stimulus, the more frequently action potentials are generated.* **(Fig. 4.5)**. This is explained by the fact that once threshold is reached, membrane events are no longer dependent upon stimulus strength. Therefore, *action potential is "all or none" in character i.e.* it fails to occur if the stimulus is *subthreshold* in magnitude or it responds to the maximum of its ability. This *All or None* relationship between the stimulus and the response is called ***All or None Law***.

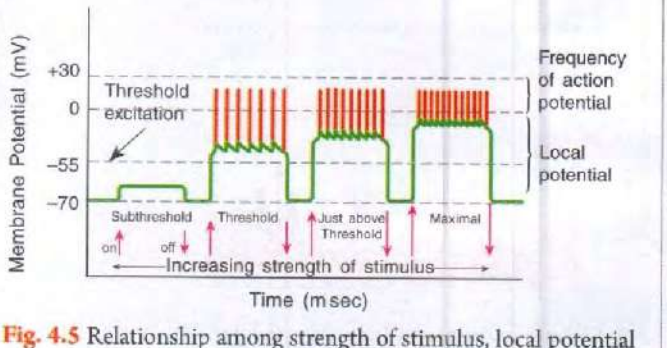


Fig. 4.5 Relationship among strength of stimulus, local potential produced and frequency of action potential.

3. Refractory period

If two successive stimuli (*i.e.* stimulus one after the another in quick succession) of more than *threshold intensity* are applied to an excitable cell, it is found that for some time after the first stimulus, the cell becomes *refractory* (non-responsive) to the second stimulus. The duration for which it remains refractory to the second stimulus is called the *refractory period*. During this period it is more difficult to elicit an action potential. There are *two types* of refractory periods:

(i) ***Absolute refractory period (ARP)*** – begins from the time the firing level is reached until the repolarization is approx. 1/3 complete. During this period no stimulus, no matter how strong,

can initiate the fresh impulse (*i.e.* second action potential) in this region; nor can an impulse generated elsewhere pass through this area **(Fig. 4.6)**.

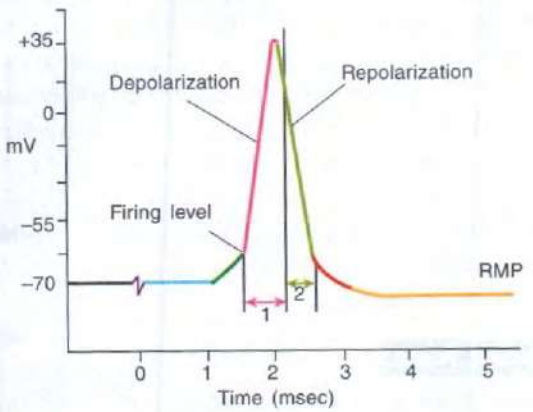


Fig. 4.6 Refractory periods: (1) 'Absolute' Refractory Period (ARP); (2) 'Relative' Refractory Period (RRP)

Causes:

(a) during the upstroke, the 'm' gates are opening rapidly; and

(b) during the early portion of the down stroke, Na^+ channels are inactivated by the 'h' gates. *No action potential can occur until the inactivation gates open.*

(ii) ***Relative refractory period (RRP)*** – begins at the end of ARP to the start of the *after depolarization*. During this period stimulus stronger than normal stimulus can cause excitation.

Cause: Because suprathreshold stimulus opens more sodium channels through 'h' gates (page 38); thus the Na^+ channels are again available for excitation when the 'h' gates open during the downstroke.

The stimulus must be greater than normal because the same Na^+ channels are still inactivated and more K^+ channels than normal are still open.

Important Note

The action potential elicited during this interval has a lower upstoke velocity and a lower 'overshoot' potential than does the normal action potential.

4. Conductivity
(*i.e.* propagation of wave of depolarization)

The action potential generated at one location on the excitable cell acts as a stimulus for the production of an action potential in the adjacent region of the membrane. The *excitation* (*activation*) impulse is conducted along the cell membrane as a wave of depolarization. The self propagating nature of action potential is due to *circular current flow* and successive depolarization to the firing level of the membrane ahead of the action potential. Once initiated, a moving impulse does not depolarize the area behind it to the firing level because this area is refractory. Direction of propagation of impulse is the same as *current flow* direction inside the nerve **(Fig. 4.7)**.

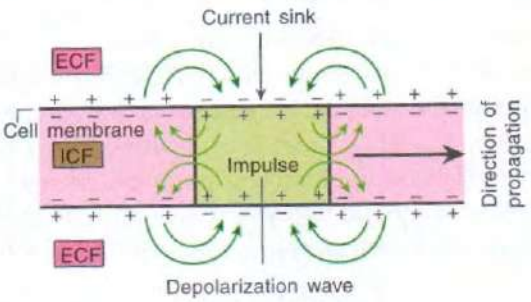


Fig. 4.7 Mechanism of conduction of action potential

The magnitude of the action potential does not change as it is conducted along the membrane, because new action potentials are being generated constantly. Furthermore, the speed of propagation varies with different tissues.

5. Accommodation

If an excitable cell is submitted to the passage of slowing rising strength of currents, the site of the membrane under stimulation fails to produce action potential *i.e.* the membrane *adapts* to the applied stimulus. This is due to the slower opening and delayed closing of the voltage-gated K^+ channels.

If depolarization occurs rapidly, the opening of the Na^+ channels overcomes the repolarizing forces; but if the induced depolarization is produced slowly, the opening of K^+ channels balances the gradual opening of Na^+ channels, and an action potential does not occur.

F. ELECTROTONIC POTENTIALS – GRADED POTENTIALS

The charges are due to flow of electrons (e^-), therefore, e^- will flow from cathode towards anode. This causes less negativity (*depolarization*) at cathode while more negativity (*hyperpolarization*) at anode. **(Fig. 4.8)**

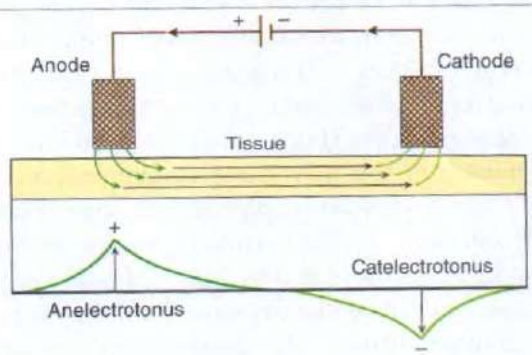


Fig. 4.8 Flow of current in a tissue

Although subthreshold stimuli do not produce an action potential, they do have an effect on the membrane potential, due to passive redistribution of ions, across the membrane. This can be demonstrated by placing recording electrodes within a few milimetres of a stimulating electrode and applying subthreshold stimuli of fixed duration.

1. ***Application of subthreshold current with cathode (negative):*** At cathode, the current flows outwards through the membrane, therefore, at cathode the inside of membrane becomes less negative (depolarized), this localized depolarising potential change which rises sharply and decays exponentially with time is called the ***Catelectrotonic Potential.*** **(Fig. 4.9)**

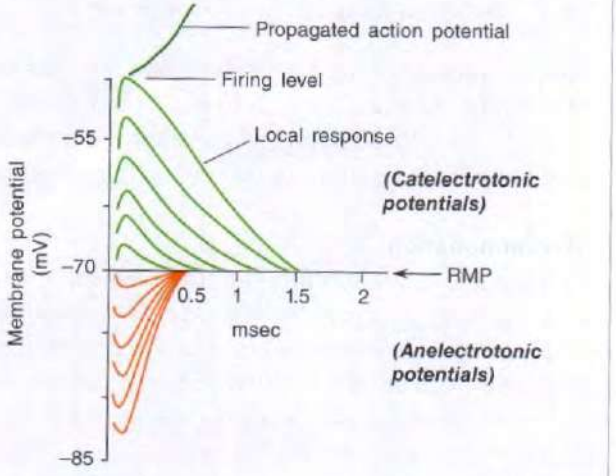


Fig. 4.9 Electrotonic potentials. Application of stimuli of 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 times threshold intensity.

2. ***Application of subthreshold current with anode (positive):*** At anode, the current flows inwards through the membrane, therefore, at anode the inside of membrane becomes more negative (hyperpolarized) producing localized hyperpolarizing potential change of similar duration, called ***Anelectrotonic Potential.***

Catelectrotonic and anelectrotonic potentials are together called as ***Electrotonic Potentials***. They are due to passive change in membrane polarization caused by addition or subtraction of charges respectively.

At low current intensities producing approx. 7 mV of depolarization or hyperpolarization, their size is proportionate to the magnitude of stimulation (called *Graded Potentials*); with stronger stimuli, this relationship remains constant tor anelectrotonic responses but not for responses at the cathode. The cathodal responses are greater than would be expected from the magnitude of the applied current. The disproportionately greater response at the cathode to stimuli of sufficient strength to produce 7-15 mV of depolarization is produced when voltage-gated Na^+ channels begin to open and is called the ***Local Response***. The point at which a run away spike potential is initiated is the ***firing level***.

At 15 mV of depolarization of cathode *i.e.* at membrane potential of –55 mV, the depolarizing forces are strong enough to overcome the repolarization processes and propagated action potential results. The current strength required for this to occur is defined as ***threshold***.

Therefore, stimulation normally occurs at the cathode because cathodal stimuli are depolarizing. Anodal current, by taking the membrane potential further away from the firing level, actually inhibit impulse formation.

Important Note

Graded potentials are changes in membrane potential that are confined to a relatively small region of the membrane and their magnitude is variable (graded). They are given various names according to the location of the potential, such as *end-plate potential* (page 156), pacemaker potential (page 176), *post-synaptic potential* (page 852), *generator or receptor potential* (page 866). The major differences between the graded potential and action potential are given in Table 4.3.

G. EXTRACELLULAR (SURFACE) RECORDING OF ACTION POTENTIAL

If both recording electrodes are placed on the surface of the axon, there is no potential difference between them at rest. When excitable cell is stimulated with threshold stimulus, an impulse is conducted past the two electrodes, a characteristic sequence of potential changes result. **(Fig. 4.10)**

1. As the wave of depolarization reaches the recording electrode nearer the stimulator, this electrode becomes negative to the other electrode, and the potential difference between the two electrodes is recorded as an upward deflection.
2. When the impulse reaches to the portion of the nerve between the two electrodes, the potential returns to zero followed by isoelectric interval.
3. When the impulse passes the second electrode, the first electrode becomes positive relative to the second, now the potential difference between the two electrodes is recorded as downward deflection.
4. When the impulse passes beyond the second electrode, the potential again returns to zero.

It is by convention that when the first electrode becomes negative relative to the second an upward deflection is recorded. Therefore, the record shows an upward deflection followed by an isoelectric interval and then a downward deflection. This sequence is called a ***Biphasic action potential***. The duration of the isoelectric interval is

Table 4.3: Differences between graded potential and action potential

Graded potential	Action potential
1. It is a change in membrane potential that is confined to a relatively small region of the membrane (1-2 mm) and is *non-propagating* in nature, *i.e.* it remains confined locally and declines with time.	1. It spreads over the membrane covering relatively a much larger area and is *propagating* in nature *i.e.* it travels for considerable distance over the membrane without decrement.
2. It is *initiated* either spontaneously (pacemaker potential) or by neurotransmitter (end-plate potential, post-synaptic potential) or by environmental stimulus (generator or receptor potential).	2. It is initiated by membrane depolarization. Only nerve, muscle and some glands have cell membranes capable of producing action potentials.
3. It shows graded response *i.e. summation is possible*, therefore, its magnitude is proportional to the strength of stimulus *i.e.* it does not obey 'All or None' law.	3. Summation of *action potential* is never possible; therefore, it obeys 'All or None' law.
4. It can *either be a depolarizing or hyperpolarizing response.*	4. It is *always a depolarization* with an overshoot.
5. It has *no threshold.*	5. It has a *threshold* that is usually 10 to 15 mV depolarized relative to the resting potential.
6. It has *no refractory period.*	6. It has *a refractory period.*
7. It is important in signaling over *short distances.*	7. It is the *long distance signal* of nerve and muscle membrane.

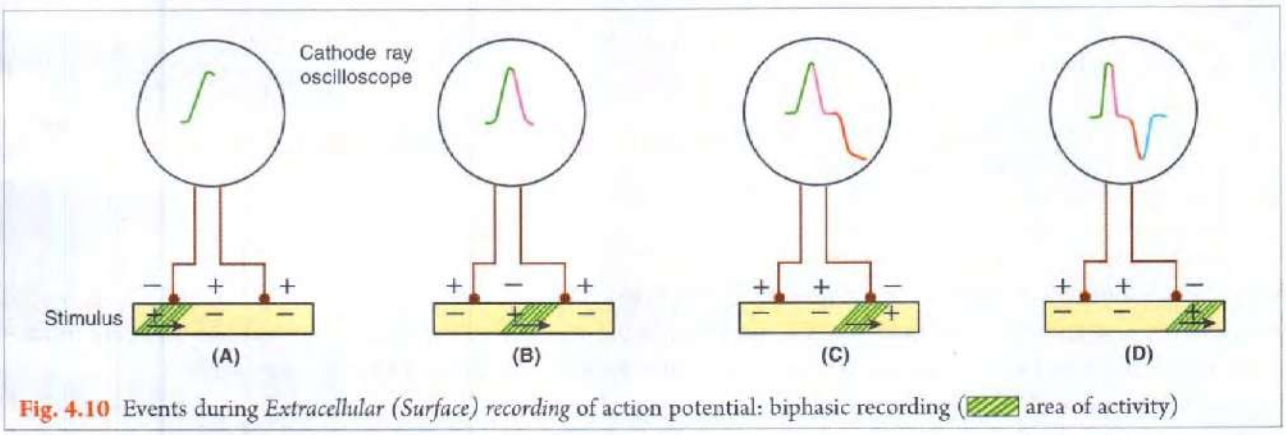


Fig. 4.10 Events during *Extracellular (Surface) recording* of action potential: biphasic recording (▨ area of activity)

inversely proportional to the speed of conduction of the tissue and directly proportional to the distance between the two recording electrodes.

Important Note

Biphasic action potential can record only changes in membrane potential (*i.e.* action potential) but not RMP, therefore, an accurate analysis of the action potential as described in the case of intracellular recording is not possible.

Physiological significance

If the axon under one of the external electrode is damaged *e.g.* by crushing or cutting the nerve (in damaged area polarity is abolished), then the damaged area becomes negative relative to the healthy portion at rest. Therefore, a steady potential difference between the two electrodes is recorded at rest, called the ***Injury (Demarcation) Potential.*** **(Fig. 4.11)** Negativity of the damaged area is due to the breakdown of the membrane. On stimulation with threshold stimulus while the demarcation potential

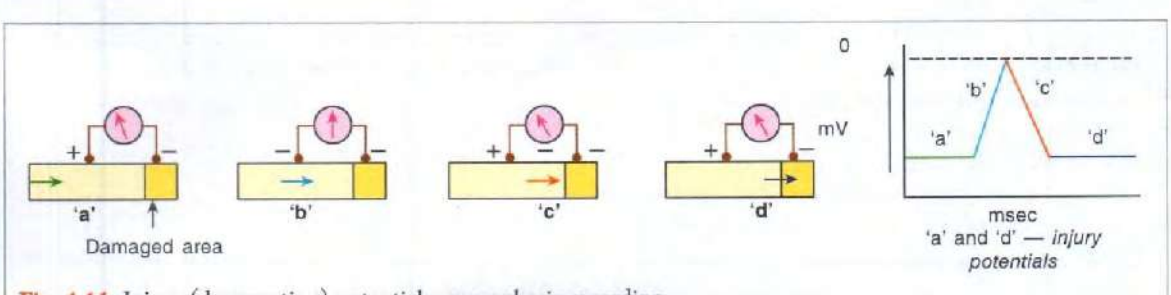


Fig. 4.11 Injury (demarcation) potential: monophasic recording

is being recorded, as wave of depolarization reaches the electrode nearest to the stimulator, this electrode too becomes negative thus neutralizing the demarcation potential *i.e.* both the electrode become equipotential (potential difference becomes zero), thus recording zero potential. When the impulse passes to the portion of the nerve between the two electrodes, the potential returns to original negative potential *i.e.* injury potential is recorded. When the impulse reaches the electrode overlying the damaged area, no potential is recorded because impulse stops at the damaged area *i.e.* on stimulation only single upward deflection is recorded and is called as ***Monophasic action potential.***

Clinical use

Extracellular recordings are useful in clinical practice, when the electrical activity of excitable tissues must be monitored; for example

(i) *Electroencephalograms (EEGs)* are used to aid in diagnosis of brain diseases,

(ii) *Electrocardiograms (ECGs)* are used to detect damage to the heart, and

(iii) *Electromyograms (EMGs)*, recordings from skeletal muscles, are used to aid in the diagnosis of neuropathies and myopathies.

Study Questions

1. **Give physiological significance of:**
   (i) Gibbs Donnan membrane equilibrium
   (ii) Resting membrane potential
   (iii) Equilibrium potential
   (iv) Mechanism of development of action potential
   (v) Chronaxie and Rheobase
   (vi) All or none law
   (vii) Electrotonic potential
   (viii) GHK equation
2. **Differentiate between:**
   (i) Intracellular and extracellular recording of action potential
   (ii) Anelectronic and Catelectronic potentials
   (iii) Graded potential and action potential
   (iv) Mono and bi-phasic action potential
   (v) Absolute and relative refractory period.
3. **What causes differences in the ionic composition of ECF and ICF?**
4. **How uneven distribution of ions between ICF and ECF compartments is constantly maintained under resting conditions?**
5. **What will happen and why to resting membrane potential with change in the ECF ionic composition.**
6. **Explain ionic basis of action potential and electrotonic potential.**
7. **Describe briefly the properties of action potential.**
8. **Draw well labelled diagram:**
   (i) Intracellular recording of action potential
   (ii) Relationship among strength of stimulus, local potential produced and frequency of action potential
   (iii) Mechanism of conduction of action potential in a neuron

MCQs

1. **Resting nerve membrane is more permeable to K^+ than to Na^+ by:**
   (a) 1-5 times (b) 20-50 times (c) 50-100 times (d) 200-500 times
2. **If the intracellular concentration of a permeable solute is much greater than the extracellular concentration, which of the following is *not true*?**
   (a) ATP is required to maintain the concentration gradient (b) The solute is transported actively into the cell
   (c) The solute is sodium (d) The solute diffuses passively out of cells
3. **Normal cell volume and pressure depends on:**
   (a) Gibbs-Donnan effect
   (b) Operation of $Na^+ - K^+$ pump
   (c) Asymmetrical distribution of ions across the cell membrane
   (d) Presence of more osmotically active particles in the cell
4. **Magnitude of resting membrane potential from tissue to tissue ranges from ..... mV**
   (a) –50 to –70 (b) –40 to –90 (c) –70 to –100 (d) –10 to –100

5. **The magnitude of the membrane potential at any given moment can be determined with considerable accuracy by:**
   (a) Nernst equation (b) Goldman-Hodgkin-Katz (GHK) equation
   (c) Gibbs Donnan membrane equilibrium (d) Equilibrium potential
6. **Presence of Ca^{2+} on nerve membrane may play a significant role in:**
   (a) Operation of sodium-potassium pump (b) Regulation of K^+ outflow
   (c) Keeping Na^+ gates closed (d) Preventing protein anion from going out
7. **A decrease in extracellular concentration of calcium causes:**
   (a) Decreased excitability (b) Hyperpolarization
   (c) Decreased membrane stability (d) Decreases membrane permeability to Na^+
8. **Value of which of the following is considered as unit of excitability:**
   (a) Rheobase (b) Chronaxie (c) Utilization time (d) Refractory period
9. **All or none law refers to:**
   (a) Resting potential (b) Spike potential
   (c) Excitatory postsynaptic potentials (d) Strength of contraction
10. **Absolute refractory period:**
    (a) Begins from the time the firing level is reached until the repolarization is complete
    (b) During this period no stimulus, no matter how strong, can initiate the fresh impulse
    (c) During this period stimulus stronger than usual can cause excitation
    (d) During this period more sodium channels open up
11. ***True* about the extracellular recording of action potential:**
    (a) One electrode is placed on the surface and other is inserted into the interior of the cell
    (b) It cannot record changes in membrane potential
    (c) It cannot record resting membrane potential
    (d) It can provide a record for accurate analysis of the action potential
12. **The differences in the permeability of resting cell membrane to various small ions is determined by:**
    (a) Atomic weight of the ions (b) Size of the hydrated ions
    (c) Size of the ions as such (d) Membrane potential
13. **Resting membrane potential is also referred as:**
    (a) Equilibrium potential (b) Generator potential (c) Steady potential (d) Spike potential
14. **Genesis of resting cell membrane potential is due to all *except*:**
    (a) Existence of Na^+ pump expelling Na^+ from the cell (b) Impermeable anions being inside the cell
    (c) Existence of K^+ pump transferring K^+ into the cell (d) K^+ pump being considerably weaker than Na^+ pump
15. **Nernst equation deals with:**
    (a) Oxygen uptake by the body (b) Forces acting on ions across the cell membrane
    (c) Cellular ATP levels (d) Plasma bicarbonate level
16. **Resting membrane potential is close to the isoelectrical potential for:**
    (a) Na^+ (b) K^+ (c) Ca^{2+} (d) Cl^-
17. **What provides most of the energy that is used to maintain a normal resting membrane potential of about 70 millivolts inside the nerve?**
    (a) The chloride pump (b) The sodium potassium pump
    (c) The calcium pump (d) Diffusion of chloride ions
18. **If the resting membrane potential of a nerve cell falls, there is a net:**
    (a) Gain of Na^+ into the cells (b) Loss of K^+ from cells
    (c) No loss of K^+ from cells (d) Gain of Cl^- into cells
19. **The repolarization of an action potential is associated with all of the following *except*:**
    (a) Loss of positive charges from inside the cell (b) Return of the membrane potential towards its resting value
    (c) Closure of sodium channels in the cell membrane (d) Decreased potassium permeability of the cell membrane
20. **Stimulation normally occurs at the cathode because:**
    (a) Cathodal stimuli are hyperpolarizing (b) Cathodal stimuli are depolarizing
    (c) Anodal current inhibits impulse formation (d) Electrons flow from anode towards cathode

Answers

1. (c)	2. (c)	3. (b)	4. (d)	5. (b)	6. (c)	7. (c)	8. (b)	9. (b)	10. (b)
11. (c)	12. (b)	13. (c)	14. (d)	15. (b)	16. (d)	17. (b)	18. (a)	19. (d)	20. (b)

Unit II

BLOOD

Chapter 5: Composition and Functions of Blood

Composition and functions of blood

Serum

Chapter 6: The Plasma Proteins

Origin; forms and their functions

Relation of diet to plasma proteins (Whipple's experiment)

Variations and functions

Chapter 7: Haemoglobin

Structure; some important definitions

Normal values; functions; free haemoglobin; synthesis; catabolism; varieties (HbA, HbF: thalassaemia; HbS)

Chapter 8: Erythrocyte: Red Blood Corpuscle (RBC)

General structure

Variations in size, shape and structure of RBC

RBC indices (blood standards): MCV; MCH; MCHC; CI

Haemopoiesis: Theories; interleukins (ILs); colony stimulating factors (CSFs)

Erythropoiesis: Stages and regulation

Anaemias: grading; classification; pernicious anaemia; folic acid deficiency anaemia; iron deficiency anaemia; congenital spherocytosis

Chapter 9: Jaundice

Chemistry of bilirubin formation

Fate of bilirubin

Types of jaundice (hemolytic; hepatic and obstructive)

Physiological jaundice: Jaundice of newborn; phototherapy

Chapter 10: Leucocyte: White Blood Corpuscle (WBC)

General: total leucocyte count; leucopenia; leucocytosis; leukaemia

Structure, functions and variations

Physiology of phagocytic mechanism

Leucopoiesis: stages, regulation, senile leucocytes

Chapter 11: Platelets or Thrombocytes

Structure; count and variations; thrombopoiesis; functions

Chapter 12: Coagulation of Blood

Definition; mechanism; physiology of clotting mechanism; Anticoagulant mechanism (fibrinolytic system); anticoagulants; Haemorrhagic (bleeding) disorders: hemophilia; purpura

Chapter 13: Blood Groups

Classical ABO; Rhesus (Rh) blood groups: determination, inheritance and hemolytic diseases

Uses: blood transfusion; investigation – a case of paternity dispute

Blood storage

Chapter 14: Lymphoid Tissues and Lymph

Tissue macrophage system; lymphocytes; plasma cells

Functions of spleen

Lymph

Chapter 15: Immunity (The Immune System)

Natural (the complement system)

Acquired (immunoglobulins; cytokines)

Regulation of immune response

Immunological tolerance: Recognition of self; Autoimmunization

Tissue transplant (graft)

Chapter 5

Composition and Functions of Blood

I. Composition of Blood

II. Functions of Blood

III. Serum

COMPOSITION OF BLOOD

Total blood volume : 5–6 litres (8% of body weight or 80 mL/kg body weight)
Specific gravity : 1050–1060
Viscosity : 4–5 times that of water
pH : 7.4 ± 0.05; alkaline

If *anticoagulated* sample of blood is allowed to stand in a narrow tube, on settling it separates out into **cells** and **plasma** (Fig. 5.1).

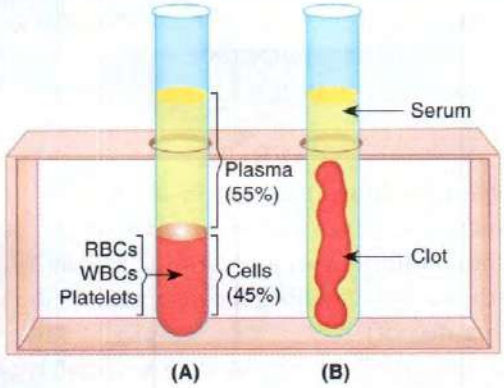


Fig. 5.1 (A) Anticoagulated blood showing proportion of plasma and cells. (B) Blood clot in a serum.

A. CELLS

The cellular elements of blood represents **45%** of the total blood volume, called ***Packed Cell Volume (PCV)*** or ***Haematocrit***. It includes:

1. **Erythrocytes** or **Red Blood Corpuscles (RBC's)**
   Normal count: 5 million/µL (5×10^6/µL).
2. **Leucocytes** or **White Blood Corpuscles (WBCs)**
   Normal count: 4,000–11,000/µL ($4\text{-}11 \times 10^3$/µL).
3. **Platelets** or **Thrombocytes**
   Normal count: 1.5-4 lacs/µL ($0.15 - 0.4 \times 10^6$/µL).

Note

µL was previouly called as cumm.

B. PLASMA

1. Plasma is a clear, straw coloured fluid portion of the blood and represents **55%** of the total blood volume (about 5% of body weight). It contains:
   (i) 91% water; and
   (ii) 9% solids. The solids comprise:
      (a) 1% **inorganic molecules**, and
      (b) 8% **organic molecules**.
2. The major inorganic molecules are:
   (i) Na^+, Ca^{2+}, Cl^-, HCO_3^- (mainly extracellular).
   (ii) K^+, Mg^{2+}, Cu^{2+}, PO_4^{3-}, $Protein^-$ (mainly intracellular).
   (iii) Fe^{2+}, Fe^{3+}.
3. Of 8% total organic molecules:
   (i) 7% are **plasma proteins**; and
   (ii) 1% are other substances like **Non-protein Nitrogenous (NPN)** substances, sugar, fats, enzymes and hormones.

Plasma Proteins

Normal value: 6.4–8.3 gm/dL

Components

1. 55% Albumin : 3–5 gm/dL (Average: 4.8 gm/dL)
2. 38% Globulin : 2–3 gm/dL (Average: 2.3 gm/dL)

(i) 13% α–Globulin : 0.78–0.81 gm/dL
(ii) 14% β–Globulin : 0.79–0.84 gm/dL
(iii) 11% γ–Globulin : 0.66–0.70 gm/dL
A/G ratio; Albumin: Globulin : : 1.7 : 1
3. 7% Fibrinogen : 0.3 gm/dL
4. Prothrombin : 40 mg/dL

Non-Protein Nitrogenous (NPN) Substances

Normal: 28–40 mg/dL

These are derivatives of food and in parts are the waste products of tissue catabolism. These include:

1. Urea : 20–40 mg/dL
2. Uric acid : 2–4 mg/dL
3. Creatine : 1–2 mg/dL
4. Creatinine : 0.6–1.2 mg/dL
5. Xanthine : Traces
6. Hypoxanthine : Traces

Other Substances

These include:

1. Neutral fats (triglycerides): 30–150 mg/dL
2. Phospholipids *e.g.* Lecithin, sphingomyelin, cephalin etc. : 150–300 mg/dL
3. Glucose (fasting) : 70–90 mg/dL
4. Cholesterol : 120–200 mg/dL

FUNCTIONS OF BLOOD

1. **Respiratory**: Blood transports oxygen from lungs to the tissues and of carbon-dioxide from the tissues to the lungs.
2. **Nutritive**: Blood conveys absorbed food materials, glucose, amino acids, fatty acids, vitamins, electrolytes and trace metals from the alimentary canal to the tissues for utilization and storage.
3. **Excretory**: Blood transports the metabolic wastes *e.g.* urea, uric acid, creatinine etc. to the kidney, skin and intestine for their removal.
4. **'Homeostatic' for water, pH and electrolyte concentration**: Blood forms *internal environment* of the cell *i.e.* ***Millieu Interieur*** (page 3, 28) in terms of volume, composition, concentration, pH and temperature, which is regulated to normal physiological limits with respect to minor changes in the body. This mechanism is called ***Homeostasis*** *(W.B. Cannon)*. Buffering power of haemoglobin helps to maintain constancy of blood pH.
5. **Regulation of body temperature**: Blood preserves the very narrow range in body temperature. How?
   Blood whose major constituent is water has:
   (i) *High specific heat* – This buffers sudden change (rise or fall) in body temperature.
   (ii) *High conductivity* – This helps to take out heat from an organ for uniform distribution throughout the body.
   (iii) *High latent heat of evaporation.*
6. **Chemical for communication and protection**
   (i) Concentration of hormones and various substances in the blood is regulated through feedback mechanisms.
   (ii) Within blood circulates the entire complex of humoral antibodies important in defence against infection, initiation of inflammation and regulation of ***Haemostasis*** (clotting mechanism).
7. **Plasma protein functions**
   (i) Exerts the osmotic pressure which influences the exchange of fluid between blood and tissues.
   (ii) Acts as a reservoir of proteins.
   (iii) Combines with many substances *e.g.* iron, thyroxine and steroid hormones to form transportable complexes from which the active components are released at the appropriate sites.

(For details refer to page 54.)

SERUM

If the blood is allowed to clot in a test tube, then the clot retracts and gives out ***serum***. Therefore, serum is plasma minus fibrinogen and clotting factors (II, V and VIII), because these factors get consumed during clotting (remaining do not).

Serum has a higher *serotonin (5 hydroxytryptamine – 5HT)* content because of the breakdown of platelets during clotting.

Study Questions

1. Give physio-clinical significance of Millieu interieur and Homeostasis
2. Enumerate functions of blood.
3. Explain the role of blood in regulation of body temperature.

MCQs

1. **Amount of total blood volume in an individual is approximately:**
   (a) 50 mL/kg body weight (b) 60 mL/kg body weight (c) 70 mL/kg body weight (d) 80 mL/kg body weight
2. **Total blood volume (% of body weight) is:**
   (a) 8 (b) 20 (c) 40 (d) 80
3. **Haematocrit is ratio of:**
   (a) WBC to plasma (b) Platelets to plasma (c) RBCs to plasma (d) Total blood cells to plasma
4. ***True* about plasma in blood is:**
   (a) It contains more of inorganic than organic molecules (b) It represents 45% of total blood volume
   (c) It is a clear, colourless fluid portion of the blood (d) It contains 91% water and 9% solids
5. **Normal A/G ratio in blood is:**
   (a) 1.7 : 1 (b) 1 : 1.7 (c) 7.1 : 1 (d) 1 : 7.1
6. **Millieu interieur refers to:**
   (a) Internal environment of the cell
   (b) Fluid which is present within the cell
   (c) Haemoglobin that helps to maintain constancy of blood pH
   (d) Haemostasis
7. **Serum does not contain:**
   (a) Calcium (b) Prothrombin (c) Factor VII (d) Factor X
8. **Normal blood pH is:**
   (a) 7.20 (b) 7.30 (c) 7.40 (d) 7.50
9. **The hematocrit of 38% means that in the sample of blood analysed:**
   (a) 38% haemoglobin is in the plasma
   (b) 38% of the total blood volume is made up of blood plasma
   (c) 38% of the total blood volume is made up of red, white blood cells and platelets
   (d) 38% of the haemoglobin is in red blood cells

Answers

1. (d) 2. (a) 3. (d) 4. (d) 5. (a) 6. (a) 7. (b) 8. (c) 9. (c)

Chapter 6

The Plasma Proteins

I. Origin of plasma proteins
II. Forms of plasma proteins and their functions
III. Relation of diet to plasma proteins (Whipple's experiment)
IV. Variations in plasma protein concentration
V. Functions of plasma proteins

ORIGIN OF PLASMA PROTEINS

1. **In Embryo:** Mesenchymal cells through a process of secretion or dissolution of their substances, form plasma proteins. First the albumin is synthesized and rest of plasma proteins afterwards.
2. **In Adults**
   (i) Albumin from liver mainly
   (ii) Fibrinogen also from the liver
   (iii) Globulin from:
      (a) *Tissue macrophages* (*i.e.* reticulo-endothelial cells, page 114) – Liver (specially synthesize α and β globulin), spleen and bone marrow.
      (b) *Plasma cells* – These are large, oval and very active cells found in medullary cords of lymphoid follicles and small lymphocytes (page 115).
      (c) *Lymphocytes* synthesize γ-globulin.

FORMS OF PLASMA PROTEINS AND THEIR FUNCTIONS

Normal total plasma protein concentration: 6.4–8.3 gm/dL of blood.

Serum proteins means all plasma proteins minus fibrinogen.

The major forms of plasma proteins and their functions are summarized in Table 6.1.

ALBUMIN

Refer to Table 6.1.

GLOBULIN

Normal plasma concentration: 2-3 gm/dL (Average: 2.3 gm/dL).

Table 6.1: Forms of plasma proteins and their functions

Type	Normal plasma level	Functions
55% : 1. Pre-albumin (MW-60000)	0.03 gm/dL	Binds Thyroxine (T_4) and tri-iodothyronine (T_3)
2. Albumin (MW-69000)	3-5 gm/dL (Av: 4.8 gm/dL)	(i) Controls colloidal osmotic pressure (page 55). (ii) *Binding and Carrier protein*: It helps in transport of anions, cations, dyes, drugs, hormones, fatty acids, metals, amino acids, enzymes and bilirubin.
38% : Globulin (MW-Variable)	2-3 gm/dL (Av: 2.3 gm/dL)	See below.
7% : Fibrinogen (MW-3,50,000)	200–450 mg/dL	Helps in blood clotting. (precursor to fibrin: page 95)
Prothrombin (MW-68000)	40 mg/dL	Helps in blood clotting. (precursor to thrombin: page 97)

Albumin: Globulin : : 1.7 : 1 (A/G Ratio) (MW: Molecular weight)

Types

	Normal level
(i) 13% α-globulin (α_1; α_2)	: 0.78–0.81 gm/dL
(ii) 14% β-globulin (β_1; β_2)	: 0.79–0.84 gm/dL
(iii) 11% γ-globulin (γ_1; γ_2)	: 0.66–0.70 gm/dL

Forms of globulin

1. *Glycoprotein:* Carbohydrate plus protein.
2. *Lipoprotein:* α_2-globulin plus lipid; it is a water-soluble complex with the following *subtypes:*
   (i) *High density lipoprotein (HDL)* or α-lipoprotein. It contains 50% protein with large amount of cholesterol and phospholipids.
   (ii) *Low density lipoprotein (LDL)* – contains large amount of glycerides.
   (iii) *Very low density lipoprotein (VLDL).*
   (ii) and (iii) are also called *'β'-lipoprotein;* they have higher proportion of fat in the form of triglycerides or cholesterol.
   (iv) *Chylomicrons* (page 263) – contain 2% protein and 98% triglycerides.

Important Notes

1. **HDL levels are increased in individuals who exercise and those who drink alcohol in moderation, whereas they are decreased in smokers, obese and sedentary workers.**
2. **In healthy individuals, proportion of HDL is high but in coronary artery disease (CAD) patients, proportion of 'β'-lipoproteins (LDL and VLDL) increases.**

Lipoproteins are used in lipid metabolism and *increase in*:
(a) Atherosclerosis,
(b) Obesity and
(c) Liver diseases

3. *Transferrin:* α_2–β globulin (mainly β-globulin), MW-90,000.
   Normal plasma concentration: 3.0–6.5 mg/ dL. It has the specific property of iron binding; each transferrin binds 2 atoms of ferric ions.
   Functions:
   (i) regulates and controls iron absorption from GIT;
   (ii) protects against iron intoxication;
   (iii) helps in iron transport.
4. *Haptoglobins:* α_2 globulin; MW-90,000.
   Normal plasma level: 40–180 mg/dL.
   It forms stable complexes with free haemoglobin, therefore:
   (i) prevents loss of iron through urinary excretion;
   (ii) protects the kidney from damage by haemoglobin;
   (iii) regulates the renal threshold for haemoglobin.
5. *Ceruloplasmin:* α_2–β globulin (mainly α_2-globulin), MW-16,000.
   Normal plasma level: 15–60 mg/dL.
   It binds with copper and helps in its transport and storage.

Important Note

"WILSON'S DISEASE" – It is due to deficiency of ceruloplasmin, therefore, free copper increases in circulation which gets deposited in brain and liver to cause their destruction (*hepato-lenticular degeneration*, page 998); also copper is lost in urine.

6. *Fetuin:* present in foetus and newborns. It is a growth promoting protein and has MW-45,000.
7. *Coagulation factors:* α, β globulin (page 97).
8. *Angiotensinogen:* α_2-globulin (page 506).
9. *Haemagglutinins i.e.* antibodies against red cell antigens.
10. *Immunoglobulin (Ig):* γ-globulin. (page 126).

RELATION OF DIET TO PLASMA PROTEINS

[WHIPPLE'S EXPERIMENT (WHIPPLES GEORGE H. 1956)]

This can be studied in the *standard plasma depleted dog.* The whole blood is withdrawn from the animal and the cells, suspended in the same volume of ringer locke solution (*i.e.* a protein free fluid), are re-injected into the animal. This procedure **(plasmapheresis)**, if repeated daily, leads to progressive reduction in the concentration of plasma protein, because rate of protein withdrawal exceeds the rate of regeneration. The depletion is continued for some weeks, till plasma protein concentration has fallen to 4 gm/dL. Why?

1. To exhaust the protein reserves and, thereafter,
2. To find out the rate of regeneration of plasma proteins on a standard diet.

Findings

1. Sudden decrease of plasma proteins upto 4-5 gm/dL; after 15 minutes plasma proteins start increasing due to mobilization of *labile protein reserve* and within 2-7 days normal level is reached if good diet containing all *essential amino acids* (page 619) is given. This shows plasma proteins are normally formed from food proteins but in protein starvation they may be formed from tissue proteins. The presence of infection depresses protein regeneration.
2. When plasma protein concentration becomes 4 gm/dL, exhaustion of protein reserves occurs; and
3. When plasma protein concentration becomes less than 2 gm/dL, it results in *shock* (page 387) and death of the

animal. If after withdrawal of the whole blood, the cells are treated with serum and then re-injected, it causes no shock because of less loss of plasma proteins.

Conclusion. Whipple's suggested that proteins of cells are of 3 types:

1. *Fixed cell proteins or indispensable cell proteins.* These are not mobilized even when serum plasma protein level is less than 2 gm/dL. It is required for all essential metabolic activity of tissues and plays no role in compensatory mechanisms.
2. *Dispensable reserve proteins* can be called upon for energy and other purposes in starvation and malnutrition etc. Increase breakdown of endogenous proteins from skeletal muscle and glands serves this purpose.
3. *Labile reserve proteins.* When plasma protein level decreases they move out readily (immediately) into the blood stream and compensate for protein loss such as in haemorrhage, burns etc. *Main sites:* Liver, tissue macrophage system and other sites of protein synthesis.

VARIATIONS IN PLASMA PROTEIN CONCENTRATION

1. **Decrease (Hypoproteinaemia)** – Haemorrhage results in loss of all forms of plasma proteins. After haemorrhage, fibrinogen, globulin and albumin are regenerated in that order with complete restroration effected in a few days (page 406); burns; pregnancy; malnutrition, starvation.
2. **Increase (Hyperproteinaemia)** – This is because of loss of more water from the plasma *e.g.* secondary to extensive burns (page 389), dehydration and diabetes insipidus (page 674); hemolysis; leukaemia.
3. **Decrease in Albumin**

   Physiological
   - (i) Infancy and newborns (normal plasma protein level: 5.1–5.5 gm/dL) because of hepatic immaturity.
   - (ii) Pregnancy (during 1st six months). Globulin also decreases.

   Pathological
   - (i) *Impaired protein synthesis due to:*
     - (a) Hepatitis
     - (b) Cirrhosis of liver
     - (c) Chronic diseases
     - (d) Severe malnutrition and fasting causes poor supply of proteins
     - (e) Malabsorption
   - (ii) *Excessive loss due to:*
     - (a) Burns
     - (b) Nephrosis. It causes increased loss of albumin in urine.
4. **Increase in Albumin**
   - (i) Dehydration
   - (ii) Congestive cardiac failure
5. **Increase in γ-globulin** – Mainly due to extensive tissue destruction, seen in:
   - (i) Multiple myeloma (page 130)
   - (ii) Tuberculosis
   - (iii) Lymphatic leukaemia (page 130)
   - (iv) Cirrhosis of liver and Acute hepatitis.
   - (v) Nephritis
6. **Decrease in γ-globulin**
   - (i) Nephritis
   - (ii) Hypogammaglobulinaemia

Important Note

When the body tissues are extensively damaged, though plasma albumin falls, the plasma immunoglobulin rises probably as a result of plasma cells hyperplasia (page 116). Thus causing reversal of A/G ratio.

7. **Fibrinogen** – Solely manufactured in the liver.

   Decreases in:
   - (i) congenital (rare)
   - (ii) carcinoma prostate
   - (iii) extensive cardiac or pulmonary surgery. (fibrinogen decreases due to fibrinolysis *i.e.* dissolution of blood clot.)
   - (iv) intra-vascular coagulation.

   Increases in:
   - (i) pregnancy, menstruation
   - (ii) malaria
   - (iii) tissue injury
   - (iv) acute or chronic infections

FUNCTIONS OF PLASMA PROTEINS

1. **Helps in coagulation of blood** due to presence of fibrinogen, prothrombin and other coagulation factors which are protein in nature.
2. **Helps to maintain colloidal osmotic pressure (COP)** across the capillary wall; normally it is 25-30 mmHg. How?
   - (i) Osmotic pressure across the capillary wall can be exerted both by (a) the crystalloids *e.g.* urea, Na^+, glucose etc., and (b) the colloids *e.g.* plasma proteins.

     However, capillary wall is completely permeable to crystalloids, therefore, crystalloids hardly contribute to capillary osmotic pressure and proteins exert an osmotic force of 25 mmHg across the capillary wall.

Note

COP due to the plasma colloids is called the *oncotic pressure*, refer to page 17.

(ii) COP is inversely proportional to the molecular size and shape, and is directly related to the concentration of molecules. Therefore, 80% of COP is due to albumin because of least molecular weight (*i.e.* molecule size) and maximum concentration.

(iii) COP across the capillary wall helps to maintain the exchange of fluid at tissue level. The rate of fluid exchange (*i.e. filtration–absorption*) at any point along a capillary depends upon a balance of forces, called *Starling forces* (*E.H. Starling*, 1896); which are (Fig. 6.1):

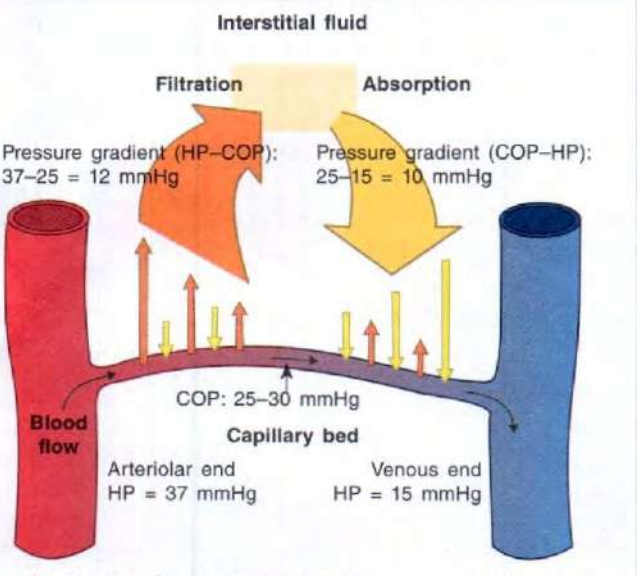


Fig. 6.1 *Starling forces:* Pressure gradient across capillary wall (in most vascular beds) (COP: colloidal osmotic pressure; HP: hydrostatic pressure)

(a) Hydrostatic pressure across capillary wall – it favours filtration.

(b) COP across capillary wall – it favours absorption.

(c) Hydrostatic pressure in interstitial fluid: Normal 2-3 mmHg.

(d) Interstitial fluid osmotic pressure: Normal 3-4 mmHg.

(c) and (d) essentially cancel each other, therefore, forces which determine fluid exchange at tissue level are (a) and (b).

Hydrostatic pressure at arteriolar end is 37 mmHg, therefore, some fluid is forced out of capillary bed: 37 – 25 = 12 mmHg.

Hydrostatic pressure at venous end is 15 mmHg, therefore, some fluid will be pulled back by osmotic forces: 25 – 15 = 10 mmHg. (Also refer to page 355) (*For whole body capillary filtration coefficient: Refer to pages 356 and 517*).

Applied

(a) *Hypoproteinaemia* (*i.e.* decrease in plasma protein level) causes decrease in COP, therefore, increase filtration occurs at arterial end and decrease in absorption of fluid at venous end, resulting in *abnormal collection of fluid in interstitial spaces*, called ***OEDEMA***.

(b) When capillary permeability is increased *e.g.* in anoxia, urticaria, inflammation etc., all the proteins escape much more readily from the capillary into interstitial spaces producing *oedema*.

3. **Helps in maintaining viscosity of blood.** How?

The viscosity of a protein depends on:

(i) the shape of the protein molecules (mainly), and

(ii) the size of the protein molecules.

The less symmetrical the molecule (like fibrinogen), the greater is its viscosity. Since 80% of total plasma protein concentration is due to albumin, and fibrinogen is present in traces, blood viscosity is maintained at low level. Normally viscosity of blood is 4-5 times that of water.

4. **Helps in maintaining systemic arterial blood pressure constant.**

The resistance to flow of fluid at constant velocity through a capillary depends almost entirely on viscosity of fluid. The arterial blood pressure *i.e.* resistance to blood flow is directly proportional to viscosity of blood. However, plasma proteins maintain the blood pressure constant by maintaining viscosity of blood.

5. **Provides stability to blood** due to presence of globulin and fibrinogen. If blood loses its stability, it will lead to ***Rouleaux*** formation of RBCs *i.e.* RBCs pile one over another.

6. **Helps in maintaining the acid-base balance in the body.**

Plasma proteins act as buffers. Their buffering capacity is 1/6th (about 15%-16%) of total buffering capacity of blood. They are ***amphoteric*** in nature *i.e.* behave as acids or bases depending on the conditions and thereby maintain the blood pH at 7.4, by accepting or donating H^+.

Mechanism: At physiological blood pH of 7.4, plasma proteins exist in an 'ionised' form *i.e.*

(i) 'C' terminal end is in the form of 'COO^-, and

(ii) 'N' terminal end is in the form of $-NH_3^+$.

Therefore, 'C' terminal end can buffer the change that may follow addition of an acid whereas 'N' terminal end can buffer the change that may follow addition of an alkali (Fig. 6.2).

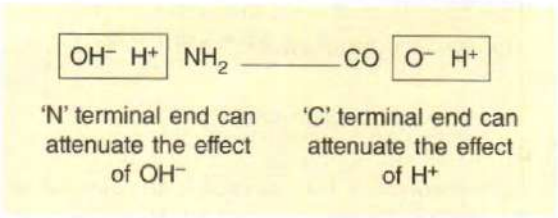


Fig. 6.2 Buffering action of plasma proteins

7. Leucocytes can manufacture a few substances called ***Trephones*** or ***Carrel*** from plasma proteins, which help in the nutrition of tissues.
8. **Immune function.** γ-globulin produce antibodies which provide *immunity* to the body.
9. **Transport function.** Plasma proteins combine loosely with many agents *e.g.*
   (i) hormones (thyroxine, cortisol)
   (ii) drugs
   (iii) metals
      (a) 'transferrin' (α_2 β_1 globulin) with two atoms of Fe^{3+}, is carried to the site of storage.
      (b) 'ceruloplasmin' (α_2 β_1 globulin) with copper, circulates in blood.
10. **Reservoir function.** Plasma proteins form loose bond with the hormones, drugs and metals etc. to serve as a 'reservoir', from which the same are released slowly. This plays a beneficial role during starvation.

Study Questions

1. Give physio-clinical significance of
   (i) Whipple's experiment (ii) Osmotic and Oncotic pressure (iii) Lipoprotein complexes
2. Write short notes on:
   (i) Origin of plasma proteins (ii) Hepato-lenticular degeneration
   (iii) Role of diet to plasma proteins (iv) Variations in plasma protein concentration
   (v) Plasmapheresis (vi) Oedema
3. Depict diagrammatically Starling forces that determine filtration-absorption across the capillary wall.
4. Give an account of forms of plasma proteins and their functions.
5. What will happen and why if total plasma protein concentration decreases?

MCQs

1. Which is *true* value for normal plasma level:
   (a) Albumin: 2-3 gm/dL (b) Globulin: 3-5 gm/dL
   (c) Fibrinogen: 0.3gm/dL (d) Prothrombin: 0.03 gm/dL
2. Lipoprotein with maximum fat content is:
   (a) High density lipoprotein (HDL) (b) Low density lipoprotein (LDL)
   (c) Very low density lipoprotein (VLDL) (d) Chylomicrons
3. In a healthy individual sudden decrease of total plasma proteins upto 4-5 gm/dL produces:
   (a) Protein shock (b) No ill-effects on the body
   (c) Exhaustion of protein reserves (d) Generalised oedema
4. Protein shock occurs when total plasma protein concentration becomes less than:
   (a) 2 gm/dL (b) 3 gm/dL (c) 4 gm/dL (d) 5 gm/dL
5. Following a haemorrhage, the order in which plasma proteins are regenerated:
   (a) Albumin, globulin, fibrinogen (b) Globulin, fibrinogen, albumin
   (c) Fibrinogen, globulin, albumin (d) Fibrinogen, albumin, globulin
6. Total plasma protein levels are low during infancy due to:
   (a) Low protein intake
   (b) Increased protein loss in urine
   (c) Hepatic immaturity
   (d) Total plasma protein levels are higher in infants as compared to adults
7. Oncotic pressure of plasma is due to:
   (a) Albumin (b) Preallbumin (c) Electrolytes (d) Fibrinogen

8. **Which one of the following is *not* a crystalloid?**
   (a) Urea (b) Na^+ (c) Glucose (d) Albumin
9. **Albumin fraction in plasma contributes to colloidal osmotic pressure to the extent of:**
   (a) 20% (b) 50% (c) 80% (d) 100%
10. **Calculate the net filtration pressure across the capillary wall.**
    Interstitial fluid hydrostatic pressure = 5.3 mmHg
    Plasma colloid osmotic pressure = 18 mmHg
    Capillary hydrostatic pressure = 17 mmHg
    Interstitial fluid colloid osmotic pressure = 6 mmHg
    (a) +0.5 mmHg (b) –0.3 mmHg (c) –0.5 mmHg (d) +0.3 mmHg
11. **Which of the following promotes the rouleaux formation?**
    (a) Albumin (b) Prealbumin (c) Fibrinogen (d) Prothrombin
12. **Buffering capacity of plasma proteins is ..... of total buffering capacity of blood:**
    (a) 10% (b) 15% (c) 20% (d) 25%
13. **In coronary artery disease patient which form of lipoprotein complex increases?**
    (a) High density lipoprotein (HDL (b) Low density lipoprotein (LDL)
    (c) Very low density lipoprotein (VLDL) (d) (b) and (c)
14. **Which statement is *not true* of plasmapheresis?**
    (a) It is a procedure of separation of plasma from the blood
    (b) It is done to exhaust the protein reserves
    (c) It is done to find out the rate of regeneration of plasma proteins on a standard diet
    (d) It is most commonly employed procedure during dialysis
15. **Increase in gamma globulin is mainly seen in:**
    (a) Tissue destruction (b) Severe malnutrition
    (c) Fasting (d) Malabsorption
16. **Increased capillary protein permeability will often cause:**
    (a) Decreased interstitial fluid protein concentration (b) Increased lymph flow
    (c) Increased capillary hydrostatic pressure (d) Interstitial oedema

Answers

1. (c) 2. (c) 3. (b) 4. (a) 5. (c) 6. (c) 7. (a) 8. (d) 9. (c) 10. (b)
11. (c) 12. (b) 13. (d) 14. (d) 15. (a) 16. (d)

Chapter 7

Haemoglobin

I. Structure
II. Some important definitions: Oxyhaemoglobin; carbamino-haemoglobin; reduced haemoglobin; carboxyhaemoglobin; methaemoglobin
III. Normal values
IV. Functions of haemoglobin
V. Disadvantages of 'free' haemoglobin
VI. Synthesis of haemoglobin
VII. Catabolism of haemoglobin
VIII. Varieties of haemoglobin: HbA; HbF-thalassaemia; HbS

The red, oxygen carrying pigment in the RBCs of vertebrates is *haemoglobin*. It consists of the protein *globin* (polypeptide) united with the pigment *haem* (heme).

STRUCTURE

1. ***Haem*** is an iron containing porphyrin, called iron-protoporphyrin IX. The porphyrin nucleus is *tetrapyrrole i.e.* it consists of 4 'pyrrole rings' joined together by 4 methine (= CH–) bridges. The pyrrole rings are numbered I, II, III and IV; the carbon atoms of the methine bridges are labelled α, β, γ and δ; the position on pyrrole rings to which side chains are attached are numbered 1 to 8. The side chains at 1, 3, 5 and 8 position are methyl ($-CH_3$); 2 and 4 are vinyl ($-CH = CH_2$); 6 and 7 are propionic acid ($-CH_2.CH_2.COOH$). (Fig. 7.1)
2. The ***iron*** in haem is in the ferrous (Fe^{2+}) form. The iron is attached to the 'N' of each pyrrole ring. Each Fe^{2+} combines loosely and reversibly with one molecule of oxygen. Combination of haem with oxygen is called *oxygenation* and **not** oxidation, because, after combination with oxygen, iron in the haem stays in Fe^{2+} state. *Therefore, the oxygen does not become ionic oxygen but is carried as molecular oxygen.*
3. ***Globin*** is a protein built from 4 polypeptide chains, two 'α' and two 'β' chains. Therefore, the normal adult haemoglobin (HbA) is written as **HbA** ($\alpha_2\beta_2$). Of two α-chains each contains 141 amino-acids and of two β-chains each contains 146 amino-acids. Each polypeptide chain is associated with one haem group. Thus, there are 4 haem to the one molecule of haemoglobin, contains 4 iron atoms and can carry 4 molecules (8 atoms) of oxygen.
4. Oxygenation of 1st haem molecule in haemoglobin, increases the affinity of 2nd haem for oxygen and oxygenation of 2nd haem increases the affinity of the 3rd and so on. Therefore, the affinity of haemoglobin for the 4th oxygen molecule is many times that of the 1st. This shifting affinity of haemoglobin for oxygen results in:
   (i) *Sigmoid* shape of ***oxygen-haemoglobin dissociation curve*** (page 429).
   (ii) Haemoglobin reacts with oxygen very rapidly requiring less than 0.01 second. Similarly, deoxygenation of haemoglobin is also very rapid.
5. *Molecular weight* of haemoglobin is 68,000.

SOME IMPORTANT DEFINITIONS

1. OXYHAEMOGLOBIN

Haemoglobin reacts with oxygen to form *oxyhaemoglobin* and is represented as HbO_2.

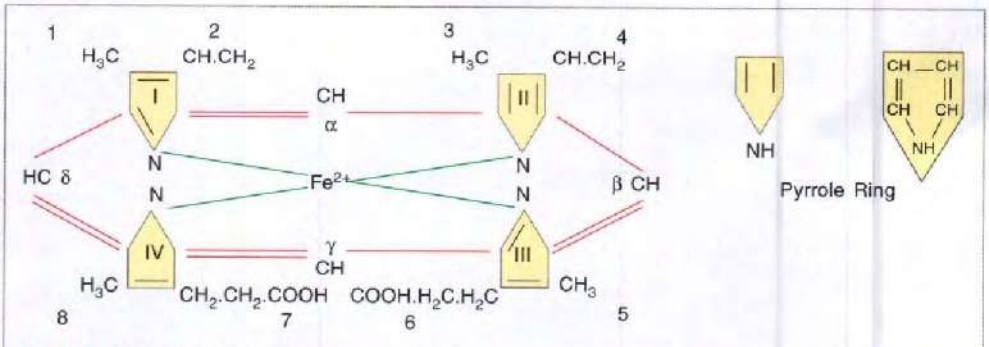


Fig. 7.1 Structure of haemoglobin molecule

The affinity of haemoglobin for oxygen is influenced by pH, temperature and concentration of 2, 3, diphosphoglycerate (2,3 DPG) in the RBCs, a product of metabolism of glucose. As concentration of 2,3 DPG rises, the affinity of haemoglobin for oxygen falls and the *oxygen-haemoglobin dissociation curve* is shifted to the right; as a result more oxygen is released by blood to the tissues.

Important Notes

1. At high altitude (*e.g.* 5000 metres above sea level) 2,3 DPG concentration in RBCs increases by 50% and this makes more oxygen available to the tissues.
2. Stored blood loses its 2,3 DPG and oxygen affinity of haemoglobin increases resulting in less release of oxygen.

2. CARBAMINO-HAEMOGLOBIN

Carbon dioxide reacts with haemoglobin to form *carbamino-haemoglobin.*

$$CO_2 + HbNH_2 \longrightarrow HbNH\ COOH$$

3. REDUCED (DEOXYGENATED) HAEMOGLOBIN

Haemoglobin from which oxygen has been removed is called reduced or deoxygenated haemoglobin and is represented as ***Hb***.

4. CARBOXY HAEMOGLOBIN or CARBON MONOXY HAEMOGLOBIN

Carbon monoxide (CO) reacts with haemoglobin to form *carboxy haemoglobin* or *carbon monoxy haemoglobin*. The affinity of haemoglobin for CO is 210 times than its affinity for oxygen which consequently displaces oxygen on haemoglobin, reducing the oxygen carrying capacity of blood.

5. METHAEMOGLOBIN

When either reduced or oxygenated haemoglobin is exposed to various drugs or oxidising agents, the ferrous (Fe^{2+}) is oxidised to ferric (Fe^{3+}) form and the compound is called *methaemoglobin*. It is represented as ***HbOH***.

Disadvantages

(i) It cannot unite reversibly with gaseous oxygen.
(ii) It is dark coloured and when it is present in large quantities (more than 1.5% gm/dL) in circulation it ***resembles cyanosis*** *i.e.* blue colouration of skin (Also refer to page 461).

Some oxidation of haemoglobin to methaemoglobin occurs normally, but an enzyme in RBCs, the NADH (dihydronicotinamide adenine dinucleotide)-methaemoglobin reductase system, converts methaemoglobin back to haemoglobin. Congenital absence of this system causes ***hereditary methaemoglobinemia***, a fatal condition. (Fig. 7.2)

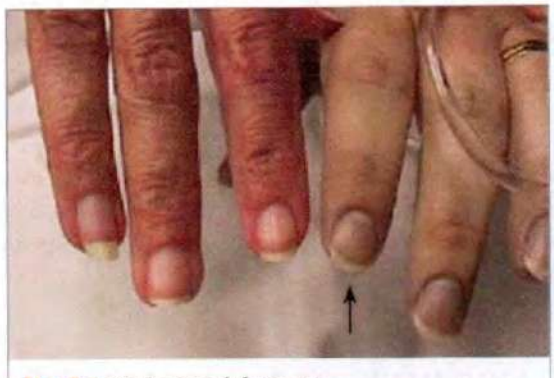


Fig. 7.2 Methaemoglobinemia
(**Note:** Dark coloured fingers on the right)

NORMAL VALUES

1. **At birth**: 23 gm/dL, because RBC count is more.
2. **At the end of 3 months**: 10.5 gm/dL, as an infant is totally on milk feed which is devoid of iron.
3. After 3 months, haemoglobin increases gradually and **at the end of 1 year** it becomes 12.5 gm/dL.
4. **Adults**
   Males : 14-18 gm/dL (Average: 15.5 gm/dL)
   Females: 12-15.5 gm/dL (Average: 14 gm/dL)
   Clinically 14.8 gm/dL haemoglobin irrespective of sex is regarded as 100% haemoglobin.

When blood is equilibrated with 100% oxygen (pO_2 = 760 mmHg), the normal haemoglobin becomes 100% saturated.

1 gm/dL haemoglobin when fully saturated combines with 1.34 mL oxygen, therefore, haemoglobin concentration is an index of ***oxygen-carrying capacity*** of blood. Normal values – *males*: 21 mL/dL, *females*: 18 mL/dL.

FUNCTIONS OF HAEMOGLOBIN

1. Facilitate transport of oxygen from lungs to the tissues.
2. Facilitate transport of CO_2 from the tissues to the lungs (refer to page 434).
3. It acts as an excellent acid-base buffer, being a protein (page 55). It is responsible for 70% buffering power of whole blood.
4. It has additional nitric oxide (NO) binding site on the β-chain which is increased by O_2. Therefore, haemoglobin binds with NO in the lungs and releases it in the tissues where it promotes vasodilation.

DISADVANTAGES OF 'FREE' HAEMOGLOBIN

(Why haemoglobin is contained within the RBCs?)

1. If haemoglobin was dissolved in the plasma (***called free haemoglobin***) it would lead to:

(i) increase in the viscosity of plasma, hence of whole blood, causing BP to rise, and
(ii) increase in the osmotic pressure of plasma to 100 mmHg.
(i) and (ii) interfere with the mechanism of fluid exchange between capillaries and tissue spaces (page 55).

2. Loss of *free* haemoglobin by the kidneys in urine *(haemoglobinuria)* also results in kidney damage.
3. *Free* haemoglobin is taken up and rapidly destroyed by the tissue-macrophage system.

SYNTHESIS OF HAEMOGLOBIN

Synthesis of haemoglobin requires the provision of nutrients *e.g.* proteins, vitamins, minerals (specially iron). It only takes place in the developing RBCs (erythroid series, page 69).

Factors controlling haemoglobin formation

1. *Role of proteins* – A low protein intake decreases haemoglobin regeneration even in the presence of excess of iron; the limiting factor here is lack of *globin* formation.
2. *Role of Minerals*
   (i) *Iron*
      (a) it helps in formation of *haem;*
      (b) *iron content of haemoglobin* is 0.33%, therefore 100 mL of blood containing 15 gm of haemoglobin contains 15 × 0.33/100 = approx. 50 mg of iron.
      (c) as life span of RBC is 120 days, therefore, 0.8% (1/120 × 100) of total blood haemoglobin contained in 50 mL (6 litres × 0.8%) of blood, is destroyed daily, releasing approx. 25 mg of iron. This iron is reused for fresh synthesis of haemoglobin.
   (ii) *Copper* – helps in promoting the absorption, mobilization and utilization of iron. Very little copper is required; adequate amount occurs in diet and most iron preparations contain traces of copper.
   (iii) *Cobalt* is necessary for manufacture of vitamin B_{12} by bacterial action in the lumen of GIT. It also increases the production of a hormone *erythropoietin* (page 68) which, in turn, stimulates the development of RBCs.
   (iv) *Calcium* – increases iron absorption from GIT.
3. *Role of vitamins* – Vitamin C, vitamin B_{12} and folic acid help in synthesis of nucleic acid which in turn, is required for the development of RBCs.

CATABOLISM OF HAEMOGLOBIN

Old RBCs are destroyed in *'tissue-macrophage system'* (previously called 'reticulo-endothelial system' page 114).

The fate of haemoglobin after its destruction is shown in Fig. 7.3.

Note

Approx. 0.3 gm of haemoglobin is destroyed and 0.3 gm synthesized every hour.

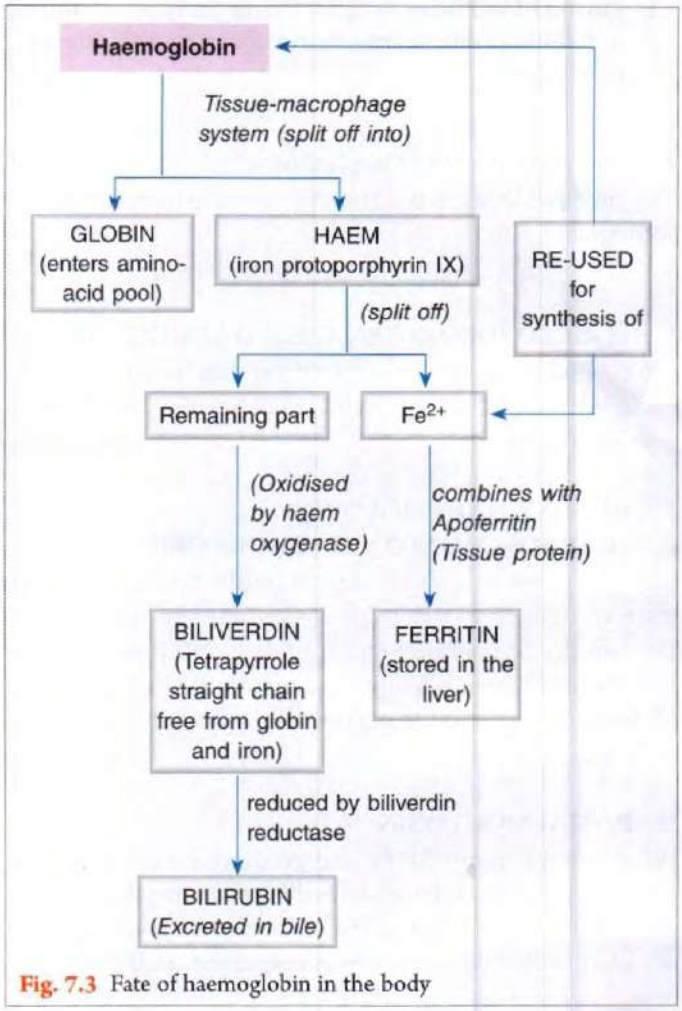


Fig. 7.3 Fate of haemoglobin in the body

VARIETIES OF HAEMOGLOBIN

Several varieties of haemoglobin occur in human, in all the 'haem' moiety is the same; physical and chemical differences being due to variations in the composition of the peptides of the 'globin' fraction. The amino-acid sequence in the polypeptide chains of haemoglobin is determined by 'globin' genes.

Important Note

In diabetes mellitus (DM) patients, small amount of HbA is non-enzymatically glycosylated to form haemoglobin A_{1C} (HbA_{1C}). It has a glucose attached to the terminal valine in each β-chain. HbA_{1C} concentrations are measured as an index of control of DM (page 751).

1. ADULT HAEMOGLOBIN (HbA)

It is of two types:

(i) ***Haemoglobin A*** ($\alpha_2\beta_2$) – predominately seen; as described above (page 58).

(ii) ***Haemoglobin A_2*** ($\alpha_2\delta_2$). Here β chains are replaced by δ chains. The δ chains also contain 146 amino-acid but 10 individual amino-acid differ from those in the β chain. Approx. 2.5% of the total haemoglobin is HbA_2 in normal adults. It produces no abnormality and is regarded as normal haemoglobin.

HbA appears in foetus, after 5 months (20 weeks) of intra-uterine life, when bone marrow begins to function as a haemopoietic agent.

Age	Amount of HbA, of the total haemoglobin
At 20 weeks of intra-uterine life	6% (rest is HbF)
At birth	20%
At 2 months (post-natal)	50%
At 4 months (post-natal)	90%
More than 1 year	> 99% (< 1% is HbF)

2. FOETAL HAEMOGLOBIN (HbF – $\alpha_2\gamma_2$)

Salient features

(i) Its structure is same as of HbA, except that the β-chains are replaced by γ-chains. γ-chains also contain 146 amino-acids but have 37 amino-acids that differ from those in the β-chains.

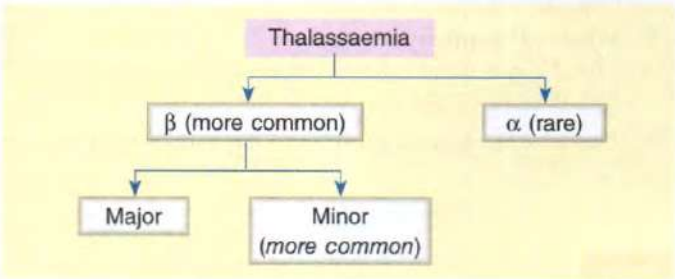
(ii) It is much *more resistant* to the action of *alkalies* than HbA. This property is made use of in a photoelectric calorimetric method to estimate HbF in the presence of HbA.

(iii) HbF has *greater affinity for oxygen*, because of poor binding of 2,3-DPG to the γ-polypeptide chain, therefore, it can take much larger volume of oxygen than HbA at low oxygen pressure. This facilitates the movement of oxygen from maternal to foetal circulation. HbF is 70% saturated at 20 mmHg of pO_2 pressure (pO_2), whereas HbA is only 30-35% saturated at this pressure.

(iv) Its *life span* is less (about 80 days) as compared to that of HbA (120 days).

(v) At birth HbF predominates, (approx. 80%), it gradually disappears 2-3 months after birth.

Hence, persistence of HbF beyond the age of 4-6 months after birth should raise the suspicion of disordered synthesis of HbA due to deficient production of α or β-chains (called α or β ***Thalassaemia*** respectively).



The main differentiating features between the major and minor β-thalassaemia are give in Table 7.1.

3. HAEMOGLOBIN-S (HbS)

It is inherited as Mendelian dominant. Here, in each β-polypeptide chain of HbA *at position 6, one glutamic acid is replaced by a valine.* Therefore, when HbS is reduced (*e.g.*

Table 7.1: Main differentiating features between major and minor β-thalassaemia

		Major β-thalassaemia (also called Cooley's anaemia or Mediterranean anaemia)	Minor β-thalassaemia
1.	Prevalence	: Less common	More common
2.	Inheritance	: 'Homozygous' transmission (*i.e.* when identical abnormal genes are inherited from both parents and all of the haemoglobin is abnormal).	'Heterozygous' transmission (*i.e.* when an abnormal gene is inherited from one parent directs the formation of abnormal haemoglobin).
3.	Anaemia	: Moderate to severe	Mild
4.	Basic defect	: Total absence of β-chain synthesis	Partial synthesis of β-chain
5.	HbF levels	: Markedly increased	Normal or slightly increased
6.	Life span	: Shorter; average age of death 17th year.	Longer; the patient survives upto adult life and can transmit the gene to the offsprings

as in low O_2 tension or low pH at tissue levels), it becomes much less soluble than HbA, haemoglobin precipitates into crystals within RBCs causes the following:

(i) It damages cell membrane producing increased fragility of RBCs.

(ii) Crystals elongate and RBCs become *sickle* shaped (*reaping hook shape* - **Fig. 8.10**, *page 74*) which decreases the blood flow to tissues due to phenomenon of *sickling* (increased blood viscosity).

(i) and (ii) cause RBCs to become more fragile producing severe anaemia, called ***Sickle Cell Anaemia***. Finally, patient dies in a few days due to severe anaemia and secondary infection.

4. MISCELLANEOUS HAEMOGLOBINS

Other haemoglobins are Haemoglobin C, E, I, J and M. HbC is same as HbS but it is not associated with sickling. All these abnormal haemoglobins cause hemolytic anaemias.

SUMMARY

Thus, there are two major types of inherited disorders of haemoglobin in humans:

1. *'Haemoglobinopathies'* in which abnormal polypeptide chains are produced. One of the example HbS.
2. *'Thalassaemias'* in which the polypeptide chains are normal in structure but produced in decreased amounts or absent because of defect in the 'globin' genes.

Study Questions

1. **Write short notes on:**
   (i) Free haemoglobin
   (ii) Haemoglobinopathies
   (iii) Foetal haemoglobin
   (iv) Thalassaemia
   (v) HbA_{1C}
   (vi) Functions of haemoglobin
   (vii) Abnormal haemoglobins
   (viii) Cooley's (or Mediterranean) anaemia
2. **What will happen and why?**
   (i) If iron in haem is present in ferric form?
   (ii) If HbF persists during adult life?
   (iii) If haemoglobin was dissolved in plasma?
   (iv) If glutamic acid in β-chain of HbA gets replaced by valine
3. **Describe the factors controlling haemoglobin formation.**

MCQs

1. **Haemoglobin iron combines with:**
   (a) Molecular oxygen rather than ionic oxygen
   (b) Both molecular as well as ionic oxygen
   (c) Oxygen attached to 2,3 DPG
   (d) Superoxide radical
2. **Each haemoglobin molecule carries how many molecules of oxygen?**
   (a) 2 (b) 4 (c) 6 (d) 8
3. **Affinity of haemoglobin for oxygen is influenced by all of the following *except*:**
   (a) pH (b) K^+ (c) Temperature (d) 2,3 DPG
4. **Increase in affinity of haemoglobin for oxygen is associated with:**
   (a) More release of oxygen by blood to the tissues
   (b) Shifting of oxygen-haemoglobin dissociation curve to the right
   (c) Rise in 2, 3 DPG concentration in the RBC
   (d) Less oxygen available to the tissues
5. **Exposure of haemoglobin to oxidising agents results in:**
   (a) Ferric form to oxidise to ferrous form of iron
   (b) Formation of methaemoglobin
   (c) Cyanosis
   (d) Formation of deoxygenated haemoglobin
6. ***Not True* about free haemoglobin:**
   (a) Increases the viscosity of the blood
   (b) Interfere with fluid exchange across the capillary wall
   (c) Damages the kidney
   (d) Decreases osmotic pressure of plasma
7. **Index of oxygen carrying capacity of blood is:**
   (a) 2,3 DPG levels in the RBCs
   (b) Iron content of haemoglobin
   (c) Haemoglobin concentration in the blood
   (d) Total iron content in the blood
8. ***Not correct* about tissue macrophage system:**
   (a) Also called as reticulo endothelial system
   (b) Destroys all the old blood cells
   (c) Ingests large foreign colloidal particles
   (d) Plays important role in immune response

9. **More than 90% of foetal haemoglobin (HbF) is normally replaced by adult haemoglobin (HbA):**
   (a) Soon after birth
   (b) 1-2 months after birth
   (c) 3 months after birth
   (d) 4 months after birth
10. **Main differentiating features between major and minor β-thalassaemia is that the former:**
   (a) Is more common
   (b) Inherited as heterozygous transmission
   (c) Life span is longer
   (d) Hb-F levels are markedly increased.
11. **Sickle cell anaemia, *false* statement is:**
   (a) RBCs fragility increases
   (b) RBCs are sickle shaped
   (c) Blood flow to tissues decreases
   (d) Manifests as mild anaemia
12. **Hereditary methaemoglobinemia:**
   (a) Is a fatal condition
   (b) Occurs normally due to some oxidation of haemoglobin to methaemoglobin
   (c) Is a reversible blood disorder
   (d) Methaemoglobin level in the blood rises upto 1.5 gm/dL

Answers

1. (a) 2. (b) 3. (b) 4. (d) 5. (b) 6. (d) 7. (c) 8. (d) 9. (d) 10. (d) 11. (d) 12. (a)

Chapter 8

Erythrocyte – Red Blood Corpuscle (RBC)

I. General structure
II. Variations in size, shape and structure of RBC
III. Haemopoiesis: Theories of haemopoiesis; Interleukins (ILs); Colony stimulating factors (CSFs)
IV. Erythropoiesis: Stages; Regulation
V. Anaemias
Grading
Classification: Pernicious anaemia; Folic acid deficiency anaemia; Iron deficiency anaemia; Congenital spherocytosis

GENERAL STRUCTURE

1. **RBC** is a circular, biconcave, non-nucleated disc.
Advantages of biconcave disc:
(i) Allows considerable alteration in cell volume. Thus, can withstand considerable changes of osmotic pressure and resist hemolysis.
(ii) Allows easy folding of RBC on itself when it passes through capillaries.
(iii) Haemoglobin remains distributed in the centre of the RBC which facilitates optimal and quick exchange of gases (*i.e.* oxygen and carbon dioxide).

Important Note

The mature RBC has no nucleus, no mitochondria and no ribosome, still it can live for 120 days and can carry out its normal activities. How?

RBC depends entirely on glucose metabolism for its energy supply.Glucose is transported easily across the cell membrane by *facilitated diffusion* (*i.e.* carrier mediated passive process, page 15). Although the cell has no mitochondria but it has cytoplasmic enzymes for metabolizing glucose and other substances and for utilization of oxygen. As these metabolic systems become progressively less active with time, it limits the life span of RBC.

2. **Structure:** RBC contains haemoglobin, which takes pink colour with Leishman's stain. Its cell membrane contains circular pores, which are concerned with ingress or egress of water and electrolytes. Below the cell membrane is a contractile layer of lipoprotein – *'spectrin'*, which is arranged in a fibrillar manner. It maintains the shape and flexibility of RBC membrane and also contains specific blood group substance, the *antigen*.

3. **Composition (Fig. 8.1)**
(i) 62.5% water
(ii) 35% Haemoglobin (29.5 ± 2.5 pg/RBC)
(iii) 2.5%:
(a) Sugar - glucose
(b) Lipids – cephalin, cholesterol and lecithin
(c) Protein – ***Glutathiones***, albumin like insoluble protein, acts as a reducing agent, thus prevents damage of haemoglobin.
(d) Enzymes of glycolytic system; carbonic anhydrase and catalase.
(e) Vitamin derivatives, and
(f) Ions – Na^+, K^+, Ca^{2+}, PO_4^{3-} and SO_4^{2-}.

4. *Diameter:* 6.5–8.8 µm (average 7.3 µm)

5. *Thickness:* at periphery 2–2.4 µm; at the centre 1.2–1.5 µm

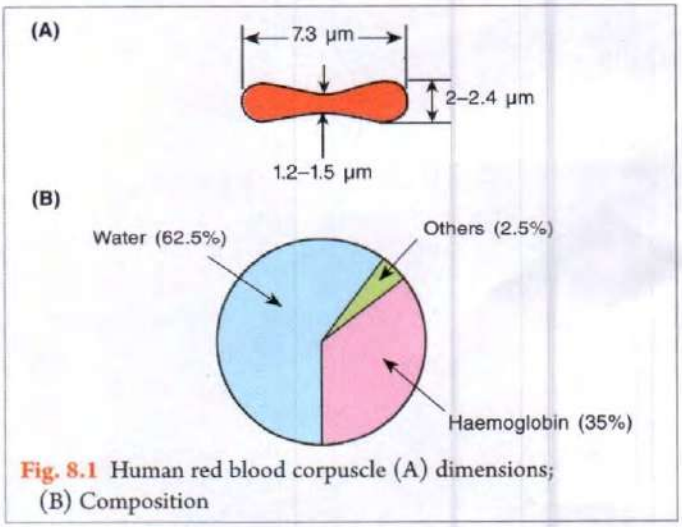


Fig. 8.1 Human red blood corpuscle (A) dimensions; (B) Composition

6. *Surface area:* 140 μm^2
7. *Volume:* 78–94 μm^3 or fL (86 ± 8 μm^3)
8. *Count:*
   At birth; 6-7 million/μL
   Adults – Male: 5–6 million/μL
   (average 5.5 million/μL)
   Females: 4.5–5.5 million/μL
   (average 4.8 million/μL)
   Clinically 5 million/μL is taken as 100% RBC count.

Note

μL was previously called as cumm.

9. *Life Span:* 120 days; determined by—
   (i) Injecting immunological distinct RBCs, then performing agglutination tests at regular intervals.
   (ii) Injection of radioactive iron (RA-Fe) or glycine labelled with isotopic ^{15}N (heavy nitrogen) or RBC tagged with radio-active chromium (^{51}Cr). Basic principle underlying the method is the fact that tagged RBCs lose radio-activity only when they are destroyed. Hence, if radio-activity in RBCs is measured periodically it declines steadily. The time after which radio-activity disappears from the RBC completely gives the normal life span of RBC.
10. *Site of destruction:* Tissue macrophage system (page 114).
11. *Functions*
   (i) That of haemoglobin (page 59).
   (ii) Helps in identifying blood groups as it contains blood group specific substance *i.e. antigen* on its surface.

VARIATIONS IN SIZE, SHAPE AND STRUCTURE OF RBC

1. **Anisocytosis** – Variation in the size of RBCs.
2. **Poikilocytosis** – Variation in the shape of RBCs.
3. **Spherocytosis** – Spherical RBCs; more fragile.
4. **Anaemia** – Reduction in number of RBCs less than 4 million/μL or their content of haemoglobin less than 12 gm/dL or both.
5. **Polycythemia** – RBC count increases more than 6 million/μL.
   Causes:
   A. ***Physiological***
   (i) at birth
   (ii) at high altitude due to chronic hypoxia
   B. ***Pathological***
   (i) congenital heart diseases which produces hypoxia
   (ii) dehydration
   (iii) shock
   (iv) tumour of bone marrow, called ***Polycythemia Vera*** (**Erythraemia**). In this condition RBC count may be increased upto 7–8 million/μL and is associated with *presence of immature RBCs* in the peripheral blood.
6. **At Birth**
   (i) RBCs are larger in size and RBC count is 6-7 million/μL.
   (ii) PCV (packed cell volume) is 54%, because RBC count is more.
   (iii) Reticulocytes are 2-6% of RBC count in the circulation; they decrease to <1% during the 1st week after birth, at which level they remain throughout life.

HAEMOPOIESIS

Definition: It is the development of blood cells *i.e.* RBCs, WBCs and platelets. Therefore, the term haemopoiesis includes:

1. ***Erythropoiesis*** *i.e.* development of RBCs
2. ***Leucopoiesis*** *i.e.* development of WBCs; and
3. ***Megakaryocytopoiesis*** *i.e.* development of platelets.

THEORIES OF HAEMOPOIESIS

1. *Monophyletic Theory* – most acceptable. According to this theory different types of blood cells arise from a single *ancestral cell*, called ***pluripotent stem cell*** present in the bone marrow. **(Fig. 8.2)**

Note

75% of the cells in the bone marrow belong to the WBCs producing *myeloid* series (*i.e.* WBCs and their precursors), and only 25% are of *erythroid* series (*i.e.* RBCs and their precursors).

2. *Polyphyletic Theory* – There are separate stem cells present in the bone marrow for each main variety of blood *i.e.* granulocytes, monocytes, lymphocytes, erythrocytes and platelets.

Monophyletic Theory' of Haemopoiesis

In health, the proliferation and maturation of blood cells that enter the blood from bone marrow is regulated with great precision by: (a) *Interleukins (ILs);* and (b) *Colony-stimulating factors (CSFs).*

Interleukins (ILs) are hormone like *chemical messengers* with known amino-acid sequence. They are produced by lymphocytes, macrophages and other body cells *e.g.* endothelial cells, neurons, glial cells activated T-cells, fibroblast etc. IL are the same as ***cytokines***, but in cytokines

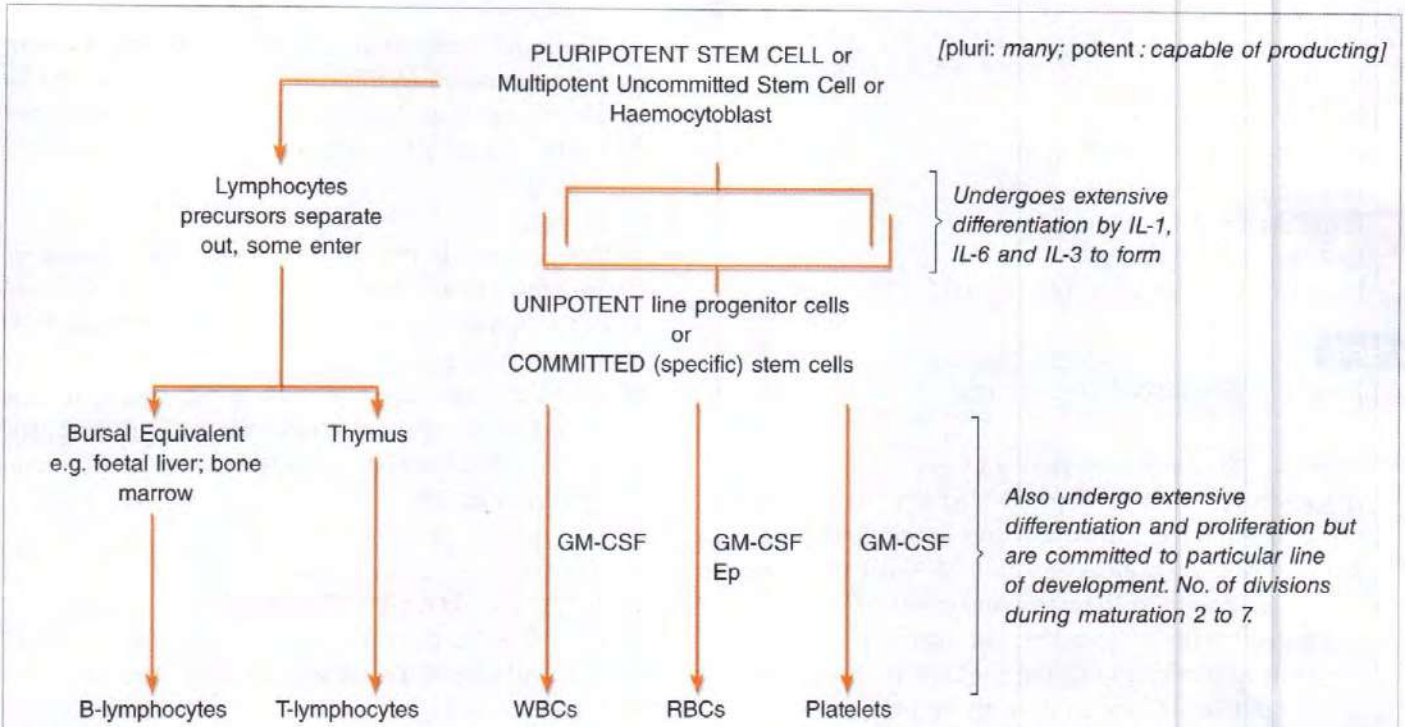


Fig. 8.2 Monophyletic theory of haemopoiesis (IL: Interleukins; Ep: Erythropoietin; GM-CSF: Granulocyte macrophage colony stimulating factors. Important: Differentiated mature cells in peripheral blood lose the power of further division, exception is lymphocyte.

amino-acid sequence of a factor is not known. Initially, the messengers secreted by lymphocytes were often called *lymphokines*. However, since they are produced by other cells as well, therefore, more appropriate term *cytokines* is used now. Cytokines play an important role in regulation of the immune system (details page 127).

Colony stimulating factors (CSFs) are growth factors or hormones. They cause appropriate single stem cell to proliferate in culture medium *e.g.* soft agar, forming colonies of daughter cells.

The principle 'ILs' and 'CSFs' with their functions are given in Table 8.1.

IL-1, IL-6 and IL-3 act in sequence to convert *multipotent uncommitted* stem cell to *committed* stem cells. 'CSF' and 'IL' have overlapping action. Furthermore, they activate and sustain mature blood cells.

Note

In health, number of all types of cells in peripheral blood are kept constant within narrow limits by 'feedback control mechanisms' (page 3). This shows that the rate of formation, release and destruction of blood cells is balanced.

Table 8.1: Principal Interleukins (ILs) and Colony Stimulating Factors (CSFs)

IL-1, IL-3 (or multi-CSF) and IL-6	:	Increases secretion of CSF and count of all blood cells except lymphocytes
IL-4	:	Increases basophil production
IL-5	:	Increases eosinophil production
IL-11	:	Increases production of granulocytes, RBCs and platelets
G-CSF (granulocyte-CSF)	:	Increases production of granulocytes
M-CSF (macrophage-CSF)	:	Increases production of monocyte
GM-CSF (granulocyte-macrophage CSF)	:	Increases production of neutrophil, monocyte, eosinophil, RBCs and platelets.
Ep-(Erythropoietin)	:	Increases production of RBCs
Thrombopoietin	:	Increases production of platelets

ERYTHROPOIESIS

(A) **During intrauterine life** – 3 stages.

1. *Mesoblastic stage.* In early embryo upto 3 months of foetal life, RBCs are formed from mesoderm of yolk sac or *area vasculosa*. Since erythropoiesis occurs within the blood vessel, therefore, this stage is also called ***intravascular erythropoiesis.*** **(Fig. 8.3)**

Mesoderm consists of syncytium or nucleated mass of protoplasm without distinct cell outline which gives rise to network of capillary vessels lined by endothelium and containing plasma (formed by liquefaction of cytoplasm).

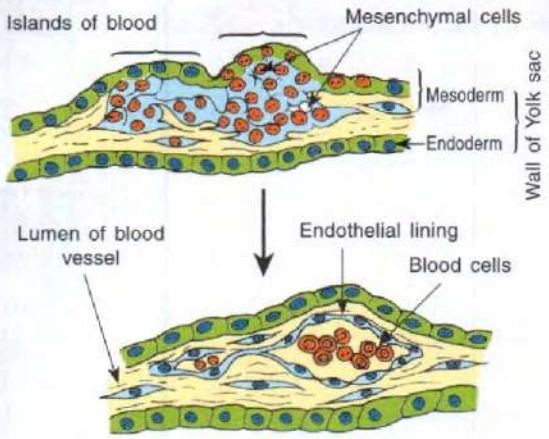


Fig. 8.3 Development of blood vessels and blood cells

Endothelial cells proliferate and differentiate to form masses of nucleated haemoglobin bearing cells; these cells get detached from capillaries and finally lose their nuclei to give rise to non-nucleated discs.

2. *Hepatic stage.* After 3 months of foetal life, liver and spleen are the site of blood formation. Nucleated RBCs develop from the mesenchyme between the blood vessels and the tissue cells.

3. *Myeloid stage.* From the middle of foetal life, erythropoiesis occurs in the bone marrow.

Hepatic and *myeloid stages* are ***extravascular erythropoiesis***.

(B) **In children**, erythropoiesis occurs in:
(1) All bones with red marrow (mainly),
(2) Liver, and
(3) Spleen.

(C) **In adults**, *i.e.* after 18-20 years of age, from red bone marrow which includes:
(1) ends of long bones like humerus and femur, because shaft is converted to yellow marrow;
(2) skull; (3) vertebrae; (4) ribs; (5) sternum, and;
(6) pelvis

If marrow gets destroyed, then liver and spleen again become important sites of blood formation.

(Differences between red and yellow bone marrow are given in Table 8.2.)

STAGES OF ERYTHROPOIESIS

Stages of erythropoiesis are summarized in Table 8.3 and Fig. 8.5.

During the development of erythrocytes as the cell attains maturity, it shows the following ***characteristic features***

1. Reduction in the cell size.
2. Cytoplasm increases in amount and nucleus decreases in size.
3. Staining reaction of cytoplasm changes from deep basophilic to polychromatophilic (acidophilic plus basophilic) and finally to acidophilic; due to gradual reduction in RNA amount.
4. Initially, nucleus is very big in size with open chromatin and containing many nucleoli; with maturity of RBC, chromatin material condenses and degenerates finally leading to disappearance of nucleus and nucleoli.

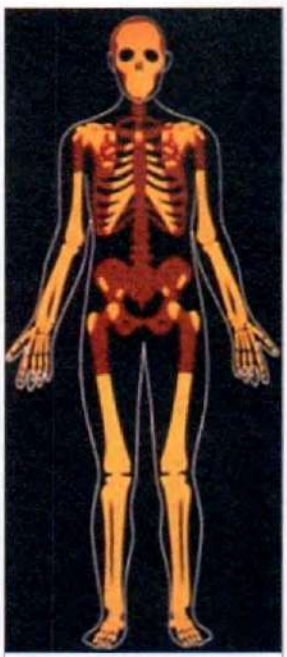


Fig. 8.4 Red and Yellow bone marrow

REGULATION OF ERYTHROPOIESIS

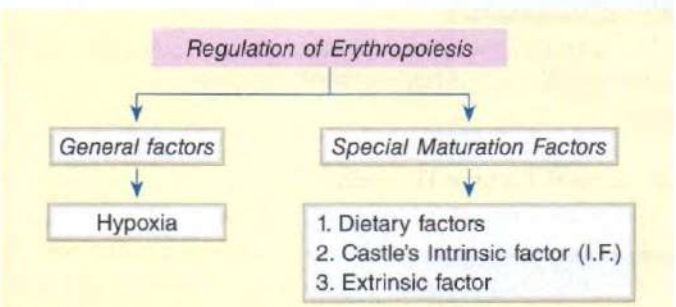


Table 8.2: Red bone marrow versus yellow bone marrow **(Fig. 8.4)**

Red Bone Marrow	Yellow Bone Marrow
1. It consists of numerous blood cells of all kinds and their precursors (erythroid and myeloid)	1. It consists of fat cells, blood vessels and a minimal framework of reticulum cells and fibers.
2. It is also called 'active' marrow.	2. It is also called 'inactive' marrow.
3. It includes the ends of long bones and other sites (see above).	3. It includes shaft of bones.

Important Note

Bone marrow cells (haemocytoblasts) are few in number and can be stimulated to form any cell in the body. They are also capable of completely replacing the bone marrow when injected into a host whose own bone marrow has been completely destroyed.

Table 8.3: Stages of Erythropoiesis (Also refer to Fig. 8.5)

Terminology	Cell size	Nucleus	Cytoplasm		Mitosis
			Staining	Haemoglobin	
Haemocytoblast (Stem cell)	19-23 µm	Very big – occupies almost whole of the cell with open chromatin, containing 4-5 nucleoli; deep basophilic	Rim all around the nucleus; deep basophilic	Absent	Present (++)
Proerythroblast	15-20 µm	Occupies 3/4 of cell volume; 2-3 nucleoli; chromatin open	Slightly more in amount; deep basophilic	-do-	Active mitosis (++)
Early normoblast	14-16 µm	Size decreases; no nucleoli; chromatin condenses	Further increase in amount; less basophilic	-do-	-do-
Intermediate Normoblast (7 Days)	10-14 µm	Nucleus size further decreases; chromatin further condenses	Marked cytoplasm, polychromatophilic staining	*Starts appearing*	-do-
Late Normoblast					
(i) Early	8-10 µm	Nucleus very small with chromatin dot *cart wheel appearance*	Increases markedly	Further increases in amount	*Stops here*
(ii) Late	7-8 µm	Nucleus degenerates, becomes uniformly deeply stained pyknotic	Further increases; more acidic, less basophilic	-do-	Absent
Reticulocyte (2 Days)	7-8 µm	No nucleus; remnants of RNA present	Acidiophilic	-do-	-do-
Erythrocyte	7.2-7.4 µm	Nil	-do-	-do-	-do-

Important Note

Normal *(Normoblastic)* bone marrow contains 30% proerythoblast and early normoblast cells and 70% of intermediate and late normoblast cells.

A. General Factors: Hypoxia

Hypoxia means lack of oxygen at tissue level.

It is the most potent stimulus for the production of RBCs. Hypoxia causes stimulation of bone marrow thereby increases RBC production. This effect is mediated by *erythropoietin*.

Erythropoietin or Haemopoietin or Erythrocyte stimulating factor (ESF) or Erythropoiesis stimulating hormone (ESH)

It is a glycoprotein, 74% protein and 26% carbohydrate; contains 165 amino-acids, molecular weight 46,000; half life: 5 hours.

Sources and metabolism

1. Mainly (85%) secreted by kidneys probably by interstitial cells (or cells in the endothelium) of the peritubular capillaries.
2. 15% produced by extra renal sources like liver parenchymal cells and cells of tissue macrophage system (page 114). The hypoxic stimulus has to be much greater than in normal persons to trigger extra renal erythropoietin production. *Inactivated* in the liver and kidneys; main site of *excretion*-urine.

Formation and Release

Hypoxia of kidneys causes release of ***Renal Erythropoietic Factor*** (REF) or *Erythrogenin* which acts on a plasma substrate, *Erythropoietinogen*, an α-globulin, to form erythropoietin. Hypoxia also increases this specific globulin formation from the liver.

Note

Erythropoietin production reaches its peak within 24 hours of hypoxic stimulus.

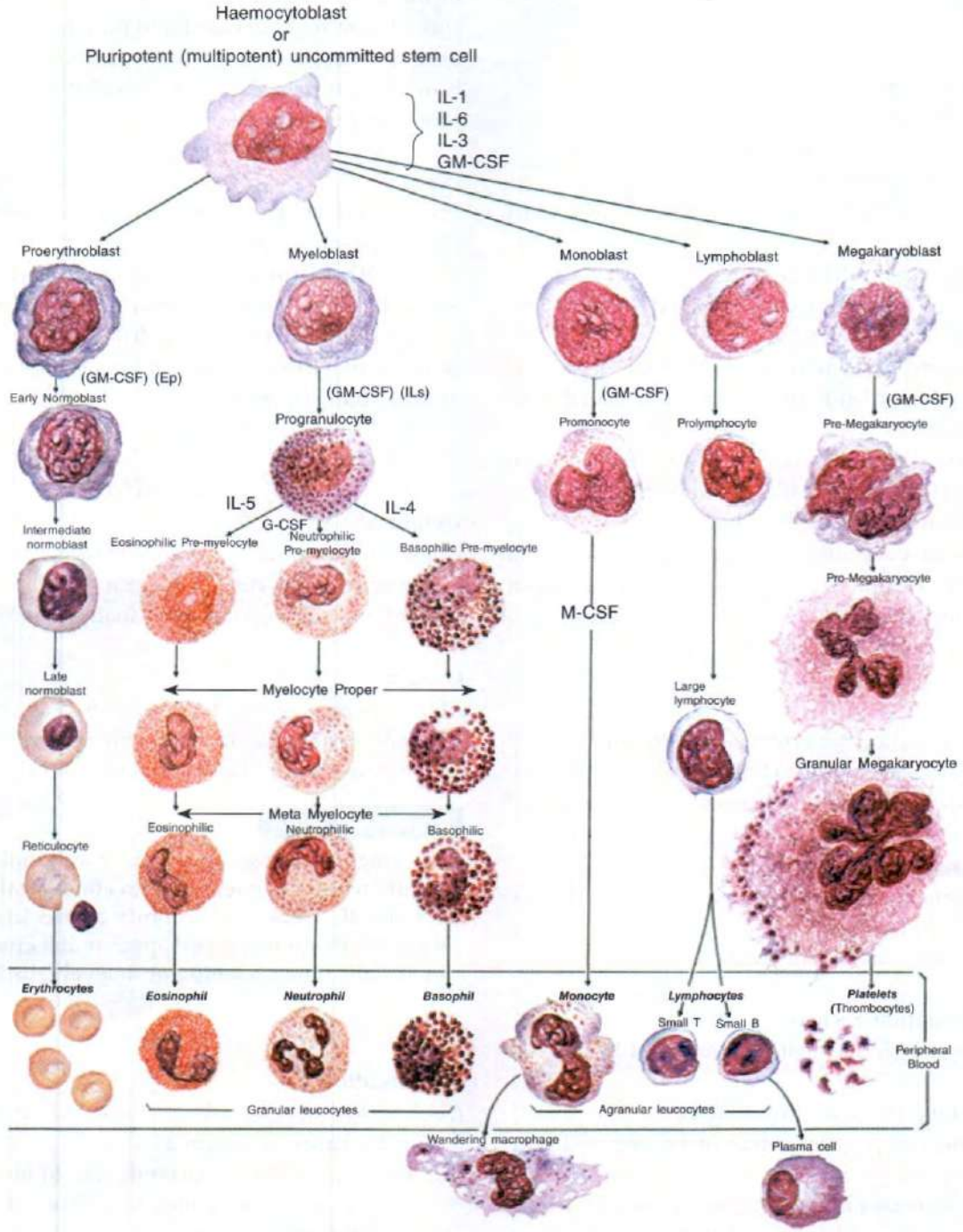
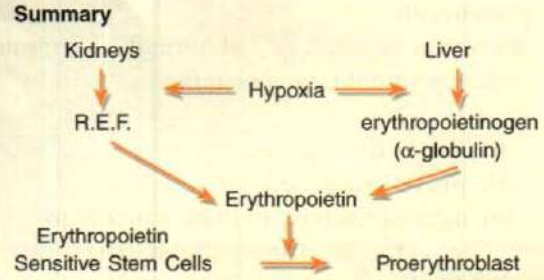


Fig. 8.5 Haemopoiesis: Origin and development of blood cells (IL: Interleukins; Ep: Erythropoietin; GM-CSF: Granulocyte macrophage colony stimulating factor)

Summary



Mode of Action

(i) Causes early differentiation of *erythropoietin sensitive stem cells* in the bone marrow into proerythroblast and subsequently to mature RBCs. Thus, increasing RBC count in 2-3 days.

(ii) Increases release of reticulocytes from the bone marrow.

(iii) Increases synthesis of RNA, DNA, globin and ferritin which increases 'haem' synthesis, thus increasing haemoglobin synthesis in already existing normoblasts.

Factors affecting erythropoietin production

1. ***Increase***
   (i) Hypoxia due to:
      (a) haemorrhage
      (b) high altitude (secondary to alkalosis)
      (c) cardio-respiratory disturbances
      (d) methaemoglobin excess
   (ii) Vasoconstrictor agents (catecholamines) due to renal hypoxia.
   (iii) Nucleotides *e.g.* cAMP, NAD and NADP.
   (iv) Products of RBC destruction, called *haemolysates.*
   (v) Hormones
      (a) Thyroxine *i.e.* why *thyrotoxicosis* leads to polycythemia and *myxoedema* is associated with anaemia.
      (b) Anterior pituitary hormones – TSH; ACTH; GH; LH; FSH and prolactin. Therefore, pituitary disturbances produce anaemia.
      (c) Androgens: stimulate erythropoiesis by increasing REF production and also potentiate the action of erythropoietin.
   (vi) Others *e.g.* cobalt salts.
2. ***Decrease***
   (i) Oestrogen–
      (a) decreases hepatic synthesis of globulin
      (b) depresses the erythropoietic response to hypoxia. *This is why, in females, RBC count is less as compared to males.*
   (ii) Chronic renal diseases
   (iii) Protein deficiency
   (iv) Cirrohosis of the liver
   (v) Chronic inflammatory diseases

B. Special Maturation Factors

(Factors responsible for final maturation of RBCs)

Dietary Factors

(i) Proteins help in 'globin' formation.
(ii) Iron, manganese, copper, cobalt, nickel help in 'haem' formation.
(iii) Calcium increases iron absorption from GIT.
(iv) Vitamins C, B_{12} and folic acid help in synthesis of nucleic acid.

Castle's Intrinsic Factor (IF)

It is a glycoprotein; MW: 50,000 to 60,000; produced by the parietal (oxyntic) cells of the stomach, therefore, it decreases due to:

(i) big gastric ulcer
(ii) malignancy of stomach and
(iii) gastrectomy (removal of stomach).

I.F. helps in absorption of vitamin B_{12} from ileum. How? One molecule of I.F. binds with one molecule of B_{12} to form ***I.F.-B_{12} complex*** which then binds to specific receptor in the ileal mucosa. 'I.F.' is split off from this complex and vitamin B_{12} is released into portal blood. This process does not require energy but is calcium dependent and is optimal at pH 7.0. *'I.F.-B_{12} complex'* is resistant to GIT proteolytic enzymes.

Extrinsic Factors

These are present in certain foods and are essentially vitamin B_{12} and folic acid **(Table 8.4)**.

I.F. with extrinsic factor form ***haematinic principle*** which helps in the maturation of RBCs (for conversion of pro-erythroblast to mature RBC). Therefore deficiency of any one of them causes *maturation failure* and will lead to ***Megaloblastic Anaemia.***

ANAEMIAS

Definition

Anaemia is a clinical condition characterized by reduction in the number of RBCs less than 4 million/μL or their content of haemoglobin less than 12 gm/dL or both.

Grading

Mild Anaemia : Haemoglobin 8-12 gm/dL
Moderate Anaemia : Haemoglobin 5-8 gm/dL
Severe Anaemia : Haemoglobin less than 5 gm/dL

Important Note

In long standing anaemia, exertional dyspnoea occurs when the haemoglobin concentraion is about 7.5 gm/dL, weakness becomes appreciable at about 6 gm/dL; dyspnoea at rest appears at 3 gm/dL and the heart fails when haemoglobin level falls to 2 gm/dL.

Classification

(A) ***Etiological or Whitby's Classification.*** This is based on the cause of anaemia.

1. *Haemorrhagic* – Anaemia due to blood loss
   (i) Acute *i.e.* sudden loss of blood
   (ii) Chronic – slow loss of blood due to piles; worm infestation; peptic ulcer; during menstruation etc.
2. *Dietary deficiencies* due to iron, vitamins and proteins etc.
3. *Dyshaemopoiesis* or abnormal haemopoiesis resulting in ***aplastic anaemia.***
   Causes:
   (i) X-rays irradiations
   (ii) γ-rays irradiation
   (iii) hypersensitivity of bone marrow to
      (a) cytotoxic drugs, sulpha drugs
      (b) chemicals.

Table 8.4: Extrinsic factors: Vitamin B_{12} and Folic acid compared

Features		Vitamin B_{12}	Folic Acid (pteroylglutamic acid)
(i)	Source	Absent in plants. It is also synthesized by bacteria in GIT.	Green leafy vegetables; yeast; liver etc. Food folate is rapidly destroyed by heating.
(ii)	Daily requirements	2 µgm	100-200 µgm
(iii)	Normal plasma concentration	300-400 pg/mL	3-20 ng/mL
(iv)	Site of absorption	Only from ileum in the presence of I.F.	Jejunum
(v)	Storage	In liver it is approx. 1000 times its amount which is present in normal mixed diet taken daily; therefore, *many months of defective vitamin B_{12} absorption will only produce its deficiency.*	Liver, RBCs and WBCs (Total body stores 2-3 mg)
(vi)	Excretion	30-40% of daily intake in faeces, also excreted in urine	Urine
(vii)	Functions	(a) Promotes the maturation and normalization of RBCs (plays an essential role with folic acid in synthesis of nucleic acid-DNA). (b) Increases WBC and platelet count through its action on bone marrow (c) Maintains normal activity of nervous system (d) Helps in myelination of nerve fibres.	(a) As of Vitamin B_{12} (b) It is reduced to tetrahydrofolic acid and then acts as a co-enzyme in different biological systems

Note

***Dyshaemopoiesis* is characterized by decrease in all types of blood cells – RBCs, WBCs and platelets.**

4. *Hemolytic anaemias* – anaemias due to excessive destruction of RBCs.
   (i) *Intrinsic (intra-corpuscular) defects* – hereditary in nature:
      (a) Congenital (or familial or hereditary) spherocytosis.
      (b) Haemoglobinopathies – sickle cell anaemia and thalassaemia.
      (c) Erythroblastosis foetalis.
      (d) Glucose-6-phosphate dehydrogenase (G6PD) deficiency.
   (ii) *Extrinsic (extra-corpuscular) defects* – acquired in nature:
      (a) Antigen-antibody reaction.
      (b) Infection *e.g.* malaria.
      (c) Drugs/poisons *e.g.* quinine, aspirin, burns, snake venom etc.
      (d) Hypersplenism, it causes over activity of normal destructive mechanism.

(B) Morphological or Wintrobe's Classification. This is based on the size of RBC and its haemoglobin concentration **(Table 8.5)**.

PERNICIOUS ANAEMIA or ADDISON'S ANAEMIA

(Pernicious means destructive or injurious.)

Cause

This is due to lack of intrinsic factor with a consequent failure in the absorption of vitamin B_{12}. The primary lesion is atrophy of gastric mucosa which contains oxyntic cells.

Table 8.5: Morphological or Wintrobe's classification of anaemia

	Normochromic	Hypochromic
(a) Normocytic	(i) After acute haemorrhage (ii) All haemolytic anaemias except Thalassaemia (iii) Aplastic Anaemia	After chronic haemorrhage
(b) Macrocytic	All megaloblastic anaemias due to deficiency of Vitamin B_{12}; folic acid or intrinsic factor	Secondary to liver disease
(c) Microcytic	Chronic infections	(i) Iron deficiency anaemia (ii) Thalassaemia

Note

Iron deficiency anaemia is characterized by *microcytic hypochromic* type of RBCs (page 73).

Characteristic features

1. Bone Marrow

Anaemia produces hypoxia which results in stimulation of erythropoiesis in bone marrow with maturation arrest, therefore, bone marrow becomes hyperplastic and gets replaced by:

(i) 70% proerythroblasts and early normoblasts (normal: 30%), and

(ii) 30% intermediate and late normoblasts (normal: 70%)

This over activity of bone marrow is called ***Megaloblastic hyperplasia*** of bone marrow. **(Fig. 8.6)**.

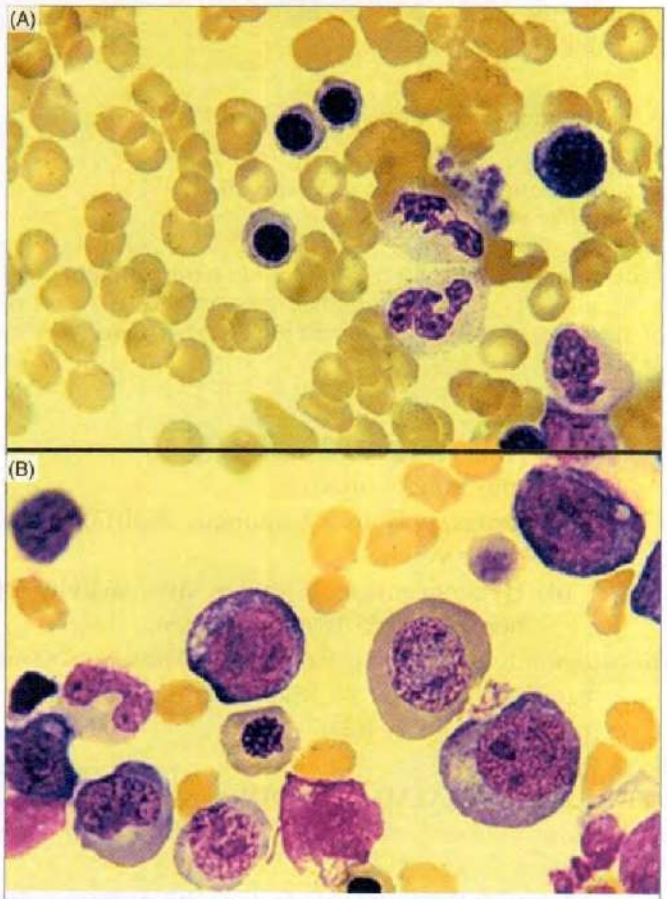


Fig. 8.6 (A) Normal Bone Marrow and (B) Megaloblastic Bone Marow ***Megatoblastic erythroblasts*** can be seen

2. Blood Changes

(i) RBC: ***Macrocytic normochromic*** **(Fig. 8.7)**

(a) Count decreases markedly; less than 1 million/μL

(b) Haemoglobin content decreases less than 12 gm/dL

(c) Diameter increases to 8.2 μm (normal 7.2 μm)

(d) MCV increases to 95-160 fL (normal 78-94 fL)

(e) MCH increases to 50 pg (normal 28-32 pg)

(f) MCHC usually normal (35 ± 3%), because both MCV and MCH increase; it may decrease in late stages.

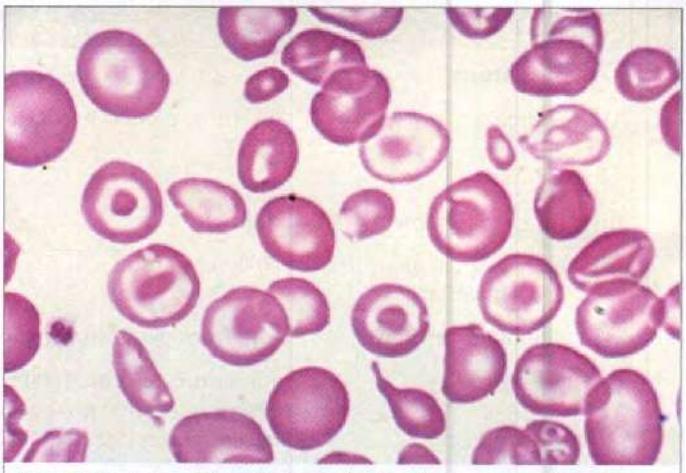


Fig. 8.7 Peripheral blood picture in Pernicious Anaemia. ***Macrocytic normochromic*** RBCs with anisocytosis and poikilocytosis

(g) Peripheral smear shows nucleated RBCs with marked anisocytosis and poikilocytosis.

(h) Reticulocyte count increases more than 5% (normal less than 1%).

(i) Excessive destruction of RBCs in spleen, liver and bone marrow produces:

- decrease in life span of RBCs;
- serum bilirubin increases more than 1 mg/dL (normal 0.2-0.8 mg/dL), this produces low grade ***hemolytic jaundice*** and increased urine urobilinogen excretion;
- increase serum iron (normal 60-160 μg/dL), because iron is not utilized by immature RBCs.

(ii) WBCs and platelets both decrease because encroachment of megaloblastic tissue on the space available in the bone marrow. In addition, vitamin B_{12} is also required for their development.

3. Changes in GIT

(i) Deficiency of intrinsic factor

(ii) Atrophy and destruction of gastric mucosa containing oxyntic cells produces marked or complete lack of HCl in gastric juice (***Achlorhydria***).

(iii) Soreness and inflammation of the tongue.

(iv) Loss of appetite and apathy (mental laziness).

(v) Diarrhoea.

4. Changes in Nervous System

In advanced cases demyelination of white fibres of the spinal cord occurs, affecting the dorsal (posterior) columns chiefly; and later the lateral columns, called ***Subacute Combined Degeneration of Spinal Cord***. This is associated with tingling and numbness in hands and feet; motor and psychological disturbances.

5. Laboratory Investigations

(i) Plasma concentration of vitamin B_{12} decreases to 1/10th of normal (normal: 300-400 pg/mL).
(ii) Vitamin B_{12} excretion in faeces increases to 90% (normal: 30-40%).
(iii) Urinary excretion of vitamin B_{12} decreases, because of poor absorption of vitamin B_{12} from intestine and its low plasma levels.

Signs of improvement after beginning treatment with Vitamin B_{12}

1. ***Reticulocytic response*** *i.e.* increase in number of circulating reticulocytes upto 30-40%. This is due to the proliferation of bone marrow and numerous young RBCs pass into the circulation. This commences within 4-5 days and reaches a peak in 7-10 days. Extent of reticulocytic response is inversely proportional to RBC count before treatment.
2. *Normoblastic reaction* of the bone marrow. Within 6-9 hours of treatment with Vitamin B_{12}, formation of nuclear DNA component promotes the maturation of proerythroblast and early normoblast into intermediate and late normoblast.
3. There is no longer excessive rapid destruction of RBCs, simultaneously serum bilirubin returns to normal.
4. Increase in WBC and platelet count.
5. Cell metabolism increases in general, therefore, patient feels stronger and characteristic symptoms *e.g.* loss of appetite, apathy and digestive disturbances disappear.

Important Note

Treatment of pernicious anaemia with vitamin B_{12} prevents further progression of CNS lesion but cannot reverse the damage already done and changes in the stomach remain unaffected. That is why this anaemia is called *pernicious (destructive or injurious) anaemia.*

FOLIC ACID DEFICIENCY ANAEMIA

Folic acid deficiency also produces ***Megaloblastic anaemia*** as seen with vitamin B_{12} deficiency, except that neuropathy occurs only with Vitamin B_{12} deficiency.

Causes of folic acid deficiency

1. Less dietary intake.
2. Poor absorption *e.g.* in steatorrhoea and sprue (pages 246-247).
3. Increased demand *e.g.* pregnancy.
4. Antifolate drugs *e.g.* anti-cancer drugs (methotrexate).

What will happen if Folic acid is given to Pernicious Anaemia patients?

Both folic acid and vitamin B_{12} plays an important part in number of biochemical reactions and synthesis of nucleic acids. Vitamin B_{12} deficiency interferes with normal folate metabolism and results in functional folate deficiency inspite of adequate folate intake.

Clinical administration of either folic acid or vitamin B_{12} will cure sprue and allied condition and both will improve the blood picture of pernicious anaemia. But while Vitamin B_{12} also protects against 'neuropathy', folic acid has no such effect and *may even accelerate the development of nervous lesions.*

IRON DEFICIENCY ANAEMIA

Commonest anaemia in India.

Definition

Any anaemia which responds to adequate dosage of iron is called iron deficiency anaemia.

Causes

1. Decrease intake – milk fed infants.
2. Increased loss:
   (i) Acute haemorrhage
   (ii) Chronic haemorrhage: worm infestation, peptic ulcer, piles, increased menstrual blood loss etc.
3. Increased demand: Infancy, childhood, pregnancy, menstruation.
4. Defective utilization due to decreased absorption in diseases of stomach and duodenum.

Characteristic features

1. RBC – ***Microcytic hypochromic*** **(Fig. 8.8)**
   (i) Count decreases or normal
   (ii) MCV, MCH, MCHC and CI decrease.

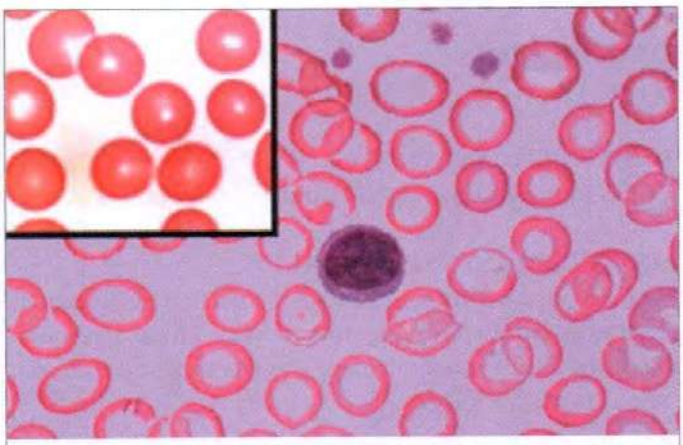


Fig. 8.8 Peripheral blood picture in Iron Deficiency Anaemia. ***Microcytic hypochromic*** RBCs with anisocytosis and poikilocytosis [Inset: Normal RBCs]

(iii) Life span – normal.
(iv) Peripheral smear shows anisocytosis and poikilocytosis.

2. Bone Marrow – ***Normoblastic hyperplasia***
3. WBC and platelets – normal count.
4. Investigations
   (i) Serum bilirubin less than 0.4 mg/dL
   (ii) Serum iron decreases (normal: 60-160 µgm/dL)
   (iii) Total iron binding capacity (TIBC) – increases (normal: 150-350 µgm/dL).
5. Nails – dry, soft, spoon shaped; later develop longitudinal striations. **(Fig. 8.9 A and B)**
6. Tongue – angry red. **(Fig. 8.9 C)**
7. Cardio vascular/Respiratory System – Early breathlessness; palpitations; repeated chest infections.
8. Nervous Sysem – Irritability; loss of concentration; headache; generalized body ache; impotence.

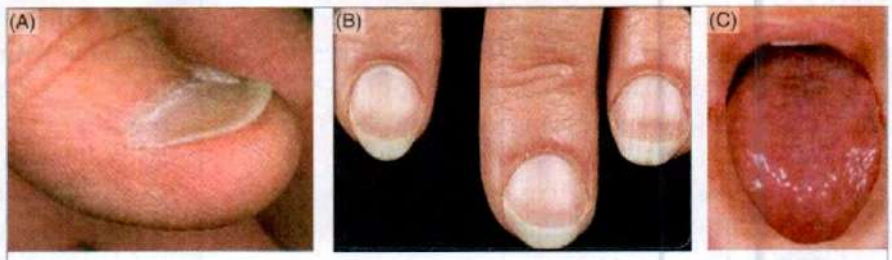


Fig. 8.9 Nails (A, B) and tongue (C) in iron deficiency anaemia

CONGENITAL SPHEROCYTOSIS or HEREDITARY (FAMILIAL) SPHEROCYTOSIS

This is inherited as an autosomal dominant character. Here, below the cell membrane, contractile layer of lipoprotein – *spectrin* is defective due to genetic glycolysis defect. Therefore, RBCs become very small in size and spherical (**microspherocytes**). As biconcavity is lost, many advantages of RBCs are lost (page 62); thus, on passing through capillaries RBCs are easily ruptured even by slightest compression. Life span decreases to 15-20 days.

RBCs are more fragile in hypotonic saline solution. The disease is characterized by splenomegaly and raised serum bilirubin producing hemolytic jaundice (page 72).

THALASSAEMIA

(page 61).

SICKLE CELL ANAEMIA

(For details, refer to page 61, and **Fig. 8.10**).

ERYTHROBLASTOSIS FOETALIS

Here Rh positive RBCs in foetus are attacked by *'sensitized'* antibodies from an Rh negative mother. These antibodies make the RBCs fragile causing severe anaemia in foetus (see also page 106).

GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD) DEFICIENCY ANAEMIA

Enzyme G6PD is required in the formation of NADPH which, in turn, maintains *glutathione* in the reduced state. Decrease in concentration of reduced glutathione, damages the RBC membrane producing hemolytic anaemia and also inhibits the killing of bacteria by granulocytes, thereby predisposes to severe infections.

Sulphonamides and antimalarial drugs can produce G6PD deficiency in normal individuals.

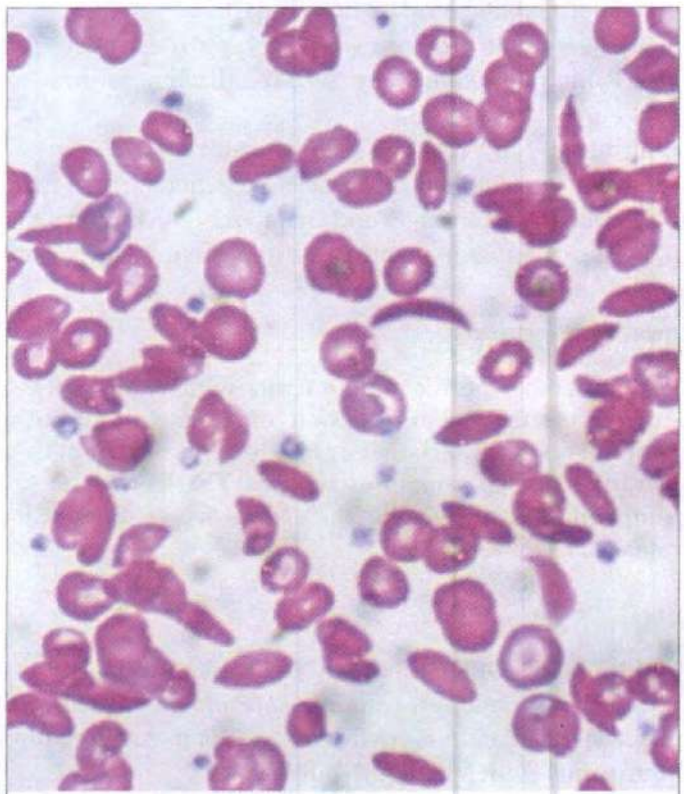


Fig. 8.10 Peripheral blood picture in Sickle Cell Anaemia. ***Sickle shaped RBCs*** with anisocytosis and poikilocytosis

Study Questions

1. **Write short notes on:**
   (i) Haemolytic anaemia
   (ii) Achlorhydria.
   (iii) Spectrin
   (iv) Interleukins and Colony stimulating factors
   (v) Regulation of Erythropoiesis
   (vi) Erythropoietin
   (vii) Haematinic principle
   (viii) Subacute combined degeneration of spinal cord
   (ix) Reticulocytic response
   (x) Erythroblastosis foetalis
   (xi) G6PD anaemia
   (xii) Osmotic fragility of RBCs
2. **Give physiological basis of:**
   (i) why anaemia can never be hyperchromic?
   (ii) Anaemia in kidney or liver disease
   (iii) How a RBC without a nucleus can carry out its normal functions for 120 days?
3. **Differentiate between:**
   (i) Haemopoiesis and erythropoiesis
   (ii) Red and yellow bone marrow
   (iii) Intravascular and extravascular erythropoiesis
   (iv) Vitamin B_{12} and folic acid deficiency anaemia
   (v) Blood picture in vitamin B_{12} and iron-deficiency anaemia
   (vi) Polycythemia and polycythemia vera
   (vii) Normoblastic and megaloblastic bone marrow
4. **Give the stages of erythopoiesis with characteristic features.**
5. **How erythropoietin is formed? Give its mode of action.**
6. **Give basis of morphological classification of anaemias with characteristic features.**
7. **What will happen**
   (i) If folic acid is given to pernicious anaemia patient?
   (ii) If immature RBCs appear in peripheral blood
8. **Give characteristic features including peripheral blood picture of:**
   (i) Iron deficiency anaemia.
   (ii) Pernicious anaemia.

MCQs

1. **Glutathiones within the RBCs:**
   (a) Acts as an enzyme of glycolytic system
   (b) Prevents damage of haemoglobin
   (c) Required in the formation of NADPH
   (d) Maintains the shape and flexibility of RBCs
2. **Which is *not* a function of RBC?**
   (a) Helps in identifying blood groups
   (b) Helps transport of gases
   (c) Provides stability to blood
   (d) Helps in maintaining acid base balance in the body
3. **Normal reticulocyte count at birth is:**
   (a) 1-2%
   (b) 2-6%
   (c) 6-10%
   (d) 30-40%
4. **Interleukins:**
   (a) Are hormones like chemical messengers
   (b) Amino acid sequence is not known
   (c) Are produced by macrophages only
   (d) Are also called as lymphokines
5. **Colony stimulating factor increases production of all *except:***
   (a) Lymphocytes
   (b) Neutrophils
   (c) Basophils
   (d) Macrophages
6. **In a normal individual, erythropoiesis is completed in ..... days:**
   (a) 3
   (b) 7
   (c) 14
   (d) 20
7. **RBC count is less in young females compared to males of same age because:**
   (a) Increased blood loss during menses
   (b) Females are less active and less muscular than the males
   (c) Oestrogens depresses the erythropoiesis
   (d) Low thyroxine levels
8. **Pituitary disturbances produce anaemia due to:**
   (a) Decreased renal erythropoietin factor production
   (b) Depressed erythropoietic response to hypoxia
   (c) Decreased erythropoietin release
   (d) Decreased hepatic synthesis of globulin
9. **Normal bone marrow contains ..... % proerythroblast and early normoblast, and ..... % of intermediate and late normoblast cells:**
   (a) 30; 70
   (b) 70; 30
   (c) 50; 50
   (d) 80; 20

10. **All of following help in the maturation of RBCs, *except*:**
    (a) Castle's intrinsic factor (b) Iron (c) Vitamin B_{12} (d) Folic acid
11. **Iron deficiency anaemia is:**
    (a) Normocytic normochronic (b) Normocytic hypochromic
    (c) Microcytic hypochromic (d) Macrocytic hypochromic
12. **All hemolytic anaemias are normocytic normochromic *except*:**
    (a) Congenital spherocytosis (b) Sickle cell anaemia
    (c) Thalassaemia (d) Erythroblastosis foetalis
13. **Treatment of a pernicious anaemia patient with folic acid will improve all *except:***
    (a) Nervous lesions (b) Blood picture (c) Changes in GIT (d) General symptoms
14. **Patient with iron deficiency anaemia tends to have all, *except*:**
    (a) Increase in total iron binding capacity (b) Normal life span of RBCs
    (c) Pallor of mucous membranes (d) A low pO_2 in arterial blood
15. **Biconcave shape and flexibility of RBC is maintained by:**
    (a) Glutathione (b) G-6-P-D (c) Spectrin (d) Actomyocin
16. **Normal myeloid-erythroid ratio in the bone marrow is:**
    (a) 1 : 1 (b) 1 : 2 (c) 3 : 1 (d) 3 : 2
17. **The commonest site of haemopoiesis in a 3 months old foetus is:**
    (a) Liver (b) Spleen (c) Bone marrow (d) Gut
18. **Which bone does not contain the red marrow?**
    (a) Vertebrae (b) Ribs (c) Sternum (d) Clavicle
19. **Major stimulus to trigger extra renal erythropoietin poduction is:**
    (a) Generalised severe hypoxia (b) Renal failure
    (c) Hepatic insufficiency (d) Bone marrow hypoxia
20. ***True* about pernicious anaemia:**
    (a) Caused by lesion of gastric mucosa which contains chief cells
    (b) Associated with normoblastic hyperplasia of bone marrow
    (c) Macrocytic hyperchromic type of RBCs
    (d) Also called destructive/injurious anaemia
21. **Erythroblastosis foetalis is:**
    (a) Destruction of RBCs of mother by foetal Rh antibodies
    (b) Haemolysis in foetus due to maternal Rh antibodies
    (c) Haemolysis in foetus due to maternal ABO antibodies
    (d) Destruction of RBCs of mother by foetal ABO antibodies

Answers

1. (b)	2. (c)	3. (b)	4. (a)	5. (a)	6. (b)	7. (c)	8. (c)	9. (a)	10. (b)
11. (c)	12. (c)	13. (a)	14. (d)	15. (c)	16. (c)	17. (a)	18. (d)	19. (a)	20. (d)
21. (b)									

Chapter 9

Jaundice

I. Chemistry of bilirubin formation
II. Fate of bilirubin
III. Types of jaundice and their characteristic features (Hemolytic; Hepatic; Obstructive)
IV. Physiological Jaundice
Jaundice of newborn
Phototherapy
V. Junctional Complexes

Jaundice is a *yellow discolouration* of the skin, eyes and other body tissues caused by the presence of an excessive accumulation of bilirubin in the plasma and tissue fluids (Fig. 9.1). In adults, normal serum bilirubin is 0.2-0.8 mg/dL (average 0.5 mg/dL). Clinically jaundice occurs when serum bilirubin exceeds 2 mg/dL.

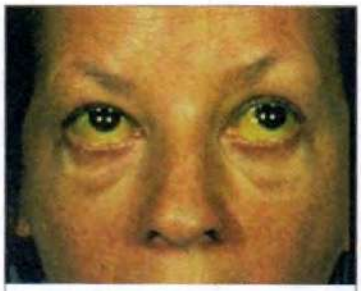


Fig. 9.1 Jaundice.
(**Note:** Yellow discolouration of the skin and eyes)

Important Note

The first site where jaundice is clinically detected is the sclera because:

(i) The sclera is white, a yellow discolouration is more obvious than a similar discolouration of brownish skin.

(ii) Sclera has a protein 'elastin' which has an extremely high affinity for bilirubin.

CHEMISTRY OF BILIRUBIN FORMATION

Page 60.

FATE OF BILIRUBIN

Refer Fig. 9.2.

1. Uptake

Bilirubin formed by the destruction of RBCs is ***free or unconjugated*** bilirubin. It is lipid soluble and bound to *albumin* in plasma (protein conjugation), which prevents its excretion by the kidneys in urine.

2. Conjugation

On reaching the liver, *free* (*unconjugated*) bilirubin is split from albumin and enters the hepatic cells. In the hepatic cells it undergoes conjugation with 'uridine diphosphate glucuronic acid' (UDPGA) in a reaction catalyzed by enzyme 'glucuronyl transferase' to form *Bilirubin Mono and Di-Glucuronides*. These are water soluble *i.e.* *Conjugated Bilirubin*, also called *Chole bilirubin*.

3. Excretion

Excretion of *conjugated* bilirubin by the hepatic cells into the bile canaliculi is an active process *i.e.* it is transported against a concentration gradient into bile canaliculi. This is the rate limiting step in the liver for handling of bilirubin and needs energy. Some of it escapes into the general circulation and is excreted via kidneys in urine as *urine bilirubin;* while most of it passes via the bile ducts to the small intestine.

4. Degradation

When this 'conjugated' water soluble bilirubin reaches the large intestine it is degraded by colonic bacteria. Glucuronic acid is split from bilirubin, and bilirubin undergoes reduction forming a colourless compound *Stercobilinogen*, also called *Urobilinogen*.

5. Re-Excretion

Some 20% of stercobilinogen (urobilinogen) is reabsorbed into the portal circulation to reach the liver.

A. A part of which is re-excreted by the liver into the bile and returns to the small intestine by ***enterohepatic circulation.***

B. The remaining absorbed stercobilinogen (urobilinogen) enters the general circulation. It gets filtered by the

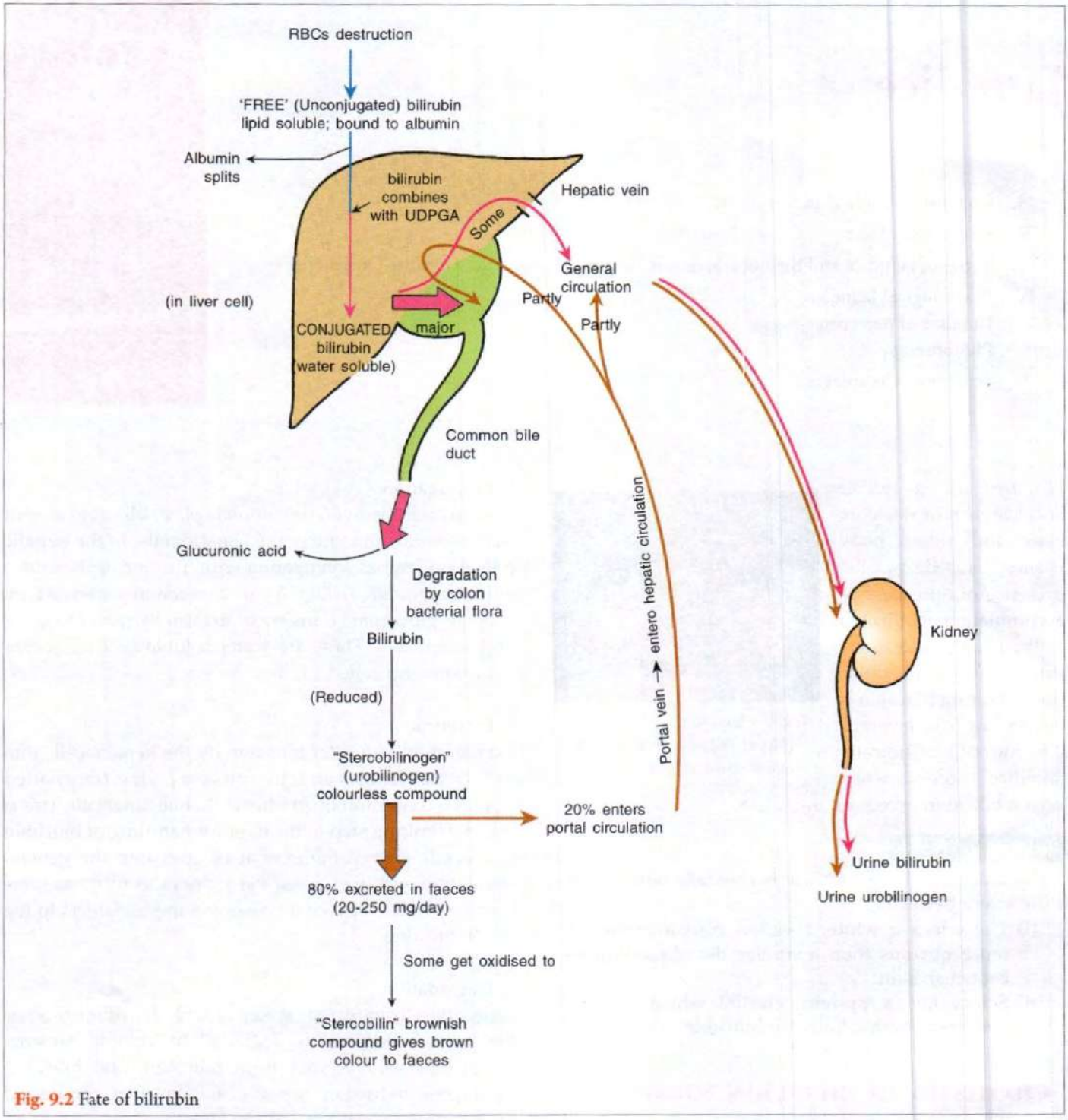


Fig. 9.2 Fate of bilirubin

kidneys and is excreted in urine as ***urine urobilinogen.*** If urine is allowed to stand, urobilinogen is oxidised to ***urobilin.***

The remaining 80% of stercobilinogen which is not reabsorbed from the intestine is excreted in amounts of 20-250 mg/day in the faeces. Some of this stercobilinogen is oxidised to a brownish compound ***stercobilin*** which is responsible for the brown colour of the faeces.

The ***total serum bilirubin*** includes conjugated bilirubin (mainly) plus free (unconjugated) bilirubin.

One of the tests which helps in determining the type of bilirubin present in the serum is ***The van den Bergh Test.***

Principle: "Diazo" reagent (mixture of sulphanilic acid, hydrochloric acid and sodium nitrite) on reacting with serum containing excess of 'conjugated bilirubin'

(water soluble) within 30 seconds *i.e.* almost immediately gives a reddish violet colour. This is said to be ***Direct*** positive reaction.

However, when Diazo reagent reacts with serum containing an excess of 'unconjugated' bilirubin (lipid soluble), no colour develops until some solvent like alcohol is added. This is called ***Indirect*** positive reaction.

TYPES OF JAUNDICE

The various types of jaundice and characteristic features of each type are given in **Table 9.1**.

PHYSIOLOGICAL JAUNDICE

Usually mild form of jaundice appears in some newborn children on the 2nd or 3rd day of life, called ***Jaundice of Newborn or Neonatal Jaundice.***

Causes

1. Excessive destruction of RBCs after birth causing increase in serum bilirubin.
2. Due to *hepatic immaturity.* During intrauterine life bilirubin formed is mainly eliminated via the placenta and to lesser extent by liver. Immediately after birth the liver has to eliminate all the bilirubin formed, therefore, liver is unable to deal adequately with this increased

Table 9.1: Jaundice: Types and Characteristic Features

	Test	Pre-Hepatic (Hemolytic) Jaundice	Hepatic (Hepatocellular) Jaundice	Post-Hepatic (Obstructive) Jaundice
1.	Cause	Excessive breakdown of RBCs such that even normal liver cannot conjugate all of it resulting in *increased* serum *unconjugated billirubin.*	Infective (viral or bacterial) or toxic damage to liver. Therefore, it can't conjugate bilirubin efficiently. Moreover, conjugated bilirubin is also unable to get excreted completely with bile, causing *increase in serum level of both conjugated and unconjugated bilirubin.*	Obstruction of bile ducts (stone or tumour), therefore, conjugated bilirubin can't flow through the biliary tract freely resulting in increased serum conjugated bilirubin.
2.	*van den Bergh Test*	*Indirect positive* reaction.	'Biphasic' reaction.	*Direct positive* reaction.
3.	Blood Examination	Anaemia, reticulocytosis and abnormal RBCs in peripheral smear.	Normal.	Normal
4.	Plasma albumin, globulin levels and A/G ratio	(i) Serum albumin level: normal. (ii) Serum globulin level: normal (iii) A/G ratio: normal	Albumin decreases as it is formed in the liver and globulin increases, therefore, A/G ratio decreases.	Albumin — usually normal, may reduce in late stages; globulin level normal; normal A/G ratio.
5.	*Thymol-turbidity* [Serum with thymol barbitone gives: (a) faint opalescencent colour when γ-globulin level is normal, (b) turbid solution, when γ-globulin level increases.]	Nil	*Markedly increased.*	Usually slight.
6.	Serum Alkaline phosphatase level (it is formed in the liver and bones and gets excreted in bile). Normal: 5-13 KA° units/dL.	Normal	Initially, slightly *increases*; later markedly increases because swollen liver cells block bile canaliculi and prevent its excretion in bile.	*Markedly increases*, because of complete obstruction to bile ducts.
7.	Urine bilirubin.	Absent, because unconjugated bilirubin is protein bound; that is why also called *Acholuric Jaundice.*	Present, conjugated bilirubin is water soluble *(Bilirubinuria)* causing deep yellow urine.	Same as in hepatic jaundice. Deep yellow urine.

Table 9.1: Jaundice: Types and Characteristic Features

	Test	Pre-Hepatic (Hemolytic) Jaundice	Hepatic (Hepatocellular) Jaundice	Post-Hepatic (Obstructive) Jaundice
8.	Urine urobilinogen.	Increases, because of increased formation of stercobilinogen.	Decreases, secondary to decrease in stercobilinogen level.	Absent, if obstruction is complete.
9.	Faecal stercobilinogen. Normal 20-250 mg/day.	Markedly increased, because higher than normal quantities of conjugated bilirubin is delivered to the intestine producing **'dark brown' coloured stools**.	Reduced, because conjugated bilirubin excreted in bile is reduced causing *'pale' faeces*.	Absent, producing *'clay' coloured faeces*.
10.	Faecal fat. A normal healthy individual after ingestion of 100 gm. of fat is expected to excrete 5-6 gm of fat/day in faeces. Normal: 5-6% of total fat intake/day.	Normal	Increased upto 40-50% due to inadequate emulsification and absorption of fats producing bulky, pale, greasy and foul smelling faeces, called *'Steatorrhoea'*.	Same as in hepatic jaundice.
11.	Liver function tests.	Normal	Impaired.	Normal or mildly impaired.

amount of bilirubin during the first 10 days of life causing development of jaundice.

That is why serum bilirubin continues to rise after birth to a peak which is generally reached during the 1st week and then it declines. It is more common and of greater severity in premature babies. *In infants, when serum bilirubin rises beyond 5 mg/dL, clinical jaundice appears.*

PHOTOTHERAPY

Exposure of the skin to white light converts bilirubin to *lumirubin* which has a shorter life than bilirubin (Fig. 9.3). It acts by photoisomerisation of bilirubin to soluble forms, which are easily excreted. Therefore, phototherapy (exposure to light) is of value in treating infants with jaundice (irrespective of its cause).

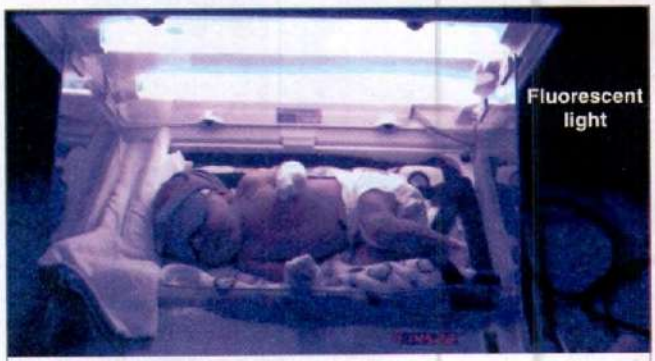


Fig. 9.3 Phototherapy of a baby with mild jaundice

Study Questions

1. **Give physiological basis of:**
   (i) Clinical jaundice
   (ii) Acholuric jaundice
   (iii) Steatorrhoea
   (iv) Pale faeces
2. **Write short notes on:**
   (i) Van Den Bergh Test
   (ii) Neonatal jaundice
   (iii) Phototherapy
   (iv) Hemolytic Jaundice
   (v) Faecal fat
3. **Draw line diagram to show fate of bilirubin after its formation.**
4. **Define jaundice. Give its types with main characteristic features.**

MCQs

1. **Conjugation of bilirubin occurs in:**
   (a) Hepatocytes (b) Granulocytes (c) Lymphocytes (d) Erythrocytes
2. **Conjugated bilirubin is excreted by hepatic cells into bile canaliculi as:**
   (a) An active process (b) A passive process (c) Diffusion (d) Carrier mediated process
3. **Jaundice is defined as:**
   (a) Increase in level of S.bilirubin above 1 mg/dL
   (b) Passage of high yellow coloured urine
   (c) Yellow discolouration of skin, eyes and other body tissues
   (d) Excessive accumulation of bilirubin in the plasma and tissue fluids
4. ***Not true* about stercobilinogen:**
   (a) Also called urobilinogen
   (b) Formed when conjugated bilirubin is degraded by colonic bacteria
   (c) A colourless compound
   (d) 80% of the total formed is reabsorbed into portal circulation
5. **Phototherapy given for treating jaundice:**
   (a) Converts lumirubin to bilirubin (b) Acts by photoisomerisation of bilirubin to soluble forms
   (c) Helpful only in obstructive jaundice (d) Any type of the light can be used
6. **Major urinary form of bilirubin in severe obstructive jaundice is:**
   (a) Free bilirubin (b) Both free and conjugated bilirubin
   (c) No bilirubin will be present in the urine (d) Bilirubin conjugated with glucuronic acid only
7. **In a 30-year old jaundiced patient, investigations show reticulocytosis, absence of urinary bilirubin with normal liver function tests. The most likely diagnosis is:**
   (a) Hemolytic jaundice (b) Hepatic jaundice
   (c) Obstructive jaundice (d) Physiological jaundice
8. ***True* about bilirubin:**
   (a) Clinically, jaundice occurs if level is more than 2 mg/dL
   (b) Is same as biliverdin
   (c) Normal serum level is 1.5 mg/dL
   (d) Is secreted in stool as such
9. **Regarding conjugated bilirubin, *false* is:**
   (a) Also called as cholebilirubin (b) It is lipid soluble and bound to albumin
   (c) Formed from free bilirubin and UDPGA in the liver (d) Normally excreted in urine in small amounts
10. **Normal amount of stercobilinogen excreted in faeces is:**
    (a) 10-20 mgm/day (b) 20-250 mgm/day (c) 250-500 mgm/day (d) 1 gm/day
11. **Clinically jaundice appears in infants when serum bilirubin rises beyond:**
    (a) 1 mg/dL (b) 2-3 mg/dL (c) 4 mg/dL (d) 5 mg/dL
12. **A person complains of passing clay coloured, bulky, greasy and foul smelling faeces for last 2-3 days, most likely he is having:**
    (a) Malabsorption syndrome (b) Pancreatic insufficiency
    (c) Hepatitis (d) Obstruction of bile ducts

Answers

1. (a) 2. (a) 3. (c) 4. (d) 5. (b) 6. (d) 7. (a) 8. (a) 9. (b) 10. (b) 11. (d) 12. (d)

Leucocyte— White Blood Corpuscle (WBC)

Chapter 10

I. General: Total leucocyte count; Leucopenia; Leucocytosis; Leukaemia

II. Structure, functions and variations of neutrophils; eosinophils; basophils; lymphocytes and monocytes

III. Physiology of phagocytic mechanism

IV. Leucopoiesis: Stages; Regulation; Senile leucocytes

GENERAL

The different types of leucocytes present in the circulation are:

A. *Granulocytes* *i.e.* WBC with granules in their cytoplasm

		Percentage	*Absolute Count*
1.	Neutrophils	50-70%	3000-6000/μL
2.	Eosinophils	1-4%	150-300/μL
3.	Basophils	< 1%	10-100/μL

B. *Agranulocytes* (Agranular)

1.	Lymphocytes	20-40%	1500-2700/μL
2.	Monocytes	2-8%	300-600/μL

Total Leucocyte Count (TLC)

At birth : 20,000/μL; count decreases after 2nd week, reaching normal adult value at 5-10 years.

In adults : 4,000-11,000/μL.

Leucopenia – TLC decreases less than 4000/μL.

Causes

1. Starvation
2. Typhoid (enteric) fever
3. Viral or protozoal infection
4. Bone marrow depression.

Leucocytosis – TLC increases above 11,000/μL.

Causes

1. Newborn (normal – 20,000/μL)
2. In the evening (**Note**: minimum count is seen in the morning)
3. Exercise
4. After injection of Epinephrine or nor-epinephrine
5. Stress
6. Pregnancy, menstruation, lactation
7. Administration of steroids
8. Any pyogenic (acute/chronic pus forming) *or* pyrogenic (fever producing) infection.

Leukaemia is a ***cancerous condition*** of blood in which TLC is usually more than 50,000/μL and associated with *presence of immature WBCs in the peripheral smear.*

Note

μL was previously called as cumm.

STRUCTURE, FUNCTIONS AND VARIATIONS

Structure as seen by *Leishman's staining*; haematoxylin-eosin stain.

A. NEUTROPHIL or POLYMORPHONUCLEAR LEUCOCYTE (Fig. 10.1)

Size: 10-14 μm diameter

Nucleus

(i) Purple in colour.

(ii) Multilobed (1-6 lobes), that is why also called ***polymorphonuclear*** leucocyte.

(iii) Young cells have single *'horse shoe shape'* nucleus.

(iv) As the cells grow older nucleus becomes multilobed. Lobes are connected with one another by chromatin threads. More the number of lobes, the more mature is the neutrophil.

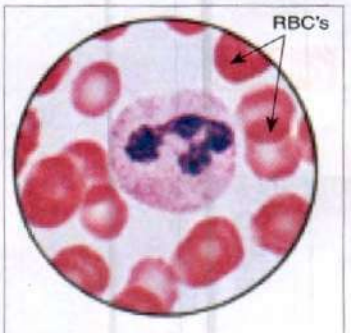


Fig. 10.1 Neutrophil

Cytoplasm: Slight bluish in colour, granular.

Granules

(i) *'fine'* sand like particles, called *'pin-point'* granules.

(ii) 'neutrophilic' in nature (red-brown or purplish in colour), *i.e.* take both the acidic and the basic stains, therefore, the cell is called a *neutrophil.*

(iii) Contain large amounts of proteins and traces of lipids and nucleic acids.

(iv) Also contain varieties of enzymes which include: glycosidases, sulphatases, phosphatases, nucleases (ribonuclease and deoxy ribonuclease) and proteolytic enzymes. They can 'lyse' any type of substance, the granules are thus regarded as ***lysosomes.***

(v) In addition, all granulocytes liberate histamine and peroxidase enzyme which aids in killing ingested bacteria. (Also refer to page 86)

Life span: 6-30 hours (half life: 6 hours). Once they leave the circulation they enter the tissues to become 'tissue macrophages'. Some enter the GIT and are excreted in faeces and some are excreted in respiratory secretions.

Metabolism

(i) metabolism of cytoplasmic glycogen forms glucose, which oxidises to release energy; this energy is utilized in phagocytic processes and to carry out amoeboid movements;

(ii) the capacity of neutrophils to function in anaerobic conditions helps in removal of cell debris or bacteria from necrotic tissues;

(iii) neutrophils also show active metabolism of neutral lipids and phospholipids during phagocytosis.

Functions

1. ***Phagocytosis*** – whenever the body gets invaded by bacteria, neutrophils are the first cells to seek out to ingest and kill the bacteria. They have been thus called the body's *first line defence* against bacterial infections. (Details page 85)
2. They contain a fever-producing substance, ***endogenous pyrogen*** which is an important mediator of febrile response to bacterial pyrogens.

Neutrophilia means increase in neutrophils, causes are:

A. Physiological

1. Exercise
2. After injection of epinephrine
3. Pregnancy, menstruation and lactation.

In healthy individuals, 2-3 × 10^{10} neutrophils are circulating in the blood at one time, called *circulating granulocyte pool* (CGP); the same number of neutrophils are 'marginated' and are attached to endothelial cells by *selectins* in the blood vessel walls or 'sequestered' in closed capillaries (*i.e.* ***marginated or sequestered neutrophils***), called *Marginated Granulocyte Pool* (MGP). Therefore, CGP = MGP; together (CGP + MGP) forms *Total blood granulocyte pool* (TBGP).

Whenever, marginated or sequestered neutrophils come into circulation, it results in ***transient increase in neutrophils***. This is seen with:

(i) exercise
(ii) injection of epinephrine/nor-epinephrine
(iii) splenic contraction.

B. *Pathological*

1. Acute pyogenic (pus forming) infections
2. Following tissue destruction *e.g.*
   (i) burns
   (ii) after haemorrhage
   (iii) myocardial infarction
   (iv) after surgery.

Neutropenia means decrease in neutrophils, causes are:

1. In children (normal count: 40%)
2. Typhoid/paratyphoid fever
3. Viral infection
4. Bone marrow depression.

B. EOSINOPHIL (Fig. 10.2)

Size: 10-14 µm diameter

Nucleus:

(i) Purple colour.

(ii) Usually (85%) cells – *'bilobed'*, the two lobes are connected with chromatin thread thus producing *'spectacle'* appearance.

(iii) Remaining 15% cells have *'trilobed'* nucleus.

Cytoplasm:

(i) Acidophilic, therefore, appears light pink in colour.

(ii) Granular.

Granules:

(i) Coarse.

(ii) Stain bright red with acidic (eosin) dye.

(iii) Granules do not cover the nucleus.

(iv) They contain:
   (a) very high peroxidase content, (histaminase content is maximum)
   (b) 'lysozymes' *i.e.* most of the enzymes found in neutrophil granules.

Functions

1. Mild phagocytosis because less motile than neutrophils.
2. Eosinophils collect at the sites of allergic reactions and limit their intensity

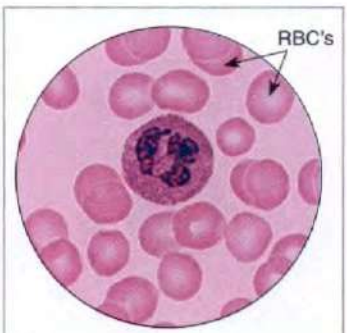


Fig. 10.2 Eosinophil

by degrading the effects of inflammatory mediators (*e.g.* histamine, bradykinin) and inhibit mast cell (page 100) or basophil degranulation.

3. They enter the tissues and are specially abundant in the mucosa of respiratory tract, gastrointestinal and urinary tract, where they provide local mucosal immunity.
4. Eosinophils attack parasites that are too large to be engulfed by phagocytosis. Eosinophil granules release chemicals (peroxidase) which are toxic to larvae of parasites.

Eosinophilia *i.e.* increase in eosinophils.

Causes

1. Allergic conditions *e.g.* bronchial asthma.
2. Parasitic infestation *e.g.* worms.
3. Skin diseases.

Eosinopenia *i.e.* decrease in eosinophils. Seen after injection of ACTH or corticosteroids because of increased sequestration of eosinophils in the lungs and spleen and by their destruction in the circulating blood.

C. BASOPHIL (Fig. 10.3)

Size : 10-14 µm diameter
Nucleus : As in Eosinophil.
Cytoplasm : Slight basophilic, therefore, appears blue; granular.

Granules

(i) Coarse
(ii) Stains purple or blue with basic (methylene blue) dye.
(iii) Granules are plenty in number and overcrowd the nucleus resulting in obscure boundary of the nucleus.
(iv) Contain histamine (an inflammatory mediator) and heparin (an anti-coagulant).
(v) The granules also contain *Eosinophil chemotactic factor of anaphylaxis (ECF-A).*

Functions

1. Mild phagocytosis.
2. Liberates histamine and ECF-A which leads to allergic manifestations. *ECF-A* attracts eosinophil into the region of antigen-IgE interaction (page 126). ECF-A is a chemical mediator of immediate hypersensitivity reactions. These ranges from mild urticaria (localized skin allergic reactions) to severe anaphylactic shock.

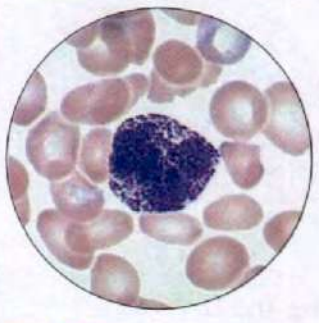


Fig. 10.3 Basophil

3. Liberates heparin which:
   (i) acts as anti-coagulant and keeps the blood in fluid state in the body;
   (ii) activates a hormone, lipoprotein lipase which facilitates absorption of triglycerides after meals.

Basophilia *i.e.* increase in basophils.

Causes

1. Chickenpox
2. Smallpox
3. Tuberculosis
4. Influenza.

Basopenia *i.e.* decrease in basophils.

Causes

1. After administration of glucocorticoids.
2. Drug induced reactions.

D. LYMPHOCYTES (Fig. 10.4)

They are of two types:

1. ***Large lymphocytes:*** 10-14 µm diameter; precursor of small lymphocytes.
2. ***Small lymphocytes (B-type):*** 7-10 µm diameter; responsible for 'antibody' production (page 115).

Both large and small lymphocytes have the same structure.

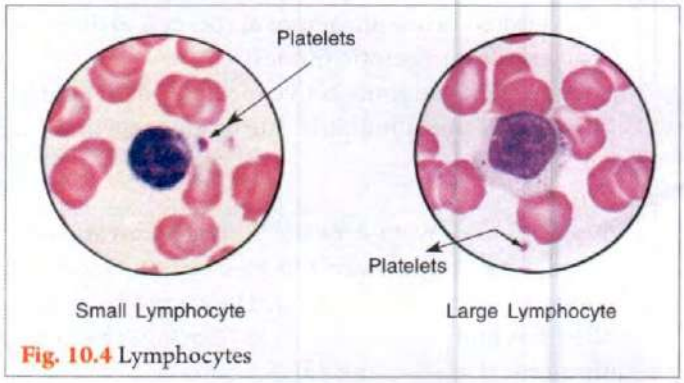


Fig. 10.4 Lymphocytes

Nucleus

(i) Single; very big; purple in colour.
(ii) Shape: round, oval or indented.
(iii) Central in position and occupies whole of the cell leaving marginal cytoplasm at one end of it or all around it.
(iv) Nuclear chromatin is coarse and lumpy (shapeless).

Cytoplasm:

(i) Pale blue
(ii) Scanty, its amount is always less than the amount of the nucleus.

Functions

Produce antibodies *i.e.* immune substances, specially in delayed hypersensitivity (for details, refer to page 115).

Lymphocytosis *i.e.* increase in lymphocytes.

Causes

1. In children – lymphocytes (60%) are more than neutrophils (40%), called ***Relative Lymphocytosis***.
2. Chronic infections *e.g.* tuberculosis (TB).
3. Lymphatic leukaemia.
4. Viral infections.

Lymphopenia *i.e.* decrease in lymphocytes.

Causes

1. Hypoplastic bone marrow
2. AIDS (acquired immuno deficiency syndrome, page 130).

E. MONOCYTE—LARGEST WBC (Fig. 10.5)

Size : 10-18 μm diameter with irregular cell outline.

Nucleus

(i) Pale staining.
(ii) Single.
(iii) Round or indented (kidney shaped).
(iv) Eccentric in position *i.e.* present on one side of the cell.
(v) Nuclear chromatin is finely reticular.

Cytoplasm : Usually pale blue; clear.

Granules : Sometimes contains fine purple dust like granules, called ***Azur granules*** which may be few or numerous.

Functions

1. Active phagocytosis. Monocytes follow the neutrophils in the areas of infections or inflammation and constitute a ***second line defence***. Phagocytic mechanism is the same as seen in neutrophils (see below).
2. Monocytes enter the circulation from bone marrow but after 72 hours they enter the tissues to become *'tissue macrophages'*. All tissue macrophages (page 114) come from circulating monocytes.
   Life span: Approx. 3 months
3. Monocytes may also kill tumour cells after sensitization by lymphocytes.
4. They synthesize complement and other biologically important substances like prostaglandin E and clot promoting factors.

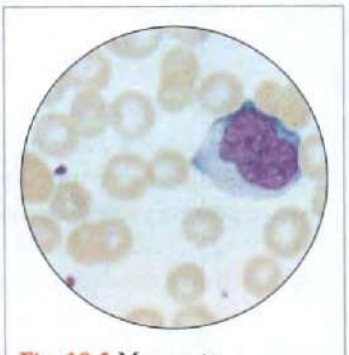


Fig. 10.5 Monocyte

Monocytosis *i.e.* increase in monocytes.

Causes

1. Tuberculosis
2. Syphilis
3. Some leukaemias.

Monocytopenia *i.e.* decrease in monocytes.

Causes: Hypoplastic bone marrow.

PHYSIOLOGY OF PHAGOCYTIC MECHANISM

Neutrophils play an important role in inflammation, particularly bacterial. The following events are seen during phagocytosis.

1. **Diapedesis.** Neutrophils are motile cells, therefore, they emigrate from the blood stream into the tissues by passing through the junction between endothelial cells, a process called *'diapedesis'* **(Fig. 10.6)**.

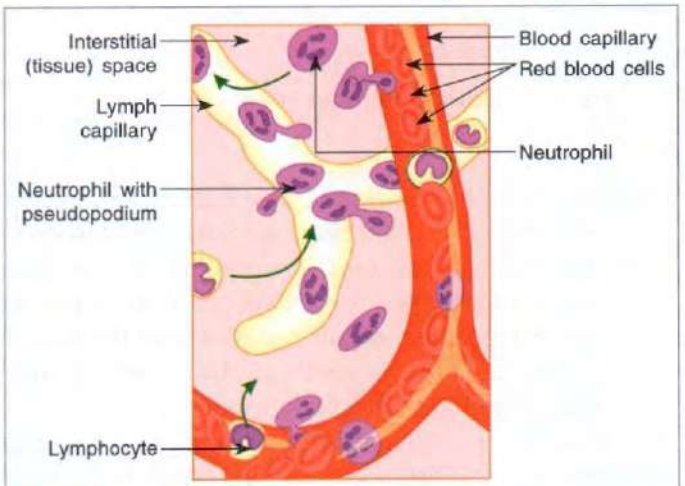


Fig. 10.6 Mechanism of diapedesis

2. **Chemotaxis.** Bacterial products interact with plasma proteins to produce 'agents' that attract neutrophils to the site of injury or inflammation, called *'chemotaxis'*. As a result of chemotaxis, neutrophils adhere to each other forming clumps and become immobile. The chemotactic agents include:
   (i) components of complement system-C5a (page 121)
   (ii) leukotrienes (originate from free fatty acids), and
   (iii) polypeptides from lymphocytes, basophils and mast cells.
3. **Opsonization.** ***Opsonins*** are antibodies against bacteria. The principal opsonins are immunoglobulin of IgG group and complement proteins. They coat the bacteria and make them tasty to the phagocytes, a process called *'opsonization'*.
4. **Phagocytosis**
   (i) Coated bacteria then bind to the receptors on the neutrophil cell membrane and get phagocytosed by a process of 'endocytosis', forming antigen antibody complex. **(Fig. 10.7)**
   (ii) This way neutrophils also phagocytize many foreign inanimate substances *e.g.* carbon particles, sodium urate crystals, etc.
   (iii) Phagocytosis occurs at pH between 6 to 8 and is *not* dependent on O_2. It is aided by bivalent cations and is prevented by calcium chelating agents.

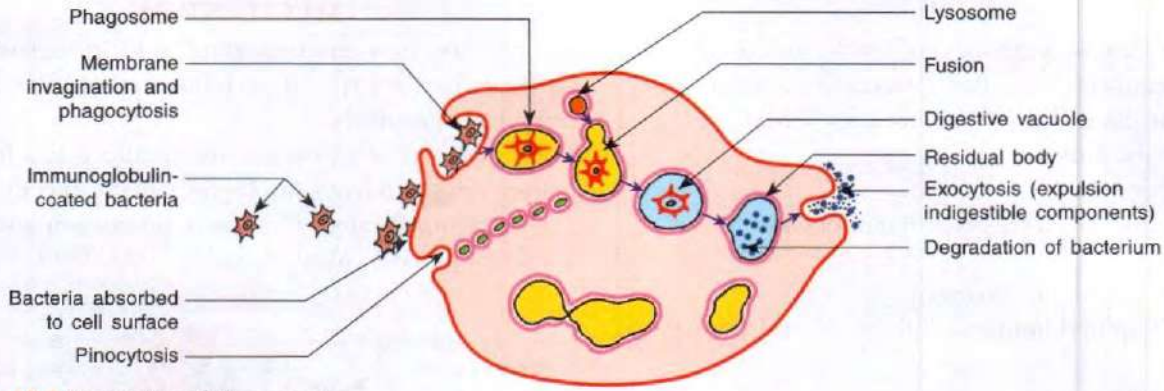


Fig. 10.7 Mechanism of phagocytosis

(iv) A neutrophil can phagocytize 5-20 bacteria before the neutrophil itself become inactivated and dies.

(v) Macrophages are more powerful phagocytes than neutrophils. They can phagocytize as many as 100 bacteria; can engulf particles upto the size of a RBC; and have the ability to phagocytize necrotic tissues and even dead neutrophils.

5. **Degranulation.** After phagocytosing the bacteria, bacteriocidal substances (called ***defensins***) such as lysozymes and peroxidases are released from the lysosomal granules into the digestive pouch (*degranulation*), where they kill and digest the bacteria. How?

   Associated with degranulation, the cell membrane bound enzyme *NADPH oxidase* is activated. This causes sharp increase in O_2 uptake and metabolism in the neutrophil (***the respiratory burst***) with generation of superoxide radical (O_2^-) and hydrogen peroxide (H_2O_2) as follows:

$$NADPH + H^+ + 2O_2 \longrightarrow NADP^+ + 2H^+ + 2O_2^-$$

$$2O_2^- + 2H^+ \xrightarrow[\text{Cytoplasmic enzyme superoxide dismutase}]{\text{Catalyzed by}} H_2O_2 + O_2$$

Free radical O_2^- and H_2O_2 are both oxidants that react to form hydroxyl radical (OH^-), a very potent bactericidal agent.

$$O_2^- + H_2O_2 \xrightarrow{\text{Catalase}} OH^- + OH^- + O_2$$

Note

In addition, neutrophils also discharge myeloperoxidase and elastase enzymes that help destroy invading organisms.

6. **Inflammatory Response.** Release of lysosomal enzymes, histamine and 5-HT into ECF produces *inflammatory response.*

7. **Limiting Inflammation.** Fusion of phagocytic vacuole with lysosome causes release of:
   (i) 'Thromboxanes' produces
      (a) vasconstriction and
      (b) platelet aggregation (page 93).
   (ii) 'Prostaglandins' produces: anti-inflammatory effect.
   (i) and (ii) limit the inflammation.

Applied: Disorders of Phagocytic Functions

Patients with these disorders are prone to infections. These are mild when only the neutrophil system is involved, but severe when the *'monocyte tissue macrophage system'* is also involved.

1. *Agranulocytosis i.e.* decrease in granulocytes. This increases susceptibility to infections.
2. *Myeloid leukaemia i.e.* presence of immature WBCs in circulation. As immature cells do not possess full phagocytic activity, therefore, patient becomes more prone to infections.
3. *Neutrophil hypomotility* – In this condition actin in neutrophils does not polymerize normally and neutrophils move slowly.
4. *Chronic granulomatous disease of childhood.* It is a fatal hereditary condition, specially affecting males. There is failure to generate superoxide radical (O_2^-) both in neutrophils and monocytes. Therefore, inability to kill many phagocytosed bacteria.
5. *Congenital neutropenia* – idiopathic, producing maturation arrest of neutrophils.
6. *Congenital myeloperoxidase deficiency*: Neutrophils also produce the enzyme myeloperoxidase which helps catalyzing the formation of oxidants, therefore, with its deficiency microbial killing power is reduced

Table 10.1: Stages of Granulopoiesis

	Stage	Cell size	Nucleus	Cytoplasm	Others
1.	Primitive WBC (stem cell)	18-23 µm	Large, spherical, many nucleoli; open chromatin.	Basophilic; thin rim of cytoplasm all around the nucleus.	Mitosis +++
2.	Myeloblast	16-20 µm	Large, pale or purple blue; many nucleoli; fine stippled chromatin.	Amount increases; narrow blue rim without granules.	Mitosis +++ Show active protein synthesis; highly developed endoplasmic reticulum.
3.	Myelocyte-A *(Pre-myelocyte)*	14-18 µm	Size decreases, nucleoli disappears, chromatin condenses.	Amount increases further.	Mitosis ++
4.	Myelocyte-B *(Myelocyte proper)* *i.e.* Neutrophil, Eosinophil and basophil.	12-16 µm	Round, nucleus size further decreases.	Amount increases, less basophilic; *granules appear* with special staining reaction; maximum granules in neutrophils, scanty in eosinophils and basophils.	Multiplication is maximum.
5.	Myelocyte-C *(Metamyelocyte)*	10-14 µm	Nucleus size decreases, becomes indented and lobed.	Amount further increases and becomes more liquid; *granules show amoeboid movements* (maximum in neutrophils).	*Mitosis stops.*
6.	Mature WBC (Neutrophil; Eosinophil; Basophil)	(See to pages 82 to 85 under individual cell description and Fig. 10.9 on page 88)			

3 Days

but *not* absent because most of the oxygen-dependent bactericidal mechanisms remain intact.

LEUCOPOIESIS: DEVELOPMENT OF LEUCOCYTES (WBCS)

A. Granulopoiesis – duration is 3 days. Granulocytes develop mainly and exclusively in the red bone marrow. It is wholly *'extravascular'* process (page 67). Between the fat cells of the marrow are branched *reticulum cells* (*i.e.* irregular in outline, free from granules or mitochondria with faint basophilic cytoplasm). Reticulum cells multiply by mitosis forming primitive WBCs (stem cells) → myeloblast → premyelocyte → myelocyte proper → metamyelocyte → mature WBCs (Table 10.1).

B. Agranulocytes also develop to a slight extent in the bone marrow but their main site of origin is in the *'lymphoid tissues'* (*i.e.* thymus, spleen, lymph nodes, peyer's patches, tonsils etc.) from 'stem cells' that originally come from the bone marrow. In Humans, 3.5×10^{10} lymphocytes per day enter the circulation via the thoracic duct alone. However, at any given time only about 2% of the total body lymphocytes are in the peripheral blood and rest are in the lymphoid organs (details page 114).

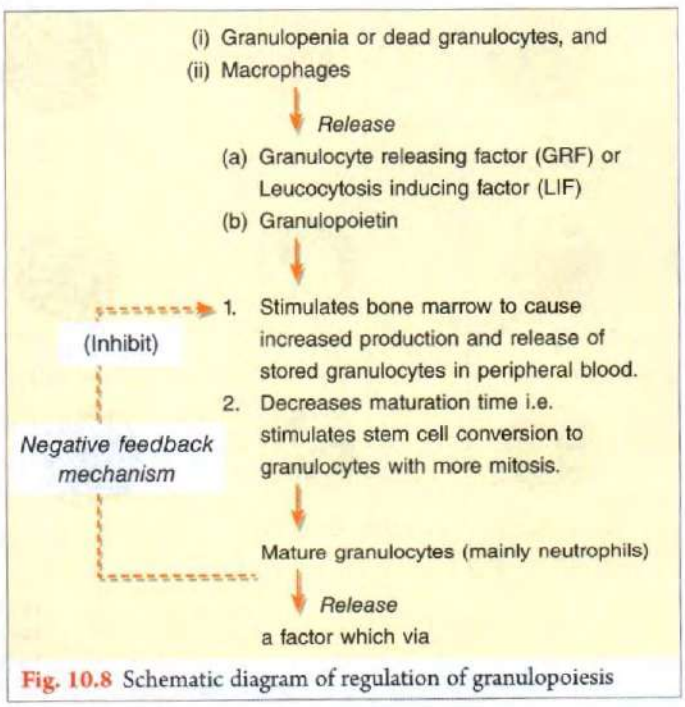


Fig. 10.8 Schematic diagram of regulation of granulopoiesis

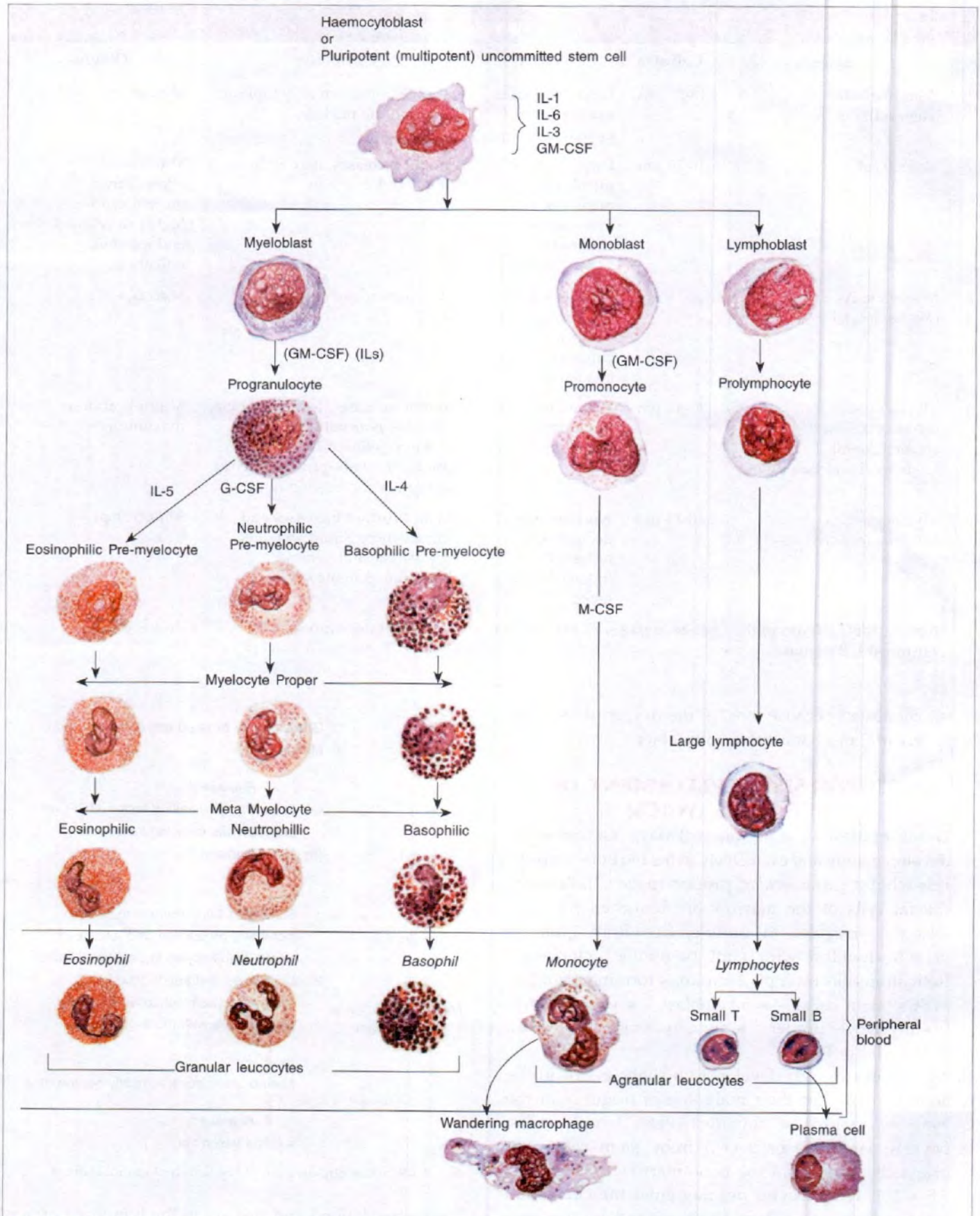


Fig. 10.9 Leucopoiesis-origin and development of WBCs (IL: Interleukins; Ep: Erythropoietin; GM-CSF: Granulocyte macrophage colony stimulating factor)

Regulation of Granulopoiesis

For every circulating granulocyte, 50-100 mature cells are held in the bone marrow reserve. The relative constancy of blood granulocyte count is maintained by an efficient 'feedback' mechanism by circulating factors. How? (Fig. 10.8).

SENILE LEUCOCYTES

Old WBCs are characterized by:

1. Loss of mobility.
2. Nucleus seen lying free; nuclear lobulation increases.
3. Poorly stained granules.
4. Cells break up readily while making blood smear.

Study Questions

1. **Write short notes on:**
   (i) ECF-A
   (ii) Relative lymphocytosis
   (iii) Respiratory burst
   (iv) Endogenous pyrogen
   (v) Variation in leucocyte count
   (vi) First and second line of defence
   (vii) Regulation of granulopoiesis
   (viii) Physiology of phagocytic mechanism
   (ix) Disorders of phagocytic function.
2. **Name various types of leucocytes found in the blood. Describe the major functions of each type.**
3. **Give the main causes of variations associated with different types of leucocytes.**
4. **Describe the role of eosinophils in control of allergic reactions.**
5. **Depict diagramatically**
   (i) Mechanism of phagocytosis by a neutrophil
   (ii) Regulation of granulopoeses

MCQs

1. **Total leucocyte count at birth is:**
   (a) 4000-11000/μL (b) 11000-15000/μL (c) 15000-18000/μL (d) 20000/μL
2. **Neurophil granules are regarded as lysosomes because:**
   (a) Of fine sand like particles
   (b) They take both acidic and basic stains
   (c) They liberate peroxidase enzyme
   (d) They contain variety of enzyme that can digest any type of substrate
3. **Neutrophil count tends to fall in all *except*:**
   (a) During acute bacterial infection
   (b) Typhoid fever
   (c) In pernicious anaemia
   (d) Drugs depressing bone marrow
4. **Eosinopenia is seen in:**
   (a) Bronchial asthma (b) Worms infestation (c) Cushing syndrome (d) Urticaria
5. ***False* about a lymphocyte:**
   (a) Small lymphocyte is more mature than a larger one
   (b) Both large and small lymphocyte have the same structure
   (c) Small lymphocyte percentage is more in peripheral blood
   (d) Large lymphocyte may survive for 2-4 years
6. **The term "relative lymphocytosis" refers to:**
   (a) Increase in lymphocytes
   (b) Lymphocytes are more than neutrophils in children
   (c) Increase in lymphocytes in response to chronic infection
   (d) Lymphatic leukaemia
7. **Neurophils differ from monocyte in that:**
   (a) Neutrophils kill and digest bacteria
   (b) Neutrophils enter the tissues to become tissue macrophages
   (c) Neutrophils constitute a second line defence
   (d) Neutrophils may also kill tumour cells

8. **Sequence of events involved during phagocytic mechanism are:**
   (a) Chemotaxis – Diapedesis – Opsonization – Phagocytosis
   (b) Diapedesis – Opsonization – Chemotaxis – Phagocytosis
   (c) Diapedesis – Chemotaxis – Opsonization – Phagocytosis
   (d) Phagocytosis – Diapedesis – Chemotaxis – Opsonization
9. **Hereditary condition in which neutrophils fail to generate superoxide radical (O_2^-):**
   (a) Myeloid leukaemia
   (b) Agranulocytosis
   (c) Chronic granulomatous disease of childhood
   (d) Neutrophil hypomotility
10. **Which is *not* a physiological cause of leucocytosis?**
   (a) Newborn (b) Stress (c) Pyogenic infection (d) Exercise
11. ***Not true* about leukaemia is:**
   (a) Cancerous condition of blood
   (b) Always associated with leucocytosis
   (c) Associated with immature WBCs in the peripheral smear
   (d) Associated with tremendous proliferation of myeloid series of cells in the bone marrow
12. **Which is *not* a function of neutrophil?**
   (a) Phagocytosis (b) First line defence against all infections
   (c) Produce febrile response (d) Kill the bacteria
13. **Chemical mediator of immediately hypersensitivity reaction is contained in the granules of:**
   (a) Neutrophils (b) Eosinophils (c) Basophils (d) Mast cells
14. **Which one of the following statements concerning the monocyte is *incorrect*?**
   (a) More common in blood than eosinophil and basophil
   (b) Produced in the adult by the bone marrow and lymph nodes
   (c) Unlike neutrophil does not accumulate outside circulation in area of inflammation
   (d) Not classified as a granulocyte
15. **Which is *not true* about phagocytosis by a neutrophil?**
   (a) Optimal pH ranges from 6 to 8
   (b) Not dependent on oxygen
   (c) Can phagocytose 5-20 bacteria
   (d) Neutrophils are more powerful phagocytes than macrophages
16. **Which of these is *not* present in eosinophil granules?**
   (a) Lysozymes (b) Histamine (c) Ribonuclease enzyme (d) Peroxidase enzyme

Answers

1. (d) 2. (d) 3. (a) 4. (c) 5. (c) 6. (b) 7. (a) 8. (c) 9. (c) 10. (c)
11. (b) 12. (b) 13. (c) 14. (c) 15. (d) 16. (b)

Chapter 11

Platelets or Thrombocytes

I. Structure
II. Count and variations
III. Thrombopoiesis
IV. Functions

STRUCTURE

(Platelets = small plate; thrombo = lump or clot; cytes = cells)

A. GENERAL

1. Platelets are the smallest blood cells, colourless, spherical, oval or rounded granulated bodies;
2. 2-5 μm in diameter with an average volume 5.8 fL (μm^3);
3. Leishman staining shows a faint blue cytoplasm with distinct reddish purple granules; nucleus is **not** present.

B. UNDER ELECTRON MICROSCOPE

1. *Platelet membrane: Salient features*
   (i) It has identical structure with the cell membranes, thickness: 60A° = 6 nm;
   (ii) it shows extensive invagination with a complicated canalicular system in contact with the E.C.F. (Fig. 11.1);
   (iii) the main lipids in lipoprotein layer of cell membrane are: phospholipids, cholesterol and glycolipids;
   (iv) it contains various receptors meant for combining with specific substances like:
   - collagen
   - fibrinogen and
   - *von-Willebrand's factor* – It is a large circulating molecule produced by endothelial cells. It plays important role in platelet adhesion (page 93) and regulates circulating level of factor VIII (page 97); and

   (v) it also contains precursors of various substances like: thromboxane A_2, prostaglandins, leukotrienes and *platelet factor 3 and 4.*

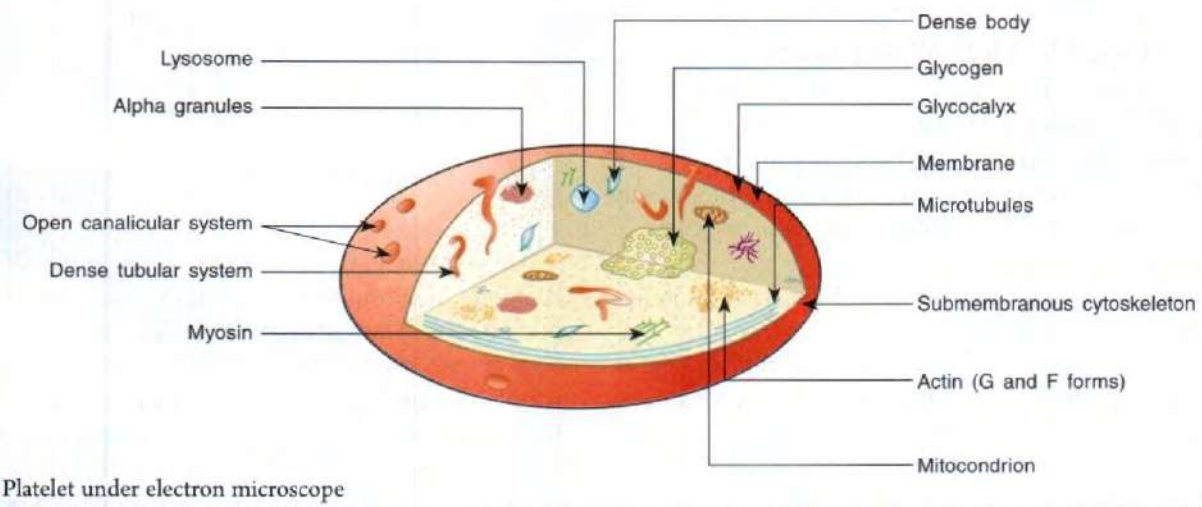


Fig. 11.1 Platelet under electron microscope

2. *Cytoplasm contains:*
   (i) Golgi apparatus;
   (ii) Endoplasmic reticulum;
   (iii) Few mitochondria;
   (iv) Microvesicles and microtubules. Microvesicles are arranged in a form of a ring at the periphery of the cell;
   (v) Contractile proteins like actin and myosin (previously called *'thrombosthenin'*); helps in clot retraction;
   (vi) Glycogen;
   (vii) Lysosomes, and
   (viii) Granules – these are of 2 types:
      (a) *Dense granules* – contain:
         • non-protein substances like phospholipid, triglycerides, cholesterol, etc.
         • 5-hydroxytryptamine (5-HT, serotonin) – vasoconstrictor agent
         • ADP – helps platelet aggregation
         • ATP – stores energy, and
         • other adenine nucleotides.
      (b) *α-granules* (granules with clear interior) – contain secreted proteins which include:
         • clotting factors, and
         • ***platelet derived growth factor (PDGF)***; it stimulates wound healing and is a potent mitogen for vascular smooth muscle (*i.e.* stimulates mitosis in the vascular wall). Thus it helps in repair of the damaged vessel wall. PDGF is also produced by macrophages and endothelial cells.

Important Note

Platelets cannot synthesize 5-HT, they obtain their 5-HT while passing through the GIT, refer page 762.

COUNT AND VARIATIONS

1. *Normal count* is 1.5 to 4 lacs/μL (average: 2.59 lacs/μL). Its count is very much constant.
2. The circulating platelets represent approx. 60-75% of the platelet pool of the body, the remaining are mostly in the spleen. Therefore, spleen acts as a reservoir of platelets.
3. *Life span:* 8-12 days. Studied by transfusing platelets labelled with ^{51}Cr or ^{32}P.
4. *Destruction:* mainly in the spleen. In ***hypersplenism*** (overactivity of spleen), the platelets may almost disappear from circulation.
5. *Variations*
   (A) ***Thrombocytosis*** *i.e.* increase in platelet count.
   Causes:
      (i) after administration of epinephrine due to splenic contraction
      (ii) after trauma *e.g.* surgery, injury, child birth etc.
      (iii) splenectomy (removal of spleen)
      (iv) thrombocytopheresis *i.e.* experimental removal of platelets from the circulation
      (v) stress causes increased epinephrine release resulting in spleen contraction.
   (B) ***Thrombocytopenia*** *i.e.* decrease in platelet count.
   Causes:
      (i) bone marrow depression
      (ii) hypersplenism
      (iii) viral infection *e.g.* dengue fever (particularly attack platelets)
      (iv) drug hypersensitivity.

   It leads to ***purpura*** which is associated with bleeding disorders (page 102).

THROMBOPOIESIS *or* MEGAKARYOCYTOPOIESIS *i.e.* DEVELOPMENT OF PLATELETS

Site of origin : bone marrow

Steps :

Pluripotent stem cell
↓
Committed stem cell (polyploid precursor cell)
↓
Megakaryoblast (Stage I)
↓
Pro-megakaryocyte (Stage II)
↓
Granular megakaryocyte (Stage III)
↓
Platelets

(Refer to **Fig. 8.5** page 69)

Megakaryocytes are giant cells with 35-160 μm diameter; multinucleated (contain irregular ring of lobed nuclei) with dense granular cytoplasm. Platelet formation begins with the formation of micro-vesicles which join to form a demarcation membrane for platelets. Platelets are formed within the cytoplasm of granular megakaryocyte by pinching off bits of cytoplasm and are released into the circulation when the cell dies. One megakaryocyte forms 2,000-4,000 platelets by this process.

Platelets production is precisely regulated by 'feedback' mechanisms by ***Colony stimulating factor*** (page 66) and ***thrombopoietic stimulating factor*** (TSF) or ***thrombopoietin***, produced by the liver and kidneys. It is probably released in circulation following excessive destruction of platelets or when the thrombocytopenia develops. It promotes the production of megakaryocytes from committed stem cells.

FUNCTIONS

1. **Haemostasis** *i.e.* spontaneous arrest of bleeding by physiological process (page 95). Haemostasis mechanism includes:
   (i) ***Platelet adhesion*** *i.e.* when a blood vessel is injured, platelets adhere to the exposed collagen, laminin and von-Willebrand factor present on the endothelium cells in the vessel wall.
   (ii) ***Platelet activation*** – platelet binding to exposed collagen initiate platelet activation, also produced by ADP and thrombin.
   The activated platelets:
   (a) change the shape *i.e.* put out pseudopodia
   (b) discharge their granule contents, and
   (c) stick to each other, called ***platelet aggregation***.
   (iii) ***Platelet aggregation*** is also increased by *'platelet activating factor'* (PAF), a cytokine secreted by neutrophils, monocytes and platelet cell membrane lipids.

Important Note

Normally platelets are present in an inactive state in the circulation, unless they become active, there can be no haemostasis.

(iv) Platelet aggregation activates phospholipase 'C', which in turn activates phospholipase A_2; this causes release of 'arachidonic acid' from membrane phospholipids, and which in turn gets converted to *thromboxane* A_2 and *prostacyclin*.

(a) ***Thromboxane*** A_2 causes:
- further increase in platelet aggregation, which along with platelet adhesion helps in formation of ***temporary haemostatic plug***; this causes stoppage of bleeding from the injured blood vessel and maintains the integrity of the vascular tree.
- release of platelet contents *i.e.* nor-epinephrine and 5-HT, both are vasoconstrictor agents, therefore, plays important role for haemostasis in contracting large blood vessels.

(b) ***Prostacyclin*** – inhibits thromboxane A_2 formation and thus prevents further platelet aggregation, keeping platelet plug localised (*i.e.* prevents intravascular spread of clot).

Important Note

Aspirin by inhibiting thromboxane A_2 formation prevents platelet aggregation., Therefore, aspirin in low doses is of value in preventing myocardial infarction and stroke (also refer to page 99).

2. **Blood Coagulation**: The loose aggregation of platelets in the temporary haemostatic plug is bound together and converted into ***definitive haemostatic plug*** by fibrin. The clotting mechanism responsible for formation of fibrin involves a complex series of reactions in which platelets play a major role (page 93).
3. **Clot Retraction**: Within 5-30 minutes of fibrin clot formation, clot retracts *i.e.* it contracts down to 40% of its original volume. This is produced by contraction of attached platelet pseudopodia which contain the contractile actomyosin like protein, *thrombosthenin*. The compact clot is a more effective haemostatic plug.
4. **Phagocytic function**: platelets help in 'phagocytosis' of carbon particles, viruses and immune complexes.
5. **Storage and transport function**: platelets store 5-HT and histamine, which are released when the platelets disintegrate and act on the blood vessel. Platelets can take up 5-HT against a concentration gradient.

Study Questions

1. Define Haemostasis. Describe the role of platelets in its occurrence.
2. Describe briefly the electron microscopic structure of a platelet. Give its functions.
3. Mention physioilogical basis of aspirin in prevention of myocardial infarction and transient ischaemic attacks (TIA).
4. Write short notes on:
   (i) Platelet granules
   (ii) Normal platelet count and its variations
   (iii) Functions of platelets
   (iv) Platelet activation factors
   (v) von-Willebrand factor
   (vi) Clot retraction
   (vii) Platelets derived growth factor
   (viii) Haemostatic plug
   (ix) Development of platelets
   (ix) Thromboxane A_2

MCQs

1. **Smallest blood cell is:**
   (a) Small lymphocyte (b) RBC (c) Platelet (d) Neutrophil
2. **Which is *wrong* regarding von-Willebrand's factor?**
   (a) Regulates circulating level of factor VIII (b) Produced by endothelial cell
   (c) Prevents platelet adhesion with collagen (d) Factor VIII gets activated after separating from it
3. **Platelet derived growth factor (PDGF):**
   (a) Also called platelet factor 3 and 4 (b) Stimulate wound healing
   (c) Inhibit mitosis in the vascular wall (d) Helps in clot retraction
4. **Thrombocytopenia commonly occur in:**
   (a) Splenectomy (b) After surgery (c) Dengue fever (d) Stress conditions
5. **Which is *not* a function of platelets?**
   (a) Haemostasis (b) Phagocytosis of viruses (c) Clot retraction (d) Synthesize 5-HT
6. **Temporary haemostatic plug is converted into definitive plug by:**
   (a) Platelets (b) ATP (c) Fibrin (d) Serotonin
7. **Platelets aggregation:**
   (a) Means platelets adhere to the exposed collagen in the vessel wall
   (b) Increased by thromboxane A_2
   (c) Increased by prostacyclin
   (d) Converts temporary haemostatic plug into definitive plug
8. **A reliable screening test for platelet function is:**
   (a) Clotting time (b) Prothrombin time (c) Thrombin time (d) Clot retraction time
9. **Aspirin inhibits:**
   (a) Platelet activating factor (b) Prostacyclin synthesis
   (c) Thromboxane A_2 (d) Phospholipase A_2
10. **Which one of the following is released by blood platelets during haemorrhage to produce vasoconstriction?**
    (a) Serotonin (b) Histamine (c) Thrombosthenin (d) Bradykinin
11. **Thrombosthenin is:**
    (a) Coagulation factor (b) Contractile protein
    (c) Thrombosis promoting protein (d) Platelets derived growth factor
12. **The life span of platelets is:**
    (a) 4 days (b) 8-12 days (c) 20-30 days (d) 90 days
13. **Platelet adhesion is dependent upon *all except*:**
    (a) Ca^{2+} (b) ADP (c) Collagen (d) Stasis of blood

Answers

1. (c) 2. (c) 3. (b) 4. (c) 5. (d) 6. (c) 7. (b) 8. (d) 9. (c) 10. (a)
11. (b) 12. (b) 13. (d)

Chapter 12

Coagulation of Blood

I. Definition
II. Mechanism of haemostasis
III. Physiology of clotting mechanism
IV. Why blood does not clot in circulation?
V. Anti-coagulant mechanism: Fibrinolytic system
VI. Anti-coagulants: Natural; Synthetic
VII. Haemorrhagic (bleeding) disorders

DEFINITION

Spontaneous arrest or prevention of bleeding by physiological processes is called ***haemostasis***.

MECHANISM OF HAEMOSTASIS

Injury to vessel wall

↓ *initiates series of events**

Formation of clot

↓

Seals off the damaged blood vessel

↓

prevents further loss of blood

***Series of events involved in haemostasis**

Three major events which get involved during haemostasis are:

A. Constriction of injured blood vessel due to
   1. local myogenic contraction of the blood vessel
   2. nervous reflexes that originate from injured tissues; and
   3. release of 5-HT and other vasoconstrictor substances from the platelets.

B. Formation of a ***temporary haemostatic plug*** of platelets.

C. Conversion of temporary haemostatic plug into the ***definitive haemostatic clot***.
   (details as per schematic **Fig. 12.1 and 12.2**)

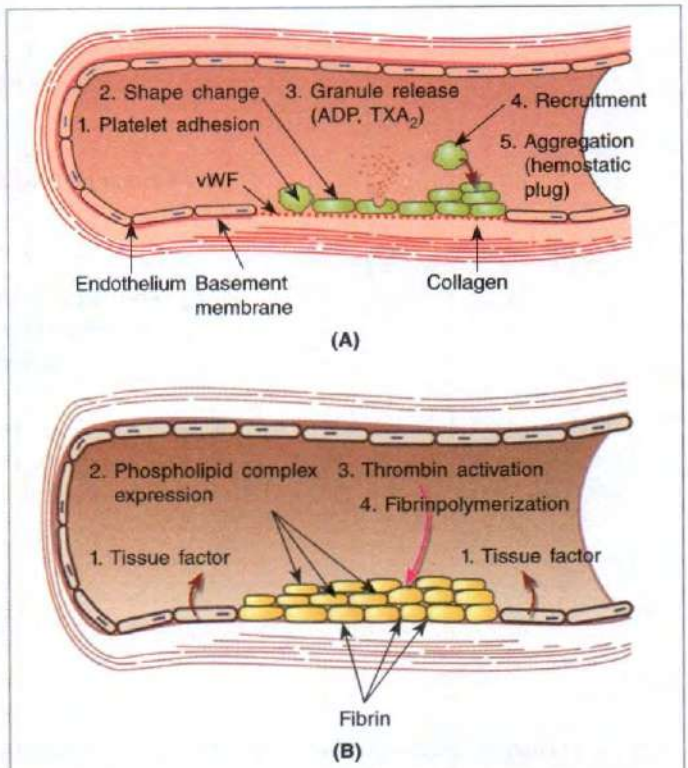


Fig. 12.1 Temporary (A) and Definitive (B) haemostatic plug. (TXA_2:Thromboxane A_2; vWF: von-Willebrand factor)

PHYSIOLOGY OF CLOTTING MECHANISM

BLOOD CLOTTING FACTORS

Refer **Table 12.1**.

The clotting mechanism responsible for the formation of fibrin involves a 'complex series' or *'cascade' of amplification reactions*. Here small quantities of 'inactive' enzymes are activated, and the activated enzymes in turn successively activate increasing quantities of other inactive enzymes (*Macfarlane, R.G. 1967*).

The fundamental reactions involved in the clotting of blood are:

1. **Formation of Fibrin** *i.e.* conversion of 'soluble' plasma protein 'fibrinogen' to 'insoluble fibrin' by an enzyme 'thrombin'. **(Fig. 12.3)**:

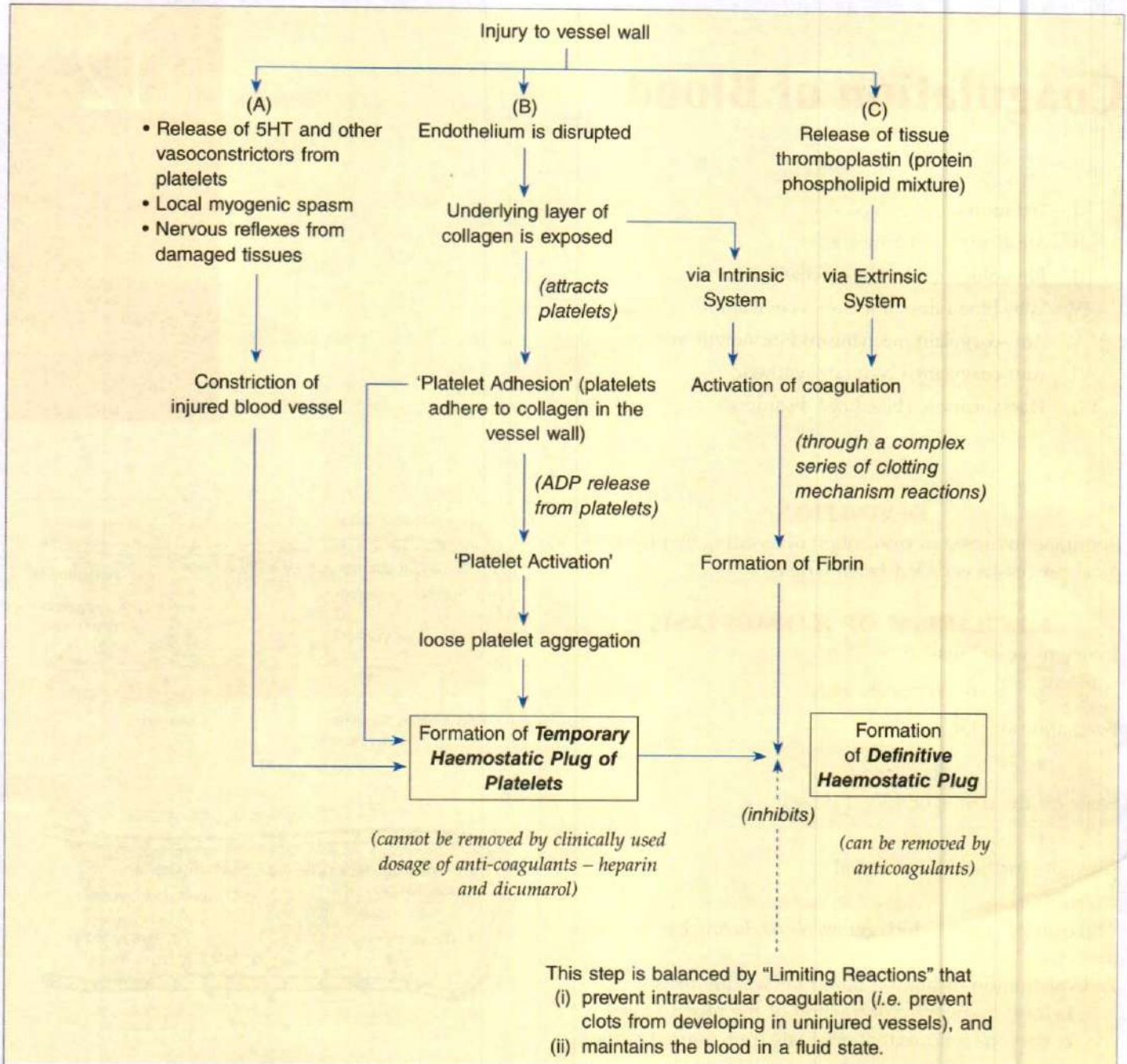


Fig. 12.2 Schematic diagram to show series of events involved in haemostasis

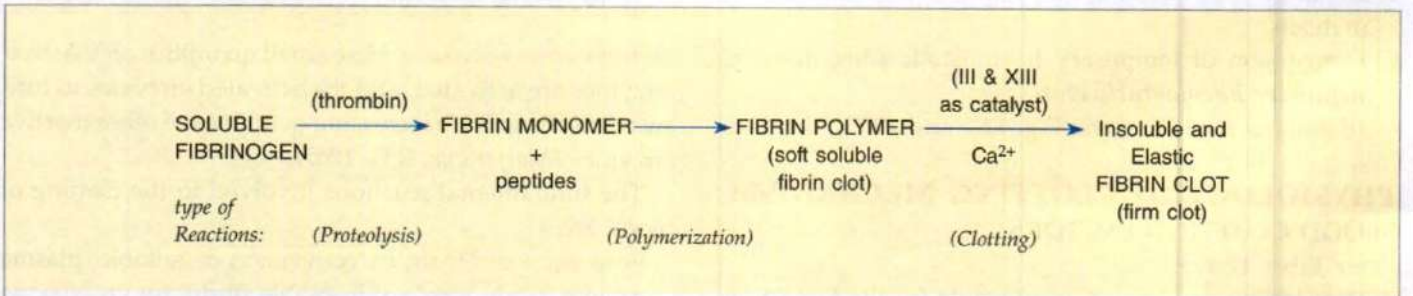


Fig. 12.3 Formation of fibrin

Table 12.1: Blood Clotting Factors (Designated by Roman numerals)

International Nomenclature	Description
Factor I	*Fibrinogen*, MW 3,30,000; plasma level: 200-450 mg/dL.
Factor II	*Prothrombin*, MW 69,000; plasma level: 40 mg/dL.
Factor III	*Thromboplastin* or tissue factor or tissue extract.
Factor IV	*Ionic calcium*
Factor V	*Labile factor*/proaccelerin/accelerator globulin. It gets consumed during clotting and is, therefore, absent from serum.
Factor VI	—
Factor VII	*Proconvertin*/serum prothrombin convergen accelerator (SPCA)/stable factor or *Autoprothrombin I.* Not consumed during clotting, therefore, is present in serum as well as in plasma.
Factor VIII	*Antihemophilic factors* (AHF)/Antihemophilic globulin (AHG)/*antihemophilic factor-A (AHF-A),* absent from serum; half life 10-20 hours.
Factor IX	*Christmas factor*/plasma thromboplastin component (PTC)/autoprothrombin II/AHF-B.
Factor X	*Stuart prower factor/autoprothrombin-C.* Present in plasma and serum.
Factor XI	*Plasma thromboplastin antecedent* (PTA)/AHF-C. Present in plasma and serum.
Factor XII	*Hageman factor*/glass factor/*contact factor,* present in plasma and serum.
Factor XIII	*Fibrin stabilizing factor*/fibrinrase/*Laki-Lorand factor.*

Fibrin initially is a loose mesh of interlacing strands and is converted by the formation of covalent cross-linkages to a dense, tight aggregate. This latter reaction is catalyzed by factor III and XIII and requires Ca^{2+}.

2. **Formation of Thrombin**: There is no circulating thrombin in normal blood but its inactive precursor '*prothrombin*', an α_2-globulin (MW 69000) is present in plasma in a concentration of approx. 40 mg/ dL. Prothrombin is formed in the liver in the presence of adequate amounts of vitamin K. Its concentration decreases in liver diseases. It is converted to thrombin (MW 33000) by the action of *prothrombin activator.*
3. **Formation of Prothrombin Activator**: It is formed in two ways by *Extrinsic* and *Intrinsic* systems.
   Extrinsic system is triggered by injury to vessel wall or other body tissues resulting in formation of *'extrinsic prothrombin activator' (EPA);* whereas *Intrinsic system* is triggered when blood is exposed to the collagen fibres or change in blood constituents, resulting in formation of *'intrinsic prothrombin activator' (IPA).* **(Fig. 12.4)**
   In both the cases the ***key reaction*** is conversion of factor X to its active form, Xa, which then interacts with factor V, Ca^{2+}, platelet, phospholipids to form *prothrombin activator.* In this stage factor V acts as a co-factor and phospholipids provide a surface on which the reagents are concentrated.
4. **Formation of Active Factor X (Xa)**: Factor X can be activated by reactions in either of two systems, an extrinsic and an intrinsic system.
   (i) Activation of 'Extrinsic' system causes release of ***tissue thromboplastin,*** a protein-phospholipid mixture (that functions as a proteolytic enzyme) from vessel walls and a variety of other tissues when they are damaged. Factor III activates factor VII (VIIa); VIIa activates factor X (Xa) in the presence of Ca^{2+}, factor III and platelet phosphilipids.
   (ii) Activation of 'Intrinsic' system causes the conversion of inactive factor XII to its active form XIIa. XIIa then activates factor XI (XIa) which in turn activates factor IX (IXa). IXa forms a complex with VIIIa; this complex in the presence of platelet phospholipids and Ca^{2+} activates factor X to form Xa.

Note

Factor VIII gets activated when it is separated from von-Willebrand factor (page 91).

Once Xa is formed clotting takes place within few seconds. The clot itself initiates a vicious circle (***positive feedback***) to promote more clotting.

5. **Clot Retraction**: 'EPA' and/or 'IPA' catalyze the conversion of prothrombin to thrombin. Thrombin acts on fibrinogen to form fibrin clot. Within 5-30 minutes clot retracts *i.e.* it contracts down to 40% of its original volume with the liberation of yellowish fluid called ***serum.*** Clot retraction is impaired in disease conditions which have a low platelet count.

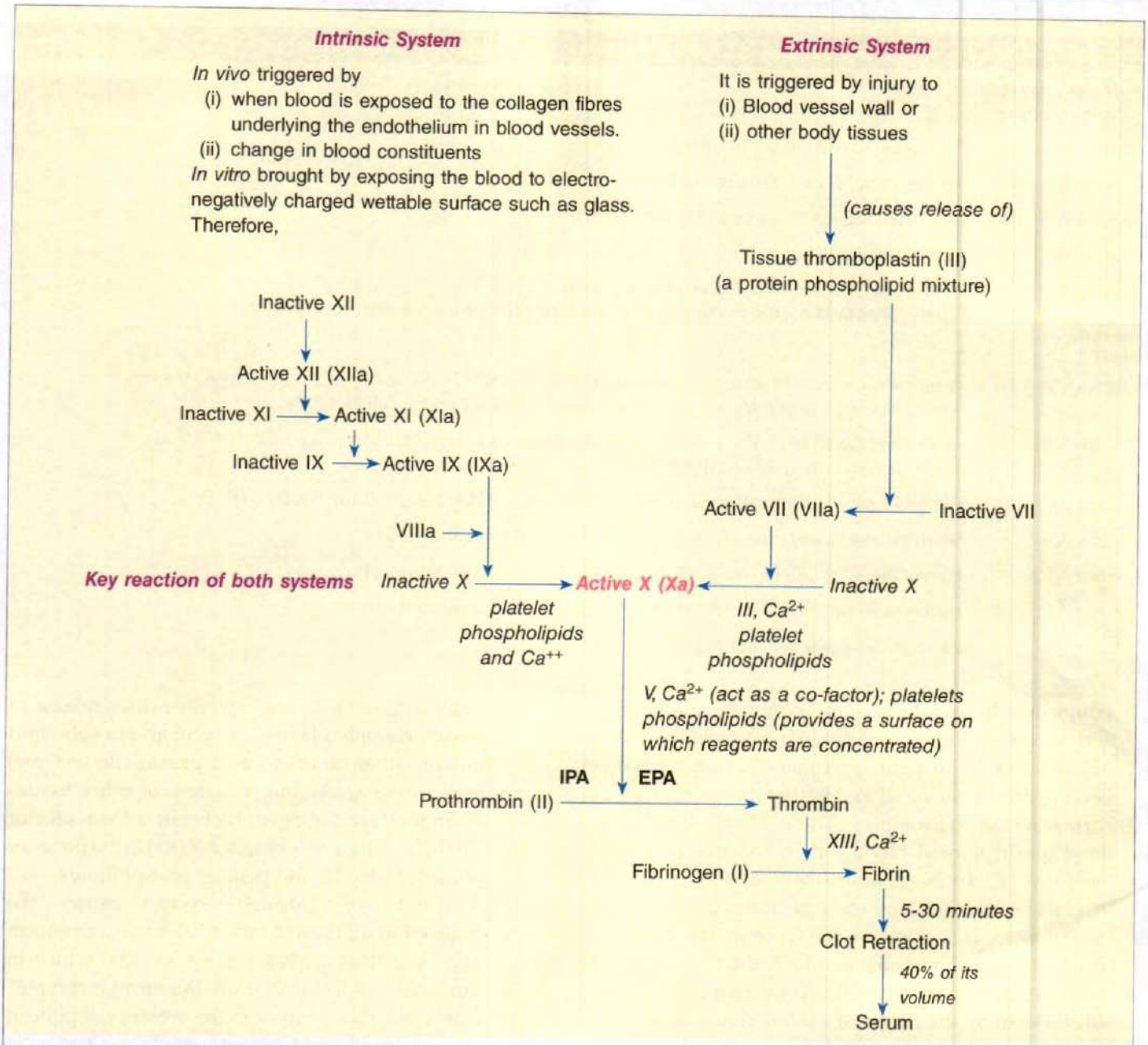


Fig. 12.4 Schematic diagram of clotting mechanism (IPA and EPA: Intrinsic and Extrinsic prothrombin activator respectively)

Important Note

In the extrinsic system the reactions leading to the formation of Xa are rapid and simple whereas in the intrinsic system reaction are more complicated and takes several minutes (1 to 6 minutes) for completion. However, after blood vessel rupture clotting occurs by both pathways simultaneously.

6. **Limiting Reactions** ***(Prevention of intravascular thrombosis)*** *i.e.* reactions which prevent spread of clot in the injured vessel after blood coagulation. These reactions include:
   (i) Removal of some activated clotting factors, specially IX, X, XI and XII from the circulation by ***antithrombin III*** (a circulating *protease inhibitor* secreted by the liver).
   (ii) Reduction in the supply of clotting factors to the degree that they are used up during clotting.
   (iii) Interaction between 'Thromboxane A_2' and 'prostacyclin' (page 93).
   Thromboxane A_2 promotes platelet aggregation and vasoconstriction (***platelet aggregating effect***), whereas prostacyclin inhibits platelet aggregation and promotes vasodilatation (***platelet anti-***

aggregating effect). Balance between these two causes:

(a) localized platelet aggregation at the site when a blood vessel is injured and consequent clot formation;

(b) preventing excessive extension of clot and maintaining blood flow around it.

Important Note

Administration of aspirin in low dosage shifts the balance towards prostacyclin and also inhibits platelet aggregation (page 93). Thus it has been shown to be of value in preventing myocardial infarction (MI), unstable angina, strokes and transient ischaemic attacks (TIA).

(iv) Presence of natural anticoagulants in the circulation *i.e.* heparin and protein C (see below).

7. **Role of Calcium**. Calcium acts as a catalyst in many stages of cascade reactions. However, calcium deficiencies do not produce coagulation disorders because only traces of calcium are required for coagulation. Even if very severe calcium deficiency occurs, it may produce other symptoms like *tetany* (page 710) before coagulation disorders develop.

WHY BLOOD DOES NOT CLOT IN CIRCULATION?

1. **Endothelial factors**:
   (i) Smoothness of endothelial lining prevents platelets adhesion and extension of clot into blood vessel.
   (ii) Negatively charged particles (such as glycocalyx, a mucopolysacoharide) present over endothelial lining, repel the clotting factors *i.e.* protein anions, thereby prevent clotting.
2. **Velocity of circulation**; if decreases leads to clotting.
3. Presence of **natural anticoagulants** in the blood *e.g. heparin* and *protein 'C'*.
   All endothelial cells except those in the cerebral microcirculation produce ***thrombomodulin***, a thrombin-binding protein that converts thrombin into *protein C activator*. This activates *protein C*, (a naturally occurring anti-coagulant protein) along with its cofactor *Protein S*, inactivates factors V and VIII and inactivates an inhibitor of "tissue plasminogen activator", increasing the formation of ***plasmin (fibrinolysin)***. **(Fig. 12.5)**
4. Simultaneous ***activation of Fibrinolytic System*** along with clotting mechanism (see below).
5. In the event of spontaneous clot formation, liver removes the activated clotting factors from the circulation (see above).

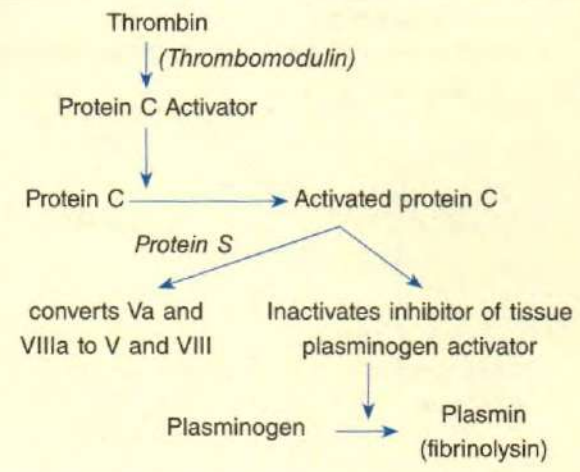


Fig. 12.5 Steps involved in the formation of plasmin (fibrinolysin)

APPLIED: THROMBOSIS

Definition

Formation of clots inside blood vessels is called ***Thrombosis***. The bits of thrombus when break off and travel in the blood stream, is called ***Emboli***.

Causes

1. *Sluggish blood flow*. It allows activated clotting factors to accumulate instead to be washed away. This is commonly seen in leg veins after delivery and surgery.
2. *Damage to tunica intima* by *atherosclerotic plaques*. They are particular prone to occur in the coronary and cerebral blood vessels.
3. *Mutation* in the gene for factor V, thus it fails to get activated by activated protein C (see above).
4. Mutation in antithrombin III and protein S (see above).

Complication

Thrombi/emboli frequently occlude the blood supply to the organ in which they form and damage it. This is commonly seen secondary to extensive tissue damage or septicemia resulting in *Disseminated intravascular coagulation*.

ANTICOAGULANT MECHANISM: FIBRINOLYTIC SYSTEM (FIG. 12.6)

Factors that initiate clotting mechanism also stimulate the dissolution of the blood clot, called ***fibrinolysis***. Fibrinolysis is due to the action of proteolytic enzyme *'fibrinolysin'* or *'plasmin'*. It is present in the circulation as inactive 'plasminogen' (*profibrinolysin*) which gets converted to "plasmin" by the action of 'thrombin' and *tissue plasminogen activator* - TPA (released by tissue damage). Plasmin lyses fibrin and fibrinogen, with the production of *fibrinogen degradation products* (FDP) that inhibit thrombin. However, this process of fibrinolysis is much slower than clotting process.

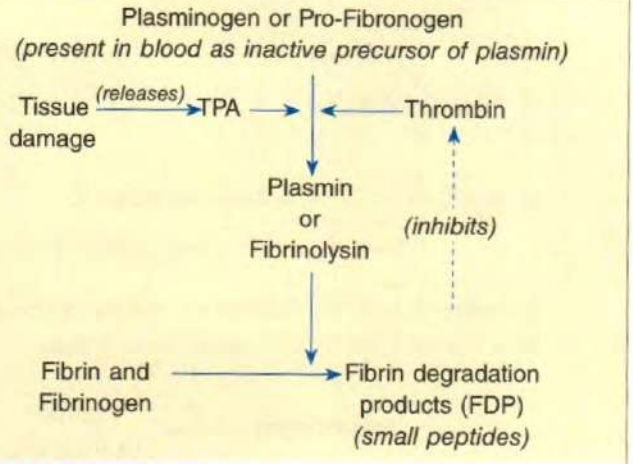


Fig. 12.6 Fibrinolytic system
(TPA: Tissue plasminogen activator)

Important Notes

(i) *Human TPA* (it is used clinically and can be produced by recombinant DNA techniques).
(ii) *Urokinase* (an enzyme produced by kidney cells)
(iii) Streptokinase (a bacterial enzyme by *streptococcus* bacteria)

These 3 enzymes (i, ii and iii) are fibrinolytic and are used in the treatment of early MI and TIA (also see to page 99).

Factors Affecting Fibrinolytic System

(A) *Promoted by*

1. Stress and strains (physical or mental) *e.g.* violent exercise, surgical operation; that is why in violent sudden death, the blood is in fluid state and incoagulable as a result of fibrinolysis.
2. After administration of epinephrine, corticosteriods and phenformin (an anti-diabetic agent).
3. Tissue activators (occur in microsomes) are widely distributed throughout the body cells and body fluids *e.g.* urine contains a plasminogen activator, *urokinase* produced by kidney cells.

(B) *Inhibited by* *'antiplasmin'* which prevents activation of plasminogen, *e.g.*

1. E-amino caproic acid (EACA)
2. Aprotinin or Trasylol (*trypsin inhibitor*).

Physiological Significance of Fibrinolytic System

1. In physiological conditions the clotting system of plasma is continually forming small amounts of fibrin which is deposited to form a thin layer on vascular endothelium, and that the fibrinolytic system is constantly in action to prevent excessive fibrin formation, therefore,
   (i) if clotting system predominates it leads to intravascular thrombosis; and
   (ii) if fibrinolytic system predominates it leads to tendency of bleeding.

   The above (i) and (ii) normally remain balanced.
2. Plasmin can form 'kinins' (*e.g.* bradykinin, kallidin) which contribute to the vascular and sensory features (pain) of the inflammatory response to injury.
3. It also plays a role in cell movement and in ovulation producing defects in growth and fertility.

ANTICOAGULANTS

Types

A. Natural Anticoagulants

1. *Heparin*

A powerful anticoagulant first isolated from liver (hence its name), also present in many other organs *e.g.* lungs. It is a polysaccharide containing many sulphate groups with MW between 15000-18000. It facilitates the action of antithrombin III (page 99), thereby inhibiting the active forms of clotting factors IX, X, XI and XII. This is responsible for its anti-coagulation property.

Origin: Heparin is secreted by –
(i) granules of circulating basophils
(ii) granules of ***mast cells***. Its *characteristic features* are:
   (a) these are granulated wandering cells and are found in large numbers in tissues that are rich in connective tissue; and are arranged in close proximity to the walls of small blood vessels.
   (b) they contain IgE (***Reagin***) receptors on their surfaces and discharge the content of their granules when IgE-coated antigen binds to the receptors, thus mediate allergic and inflammatory reactions (page 126);
   (c) they also contain heparin, histamine and proteases;
   (d) they may aid in defence against parasitic infestation and response to natural immunity.

Destruction: by an enzyme *Heparinase* in the liver.

Uses:
(i) responsible for fluidity of blood
(ii) post-operatively prevents spread of intravascular thrombosis.

2. *Antithrombin* or *Heparin co-factor II*. It inhibits thrombin (page 97).
3. *Protein C* (page 99).

B. Synthetic Anticoagulants

1. *Vitamin K Antagonists:* effective orally. These include:
   (i) coumarin derivatives *e.g.* Dicoumarol
   (ii) Warfarin
   (iii) phenindione, and

(iv) Nicoumalone, etc.

Mode of action: by substrate competitive inhibition of vitamin K in liver *i.e.* they occupy vitamin K receptor sites in the liver and prevent vitamin K to carry out its normal physiological function (page 102). Vitamin K deficiency thus produced results in deficiency of prothrombin, factor VII, IX, X, protein S and protein C, and hence decrease in blood coagulability.

2. Clotting can be prevented in vitro if Ca^{2+} is removed from the blood:
   (i) by addition of substances which form insoluble salts with Ca^{2+} such as sodium citrate or sodium oxalate or sodium edetate (EDTA-ethylene diamine tetra acetic acid);
   (ii) by *chelating agents* which bind Ca^{2+}.
   However, in vivo, a plasma Ca^{2+} level low enough to interfere with blood clotting is incompatible with life.
3. *Malayan (Malaysian) Pit Viper*
   (i) *In vitro*, it has a direct anticoagulant effect on fibrinogen by forming imperfect fibrin polymer.
   (ii) *In vivo*,
      (a) by *Defibrination* (destruction of fibrinogen), and
      (b) by stimulating conversion of plasminogen to plasmin *i.e.* stimulation of fibrinolytic system.
4. *Arvin (Ancord)* – also a type of snake venom. It is a glycoprotein, used therapeutically; anti-coagulation is due to 'defibrination' which produces ***fibrinogenopenia***.
   Other causes of 'fibrinogenopenia' include:
   (i) Congenital, and
   (ii) During pregnancy; secondary to embolism from prematurely separated placenta.
5. *Cold:* Keeping blood at 5-10°C, postpones coagulation but does not absolutely prevents its occurrence. Ice when applied to the surface of body for arresting haemorrhage, prevents bleeding by inducing reflex vasoconstriction.

HAEMORRHAGIC (BLEEDING) DISORDERS

Common major causes of bleeding disorders can be classified as:

A. Defective blood clotting due to:
   (i) deficiency of clotting factors (I, II, V, VIII, IX, X);
   (ii) deficiency of vitamin K;
   (iii) anticoagulant overdose.

B. Defective capillary contractility – ***PURPURA***

C. Combined defects.

A. DEFECTIVE BLOOD CLOTTING

In this disorder a firm clot is not formed following an injury during period of capillary contraction. When the capillaries finally open up once more, oozing will recur.

Causes

1. Deficiency of clotting factors **(Table 12.2)**
2. Vitamin K deficiency
3. Anticoagulant overdose.

Hemophilia A (Classical Hemophilia)

It is of interest because it is relatively common.

Cause

It is caused by an abnormality or deficiency of factor VIII. It is an inherited sex-linked anomaly due to an abnormal gene on X-chromosome. It is invariably transmitted by females (who themselves show no symptoms) to males who manifest signs of the disease. The gene responsible for hemophilia is present in the X-chromosomes. In the presence of another normal X-chromosome the gene acts as a recessive *i.e.* the individual has no sign of hemophilia but can transmit the disease. Certain constituents of the normal X-chromosome may be responsible for this. **(Fig. 12.7)**

Important Note

If a hemophilic man marries a hemophilic carrier, it is possible to have a female hemophilic child, but this occurrence is very rare.

Table 12.2: Major bleeding disorders due to deficiency of clotting factors

Deficiency of factor	Clinical Syndrome	Cause
I	Afibrinogenemia or Fibrinogenopenia	See text
II	Hypoprothrombinemia	Decreased hepatic synthesis of factor II
V	Parahaemophilia	Congenital
VIII	Hemophilia A (classical hemophilia)	See text
IX	Hemophilia B (christmas disease)	Congenital
X	Stuart-Prower factor deficiency	Congenital
Von-Willebrand's factor (page 91)	Von-Willebrand's disease	Congenital or Acquired

Diagnosis

(i) The condition is characterized by a marked increase in the coagulation time (CT); normal CT is 3-8 minutes.

(ii) The bleeding time (BT) is normal; normal BT is 2-5 minutes. Therefore, minute breaks in the skin are sealed by contraction of the capillaries.

(iii) Normal blood collected after venopuncture clots in 5-10 minutes, while hemophilic blood may take 1-12 hours and may form only the soft clot.

Treatment

(i) Fresh blood transfusion, because factor VIII is lost rapidly on storage; *or*

(ii) Injecting factor VIII and IX, prepared from fresh frozen plasma *i.e.* 'cryoprecipitates'; *or*

(iii) Injecting thrombin or thromboplastin.

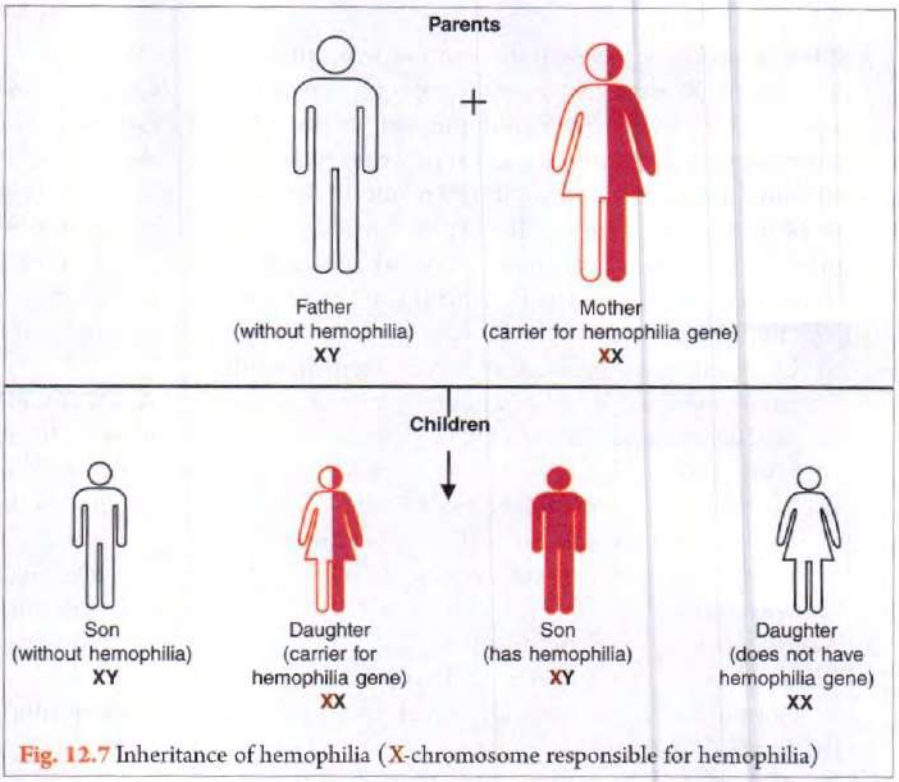


Fig. 12.7 Inheritance of hemophilia (X-chromosome responsible for hemophilia)

Vitamin K Deficiency

Vitamin K is required for the synthesis of prothrombin (factor II) and factors VII, IX and X in the liver. Vitamin K in liver acts on certain receptor sites to form all the factors. Anticoagulants act by substrate competition by occupying vitamin K receptor sites.

Sources of vitamin K

Green leafy vegetables, cereals, animal tissues, synthesized by many bacteria normally present in the intestine.

Vitamin K is absorbed from small intestine in the presence of adequate amounts of bile salts. Its deficiency is characterized by prolongation of clotting time and serious haemorrhages may occur.

Causes of vitamin K deficiency

(i) *Obstructive jaundice*: Absence of bile from bowel depresses fat absorption and consequently vitamin K absorption from the GIT and, therefore, prothrombin and factor VII are decreased and prothrombin time is prolonged. In severe cases clotting time may be prolonged and can cause serious haemorrhages.

(ii) *Chronic diarrhoeas e.g.* ***sprue*** causes:

(a) defective fat absorption causing decreased absorption of vitamin K, and

(b) abnormal state of intestinal bacteria secondary to antibiotic administration producing defective vitamin K synthesis.

(iii) *Liver diseases e.g.* hepatitis, cirrhosis, malignancy cause failure of prothrombin formation; therefore, even parenteral administration of vitamin K is ineffective.

(iv) ***Haemorrhagic states in infants:*** Normal prothrombin level in infants is 1/3 – 1/6 of normal adult level because bacterial flora in the GIT takes several days to one month to develop satisfactorily. Therefore, vitamin K deficiency is even more marked in premature infants. Usually plasma prothrombin level returns to normal during the second week after birth. If the level is less than 1% of normal it may produce severe haemorrhagic states.

Treatment

Injecting vitamin K usually causes complete recovery in less than 48 hours.

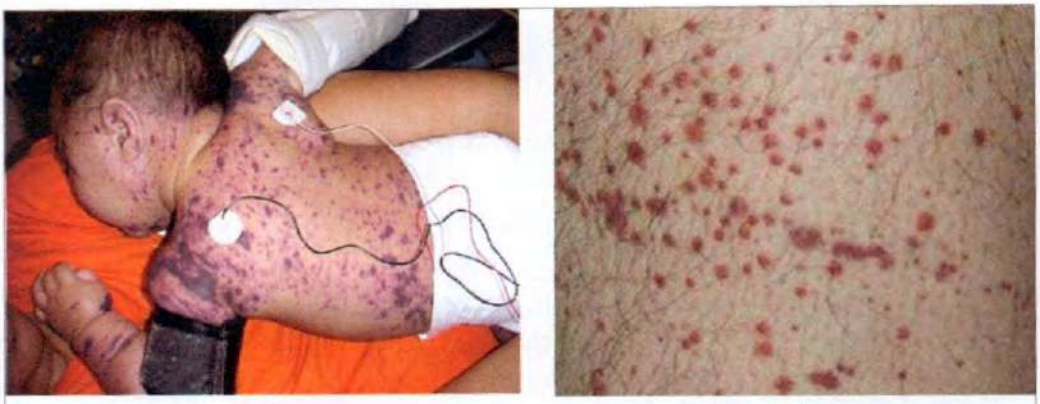
B. DEFECTIVE CAPILLARY CONTRACTILITY

The clinical condition in which the capillary abnormality results in bleeding is known as ***Purpura***. It is characterized by spontaneous haemorrhages beneath the skin, mucous membrane and in internal organs. Severe purpura with haemorrhages in the skin and from mucous membrane is called ***Purpura Haemorrhagica***.

PURPURA

Types of Purpura: Two types: **(Fig. 12.8)**

1. **Primary (Idiopathic)** – congenital or hereditary and usually occurs in children.
2. **Secondary (Symptomatic)** – due to allergies; infections *e.g.* sub-acute bacterial endocarditis (SABE) and typhus; drugs *e.g.* iodine, bismuth, ergot, quinine, phenothiazine; cachectic states *e.g.* cancer.



(A) (B)

Fig. 12.8 Purpura: Primary (A) and Secondary (B)

Diagnosis

1. *Clotting Time (CT):* Normal (3-8 minutes).
2. *Bleeding Time (BT):* Increases (normal: 2-5 minutes).
3. *Capillary endothelium resistance:* decreases, causing increased capillary fragility.
   Evidense: If firm pressure is applied to the skin (by inflating a blood pressure cuff at 60 mmHg for 2 minutes) the local capillaries leak blood, leading to appearance of a crop of minute haemorrhage (*petechiae*).
4. *Skin Microscopy*
   (i) *In primary purpura* – skin capillaries are very irregular and distorted in form, sometimes branching; after puncture these vessels remain patent, therefore, free bleeding proceeds from the needle track for several minutes.
   (ii) *In secondary purpura* the capillaries are anatomically normal but because of presence of toxic agents or other causes they do not contract effectively in response to injury.
5. *Platelet Count:* Normal 1.5–4.0 lacs/μL.
   In many cases of purpura there is reduction in platelet count, called ***Thrombocytopenic Purpura***. Also occurs in hypersplenism because platelets are destroyed in spleen.
6. With low platelet count, clot retraction is deficient and there is poor constriction of ruptured vessels. The clot formed is soft, friable and does not retract well. This results in *easy bruisability* and *multiple subcutaneous haemorrhages.*

Forms/Classification

1. *Thrombocytopenic Purpura:* Purpura with low platelet count. It results in poor clot retraction and poor constriction of injured blood vessels, therefore, it is characterized by easy bruisability and subcutaneous haemorrhages. Clinically, it is seen as:
   (i) *Mild Purpura:* platelet count less than 50,000/μL.
   (ii) *Moderate Purpura:* platelet count less than 10,000/μL. It is characterized by 'severe bleeding'.
   (iii) *Fulminating Purpura:* platelet count less than 1000/μL.
2. *Athrombocytopenic Purpura:* purpura with normal platelet count.
3. *Thromboasthenic Purpura:* It is due to abnormal circulating platelets but platelet count is normal.
4. *Haemorrhagic Telangiectasis:* It is entirely due to a localized capillary abnormality. There is group of dilated capillaries in the skin or mucous membrane; these do not contract with stimuli which affect normal capillaries. Profuse bleeding follow rupture of these vessels.

Treatment

1. Injecting ACTH or corticosteroids, decreases fragility of capillaries; helpful in primary thrombocytopenic purpura.
2. Splenectomy (removal of spleen) helps by decreasing platelet destruction in spleen. It cures approx. 70% of severe primary thrombocytopenic purpura.

Study Questions

1. **Draw line diagram to show:**
   (i) Series of events involved in haemostasis (ii) Clotting mechanisms (iii) Formation of plasmin
   (iv) Fibrinolytic system (v) Inheritance of hemophilia

2. **Write short notes on:**
   (i) Cascade amplification reaction (ii) Thrombomodulin (iii) Protein C
   (iv) Heparin (v) Human TPA (vi) Mast cells
   (vii) Reagins (viii) Chelating agents (ix) Anticoagulants
   (x) Bleeding disorders (xi) Effects and causes of vitamin K deficiency
3. **Explain/Give physiological basis:**
   (i) Why calcium deficiency does not produce coagulation defects?
   (ii) Role of aspirin in prevention of a stroke.
   (iii) Why blood does not clot in circulation?
   (iv) Why clot does not spread in the injured vessel after blood coagulation?
   (v) Is it possible to have a female hemophilic child?
   (vi) Why is blood clotting abnormal in an individual with vitamin K deficiency?
   (vii) Purpura and its various forms.
4. **Mention the role of calcium in clotting mechanism.**
5. **Give steps involved in formation of fibrinolysin.**
6. **Give physiological significance of fibrinolytic system.**
7. **Name natural anticoagulants and give their functioning in the body.**
8. **How a balance is maintained between the clotting mechanism and fibrinolytic system in the body?**
9. **Name the tests to determine bleeding disorders.**

MCQs

1. **The conversion of fibrinogen into fibrin occurs by:**
   (a) Prothrombin (b) Thrombin
   (c) Thromboplastin (d) Platelets
2. ***Not true* of temporary haemostatic plug:**
   (a) It is a loose aggregation of platelets (b) Can be removed by anticoagulants
   (c) Seen following an injury to vessel wall (d) Gets converted to definitive plug by fibrin
3. **Role of platelets phospholipids in blood coagulation is:**
   (a) Provides a surface on which reagents are concentrated (b) Helps platelets adhesion
   (c) Helps platelets activation (d) Helps platelets aggregation
4. **In clotting mechanism via intrinsic and extrinsic pathway, the key reaction is:**
   (a) Formation of thrombin (b) Formation of fibrin
   (c) Formation of prothrombin activator (d) Conversion of factor X to its active form
5. **Administration of aspirin in low dosage shown to be of value in preventing myocardial infarction by:**
   (a) Inhibiting platelets aggregation (b) Preventing platelets adhesion
   (c) Initiating platelets activation (d) Retracting the blood clot
6. **Calcium deficiencies *do not* produce coagulation disorders because:**
   (a) Only traces of calcium are required for coagulation
   (b) If deficiency is very severe, it may produce bleeding symptoms
   (c) Calcium acts as a co-factor
   (d) All other clotting factors are intact
7. **Blood normally does not clot in circulation; *false* statement is**
   (a) Clotted blood liberates certain substances which prevents further clotting
   (b) Removal of activated clotting factors from the circulation
   (c) Activation of fibrinolytic system that limits clotting
   (d) Presence of clotting factors in small amounts.
8. **Human TPA (tissue plasminogen activator) used clinically in treatment of early myocardial infarction acts by:**
   (a) Activation of fibrinolytic system (b) Stimulating heparin release from liver
   (c) Removing activated clotting factors from the circulation (d) Inhibiting thrombin
9. **Heparin acts by inhibiting:**
   (a) Active form of clotting factor VIII (b) Vitamin K synthesis in liver
   (c) Calcium (d) Action of thrombin

10. Haemophilia is:
(a) Autosomal dominant (b) Autosomal recessive (c) X-linked recessive (d) X-linked dominant

11. Lack of Vitamin K causes deficiency of all *except*:
(a) Prothrombin (b) Fibrinogen (c) Factor VII (d) Factor X

12. Primary purpura is:
(a) Congenital disorder (b) Due to allergies
(c) Clotting time is prolonged (d) Capillaries are normal

13. Bleeding in thrombocytopenic purpura usually occurs when platelet count is reduced below:
(a) 1.5 lac/μL (b) 75,000/μL (c) 50,000/μL (d) 25,000/μL

14. Haemostasis is normally associated with all of the following *except*:
(a) Polymerization of plasma prothrombin molecule (b) Blood coagulation
(c) Vascular spasm (d) Formation of a platelet plug

15. The *main* feature of enzyme cascade systems is:
(a) Amplification
(b) Activated enzymes in turn activate other inactive enzymes
(c) Negative feedback regulation
(d) Counter regulation

16. Constriction of blood vessel following injury is due to:
(a) Anoxia (b) Serotonin (c) Endothelin (d) Prostacyclin

17. Once active form of factor X is formed, clotting takes place within:
(a) Few milliseconds (b) Few seconds (c) Few minutes (d) 5-10 minutes

18. Fibrinolytic system gets activated by all of the following conditions, *except*:
(a) Violent sudden death (b) Stress and strain (c) Glucocorticoids (d) Trypsin inhibitor

19. Anticoagulants that prevent coagulation when placed in a blood sample outside the body include all *except*:
(a) Citrates (b) Oxalate (c) Heparin (d) Coumarins

20. Hemophilia B results due to lack of which of the following factors of coagulation?
(a) Factor V (b) Factor VIII (c) Factor IX (c) Factor X

Answers

1. (b)	2. (b)	3. (a)	4. (d)	5. (a)	6. (a)	7. (d)	8. (a)	9. (d)	10. (c)
11. (b)	12. (a)	13. (c)	14. (a)	15. (b)	16. (b)	17. (b)	18. (d)	19. (d)	20. (c)

Chapter 13

Blood Groups

I. Classical 'ABO' blood groups
Landsteiner's law
• Determination of classical blood groups • Inheritance of classical blood groups
II. Rhesus (Rh) blood group
Rh incompatibility (hemolytic diseases)
III. M and N blood groups
IV. Uses of blood grouping tests
Blood transfusion, investigation – a case of paternity dispute
V. Blood storage

The membrane of human RBCs contains a variety of blood group specific *antigens*, also called ***agglutinogens***. More than 30 such antigens are known but a few of them are of practical significance. These antigens enable the blood group of different individuals to be differentiated.

The chief blood groups are:

I. *Classical 'ABO' blood groups*
II. *Rhesus (Rh) blood group*
III. *M and N blood group*

CLASSICAL 'ABO' BLOOD GROUPS

The individuals are divided into 4 major blood groups depending on the presence or absence in their RBCs membrane of the blood group specific substance called A, B and O. The groups are correspondingly called as given in Table 13.1.

A and B are group specific substances, polysaccharide in nature. They are called antigen (*agglutinogen*) *i.e.* in the presence of a suitable antibody (*agglutinin or 'isohaemagglutinin'*) cause clumping of RBCs (*agglutination*). (Fig. 13.1)

The agglutinin acting on agglutinogen A is called 'α' or 'Anti-A'; the agglutinin acting on agglutinogen B is called 'β' or 'Anti-B'. Group specific substance 'O' does not normally act as an agglutinogen and there is no corresponding agglutinin; that is why group 'O' RBCs are not agglutinated by agglutinins α or β. *The agglutinins α and β are globulins of IgM type and cannot cross the placenta* (page 126).

Based on these facts *Karl Landsteiner* in 1900 framed a law, called ***Landsteiner's Law***. It has two major components:

1. If an agglutinogen is present in the RBCs of an individual, the corresponding agglutinin must be absent from the plasma;
2. If the agglutinogen is absent in the individual RBCs, the corresponding agglutinin must be present in the plasma.

Exception to the 2nd part are: absence of Rh, M and N agglutinogens from the RBCs which are not accompanied by presence in the plasma of anti-Rh, anti-M or anti-N agglutinins.

Taking into account both agglutinogen and agglutinin, therefore, the full description of four blood groups is A_β; B_α; AB and $O_{\alpha\beta}$. The agglutinin α is subdivided into:

α_1 – agglutinate only A_1

α proper – agglutinate both A_1 and A_2.

Table 13.1: Classical 'ABO' blood groups and their frequency of distribution

Antigen (Agglutinogen) present on the RBC membrane	Blood Group	Frequency of distribution		Sub-groups
		Britain or United States	India	
A	A	42%	21%	A_1 (75%); A_2 (25%)
B	B	9%	39%	
Both A and B	AB	3%	9%	A_1B and A_2B
Neither A nor B	O	46%	31%	

DETERMINATION OF CLASSICAL BLOOD GROUPS

These can be determined by mixing a drop of isotonic saline suspension of subject's RBCs with a drop of serum A and serum B separately on a glass slide; and seeing whether agglutination occurs or not. The results are diagnostic and are shown in the Fig. 13.1.

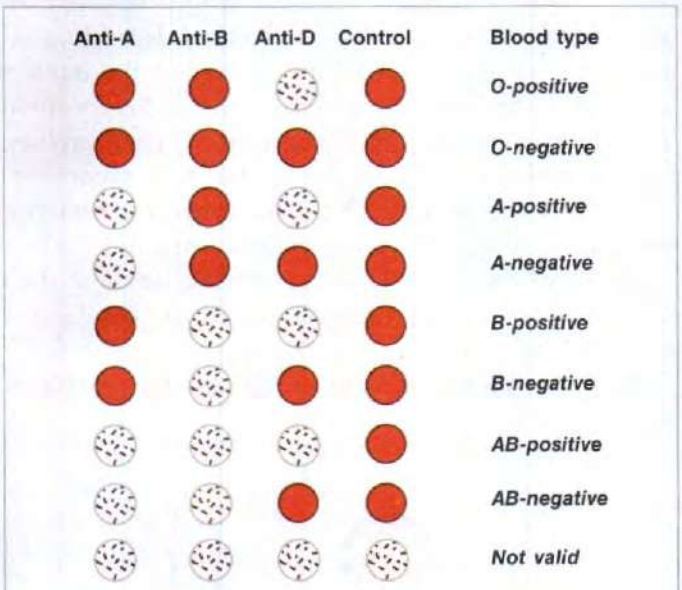


Fig. 13.1 Detemination of ABO and Rh blood types

Note

With high agglutinin titre the cells are massed into a few large clumps; with weaker agglutinin titre more numerous but smaller clumps are formed.

INHERITANCE OF CLASSICAL 'ABO' BLOOD GROUPS

1. The Agglutinogen A and B are inherited as Mendelian dominant and first appear in the sixth week of foetal life. Their concentration at birth is 1/5th the adult level and it progressively rises during puberty and adolescence.
2. Group specific substances A and B are not limited to the RBCs but are also found in many organs like salivary glands and pancreas (++); kidney, urine, liver and lungs (+); testes, semen and amniotic fluid.
3. The antigens very similar to A and B are common in intestinal bacteria and food to which infants are exposed; and infants rapidly develop antibodies against those antigens which are not present in their own RBCs *i.e.* either α or β or αβ or none.
4. The specific agglutinins are present in the plasma and appear at 10th day, rise to peak at 10 years; and then decline.
5. The specific agglutinins act best at low temperature (between 5°C to 20°C, called *Cold Antibodies*) and against well-diluted cells; with weak serum and high cell concentrations the cells may "mop up" agglutinins without being agglutinated.
6. The 4 classical ABO blood groups depend on 3 genes, named after the corresponding factor A, B and O. Each person's blood group is determined by the two genes which he receives from each parent. (Fig. 13.2)

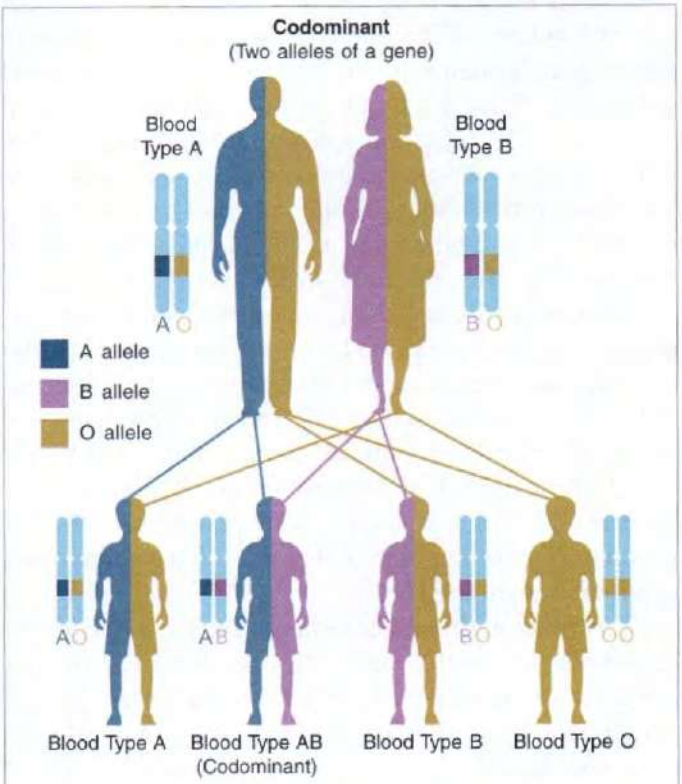


Fig. 13.2 Inheritance of blood groups

Note

Genes A and B may be demonstrated by the use of anti A or anti B serum. The presence of 'O' gene is not easily demonstrated; and to anti-A serum AA and AO cells react alike, both serologically being group A.

RHESUS (Rh) BLOOD GROUP

Discovered by *Landsteiner and Weiner* in 1940.

RBCs of ***Rhesus monkeys*** (monkeys with red ischial callosity) when injected into rabbits, the rabbits respond to the presence of an antigen in these cells by forming an antibody which agglutinates Rhesus RBCs. If the immunized rabbit's serum is tested against human RBCs, agglutination occurs in 85% of people , these are called *Rh '+' (positive)* and their serum contains no Rh antibody. No agglutination occurs in 15%, these are called *Rh '–' (negative)* and their serum also contains no Rh antibody. *The Rh blood group system has not been detected in tissues other than RBCs.*

Distribution of Rh negative percentage of individuals in India

1. Jammu and Kashmir : 10–12%
2. Punjab : 5–6%
3. Delhi : 4.84%
4. South India : 2.3%

The Rh antigen is called 'D', and its antibody is called ***anti-D***. *In Rh system, Rh antibodies are of the IgG type and antigen-antibody reaction occurs best at the body temperature.* Therefore, the Rh antibodies are called ***warm antibodies***. These antibodies being IgG type ***can cross the placenta***. Blood group antigens are the results of the action of genes which are present in the chromosomes. The gene corresponding to the antigen D is also called D; when D is absent from a chromosome, its place is occupied by the alternate form (*Allelomorph*) called 'd'. Rh gene is inherited from both the father and the mother. If gene D is carried by both sperm and ovum the resulting gene composition (genotype) of the offspring is DD; if the gametes carry D and d respectively the result is Dd; if both gametes carry d the result is dd.

DD (homozygous) and Dd (heterozygous) are both Rh positive; dd (homozygous) is Rh negative. Of 85% Rh positive 35% are DD, 48% Dd and 2% have some other genotype containing D.

Rh negative individuals (whose RBCs contain no D agglutinogens), anti-D antibodies (agglutinins) are not naturally present in the plasma, but the production of anti-D antibodies in these Rh negative individuals may be evoked by:

1. transfusion with Rh positive blood *i.e.* D-positive RBCs (0.5 mL may be sufficient);
2. entrance of D-positive RBCs from Rh positive foetus into the maternal circulation of Rh negative mother.

Important Note

C, D and E are three common types of Rh antigens (or Rh factor). The type D antigen is widely prevalent in the population and is more antigenic than the other Rh antigens.

Rh factor and hemolytic disease

The child of a Rh negative mother (genotype dd) and a Rh positive father (genotype DD) must be Rh positive (Dd). If Rh positive father is Dd the offspring may be Rh positive (Dd) or Rh negative (dd).

If mother is Rh negative and foetus is Rh positive, serious complications may occur. RBCs containing 'D' antigen may pass the placenta from the foetus to the mother, either during pregnancy or small amount of foetal blood leaks into maternal circulation at the time of delivery. The mother responds by forming anti-D which returns to foetal circulation and tends to destroy foetal RBCs. The degree of damage done to the foetus depends on the magnitude of maternal anti-D response and the ability of maternal Rh antibodies to cross the placenta.

Since sensitization of Rh negative mother by carrying an Rh positive foetus generally occurs at birth, ***the first child is usually normal***; however, serious results may occur in the second or later pregnancies depending on the degree of sensitivity of the mother. If the mother has been immunized previously by a Rh positive transfusion at any time, even in childhood, a dangerously high response may occur during the first pregnancy.

The changes in the foetus are termed ***Hemolytic Disease***, because, they are due to the destruction of RBCs by maternal anti-D. *ABO incompatibilities rarely produce hemolytic disease of new born*, because, α *and* β *antibodies are of IgM and cannot cross the placenta.*

The various forms of *hemolytic disease* of the newborn are: **(Fig. 13.3)**

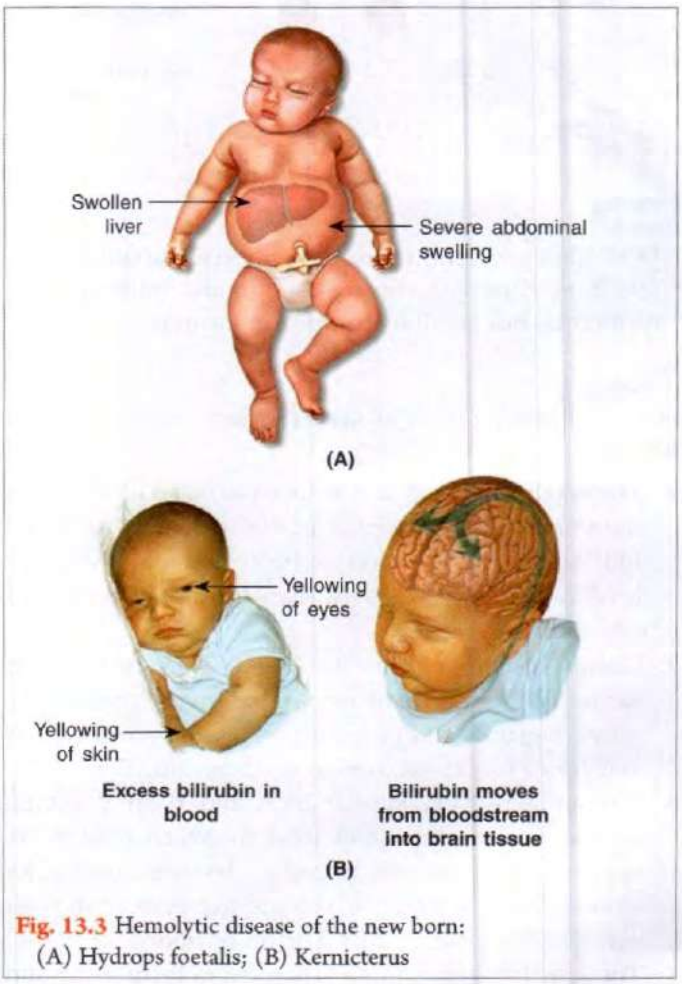


Fig. 13.3 Hemolytic disease of the new born: (A) Hydrops foetalis; (B) Kernicterus

1. ***Hydrops Foetalis:*** The foetus is grossly oedematous, it either dies in utero or if born prematurely or at term, it dies within a few hours.

2. *Icterus Gravis Neonatorum: Characteristic features:*
   (i) The infant born at term is jaundiced or becomes so in 24 hours, due to excessive destruction of RBCs (***hemolytic jaundice***).
   (ii) There may be no anaemia at birth, but develops in a few days. Because at birth, excessive destruction of RBCs is compensated for by an intense normoblastic response of the marrow, associated with high reticulocyte count and presence of many nucleated RBCs in the circulation (***Erythroblastaemia*** or ***Erythroblastosis Foetalis***).
   Free anti-D (derived from the mother) is present in the infant's blood for at least one week after birth and continues to destroy the infant's cells, though at diminished rate at this time.
3. There may be severe neurological lesions involving basal ganglia. As blood brain barrier is not developed in foetus and newborn infants, bile pigments enter the brain and they secondarily become stained bright yellow with bile pigments ***(kernicterus)*** (page 998). It usually develops when serum bilirubin level exceeds 18 mg/dL.
4. Liver may also be severely damaged and death may occur from liver failure.

Treatment: Exchange blood transfusion soon after birth *i.e.* removing small quantities of infant's blood successively from IVC (by passing a polyethylene catheter along the umbilical vein) and replacing an equal volume of compatible Rh negative blood. Thus, infant Rh positive RBCs prone to destruction are removed from the circulation.

Prevention of Rh hemolytic disease

Destruction of Rh positive foetal cells in the maternal blood can be brought about by administering a single dose of anti-Rh antibodies in the form of *Rh-immunoglobulin* soon after child birth. This prevents active antibodies formation by the mother.

(Many of the other blood group antigens like *Lutheran, Kell* and *Kidd* are also known).

M AND N BLOOD GROUPS

M and N factors depend on two minor genes. Each person carries two of the gene of the M and N group *i.e.* M + M (= M); N + N (= N) or M + N (= MN). These are antigenic to rabbits.

USES OF BLOOD GROUPING TESTS

1. In blood transfusion.
2. In pregnancy (Rh incompatibility): see above.
3. Investigating cases of paternity dispute.
4. Medicolegal value.
5. Blood group antigens help in cell recognition.

BLOOD TRANSFUSION

Indications: Alteration in blood either in quantity or quality that interferes with the normal functions of the body.

1. Blood loss: accidents, surgical operations.
2. Blood disorders: hemophilia, purpura, clotting defects.
3. Blood diseases: severe anaemia, leukaemia, blood dyscrasias.
4. Poisoning *e.g.* carbon monoxide poisoning.
5. Acute infections or fever, when γ-globulins are needed.
6. Pre or post-operatively in building up and making up the loss.
7. Shocks.

Basic rules to be observed

1. The *plasma of the donor* which contains the agglutinins can *usually be ignored* because:
   (i) The donor's plasma in the transfusion is usually diluted so by the much larger volume of recipient's plasma that it rarely causes agglutination even when the titre of agglutinins against the recipient's cells is high.
   (ii) Donor's agglutinins are also neutralized by soluble agglutinogen which are found free in the recipient's body fluid.
   However, when the recipient's plasma has agglutinins against the donor's RBCs, the cells agglutinate and hemolyse. Therefore, account need only be taken of the effect of the serum agglutinins of the recipient on the cells (agglutinogens) of the donor.
2. *No Rh negative female at any age before menopause should ever be given a Rh positive blood transfusion,* otherwise she becomes sensitized by the injected Rh positive blood and forms anti-D antibodies (she is likely to destroy subsequently any Rh positive foetus). In other words, the transfusion may make her permanently childless.
3. For effect of transfusing cell of any group into a recipient of any group (intra-group transfusion), refer to **Table 13.2**.

Conclusion

1. Group A and group B can only safely receive blood from their own group and group O.
2. Persons of group AB have no circulating agglutinins and can, therefore, be given blood of any type without developing a transfusion reaction, called ***Universal Recipients***.
3. Persons of group O contain no agglutinogen and their blood can be given to anyone, therefore, its RBCs are

not agglutinated by the members of group, called ***Universal Donors.***

The classical terms, *universal recipient and universal donor are no longer valid* as complication can be produced by the existence of Rh and other factors. Therefore, only safeguard against blood transfusion complication is *Direct Cross Matching i.e.* to match the serum of the recipient directly against the RBCs of the donor and again to match the RBCs of the recipient against the serum of the donor; agglutination in either case indicates incompatibility.

4. If time does not permit the grouping and cross-matching of the recipient, 'O' Rh negative blood should be used. *In case of extreme emergency* like war casualties, train accidents etc., 'O' Rh positive blood should be given (in case if 'O' Rh negative blood is not available).

Important Note

To eliminate the risk of transfusion reactions, patient's own blood can be withdrawn (upto 1–1.5 L) 3–4 weeks in advance of elective surgery and then reinfused during the surgery, a procedure called *Autologous Transfusion*. Anaemia resulting from withdrawal of blood can be made good with appropriate iron therapy over a period of 3–4 weeks.

Dangers/Hazards

1. **Effects of Incompatible (mismatched) blood transfusion (Fig. 13.4)**

Signs and symptoms occur because the recipient serum contains antibodies (α, β or anti-D) which agglutinate the donor's RBCs. The RBCs are first agglutinated and then undergo hemolysis. The following types of clinical reaction occur:

(i) *Inapparent hemolysis:* Injected RBCs are rapidly destroyed, the recipient's blood returning within a week or less to its pre-transfusion state.

(ii) *Post-transfusion jaundice:* Hemolysed RBCs cause increased release of haemoglobin which gets metabolised to bilirubin producing 'hemolytic jaundice'.

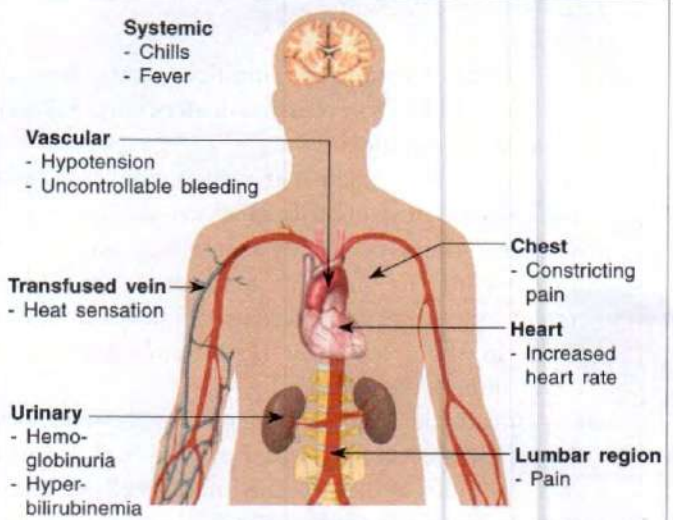


Fig. 13.4 Summary: Main symptoms of mismatched blood transfusion

(iii) Severe reaction with *haemoglobinuria* and renal failure.

(a) After few mL of blood has been introduced, patient complains of violent pain in the back or elsewhere and tightness in the chest; because, agglutinated RBCs form clumps which block capillaries.

(b) *Oliguria* (decreased urinary output) due to:
- fall in arterial blood pressure below 70 mmHg, and
- local vascular disturbances in kidneys

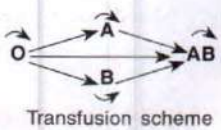
These cause marked fall in glomerular filtration rate (GFR).

If urine is acidic and GFR is slow, the hemoglobin which passes through glomeruli is precipitated in the tubules as *'acid haematin'*. This obstructs the lumen of tubules producing renal tubular damage. Later, *anuria* sets in (*i.e.* complete absence of urine output) due to vascular disturbances involving the glomeruli.

Table 13.2: Effect of inter-group blood transfusion

Recipient Blood group	Agglutinin in serum	RBCs of Donor			
		AB	A	B	O
AB	Nil	–	–	–	–
A	β	+	–	+	–
B	α	+	+	–	–
O	α β	+	+	+	–

'+': Agglutination of RBCs and incompatibility; '–': No agglutination of RBCs and compatibility



Transfusion scheme

Still later,
Renal failure
↓
increases nitrogenous substances and potassium in the body (uraemia)
↓ *within 8-10 days*
Lethargy, coma and death

(Death occurs, if more than 350 mL of incompatible blood is transfused)

2. ***Mechanical overloading*** of circulation produces 'hypervolemia', specially in patients with cardiac damage.
3. ***Chemical risks***
   (i) As stored blood cells lose K^+ to the external plasma, therefore, after excessive transfusion *e.g.* replacement transfusion for erythroblastosis foetalis, death occurs due to hyperkalemia (page 178).
   (ii) With massive transfusion of citrated blood, normal conversion of citrate to bicarbonate by the tissue cells may be delayed as a result, therefore,
      (a) patient may suffer from lack of ionized calcium (more in patients with liver disease or induced hypothermia) causing *'tetany'*;
      (b) 'alkalosis' develops in patients with defective kidney functions.

   This is why, intravenous calcium gluconate is given with citrated blood transfusion.
4. ***Pyrogenic reactions*** like fever with chills and rigors.
5. **Allergic reactions** *e.g.* rash, anaphylactic shock, angioneurotic oedema, urticaria, serum sickness etc.
6. ***Transmission of diseases*** like malaria, syphilis, AIDS, jaundice (viral hepatitis) etc.

INVESTIGATION – A CASE OF PATERNITY DISPUTE

This is based on the knowledge of inheritance of classical and other blood groups. A baby must receive one of the three possible genes (A, B or O) from each parent. Further, each parent transfers one or two genes to the child: an 'A' parent (genotype AA or AO) can give A or O; a 'B' parent (genotype BB or BO) can give B or O; an AB parent can give A or B and 'O' parent (OO) can give 'O' only **(Table 13.3).**

Table 13.3: Investigation: A case of paternity dispute

If the baby's group is	Parents must have given it	So if mother was	Father could not have been
O (M)	O+O (M+M)	No matter which	AB (N)
AB (N)	A+B (N+N)	No matter which	O (M)
A (MN)	A+O or A+A (M+N)	B or O (N)	B or O (N)
B (MN)	B+O or B+B (M+N)	A or O (M)	A or O (M)

Brackets indicate supplementay blood groups

BLOOD STORAGE

Characteristic features

1. For better long term preservation of blood cells and for transfusion purposes citrate is used in combination with dextrose in the following forms:
   A. ***Acid-Citrate-Dextrose (ACD) solution***—(Trisodium citrate, citric acid and dextrose; its pH is 5·4. For use, add 10 volumes of blood to 1·5 volumes of solution).
   B. ***Citrate-Phosphate-Dextrose-Adenine (CPD-A) solution***–(Trisodium citrate, citric acid, dextrose and adenine; its pH is 5·6–5·8. For use, add 7 volumes of blood of to 1 volume of solution).

   Dextrose *acts by*:
   (i) liberating lactic acid which by decreasing the pH helps in survival of RBCs both in vitro and in vivo.
   (ii) providing a substrate for the metabolism, which is still required even at 4°C and thus helps in cell survival (Also see to page 431).

   Under such conditions blood can be stored for 14 days. 80% cell survive for 24 hours after transfusion and thereafter surviving cells are destroyed at a rate of 1% per day.
2. *Stored blood* is **not** a suitable medium for transfusing WBCs and platelets to a recipient, because blood stored for longer than 24 hours contains virtually no viable WBCs and platelets. (Also see page 102)
3. Cold storage *decreases cell metabolism*, thereby decreases active transport and cations move with concentration gradient. Therefore,
   (i) *cell loses* K^+, increasing plasma K^+ concentration from 4-5 mEq/L to 20-30 mEq/L in 2 weeks.
   (ii) *cell gains* Na^+, increasing intra cellular Na^+ concentration from 12 mEq/L to 30-40 mEq/L.

Important Notes

1. **The child's blood group may not be set in its true ABO type until as late as 1 year after birth.**
2. **Blood grouping test can never prove that any suspected person is the actual father; they can only show that he could not possibly have been the father or that he (like many others) might have been.**
3. ***DNA finger printing* is of great value in determining paternity to almost 100%.**

(i) and (ii) cause net increase in cell total base and water, as a result cells become shorter and fatter (*more spherocytic*). This causes increase in their hemolysis in hypotonic solution and cells may rupture in vitro even in 0.8% NaCl solution.

(iii) Spontaneous hemolysis of cells takes place to an increasing degree while in contact with their own plasma in the blood bank.

4. If stored cells are transfused, they become normal (*reconditioned*) in less than 48 hours, with reference to Na^+ and K^+ content, volume, shape and saline fragility.

Study Questions

1. **Write short nots on:**
   (i) Landsteiner's Law
   (ii) Rh factor
   (iii) Hemolytic disease of newborn
   (iv) Uses of blood group tests
   (v) Direct cross matching
   (vi) Dangers of incompatible blood transfusion
   (vii) Storage of blood
   (viii) Heterologus and autologus transfusion
   (ix) Prevention of Rh hemolysis diseases
   (x) Warm and cold antibodies
2. **Give physiological basis of:**
   (i) How α and β agglutinins are produced in persons who do not have the respective agglutinogens in their RBCs?
   (ii) In a Rh negative mother carrying an Rh positive foetus, the first child is usually normal.
   (iii) In case of extreme emergency, 'O' Rh negative blood should be transfused.
   (iv) I.V. calcium gluconate is given with citrated blood transfusion.
   (v) Blood grouping can never prove that any suspected person is the actual father.
   (vi) ABO incompatabilities rarely produce hemolytic disease of newborn.
   (vii) Stored blood is not suitable for transfusing WBCs and platelet to a recipient.
   (viii) The terms 'universal donor' and 'recipient' are no longer valid.
   (ix) Kernicterus, Hydrops foetalis, Icterus gravis neonatorum and Erythroblastosis foetalis
3. **How the blood groups are determined? Give their frequency of distribution in India.**
4. **How much blood is sufficient to produce anti-D antibodies in an Rh negative individual?**
5. **Give the basic rules needed to be observed for blood transfusion.**
6. **At what age after birth a child's blood group is set in its true ABO type? Explain.**
7. **What changes blood cells undergo during cold storage?**
8. **Draw labelled diagram to show inheritance of blood groups.**

MCQs

1. **The least frequent blood group in India is:**
   (a) A (b) B (c) AB (d) O
2. **Blood group 'O' RBCs are agglutinated by:**
   (a) Agglutinin α (b) Agglutinin β (c) Both α and β (d) Neither α nor β
3. **Classical 'ABO' blood groups agglutinogen first appear:**
   (a) In the 6th week of foetal life (b) At birth
   (c) 1 year of life (d) 10 years of life
4. **With respect to blood groups, maximum titre of agglutinin is usually reached:**
   (a) At birth (b) 1-3 years of age (c) 4-7 years of age (d) 8-10 years of age
5. **Rh negative individuals are those:**
   (a) Whose serum contains no Rh antibody
   (b) Whose RBCs contain antigen D
   (c) With absence of Rh antigen on their RBCs membrane and their serum also contains no Rh antibody
   (d) In whom production of anti-D antibodies can be provoked by other blood groups
6. **Absence of anti-A and anti-Rh agglutinin in the plasma means that subject is:**
   (a) A-positive or AB-positive (b) A-negative or AB-negative
   (c) A-positive, AB-positive, A-negative or AB-negative (d) Type O-positive

7. **Prevention of erythroblastosis in Rh-positive babies with the Rh-negative mother is by:**
   (a) Passive immunizing the mother against Rh-positive factor soon after child birth
   (b) Above immunization to be carried out during the pregnancy
   (c) Destruction of Rh-positive cells in foetus by anti-Rh antibodies
   (d) Fresh blood transfusion to the baby immediately after birth
8. **Which one of the following changes would *not* occur in blood after 7 days of storage?**
   (a) Decreased concentration of dextrose and increased concentration of lactic acid
   (b) Decreased concentration of plasma potassium
   (c) An increased prothrombin time
   (d) A decreased concentration of platelets
9. ***True* about cold antibodies:**
   (a) Act best at low temperature between 20°C to 37°C (b) Act against undiluted RBCs
   (c) Are globulin of IgM type (d) Can cross the placenta readily
10. **Commonly seen earliest sign of blood transfusion reaction is:**
   (a) Jaundice (b) Haemoglobinuria
   (c) Violent pain in the back or elsewhere (d) Skin rashes
11. **Death usually occurs if more than ..... mL of incompatible blood is transfused:**
   (a) 50 mL (b) 150 mL (c) 250 mL (d) 350 mL
12. **A man with blood group B cannot be the biological father of a child with an AB blood type if:**
   (a) The mother is type O (b) The mother is type A
   (c) The mother is type AB (d) The mother is Rh-positive
13. **Addition of glucose to stored blood is to:**
   (a) Prevents hemolysis (b) Provides nutrition
   (c) Increases haemoglobin content (d) Decreases the pH
14. **Agglutinin α and β are globulin of:**
   (a) IgG type (b) IgA type (c) IgM type (d) IgD type
15. **Blood group antigens are:**
   (a) Carried by sex chromosomes (b) Attached to plasma proteins
   (c) Attached to haemoglobin molecule (d) Found in saliva
16. **Minimum amount of Rh positive blood required for transfusion to provoke Rh-anti-bodies in a Rh negative individual is ..... mL:**
   (a) 0.5 (b) 10 (c) 50 (d) 100
17. **Which one of the following represents the most potentially dangerous situation?**
   (a) Rh-positive mother with 2nd Rh-negative child (b) Rh-negative mother with 2nd Rh-positive child
   (c) Rh-positive mother with 1st Rh-negative child (d) Rh-negative mother with 1st Rh-positive child
18. **Rh-negative mother carrying Rh-positive foetus, first child born is usually normal because:**
   (a) Mother does not have agglutinogen D
   (b) Anti-Rh titre developed is not sufficiently high to destroy the foetal RBCs
   (c) Foetal agglutinogen D cannot cross the placenta
   (d) Anti-Rh titre developed in the mother's plasma cannot cross the placenta to destroy the foetal RBCs
19. **In cross-matching before blood transfusion:**
   (a) Donor's RBCs are mixed with recipient plasma (b) Donor's RBCs are mixed with recipient RBCs
   (c) Donor's plasma is mixed with recipient RBCs (d) Donor's plasma is mixed with recipient plasma
20. **"Fresh" blood refers to blood which is administered:**
   (a) Immediately (b) Within 12 hours (c) Within 24 hours (d) Within 72 hours

Answers

1. (c)	2. (d)	3. (a)	4. (d)	5. (c)	6. (c)	7. (a)	8. (b)	9. (c)	10. (c)
11. (d)	12. (a)	13. (d)	14. (c)	15. (d)	16. (a)	17. (b)	18. (b)	19. (a)	20. (c)

Chapter 14

Lymphoid Tissues and Lymph

I. Lymphoid Tissues
   A. Tissue macrophage system
   B. Lymphocytes
   C. Plasma cells
II. Functions of Spleen
III. Lymph

LYMPHOID TISSUES

Lymphoid tissues are of fundamental importance in all types of immune response.

Sites

Bone marrow, spleen, lymph nodes, tonsils, peyer's patches, appendix, thymus.

It contains 3 types of cells:

I. Tissue macrophages
II. Lymphocytes (90% are small; 10% are large)
III. Plasma cells

A. TISSUE MACROPHAGES

These are special group of ***phagocytic cells*** found in different parts of the body having common characteristic features to ingest (*i.e.* phagocytose) large (macro) foreign colloidal particles. Therefore, they are called *macrophages*. They are scattered all over the body tissues, thus together they constitute ***tissue macrophage system*** (previously called as *reticuloendothelial system - RES*). Examples include: **(Fig. 14.1)**

1. *Littoral cells* – the cells that form part of the lining of blood sinuses in the bone marrow.
2. *Kupffer's cells* – the cells that lie at frequent intervals along the vascular capillaries in the liver.
3. **Reticulum cells** found in both the red and white pulp of the spleen.
4. **Lymph nodes** that line the lymphatic paths.
5. **Pulmonary alveolar macrophages** (PAM).
6. **Osteoclasts** in the bones.

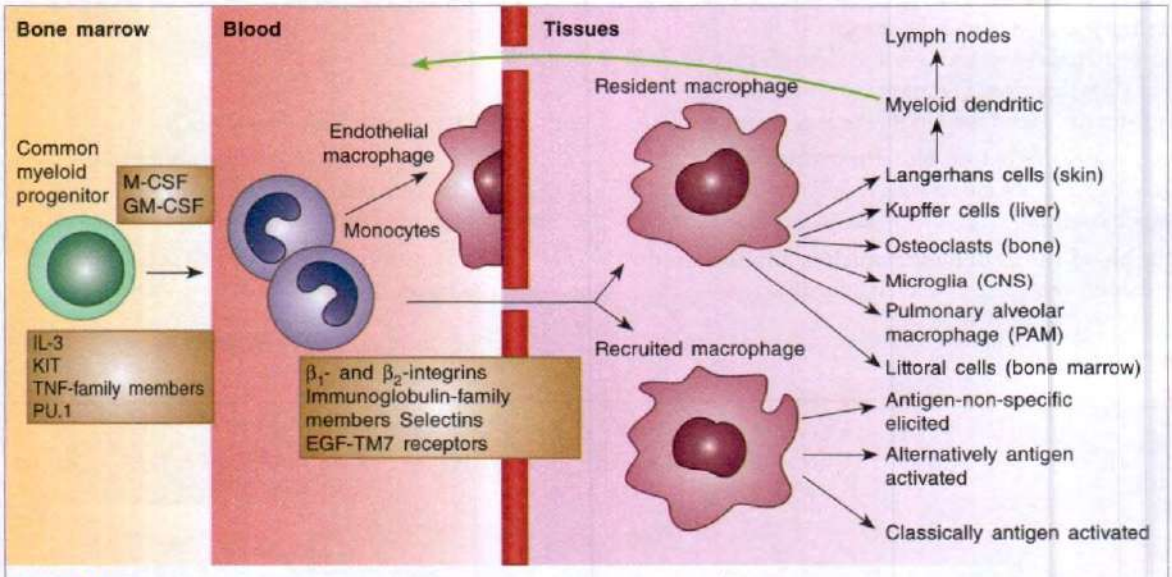


Fig. 14.1 Tissue macrophage system (Abbreviations as given in the text)

7. Microglia in the brain.
8. In Subcutaneous tissues.

Functions

1. They ingest and destroy RBCs; form and release bilirubin. They also destroy WBCs and platelets.
2. They ingest bacteria and are, therefore, concerned with the defence of the body against infection. They rapidly increase in number under these conditions with resulting enlargement of the organs which are rich in these cells *e.g.* spleen, lymph nodes etc.
3. They ingest and 'process' antigen which then stimulate antibody formation in *plasma cells* (page 116).

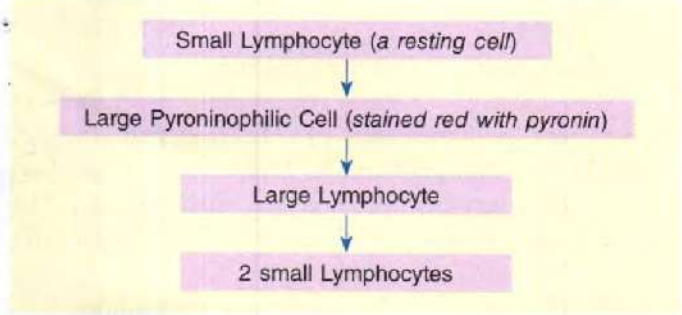
Tissue macrophage system functions as a ***physiological unit*** *i.e.* if any part of it is put out of action, the rest of the system undergoes compensatory hypertrophy and makes good the deficiency.

B. LYMPHOCYTES

They circulate in the lymph and blood; non-phagocytic, move in a characteristic way and do not respond to chemotactic stimuli (compared to other WBCs).

Formation and Development

Lymphocyte proliferation occurs in lymphoid tissues, in response to antigenic stimulation.



Role of thymus

During embryological development it is the first organ to contain lymphoid tissue, therefore, following its removal in neonates lymphoid tissues of other sites fail to develop normally, thus lymphocytes are markedly decreased in circulation.

Mechanism of action

1. It acts on bone marrow and causes
   (i) promoted differentiation of stem cells into lymphocytes, and
   (ii) migration of lymphocytes to other lymphoid tissues to complete their development.
2. It secretes humoral factors, one of these is ***thymosin*** which checks the development of lymphocytes.
3. It seems to condition the lymphocytes so that they respond to antigenic stimulation; such lymphocytes are called ***immunologically competent lymphocytes*** (also refer to page 757).

Factors affecting lymphocyte production

Inhibition by

(i) X-rays
(ii) Administration of cortisol/glucocorticoids

Stimulation by

(i) Thyroid hormones
(ii) Pyridoxine and folic acid
(iii) A substance in the plasma of lymphatic leukaemia patients

Life Span

Two population of lymphocytes exist in the blood:

1. ***B-lymphocytes*** (20-30%), which survive for few days or weeks; and
2. ***T-lymphocytes*** (60-80%), which survive for 2-4 years.

Location of B and T lymphocytes

1. *Peripheral blood* and thoracic duct contain:
   (i) 60-80% T-lymphocytes cell, and
   (ii) 20-30% B-lymphocytes cell
2. *Lymph Nodes* contain T-lymphocytes in the paracortical region and B-lymphocytes in subcapsular region, in germinal centres and in medullary cords.
3. *Spleen* contains T-lymphocytes in periarteriolar sheaths and B-lymphocytes in germinal centres, red pulp and peri-arteriolar sheaths.

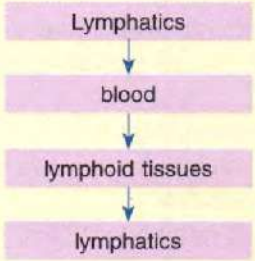
(For details, also refer to page 122)

The Circulation of Lymphocytes

Lymphocytes formed in lymphoid tissues pass into afferent lymphatics and enter the blood stream via the thoracic duct and right lymphatic duct.

The small lymphocytes in peripheral blood (mostly T-lymphocytes) have a special affinity for the endothelium of the post capillary venules in lymphoid tissues. From here they enter efferent lymphatics and finally the thoracic duct, which conveys them into the blood stream where the re-circulation is completed.

Summary



This re-circulation of lymphocytes keeps the blood lymphocyte count relatively constant.

C. PLASMA CELLS

Plasma cells are found in the medullary cords of lymphoid follicles; the small lymphocytes give rise to *"plasma blast" cells* which undergo replication and differentiation to form ***plasma cells***. Life span: 2-3 days.

These cells are oval in structure; 14 × 10 µm size; eccentric nucleus and a deep basophilic cytoplasm which contains abundant endoplasmic reticulum, polyribosomes and golgi apparatus. Therefore, it is a very active cell which forms and secretes *antibodies* (immunoglobulins, page 126) into the blood and other body fluids.

Any one plasma cell only manufactures antibodies of a single kind *i.e.* those which are specific for the antigen that caused the initial activation of the B-lymphocyte from which that plasma cell was derived.

FUNCTIONS OF SPLEEN

Spleen functions are mostly shared with other organs and tissues, therefore, it is ***not essential for life***. Its functions include:

1. **Formation of RBCs**
   (i) during 2nd trimester of uterine life; and
   (ii) in adults after destruction of red bone marrow.
2. Contains lymphoid tissues which **form lymphocytes and plasma cells.**
3. Being a part of tissue macrophage system, it **destroys** aged RBCs, platelets and WBCs, therefore, splenectomy causes:
   (i) appearance of nucleated RBCs in circulation with increase in percentage of reticulocytes, and
   (ii) thrombocytosis and leucocytosis.
4. **Participates in defence** reaction against toxins (diphtheria, tetanus); bacteria and large parasites by the formation of antibodies and by phagocytosis. Therefore, in the absence of spleen, bacterial infections are more common and severe. Moreover, malaria has a higher mortality rate, because the deformed RBCs that contain the malaria parasite are not removed.
5. **Reservoir of RBCs.** *Proof*: contraction of spleen following injection of epinephrine, increases RBC release into peripheral blood.

Clinical Significance

Splenectomy is of therapeutic value in human in cases where the spleen is the predominant site of RBCs destruction *e.g.*

(i) hereditary spherocytosis
(ii) hypersplenism which causes destruction of RBCs, WBCs and platelets
(iii) auto-immune hemolytic states.

LYMPH

The lymphatics at the periphery are a closed system of tubes. Their walls are formed by a single layer of cells without basement membrane under the endothelium, and junction between endothelial cells are open with no tight intercellular connections. These tubes rapidly join together to form bigger lymphatic vessels **(Fig. 14.2)**. The lymph vessels contain valves and regularly traverse lymph nodes along their course. All the lymph from the body is finally collected into two big channels:

1. Right lymphatic duct, which opens into right subclavian vein, and
2. Thoracic duct which opens into left subclavian vein.

Lymphatic system exists in all the organs with the ***exception*** *of CNS and cornea.*

Chemistry of Lymph

Lymph is the modified "tissue fluid", transparent, yellowish in colour, faintly alkaline in reaction and clots slowly. Its colloidal osmotic pressure is less than that of plasma.

1. ***Protein*** content varies with the different tissues but slightly less than that of plasma proteins.

Organ	*Protein content*
(i) Liver	6 gm/dL
(ii) Intestine, heart, lungs, thoracic duct	approx. 4 gm/dL
(iii) Skin and skeletal muscle	2 gm/dL
(iv) Legs	1–1.5 gm/dL
(v) Choroid plexuses, ciliary body	zero

2. ***Lipids*** – more in intestinal region and less in thoracic duct, because lymphatics of intestinal villi (*lacteals*) absorb and transport lipids. Lipoprotein absorption from GIT is responsible for giving *milky* colour to the lymph, called ***Chyle***. This is seen usually after a fatty meal.
3. ***Carbohydrates*** – less than plasma concentration.
4. ***Coagulation factors*** – More in liver lymph as compared to the peripheral lymph, because they are formed in the liver. In vitro, lymph clots on standing due to clotting factors present in it.
5. ***Cellular components***
   (i) Contains large number of lymphocytes of all sizes with variable degree of maturity.
   (ii) Contains no granulocytes (rarely monocyte and macrophages are found).
   (iii) Contains few RBCs and platelets.
6. ***Others*** – Na^+, K^+, Cl^-, SO_4^{2-}, Ca^{2+}, phosphorus, NPN substances, urea, creatinine etc.

Formation and Flow of Lymph

Lymph is formed from tissue fluids, therefore, its formation is based on *transcapillary exchange i.e.* rapid

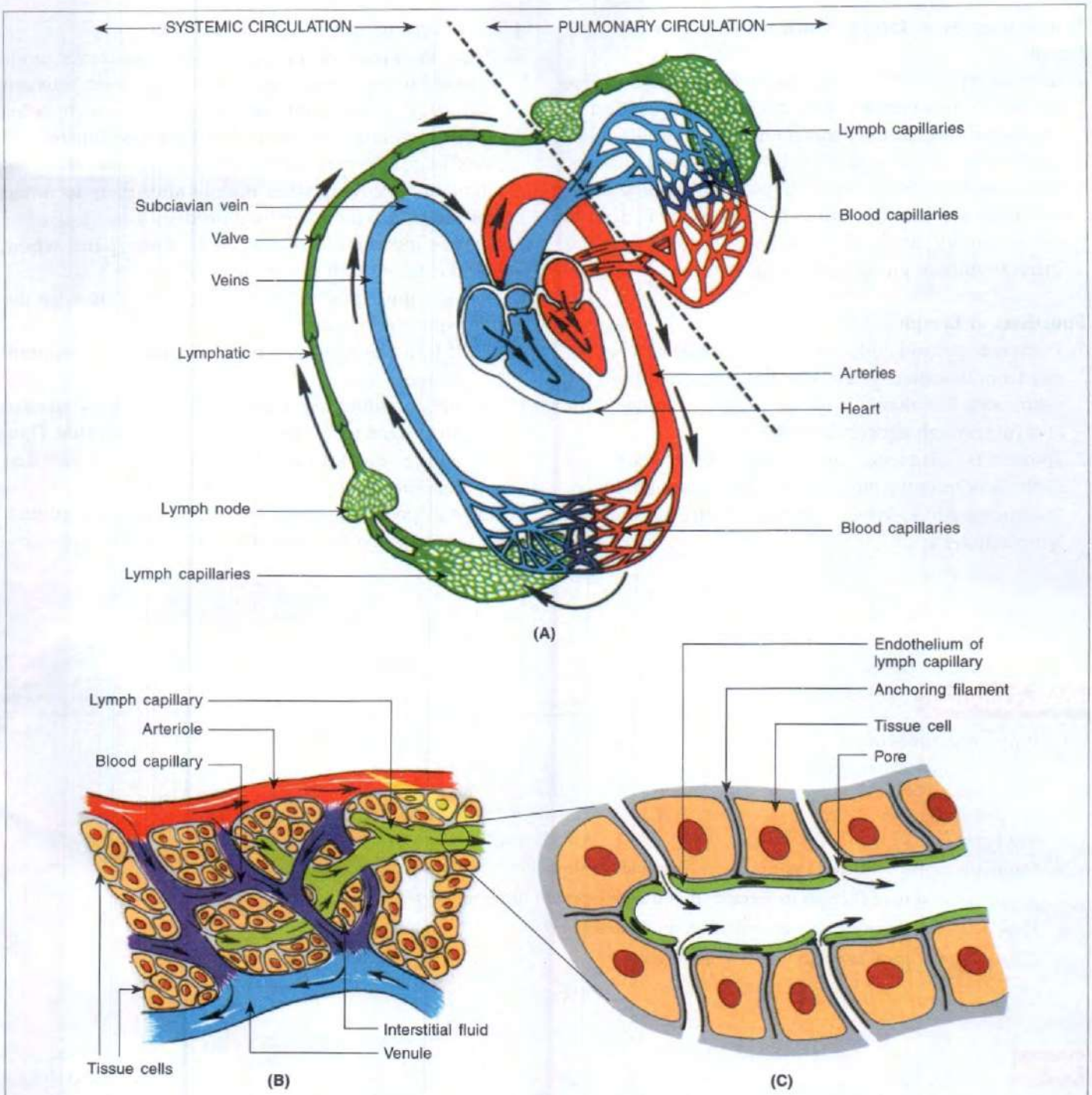


Fig. 14.2 Lymphatic System. (A) Its relationship to the cardiovascular system. (B) Relationship of lymph capillaries to tissue cells and blood capillaries. (C) Appearance of a lymph capillary. (→: direction of flow)

and continuous exchange between intravascular and extravascular compartments (page 55).

Only 2-4 litres of lymph seeps back into the blood stream in 24 hours and lymph flow is very slow, approx. 0.5–1 mL/min (in the thoracic duct).

Factors Maintaining Flow of Lymph Towards the Heart

1. Contractile valves in lymphatics prevent the retrograde flow of lymph.
2. *Muscle pump i.e.* rhythmic contraction of skeletal muscles.
3. *Peristalsis* in the GIT.
4. Rhythmic contraction of the walls of large lymphatics. Rate of these contractions increases in direct proportion to the volume of lymph in the vessels.
5. *Interstitial fluid pressure* is 1.9 cm of H_2O whereas pressure in the lymphatic system is 1.3 cm of H_2O.
6. *Negative intrathoracic pressure* during inspiration.
7. *Suction effect* of high velocity of blood in veins in which the lymphatics finally terminate.

Lymphagogues *i.e.* factors which increase flow of lymph

1. Increase in pressure in capillaries at venous end due to venous obstruction. This decreases absorption of interstitial fluid at the venous end of the capillaries.
2. Increase in capillary permeability *e.g.* bacterial toxins, increased local temperature, hypoxia, histamine etc.
3. Decrease in osmotic pressure of plasma due to administration of hypotonic saline.
4. Increase muscle pump activity *e.g.* exercise.

Functions of Lymph

1. *Transports proteins:* approx. 95% of proteins lost per day from vascular system into the interstitial fluid are returned to the blood via lymphatics by (i) pinocytosis and (ii) through endothelial gaps.
2. Transports absorbed long chain *fatty acids* and cholesterol from the intestine via lymphatics into blood.
3. Transports *RBCs, WBCs* and bacteria to the regional lymph nodes.
4. Transports *antibiotics.*
5. Helps in formation of maximally *concentrated urine* by maintaining appreciable osmotic gradient between medullary interstitium and vasa recta which helps efficient operation of counter current mechanism.
6. Some large enzymes *e.g.* histamine and lipase, reach the circulation by lymphatics mainly after their secretion from cells into the interstitial fluid.
7. *Supplies* nutrition and oxygen to those parts where blood cannot reach *e.g.* cartilage etc.
8. *Enhances* the efficiency of the immune system by the following mechanisms:
   (i) it transports antigen to the organ of the immune system;
   (ii) the continual movement of lymphocytes exposes an antigen to a large sample of lymphocytes. Thus antigen quickly encounters any lymphocytes that can specifically react with it;
   (iii) it disperses the 'memory' lymphocytes, in readiness for any second encounter with the antigen.

Study Questions

1. **Write short notes on:**
   (i) Tissue macrophage system
   (ii) Thymosin
   (iii) Plasma cells
   (iv) Functions of spleen
   (v) Lymphagogues
   (vi) Immunologically competent lymphocytes
   (vii) Functions of lymph
   (viii) B and T lymphocytes.
2. **Name the organs which do not possess lymphatic system. Give its physiological significance.**
3. **Mention the role of thymus in formation and development of lymphocytes.**
4. **How do tissue macrophages contribute to body defences?**
5. **Give physio-clinical significance of splenectomy.**

MCQs

1. **All types of immune response are fundamentally mediated by:**
   (a) Tissue macrophages (b) Lymphoid tissues (c) Lymphocytes (d) Plasma cells
2. **Which is *not* a function of tissue macrophage system?**
   (a) All blood cells get destroyed here
   (b) Concerned with the defence of the body against infection
   (c) Capable of phagocytosing large foreign colloidal particles
   (d) Forms and secretes antibodies
3. **If thymus is removed a few years after birth:**
   (a) Cellular immunity will be lost
   (b) Humoral immunity will be lost
   (c) Both cellular and humoral immunity will be lost
   (d) Both cellular and humoral immunity will be present
4. **Percentage of T-lymphocytes in peripheral blood is:**
   (a) 20-30% (b) 40-60% (c) 60-80% (d) Above 85%

5. *Not true* about plasma cells is:
   (a) Very active cells
   (b) A particular cell is capable of forming many types of antibodies
   (c) Forms and secrets immunoglobulin
   (d) Formed in the lymph nodes
6. *True* about spleen is that:
   (a) It is not essential for life
   (b) Normally forms RBCs during adult life
   (c) Its removal causes thrombocytopenia
   (d) Its removal causes leucopenia
7. Milky colour of the lymph in intestinal region is due to:
   (a) Carbohydrates
   (b) Cellular components
   (c) Lipids
   (d) NPN substances
8. The most important physiological function of the lymphatic system is to:
   (a) Transport fluid and proteins away from the interstitium
   (b) Concentrate proteins in the lymph
   (c) Remove particulate materials from the interstitium
   (d) Create negative pressure in the free interstitial fluid
9. T-lymphocytes survive for:
   (a) Few days or weeks (b) Few months (c) 1-2 years (d) 2-4 years
10. *True* about lymph capillaries is:
    (a) Have smaller diameter than blood capillaries
    (b) Less permeable than blood capillaries
    (c) Have no endothelial lining
    (d) Have a discontinuous basement membrane
11. Lymph of which organ has highest protein concentration upto 6 gm/dL?
    (a) Liver (b) Intestine (c) Thoracic duct (d) Legs
12. Total quantity of lymph entering circulation daily is:
    (a) 1 litre (b) 2 litres (c) 2-4 litres (d) 4-6 litres

Answers

1. (b) 2. (d) 3. (d) 4. (c) 5. (b) 6. (a) 7. (c) 8. (a) 9. (d) 10. (d)
11. (a) 12. (c)

Chapter 15

Immunity (The Immune System)

I. Introduction
II. Classification
Natural immune System (the complement system; C-reactive protein; interferons; NK cells)
Acquired immune system (immunoglobulins; cytokines)
III. Regulation of immune response
IV. Immunological tolerance: Recognition of self; Auto-immunization
V. Tissue Transplant/Graft

INTRODUCTION

Body is protected against invading organisms by physical barriers like skin and other epithelial linings which constitute the ***first line of defence***. (Fig. 15.1). If these mechanisms fail to control the invading organisms, then the ***second line of defence***, *the immune system* is activated.

Lymphoid tissues (page 114) are fundamentally involved in the process of immunity *i.e.* body protection power against invasion by undesirable agents like microorganisms,viruses, tumour cells and parasites.

The responses of the immune system of each division (natural and acquired) are divided into two types: *Humoral* and *Cellular* (cell mediated). The Humoral responses are effected by elements free in the serum or body fluids; whereas cell mediated responses involve cells directly eliminating the invading organisms. (The main differences between natural and acquired immune system are given in Table 15.1)

THE NATURAL (OR INNATE) IMMUNE SYSTEM

A. Natural Humoral Responses

These are effected by soluble factors in the serum and body fluids, *e.g.*

1. The complement system
2. C-reactive protein
3. Interferons
4. Natural killer cells

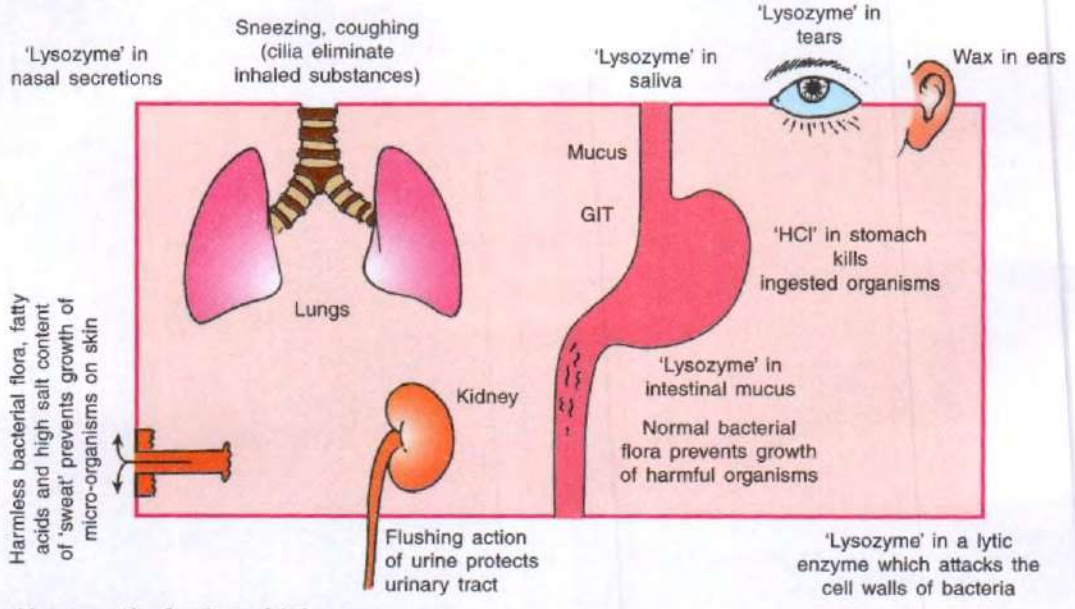


Fig. 15.1 Physical barriers: The first line of defence

CLASSIFICATION OF IMMUNE SYSTEM

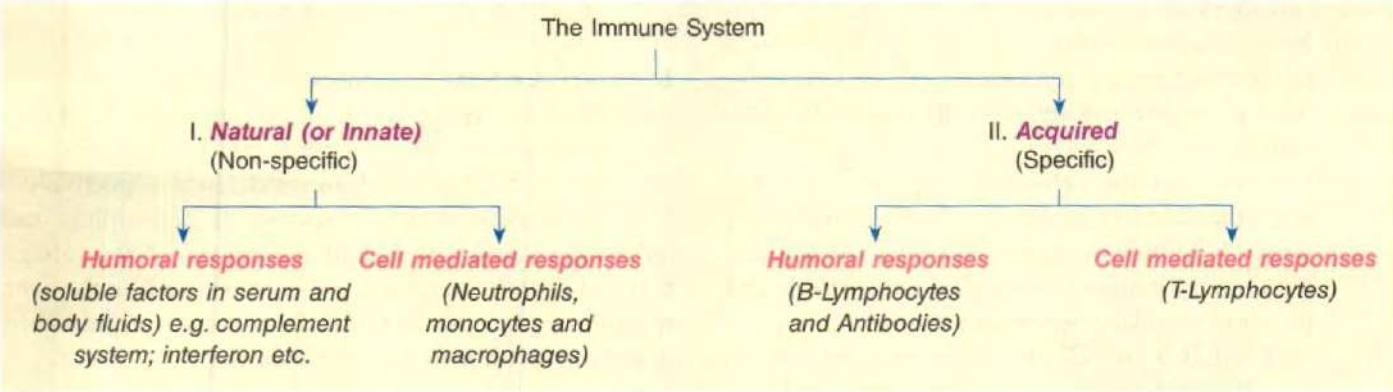


Table 15.1: Natural and Acquired immune systems compared

Natural Immune System	Acquired Immune System
1. It is available since birth	1. It is acquired after birth after exposure to micro-organisms.
2. *'Not specific'* to a particular micro-organism.	2. *'Specific'* for each species of micro-organisms and shows *'immunological memory'* (*i.e.* 2nd exposure to same foreign substance produces a more rapid and greater response).
3. It is able to recognise and respond immediately to any foreign cell or particle.	3. It is more complex and requires time to be fully developed.

1. The Complement System

The cell-killing effects of circulating antibodies and cellular immunity are mediated by a system of plasma enzymes, called the ***Complement System***.

The enzymes are identified by the numbers C1 to C9. C1 is made up of 3 sub-units, C1q, C1r and C1s, therefore, there are 11 proteins in the system. **(Fig. 15.2)**

Activation of this system triggers a sequence of *'cascade' reactions* that activates other components of the system. The system gets activated by three pathways:

(i) *Classical pathway* – initiated by antibody binding to antigen. C1 binds to the antibody-antigen complex (*i.e.* immune complex) and thus triggers a sequence of events that activates C3.

(ii) *Mannose binding lectin pathway* initiated when lectin binds mannose groups in bacteria; and

(iii) *Alternative* or *properdin pathway* – initiated by polysaccharides on bacterial cell wall (endotoxin), yeast cell wall (zymosan) and tumour cells. Interaction of factor I with polysaccharides in cell membrane of invading cells triggers reactions that activate C3 and C5. ***Properdin*** (a circulating protein) stabilizes the activating enzyme complex.

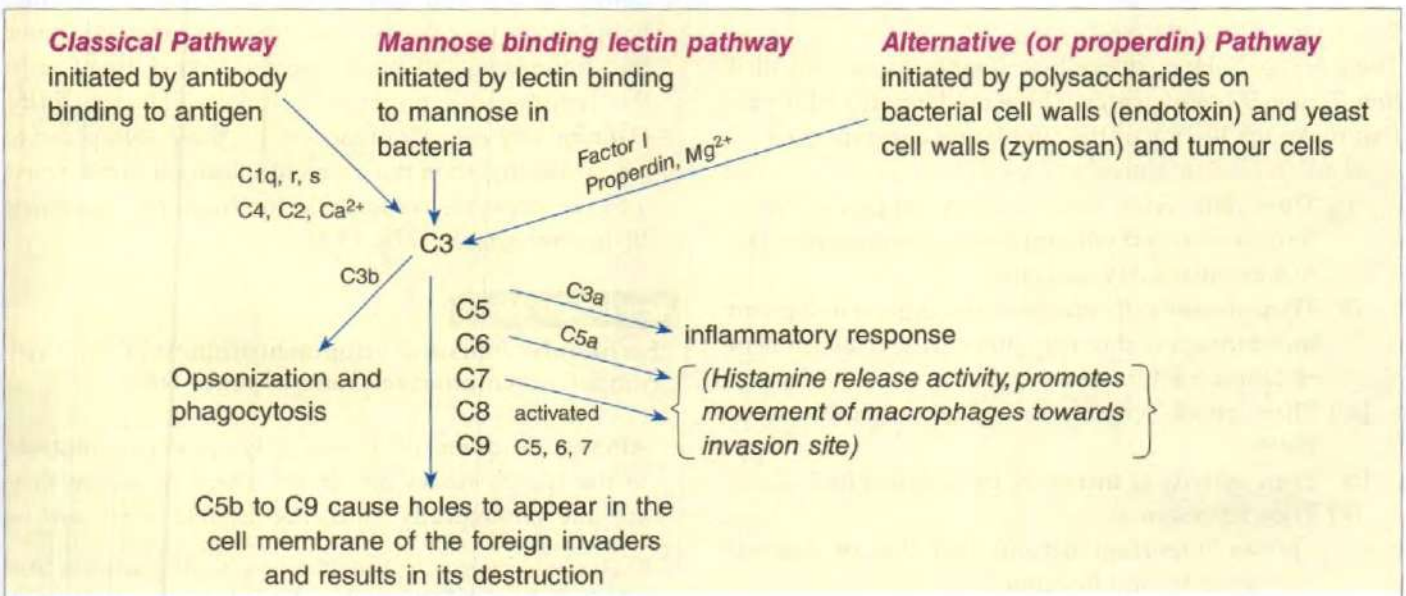


Fig. 15.2 The complement system

Once activated, the complement system helps in lysing foreign invaders by the following mechanisms:

(i) Insertion of pore-forming molecules (***perforins***) in the cell membranes of foreign invaders. Ions move through these pores and the cells become lysed by osmosis.

(ii) Formation of the activated fragments C3a and C5a from C3 and C5 respectively. These fragments release histamine from granulocytes, mast cells and platelets. Histamine dilates the blood vessels and increases capillary permeability.

(a) C5b, C6 and C7 are chemotactic and attract leucocytes to the site of the antigen-antibody reaction.

(b) C3b is responsible for opsonization and phagocytosis of bacteria (page 85). It also initiates reactions that activate the rest of the complement enzymes.

2. C-Reactive Protein

Entry of foreign invaders (antigens) activates concentration of many plasma proteins, specially of *C-reactive protein (CRP)* which coats the invading antigen. CRP-coated organisms activate the complement system which, in turn, facilitates phagocytosis.

Normal plasma level: < 1 mg/dL

3. Interferons

Virally infected cells release *'interferons'* into ECF which:

(i) forms a protective ring of uninfectable cells, thus limits the spread of infection;

(ii) inhibits protein synthesis by promoting degradation of mRNA thereby inhibits replication of viruses.

4. Natural Killer Cells (NK Cells)

These are special type of cytotoxic lymphocytes, also called ***non-T, non-B lymphocytes***. These are large lymphocytes that make up 10-15% of the circulating agranulocytes.

Characteristic features:

(i) They kill cells without any apparent prior sensitization and without the involvement of major histocompatibility antigen.

(ii) They destroy cells that have undergone malignant transformation, thus help prevent the establishment of cancerous tumours.

(iii) They attack viruses and kill antibody-coated viruses.

(iv) Their activity is increased by interleukin-2 (IL-2).

(v) They represent:

(a) an important natural first line of defence against viral infections

(b) combating the spread of disease while more specific T and B cell responses are activated.

(vi) They may represent a primitive immune system from which T and B cell system evolved.

B. Natural Cellular Responses

Foreign substances entering the blood stream are dealt with by *circulating phagocytes e.g.* neutrophils and monocytes: Cell mediated 1st and 2nd line of defence respectively.

If initial inflammatory response by neutrophils and monocytes does not prevent the spread of the foreign material further then, the 'fixed' macrophages, in the liver, spleen, lymph nodes and other body tissues may succeed in eliminating it from the tissue fluids.

THE ACQUIRED IMMUNE SYSTEM

If the invaders overcome the natural immune system, then the acquired immune system comes into play. The body has two principal acquired immune defence systems **(Fig. 15.3)**. Both react to antigens *i.e.* protein or polysaccharides substances which are able to induce the synthesis of antibodies.

1. ***Humoral Immunity*** is due to circulating antibodies in the γ-globulin fraction of plasma protein. It is mediated by B-lymphocytes, and they *activate the complement system* to neutralize antigens (page 121). It is a major defence against bacterial infections.
2. ***Cellular Immunity*** is responsible for delayed allergic reactions, rejection of transplants of foreign tissue and lysis of tumour cells. It is mediated by T-lymphocytes and constitutes a major defence against infections due to viruses, fungi and few bacteria *e.g.* tubercle bacillus. It also helps defend against tumours.

Development of the Acquired Immune System

1. During the foetal development and neonatal life, lymphocyte precursors from the bone marrow enter the thymus gland and become transformed into the lymphocytes responsible for ***cellular immunity (T-lymphocytes)***. Simultaneously these lymphocyte precursors that enter the liver and spleen get transformed into lymphocytes responsible for ***Humoral immunity (B-lymphocytes)***.* **(Fig. 15.4)**

Important Note

For role of thymus in development of immunologically competent lymphocytes, refer to pages 115 and 757.

Afterwards many of T and B lymphocytes migrate to the lymph nodes and bone marrow, where they are morphologically indistinguishable but can be

* *'B' is named because in birds the lymphocyte precursors that populate the bursa of Fabricius, a lymphoid structure near the cloaca, become transfered into the lymphocytes responsible for humoral immunity.*

Summary: The acquired immune defence systems

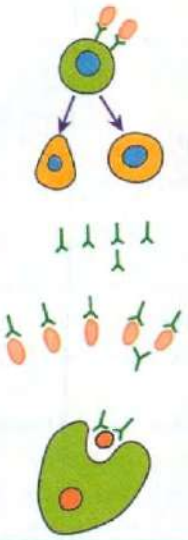
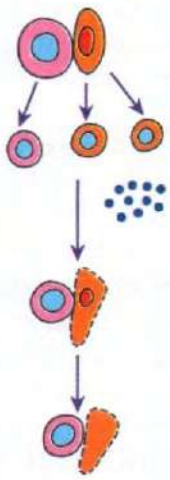
Features	*Humoral response*	*Cellular response*
1. Type of response	Involves antibodies	Does not involve antibodies
2. Major cells involved	B-Lymphocytes	T-Lymphocytes
3. Antigen reacted to	Extracellular phases of bacterial and viral infections	Fungi; parasites; intracellular viral infections; tumours and cancer cells; foreign tissues such as transplanted organs.
4. Effect of stimulation	 *B-Lymphocyte* encounters bacterium → Plasma Cells formation → Secretion of Antibodies → Antibodies attack bacteria → Bacteria eliminated by phagocytosis	 *T-Lymphocyte* encounters cancer cells → Activated T-Lymphocytes → Release of Lymphokines → Cytotoxic-T Lymphocyte attack cancer cells → Elimination of cancer cell

Fig. 15.3 The acquired immune system

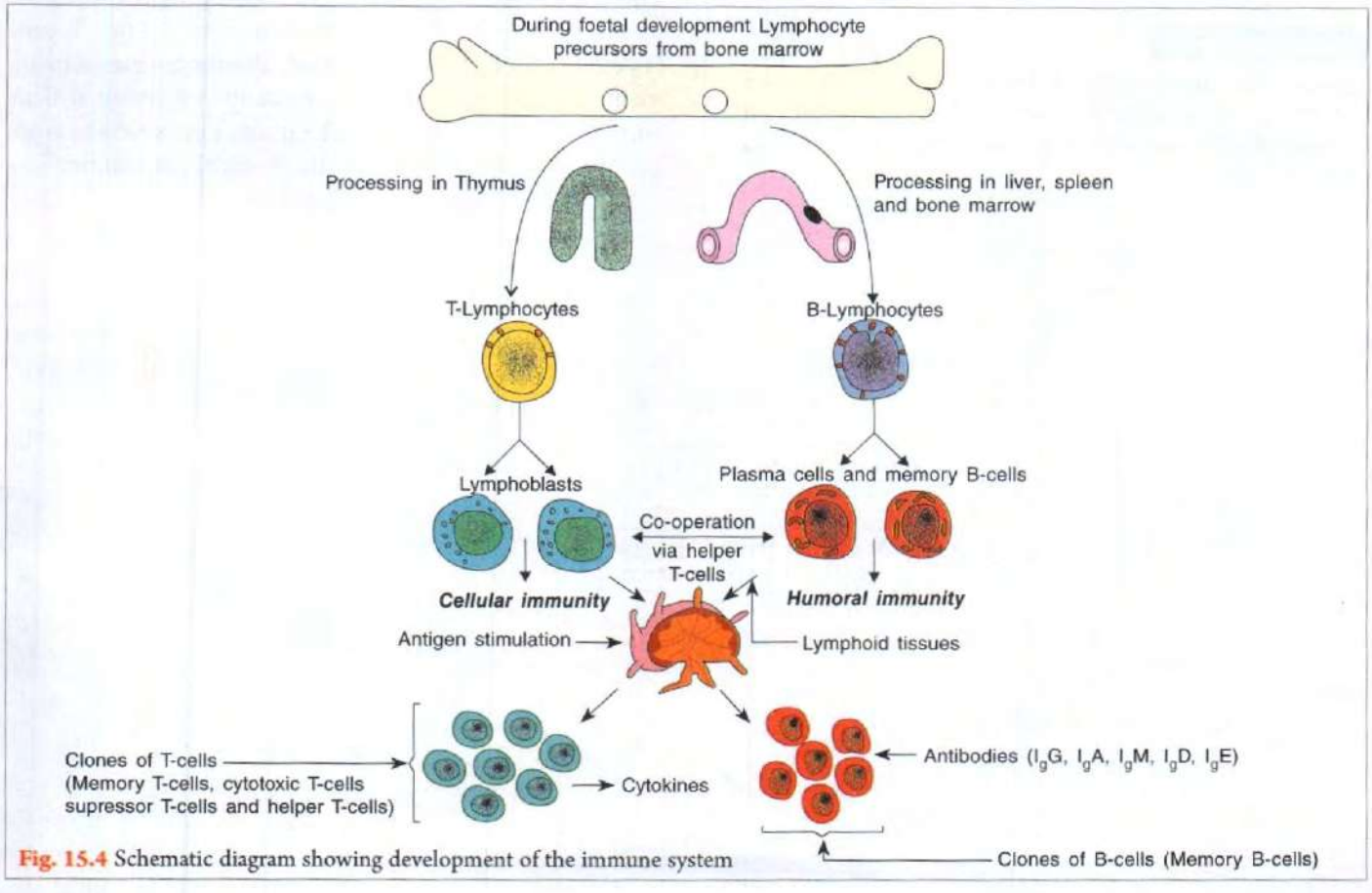


Fig. 15.4 Schematic diagram showing development of the immune system

identified by special techniques. Both types are present throughout life.

2. There is also a slow continuous production of new lymphocytes from stem cells with processing in the bone marrow in adults. When appropriate contact with an antigen occurs, both types of lymphocytes become activated and differentiate further. This takes place in the organs of the immune system such as the lymph nodes and spleen.
3. B-lymphocytes differentiate into *Plasma Cells* (page 116) and *Memory B Cells*. Plasma cells form and secrete protein *immunoglobulin* (IgG, IgA, IgM, IgD and IgE). Similarly, T-lymphocytes differentiate into 4 different varieties of T-cells:
   (i) helper/inducer T cells; (ii) suppressor T cells; (iii) cytotoxic (effector) T cells or killer cells; and (iv) memory T cells
4. *Cytotoxic* and *suppressor T cells* have on their surface the glycoprotein CD8 (*cluster of differentiation*) and are, therefore, called T_8 cells. *'Helper'/'Inducer' T cells* have on their surface the glycoprotein CD4 and are, therefore, called T_4 cells. CD8 and CD4 are *co-receptors* (*i.e.* ***T-cell receptor***) for ***major histocompatibility complex (MHC)*** class I and II molecules respectively. (MHCs are a family of membrane protein complexes encoded by a specific set of genes. Every nucleated cell of the body has MHC on its membrane)

Important Note

There are three types of cytotoxic lymphocytes in the body: αβ T cells, γδ T cells and natural killer cells (page 122). These T-cells are prominent in the mucosa of the GIT.

5. The *Helper and Suppressor T cells* are involved in the regulation (neither excess nor deficient) of antibodies production by B-lymphocytes. There are two types of helper T cells:
   (i) ***T helper 1 (T_H 1) cells*** secrete IL-2 and γ-interferon (concerned with cellular immunity).
   (ii) ***T helper 2 (T_H 2) cells*** secrete IL-4 and IL-5 (concerned with humoral immunity) (also refer to **Table 15.3**, page 127).
6. *The Cytotoxic T-cells* are responsible for *delayed allergic reactions*, rejection of transplant of foreign tissues and lysis of tumour cells. They combine directly with target cells that have the antigen which initially stimulated them to bring about their destruction by inserting perforins and by initiating apoptosis.
7. *Memory B and T Cells* are cells that have been exposed to an antigen and are readily converted to *effector cells* by a later encounter with the same antigen. Unlike other lymphocytes, they persist in the body for months or even years/life long, such as immunity to measles.

A. Acquired Humoral Responses

When an antigen *e.g.* bacteria or other foreign proteins enters the body, it is ingested by macrophages and partially digested. Antigen peptide fragments then combine with MHC-II and move to the cell surface. This processed antigen with the macrophage then binds to *receptors* on T-Lymphocytes activating helper T cells (T_4 cells). The T_4 cells then activate B-lymphocytes causing them to proliferate and transform into *Memory B Cells* and *Plasma Cells* **(Fig. 15.5)**. The plasma cells secrete large quantities of antibodies into the general circulation. The

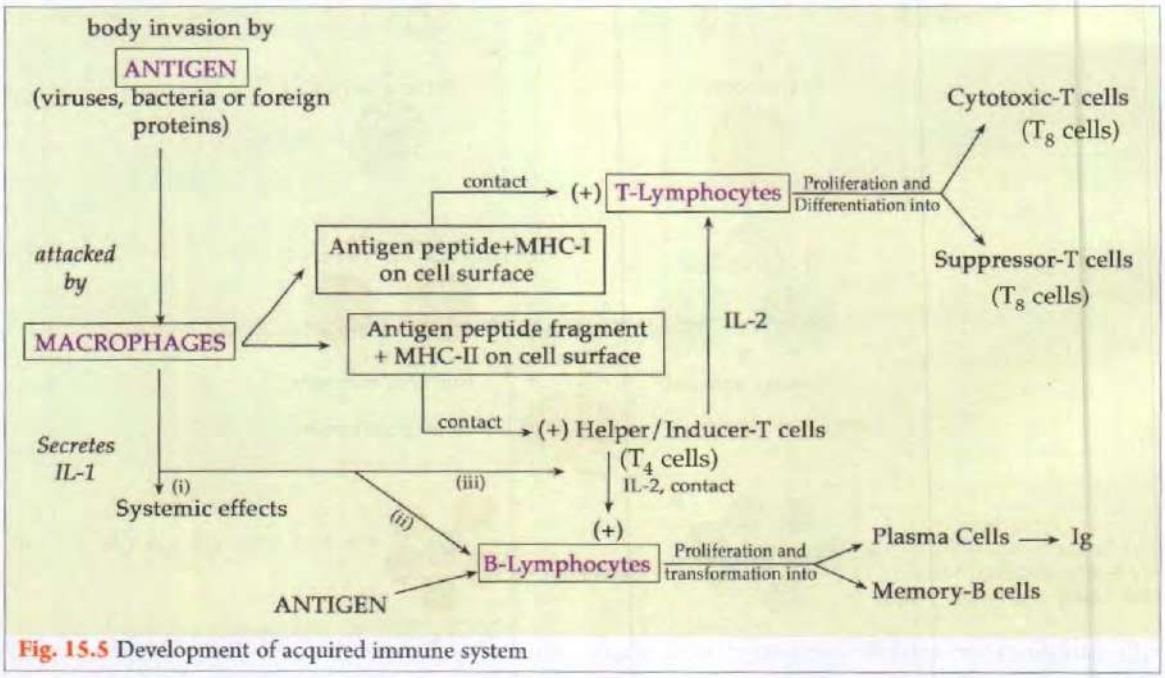


Fig. 15.5 Development of acquired immune system

antibodies circulate in the γ-globulin fraction of the plasma and are, therefore, called ***Immunoglobulins***. For efficient production of antibodies against proteins or polypeptides, B-lymphocytes require contact with helper/inducer T_4 cells. Macrophages by liberating IL-1 cause further activation of helper T_4 cells and B-lymphocytes.

Antigens can also be processed and presented to T_4 cells by:

(1) other types of antigen-presenting cells which include the B-lymphocytes themselves;
(2) the Langerhans cells of the skin, and
(3) specialized cells – dendritic cells in the lymph nodes and spleen.

Primary and Secondary Antibody Responses

When antigens *e.g.* microorganisms, are encountered for the first time there is a ***Primary response*** in which a low level of antibodies can be detected in the blood after about 2 weeks. Although the response may be sufficient to limit the antigen, the antibody level then fall. The second exposure to the *same* antigen produces a ***Secondary response*** in which there is a rapid response by memory B-cells resulting in a marked increase in antibody production and it declines more slowly **(Fig. 15.6)**.

The secondary response may overcome a potential pathogen before it can cause the symptoms of infection *i.e. it provides acquired antibody mediated immunity.* This principle is used in active immunization against infectious diseases.

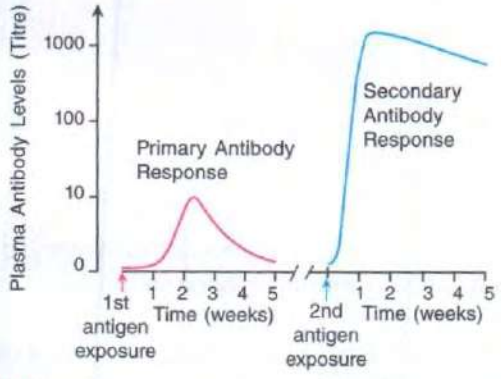


Fig. 15.6 Primary and secondary antibody response to a given antigen.

How Body Recognises so many Different Antigens? Clonal Selection Theory (Burnet, F.M. 1969)

The number of different antigens recognized by lymphocytes in the body is extremely large and develops without exposure to the antigen.

Stem cells differentiate into many million different T and B lymphocytes, each with the ability to respond to a particular antigen. When the antigen first enters the body, it is processed by antigen-binding cells and binds to the appropriate lymphocytes by the process described above. These cells are stimulated to divide, forming *clones of cells* that respond to this antigen (***clonal selection***). A clone is the population of cells descended by asexual reproduction from a single cell.

Immunoglobulins (Ig)

Structure

1. Immunogobulins contains 4 polypeptide chains, linked by disulphide bonds. There are two identical low molecular weight chains (*light chains*) and two identical high molecular weight chains (*heavy chains*). **(Fig. 15.7)**

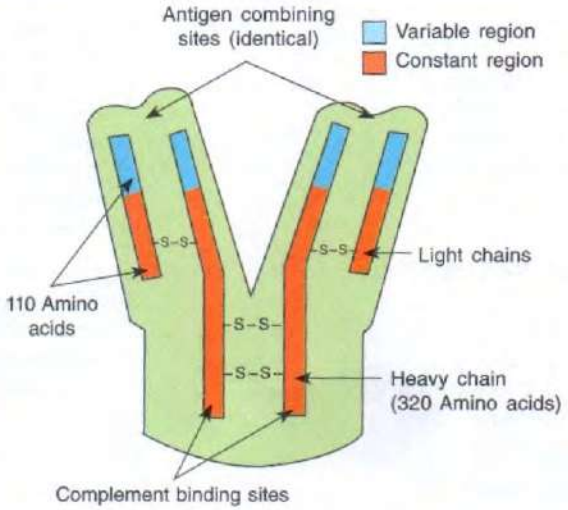


Fig. 15.7 Structure of an immunoglobulin molecule

2. Both the heavy and light chains are divided into 'constant' and 'variable' regions.
3. The amino-acid sequences in the 'constant' regions of the different Immunoglobulins are very similar. The 'constant' regions are involved in the effector functions of antibodies.
4. The arrangements of the amino acids in the 'variable' regions are distinct for each immunoglobulin. This variability allows for a vast number of unique antibodies each of which binds with specific affinities for different antigens.

Types

Immunoglobulins can be divided into 5 classes based on differences in the 'constant' regions of their heavy chains. There are five types of heavy chains γ (gamma), α (alpha), μ (mu), δ (delta) and ε (epsilon); and these give rise to the five classes of immunoglobulins, IgG, IgA, IgM, IgD and IgE respectively. Each of these classes has different effector functions as shown in **Tables 15.2** and **15.3**.

Table 15.2: The major Immunoglobulins (Ig) with their characteristic functions

Type of Ig	Functions
1. **IgG** (i) 71% (ii) 0.8–1.6 gm/dL (Av.1.3 gm/dL) (iii) 1,60,000 (iv) γ (gamma) (v) κ (kappa) or λ (lamda)	(a) Antibodies of *secondary immune response*, because get stimulated by natural infection or artificial immunization with viruses or bacteria, therefore, produce major antiviral, antibacterial and antitoxin activity in serum. (b) These antibodies are distributed equally between blood and tissue fluids. (c) They *cross the placenta* from mother to foetus by active transport *e.g.* antibodies of Rh blood group system and are found in milk, saliva, nasal and bronchial secretion. (d) They can serve as *opsonin and promote chemostactic activity* of WBC's
2. **IgA** (i) 22% (ii) 140-420 mg/dL (Av. 250 mg/dL) (iii) 1,70,000 (iv) α (alpha) (v) κ or λ	(a) Occur in plasma and are also secreted in tears, saliva, intestinal juices, respiratory secretions and colostrum. (b) They *can't cross the placenta.* (c) Lyse bacteria in the presence of lysozyme, which also occurs in the secretions containing IgA, thereby protect mucous surfaces *i.e. provide localized protection.*
3. **IgM** (i) 7% (ii) 50-200 mg/dL (Av. 120 mg/dL) (iii) 1,000,000 (iv) μ (mu) (v) κ or λ	(a) Because of their large size they are predominantly intravascular and *produced in primary immune response e.g.* α, β antibodies of ABO blood groups. (b) Each IgM molecule possesses at least 5 identical combing sites, therefore, these antibodies can adhere to surfaces of cells with large numbers of similar antigenic sites. (c) Activate *complement system*, promote phagocytosis and cause cell lysis by digesting holes in the cell membrane at the site of antibody attachment. (d) More effective than IgG antibodies in lysing cells.
4. **IgE: Reagins** (i) Traces (ii) 0.03 mg/dL (iii) 1,85,000 (iv) ε (epsilon) (v) κ or λ	(a) Heat labile, skin sensitizing antibodies, therefore, also called *reagins.* (b) When specific antigen is brought into contact with IgE-coated mast cells (page 100) or blood basophils; these cells undergo degranulation with release of chemical mediators and E.C.F.-A (page 84). Thus play important role in allergies, parasitic infestations and anaphylactic type of *immediate hypersensitivity disorders.*
5. **IgD** (i) Traces (ii) 3 mg/dL (iii) 1,50,000 (iv) δ (delta) (v) κ or λ	Present on the surface of B-lymphocytes with IgM, therefore, *involved in antigen recognition.*

(i) Percentage of total Ig (ii) Normal plasma level (iii) Molecular weight (MW) (iv) Heavy chain (v) Light chain

There are two types of 'light' chains, κ (kappa) and λ (lamda). Both light chains in any antibody are either κ or λ type.

B. Acquired Cellular Responses

Cellular immunity is mediated by T_8-cells. These cells are activated:

(i) when they are presented with antigens and MHC-I proteins on the surfaces of antigen-presenting cells;

(ii) when exposed to interleukin-2 (IL-2).

T-lymphocytes proliferate and differentiate into cytotoxic-T cells in about 2 weeks time **(Fig 15.5)**. T-cells attack and destroy cells that have the antigen which activated them *i.e.* highly specific immune reaction. They kill by inserting pore forming molecules (*perforins*) in the membranes of their target cells.

How is the actual killing of the antigen brought about?

Lymphocytes, macrophages and other lymphoid tissue cells involved in immune responses communicate as follows:

1. In part by hormones like chemical messengers called *Interleukins* (IL) and *cytokines;*
2. In part by *the complement system* (page 121); and
3. By *Natural killer cells* (page 122).

Role of *Interleukins* (IL) and *Cytokines:* (pages 66 and 127)

REGULATION OF IMMUNE RESPONSE

A. Local Factors

1. *The Antigen:* As long as the antigen persists, the response continues.
2. *The Response:* The 2 major immune systems *i.e.* humoral and cellular, regulate their own responses through

Table 15.3: Principal cytokines and their functions

	Cytokines	Sources	Principal Actions
1.	IL-1 (α and β)	Macrophage, T-lymphocyte, keratinocytes, glial cells etc.	α and β act on same receptor, many of the effects are those that are seen at the start of an infection. (i) CNS: fever, anorexia (ii) Metabolic: ↑ protein synthesis, ↑ Na^+ excretion (iii) Blood: (a) ↑ secretion of colony stimulating factors (b) ↑s count of all blood cells except Lymphocytes (iv) CVS: ↑s capillary permeability, ↑s WBCs adherence, hypertension.
2.	IL-2	T_4-Lymphocytes	Proliferation, *i.e.* ↑ synthesis and maturation of T_8 and B-Lymphocytes. Also activate N-K cells (page 122)
3.	IL-3	T-Lymphocytes	↑s secretion of 'colony stimulating factors' and count of all blood cells except Lymphocytes
4.	IL-4	T4-Lymphocytes	Differentiation of B-lymphocytes, basophils.
5.	IL-5	T-Lymphocytes	B-cell activation; eosinophil differentiation.
6.	IL-6	Fibroblasts, tumour cells, macrophages	↑s synthesis and secretion of immunoglobulins by B-lymphocytes
7.	IL-7	Bone marrow cells	Proliferation of T and B lymphocytes.
8.	Tumour Necrosis factor (TNF)	Macrophages, Mast cells	Lysis of bone; fever, hemorrhagic necrosis in tumours.
9.	α-interferon	Leukocytes	Antiviral; antiproliferative, induces MHC-I antigens on lymphocytes; ↑s NK cell activity (therefore, may be of value in treatment of cancer).
10.	β-interferon	Fibroblasts	Antiviral; anti-proliferative.
11.	γ-interferon	T-Lymphocytes, NK cells	Activates macrophages; induces MHC-II antigens on macrophages.
12.	Platelet derived growth factor (PDGF)	Platelets, macrophages, endothelial cells	Mitogen for vascular smooth muscle; faster wound healing.
13.	Platelet-activating factor (PAF)	Neutrophils, monocytes, platelets	Platelet aggregation; inflammation; ↑s chemotactic activity; bronchoconstriction; ↑s capillary permeability.

↑s: increases

(IL = Interleukins, also refer to page 66)

feedback mechanisms, *e.g.* IgM antibodies which appear during humoral immune response exert 'negative feedback', thus preventing uncontrolled population of one type of antibodies far in excess of requirements.

3. *Suppressor T cells,* which develop more slowly than cytotoxic T cells, help terminate the immune response by dampening the immune responses of T and B cells. This includes turning off the helper/inducer cells.

B. General Factors

1. Effect of hormones on immune response

Inhibit	*Stimulate*
(i) Glucocorticoids	(i) Growth hormone
(ii) Oestrogens	(ii) Thyroxine
(iii) Androgens	(iii) Insulin
(iv) Progesterone	

2. Genetic factors: Some individuals are more susceptible to infections. This may be due to a genetic tendency for poor immune response.

IMMUNOLOGICAL TOLERANCE: RECOGNITION OF SELF

Why animals do not usually make an immunological response to their own proteins or tissue cells, although these are excellent antigens in other species?

The capacity to make an immunological response to foreign antigen develops late in foetal life or even after birth. All potential antigens with which the cells are in contact during the period of immunological immaturity are recognized as ***self*** while materials with which first contact is made after this period are recognized as ***Not self*** and will evoke an immunological response.

The ability to recognise *self* is due to the following mechanisms:

1. **Clonal Anergy** *i.e.* mechanism preventing destruction of *self*. When B and T lymphocytes (precursors of humoral and cellular immunity) in foetal life are exposed to potentially antigenic materials in the tissues, they are subsequently unable to make a specific immune response to these materials *i.e.* B and T lymphocytes enter a prolonged hyporesponsive state *(Clonal Anergy or Immunological Silence)*.
   Thus, all potentially antigenic material encountered in foetal life, whether *self* or *not self* elicit no response either then or subsequently at least as long as it persists in the tissues.
2. **Clonal Abortion** (or *Negative selection*)*i.e.* probably many of the *not self* (anti-self) T-lymphocytes are eliminated in the thymus during their early development.
3. Suppressor T cells keep the development of *not self* antibodies in check.
4. In a few tissues *e.g.* the lens of the eye, *self* antigens are almost isolated from the cells of the immune system.

Applied

However, immune tolerance sometimes fails and allows the production of antibodies against or sensitization by products of animal's own tissues. This is called *Auto-Immunization* and *Auto-Sensitization*. These antibodies are called *Auto-antibodies*. How?

Auto-immunization might occur when new antigenic materials are formed at any time after the period of immunological immaturity. Some potential antigens are *anatomically segregated* so that there is normally a barrier between them and immunologically competent cells. A breakdown of this barrier at any time after early infancy leads to *auto-antibody* formation (see below).

Deficiency of suppressor T cells is probably the cause of ***auto-immune diseases.***

Auto-immune diseases	Auto-antibodies are formed against
1. Insulin dependent (Type I) diabetes mellitus	Pancreatic islet β-cells
2. Myasthenia gravis	Nicotinic cholinergic receptors
3. Multiple sclerosis	Myelin basic proteins
4. Hashimoto's thyroiditis	Thyroid gland cells
5. Grave's disease (hyperthyroidism)	TSH receptors
6. Rheumatoid arthritis	Collagen tissues

TISSUE TRANSPLANT/GRAFT

TYPES OF GRAFTS (Fig. 15.8)

1. *Homograft (Allograft)* – graft from one person to another.
2. *Autograft* – graft from one site to another in the same person.
3. *Heterograft (xenograft)* – graft from one animal species to another.

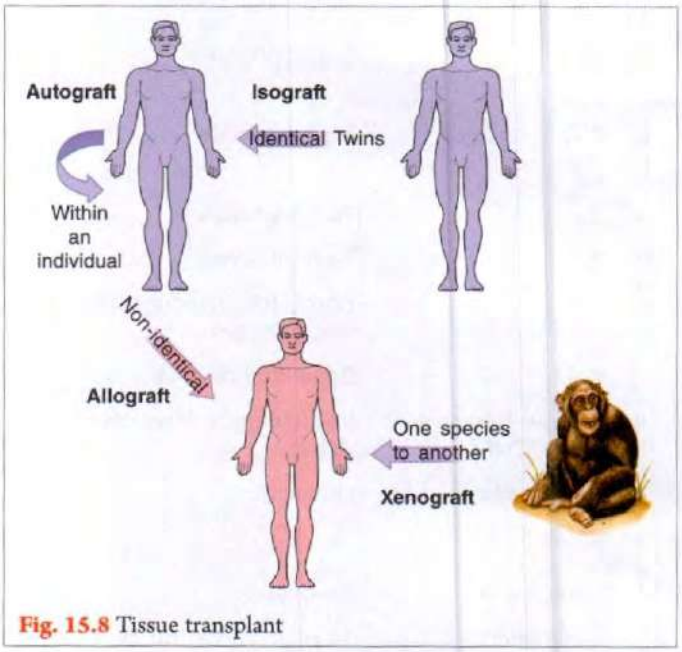


Fig. 15.8 Tissue transplant

When tissues such as skin, kidneys or heart are transplated from a donor to a recipient of the same species, the transplants "take" and function for the first few days but after a week or so they become necrotic and are "rejected" because the recipient develops an immune response to the transplanted tissue. The T-Lymphocyte system is responsible for the rejection of the transplanted tissue. How?

Some of the T-lymphocytes possess recognition sites for foreign transplantation antigens on cell surfaces; contact with antigen causes lymphocytes to become large 'pyroninophilic' cells (page 115) which divide and form daughter lymphocytes with similar recognition site (this occurs in regional lymph nodes). These immunologically activated small lymphocytes leave the lymphoid tissue via the efferent lymph and enter the blood. When they reach the transplanted tissue they react with it, reducing its blood supply and bring about its destruction ***(Homograft Reaction)***. **(Fig. 15.9)**

After first graft has been rejected, if a second graft from the same donor is applied, the tissue transplant rejection is greatly speeded up and the graft is "sloughed off" in 3-4 days. This *second set reaction* is due to previous immunization and is associated with rapid invasion

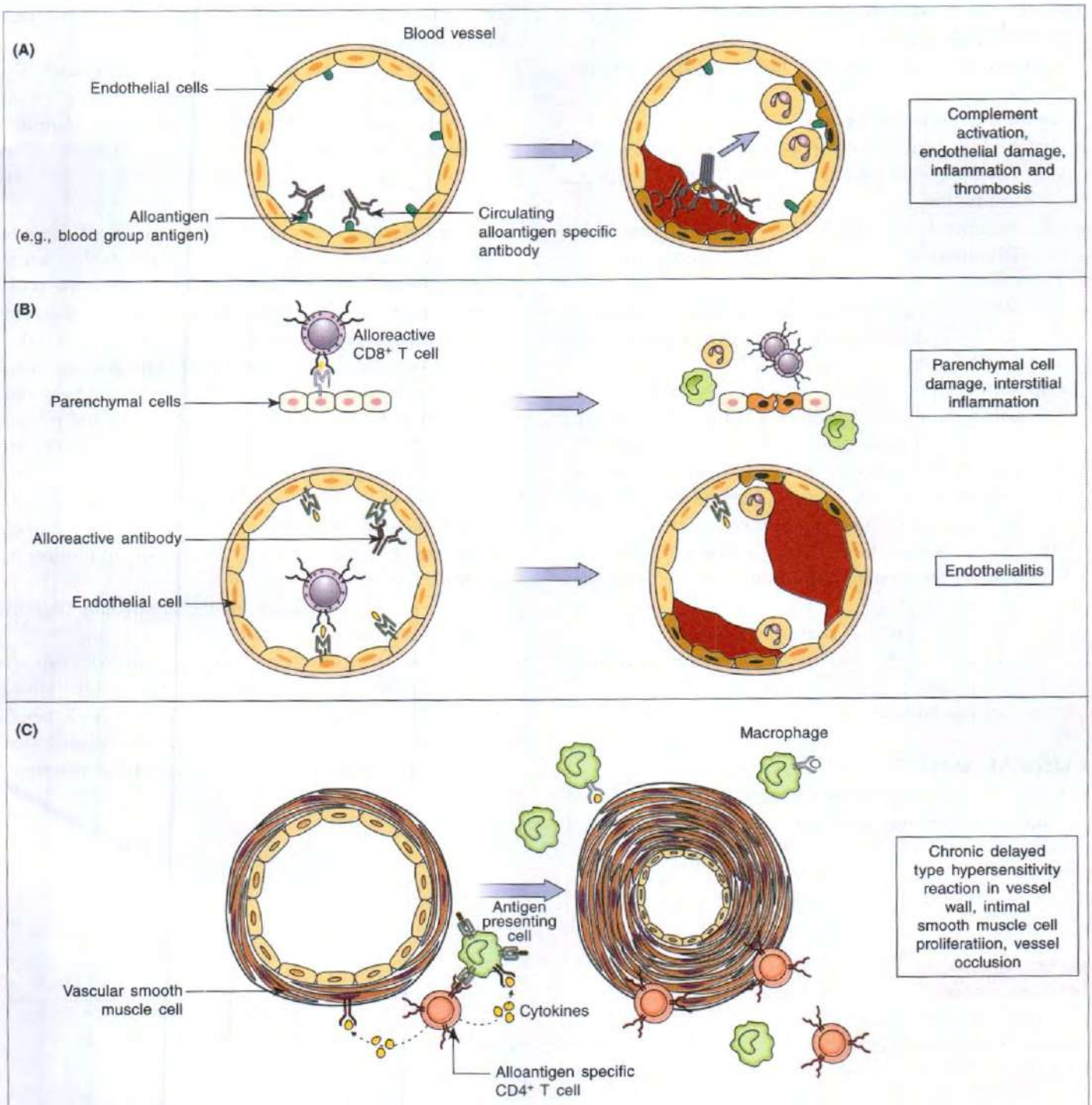


Fig. 15.9 Graft rejection: (A) Hyperacute rejection; (B) Acute rejection; (C) Chronic rejection

by lymphocytes, plasma cells and polymorphs. This is generally true even if the donor and recipient are close relatives; and the only transplants that are never rejected are those from an identical twin.

Note

Individuals with same genetic constitution are called syngeneic, those with different genetic constitution are called allogeneic.

Biological significance of homograft reaction lies in its ability to reject occasional mutant cells formed during the normal course of cell division in the body. Rejection of mutant cells may be an important process for the preservation of an unchanged genetic constitution.

Important Note

Probably cellular immunity mechanism declines with age, which accounts for increased incidences of tumours with advancing age.

PREVENTION OF THE REJECTION OF TISSUE TRANSPLANT

1. If donor and recipient are identical (homozygotic) twins.
2. ***Immune-suppressive agents:***
   (i) X-rays irradiation to destroy most of T-lymphocytes producing tissues *i.e.* total lymphoid irradiation.
   (ii) Antimetabolic drugs *e.g.* Azathioprine kills T-lymphocytes by killing all rapidly dividing cells.
   (iii) Steroids or glucocorticoids, inhibit production of IL-2 by T_4 cells, thereby inhibiting cytotoxic T cells proliferation.
   (iv) Anti-lymphocytic serum (ALS) or anti-lymphocytic globulin (ALG) *i.e.* antibodies against T-lymphocytes. These are prepared by injection of lymphocytes of one species into an animal of another species, prevent lymphocytes from participating in immune reaction.
   (v) Drugs which bind with immuno-suppressant binding proteins (immunophilins) in the cytoplasm of lymphocytes, *e.g.*
      (a) Cyclosporin A (a fungi extract)
      (b) FK-506 (a macrolide antibiotic of fungal origin)
      (c) Rapamycin

CLINICAL ASPECT

1. Failure of the lymphocyte precursors to develop or to migrate to thymus, liver and spleen causes absence of cellular and humoral immunity, with marked susceptibility to infections.
2. *DiGeorge Syndrome* – disorder characterized by congenital absence of thymus, thymic hypoplasia, as a result cellular immunity is absent but humoral immunity is present.
3. *Agamma-globulinaemia or hypogamma-globulinaemia*
   Congenital disorder due to abnormality of Helper and Suppressor T cells, therefore, there is deficiency of peripheral lymphoid tissue and B-lymphocyte with absence of plasma cells and marked reduction of serum IgG levels. As a result, after maternal antibodies (IgG) have disappeared from the blood (by 3-4 months), children with this condition are susceptible to bacterial infections but are relatively resistant to viral and fungal diseases. This shows that cellular immunity brought by T-lymphocytes is normal.
4. Most cases of *chronic lymphatic leukaemia* are due to uncontrolled proliferation of B-lymphocytes; and that of ***Acute lymphocytic leukaemia*** are due to T-lymphocyte malignancies.
5. *Multiple myeloma* is due to malignant proliferation of clones of mature plasma cells.
6. *Acquired immuno deficiency syndrome* ***(AIDS)*** is caused by HIV (human immuno-deficiency virus). HIV binds to CD4 ⟶ decrease or loss of helper T cells (T_4 cells) ⟶ failure of proliferation of T_8 and B-lymphocytes ⟶ loss of immune functions and death from infections or cancer.

Study Questions

1. **Write short notes on:**
   (i) Complement system
   (ii) NK cells
   (iii) Interferons
   (iv) Non-T, Non-B lymphocytes
   (v) Perforins
   (vi) Plasma cells and Immunoglobulins
   (vii) Reagins
   (viii) Immunological silence
   (ix) Helper and suppressor T-cells
   (x) Immunologically competent lymphocytes
   (xi) Major histocompatibility complex (MHC)
   (xii) Cytotoxic and memory T-cells
2. **How the foreign substances entering the circulation are dealt with?**
3. **Mention the role of thymus in development of immunologically competent lymphocytes.**
4. **Give the mechanism by which body recognizes so many different antigens.**
5. **How immune response is regulated? Why we do not make an immunological response to our own body proteins? Explain.**
6. **Define the term auto-immunization. Name some auto-immune diseases and give their physiological basis.**
7. **Explain: increase incidences of tumours with advancing age.**
8. **What is AIDS? How it is caused? Which immune system gets affected with this disease?**

9. **Draw labelled diagram to depict:**
   (i) Development of immune system
   (ii) Acquired immune system
   (iii) Primary and secondary immune respponse to a given antigen
10. **List major immunoglobins. Give their characteristic functions.**

MCQs

1. **Humoral responses of natural immune system include:**
   (a) Neutrophil (b) Complement system (c) Macrophages (d) B and T-lymphocytes
2. **Natural cell mediated responses involve all of the following cells *except*:**
   (a) Neutrophils (b) Monocytes (c) Macrophages (d) T-lymphocytes
3. **C5b of complement complex is known as:**
   (a) Opsonizing complex (b) Lytic complex (c) Chemotaxic complex (d) Agglutinating complex
4. **Interferons act by:**
   (a) Coating the invading antigens, thus facilitate phagocytosis
   (b) Inhibiting protein synthesis thereby inhibit viruses replication
   (c) Lysing the foreign invaders
   (d) Formation of perforins
5. **Which statement is *not true* for natural killer cells:**
   (a) Also called non-T, non-B lymphocytes (b) They kill cells without prior sensitization
   (c) They destroy the cancer cells (d) Forms a major component of acquired immune system
6. **Most T-cell population consists of:**
   (a) Helper (or inducer) T cells (b) Memory T cells
   (c) Suppressor T cells (d) Cytotoxic (or killer) T cells
7. **Helper T_4 lymphocytes cause proliferation of:**
   (a) Neutrophils in bone marrow (b) B-lymphocytes
   (c) Tissue macrophages (d) Plasma cells
8. **Cellular immunity differs from humoral immunity in that:**
   (a) Cellular responses are affected by elements free in the serum or body fluids
   (b) Cellular immunity persists for a longer time
   (c) Cellular immunity activity is increased by interleukins
   (d) Cellular immunity checks the cells that have undergone malignant transformation
9. **Cellular response of acquired immune system become fully functional after an encounter with an antigen in about ..... time:**
   (a) 1 week (b) 2 weeks (c) 3 weeks (d) 4 weeks
10. **Immunoglobulins that provides localised protection:**
    (a) IgG (b) IgA (c) IgM (d) IgD
11. **Immunity is most suppressed in:**
    (a) Liver failure (b) Patients on ACTH therapy (c) Anaemia (d) Renal failure
12. **Which is *not* an autoimmune disorder?**
    (a) Hashimoto's thyroiditis (b) Grave's disease
    (c) Endemic goitre (d) Myasthenia gravis
13. **Increased incidence of tumours with advancing age is probably due to:**
    (a) Decline in acquired cellular immunity (b) Decline in natural cellular immunity
    (c) Decline in natural humoral immunity (d) Decline in acquired humoral immunity
14. ***True* about AIDS is:**
    (a) Caused by a fungus (b) Associated with increase in number of helper T cells
    (c) Only cellular immunity is lost (d) Both cellular as well as humoral immunity is lost
15. **Perforins are:**
    (a) Phagocytised molecules (b) Pinocytised molecules
    (c) Cell membranes barrier (d) Pore forming molecules
16. **Mast cell and basophil activation with amplification of inflammatory response is brought about by:**
    (a) C3a (b) C3b (c) C6 (d) C7

17. *True* about acquired humoral immunity is:
    (a) Constitute a major defence against infections due to viruses
    (b) Constitute a major defence against bacterial infections
    (c) Constitute a major defence against fungi
    (d) It helps defence against tumours

18. Cells previously exposed to an antigen and readily converted to effector cells by a later encounter with the same antigen are:
    (a) Helper T cells (b) Cytotoxic/effector T cells (c) Memory T cells (d) Suppressor T cells

19. Immediate hypersensitivity reaction is due to:
    (a) IgE (b) Activated T cells (c) IgG (d) All of the above

20. Anaphylactic results from liberation of all except:
    (a) Histamine
    (b) Eosinophil chemotactic factor-A
    (c) Interleukins
    (d) Bradykinin

21. Most likely cause of autoimmune diseases is:
    (a) Activation of suppressor T cells
    (b) Activation of memory T cells
    (c) Deficiency of suppressor T cells
    (d) Deficiency of memory T cells

Answers

1. (b) 2. (d) 3. (c) 4. (b) 5. (d) 6. (a) 7. (b) 8. (b) 9. (b) 10. (b)
11. (b) 12. (c) 13. (a) 14. (d) 15. (d) 16. (a) 17. (b) 18. (c) 19. (a) 20. (c)
21. (c)

Unit III

NERVE MUSCLE PHYSIOLOGY

Chapter 16: Structure and Function of Nervous Tissues

Myelinated and unmyelinated nerves; Myelinogenesis; Glial cell (neuroglia); Neurotrophins: Nerve growth factor; Metabolism in the nerve fibers; Heat production in the nerve fibers.

Chapter 17: Physiological Properties of the Nerve Fibers

Excitability; All or none law; Refractory period; Accommodation; Conductivity (saltatory conduction)

Orthodromic and antidromic conduction

Chapter 18: Nerve Fiber Types and Function

General; Classification; Properties of mixed nerves (compound action potential)

Chapter 19: Degeneration and Regeneration in Peripheral Nerves

Causes and grading of injury; Degenerative and Regenerative changes; Complications

Chapter 20: Neuromuscular Junction (NMJ)

Definition; Structure; Sequence of events at NMJ; Gradation of muscular activity; Miniature and Giant end plate potentials; Clinical importance, Applied (Myasthenia gravis, Lambert Eaton syndrome)

Chapter 21: Skeletal Muscle

Structure; contractile response: Excitation contraction coupling; Steps in muscular contraction and relaxation with molecular basis; Mode of contraction (isometric and isotonic); Motor unit; White (fast) and Red (slow) muscle fibers.

Energy source of muscular contraction.

Chapter 22: Cardiac Muscle

Structure; Properties (morphological, electrical, mechanical, metabolic)

Chapter 23: Smooth Muscle

General features; Single unit and multi-unit smooth muscles; properties of visceral smooth muscles (electrical, mechanical-plasticity); Nerve supply; excitatory junctional potential; Denervation hypersensitivity; Effect of various agents on membrane potential of intestinal smooth muscle.

Chapter 16

Structure and Function of Nervous Tissues

I. Structure of the neuron
II. Functions of the neuron
III. Myelinated and unmyelinated nerves; myelinogenesis
IV. Glial cell (neuroglia)
V. Neurotrophins: Nerve growth factor (NGF)
VI. Metabolism in nerve fibers
VII. Heat production in the nerve fibers

Structural and functional unit of the nervous system is called ***Neuron*** **(Fig. 16.1)**. The term neuron is used to describe the nerve cell and its processes, the *dendrites* and the *axon*. Nerve cell is present in the grey matter while dendrites and the axon are present in the white matter. Neurons vary considerably in shape and size (5 µm to 120 µm in diameter) in different parts of the body. The human nervous system contains approx. 10^{12} neurons.

STRUCTURE OF THE NEURON

A. NERVE CELL BODY (or SOMA or PERIKARYON)

They are of various sizes and forms—stellate, round, pyramidal, fusiform etc. Its principal constituents are similar to a generalised cell. However, after fixation with special stains its cytoplasm also reveals the presence of:

1. Nissl Granules/Bodies

These are basophilic granules/bodies composed of many thin, parallely arranged, membrane bounded cavities or cisternae which are covered by many minute particles consisting of ***Ribose nucleoproteins*** *i.e.* RNA with proteins. Granule's size and number varies with physiological condition of the cell. For *example*, fatigue, certain poisons and sectioning of axon causes Nissl granules to disintegrate into fine dust and which finally disappears (***Chromatolysis***).

2. Neurofibrillae

These are fine threads 6-10 nm in diameter and of variable length. They traverse the cytoplasmic matrix forming a loose framework of fibrils in the cytoplasm.

B. DENDRITES

These are 5-7 processes extending out from the cell body and arborize extensively after they leave the cell. They also contain Nissl granules, mitochondria and neurofibrillae. They are the ***receptive processes*** of the neuron. Impulses can be transmitted from one dendrite to another in the central nervous system (CNS).

C. AXON (AXIS CYLINDER or NERVE FIBER)

It originates from a somewhat thickened area of the cell body called ***Axon Hillock***, in which there are no Nissl granules. The cytoplasmic fluid occupying the centre of the axon is known as ***Axoplasm***. The cell membrane enveloping the cytoplasm is also continued on the axon as ***Axolemma***. A short distance from its origin (***Initial segment of the Axon***), the axon acquires a *sheath of myelin*. Axons vary from a few microns in length to as long as 90 cms. Axon is the single elongated cytoplasmic extension with the specialized function of conducting impulses away from the cell body.

Myelin Sheath is a protein-lipid complex made up of many layers of unit membrane which envelopes the axon *except* at its endings and at periodic constrictions about 1 mm apart called ***Nodes of Ranvier***. Functions of myelin sheath:

(1) Due to high insulating property it confines the nerve impulse to individual fibers and thus prevents cross stimulation of adjacent axons; and
(2) facilitates conduction of nerve impulse (page 138).

Axons (Nerve Fibers) are of 2 types:
(1) *Afferent*: nerve fibers which carry impulses to the CNS.

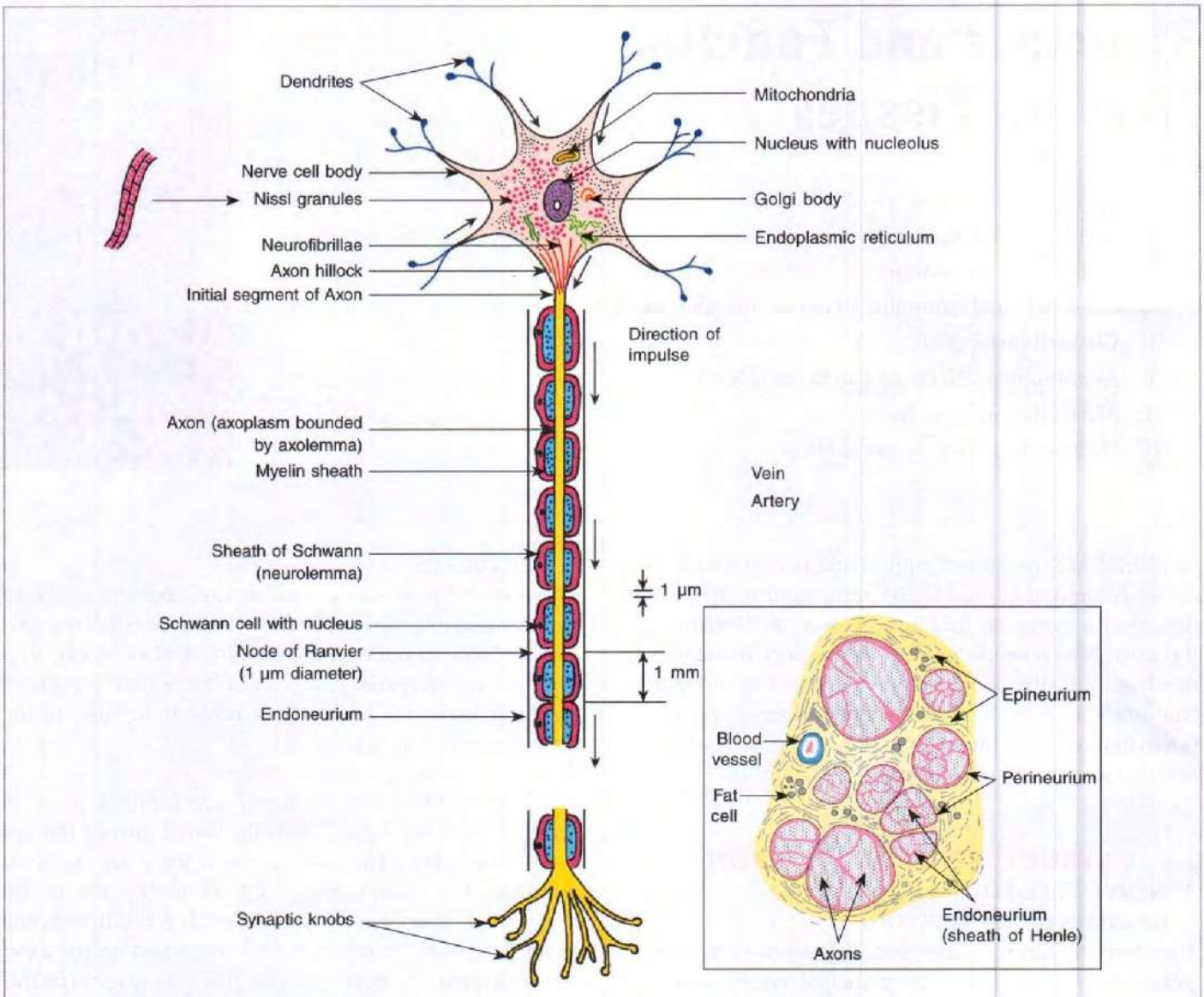


Fig. 16.1 Structure of a typical spinal motor neuron (Inset: A nerve trunk in cross section)

(2) *Efferent*: nerve fibers which convey impulses from the CNS to the periphery.

A mixed nerve contain fibers of both types.

D. SYNAPTIC KNOBS (TERMINAL BUTTONS or AXON TELODENDRIA)

The axon divides into terminal branches, each ending in a number of *synaptic knobs*. They contain granules or vesicles in which synaptic transmitter secreted by the nerve is stored.

Note

Based on the number of processes that originate from their cell body, neurons can be classified as *unipolar* (e.g. cutaneous neurons); *bipolar cell* of retina and *multipolar* e.g. motor neurons of spinal cord, Purkinje cell is cerebellum, Pyramidal cells of hippocarpus. (Fig. 16.2 A)

FUNCTIONS OF THE NEURON

1. *Nerve cell body*

(i) It maintains the functional and anatomical integrity of the axon. *Proof*: If axon is cut, the part distal to the cut degenerates ***(Wallerian Degeneration)*** (page 150). Materials responsible for maintaining integrity of the axon (mostly proteins called ***Neurotrophins***, page 139) are formed in the endoplasmic reticulum of the cell body and then transported down the axon at variable rates (upto 400 mm/day) called *anterograde transport*. There is also a *retrograde transport* at about 200 mm/day of 'nerve growth factor' (page 139) and various 'viruses' from the nerve endings to the cell body.

(ii) The proteins associated with synaptic transmitters are also synthesized in the endoplasmic reticulum of the cell body and are transported to the synaptic knobs by the process of *axoplasmic flow*.

2. *Dendrites:* It is the *receptive* process of the neuron *i.e.* transmits the impulses towards the cell body. Here non-conducted local potential changes generated by synaptic connections are integrated. (Fig. 16.2 B)
3. *Axon*
   (i) Initial segment of the axon: a site where propagated action potentials are generated.
   (ii) An axonal process: transmits propagated impulses away from the cell body to the nerve endings (*All or None transmission*).
4. *Synaptic knobs:* nerve endings where action potentials cause the release of synaptic transmitter.

Important Note

Voltage gated Na^+ channels are highly concentrated in the nodes of Ranvier and the initial segment in myelinated nerve fibers (See Table 16.1).

MYELINATED AND UNMYELINATED NERVES

Within the grey matter the axons are surrounded only by the plasma membrane, but upon leaving the grey substance they acquire myelin sheath. Therefore, a nerve fiber with myelin sheath is called *myelinated* (medullated) nerve fiber. When this fiber comes out of CNS it receives a second covering – the ***Neurolemma*** *i.e.* sheath of Schwann cell (glial like cell). While in *unmyelinated* (unmedullated) nerve fibers, when they emerge out of the grey substance, these fibers remain embedded in the Schwann cell. (Fig. 16.3 and Table 16.1)

Surrounding the 'neurolemma' of myelinated nerve fiber in peripheral nerve trunk is a thin layer of fine reticular fibers which form the ***Endoneurium*** or ***Sheath of Henle***. Bundles (fascicles) of nerve fibers are enclosed in a connective tissue capsule called ***Perineurium***. Number of fascicles are bounded together by connective tissue fibers, called ***Epineurium***. (Fig. 16.1 – inset)

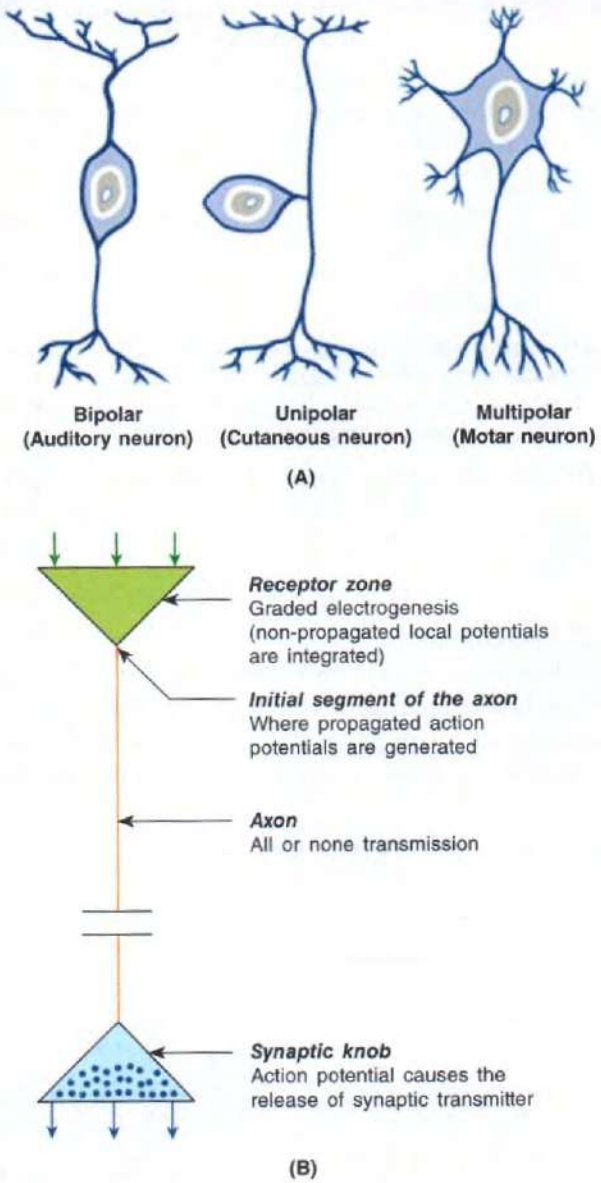


Fig. 16.2 (A) Types of neurons; (B) Functional organization of neurons.

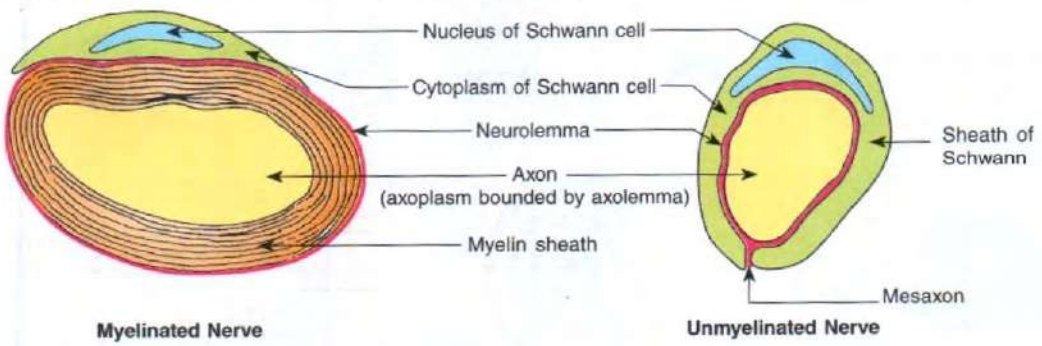


Fig. 16.3 Cross section of myelinated and unmyelinated nerves compared

Table 16.1: Myelinated and Unmyelinated nerves compared

Myelinated Nerves	Unmyelinated Nerves
1. The Schwann cell membrane is coiled many times round the axon forming the multiple layers of membrane that make up myelin.	1. Axons are simply buried (surrounded) in the Schwann cell without wrapping of myelin.
2. Examples: (i) All preganglionic fibers of autonomic nervous system (ANS) (ii) Nerve fibers in somatic nervous system more than 1 μm diameter.	2. Examples: (i) All post-ganglionic fibers of ANS. (ii) Nerve fibers in somatic nervous system less than 1 μm diameter.
3. Conduction of nerve impulse is faster (50-100 times) than the unmyelinated fiber of same diameter because of *Saltatory Conduction i.e.* leaping of impulse from node to node over inter segmental region.	3. Conduction of nerve impulse is slower because it is a continuous process.
4. **Density of voltage gated Na^+ channels:** Highly concentrated, number being 350–500/μm^2 in the initial segment of the axon and 2000–12,000/μm^2 at the nodes of Ranvier.	4. Much less (about 110/μm^2 along the axons)

MYELINOGENESIS

Neurolemma or sheath of Schwann has got a cell, *Schwann cell* which takes part in the deposition of myelin sheath round the axon, a process called *Myelinogenesis* (Fig. 16.4). It begins during second trimester of intrauterine life and is completed by the end of infancy (*i.e.* 1 year after birth). Steps involved during myelinogenesis are:

(1) Myelinogenesis begins with Schwann cell growing round the axon and completely enveloping it all along its width.

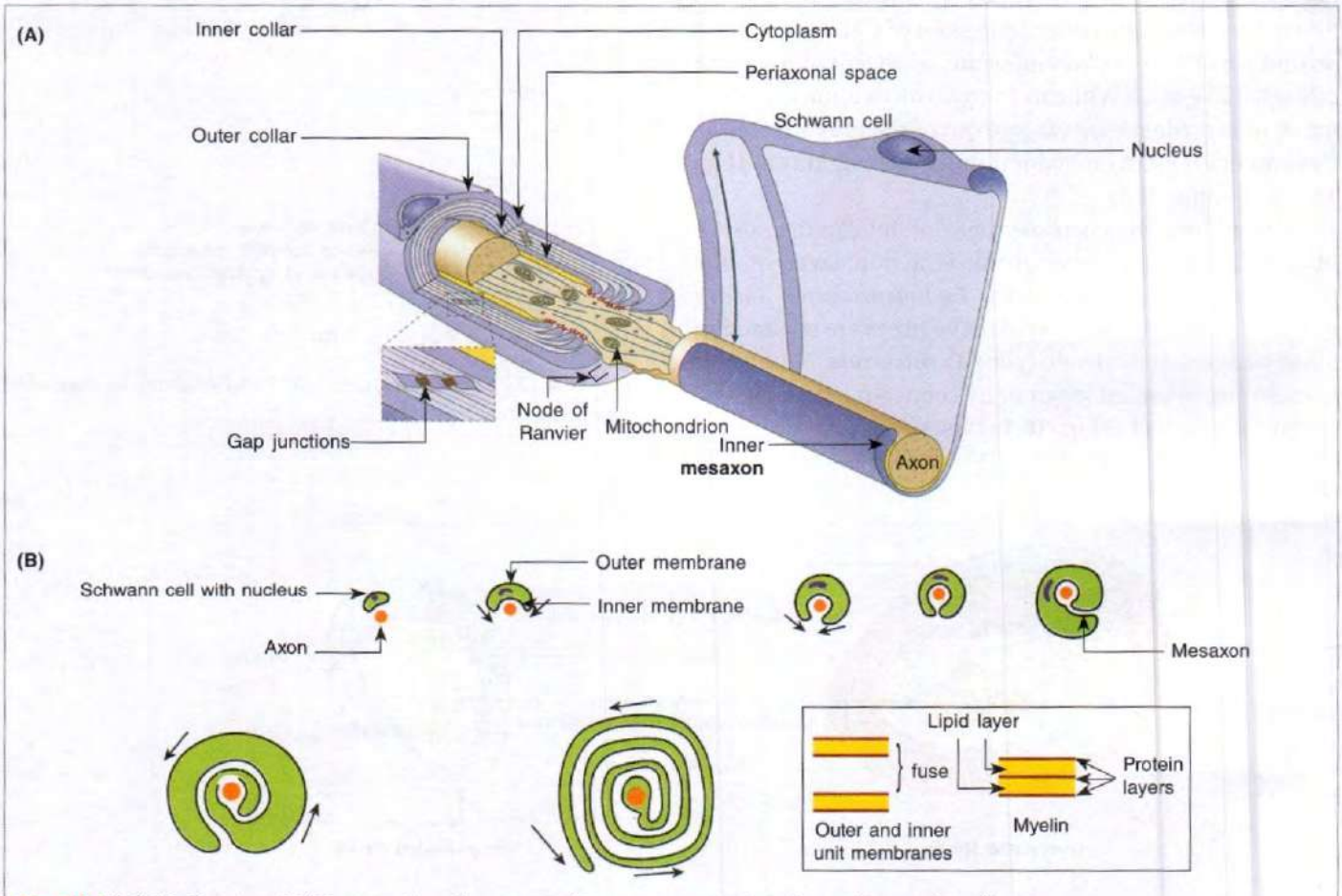


Fig. 16.4 (A) Myelination; (B) Process of myelinogenesis (**Inset**: structure of single myelin membrane)

(2) The cell membrane of the Schwann cell surrounding the axon is connected to the rest of the membrane of the Schwann cell by the double *mesaxon*.
(3) The Schwann cells now begin to rotate and so wrap around the axon as many closely packed helically arranged layers of double membranes.
(4) Each membrane of myelin is composed of two lipid layers sandwiched between layers of protein and they form the ***Myelin Sheath*** of nerve fiber. The thickness of myelin sheath is determined by the number of membrane layers wrapped around the axon.
(5) The *Nodes of Ranvier* which interrupt the myelin sheath at periodic intervals (about 1 mm apart) indicate the junction between adjacent non-syncytial Schwann cells.

Applied

*In **multiple sclerosis**, there is patchy destruction of myelin in the CNS. It is a crippling disease associated with delayed or blocked conduction in the demyelinated axons.* Loss of myelin leads to leakage of K^+ through voltage gated channels, hyperpolarization and failure to conduct action potentials. (For details refer to page 937)

GLIAL CELL (NEUROGLIA)

Glial Cell (Neuroglia) means glue, these are the cells which support the nerve cells. Glial cells are very numerous. There are approx. 10 times as many glial cells as neurons. Unlike the neurons, the glial cells are capable of multiplying by mitosis (This is particularly seen after brain injury e.g. stroke). The Schwann cells that invest the axons in peripheral nerves are also the glial cells. Glial cells are of 3 types: **(Fig. 16.5)**

(1) ***Microglia:*** They are *phagocytic cells* (like macrophages) that come from the bone marrow (*scavenger cells*) and enter the CNS from meninges and blood vessels. They remove damagaed cells and foreign invaders.
(2) ***Astrocytes:*** They are found throughout the brain joining to the blood vessels and investing synaptic structures, neuronal bodies and neuronal processes.

Types:

(i) *Fibrous astrocytes* are found mainly in white matter; and
(ii) *Protoplasmic astrocytes* are found in the grey matter.

Functions:

(i) help in support, transport mechanisms, inflammatory and reparative reactions,
(ii) help forming the blood brain barrier (page 372), and
(iii) help maintain homeostasis in the CNS extracellular fluid by maintaining optimal concentration of ions and neurotransmitters (specially glutamic acid and GABA) in the brain neurons.

(3) ***Oligodendroglia*** *(Oligodendrogliocytes):* These are the cells that form myelin around axon within CNS. The axons in the CNS do not have Schwann cells. Unlike Schwann cells, these cells form myelin on many (1 to 15) neighbouring axons.

Note

Schwann cells, astrocytes and oligodendroglia are together called *Macroglia*.

NEUROTROPHINS: NERVE GROWTH FACTOR (NGF)

Neurotrophins are number of proteins produced by the structures that the neurons innervate and by astrocytes. They bind to *tyrosine kinase associated* ***(trk) receptors*** at the endings of a neuron and are essential for survival and growth of nerves. The established neurotrophins are:

1. Nerve growth factor (NGF)
2. Brain derived neurotrophic factor (BDNF) necessary for the growth and maintenance of cutaneous mechanoreceptors
3. Neurotrophins 3, 4 and 5 (NT-3, 4, 5).

Characteristic features of nerve growth factor

(1) NGF, a protein growth factor (the first neurotrophin isolated, page 136), present in salivary glands, circulation (plasma) and many different tissues.

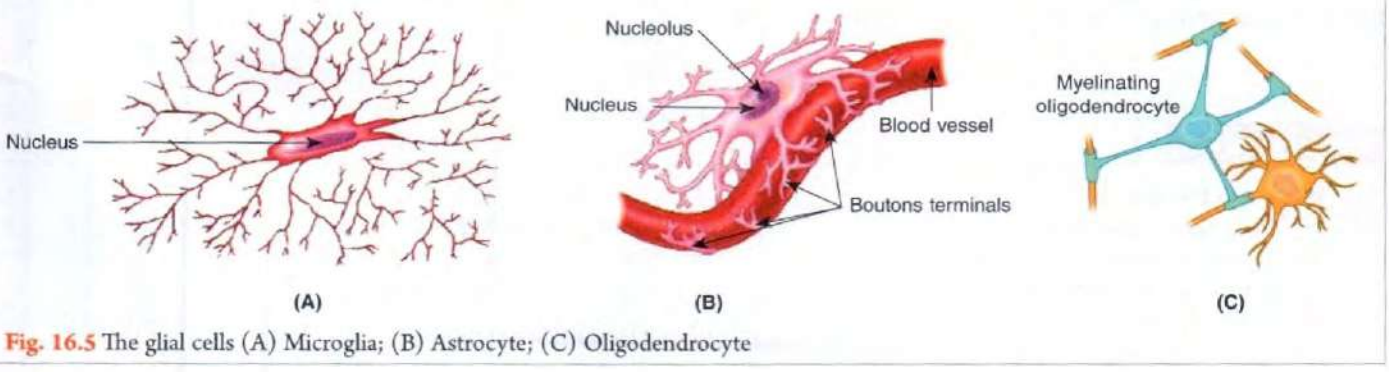


Fig. 16.5 The glial cells (A) Microglia; (B) Astrocyte; (C) Oligodendrocyte

(2) It is made up of 2α, 2β and 2γ sub-units; structure of β unit resembles that of insulin.
(3) *Functions*: It is necessary for the growth and maintenance of sympathetic neurons, some sensory neurons and cholinergic neurons in the brain.
Injection of antiserum against NGF in newborn animals leads to almost total destruction of sympathetic ganglion (***immunosympathectomy***).
(4) It is picked up by neurons in the extracerebral organs they innervate and is transported in retrograde fashion from the endings of the neurons to their cell bodies.
(5) It is also responsible for the growth and maintenance of cholinergic neurons in the basal ganglia.
(6) Other factors that promote the growth of neurons include:
(i) *Ciliary neurotrophic factors* (CNTF) produced by Schwann's cells and astrocytes. It promotes the survival of damaged and embryonic spinal cord neurons.
(ii) *Glial cell line derived neurotrophic factor* (GDNF); maintains midbrain dopaminergic neurons.
(iii) *Leukaemia inhibitory factor* (LIF);
(iv) *Insulin like growth factor I* (IGF-I);
(v) *Transforming growth factor* (TGF);
(vi) *Fibroblast growth factor* (FGF); and
(vii) *Platelet derived growth factor* (PDGF).

(Also refer to note on page 152)

METABOLISM IN THE NERVE FIBERS

Metabolic changes are constantly going on in a nerve fiber at a very low level. About 70% of total energy required is used to maintain polarization of the membrane by the action of Na^+ – K^+ pump. During maximum activity, the metabolic rate of nerve doubles (as compared to skeletal muscle where metabolic rate can increase upto 100 folds).

Characteristic features

(1) Unlike muscles, the excitability, conductivity and recovery processes can go in a nerve for a considerable period even in the absence of oxygen.
(2) Chemical changes in the nerve are roughly of the same nature as seen in the muscles, *i.e.* pyruvic acid is formed and if O_2 supply is insufficient, lactic acid accumulates.
(3) Energy requirement of the resting nerve to maintain polarization of the membrane is supplied by combustion of sugar and phospholipids mainly. During activity ATP and creatine phosphate break down (hydrolysis) and supply energy for the propagation of the nerve impulse.
(4) During activity acetyl choline is liberated by the cholinergic fibers, while epinephrine by the adrenergic fibers.
(5) The nerve fibers are rich in K^+ and vitamin B_1. Vitamin B_1 is essential for complete oxidation of pyruvic and lactic acids.

HEAT PRODUCTION IN THE NERVE FIBERS

As metabolism in the nerve fiber is very low, therefore, during rest a minute quantity of heat is produced which increases during activity.

Heat is evolved in 3 phases:
1. *Resting heat* – while the nerve is inactive.
2. *Initial heat* – during action potential.
3. *Recovery or delayed heat* – that follows the activity.

Initial Heat

1. It is approx. 10% of the total heat.
2. It is anaerobic and coincides with the spike potential.

Cause: Breakdown of ATP and creatine phosphate.

Delayed (Recovery) Heat

1. It is aerobic and is 30 times the initial heat.
2. This energy is used for re-synthesis of ATP and creatine phosphate and as such for restoring the normal excitability of the nerve fiber.
3. It comes in 2 stages:
(i) 1st stage – lasts for few seconds and the quantity of heat is small – approx. same as the initial heat.
(ii) 2nd stage – lasts for 10-30 minutes and contributes the greatest proportion of both total and delayed heat.

Study Questions

1. **Write short notes on:**
(i) Wallerian degeneration
(ii) Saltatory conduction
(iii) Multiple sclerosis
(iv) Types of neuroglia and their functions
(v) Functions of neurotrophins
(vi) Nerve growth factor
(vii) Myelinogenesis

2. **Depict diagrammatically**
   (i) functional organization of a neuron (ii) Structure of a neuron
3. **Give an account of:**
   (i) Myelinated and unmyelinted nerve fibres (ii) Nerve cells and glial cells

MCQs

1. **Chromatolysis is seen in:**
   (a) Demyelination (b) Multiple sclerosis (c) Section of axon (d) All of the above
2. **Material responsible for maintaining integrity of the axon is:**
   (a) RNA (b) DNA (c) Neurotrophins (d) Lipoprotein complexes
3. **Large myelinated nerve fibers *differ* from unmyelinated fibers in that former are characterized by:**
   (a) Saltatory conduction
   (b) Axons are burried in the Schwann cells without wrapping of myelin
   (c) Conduction is a continuous process
   (d) Examples include all postganglionic fibers of ANS
4. **Which of the following is an example of an unmyelinated neuron?**
   (a) Anterior horn cell (b) Preganglionic parasympathetic neuron
   (c) Preganglionic sympathetic neuron (d) Postganglionic parasympathetic neuron
5. **Development of myelin sheath in peripheral nervous system depends on:**
   (a) Oligodendrocytes (b) Astrocytes (c) Microglia (d) Schwann cell
6. ***Not true* about myelinogenesis:**
   (a) Process of deposition of myelin sheath around the axon
   (b) Begins 1 year after birth
   (c) In peripheral nerves, Schwann cells are responsible to carry out this function
   (d) Oligodendroglia are responsible for it within the CNS
7. **Myelin is composed of:**
   (a) A double layer of lipid molecules with proteins embedded in it
   (b) Two lipid layers sandwiched between three protein layers
   (c) Many layers of unit membranes
   (d) A single layer of cell membrane
8. **Which is *not* a glial cell?**
   (a) Schwann cell (b) Microglia (c) Astrocyte (d) Oligodendroglia
9. ***Not true* of an astrocyte:**
   (a) Found throughout the brain joined to the blood vessels
   (b) Helps forming blood brain barrier
   (c) Forms myelin around the axons within CNS
   (d) Helps in maintaining optimal concentration of ions in the brain neurons
10. **Substance necessary for complete oxidation of pyruvic and lactic acids in a nerve:**
    (a) Vitamin B_1 (b) Vitamin B_6 (c) Vitamin B_{12} (d) All of the above
11. **Which part of a neuron has the highest concentration of Na^+ channel:**
    (a) Dendrites (b) Cell body (c) Initial segment (d) Synaptic knob
12. **Nodes of Ranvier are about ..... mm apart:**
    (a) 0.01 (b) 0.1 (c) 1.0 (d) 2.5
13. **Proteins associated with synaptic transmitters are synthesized in:**
    (a) Nerve fiber (b) Endoplasmic reticulum of cell body
    (c) Dendrites (d) Synaptic vesicles
14. ***False* about dendrites is:**
    (a) There are 5-7 elongated cytoplasmic extensions from the cell body
    (b) Receptive process of the neuron
    (c) Here non-propagated local poten-tials are integrated
    (d) Local potentials generated here obey all or none law

15. **Most sensitive part of axon is:**
    (a) Dendrite
    (b) Soma
    (c) Axon tip
    (d) Initial segment of axon

16. **In multiple sclerosis:**
    (a) There is excessive deposition of myelin round the axons
    (b) Thickness of myelin increases
    (c) There is patchy destruction of myelin
    (d) It is associated with increased conduction in the axons

Answers

1. (c)	2. (c)	3. (a)	4. (d)	5. (d)	6. (b)	7. (b)	8. (a)	9. (c)	10. (a)
11. (c)	12. (c)	13. (b)	14. (d)	15. (d)	16. (c)				

Chapter 17

Physiological Properties of the Nerve Fibers

I. Excitability
II. All or none law
III. Refractory period
IV. Accommodation
V. Conductivity

The basic properties of nerve fibers are essentially similar to the properties of action potential generated in any excitable tissue (page 40) *viz. Excitability; All or None Law; Refractory period; Accommodation and Conductivity.*

EXCITABILITY

It is the property by virtue of which various cells respond to changes in the external or internal environments. It is due to the disturbances in the ionic equilibrium across the receptive zone of cell membrane.

The nerve fiber can be stimulated by a suitable stimulus, which may be *mechanical, thermal, chemical or electrical.* In experiments, 'electrical' stimulus is usually employed, because its strength and frequency can be accurately controlled. The production of a *wave of depolarization, called* ***excitation (or activation) impulse*** will show that a nerve has been excited.

Factors affecting the excitability

(1) Strength and duration of the stimulus *i.e. Strength-duration curve* (page 40).
(2) Effect of extracellular Ca^{2+}
   (i) *Decrease in ECF* $[Ca^{2+}]$ increases excitability of nerve and muscle cell by decreasing the amount of depolarization necessary to initiate the changes in the Na^+ and K^+ permeability that produces the action potential.

Note

Decrease in plasma $[Ca^{2+}]$ increases membrane permeability to Na^+.

   (ii) *Increase in ECF* $[Ca^{2+}]$ stabilizes the membrane by decreasing excitability. Moreover, Ca^{2+} entry contributes to depolarization.

ALL or NONE LAW

The action potential in a single nerve fiber is "all or none" in character *i.e.* when a stimulus is applied, either the axon does not respond with a spike production or it responds to the maximum of its ability. This *'All or None' relationship between the stimulus and response* is known as the ***All or None Law.***

It also applies to the skeletal muscle (the unit being the individual muscle fiber) and to the heart (the unit being entire atria or entire ventricle).

REFRACTORY PERIOD

If two successive stimuli of more than threshold intensity are applied to a nerve, the nerve is unable to respond to the second stimulus for quite some time *i.e.* the nerve has become *refractory* (non-responsive) to subsequent stimulation. The length of time it remains refractory to the second stimulus is called refractory period (page 40).

Important Note

This puts a limit to the frequency at which nerve can generate or conduct impulses, the maximum of which is not more than 1000/sec.

ACCOMMODATION

If a nerve is submitted to the passage of a constant strength of current, the site of the nerve under stimulation shows decrease of excitability. Therefore, accommodation consists of a rise in threshold of the tissue during stimulation (*Mechanism of accommodation* – page 40).

A similar feature at nerve endings is called ***Adaptation*** *i.e.* nerve fiber accommodates while the nerve endings adapt.

Important Note

The fibers of sensory nerves have far less power of accommodation, than those of motor nerves; fine pain fibers show almost no accommodation at all.

CONDUCTIVITY

The nerve impulse is conducted along the nerve fiber *i.e.* propagation of wave of depolarization. It is an active, self propagating process and the impulse moves along the nerve at a constant amplitude. (Fig. 17.1)

'A' in Unmyelinated Nerves

The nerve cell membrane is polarized at rest *i.e.* positivity outside and negativity along the inside of the membrane. As soon as fiber is excited at a point, the polarity is reversed for a brief period, positive charges from the membrane ahead of and behind the action potential flow into the area of negativity represented by the action potential (*current sink*). A *local circuit current* flows between the depolarized membrane and the resting membrane areas. The self propagating nature of the nerve impulse is due to circular current flow and successive depolarization to the firing level of the membrane ahead of the action potential. Once initiated, a moving impulse does not depolarize the area behind it to the firing level because this area is refractory. Direction of propagation of impulse is same as current flow direction inside the nerve (Fig. 4.7, page 41).

'B' in Myelinated Nerves

Myelin is a relatively effective insulator and current flow through it is negligible. In myelinated nerves, action potentials are generated only at the Nodes of Ranvier (about 1 mm apart), the regions where axon cell membrane is exposed to the ECF. Each node has a high concentration of voltage-gated Na^+ channels, which open with depolarization and allow Na^+ into the axon. In general, as the diameter of the axon increases, the internodal distance increases. Here the active generation of current is confined to the node but depolarizing the whole internodal length of fiber by local circuit action. Instead, depolarization jumps from one node of Ranvier to the next. Jumping of depolarization from node to node is called *Saltatory conduction* (saltatory means leaping). Therefore, it is a rapid process and can conduct much faster (50-100 times) than the unmyelinated fiber of same diameter.

ORTHODROMIC AND ANTIDROMIC CONDUCTION

An axon can conduct in either direction, when an action potential is initiated in the middle of it; two impulses travelling in opposite directions are set up by electrotonic depolarization on either side of the initial current sink.

Impulses normally pass in one direction only *i.e.* from synaptic junction or receptor along axon to their termination. Such conduction is called *Orthodromic conduction*. Conduction in the opposite direction is called *Antidromic conduction*, seen in sensory nerve supplying the blood vessels.

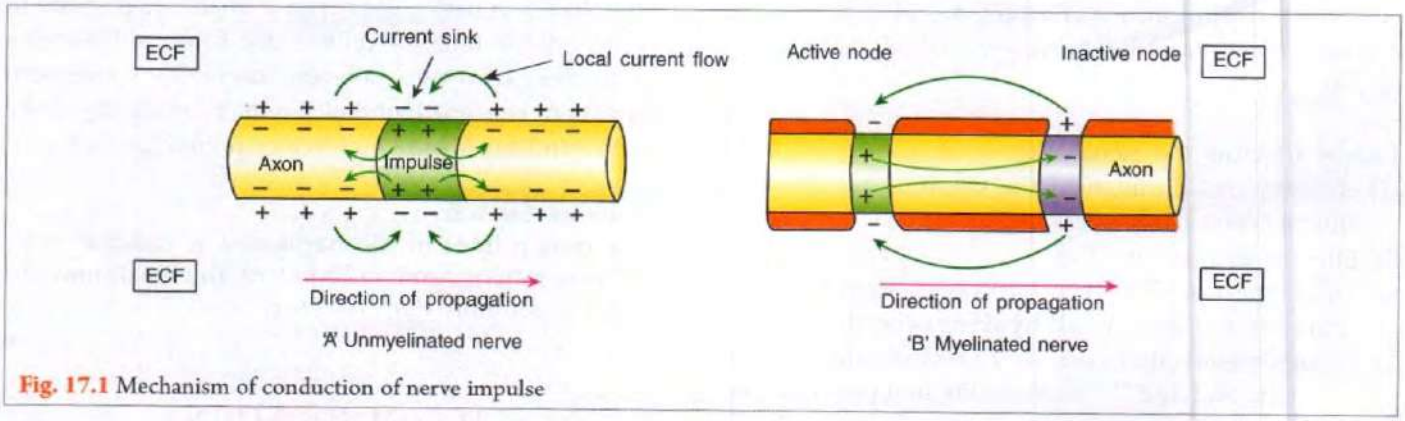


Fig. 17.1 Mechanism of conduction of nerve impulse

Study Questions

1. Describe briefly the physiological properties of the nerve fibers.
2. Write short notes on:
   (i) All or None law
   (ii) Types of conduction in a nerve fiber
   (iii) Accommodation
   (iv) Refractory period
   (v) Adaptation
   (vi) Excitability
   (vii) Orthodromic and antidromic conduction of nerve impulse
3. Give physiological significance of refractory period in a nerve.

MCQs

1. **What does indicate that a nerve has been excited?**
   (a) Production of generator potential
   (b) Production of an electrotonic potential
   (c) Production of catelectronic potential
   (d) Production of a wave of depolarization
2. **Rate of propagation of nerve impulse in a myelinated nerve is .... times as compared to unmyelinated fiber of same diameter:**
   (a) 5-10 (b) 10-50 (c) 50-100 (d) 100-150
3. ***Not true* of refractory period in a nerve:**
   (a) It is length of time it remains non-responsive to re-stimulation
   (b) Increase with the diameter of nerve fiber
   (c) It puts a limit to the frequency at which a nerve can conduct impulses
   (d) Because of it, a nerve can conduct maximum of 1000 impulses/sec.
4. **Once initiated, a moving nerve impulse does not depolarize the area behind to the firing level because:**
   (a) It travels only in one direction along axon
   (b) This area is refractory
   (c) Direction of current flow inside the nerve is reversed
   (d) All of the above
5. **Propagation of an impulse in one direction along axon to its termination is called:**
   (a) Antidromic conduction
   (b) Orthodromic conduction
   (c) Antegrade conduction
   (d) Saltatory conduction
6. **Presence of calcium on nerve membrane may play a significant role in:**
   (a) Operation of sodium pump
   (b) Regulation of potassium outflow
   (c) Keeping sodium gates closed
   (d) Preventing protein anions from going out
7. **Excitability of a nerve is increased by:**
   (a) Decrease in ECF calcium ion concentration
   (b) Increase in ECF calcium ion concentration
   (c) Increase in strength of a stimulus
   (d) Increase in duration of a stimulus
8. **All or none response in a nerve is applicable to:**
   (a) A mixed nerve (b) Only a sensory nerves (c) Only a motor nerve (d) A single nerve fiber
9. **Accommodation in a nerve is:**
   (a) Decrease of excitability to constant stimulation
   (b) Rise in threshold during stimulation
   (c) Due to slow opening of K^+ channels
   (d) All of the above
10. **Which of the following may show antidromic conduction?**
    (a) Synapse (b) Axons (c) Both (a) & (b) (d) Cell body

Answers

1. (d) 2. (c) 3. (b) 4. (b) 5. (b) 6. (c) 7. (a) 8. (d) 9. (d) 10. (b)

Chapter

18

Nerve Fiber Types and Functions

I. General

II. Classification of nerve fibers

III. Properties of mixed nerves: Compound action potential

GENERAL

1. *Greater the diameter* of a given nerve fiber
   (i) The greater is its speed of conduction (because a large fiber offers *less resistance* to local currents).
   (ii) The greater is the magnitude of spike potential.
   (iii) The smaller is the duration of spike.
   (iv) The lesser will be the threshold of excitation.
   (v) The lesser will be its refractory period. Therefore, number of stimuli that can be applied per second are more.
2. *Speed of conduction*
   (i) *In myelinated fibers*, the speed of conduction is approximately 6 times the fiber diameter. The diameter of myelinated fibers ranges from 1-20 µm, therefore, conduction velocity varies from 6-120 mts/sec.
   (ii) *In unmyelinated fibers*, the speed of conduction is proportional to the square root of the diameter. The largest unmyelinated fibers are approx. 1 µm in diameter, therefore, their maximum conduction velocity is 1 mt/sec.
3. The *Large axons* are concerned mainly with proprioceptive, pressure and touch sensations and somatic motor functions.
4. The *Small axons* are concerned with pain and temperature sensations and autonomic functions.

CLASSIFICATION OF NERVE FIBERS

I. Erlanger and Gasser's classification: Nerve fibers have been divided into A, B and C groups; 'A' group is further subdivided into α, β, γ and δ fibers (Table 18.1).

II. Numerical classification: This is sometimes used for sensory neurons and is based on the origin of nerve fibers (Table 18.2).

III. Physio-clinical classification: It has clinical as well as physiological significance and is based on sensitivity to *hypoxia*, *pressure* and *anaesthetic agents* (Table 18.3).

1. *Hypoxia* (*i.e.* O_2 lack at tissue level) to the body is associated with alteration of autonomic functions such as rise in the heart rate, blood pressure, respiration etc. as the pre-ganglionic autonomic 'B' fibers are most susceptible to it.
2. *Pressure* on a nerve can produce temporary *paralysis* due to loss of conduction in motor, touch and pressure fibers (in group 'A') while pain sensations remain relatively intact. This is commonly seen after sitting cross-legged for long periods or after sleeping with arm under the head.
3. *Local anaesthetics* are used for stitching cut wounds and for other minor surgical procedures as these agents can block transmission of pain sensations in group 'C' fibers before they affect touch fibers in group 'A'.

PROPERTIES OF MIXED NERVES

(1) Peripheral nerves are made up of many axons of various types, bound together in a fibrous envelop called the *Epineurium*. Potential changes recorded from such nerve, therefore, represent an algebric summation of the *all or none* action potential of these axons.
   (i) With *subthreshold* (subliminal) stimuli, none of the axons are stimulated and no response occurs.
   (ii) With *threshold* intensity stimuli, axons with low threshold fibres fire and a small potential change is recorded.
   (iii) With further increase in intensity of stimulating current, axons with higher threshold will also fire, producing recording of larger potential change.

Table 18.1: Erlanger and Gasser's classification

	Fiber Type	Function	Fiber Diameter (μm)	Conduction Velocity (mts/sec.)	Spike duration (Millisec.)	Absolute Refractory Period (Millisec.)
Typical myelinated fibers of spinal nerves (Motor and sensory)	Aα	Proprioception, Somatic Motor Golgi tendon organs, touch	12–20	70–120	0.4–0.5	0.4–1
	Aβ	Touch, pressure and motor functions	5–12	30–70		
	Aγ	Motor to muscle spindles	3–6	15–30		
	Aδ	Pain, temperature, crude touch	2–5	12–30		
(Myelinated efferent preganglionic) (Unmyelinated)	B	'Preganglionic' autonomic nerve fibers	< 3	3–15	1.2	1.2
	C (i) (Dorsal root)	Pain, touch, temperature and conduct impulses generated by cutaneous receptors	0.4–1.2	0.5–2	2	2
	(ii) Sympathetic	Postganglionic sympathetic nerve fibers	0.3–1.3	0.7–2.3	2	2

Table 18.2: Numerical classification for sensory neurons

Number	Origin	Fiber Type
Ia	Muscle spindle, annulospiral ending	Aα
Ib	Golgi tendon organ	Aα
II	Muscle spindle secondary ending, kinesthesia, touch, pressure	Aβ
III	Pain and temperature receptors; crude touch and pressure receptors	Aδ
IV	Pain touch, pressure, temperature	Dorsal root 'C' fibers

Table 18.3: Physio-clinical classification

Susceptibility	Most susceptible	Intermediate	Least susceptible
Sensitivity to 'Hypoxia'	B	A	C
Sensitivity to 'Pressure'	A	B	C
Sensitivity to 'Local Anaesthetics'	C	B	A

Thus, electrical response increases proportionately until the stimulus is strong enough to excite all the axons in the nerve. Such a stimulus is called *maximal* stimulus.

(iv) With *supramaximal* stimulus, no further increase in size of the potential change occurs.

(2) Appearance of multiple peaks in action potential is called ***compound action potential***.

COMPOUND ACTION POTENTIAL

It is an extracellular (biphasic) recording of action potential which is made 'monophasic' recording by injuring the part of a nerve and placing one of the external recording electrode on it. **(Fig. 18.1 and 18.2)**

Cause of Compound Action potential

A mixed nerve is made up of families of fibers with varying speed of conduction. Therefore,

1. When all the fibers are stimulated, the activity in fast conducting fibers arrives at the recording electrodes sooner than the activity in slower fibers; and
2. The farther away from the stimulating electrodes the action potential is recorded, the greater is the separation between the fast and slow fiber peaks.

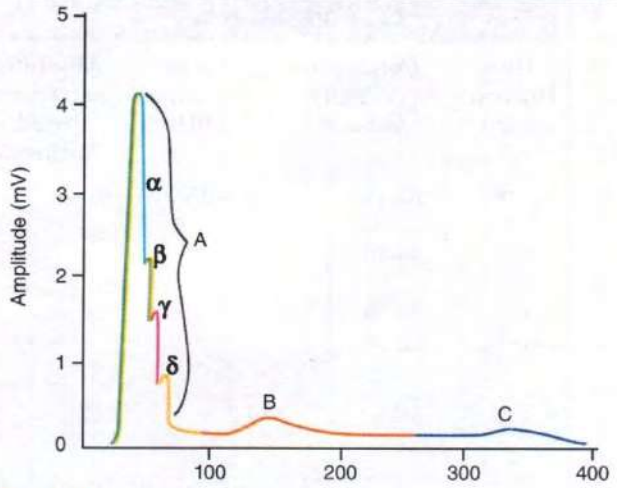


Fig. 18.1 Compound action potential

In general, Group 'A' fibers make maximum contribution to compound action potential and group 'C' the least.

Factors affecting compound action potential

1. *Type of fibers:* None of the peripheral nerves show all the components because none contain all fiber types. Therefore, the number and size of the peaks vary with the types of the fibers in the particular nerve being studied.
2. *Strength of the stimulus:* The shape of the compound action potential also depends upon the number and type of fibers stimulated by a given strength of the stimulus.

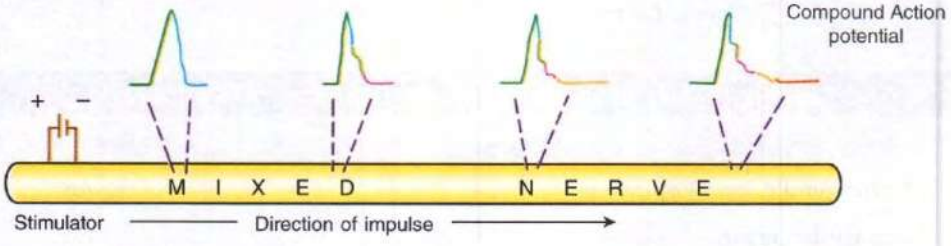


Fig. 18.2 Compound action potential as recorded at various distances from the stimulating electrode

Study Questions

1. Draw a well labelled diagram of compound action potential. Mention the factors affecting it.
2. Mention properties of mixed nerves.
3. Give physio-clinical classification of nerve fibers. Add a note an its significance.
4. Differentiate between action potential and compound action potential.

MCQs

1. Greater the diameter of a given nerve fiber, greater is the:
   (a) Speed of conduction (b) Spike duration
   (c) Magnitude of spike (d) Both (a) and (c) are correct
2. The fiber which is the thickest in human nerve is:
   (a) Touch (b) Pain (c) Temperature (d) Proprioception
3. The most susceptible nerve fiber to hypoxia is:
   (a) A (b) B (c) C (d) All are equally sensitive
4. Conduction in which type of nerve fibers is blocked maximally by pressure?
   (a) C (b) A (c) B (d) Sympathetic nerve
5. Which of the following nerve fibers is affected by local anaesthetics first?
   (a) Parasympathetic (b) A (c) B (d) C

6. **If a mixed nerve is stimulated with increasing strength of current, magnitude of potential:**
   (a) Does not change
   (b) Gradually increases to a limit
   (c) Decreases
   (d) Multiple peaks appear
7. **Which group of nerve fiber makes maximum contribution to compound action potential?**
   (a) A (b) B (c) C (d) Ib
8. ***Not true* about compound action potential is:**
   (a) Represents an algebraic summation of action potential of axons in a mixed nerve
   (b) It is an extracellular recording
   (c) Associated with appearance of multiple peaks
   (d) All of its components can be recorded from a given mixed nerve
9. **Spike duration is maximum in which nerve fiber?**
   (a) Aα (b) Aβ (c) Aγ (d) C
10. **Type B nerve fibers are seen in:**
    (a) Preganglionic autonomic
    (b) Somatic motor
    (c) Motor to muscle spindle
    (d) Sympathetic post-ganglionic
11. **Compound action potential is the property of:**
    (a) Single nerve fiber (b) Sensory nerves (c) Motor nerves (d) Mixed nerves
12. **The shape of compound action potential depends upon:**
    (a) Number and type of nerve fibers stimulated
    (b) Strength of the stimulus
    (c) Distance between the stimulating and recording electrodes
    (d) All of the above

Answers

1. (d) 2. (d) 3. (b) 4. (b) 5. (d) 6. (d) 7. (a) 8. (d) 9. (d) 10. (a)
11. (d) 12. (d)

Chapter 19

Degeneration and Regeneration in Peripheral Nerves

I. Introduction: causes and grading of injury

II. Degeneration and regeneration processes

III. Complications

INTRODUCTION

The destructive and constructive changes which occur in a nerve fiber after injury are called *degeneration* and *regeneration* respectively. The degenerative changes in distal part of cut nerve fiber were first described by *Augustus Waller* in 1862 (***Wallerian degeneration***); although there is in addition retrograde degeneration of the axon upto the nearest cell body.

Common Causes of Injury (in order of occurrence)

1. *Transection i.e.* (through and through cut)
2. Crushing
3. *Injection of toxic* or *poisonous substance* into the nerve
4. *Ischaemia i.e.* interference in the blood supply
5. *Hyperpyrexia* – increase in body temperature beyond 40.5°C (106°F).

Grading of injury (by "Sunderland")

First Degree Injuries (1°)

Most commonly seen and is secondary to ischaemia caused by direct pressure to a nerve for a limited time. Ischaemia produces local anoxia (*i.e.* complete lack of O_2) with temporary impairment of nerve function. It gets corrected within few hours to few weeks, because the axon is not destroyed but merely loses its functional properties for a short time.

Second Degree Injuries (2°)

Prolonged and/or severe pressure, damages the nerve fibers at the pressure point eventually causing death of axon locally and distally.

Third Degree Injuries (3°)

Endoneurial tubes becoming interrupted.

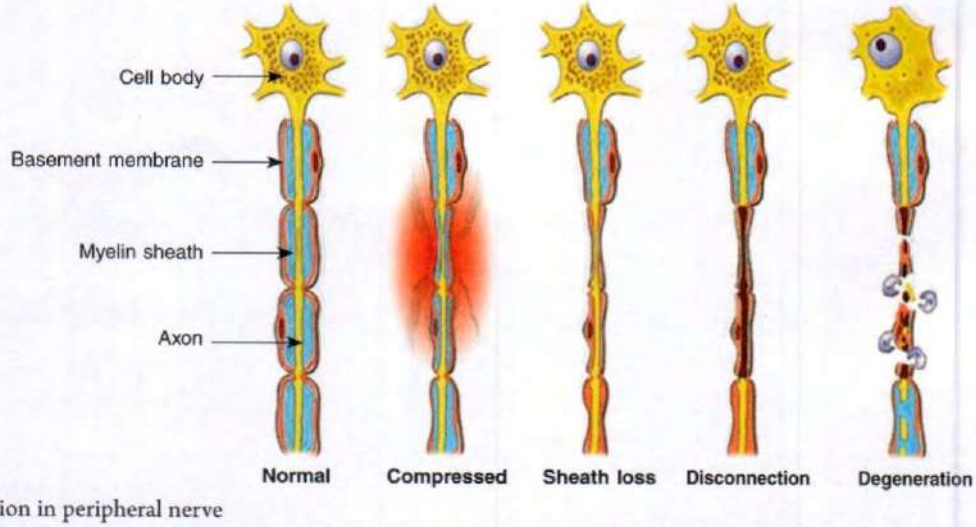


Fig. 19.1 Degeneration in peripheral nerve

Fourth Degree Injuries (4°)

Fascicles becoming disorganised.

Fifth Degree Injuries (5°)

Through and through cutting of nerve fibers *i.e.* complete transection.

DEGENERATION AND REGENERATION PROCESSES

Degenerative and regenerative processes go side by side and take place at three levels:

1. Changes in the nerve cell body (*retrograde changes*)
2. Changes in the distal stump
3. Changes at the site of injury.

A. DEGENERATIVE CHANGES (Fig. 19.1 and 19.2)

1. Changes in the Nerve Cell Body (Retrograde Changes)

Changes begin within 48 hours of the nerve section and reach to maximum by 15-20 days.

1. *Chromatolysis:* Nissl's granules disintegrate (*i.e.* break up into a fine dust) and lose their staining reaction after 15-20 days and cell becomes colourless. The process begins in the zone around the nucleus and spreads to the periphery of the cell. The dissolution of Nissl substance (RNA) occurs in order that the protein manufacturing process can be mobilized to help the neurons to survive.
2. Golgi Apparatus, mitochondria and neurofibrils: Fragmented and gradually disappear.
3. Cell draws in more fluid, loses its polygonal shape and becomes rounded cell.
4. Nucleus: increases in size and is displaced to the periphery and becomes oval. It may be completely thrown out of the cell, in which case the cell atrophies and finally dies off.

Degree of damage and chromatolysis depends on:

(i) Variety of neurons affected
  (a) At the periphery, changes as described above will occur.
  (b) In the central nervous system (CNS): No regeneration of nerve cell takes place because it has no centrosomes.
  (c) In the autonomic nervous system (ANS): Changes are difficult to demonstrate due to normal sparseness of the Nissl granules.

(ii) Distance of the lesion from the nerve cell: if less, more damage.

(iii) Nature of injury
  (a) if sharp cut, effects will be less;
  (b) if forcibly torn, death of the cell occurs.

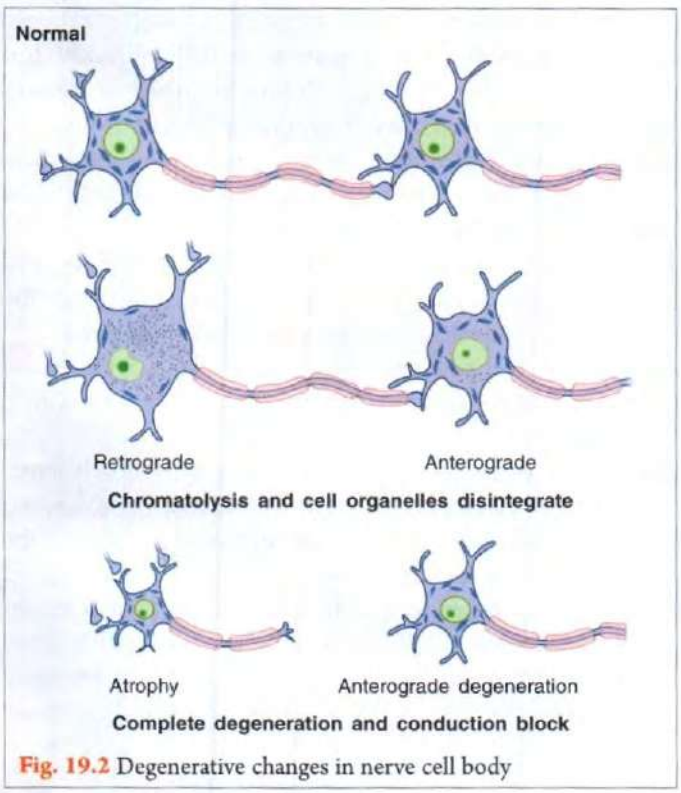


Fig. 19.2 Degenerative changes in nerve cell body

2. Changes in the Distal Stump

It undergoes complete degeneration, the process starts within 24 hours of injury. Changes in the action potential can be observed as early as 2 days after section. After the 3rd day, the ability of the nerve to conduct an impulse decreases markedly and after the 5th day, an impulse can no longer be evoked. Changes are as follows:

(1) *Axis cylinder* breaks up into short lengths and after a few days little debris (dust like particles) are left in the space formerly occupied by the axon.

(2) *Myelin Sheath* breaks up, more slowly than the axon into small oily droplets. Its destruction occurs in two stages:

(i) *Physical destruction: i.e.* upto 8-10 days little or no change in histochemistry of oily droplets is seen.

(ii) *Chemical destruction:* starts on 8th day and goes on till 32nd day. It is caused by enzymes of Schwann cells or macrophages lining the endoneurial tubes. Principal myelin lipids are:
  (a) Free cholesterol, and
  (b) Lipids containing sphingomyelin (cerebrosides and sphinosine).

At the time of disappearance of myelin, cholesterol esters appear in large quantities and free cholesterol disappears.

Note

Now if the damage is in the CNS, no further changes take place (because CNS neurons do not have the growth promoting chemicals needed for regeneration and CNS myelin is a potent inhibitor of axonal growth. But if the damage is in the peripheral nervous system – neurolemma shows the further changes.

(3) *Nucleus of Schwann cell* starts multiplying mitotically with formation of *cords of cell* which fill up the endoneurial tubes.
(4) Macrophages from endoneurium start digesting (phagocytosing) debris of axis cylinder and of myelin sheath.
(5) Once the debris are cleared, Schwann cell cytoplasm starts, proliferating and fills up the endoneurial tubes (this process takes 3 months).
(6) Ultimately all that is left of the fibers is the neuroleminal tubes enclosing a mass of granular cytoplasm containing many nuclei.

3. Changes at the Site of Injury

Schwann cells differentiate into thin elongated cells, mainly from the distal end and very little from the proximal end. They can bridge up the gap upto 3 cms. Rate of progress of growth is 1-2 mm/day. *If gap is more than 3 cm it is difficult to fill up,* because of inter-lacing of fibers. This can be fascilitated by operation procedures by removing the debris and stitching the two cut ends together for an early degeneration and regeneration.

B. REGENERATIVE CHANGES (Fig. 19.3)

1. Changes in the nerve cell body

It begins in 20 days and is completed in 80 days. Nissl granules and golgi apparatus gradually reappear, cell regains its normal size and nucleus returns to its central position. Repair of cell may even occur if the axon does not regenerate.

2. Changes in the distal stump and at the site of injury

(1) Axis Cylinder (Central Axon) from the proximal stump elongates and then grows out in all directions as rounded *pseudopod* like structures called ***Fibrils*** towards the distal stump. This results from growth promoting factors secreted by Schwann's cells and increased production of neurotrophin from the denervated distal stump.

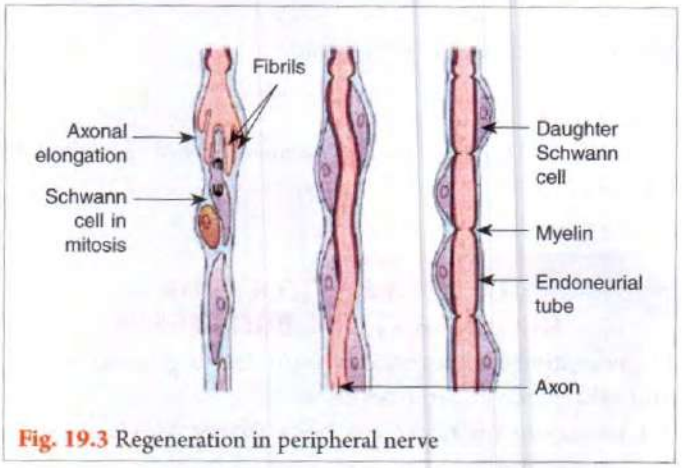


Fig. 19.3 Regeneration in peripheral nerve

Important Note

In the CNS the distal stump of a damaged axons are unlikely to form new synapses, therefore recovery is rare.

(2) Each axon gives rise to 50-100 *fibrils* which are guided by the strands of Schwann cell into the distal ends of the endoneurial tubes.
(3) 2-3 weeks after the nerve section the distal endoneurial tube contains varying numbers of developing fibrils, some none, some as many as 20-30 fibrils.
(4) Eventually all the fibrils re-innervating one tube degenerate except one which thereupon progressively gets enlarged to fill the tube.
(5) To begin with, rate of growth is 0.25mm/day, but once it enters the distal stump, the rate of growth becomes 3-4 mm/day. As it grows deeper down, the rate of growth further increases because mechanical conditions for regeneration are more suitable than those in a cut nerve end.
(6) In approx. 15 days, Schwann cell filling the endoneurial tube starts laying down myelin sheath round the successful fibril, which is completed by one year.
(7) Once the regenerated axon reaches its target, a new functional connection (e.g. neuromuscular junction) is formed.
(8) Increase in fiber diameter takes place very slowly. Final diameter attained is 80-85% of normal. It is limited by the diameter of the distal tube and the size of the parent nerve cell. Therefore, functional recovery is not full which takes longer time and may be associated with different complications if it does not reach its own cut stump. That is why, for a motor nerve, recovery may be complete but for a mixed nerve it is rarely so.

COMPLICATIONS

A. FUNCTIONAL COMPLICATIONS

1. In sensory nerve damage, an area of anaesthesia is produced.
2. Misinterpretation of sensations, because many fibers will establish connections with new kinds of endings in new situations. Therefore, difficulty in localization *e.g.* thumb base may be interpreted as its tip.
3. Fine motor control may be permanently impaired because of improper connections within the motor fibers.

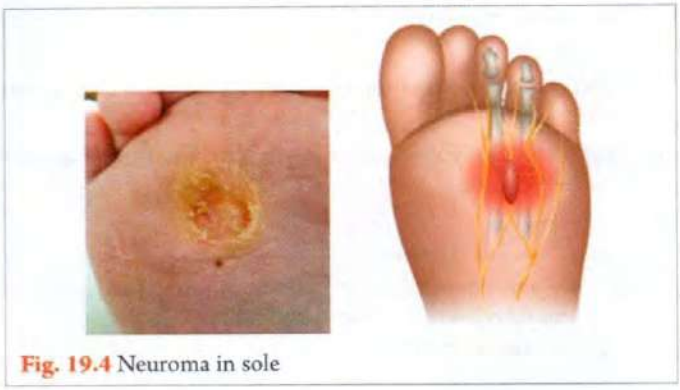


Fig. 19.4 Neuroma in sole

B. ANATOMICAL COMPLICATION

When the gap is more than 3 cm between the two cut ends of the nerve, intermashing of nerve fibers leads to painful collection at the site of injury called ***neuroma***. (Fig. 19.4)

Study Questions

1. **Describe briefly the degenerative and regenerative changes in a peripheral nerve following 5° injury:**
   (i) in the nerve cell body (ii) in the distal stump (iii) at the site of injury.
2. **Write briefly about:**
   (i) Wallerian degeneration (ii) Chromatolysis (iii) Complications following a nerve injury.

MCQs

1. **Hyperpyrexia usually beyond ..... results in nerve damage:**
   (a) 37°C (b) 38°C (c) 39.5°C (d) 40.5°C
2. **Peripheral nerves can tolerate ischaemia up to:**
   (a) 6 hours (b) 12 hours (c) 24 hours (d) 30 minutes
3. **Retrograde degenerative changes begin within ..... of nerve section:**
   (a) 6 hours (b) 12 hours (c) 24 hours (d) 48 hours
4. **First change to occur in the distal segment of cut nerves:**
   (a) Myelin sheath degeneration (b) Axonal degeneration
   (c) Mitosis of Schwann cell (d) Axonal sprouting
5. **After injury to a nerve, its ability to conduct an impulse is lost completely:**
   (a) Immediately (b) After 24 hours (c) After 3rd day (d) After 5th day
6. **The gap between two cut ends of a nerve fiber, which can be filled up by natural processes is up to:**
   (a) 0.5 cm (b) 1 cm (c) 2 cm (d) 3 cm
7. **Following injury to a nerve regeneration changes in the nerve cell body begins in:**
   (a) 10 days (b) 20 days (c) 30 days (d) 40 days
8. **After transection of a peripheral nerve, the regenerative changes usually get completed in:**
   (a) 1 month (b) 2 months (c) 3 months (d) 4 months
9. **Recovery in a nerve is completed approx. by ..... after the section:**
   (a) 3 months (b) 6 months (c) 9 months (d) 1 year
10. **Which nerve is likely to show complete functional recovery after it has been injured?**
    (a) Sensory nerve (b) Motor nerve (c) Mixed nerve (d) Autonomic nerve
11. **Wallerian degeneration refers to:**
    (a) Degenerative and regenerative changes in a cut nerve fiber
    (b) Retrograde degeneration of the axon upto the cell body
    (c) Degenerative changes in distal part of cut nerve fiber
    (d) Degenerative changes at the site of injury

12. Complete transection of a nerve is:
(a) 1° injury (b) 3° injury (c) 5° injury (d) 7° injury

13. Wallerian degeneration of ruptured nerve begins within:
(a) 6 hours (b) 12 hours (c) 24 hours (d) 6 weeks

14. After the nerve section, to begin with the rate of growth of fibrils is:
(a) 0.05 mm/day (b) 0.25 mm/day (c) 1-2 mm/day (d) 3-4 mm/day

15. Neuroma is:
(a) A cancerous condition of nerve fibers
(b) Degenerative and regenerative changes in peripheral nerves after injury
(c) Painful collection of nerve fibers at the site of injury
(d) An area of anaesthesia following sensory nerve damage

Answers

1. (d)	2. (b)	3. (d)	4. (b)	5. (d)	6. (d)	7. (b)	8. (c)	9. (d)	10. (b)
11. (c)	12. (c)	13. (c)	14. (b)	15. (c)					

Chapter 20

Neuromuscular Junction

I. Definition
II. Structure
III. Synthesis, storage and release of A-ch
IV. Sequences of events at neuromuscular junction during nerve impulse transmission
V. Miniature end plate potential
VI. Clinical importance of neuromuscular junction
VII. Applied: Myasthenia gravis; Lambert-Eaton syndrome

DEFINITION

Neuromuscular (or ***myoneural***) junction is a junction between the motor nerve and skeletal muscle fiber. (Fig. 20.1)

STRUCTURE

A. LIGHT MICROSCOPIC APPEARANCE

At termination, the axon supplying a skeletal muscle fiber loses its myelin sheath and divides into a number of terminal buttons (***End Feet***) which make a close and intensive contact with a specialized part of muscle *sacroplasm* known as *Motor End Plate*. *Only one nerve fiber ends on each motor end plate.*

B. ELECTRON MICROSCOPIC APPEARANCE

1. Underneath the nerve ending, the muscle membrane of motor end plate is thrown into folds, called ***Palisades***. These folds increase the surface area on which neuromuscular transmitter can act.
2. At terminal nerve endings there are many mitochondria and minute *Vesicles* (***Synaptic vesicles***) or granules, diameter 30-50 nm, which contain small packets of the chemical transmitter, acetyl-choline (A-ch) responsible for synaptic transmission. These vesicles are most numerous at active zones in the nerve terminal.
3. Nerve membrane which is in close approximation with the muscle membrane, is known as *pre-synaptic membrane*, while the muscle membrane is called *Post Synaptic Membrane*. The space between these two membranes is called ***Synaptic cleft*** (50-100 nm wide) which is filled with ECF.
4. The *nicotinic A-ch receptors* are found on the post-synaptic membrane near the junctional folds. They are so called because they are stimulated by both nicotine and A-ch, and inhibited by curare.
5. An enzyme ***Specific acetyl-cholinesterase*** (*true or specific cholinesterase*) is found in high concentration in *post-synaptic clefts*, which destroys (hydrolyzes) the A-ch.

SYNTHESIS, STORAGE AND RELEASE OF A-ch

1. A-ch is synthesized within the mitochondria from 'choline' in the presence of enzyme *Choline Acetyltransferase* (Choline Acetylase). In addition, it requires acetyl Co-enzyme A, ATP and glucose. With the exception of choline all components required for synthesis of A-ch are present within mitochondria or gain ready access to them.
2. A-ch once formed is temporarily stored in minute vesicles (synaptic vesicles). Each vesicle contains a quantum (packets) consisting of a few hundred or a few thousand molecules of A-ch (Approx. 10^4 molecules).
3. Nerve impulse causes fusion of vesicles with membrane of terminal nerve fiber by increasing permeability of Ca^{2+}. Ca^{2+} in ECF helps in fusion by changing ionic composition and thereby decreasing the flow of axoplasm, therefore, vesicles easily come in contact with the membrane. Mg^{2+} decreases this process. The amount of transmitter released is directly proportional to the Ca^{2+} influx.

SEQUENCE OF EVENTS AT NEUROMUSCULAR JUNCTION DURING NERVE IMPULSE TRANSMISSION

1. Nerve impulse (action potential) reaches presynaptic nerve ending.

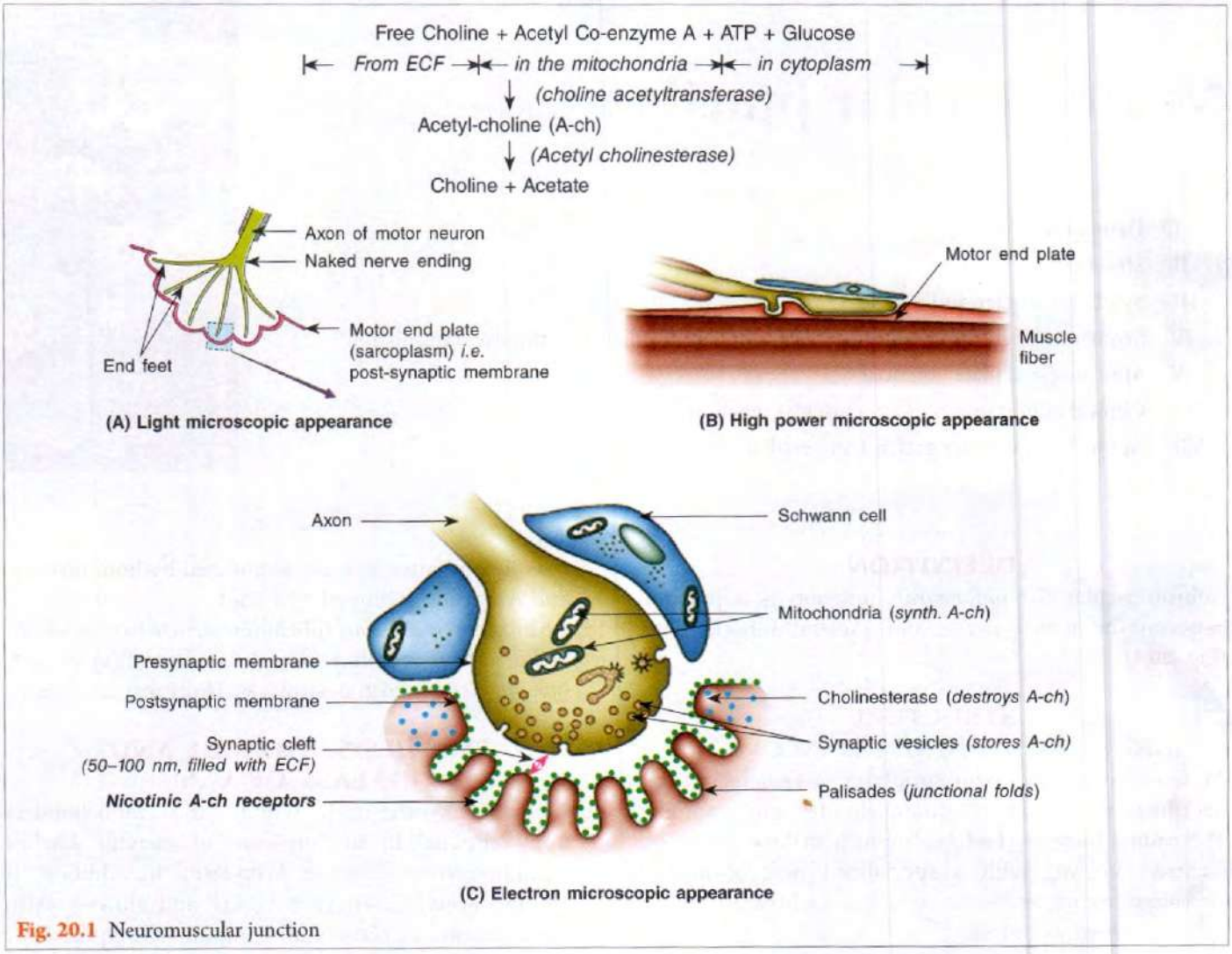


Fig. 20.1 Neuromuscular junction

2. As it reaches presynaptic membrane it causes release of A-ch into the synapse (synaptic cleft) by a process of exocytosis.
3. A-ch, after its release, diffuses within few hundred microsec. across the very short distance to the post synaptic membrane *i.e.* motor end plate.
4. A-ch attaches to ***nicotinic A-ch receptors*** on motor end plate surface and increases the permeability of motor end plate to Na^+ (mainly) and other positive ions (*e.g.* Ca^{2+}, NH_4^+, K^+).
5. Increased permeability of Na^+ (Na^+ influx) causes depolarization of the post synaptic membrane causing generation of local potential, called *end plate potential* (non-propagated depolarization). **(Fig. 20.2)**
6. Resting membrane potential (RMP) in skeletal muscle membrane is –90 mV. When end plate potential reaches a threshold of 30-40 mV, it depolarizes the surface membrane of the muscle and results in generation of action (spike) potential (magnitude 120-130 mV).
7. Spike potential thus sets up a propagated muscle action potential which can travel in both directions along the muscle membrane. Once it reaches inside the muscle cell (muscle fiber) then the muscle gives mechanical response by contraction.

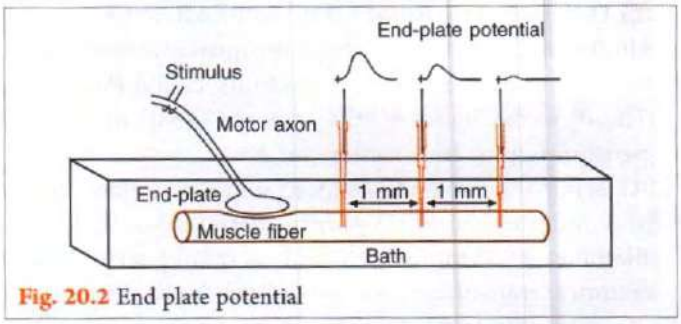


Fig. 20.2 End plate potential

Gradation of muscular activity

For graded, repeated muscular contractions (page 171) in response to different frequencies of stimulation, A-ch should be removed, therefore, repolarization occurs and second impulse can be received. *How A-ch is removed?*

1. Some A-ch gets diffused back to presynaptic region from synaptic clefts.

2. A major part of A-ch is removed by post-synaptic membrane enzyme acetyl cholinesterase which hydrolyses A-ch and results in repolarization of the membrane. (Fig. 20.3)

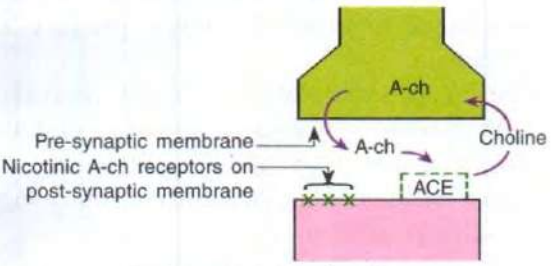


Fig. 20.3 Removal of Acetyl-choline (A-ch)
ACE: Acetyl cholinesterase

Note

Action of A-ch liberated by a single nerve impulse is very brief, thus muscle end plate becomes sensitive to subsequent release of A-ch.

Characteristic Features of Neuromuscular Junction

1. Tip of pallisade is only 50-100 nm from presynaptic ending. Therefore, A-ch diffusion is very easy, calculated time for this diffusion is a few hundred microsecs.
2. An average *motor end plate* contains about 50 million 'nicotinic' A-ch receptors.
3. When a nerve impulse arrives it causes the almost synchronous release of approx. 10^6 molecules of A-ch at a single motor nerve ending. Each nerve impulse causes fusion of 60 A-ch 'vesicles' with pre-synaptic membrane and each vesicle contains about 10^4 molecules of A-ch. This amount is enough to activate all the nicotinic A-ch receptors.
4. Artificial application of A-ch is less effective than liberation from the nerve endings. *Proof*: Intracellular application of A-ch to the motor end plate by means of electrophoresis through a micro-pipette, 10^8–10^9 molecules of A-ch are required to depolarize the membrane. This also shows that the 'nicotinic A-ch receptors' are present on the surface of motor end plate and not inside the cell.
5. Under normal functioning conditions measurable fatigue of the neuromuscular junction occurs rarely.

MINIATURE END PLATE POTENTIAL (MEPP)

During rest, very small potential changes upto 0.5 mV can be recorded from the motor end plate even if there is no impulse, called *MEPP*. (Fig. 20.4)

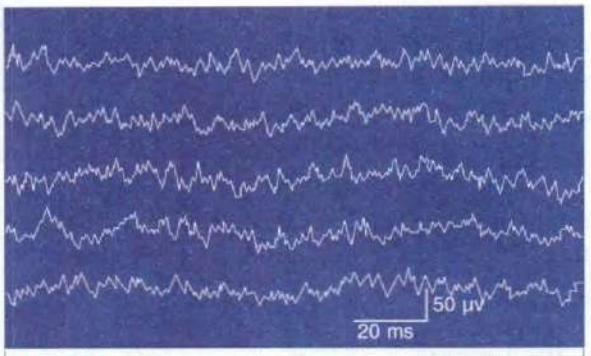


Fig. 20.4 Miniature end plate potential (MEPP)

Reason for MEPP

As axoplasm is flowing, because of random ***Brownian movements*** some impulses come to nerve terminal and cause fusion of some 'vesicles' with nerve membrane, with release of A-ch in very small amounts ($<10^6$ molecules of A-ch). Therefore, no mechanical response is seen and it can only result in production of MEPP (*i.e.* a minute depolarizing spike). (For difference between end plate potential and action potential, refer to page 43.)

In experimental animals, inhibition of MEPP results in production of *Giant End Plate Potential (GEPP)* due to release of more quantity of A-ch. This causes more depolarization of motor end plate upto 12 mV but not sufficient enough to generate threshold potential. GEPP are responsible for fibrillation in muscle treated with anti cholinesterase.

CLINICAL IMPORTANCE OF NEUROMUSCULAR JUNCTION

Blocking of neuromuscular junction produces muscle relaxation, therefore:

(1) Helps in surgical operations by providing open fields.

(2) Reduces movements during electroconvulsive treatment of psychotic patients.

Blockage of neuromuscular junction. This can be achieved in two ways:

A. By *inhibiting release of A-ch* from presynaptic membrane *i.e.* motor nerve endings *e.g.* Botulinum toxin (a bacterial toxin) which combines irreversibly with cholinergic motor nerve terminals and interferes with synthesis or release of A-ch.

B. *Drugs* which *antagonize the action of A-ch* on the post-synaptic membrane *i.e.* motor end plate. They act by two ways:

1. By Competitive Inhibition

For example, Tubocurarine, Gallamine (Flaxedil). Both A-ch and curare compete for the same 'nicotinic A-ch receptors'

site. Therefore, if amount of curare is more than A-ch it competes with A-ch for receptors on motor end plate and forms a strong complex with them.

Antagonists to curare are ***anti-cholinesterases*** (neostigmine, eserine, tetraethyl-pyrophosphate and edrophonium) which act on cholinesterase and prevent destruction of A-ch by cholinesterase, thus increase the A-ch amount and eventually lead to removal of the block.

2. By Persistent Depolarization

For example, suxamethonium (succinyl choline), decamethonium. Their duration of action is very short (5 minutes). Both these agents act by persistent depolarization to cause local energy exhaustion with no further generation of ATP, resulting in muscle relaxation. Antidote is not required because they act for 5 minutes and are used for fracture reduction. If necessary, curare is the antidote.

Suxamethonium gets destroyed itself in the body after 5 minutes by ***Pseudo-Cholinesterase*** or *non-specific cholinesterase*, found in the plasma. Its concentration is under endocrine control and is also affected by alterations in liver functions.

APPLIED

1. MYASTHENIA GRAVIS

(Myo means muscle; asthenia means weakness and gravis means severe)

(i) Myasthenia gravis is a rare ***autoimmune disease*** caused by the formation of circulating antibodies to the *nicotinic A-ch receptors*. These antibodies destroy some of the receptors and bind others to neighbouring receptors.

(ii) The size of end plate potential is reduced, therefore, it is more difficult to depolarize the muscle membrane to threshold and to produce action potential.

(iii) The disease is characterised by rapid onset of fatigue with marked generalised weakness of muscles.

(iv) The *most commonly affected muscles are*: extra-ocular muscles, facial, swallowing and mastication muscles. **(Fig. 20.5)**

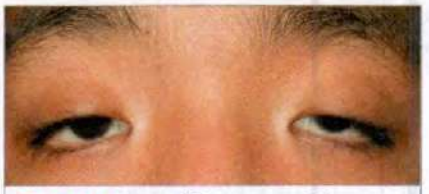


Fig. 20.5 Ptosis (drooping of the eyelid) in Myasthenia gravis

(v) In severe form patient becomes bed ridden and may even die from paralysis of respiratory muscles.

2. LAMBERT-EATON SYNDROME

In this condition, muscle weakness is caused by antibodies against one of the Ca^{2+} channels in the nerve endings at the neuromuscular junction. This decreases the normal Ca^{2+} influx that causes A-ch release. Proximal muscles of the lower limbs are primarily affected.

Study Questions

1. Give physiological significance of:
   (i) End plate potential (ii) MEPP (iii) GEPP (iv) Threshold excitation
2. Write short notes on:
   (i) Nicotinic A-ch receptors (ii) True and pseudo cholinesterase (iii) Myasthenia gravis
3. Draw well labelled diagram of neuromuscular junction. Give its physio-clinical significance.
4. How A-ch is removed at neuromuscular junction (NMJ)? Mention characteristic features of NMJ.
5. Give sequence of events at NMJ during transmission of nerve impulse.
6. How blockage of NMJ can be achieved? Why is it required?
7. Name the drugs that antagonise the action of A-ch at motor end plate. Give their mode of action.
8. Define Myasthenia gravis. Name the muscles most commonly affected by it and why?

MCQs

1. At neuromuscular junction, one nerve fiber end on ..... motor end plate:
   (a) One (b) Three (c) Five (d) Many
2. Synaptic cleft at neuromuscular junction is filled with fluid which is rich in:
   (a) Na^+ (b) K^+ (c) PO_4^{3-} (d) Protein anions

3. **What type of ions is probably most important in causing release of transmitter from vesicles at nerve endings?**
   (a) Sodium ions (b) Potassium ions (c) Magnesium ions (d) Calcium ions
4. **Value of threshold excitation in a skeletal muscle fiber is:**
   (a) 10-15 mV (b) 30-40 mV (c) 50-60 mV (d) 70-80 mV
5. **The end plate potential is characterised by:**
   (a) Propagation (b) All or none law (c) Depolarization (d) Hyperpolarization
6. **On an average motor end plate contains approx. ..... nicotinic A-ch receptors:**
   (a) 10,000 (b) 1 million (c) 10 million (d) 50 million
7. **When a nerve impulse arrives at neuromuscular junction it causes release of approx. ..... molecules of A-ch:**
   (a) 10^4 (b) 10^5 (c) 10^6 (d) 10^7
8. **Usual amplitude of miniature end plate potential is:**
   (a) 0.5 mV (b) 12 mV (c) 30-40 mV (d) 120-130 mV
9. **A patient complains of muscle weakness. On administration of neostigmine, it disappears, because:**
   (a) It blocks the action of acetylcholine (b) It competes with A-ch for receptors on motor end plate
   (c) It acts by persistent depolarization of motor end plate (d) It interferes with the action of anticholine esterase
10. **Curare in therapeutic doses:**
    (a) Competes with A-ch for same nicotinic receptors on motor end plate
    (b) Prevents propagation of action potential in skeletal muscle
    (c) Enhances the action of cholineesterase
    (d) Enhances the action of catecholamines
11. **In myasthenia gravis:**
    (a) Tolerance of acquired immunity system to one's tissue is depressed
    (b) Antibodies are formed against muscurinic A-ch receptors
    (c) Most commonly affected muscles are respiratory group of muscles
    (d) It is a common auto-immune disease
12. **Myasthenia gravis is characterized by:**
    (a) Formation of circulating antibodies to the nicotinic A-ch receptors
    (b) Sustained contraction of muscles
    (c) Most commonly affected muscles are those of the limbs
    (d) Formation of antibodies against Ca^{2+} channels
13. **All may be the causes of muscle weakness *except*:**
    (a) Myasthenia gravis (b) Lambert-Eaton syndrome
    (c) Administration of curare (d) Neostigmine in therapeutic doses
14. ***True* about pseudocholinesterase is:**
    (a) Stored in the synaptic vesicles (b) Normally found in the plasma
    (c) Gets destroyed in the body itself (d) Also called specific cholinesterase
15. **Specialized part of muscle sacroplasm is called as:**
    (a) Pre-synaptic membrane (b) End feet (c) Motor end plate (d) Palisades
16. **At neuromuscular junction the following transmitter is released:**
    (a) Acetylcholine (b) Bradykinin (c) Histamine (d) Serotinin
17. **Width of synaptic cleft is:**
    (a) 10 nm (b) 30 nm (c) 50-100 nm (d) Above 100 nm
18. **Each synaptic vesicle stores approx. ..... molecules of A-ch:**
    (a) 10^2 (b) 10^3 (c) 10^4 (d) 10^5
19. **Resting membrane potential in skeletal muscle membrane is:**
    (a) –60 mV (b) –70 mV (c) –80 mV (d) –90 mV
20. **Which of the following statements is *not* correct? Release of acetylcholine at the neuromuscular junction:**
    (a) Produces an end-plate potential (b) Increases sodium movement into the muscle cell
    (c) Always causes the muscle fiber to contract (d) Is followed by rapid destruction of acetylcholine

Answers

1. (a)	2. (a)	3. (d)	4. (b)	5. (c)	6. (d)	7. (c)	8. (a)	9. (d)	10. (a)
11. (a)	12. (a)	13. (d)	14. (b)	15. (c)	16. (a)	17. (c)	18. (c)	19. (d)	20. (c)

Chapter 21

Skeletal Muscle

I. Introduction
II. Structure
III. Electrical phenomenon and ionic fluxes in skeletal muscle fiber
IV. Contractile response
V. Mode of contraction: Isometric and isotonic contraction
VI. Properties of motor unit
VII. Energy source for muscular contraction

INTRODUCTION

Body muscles are divided into 3 types: *Skeletal, Cardiac* and *Smooth muscles*. The main differentiating features between the 3 types of muscles are given in **Table 21.1** and pages 191-192.

STRUCTURE

A. GENERAL FEATURES

1. Skeletal muscle is made up of many long thin cells called *muscle cells or muscle fibers*. They are contained in connective tissue sheets and form bundles of fibers. These are then bound in further connective tissue to form the whole muscle.
2. Muscles begin (*origin*) and end (*insertion*) in the tendons and muscle fibers are arranged in parallel between tendinous ends so that the force of contraction of the 'unit' is additive.
3. Each muscle fiber is a multinucleated, 1-40 mm long, cylindrical in shape, 50 to 100 µm diameter and surrounded by a cell membrane, the *sarcolemma*. There are no syncytial bridges between the cells. **(Fig. 21.1 and 21.2: A)**
4. The muscle fibers are made up of many fibrils called ***Myofibrils***. Each myofibril is 1-2 µm in diameter, lies parallel to one another and are *striated*.
5. The fibrils are divisible into individual filaments, made up of the ***contractile proteins***: *myosin; actin; tropomyosin* and *troponin*.
6. The cytoplasm in the muscle fibers, called ***Sarcoplasm***, contains: numerous mitochondria (sarcosomes); smooth surface endoplasmic reticulum (*sarcoplasmic reticulum*) and rich in glycogen.

B. LIGHT MICROSCOPIC APPEARANCE (Fig. 21.1 and 21.2: B)

The *cross striations* which are characteristic of skeletal muscle are due to difference in the refractive indices of various parts of muscle fiber. Therefore, the muscle fiber is seen to show *alternate dark and light cross bands*.

1. The dark band is made of highly refractile material (*i.e. Anisotropic*), therefore, looks dark and is called ***'A' band*** - (1.5 µm in length).

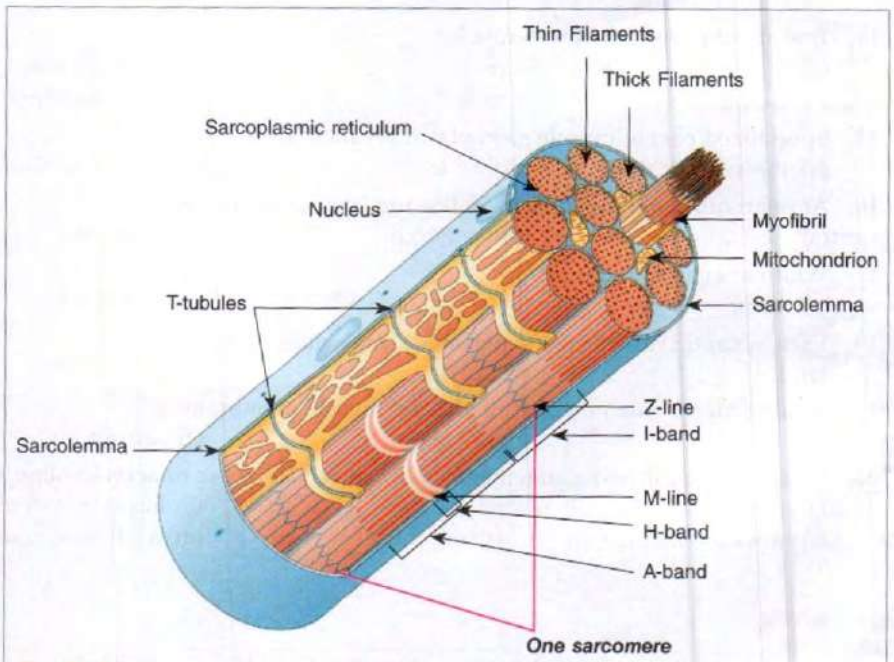


Fig. 21.1 Structure of skeletal muscle fiber

Table 21.1: Skeletal, Cardiac and Smooth muscles compared (main features only)

	Skeletal Muscle	Cardiac Muscle	Smooth Muscle
(1) Location	Most are attached to the skeleton and form 'somatic' musculature.	Present in heart only	Mostly in hollow viscera.
(2) Structure	(i) Shows well developed cross striations, thus, called *'striated'* muscle. (ii) *Non-syncytial i.e.* lacks anatomic and functional connections between individual muscle fibers.	(i) Also has well developed cross-striations. (ii) Functionally *syncytial* in character.	(i) Lacks cross-striations, therefore also called *plain* muscle. (ii) They are of 2 types: (a) *Single Unit:* functionally syncytial. (b) *Multi Unit:* functionally non-syncytial. (For details, refer to page 187.)
(3) Control	Generally under voluntary control *i.e.* why called *"voluntary"* muscles.	*Involuntary i.e.* can't be controlled at will.	*Involuntary*

2. In the centre of each 'A' band is found a slightly less refractile region called 'H' *band* (0.5 μm in length) (after the discoverer *Hensen*).
3. A 'M' ***line*** is seen in the middle of the 'H' band, due to central bulge in each of the 'A' band (the M-line is particularly pronounced during muscle contraction).
4. This line plus the narrow light area on either side of it is called the ***Pseudo-H Zone***.
5. The alternate light band is made of lower refractile material (*i.e. Isotropic*), therefore, looks lighter and is called ***'I' band*** (1 μm in length).
6. In the centre of each 'I' band is found a narrow line of highly refractile material which, therefore, looks dark called ***'Z' line*** (from German - Zwischenscheibe *i.e.* between disc).
7. The ***contractile unit*** of the muscle is the substance included between two adjacent 'Z' lines, called ***Sarcomere*** (2.5 μm in length).

Important Note

Variation in sarcomere length: At rest, i.e. when the muscle is relaxed, I band interdigitates with the 'A' band only outside the 'H' band (*sarcomere length is* 2.5 μm). *During muscle contraction,* the length of 'A' band remains constant whereas the 'Z' lines move close together causing sarcomere length to reduce to 1.5 μm. *By stretching* the muscle to the point where I and A band overlap ceases, the sarcomere length becomes 3.5 μm.

C. ELECTRON MICROSCOPIC APPEARANCE

The myofibrils are made up of two sets of protein filaments, *thick* and *thin* filaments **(Fig. 21.2: C)**.

(A) ***Thick filament:*** It is approx. twice the diameter of thin filament and made up of *Myosin*. It is responsible for the formation of ***'A' band***. There are several hundred (about 500) myosin molecules in each thick filament.

Myosin

1. Myosin filaments are 10-11 nm thick and approx. 45 nm apart, extending from one end of the 'A' band to the other.
2. Transverse section through 'A' band shows that each myosin filament is surrounded by 6 actin filaments in a regular hexagonal manner **(Fig. 21.3)**.
3. Myosin treated with proteolytic enzyme (trypsin) reveals two fragments which are arranged symmetrically on either side of centre of sarcomere **(Fig. 21.4)**. It is this arrangement that creates the light areas in the pseudo-H zone.

The myosin molecule is composed of two large polypeptide ***heavy chains*** and four smaller ***light chains***. These polypeptides combine to form a molecule that consists of *two* globular heads (containing heavy and light chains) and a long tail formed by the two twisted heavy chains **(Fig. 21.4)**.

Function: Each globular head has two important sites:
(a) *Actin binding site*, where myosin comes in contact with actin.
(b) *ATPase* (*catalytic*) site, that hydrolyzes (breaks) ATP.

The myosin molecules aggregate with their heads pointed in one direction along one half of the filament and in the opposite direction along the other half. The heads serve as the ***cross-bridges***.

(B) ***Thin filament:*** It is made up of *Actin, Tropomyosin and Troponin*. They arrange themselves to form 2 chains of globular units that form a long double helix (*i.e.* spiral chain). It is responsible for the formation of ***'I' band***. Each thin filament contains 300-400 actin molecules and 40-60 tropomyosin molecules.

1. ***Actin:*** Actin filaments are thinner, 4-5 nm in diameter and stretch from 'Z' lines to the edge of the 'H' zone. They occur in 2 forms:
   (i) *'G' Actin (Globular Actin)*: 5.5 nm diameter,

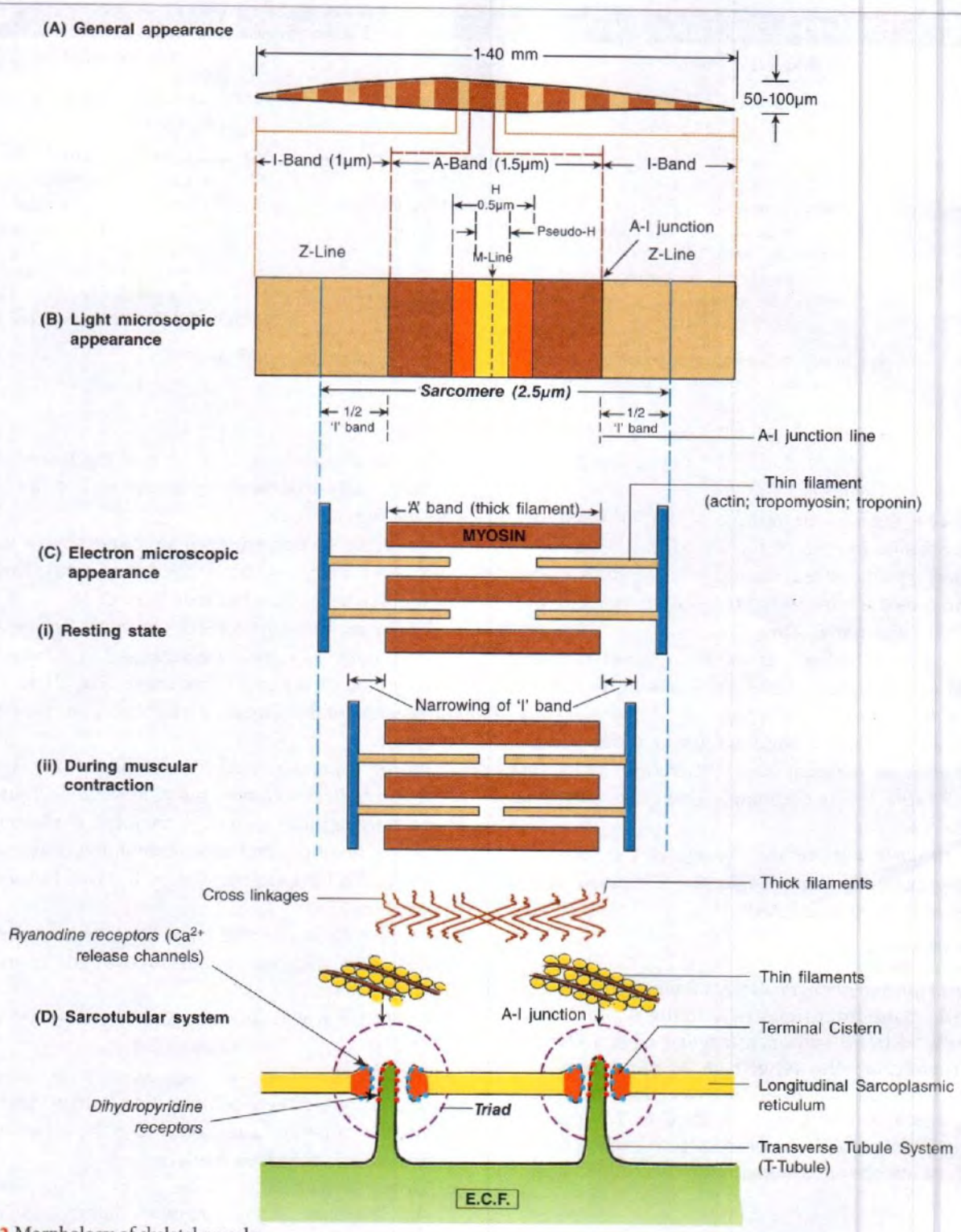


Fig. 21.2 Morphology of skeletal muscle

(A) Through 'I' band showing only thin filaments

(B) Through 'A' band. Each thick filament is surrounded by six thin filaments

(C) Through 'H' zone showing hexagonally packed thick filaments.

Fig. 21.3 Transverse Section through myofibrils

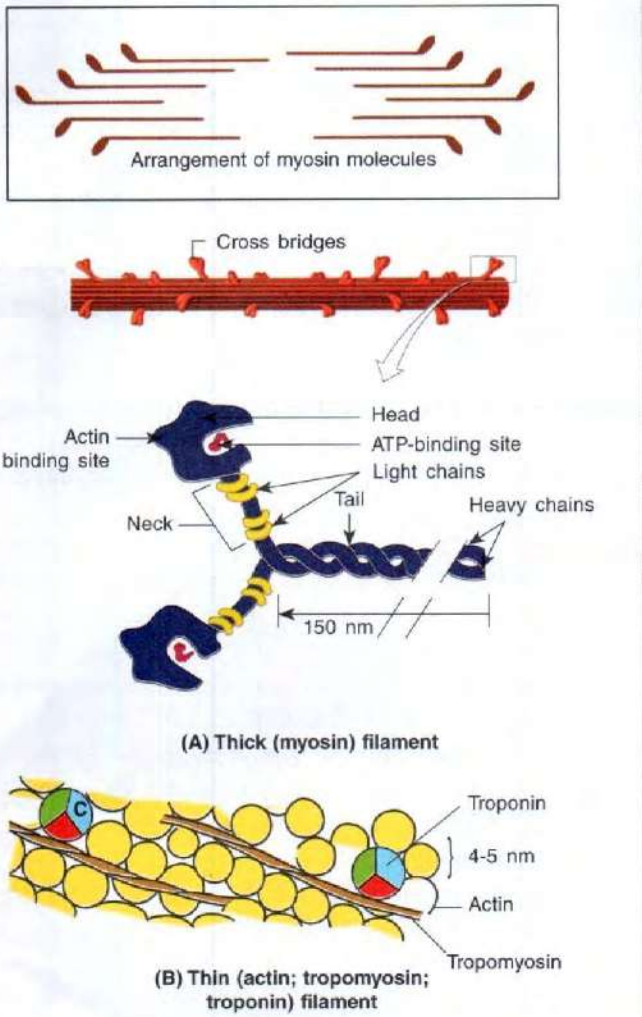


Fig. 21.4 Electron microscopic structure of thick and thin filaments

molecular weight 60,000. Each molecule binds one molecule of ATP firmly.

(ii) *'F' Actin (Fibrous Actin)*: Formed by polymerization of 'G' actin molecule with liberation of energy.

G-actin ATP → F-actin ADP + inorganic PO_4^{3-} + Energy (7 Kcal).

Note

Actinin, Titin **and** ***Desmin*** **are important structural muscle proteins;** ***Actinin*** **binds the Z-lines to actin;** ***Titin*** **connects Z-lines to the M-line and** ***Desmin*** **binds the Z-lines to the cell membrane. These protein together forms the** ***cytoskeleton*** **of the muscle cell (page 10)**

2. ***Tropomyosin:*** Tropomyosin molecules (molecular weight 70,000) are long filaments located in the groove between two chains in the actin. It covers the binding site of actin where myosin head comes in contact with actin *i.e.* prevents the interaction between actin and myosin filaments.
3. ***Troponin:*** Troponin molecules are small globular units located at intervals along the tropomyosin molecules. It is made up of 3 subunits with molecular weight ranging from 18,000 to 35,000.
   (i) *Troponin 'T':* binds the other troponin components to tropomyosin.
   (ii) *Troponin 'I':* inhibits the interaction of myosin with actin.
   (iii) *Troponin 'C':* contains binding site for Ca^{2+} that initiates muscle contraction.

At rest, i.e. when the muscle is relaxed the thin filaments interdigitate with the thick (myosin) filaments only outside the 'H' band. *During muscle contraction,* the length of 'A' band (thick filament) remains constant, whereas 'Z' lines move close together.

D. SARCOTUBULAR SYSTEM

1. It is a highly specialized system of internal conduction of depolarization within the muscle fiber. It is made up of *T-system* (transverse tubular system) and a *Longitudinal Sarcoplasmic Reticulum.* **(Fig. 21.2: D)**
   (i) ***T-System or Transverse Tubular System***
      (a) They are inwardly directed extensions of the sarcolemma into the muscle fibers at the junction between 'A' and 'I' bands (A-I junction), therefore, each sarcomere has 2 tubules. Their lumina (30 nm wide) are thus in continuity with the ECF which surrounds the muscle fibrils. Its function is the rapid transmission of the action potential from the cell membrane to all the fibrils in the muscle.
      (b) Depolarization of the T-tubule membrane activates the longitudinal sacroplasmic reticulum (see below) via ***dihydropyridine receptors.*** These receptors are voltage gated Ca^{2+} channels in the T-tubule membrane and are so called because they get blocked by the drug dihydropyridine. These receptors *serve as voltage sensor* and cause release of Ca^{2+} from the longitudinal sarcoplasmic reticulum. **(Fig. 21.5)**
   (ii) ***Longitudinal Sarcoplasmic Reticulum***
      (a) Longitudinally on either side of the tubular system are found the vesicle or sacs of the dilated longitudinal sarcoplasmic reticulum. These sacs are named ***Terminal Cisternae,*** which are rich in glycogen and Ca^{2+}. It is concerned with Ca^{2+} movements and muscle metabolism.

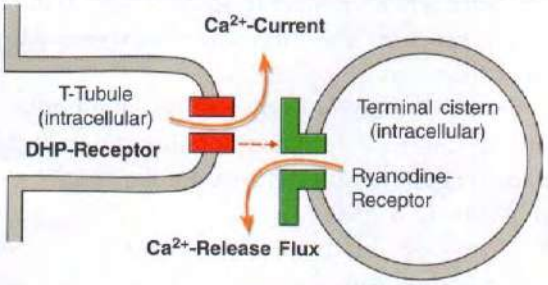


Fig. 21.5 Dihydropyridine (DHP) and ryanodine receptors

(b) The Ca^{2+} channels in the longitudinal sarcoplasmic reticulum are **not** voltage gated and allow the release of Ca^{2+} into the cell. These are called *ryanodine receptors*, as they are kept in the open position by ryanodine, a plant alkaloid.

2. There is a close proximity *i.e.* ***contiguity*** (and not the continuity) of the terminal cisternae and the T-system. Transverse tubule with two terminal cisterns is called the ***Triad*** structure which is found at the A-I junction and hence there are two *triads* per sarcomere. Triad structures are most extensive in muscles which contract and relax very rapidly.

ELECTRICAL PHENOMENON AND IONIC FLUXES IN SKELETAL MUSCLE FIBER

The electrical events and distribution of ions across the muscle fiber membrane in skeletal muscle are similar to those in nerve fiber (page 38). The differences in values between the two are given in the **Table 21.2**.

CONTRACTILE RESPONSE

The skeletal muscle is contractile in nature *i.e.* it contracts when excited. How?

Excitation (depolarization) of muscle fiber causes development of action potential (*Electrical phenomenon*) which through sequence of events produces muscular contraction (*Mechanical phenomenon*). These two phenomena are *coupled* (linked) together by coupling agent *i.e.* Ca^{2+}. Therefore, the process by which depolarization of the muscle fiber initiates contraction is called ***Excitation contraction coupling***.

Sequence of events in excitation contraction coupling

A. Steps in muscular contraction

1. Stimulation of motor nerve with threshold intensity produces propagated action potential.
2. Release of Acetylcholine (A-ch) into *synaptic cleft* which binds with "nicotinic A-ch receptors" concentrated on motor end plate causing generation of end plate potential.
3. Depolarization of muscle membrane (sarcolemma) by increasing its permeability to Na^+.
4. Generation of action potential in the muscle fiber.
5. Inward spread of action potential along T-system.
6. Spread of depolarization to terminal cistern with release of Ca^{2+} in the myofibrils.
7. Increase in concentration of Ca^{2+} in ICF by 2000 times *i.e.* from 10^{-7} moles/L to 2×10^{-4} moles/L.
8. *Role of calcium*

(i) Ca^{2+} binds to troponin C to saturation point causing tropomyosin to move laterally. This exposes the

Table 21.2: Electrical phenomenon in skeletal muscle fiber and nerve fiber compared

	Features	Skeletal Muscle Fiber	Nerve Fiber
1.	Resting Membrane potential	–90 mV	–70 mV
2.	Initial excitation threshold potential level	30-40 mV	15 mV
3.	Magnitude of action potential	120-130 mV	100-105 mV
4.	Duration of spike potential	2-4 msec.	Variable; 0.4 to 2 msec.
5.	Absolute refractory period	1-3 msec.	0.4-2 msec.
6.	Maximum number of impulses which can pass	100-200/sec.	1000/sec.
7.	Threshold of electrical stimulus	More	Less
8.	Conduction velocity of action potential	5 mts/sec. (that of a smallest myelinated nerve fiber)	Variable; (directly proportional to the diameter of nerve fiber)
9.	Chronaxie (to compare excitability)	Longer	Shorter
10.	Equilibrium potential		
	(i) Na^+	+65 mV	+60 mV
	(ii) K^+	–95 mV	–90 mV
	(iii) H^+	–32 mV	–25 mV
	(iv) Cl^-	–90 mV	–70 mV
	(v) HCO_3^-	–32 mV	–25 mV

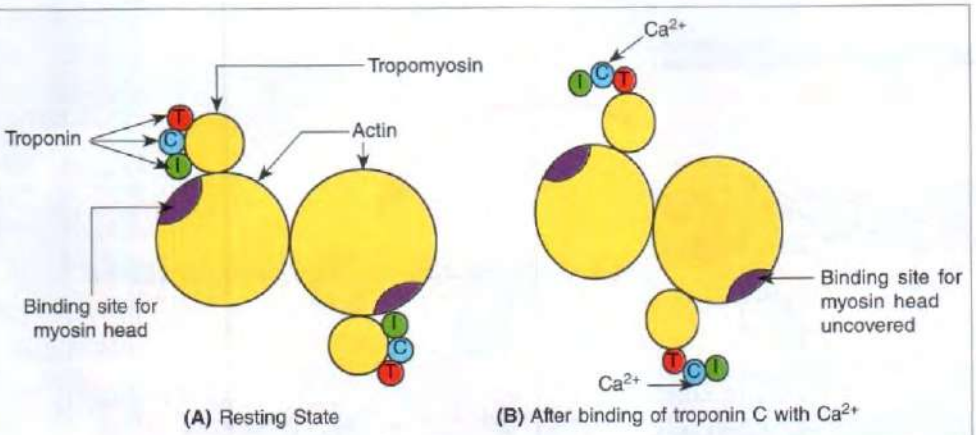


Fig. 21.6 Configuration change in thin filament

binding sites for myosin heads on actin. **(Fig. 21.6)**

(ii) Activates prosthetic group of myosin filament which acts as enzyme (ATPase) catalyzing the breakdown of ATP to produce energy for: (a) contraction of actomyosin complex; and (b) activating sliding filament system.

9. Muscular contraction.

B. Steps in muscular relaxation

1. A few milliseconds after the action potential is over, sarcoplasmic reticulum begins to reaccumulate Ca^{2+}. The Ca^{2+} ions are 'actively' pumped by *Ca^{2+}- Mg^{2+} ATPase* (*i.e. Ca^{2+} pump*) into longitudinal portion of the reticulum and from there discharges into the terminal cistern for storage.
2. Once Ca^{2+} concentration decreases in ICF sufficiently to 10^{-7} moles/L, chemical interaction between myosin and actin ceases and muscle relaxes.

Important Notes

1. If the active transport of Ca^{2+} is inhibited, relaxation does not occur even though there is no action potential. This results in sustained contraction of the muscle, called *Contracture*. A phenomenon commonly seen when the fibrous tissue replaces the muscle fibre during denervation atropy (page 171)
2. It is thus obvious from above that both contraction and relaxation of muscle are active processes and require energy which is provided mainly by ATP.

Molecular Basis of Muscle Contraction

Sliding filament theory *of A.F. Huxley and H.E. Huxley* (not related) – 1964.

The process by which the shortening of the contractile elements in the muscle is brought about is the sliding of actin filament over the thick (myosin) filaments. The sliding of filaments is brought about by interaction between the ***Cross-bridges*** of myosin and actin molecules. The force of contraction is developed by the cross-bridges in the overlap region between actin and myosin **(Fig. 21.7 and 21.8)**

Events during formation of the cross-bridges

1. In resting muscle, troponin 'I' is lightly bound to actin and tropomyosin covers the actin sites where myosin heads bind to actin. Therefore, the *"troponin-tropomyosin" complex* constitutes a ***Relaxing Protein*** which inhibits the interaction between actin and myosin.
2. Ca^{2+} released from the terminal cisterns by the action potential binds to troponin 'C' and causes:
   (i) the binding of troponin 'I' to actin is weakened, this permits tropomyosin to move laterally, uncovering the binding sites for myosin heads on actin filaments, and
   (ii) hydrolysis of ATP by ATPase activity in the myosin heads to produce energy.
3. Seven myosin binding sites on actin filaments are uncovered for each molecule of troponin that binds a Ca^{2+}.
4. The cross-bridges of the myosin molecules (which serve as the *cross linkages*) link to the actin at 90 degree angle, produce movement of myosin on

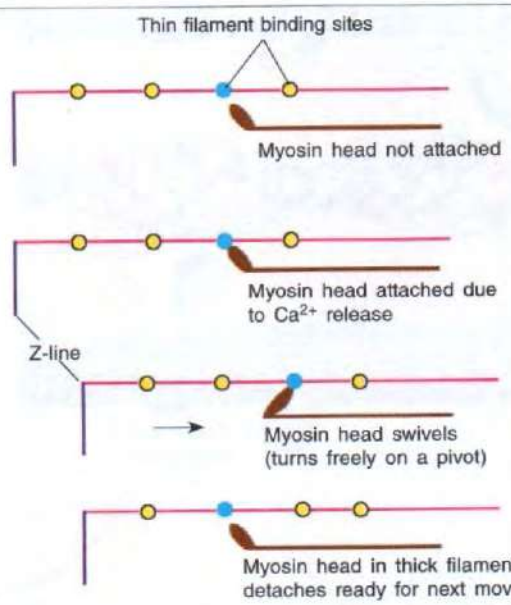


Fig. 21.7 Mechanism of interaction between cross bridges of myosin head and actin filament

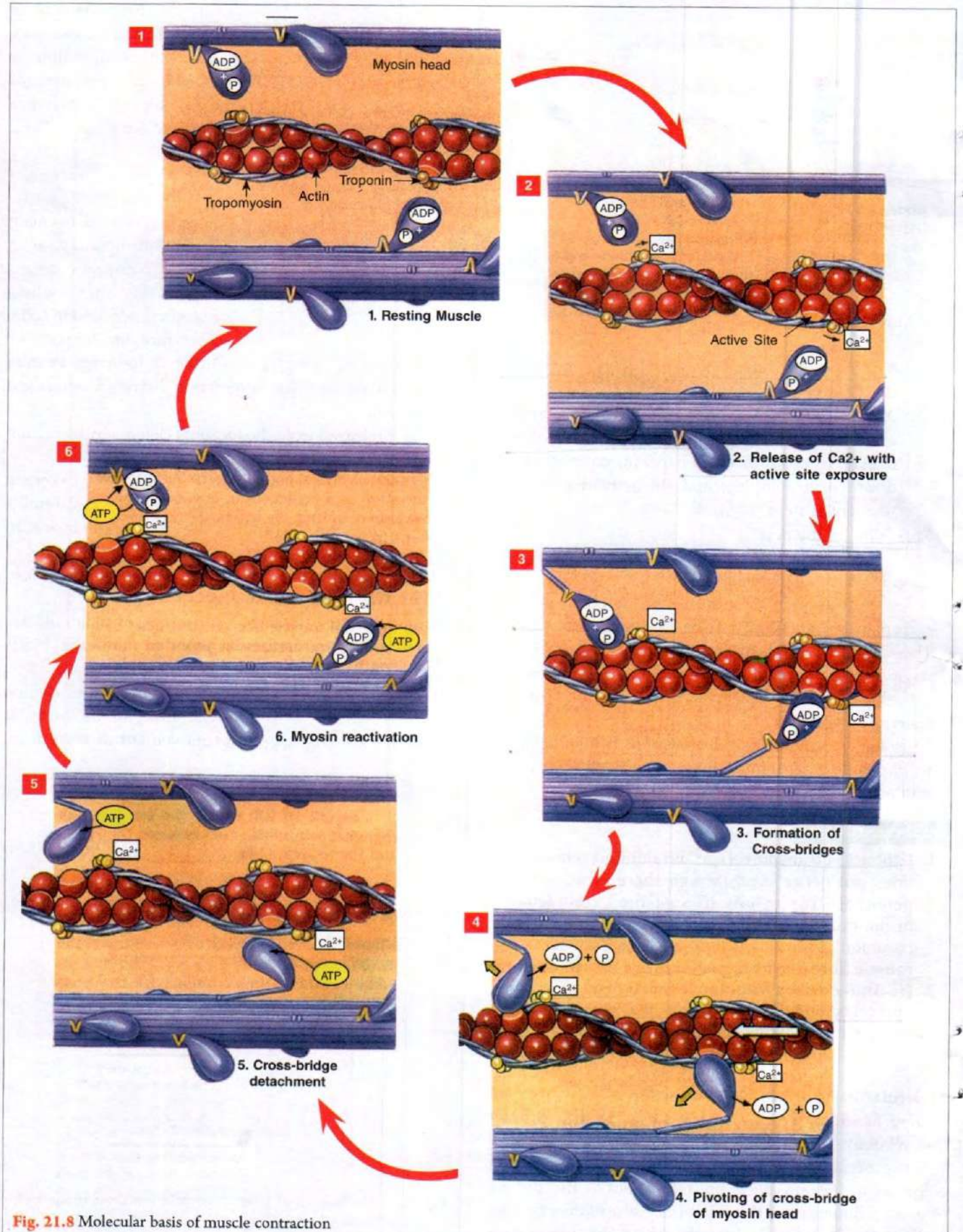


Fig. 21.8 Molecular basis of muscle contraction

actin by swiveling (revolving *i.e.* turns freely on a point), and then disconnect and reconnect at the next linking site **(Fig. 21.8)**.

Mechanism of sliding of thin filaments over thick filaments

1. The sliding during muscle contraction is produced by breaking and re-forming of cross-bridges of cross-linkages between actin and myosin, repeating the process in serial fashion for 5-6 times.
2. The width of 'A' bands is constant, whereas the 'Z' lines move closer together when the muscle contracts and farther apart when it is stretched. As the muscle shortens, the actin filament from the opposite end of the sarcomere approach each other; when the shortening is marked, these filaments apparently overlap.

Important Note

A single action potential causes the release of a standard amount of Ca^{2+} from the terminal cistern and produces a single muscle twitch (SMT). If the muscle is stimulated repeatedly more Ca^{2+} is released from the terminal cistern and intracellular Ca^{2+} concentration increases. Thus the muscle does not relax, (*Tetanus* is a state of sustained contraction of the muscle).

MODE OF CONTRACTION –ISOMETRIC AND ISOTONIC CONTRACTION

General

1. Muscle consists of *Contractile Component (CC)* which represents elasticity of thick and thin filaments. It comprises 3/5th of the total muscle protein **(Fig. 21.9)**.
2. The contractile component is:
   (i) in parallel with elastic component which represents the elasticity of the structural elements *e.g.* connective tissue sheath etc. – called *Parallel Elastic Component (PEC);*
   (ii) in series with another elastic component which represents elasticity of tendon etc. – called *Series Elastic Component (SEC).*

   'PEC' and 'SEC' together comprise 2/5th of the total muscle protein.
3. Resistance to stretch is mainly due to 'SEC' and some by 'PEC'. The myofibrils ('CC') themselves act only as a passive viscous element and have very little resistance to stretch.
4. Since muscle is not a complete elastic component, therefore it does not obey the Hook's Law; thus, when a resting muscle is stretched the tension produced is not proportional to the stretch applied.
5. ***Optimum Length*** is the length of the muscle at which it develops maximum *active tension.*
6. ***Resting Length*** of the muscle is the muscle length at which it is present under natural conditions in the body in relaxed state. The length of many of the muscles in the body at rest is the *optimum length.*
7. ***Equilibrium length*** is the length of the relaxed muscle cut free from its bony attachments.
8. ***Initial length*** is the length of the muscle before it contracts.

A. ISOMETRIC CONTRACTION

Isometric means same measure or length.

An isometric contraction occurs when the ends of the muscle do not move during contraction *i.e.* although muscle contracts, but length of the muscle remains the same and ***no external work is done***. (*i.e.* contraction that creates force without moving a load)

(i) *How muscle length remains the same?* Any shortening in muscle length which occurs due to contraction of contractile component (thick and thin filaments) gets compensated by stretching of series elastic

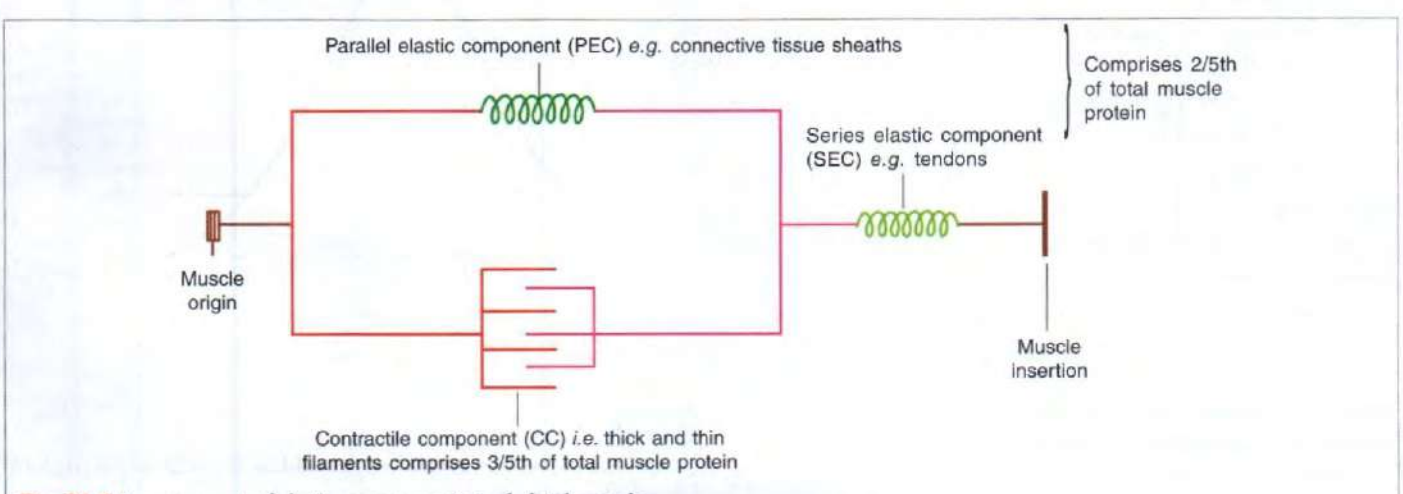


Fig. 21.9 Arrangement of elastic components in skeletal muscle

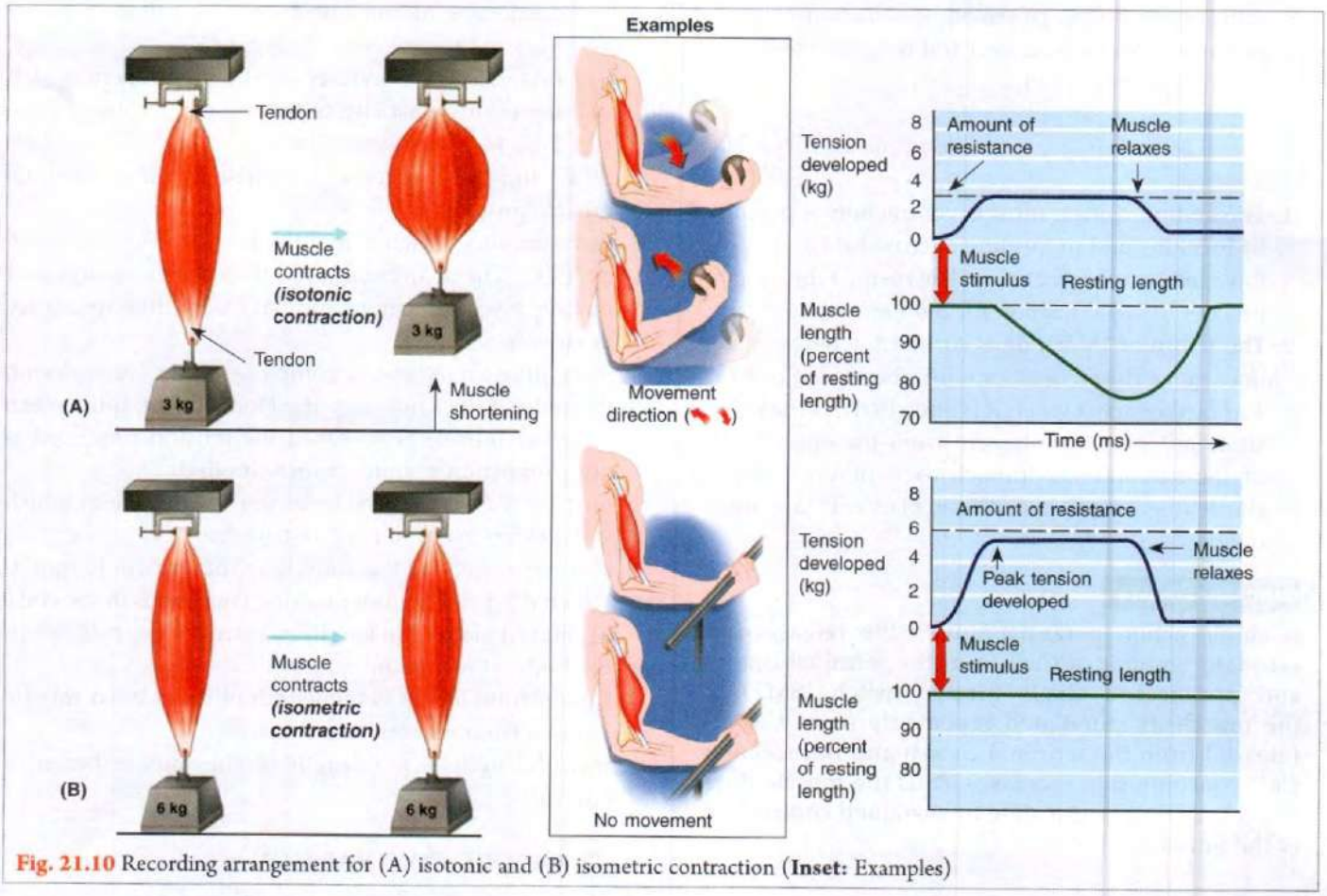


Fig. 21.10 Recording arrangement for (A) isotonic and (B) isometric contraction (**Inset:** Examples)

component, therefore, length of the muscle remains constant but the tension increases. **(Fig. 21.10)**

(ii) *Why no external work is done?* Since work done = force (tension) × distance.

As distance through which weight is moved is very small in this situation, therefore, very little external work is done by the muscle (which is negligible).

Examples:

(1) muscle which helps in maintaining posture against gravity;

(2) contraction of the arm muscles when trying to push a wall.

(3) If you pick up the weights and hold them stationary in front of you.

Experimentally, isometric contraction is studied by keeping both ends of the muscle fixed with the help of *isometric instrument;* the tension (force) developed by the muscle during isometric contraction at different muscle length can be measured or recorded with force transducer. **(Fig. 21.10)**

Relation between muscle length and tension (Length-Tension Relationship) (Fig. 21.11)

1. When a muscle is stretched *passively i.e.* without stimulation, by applying weights to its lower end, the tension developed in the muscle is called the ***passive (or resting) tension***. It is due to the stretching of parallel and series elastic components (PEC and SEC).

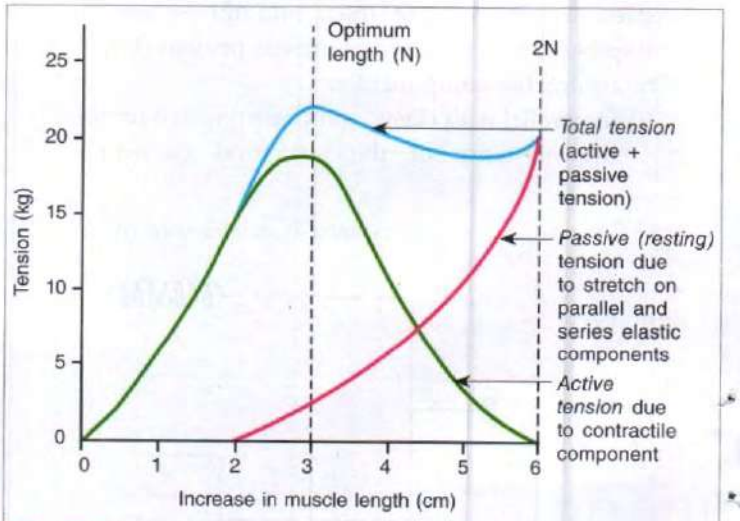


Fig. 21.11 Length tension relationship in skeletal muscle

Note

Optimum length is the length of the muscle at maximum number of cross-bridge overlap.

2. On stimulation, contractile component (CC) is transformed into an active structure which converts chemical energy into mechanical work, while the characteristics of PEC and SEC are unchanged during contraction. Therefore, the *total tension* developed after stimulation will be the *active tension* (due to presence of CC) plus the *passive tension* (due to presence of PEC and SEC).
3. *Active tension* can be indirectly determined by subtracting the passive (resting) tension from the 'total' tension.
4. Alter (increase or decrease) the muscle *'initial' length* (see above) and measure the passive tension; then stimulate with maximum intensity of current and measure the total tension developed. Plot a graph between muscle length and tension. The following observations can be made:
   (i) As muscle length increases passively, 'passive' tension increases.
   (ii) When increase in muscle length is twice the 'optimal' length, then contractile tissues can no longer contract (*i.e.* active tension becomes zero) and 'total' tension developed will be equal to the 'passive' tension.
   (iii) If muscle length is increased thrice the 'equilibrium' length, rupture of the muscle occurs.
   (iv) As Active tension = Total tension minus Passive tension, therefore, when muscle length decreases less than the 'optimal' length and is stimulated, 'passive' tension fails to develop, because there is no stretch on the muscle, then the *total* tension recorded after stimulation is the *active* tension alone.
   (v) From the graph, it is apparent that the *'active' tension is maximum when muscle is in 'optimal' length i.e. the length at which the muscle is present under natural conditions at rest in the body* (page 167).

Molecular basis of "length – tension relationship"

When muscle fibers contract isometrically, tension developed is proportionate to the number of cross-linkages beween the actin and myosin molecules. At an initial sarcomere length of 2.5 μm, the formation of cross bridges between the head of myosin and actin molecules is maximum (Fig. 21.12). Therefore,

1. When a muscle is stretched, the overlap between actin and myosin is reduced and number of cross-linkages between two proteins is reduced. This decreases the development of active forces.
2. When the muscle is shorter than the *resting length*, the thin filaments overlap and this also reduces the number of cross-linkages between the two proteins.

Important Note

The performance of contractile component is given by load (or force) - velocity relationship (Refer to Fig. 22.8 and page 181)

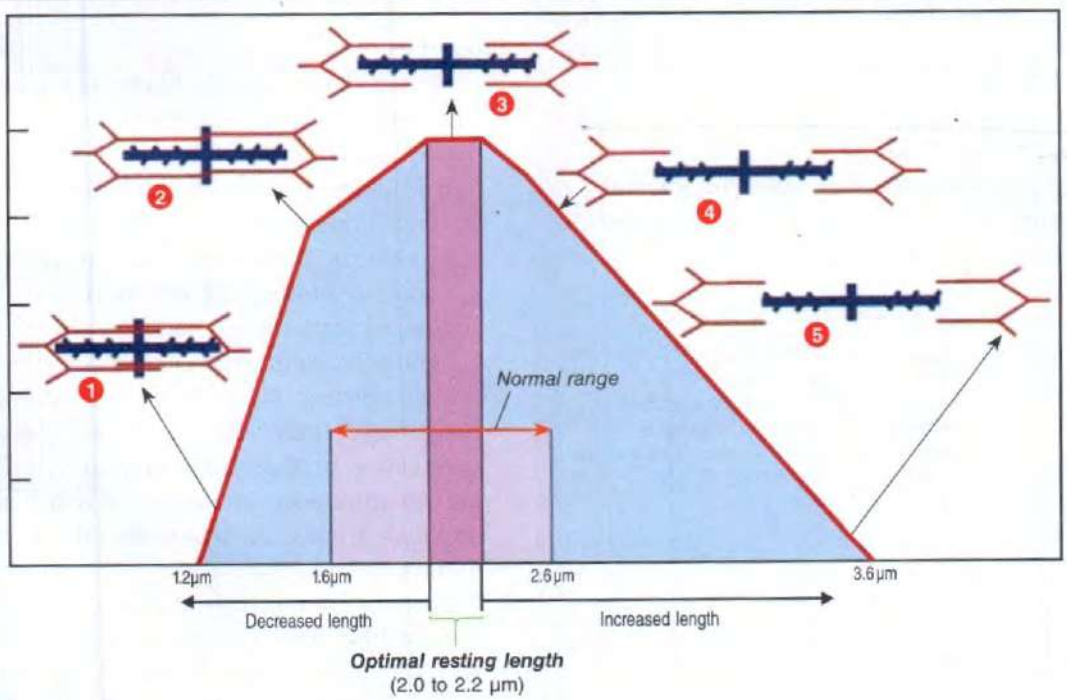


Fig. 21.12 Effect of sarcomere length and amount of '***myosin - actin overlap***' on the active tension developed. The sequence **(1-5)** represents the force developed as the sarcomere length is increased. **Note** that maximum tension develops at position **(3)** (*i.e.* at *Sarcomere length 2.0 to 2.2 μm*) when maximum cross-linkage between actin filament and cross-bridges of myosin head is possible.

B. ISOTONIC CONTRACTION

Isotonic means same tension.

An isotonic contraction occurs when a muscle shortens without a change in its tension, *i.e.* the length of the muscle changes while tension remains constant, therefore, ***external work is done*** (*i.e.* any contraction that creates force and moves a load). This form of muscle work is carried out when the legs are moved in walking and running or while lifting a load.

Experimentally, isotonic contraction is studied when muscle is subjected to ***'after load' preparation,*** *i.e.* weight will act only when whole muscle begins to shorten **(Fig. 21.10)**. Therefore, 'initial' muscle length is the same, whatever weight is hung from the lever. On stimulation, the muscle contracts, contractile component shortens and stretches series elastic component, therefore, muscle tension increases (*i.e.* the initial portion of the contraction is 'isometric'). When the force developed by the muscle just exceeds the effect of weight, the muscle as a whole begins to shorten, thereafter the tension in the muscle remains constant (isotonic) throughout the remainder of the shortening and the contraction recorded is due to shortening of the contractile component alone.

The work done by the muscle is the product of force exerted (weight lifted) and the distance moved (shortened).

The isotonic contraction varies with the magnitude of the *after load.* The effects of increase in after-load are:

(1) Duration of initial part of isometric contraction increases, because the series elastic component must stretch more to transmit the force required to lift the greater 'after load'.

(2) Velocity of shortening (as given by *Load-velocity relationship*, **Fig. 21.13**) decreases, because:
   (i) formation of cross-linkage takes longer, and
   (ii) 'opposing forces' *i.e.* forces which oppose muscle shortening, increase.

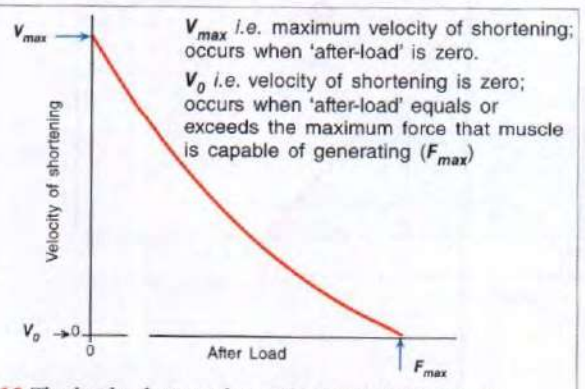


Fig. 21.13 The load-velocity relationship in skeletal muscle

PROPERTIES OF MOTOR UNIT

Definition

Each ventral horn cell along with its motor nerve (efferent fiber) is called the ***Motor Neuron***. Each single motor neuron and all the muscle fibers it supplies constitutes a ***Motor Unit*** **(Fig. 21.14)**. This is the smallest part of the muscle that can be made to contract independently.

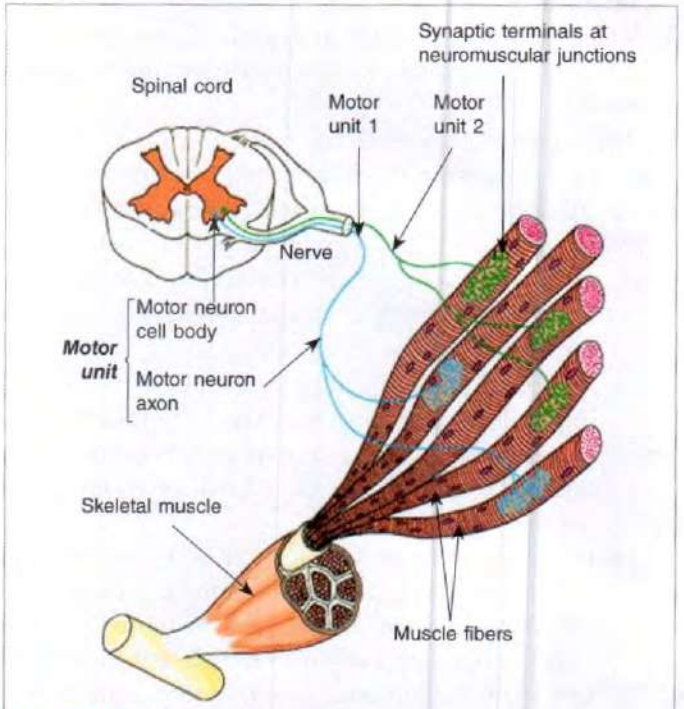


Fig. 21.14 Motor unit(s)

Properties

1. The number of muscle fibers in a motor unit varies inversely with the precision of movements performed by the part, therefore
   (i) Muscles concerned with fine graded, precise movements *e.g.* muscles of hand and those concerned with eye movements, there are 3-6 muscle fibers per motor unit.
   (ii) In leg muscles and back muscles, there are 120-165 muscle fibers per motor unit.
2. *All the efferent fibers passing to skeletal muscles are* ***excitatory.*** There are no efferent nerves which on stimulation produce relaxation of the muscle *i.e.* there are no inhibitory efferents (as compared to cardiac and smooth muscles where the efferent supply is both excitatory and inhibitory).
3. Each spinal motor neuron innervates only one kind of muscle fiber, therefore, *all the muscle fibers in a motor unit are of same type.* These are of two types, 'red' (Type I) and 'white' (Type II) muscle fibers. (Differentiating features, Refer to **Table 21.3**.)

Table 21.3: White and red muscle fibers compared (Also see to page 379)

White (fast) muscle fibers (Type II)	Red (slow) muscle fibers (Type I)
1. Here muscle fibers are large in diameter with high glycogen capacity and ATPase activity; because they are pale due to less amount of myoglobin content, therefore, called 'white' muscle fibers.	These muscle fibers are of moderate diameter and moderate glycogen capacity with low ATPase activity; because they are darker than other muscle fibers, therefore, called 'red' muscle fibers.
2. They are innervated by large, fast conducting motor neurons (***size principle*** neurons), *i.e.* having 50 times the contractile force of the smallest units, therefore, also called 'fast' muscle fibers.	They are innervated by small, slow conducting motor neurons, therefore, also called "slow" muscle fibers.
3. Their muscles have short twitch durations (7.5 msec) and are *specialized for fine rapid and skilled movements e.g.* extraocular muscles and muscles of the hand.	These muscles respond slowly (twitch duration 100 msec. and have long latency and are *adapted for long (sustained), slow, posture maintaining contractions e.g.* long muscles of limb and muscles of the back.
4. These muscles get *fatigued easily (less vascular, fewer mitochondria).*	These muscles are *resistant to fatigue* and most used muscles *(Highly vascular, abundant mtochondria).*
5. They are particularly suited for high intesity workbouts that can be sustained for only short period time.	They are required to perform work when endurance type activities are performed *i.e.* to perform low-intensity work over long periods of time such as athletes, running bicyclist and swimmers.

Important Note

The percentage of fast and slow muscle fibre within each muscle is genetically determined *i.e.* one is born with a certain ratio of fast and slow fibres. This ratio is more or less fixed and cannot be changed with physical training. However, there is a *selective hypertrophy* of fast and slow fibers, depending upon the types of training and activity programme used.

4. Depending on the type of muscle fibers they innervate, motor units are of 2 types, *'slow'* and *'fast'* motor units.
5. *Recruitment of motor units.*
   In normal individuals, at rest, there is a slow, asynchronous discharge by the motor units (*i.e.* out of phase with each other). This results in little (if any) spontaneous activity in the skeletal muscle. With minimal voluntary activity a few motor units discharge and with increasing voluntary efforts more motor units are brought into play. This process is called *Recruitment of Motor Units.* *Gradation of muscle response* (page 156), therefore, depends on:
   (i) Number of motor units activated at a time during any reflex or other kind of the act.
   (ii) Frequency of discharge in individual nerve fibers *i.e.* duration for which motor units are being stimulated. This causes gradual development of tension in a muscle fiber to the maximum.
   (iii) *'Resting' length* of the muscle (page 167).

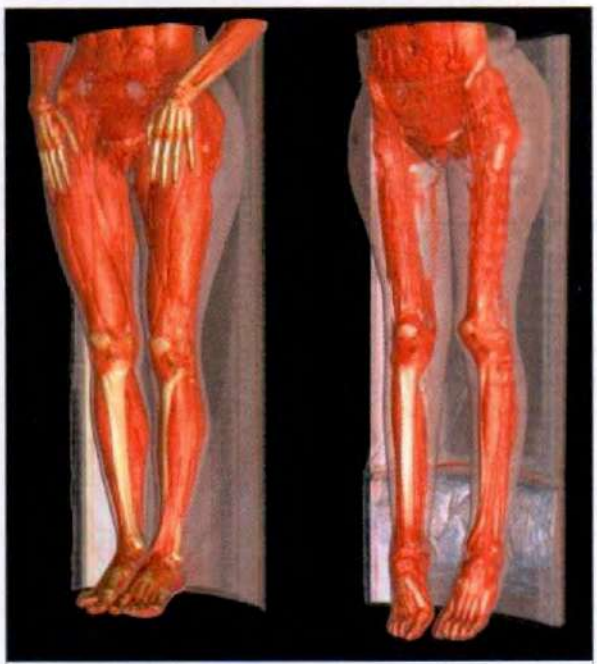
Applied Aspect

1. When the motor nerve to a skeletal muscle is cut it causes:
   (i) disuse 'atrophy' of the muscle *i.e.* shrinkage of muscle fibers, which finally gets replaced by fibrous tissue *(fibrous muscle);*
   (ii) complete paralysis of the muscle, called *flaccid paralysis;*
   (iii) appearance of fine, irregular contractions of individual fibers, called *fibrillations.*
   (iv) abnormal excitability of the muscle and increases its sensitivity to circulating A-ch *(**Denervation hypersensitivity**)* (also see to page 189).

   These effects are the classical consequences of a ***lower motor neuron lesion (LMNL)*** *i.e.* injury to the spinal and/or cranial motor neurons which directly innervate the muscles (for details, refer to page 914).
2. ***Muscular Dystrophy***
   It is a syndrome characterized by progressive muscle weakness due to mutation in ***dystrophin*** gene, which results in congenital defect in ***dystrophin-glycoprotein complex***. This is a large protein complex that connects the thin filaments to the transmembrane protein *β-dystroglycan* in the sarcolemma and thus provides support and strength to the myofibrils.
   Forms of muscular dystrophy
   (i) *Duchenne's Muscular Dystrophy,* a X-linked disease in which *dystrophin is absent* from the muscles. It manifests as great generalised muscle disability and finally death due to involvement of the myocardium (heart muscles). It is usually fatal by the age of 30 years. **(Fig. 21.15)**

(ii) *Becker's Muscular Dystrophy*, a milder form of disease in which *dystrophin is either present in small amounts or gets altered.*

3. ***Myotonia***. A condition characterized by difficulty and slowness in relaxing muscle after voluntary effort. Various forms of myotonia defined clinically are due to abnormal genes on 7, 17 or 19 chromosomes, which result in abnormalities of Na^+ or Cl^- channels.
4. ***Metabolic Myopathies***. *McArdle's syndrome* (Refer to page 601).



Normal | Duchenne's muscular destrophy

Fig. 21.15 Muscular dystrophy

ENERGY SOURCE FOR MUSCULAR CONTRACTION

Salient features

1. Muscle contraction

requires energy. The only immediate source of this energy is adenosine triphosphate (ATP).

$$ATP \longrightarrow ADP + Pi + \text{Free energy for biological work}$$

2. This ATP must be continually renewed. How? In muscle cells, ATP is produced at rest and during *light* exercise, mainly because of oxidation of stored fat in the form of "free fatty acids". As intensity of exercise increases, fats alone cannot supply energy fast enough and so much of the energy comes from the breakdown (oxidation) of glucose and its stored form, 'glycogen' ***(Aerobic glycolysis).***

$$ADP + Pi + \underset{\text{(carbohydrates and fats)}}{\text{Energy from reserve fuel}} \longrightarrow ATP$$

3. With strong muscular contractions (*e.g.* during heavy exercise), this process of aerobic glycolysis may be inadequate. There are three further processes which can take place to provide energy.

(A) Oxygen Supply

Glycogen requires oxygen for its oxidation. Muscle contains a red-pink pigment, *myoglobin* which is similar to haemoglobin. Myoglobin forms complexes with oxygen and acts as a store. When the concentration of oxygen in the cells is low, myoglobin releases its oxygen.

(B) Anaerobic Metabolism

In addition to getting ATP from the complete oxidation of glycogen, a muscle cell can break down further glycogen part of the way, with the formation of lactic acid. This reaction does not need oxygen and is called ***anaerobic glycolysis.***

Important Notes

(i) Aerobic breakdown of glucose results in 19 times more ATP production per glucose molecule than does anaerobic glycolysis (38 ATP versus 2 ATP).

(ii) *Aerobic breakdown of 6-C glucose molecule yields 38 ATP, whereas aerobic breakdown of 18-C fatty acid molecule yields 147 ATP.*

(C) Storage of High Energy Phosphate

In a single muscle fiber there is comparatively little ATP storage. It can, however, be regenerated quickly from high energy ***creatine phosphate***, also called ***phosphocreatine***, which is stored in large amounts.

$$\text{ADP} + \underset{\text{phosphate}}{\text{create}} \underset{\text{at rest or during muscle relaxation}}{\overset{\text{During exercise}}{\rightleftharpoons}} \text{ATP} + \text{creatine}$$

This reaction takes place when ATP levels are low. It can continue until all the creatine phosphate is used up. It is later restored by means of the 'converse' reaction during muscle relaxation; some ATP in the mitochondria transfers its phosphate to creatine.

4. **Applied Aspect**

When muscle fibers are completely depleted of ATP and phosphocreatine, they develop a state of extreme rigidity called ***rigor***. When this occurs after death, the condition is called ***rigor mortis***. In rigor, almost all of the myosin heads attach to actin but in an abnormal, fixed and resistant way.

5. The ***efficiency of muscle*** when it contracts is about 25%.

Mechanical efficiency

$$= \frac{\text{Output}}{\text{Input}}$$

$$= \frac{\text{Work done (W)}}{\text{Oxygen consumption } (V_{O2})}$$

$$= \frac{\text{W (Kilo pond meter/min} \div 426.7)}{V_{O2}\text{ (L/min)} \times 5}$$

[Since 426.7 kpm/min = 1 kcal, and 1 L/min V_{O_2} at STPD produces 5 kcals of energy]

In an isotonic muscle contraction, approx. 25% of the energy expended by the muscle is used to do work. The remaining 75% is degraded as heat. During an isometric muscle contraction, there is no external work done and it could be said that 100% of the energy expended by the muscle disappears as heat.

Study Questions

1. **Give physiological significance of:**
   (i) Sarcomere
   (ii) Initial, resting, optimal and equilibrium length of the muscle
   (iii) Active, passive and total tension in skeletal muscle
2. **Draw well labelled diagrams of:**
   (i) Length tension relationship in skeletal muscle
   (ii) Load velocity relationship in skeletal muscle
   (iii) Electron microscopic structure of thick and thin filaments
   (iv) Sarcotubular system
   (v) Isotonic and isometric muscle contraction
   (vi) Thick and thin filaments
   (vii) Motor unit
   (viii) Arrangement of elastic element in skeletal muscle
3. **Write briefly about:**
   (i) Sarcotubular system
   (ii) Sequence of events in excitation contraction coupling
   (iii) Role of Ca^{2+} in muscle contraction
   (iv) Cross bridges
   (v) Motor unit and its significance
   (vi) Factors determining gradation of muscle response
   (vii) Denervation hypersensitivity in skeletal muscle
   (viii) Energy source for muscular contraction and efficiency of muscle
   (ix) Muscular dystrophy
   (x) Contracture
   (xi) Rigor and rigor mortis
4. **What are modes of contraction in a skeletal muscle. Give suitable examples.**

MCQs

1. **Contractile unit of muscle is the portion of myofibril between:**
   (a) A and H band
   (b) Z line and A band
   (c) Two adjacent Z lines
   (d) A and I band
2. **Sarcotubular system:**
   (a) Exists in the kidney
   (b) Comprises proximal and distal convoluted tubules
   (c) Made up of T-tubules and sarcoplasmic reticulum
   (d) Comprises smooth and rough endoplastic reticulum
3. ***True* about skeletal muscle contractile response is:**
   (a) Contraction precedes the action potential
   (b) Decrease in Ca^{2+} concentration within the cell to 2×10^{-4} moles/L produces muscle relaxation
   (c) Both contraction and relaxation of muscle are active process
   (d) Ca^{2+} ions passively reaccumulate into terminal cisternae during muscular relaxation
4. **The troponin-tropomyosin complex is believed to play what role in muscle contractile process?**
   (a) It provides the major amount of elastic tension during the contractile process
   (b) It is believed that in the resting state it covers the active sites on the actin filament
   (c) Combination of this complex with myosin excites the activity of "power stroke"
   (d) Combination of potassium with the troponin portion of this complex is believed to trigger muscle contraction
5. **The length of the muscle at which it develops maximum active tension is called:**
   (a) Optimum length
   (b) Resting length
   (c) Equilibrium length
   (d) Initial length
6. **The muscles typically exhibiting isometric contractions are:**
   (a) Extraocular
   (b) Respiratory
   (c) Antigravity
   (d) Masticatory
7. **Tension developed in a muscle due to activation of contractile component is called:**
   (a) Passive tension
   (b) Active tension
   (c) Total tension
   (d) None of the above

8. **The muscle ruptures when it is stretched at least .... times its equilibrium length:**
   (a) 2 (b) 3 (c) 5 (d) 10
9. **An isotonic contraction differs from an isometric contraction in that in isotonic contraction:**
   (a) Muscle is less efficient (b) External work is done
   (c) Heat of activation is greater (d) Recovery heat is reduced
10. **The motor unit is:**
   (a) Muscle fibre and neurons supplying it (b) Ventral horn cells along with its motor nerve
   (c) Single motor neuron and all the muscle fibers it supplies (d) Single muscle fiber with its nerve
11. **Best method to increase the muscle strength is:**
   (a) Isometric exercises (b) Isotonic exercises (c) Aerobic isotonic exercises (d) Electrical stimulation
12. **Fast twitch skeletal muscle fibers differ from slow twitch muscles in that former:**
   (a) Have low ATPase activity
   (b) Are adapted for posture maintaining contraction
   (c) Contain more sarcoplasmic reticulum with high glycogen capacity
   (d) Resistant to fatigue
13. **Muscle fatigue is due to:**
   (a) Long latency (b) Low ATPase activity
   (c) Slow contraction of a muscle (d) Inadequate supply of ATP
14. **Duchenne's muscular dystrophy, *correct* statement is:**
   (a) A mild form of disease (b) Dystrophin is present in small amounts
   (c) Manifest as great generalised muscle disability (d) Characterized by slowness in relaxing muscle after voluntary effort
15. **Slow relaxation of muscle is known as:**
   (a) Myokinesia (b) Myotonia (c) Muscular dystrophy (d) Muscle spasm
16. **Immediate energy source for muscle contraction is:**
   (a) GTP (b) Adenosine triphosphate (c) Lactic acid (d) Creatine phosphate
17. **The chemical energy of foodstuff that can be converted into work under optimal conditions is:**
   (a) 10% (b) 25% (c) 50% (d) 75%
18. **What is *not true* of rigor mortis?**
   (a) Depleted ATP and phosphocreatine stores (b) Muscle becomes extremely rigid and contracted
   (c) Occurs after death (d) Calcium ions concentration within the muscle cells increases
19. **Sarcomere refers to the portion of myofibril between:**
   (a) A and H band (b) Z line and A band (c) Two adjacent Z lines (d) A and I band
20. **When skeletal muscle shortens in response to stimulation, there is:**
   (a) Decreased width of I and H bands (b) Decreased width of A band
   (c) Decreased width of A and I bands (d) Increased width of H zone
21. **At rest, thin filaments interdigitate with thick filaments:**
   (a) Upto the A-I junction (b) Upto the pseudo H-band
   (c) Only outside the H-band (d) Upto the M-line
22. **Ryanodine receptor controls uptake of:**
   (a) Calcium by sarcoplasmic reticulum (b) K^+ by sarcoplasmic reticulum
   (c) Na^+ by mitochondria (d) Mg^{2+} by nucleus
23. **Equilibrium length is the length of muscle:**
   (a) At which it develops maximum active tension (b) At which it is present under natural conditions in the body
   (c) Cuts free from its bony attachments (d) Before it contracts
24. **The length-tension diagram in a skeletal muscle shows a peak of maximum tension occurring with sarcomere length of:**
   (a) 0.1 – 1.1 μm (b) 1.1 – 2.0 μm (c) 2.2 – 2.5 μm (d) 3.0 – 3.5 μm
25. **Slow muscle fibers have:**
   (a) Decreased endoplasmic reticulum (b) Decreased calcium stores
   (c) Decreased glycogen (d) Increased mitochondria

Answers

1. (c) 2. (c) 3. (c) 4. (b) 5. (a) 6. (c) 7. (b) 8. (b) 9. (b) 10. (c) 11. (a) 12. (c) 13. (d) 14. (c) 15. (b)
16. (b) 17. (b) 18. (d) 19. (c) 20. (a) 21. (c) 22. (a) 23. (c) 24. (c) 25. (d)

Chapter

22

Cardiac Muscle

I. Morphological properties

II. Electrical properties

III. Mechanical properties

IV. Metabolic properties

MORPHOLOGICAL PROPERTIES

A. GENERAL FEATURES

1. Cardiac muscle is an *involuntary, striated* muscle. The individual muscle cell is 100 µm long and 15 µm broad. The fibers are branched and interlock freely with each other, but each is a complete *unit* surrounded by a cell membrane, *sarcolemma*. **(Fig. 22.1)**
2. At the point of contact of two muscle fibers, extensive folding of cell membrane occurs, called ***intercalated discs***. They provide a strong union between fibers, so that the pull of one contractile unit can be transmitted along its axis to the next, thereby help in increasing force of contraction.
3. ***Gap junction*** (nexus, page 10), a specialized intercellular junction is present in the intercalated disc along the sides of the adjacent myocardial cells. Here ions, electrical currents and other molecules can be transferred from one cell to other without coming in contact with the ECF. Thus, they provide ***low resistance bridges*** for the spread of excitation from one muscle fiber to the next and permit cardiac muscle to function as if it were a ***functional syncytium*** (*i.e.* a single cell).
4. There are two such separate *syncytia* in the heart. The *atrial* and *ventricular* syncytia, connected with each other by A-V bundle. Each syncytium obeys ***all or none*** law.
5. The cardiac muscle fibers are highly vascular *i.e.* surrounded by a very rich capillary network. They show well developed sarcoplasmic reticulum with plenty of cytoplasm, mitochondria and rich in glycogen.
6. The muscle fibers are made up of many fibrils (myofibrils), each of which is 1-2 µm in diameter, lie parallel to one another and are *striated*.

B. LIGHT MICROSCOPIC APPEARANCE

C. ELECTRON MICROSCOPIC APPEARANCE

'B' and 'C' same as in skeletal muscle (pages 161-162).

D. SARCOTUBULAR SYSTEM

Like the skeletal muscle, sarcotubular system is *well developed* in the cardiac muscle but 'T-system' penetrates the sarcomere at Z-line. Therefore, in cardiac muscle, there is *only one 'triad' per sarcomere.*

Note

in skeletal muscle there are two 'triads' per sarcomere (page 164).

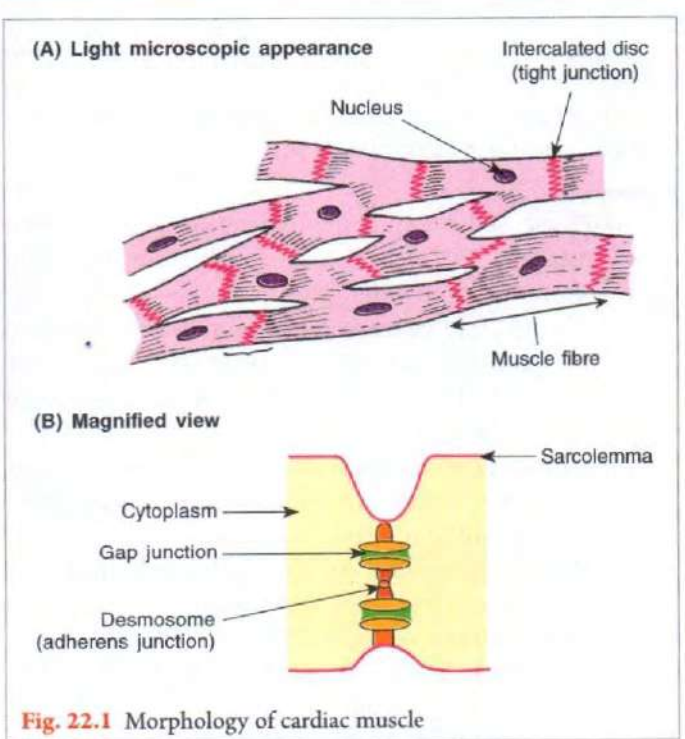


Fig. 22.1 Morphology of cardiac muscle

ELECTRICAL PROPERTIES

It includes:
A. Excitability
B. Autorhythmicity, and
C. Conductivity

A. EXCITABILITY

Cardiac muscle is excitable, *i.e.* it forms a wave of depolarization (excitation impulse) in response to a stimulus. The *'extracellular' recording* of the electrical events generated with each heart beat is called ***Electrocardiogram*** *(ECG)* (page 291).

Characteristic features as obtained with *intracellular recording* include:

1. At rest, myocardial fibers (atrial and ventricular) show a resting (polarised) membrane potential (RMP) of approx. –90 mV (negative inside with reference to outside).
2. On stimulation there occurs: **(Fig. 22.2)**

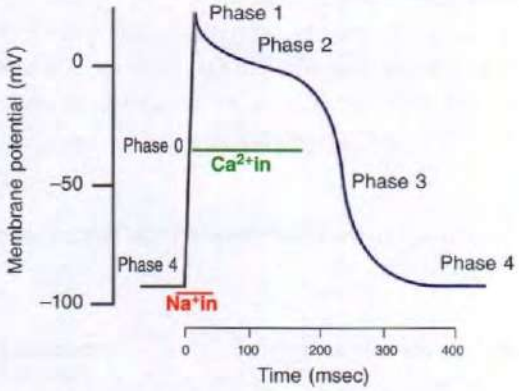


Fig. 22.2 Changes in ion permeability (conductance) during the action potential in cardiac muscle fiber

(i) ***Phase "0"*** – Rapid depolarization and potential reaches +20 to +30 mV (positive inside with reference to outside). This is due to:
   (a) 100 fold increase in Na^+ permeability resulting in Na^+ influx, which appears when membrane potential is –60 mV; but it is short-lived and self-limiting;
   (b) marked increase in Ca^{2+} permeability causing Ca^{2+} influx which appears at membrane potential of –30 to –40 mV.

   Depolarization lasts for approximately 2 msec and is followed by slow repolarization.

(ii) Repolarization occurs in 3 phases:
   (a) ***Phase 1***: A *rapid initial fall* from +30 mV to –10 mV due to:
      - 5 fold increase in K^+ permeability causing K^+ efflux (probably caused by excess Ca^{2+} influx).
      - The Na^+ permeability is rapidly reduced secondarily to Na^+ channel closure and stops at zero potential.
   (b) ***Phase 2:*** A *plateau phase* in which the membrane potential falls slowly only to –40 mV due to:
      - inactivation of Na^+ influx which starts appearing at zero potential, and
      - Ca^{2+} influx and K^+ efflux continue at a slow rate.
   (c) ***Phase 3***: A *rapid fall* during last stage in which membrane potential falls to the resting value of –90 mV due to inactivation of Ca^{2+} and Na^+ influx with rapid K^+ efflux.

(iii) ***Phase 4***: *Polarised state.* During this phase, though RMP is achieved yet resting ionic composition is restored by the activation of $Na^+ - K^+$ pump.

The duration of action potential (primarily repolarization) is 250 msec at a heart rate of 75 beats per minute; it decreases to 150 msec at a heart rate of 200 beats/min.

B. AUTORHYTHMICITY

1. The heart continues to beat for quite some time even after all nerves to it are cut or even if it is cut into pieces. This is because of the presence of the specialized *'pacemaker' tissue* in the heart that can initiate repetitive action potentials.

   'Pacemaker' tissue includes *sinu atrial node* (SAN); *atrio ventricular node* (AVN); *atrio ventricular bundle* and *purkinje fibers.*
2. The 'pacemaker' tissue is characterized by ***unstable resting membrane potential*** because of the continuous change in membrane permeability; therefore, membrane potential declines steadily after each action potential until firing level is reached and another action potential is triggered. This slow depolarization between action potential is called ***pre-potential*** or ***'pacemaker potential'*** or *diastolic depolarization.* **(Fig. 22.3)**
3. The property of spontaneous pre-potential followed by action potential is called ***autorhythmicity*** and is characteristic of the pacemaker tissue.
4. The ion channels in the cell membrane of pacemaker tissues appear to open and close spontaneously.
   (i) Once membrane potential achieves a *threshold value* of –40 mV, there occurs a rapid depolarization due to:
      (a) increase in *long lasting* Ca^{2+} influx–***iCa(L)*** (mainly), and
      (b) increase in Na^+ influx (to little extent).
   (ii) *Repolarization* starts with K^+ efflux ***(ek)*** and membrane potential falls rapidly towards the baseline.

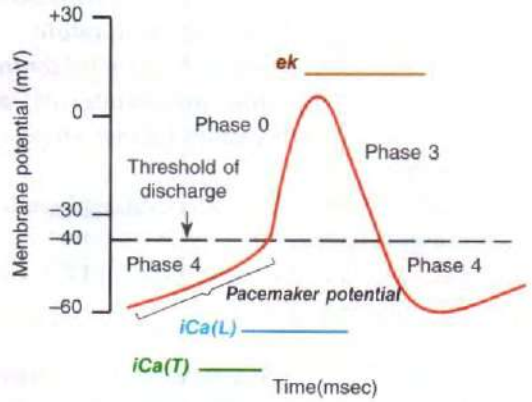


Fig. 22.3 Membrane potential of pacemaker tissue (SAN) (Abbreviations as given in the text)

(iii) *Pre-potential*: It is primarily due to slow decrease in K^+ efflux while permeability of other ions remains constant. Decrease in K^+ efflux with *transient* increase in Ca^{2+} influx–***iCa(T)*** completes the pre-potential.

Since increase in Na^+ influx contributes little to depolarization, therefore, depolarization is not sharp. This slow response is especially characteristic of SAN and AVN.

Important Note

Atrial and ventricular muscle fibers do not have pre-potentials, since K^+ permeability is constant during diastole and they discharge spontaneously only under abnormal conditions.

5. RMP in SAN is –50 mV, and in other pacemaker tissues is approx. –90 mV; whereas normal rate of rise of pre-potential in SAN is 15 to 60 mV/sec., and that in AVN and purkinje cells is slower. Therefore, the ***threshold excitation*** *is achieved earliest in SAN*. Action potential thus generated in SAN is conducted to the other cardiac cells and arrives there before their own diastolic depolarization to threshold is reached.

6. Normally the SAN generates impulses regularly at an interval of approx. 0.8 sec *i.e.* @ 75 beats/min, causing the normal sequential rhythmic beat of the various parts of the heart.

Applied Aspect

(i) *If the natural pacemaker of SAN is destroyed, then the next fastest latent pacemaker, often the AVN takes over. Rhythmic rate of AVN is 40-60 beats/min, and that of purkinje fibers is 15-40 beats/min.*

(ii) *The various chemical agents which alter the heart rate, do so by changing the slope of pre-potential. The steeper is the slope of pre-potential, the faster is the rate at which the pacemaker fires* **(Fig. 22.4)**.

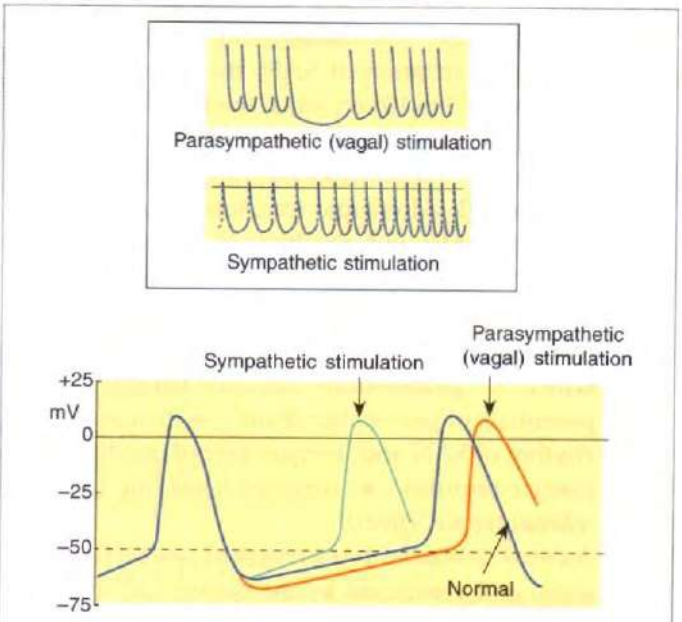


Fig. 22.4 Effect of sympathetic and parasympathetic stimulation on the pacemaker potential (**Inset** shows mechanical response)

Summary—Types of Action potential in cardiac muscle fiber **(Refer Table 22.1).**

Factors affecting membrane potential

1. ***Acetyl choline (A-ch) or vagal stimulation***; acts via

Table 22.1: Types of cardiac action potentials

Fast response	Slow response
1. *Site:* Atria, ventricles and Purkinje fibers	SAN; AVN
2. *RMP:* –90 mV. It can convert into slow response RMP in patients with coronary artery disease.	–50 to –90 mV
3. *Action potential:* (i) '0' phase—slope of upstroke, amplitude and extent of overshoot is greater. Rate of rise of overshoot is faster. (ii) Repolarization is slow and shows three phases (phase 1, 2 and 3)	(i) '0' phase has slower velocity. (ii) Phase 2 (plateau) is absent; Phase 3, velocity is more gradual; Phase 4, is characterized by pacemaker potential.
4. *Conduction velocity:* fast	Slow

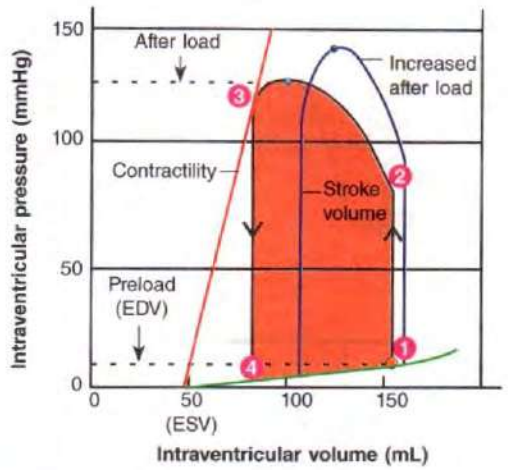


Fig. 22.9 *Pressure-volume loop in the left ventricle*
(Step 1 → 2 Isovolumetric contraction;
2 → 3 Ventricular ejection;
3 → 4 Isovolumetric relaxation;
4 → 1 Ventricular filling
ESV and EDV: End systolic and end diastolic volume;
For details refer to page 286)

the force of contraction of the ventricular muscle fibers is directly proportional to its initial length *i.e.* larger the initial length of the cardiac muscle fibers, greater will be the force of contraction of the ventricles. This is known as the ***Frank-Starling Law*** of the heart (*Otto Frank and Ernest Starling E.H.*, 1910). The increased force of contraction is probably caused by the fact that actin and myosin filaments are brought to a more nearly optimal degree of interdigitation for achieving contraction.

Limitations of Frank-Starling Law

As the muscle is stretched, the developed tension increases to a maximum and then declines as stretch becomes more extreme, because at such extreme stretch the actin and myosin filaments of cardiac muscle fibers are actually pulled apart. Such extreme stretching of ventricular muscle fibers occurs when heart rate increases to 150 beats/min, thereby decreasing duration of systole and this increases EDV.

Significance of Frank-Starling Law

(i) The law helps us to explain that blood ejected by each of the ventricle per heart beat is the same. If RV output per beat exceeds LV output, this will cause accumulation of blood in LV; the Frank-Starling law begins to operate causing more complete evacuation of LV. Thus, the output of two ventricles becomes the same again.

(ii) *It is a life saving device in cardiac failure.* How? Left ventricular failure (LVF) causes accumulation of blood within LV, thereby decreases blood supply to vital organs. However, accumulation of blood in LV initiates operation of Frank-Starling mechanism leading to greater output by LV. If accumulation of blood is too great, the Frank-Starling law will fail to operate, decreasing blood supply to vital organs and finally leads to death.

2. ***Effect of After-load*** on the force of contraction of cardiac muscle.
After-load (resistance) is low in pulmonary artery due to its intra-thoracic location. It is high in the aorta due to resistance to blood flow through the aortic valves and systemic blood vessels, called *peripheral resistance*. Therefore,
(i) When the pressure against which the heart is pumping the blood is raised, the heart puts out less blood than it receives for several beats. Blood accumulates in the ventricles and the size of the heart increases. The distended heart beats more forcefully and the output returns to its previous level.
(ii) Conversely, when the resistance is reduced, output rises transiently but the size of the heart decreases and the output falls to the previously constant level.

B. ALL OR NONE LAW

All or none relationship between the stimulus and the response is called *All or none law* (page 40). Responses in cardiac muscle are also "all or none" in character; this is because of the syncytial and interconnecting nature of cardiac muscle fibers. The "all or none" principle, however, applies to the whole of the functional syncytium in the heart, the unit being the entire atria or entire ventricle.

C. REFRACTORY PERIOD (Page 40)

1. Cardiac muscle is refractory *i.e.* non-responsive to re-stimulation during most part of action potential. Normal refractory period of ventricles is 250-300 msec and that of atria is 150 msec, that is why the rhythmic rate of contraction of atria can be much faster than that of ventricles.
2. Normal duration of *absolute refractory period* (ARP) is 180-200 msec and that of *relative refractory period* (RRP) is upto 50 msec (page 180).

Significance

(i) Since contractile response is more than half over during ARP, hence summation of contractile response is not possible *i.e.* ***cardiac muscle cannot be tetanized***. This is a protective mechanism helpful in proper blood flow to the heart.
(ii) If the ventricle is stimulated with a stimulus stronger than the normal during RRP, it responds

by contracting prematurely. This is called ventricular *extrasystole*.

D. STAIRCASE EFFECT (TREPPE)

Treppe, *German word means staircase.*

When the cardiac muscle begins to contract after a brief period of rest *e.g.* following vagal stimulation, its initial strength of contraction increases to a plateau, a phenomenon called *staircase effect* or *treppe.* **(Fig. 22.10)** It is due to:

During stoppage of the heart there occurs increase in Na^+ and decrease in K^+ concentration inside the cell, this increases Ca^{2+} influx. Therefore, progressive increase in Ca^{2+} concentration in the sarcoplasm due to increase in Ca^{2+} influx with each action potential causes progressive increase in strength of cardiac muscle contraction.

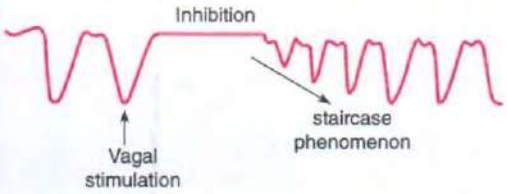


Fig. 22.10 Staircase effect

METABOLIC PROPERTIES

Salient features of cardiac muscle metabolism are:

1. Abundant blood supply. Blood flow through the myocardium is very high, 80 mL/100 gm/min; compared to skeletal muscle blood supply, 3 mL/100 gm/min.
2. Presence of numerous mitochondria (power generating units) in cardiac muscle fibers.
3. High content of ***myoglobin,*** an O_2 storing muscle pigment.
4. Normally, less than 1% of the total energy liberated is provided by anaerobic metabolism *i.e.* heart normally works under complete aerobic condition with essentially no accumulation of lactic acid.
5. Under basal (resting) conditions, of the total caloric needs of the heart (i) 60% is provided by fats; (ii) 35% by carbohydrates and (iii) 5% by ketones and amino acids.

Important Note

Circulating free fatty acids, accounts for approx. 50% of fat utilized. However, proportions of substrates utilized vary greatly with the nutritional state. For example:

(i) After ingestion of large amounts of glucose, more lactic acid and pyruvic acid are used.

(ii) During prolonged starvation, more fats are used.

The unique properties of the cardiac muscle enable it to function throughout the life of an individual without showing any sign of fatigue.

Note

Maximum efficiency of the normal cardiac muscle is 20–25%, page 173)

Study Questions

1. **Give physiological significance of:**
   (i) Intercalated disc
   (ii) Gap junction
   (iii) Low resistance bridges
   (iv) Long refractive period in cardiac muscle
   (v) Functional syncytium
   (vi) A-V nodal delay
2. **Draw well labelled diagrams:**
   (i) Types of action potential in cardiac muscle cell
   (ii) Pacemaker potential
   (iii) Correlation of mechanical and electrical events in cardiac muscle
   (iv) Normal spread of electrical activity in the heart
   (v) Effect of pre-load and after-load on myocardial contractility
   (vi) Force velocity relationship in cardiac muscle
   (vii) Effect of stimulation of sympathetic and parasympathetic nerve on pacemaker potential
3. **Write short notes on:**
   (i) Pacemaker potential
   (ii) Calcium rigor
   (iii) Idioventricular rhythm
   (iv) Frank-Starling law of heart and its significance
   (v) Length-tension relationship in cardiac muscle
4. **Give the mechanism of action with effect of following agents on the heart:**
   (i) A-ch
   (ii) Epinephrine
   (iii) K^+
   (iv) Ca^{2+}

5. **What determines myocardial contractility. Mention factors affecting it.**
6. **Give physiological basis of:**
   (i) SAN generate impulses at the fastest rate as compared to other pacemaker tissues.
   (ii) Cardiac muscle shows no sign of fatigue.
   (iii) Atrial and ventricular muscles do not show autorhythmicity.
7. **What will happen and why?**
   (i) If natural cardiac pacemaker get destroyed
   (ii) If hyperkalemia develops
   (iii) If AV node get destroyed

MCQs

1. **Intercalated discs:**
   (a) Provide a strong union between the myocardial fibres
   (b) Provide low resistance bridges for the spread of excitation from one cardiac muscle fiber to another
   (c) Permit cardiac muscle to function as a syncytium
   (d) Are also known as gap junctions
2. **Calcium enters the cardiac muscle at a very rapid rate when membrane potential falls to:**
   (a) –70 mV (b) –55 to –70 mV (c) –40 to –55 mV (d) –30 to –40 mV
3. **Long plateau phase of action potential in myocardial fibers is due to:**
   (a) Increased Na^+ conductance (b) Inactivation of Ca^{2+} and Na^+ influx
   (c) Decreased K^+ conductance (d) Increased K^+ and Ca^{2+} conductance
4. **Pacemaker potential is:**
   (a) Characterized by unstable RMP (b) Also called spike potential
   (c) Largely due to increased membrane permeability to K^+ (d) Characteristic of atrial and ventricular muscle fiber
5. **In pacemaker tissue of heart, after the impulse, action potential comes back to firing level due to:**
   (a) Increase in Na^+ permeability (b) Decrease in K^+ permeability
   (c) Increase in Ca^{2+} efflux (d) Increase in K^+ efflux
6. **Sinoatrial node generates the impulses at the fastest rate as compared to other pacemaker tissue because:**
   (a) It is located in the right atrium where pressure changes are minimum
   (b) Of larger diameter of its fibers
   (c) Its RMP is close to threshold excitation
   (d) Of better neural control
7. **Increase in K^+ concentration in ECF leads to death due to:**
   (a) Kidney failure (b) Decreased contractility of myocardium
   (c) Vasomotor centre (VMC) failure (d) Peripheral circulatory failure
8. **Delay in conduction of heart impulses maximally occurs at:**
   (a) SA node (b) AV node (c) Bundle of His (d) Left bundle branch
9. ***Not true* about pre-load is:**
   (a) The load which acts on the muscle before it contracts (b) The degree to which myocardium is stretched before it contracts
   (c) Its extent in heart is determined by end diastolic volume (d) It causes the cardiac muscle to contract isotonically
10. **After load causes:**
    (a) Complete contraction of contractile element (CE) of the muscle with stretching of series elastic elements (SEE)
    (b) Complete contraction of CE of the muscle with no further stretching of SEE
    (c) Partial contraction of CE of the muscle with stretching of SEE
    (d) Partial contraction of CE of the muscle without stretching of SEE
11. **For cardiac muscle, V_{max} can be used as a measure of:**
    (a) Excitability (b) Contractility (c) Rhythmicity (d) Conductivity
12. **In the force-velocity relationship of cardiac muscle fibers, addition of catecholamines shows:**
    (a) An increase in maximal velocity of shortening of the muscle (V_{max})
    (b) An increase in maximal isometric force developed in the muscle (P_0)
    (c) An increase in both maximal velocity of shortening and isometric force developed in the muscle
    (d) A fall in both V_{max} and P_0.

13. **The Frank-Starling Law of the heart states that:**
    (a) Output of the heart is controlled almost entirely by the activity of the heart
    (b) Blood entering the atria is pumped immediately into the ventricles
    (c) Heart rate controls the output of the heart
    (d) Within physiologic limits, contraction of myocardium is a linear function of its resting length
14. **Cardiac muscle *cannot* be tetanized because:**
    (a) Heart has abundant blood supply
    (b) It has high myoglobin content
    (c) Contractile response is more than half over during the action potential
    (d) Less than 1% of total energy liberated is provided by aerobic metabolism
15. **Under basal conditions caloric need of heart is largely met by:**
    (a) Fat (b) Protein (c) Carbohydrates (d) Ketones
16. **The property of spontaneous pre-potential followed by action potential is characteristic of:**
    (a) Atrial muscle fibers (b) Ventricular muscle fibers (c) Papillary muscles (d) Purkinje fibers
17. **The depolarization time in atrial muscle is:**
    (a) 0.05 sec (b) 0.1 sec (c) 0.2 sec (d) 0.3 sec
18. **Duration of AV nodal delay is:**
    (a) 0.1 sec (b) 0.5 sec (c) 1 sec (d) 2 sec
19. **Which statement is *not true* about AV nodal delay?**
    (a) Enable atria to empty the blood within them into the ventricles before these contract
    (b) Protects the ventricles from being driven at excessive rates
    (c) AV nodal cells have long refractory period
    (d) Sympathetic stimulation increases AV conduction time
20. **The last part of the heart to be depolarized is the:**
    (a) Mid-portion of the interventricular septum from left to right
    (b) Uppermost portion of the interventricular septum
    (c) Mid-portion of the interventricular septum from right to left
    (d) Apex of both the ventricles

Answers

1. (a)	2. (d)	3. (d)	4. (a)	5. (b)	6. (c)	7. (b)	8. (b)	9. (d)	10. (b)
11. (b)	12. (c)	13. (d)	14. (c)	15. (a)	16. (d)	17. (b)	18. (a)	19. (d)	20. (b)

Chapter
23

Smooth Muscle

I. General features: single unit and multiunit smooth muscles.

II. Properties of visceral smooth muscle (electrical, mechanical-plasticity)
Effect of various agents on membrane potential of intestinal smooth muscle.

GENERAL FEATURES

1. *Involuntary* muscle, *i.e.* not under the control of will.
2. *Unstriated* (lacks visible cross striations), therefore also called ***plain muscle***.
3. Smooth muscle cells are smaller, spindle shaped with varying dimensions *e.g.* fibers in digestive tract are 30-40 µm long and 5 µm diameter; in blood vessels, 15-20 µm long and 2-3 µm diameter; uterus 300 µm long and 10 µm diameter.
4. In general, it contains few mitochondria and depends largely on glycolysis for its metabolic needs. Sarcoplasmic reticulum is poorly developed.
5. The *contractile units* are made up of small bundles of interdigitating thick and thin filaments that are irregularly shaped and randomly arranged. Z-lines are replaced by *dense bodies* in the cytoplasm and attached to the cell membrane, and these are bounded by *α-actinin* to actin filaments. Therefore, under electron microscopy, individual myofibrils are striated but striations do not form a regular pattern. *The thin filaments lack troponin in smooth muscle.*
6. The contractions in smooth muscle have a longer duration, they are more variable and they produce less tension than in skeletal muscle.
7. They are of 2 types: *single unit* and *multiunit* smooth muscles; the main differentiating features between the two are given in Fig. 23.1 and Table 23.1.

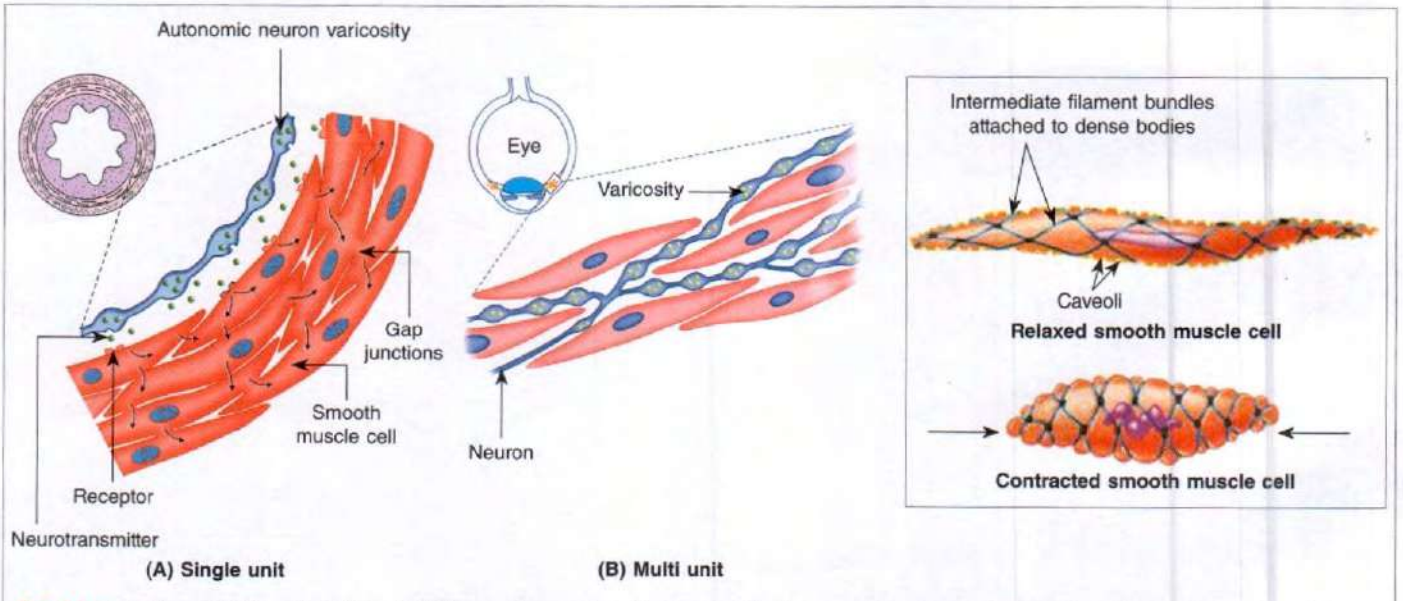


Fig. 23.1 Single unit (A) and Multi unit (B) smooth muscle cell. (**Inset:** Rhythmic contraction and relaxation of smooth muscle cells)

Table 23.1: Main differentiating features between single unit and multiunit smooth muscles

Single unit smooth muscle or Visceral smooth muscle	Multi unit smooth muscle
(i) It occurs in large sheets and has *low resistance bridges* between individual muscle cells; and function in a *syncytial* fashion, that is why called single unit smooth muscle.	It is made up of individual units without inter-connecting bridges *i.e. non-syncytial* in character. Therefore, its contractions are more discrete, fine and localized, and called multi unit smooth muscle.
(ii) *Most common Sites:* Wall of hollow viscera *e.g.* GIT; bile ducts; ureters; bronchi, uterus and urinary bladder. Also occur in some of the blood vessels.	Iris; ciliary muscle of eye; pilomotor muscle of skin, and muscles of blood vessels.
(iii) The muscles are characterized by appearance of spontaneous activity in certain areas, called *pacemakers*.	These muscles are richly innervated and each muscle fiber has its own nerve supply.
(iv) Rhythmic contraction and relaxation of these muscles is independent of their innervation. The nervous influence only modulates their activity.	These muscles only contract in response to a stimulus through their nerves by releasing chemical mediators at their endings (A-ch or Nor-epinephrine) to which they are very sensitive. Here single stimulus to the nerve causes repeated firing of action potential which *produces* ***irregular tetanic contractions*** rather than a single muscle twitch. (whereas in the skeletal muscle, a single stimulus causes generation of single action potential producing a single muscle twitch.)
(v) If these muscles are stretched, there is production of active tension.	These muscles do not respond to stretch.

Important Note

Vascular smooth muscle has properties of both single unit and multi-unit smooth muscle.

VISCERAL (or SINGLE UNIT) SMOOTH MUSCLE

A. ELECTRICAL PROPERTIES

1. These are characterized by the ***instability of its membrane potential***, that is, membrane potential has no true 'resting' value, being relatively low when the tissue is active and higher when it is inhibited (average RMP approx. –50 mV). RMP is low, because intracellular concentration of Na^+ and Cl^- is high and that of K^+ is relatively low.
2. *Initiation of spontaneous activity*: It begins with Ca^{2+} influx (mainly) and due to Na^+ influx. *Proof:* blockage of Na^+ influx does not affect the depolarization because the smooth muscle cell membrane has far more voltage-gated Ca^{2+} channels.

   Superimposed on the membrane potential are waves of various types **(Fig. 23.2)**:

   (i) ***Slow-sine wave like fluctuations*** (more common). These are few millivolts in magnitude and spikes that sometimes overshoot the zero potential line and sometimes do not. The spikes may occur on the rising or falling phases of sine wave oscillations. In many tissues spike duration is approx. 50 msec. In some tissues (such as uterus, ureters) action potential has a prolonged plateau during depolarization. This accounts for the prolonged contraction.

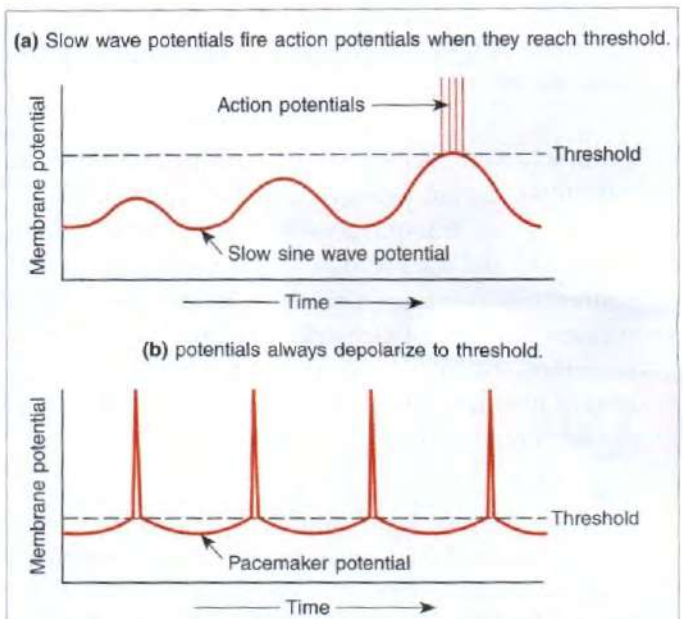


Fig. 23.2 Spontaneous electrical activity in visceral smooth muscle

(ii) ***Pacemaker potential*** (similar to those found in cardiac pacemaker). These potentials are generated in multiple foci that shift from place to place. Spike generated in the pacemaker foci are conducted for some distance in the muscle. Here action potentials are superimposed over the rising phase.

B. MECHANICAL PROPERTIES

1. It shows continuous irregular (asynchronous) contractions that are independent of its nerve supply. This maintained state of partial contraction is called ***Tonus or Tone***. It is *'myogenic'* in origin *i.e.* inherent property of the smooth muscle.
2. *Mechanical Events:* Because of continuous activity, it is difficult to study the exact relation between electrical and mechanical events. However, the muscle starts to contract approx. 200 msec after the start of the spike (*i.e.* 150 msec after the spike is over). The peak contraction is reached after 500 msec of the spike, therefore, *excitation contraction coupling is a very slow process*. This is due to slowness of attachment and detachment of the cross-bridges with the actin filament. **(Fig. 23.3)**

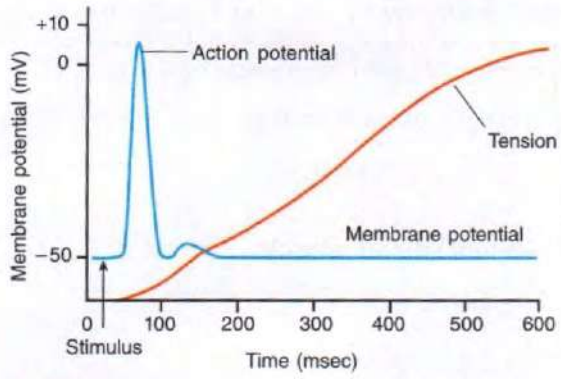


Fig. 23.3 Relation between action potential and tension in visceral smooth muscle

3. It can maintain prolonged tonic contraction for hour with little use of energy. The maximum force of contraction of smooth muscle is often greater than that of skeletal muscle due to the prolonged period of attachment of the myosin cross-bridges to the actin filament, called the ***Latch bridge mechanism***. This is of importance because visceral organs such as the GIT, urinary bladder, gall bladder etc. can maintain tonic muscle contraction almost indefinitely.

Note

In smooth muscle contraction is initiated by Ca^{2+} binds to calmodulin, and the resulting complex activates *calmodulin dependent myosin light chain kinase* (page 22). This enzyme catalyzes the phosphorylation of the myosin light chain to activate myosin ATPase, and actin slides on myosin producing contraction. Relaxation occurs when the Ca^{2+} - calmodulin complex dissociates.

4. *Unique feature of visceral smooth muscle*: It contracts when stretched in the absence of any extrinsic innervation. Stretch is followed by a decline in membrane potential, an increase in frequency of spikes and a general increase in tone.
5. *Length-tension relationship i.e.* relationship between "initial" length of muscle fiber and tension, called property of ***plasticity***.

Another special characteristic of smooth muscle is the *variability of the tension it exerts at any given length.* If a piece of visceral smooth muscle is stretched, it first exerts increased tension. However, if the muscle is held at the greater length after stretching, the tension gradually decreases. Sometimes the tension falls to a level below that exerted before the muscle was stretched. It is, therefore, impossible to correlate length and developed tension accurately and *no resting length can be assigned.* This property is referred as the *plasticity* of smooth muscle.

SUMMARY

Sequence of events in contraction and relaxation of visceral smooth muscle

A. Contraction

Binding of A-ch to muscarinic receptors
↓
Increase Ca^{2+} influx into the cell
↓
Binding of Ca^{2+} with Calmodulin (a regulating protein)
↓
Activation of calmodulin-dependent light chain kinase enzyme
↓
Phosphorylation of myosin head
↓
Activation of myosin ATPase
↓
Binding of myosin to actin
↓
Actin slides on myosin

B. Relaxation

Once $[Ca^{2+}]$ within smooth muscle cells fall
↓
Dephosphorylation of myosin by myosin light chain phosphatase enzyme
↓
Dissociation of Ca^{2+}-calmodulin complex
↓
Muscle relaxation
or
Muscle sustain contraction (due to myosin cross bridges remain attached to actin even after decrease in intracellular $[Ca^{2+}]$ (***Latch bridge mechanism***)

The *length-tension relationship* in visceral smooth muscle can be studied experimentally in intact human by recording the tension exerted by walls of the urinary bladder while it is filled with 50 mL increments of fluid (water or normal saline).

A catheter was inserted into a normal urinary bladder of a human subject and emptying it; then recording the pressure, while urinary bladder is filled with 50 mL increment of fluid. Immediately after each increment of fluid the tension was higher; but after a short period of time, it decreased. Therefore, the *"filling" curve* is not a smooth curve but a jagged line. After 700 mL had been infused into the bladder, the subject voided in 50 mL increments, and the tension was recorded after each increment. Plotting these tensions produced an *"emptying" curve* that was different from filling curve, again due to the absence of any constant relationship between fiber length and tension. **(Fig. 23.4)**

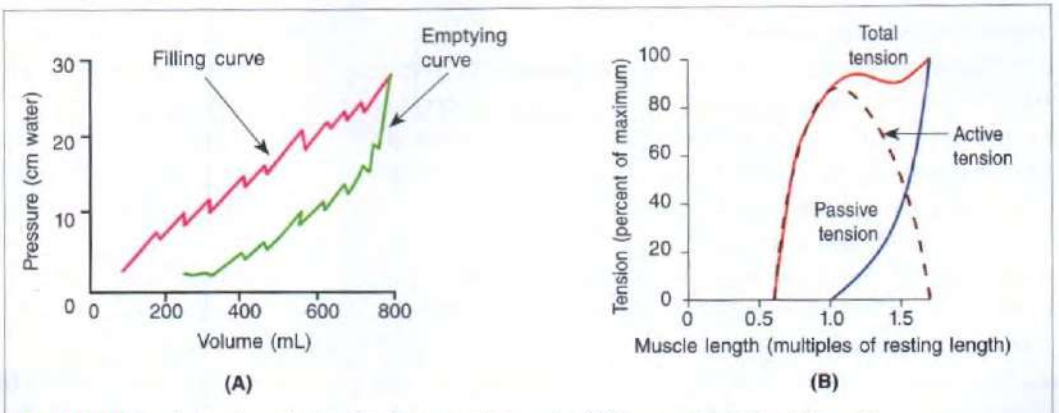


Fig. 23.4 Length-tension relationship in smooth muscle: (A) property of plasticity and (B) Graphical representation (Also refer to Fig. 21.11 on page 171)

Function of the Nerve Supply to Smooth Muscle

Smooth muscles receive dual nerve supply from two divisions of autonomic nervous system (ANS) (page 925). In some organs: adrenergic nerve stimulation increases, and cholinergic nerve stimulation decreases smooth muscle activity. In other organs reverse is seen.

In smooth muscle, nerve fibers are *not* ending in motor end plate. ANS fibers emerge out of spinal cord as preganglionic fibers. These fibers relay in the ganglia from which post ganglionic fibers arise which divide and run along the length of muscle fibers and groove it. During their course they show ***varicosities*** *i.e.* beaded appearance which contains the chemical transmitter **(Fig. 23.5)**. The varicosities and approximately 5 µm apart with upto 20,000 varicosities per neuron. Release of chemical transmitter acts on many muscle fibers causing activation of all muscle fibers upto where it is forming *syncytium*. Therefore, here single stimulus does not cause stimulation of all muscle fibers in the whole organ. Thus, repeated stimuli are needed to cause release of more chemical transmitter which stimulates all the muscle fibers.

Excitatory Junctional Potential (EJP)

In smooth muscles in which adrenergic discharge is excitatory, stimulation of the adrenergic nerve produces discrete partial depolarization that looks like small end plate potentials and are called ***EJPs***. These potentials summate with repeated stimuli. Similar ***EJPs*** are seen in tissues excited by cholinergic discharges.

Denervation Hypersensitivity

When the motor nerve to skeletal muscle is cut and allowed to degenerate, the muscle gradually becomes extremely sensitive to acetylcholine. This denervation

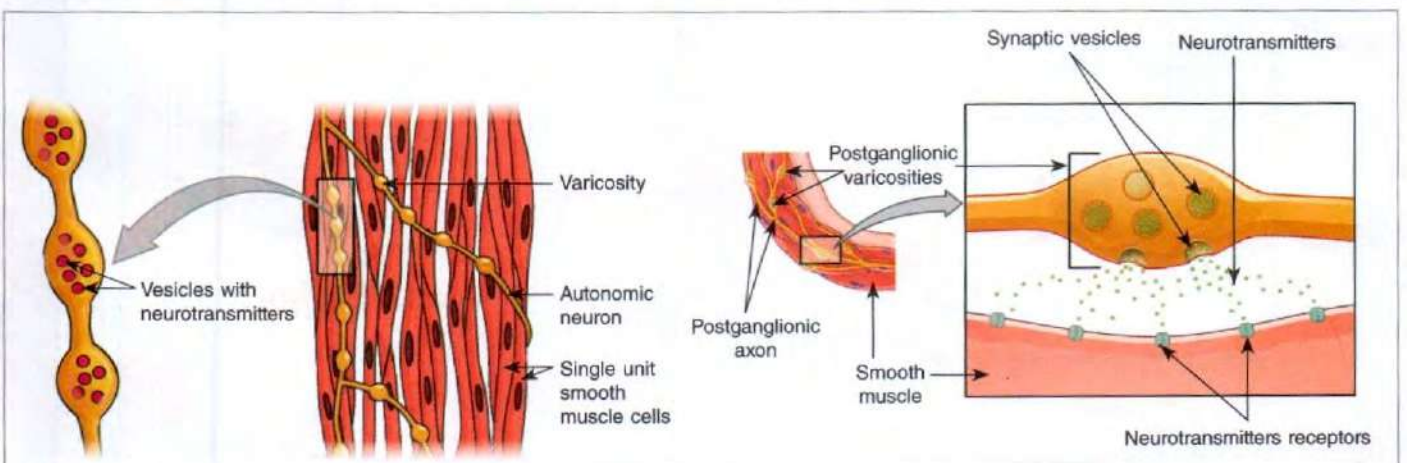


Fig. 23.5 Nerve supply to smooth muscle (**Inset:** Propagation of action potentials through gap junction)

hypersensitivity or '*supersensitivity*' is also seen in smooth muscles. Smooth muscle, unlike skeletal muscle, does not atrophy when denervated but it becomes hyperresponsive to the chemical mediator that normally activates it. Hypersensitivity is limited to the structure immediately innervated by the destroyed neurons.

Cause: It appears to be a general rule that when there is a deficiency of a given neurotransmitter, there is an increase in the number of active receptors for the transmitter (page 6 and 651). For example, in denervated skeletal muscle, there is an increase in the area of the muscle membrane sensitive to A-ch. Normally only end-plate region is depolarized by A-ch, after denervation, the sensitivity of the end-plate is not only greater but whole of the muscle membrane become sensitive to A-ch. The sensitivity returns to normal if the nerve regrows.

Similar changes are seen in smooth muscles.

Note

A typical example of denervation hypersensitivity is the wide dilatation of pupil in response of the denervated iris to nor-epinephrine. Denervated exocrine glands *(except sweet glands)* also shows hypersensitivity.

Effect of Various Agents on the Membrane Potential of Intestinal Smooth Muscle (Fig. 23.6)

1. Effect of Catecholamines

Epinephrine (Adrenaline) and Nor-Epinephrine (Nor-Adrenaline) act via both α and β adrenergic receptors.

(i) Via α, increases Ca^{2+} efflux; and

(ii) Via β, stimulates cAMP causing increase in intracellular binding of Ca^{2+}.

(i) and (ii) cause:

(a) *increase in membrane potential (hyperpolarization)*, and

(b) *decrease in spike frequency*. Thus finally leads to:

- decrease in muscle tension (tonus), therefore, baseline shifts downwards, and
- decrease in rhythmic muscle contraction, which produces muscle relaxation.

Same effect is seen by stimulation of sympathetic nerves which cause release of catecholamines at their endings.

2. Effect of Acetyl choline (A-ch)

A-ch causes increase in Na^+ and Ca^{2+} influx, producing *decrease in membrane potential (depolarization)* and *increase in spike frequency*. Therefore, muscle tension (tonus) increases and baseline shifts upwards. Moreover, increase in rhythmic muscle contraction leads to increase muscle contraction. The effect is mediated by phospholipase C and IP_3 (page 22), which increases the intrecellular Ca^{2+} concentration.

Similar effects are seen on the intestinal smooth muscles by:

(i) stimulation of cholinergic nerves which release A-ch at their endings

(ii) cold, and

(iii) stretch. (page 15)

3. Effect of ions

(i) *Ca^{2+}*

(a) Increase in Ca^{2+} concentration in ECF, increases the spontaneous muscle contraction due to Na^+ influx, thus producing increase in height of contraction.

(b) Decrease in Ca^{2+} concentration in ECF produces the opposite effect.

(ii) *Barium.* It is a direct stimulant to smooth muscle producing marked increase in frequency and force of contraction.

(iii) *Na^+.* Spontaneous activity in smooth muscle is not affected by Na^+ influx, therefore, increase in Na^+ concentration in ECF produces no effect.

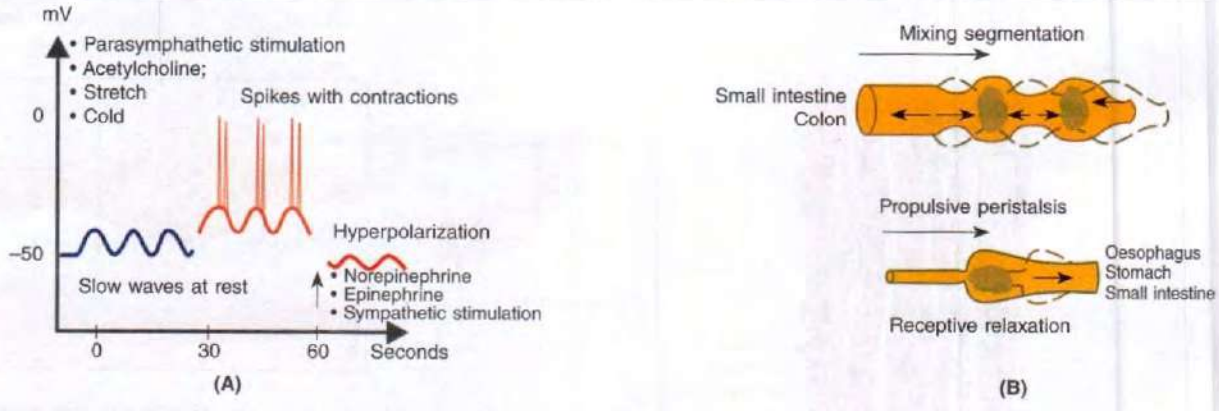


Fig. 23.6 (A) Intestinal smooth muscle potentials and (B) mechanical contractions

Summary: Characteristic properties of skeletal, cardiac and smooth muscles compared

Characteristics		Skeletal	Cardiac	Smooth
I. Morphological Properties				
1.	Distribution	Fixed to the skeleton (origins and insertions) and form 'somatic' musculature.	Heart only; not attached to bone.	(A) Single (visceral) unit smooth muscle: *Sites:* Hollow viscera *e.g.* Intestine, Bronchi, Uterus, Ureters. (B) Multi unit smooth muscle – sites: Ciliary muscle. Iris in eye, pilomotor muscle of skin; Muscle in blood vessels.
2.	Structure	It has well developed cross striations, therefore, also called *striated muscle.*	Also show cross striations.	Lack cross striations, therefore also called *'plain'* muscles.
3.	Size and Shape	Cylindrical, 1-40 mm long, 50-100 µm diameter; multinucleated cells.	Short, cylindrical, 100 µm long and 15 µm diameter; single nucleus; forms a branching net-work.	Elongated (spindle), single nucleus, variable sizes.
4.	Sarcoplasmic Reticulum	Well developed.	Well developed, more than in the skeletal muscle.	Poorly developed.
5.	Physiological Structure	*Non-syncytial i.e.* lacks anatomic and functional connection between individual muscle fibers.	It is functionally *syncytial* in character.	Functionally syncytial in character (single unit smooth muscle); non-syncytial (multi-unit smooth muscle)
6.	Sarcotubular System	Present; T-system at A-I junction; terminal cistern prominent.	Present with poorly developed terminal cistern. T-system prominent and present at 'Z' lines.	Present, but not so characteristic
7.	Nerve supply	By somatic nerves and by special nerve endings	Via two branches of ANS with ganglia and free nerve terminals.	Same as in the cardiac muscle.
8.	Control and Rhythmicity	Does not normally contract in the absence of nervous stimulation; under voluntary control. Therefore also called *voluntary muscles.*	It contracts rhythmically and spontaneously in the absence of external innervation due to presence of pacemaker tissue; *involuntary.*	Rhythmicity: two types (i) regular and (ii) irregularly discharging pacemaker (asynchronous). *Involuntary.*
9.	Blood supply and oxygen consumption	840 mL/min (3-4 mL per 100 gm/min) with moderate oxygen consumption.	Abundant, 250 mL/min (80 mL/100 gm/min) with high O_2 consumption.	350 mL/min (1.4 mL per 100 gm/min) with less O_2 consumption.
II. Electrical Properties				
10.	Resting membrane potential	–90 mV	–80 mV	–55 mV; *unstable*, superimposed with slow sine wave like fluctuations and pacemaker potential
11.	Action potential (AP)	Initial depolarization of 30 mV produces rapid depolarization and repolarization; total duration of spike 2-4 msec (duration of AP is 30-40 msec). Total amplitude: 120 mV; conduction velocity 5 mts/sec.	Initial depolarization of 15 to 20mV produces rapid depolarization (2 msec.) and slow repolarization in 3 phase (≈200 msec); total duration 250 msec at HR 75/min; total amplitude 100 mV; variable speed of conduction.	Variable; (rapid rise and fall in action potential). Total duration 50 msec. Total amplitude upto 60 mV, variable speed of conduction.

Summary: Characteristic properties of skeletal, cardiac and smooth muscles compared

Characteristics		Skeletal	Cardiac	Smooth
12.	Absolute refractory period	1-3 msec.	180-200 msec.	Not defined.
III. Mechanical Properties				
13.	Mechanical events	Contraction starts 2 msec after the start of depolarization before repolarization ends. Can summate the contractile response and hence phenomenon of *"tetanus"* is present.	More than half of mechanical contraction is over during absolute refractory period. Therefore, "tetanus" phenomenon is *not* seen. (Protective mechanism)	Muscle starts to contract approx. 200 msec. after the start of the spike (150 msec after the spike is over). The peak contraction is reached approx. 500 msec after the spike. Tetanus is seen.
14.	Duration of muscle twitch	Varies with type of muscle fibers; 7.5 msec in fast muscles, 100 msec in slow muscles.	$1^1/_2$ times the total duration of action potential.	Approx. 1000 msec.
15.	Excitation contraction coupling	Rapid process, time from initial depolarization to initiation of contraction is 10 msec.	More rapid process (time <10 msec).	Very slow process.
16.	All or none law	Applicable, true for single muscle fiber.	Applicable, true for the whole of the atria or ventricles.	Applicable, true for single muscle fiber.
17.	Length tension relationship	Maximum "active" tension is developed at the "optimal" length.	Similar to that in skeletal muscle.	Shows property of *plasticity*.
18.	Phenomenon of fatigue	Possible	None because of long absolute refractory period; more blood supply, myoglobin presence and other metabolic properties.	Possible but difficult to demonstrate.
IV. Metabolic property				
19.	Energy utilization	Under basal state 20% by fats; >60% from carbohydrates; 20% by proteins.	60% by fats, 35% by carbohydrates and 5% by ketones and amino acids.	Low, mainly provided by the fats.

Study Questions

1. **Give physiological significance of:**
   (i) Single and multi-unit smooth muscles
   (ii) Tonus and Unstable RMP in smooth muscle
2. **Write short notes on:**
   (i) Plasticity of smooth muscle
   (ii) Nerve supply to smooth muscle
   (iii) Excitatory junctional potential
   (iv) Denervation hypersensitivity in smooth muscle.
   (v) Latch bridge mechanism
3. **Give the mechanical events during contraction and relaxation of the smooth muscle.**
4. **Mention the effect of following agents on intestinal smooth muscle:**
   (i) Catecholamines (ii) A-ch (iii) Ca^{2+}
   (iv) Barium (v) Na^+
5. **Draw diagram to show:**
   (i) Relation between action potential and tension in visceral smooth muscle
   (ii) Length-tension relationship in smooth muscle.
   (iii) Spontaneous electrical activity in visceral smooth muscle

MCQs

1. **Smooth muscle does not contain:**
   (a) Actin (b) Myosin (c) Tropomyosin (d) Troponin
2. **Visceral smooth muscles are characterized by:**
   (a) Unstable RMP (b) Average RMP is approx. –80 mV
   (c) High K^+ concentration inside the cells (d) Spontaneous depolarization begins with Na^+ influx mainly
3. **In GIT smooth muscle, the action potential is generated by opening up of:**
   (a) Sodium channel (b) Calcium channel (c) Sodium-calcium channel (d) Potassium channel
4. **Single unit smooth muscle refers to:**
   (a) Single muscle fiber
   (b) Multiple muscle fibers contracting as a unit
   (c) Each muscle fiber contracting independently of the other
   (d) Single stimulus to its nerve causes repeated firing of action potential
5. **A unique characteristic of smooth muscle is that:**
   (a) It can sustain a contraction for prolonged periods (b) Calcium is not required for contraction
   (c) Repetitive contractions are not possible (d) Myosin filaments are not required
6. **Smooth muscle contraction *differs* from that of skeletal muscle by:**
   (a) Slowness of onset of contraction and relaxation
   (b) Maximum force of contraction is very low
   (c) Begin to contract immediately after the start of the spike
   (d) Excitation contraction coupling is a rapid process
7. **Property of plasticity in single unit smooth muscle refers to:**
   (a) Impossible to correlate between fiber length and tension developed accurately
   (b) No resting length can be assigned
   (c) Variability of the tension it exerts at any given length
   (d) All of the above are true
8. **Discrete partial depolarization in smooth muscles similar to end plate potential is called:**
   (a) Spike potential (b) Excitatory junctional potential
   (c) Miniature end plate potential (d) Threshold potential
9. **If smooth muscle is denervated:**
   (a) It undergoes atrophy
   (b) It becomes hypersensitive to chemical mediators
   (c) Number of active receptors for the transmitter decreases
   (d) Less amount of chemical transmitter is released at nerve ending when stimulated
10. **Stimulation of sympathetic nerve to intestinal smooth muscle results in:**
    (a) Increase in muscle tension (b) Muscle relaxation
    (c) Increase in rhythmic muscle contraction (d) Increased secretions
11. ***Not* characteristic of smooth muscle is:**
    (a) Involuntary muscle (b) Unstriated
    (c) Variable dimensions of its cells in the body (d) Well developed sarcoplasmic reticulum
12. **Membrane potential in urinary smooth muscle is:**
    (a) Spontaneously depolarizing (b) Stable at 65 mV
    (c) Hyperpolarized by stretch (d) Cannot be changed by neuro-hormones
13. **Two basic types of electrical waves in smooth muscle of the gastrointestinal tract are:**
    (a) Fast waves and spikes (b) Short and long spikes
    (c) Slow waves and spikes (d) Slow waves and fast waves

Answers

1. (d) 2. (a) 3. (c) 4. (b) 5. (a) 6. (b) 7. (d) 8. (b) 9. (b) 10. (b)
11. (d) 12. (a) 13. (c)

Unit IV

THE DIGESTIVE SYSTEM

GIT secretion composition
GIT movements.

Chapter 24: Physiological Anatomy of Gastro-Intestinal Tract (GIT)

Organisation of structure of GIT: innervation; structure of small and large intestine

Chapter 25: Physiology of salivary secretion

Salivary glands: Types, histology, innervation, composition, function, mechanism and control of salivary secretion

Applied: Aptyalism; sialorrhoea

Chapter 26: Mouth and Oesophagus

Mastication; swallowing (deglutition); upper and lower oesophageal sphincters

Applied: Aerophagia; achalasia cardia

Chapter 27: The Stomach

Structure, functions, innervation; Composition, functions, mechanism of secretion and regulation of gastric juice; regulation of gastric motility and emptying. (5m)

Applied: Total gastrectomy; pathophysiology of peptic ulcer; physiology of vomiting. (5m)

Chapter 28: Pancreas (10m)

Structure, nerve supply; composition, function and regulation of pancreatic juice; pancreatic exocrine function tests.

Chapter 29: Liver and Gall Bladder

Liver: Structure, functions of liver and signs of liver insufficiency; bile: composition, functions and control of bile secretion; bilirubin metabolism, excretion and jaundice; functions of gall bladder; cholecystectomy; gall stones.

Chapter 30: Small Intestine

Structure. Intestinal Juice: composition, functions and control; digestion in small intestine; malabsorption syndrome; movements in small intestine: adynamic ileus, ileo caecal valve and gastro-ileal reflex

Chapter 31: Large Intestine (Colon)

Structure; movements; disorders: Hirschsprung's disease, defecation

Absorption and secretion in large intestine, faeces Dietary fibers.

Chapter 32: Digestion and Absorption in the GIT

Digestion and absorption of carbohydrates, fats and proteins

Absorption of water, electrolytes, vitamins and minerals.

Chapter 33: GIT Hormones

Chapter

24

Physiological Anatomy of Gasto-Intestinal Tract (GIT)

I. Introduction

II. Organisation of structure of GIT

Innervation of GIT: Enteric nervous system

Structure of small intestine

Structure of large intestine

INTRODUCTION

Gastro-Intestinal Tract (GIT) is the portal (or gate) through which nutritive substances, vitamins, minerals and fluids enter the body. Proteins, fats, carbohydrates are broken down into *absorbable units* in the small intestine *i.e.* they can cross the mucosa and enter the lymph or blood and, therefore, become usable by the body.

The digestive tract is more than 10 metres (30 feet) long from one end to the other. It is continuous starting at the mouth, passing through the pharynx, the oesophagus, the stomach, the small and large intestines, and ending in the rectum, and finally into the anus. Associated with it are *accessory organs of digestion:* the teeth, tongue, salivary glands, liver and pancreas. **(Fig. 24.1)**

From the *mouth*, the chewed food mixed with saliva passes into the *pharynx*, then into the *oesophagus* which is a 25 cm long fibro-muscular tube lined with mucous membrane. The contraction and expansion movements of its wall called *peristaltic* movements cause the food to pass into the stomach. From the *stomach* the food passes into the *small intestine*, the first part of this being the *duodenum* which is about 25 cm long and shaped like letter 'C'. The remainder of the small intestine consists of the *jejunum* which is about 2.5 metres long and the *ileum* which is about 3.5 metres long.

The small intestine then merges with the large intestine which though wider than the small intestine is much shorter, being in total 1.5 to 1.8 metres long. The *large intestine* is divided into nine parts. Starting with the *caecum* into which the *ileum* opens, the opening is being guarded by the 'ileo-caecal valve' which allows onflow but prevents backflow of intestinal contents. Then there is the *vermiform appendix* which is about 7.5 cm in length and terminates in a blind end. Next there is the *ascending colon* which passes up along the right side of the abdomen to bend sharply at the *right hepatic flexure* and leads into the *transverse colon*. This further bends at the *left splenic flexure* and the *descending colon* which goes down the left side of the abdomen to the *sigmoid colon* and the *rectum* which is 12.5–15 cm long, the exit of which, guarded by two sphincter muscles, is known as the 'anus'.

Functions of digestive system

The GIT is responsible for breaking down food and supplying the body with water, nutrients, and electrolytes needed to sustain life. This is brought about by the following processes:

1. ***Ingestion*** involves
   - (i) placing the food into the mouth;
   - (ii) chewing the food into smaller pieces (***mastication***);
   - (iii) moistening the food with salivary secretions;
   - (iv) swallowing the food (***deglutition***).
2. ***Digestion.*** During digestion, food is broken down into small particles by grinding action of the GIT and then degraded by digestive enzymes into usable nutrients.
   - (i) Starches are degraded by 'amylases' into monosaccharides.
   - (ii) Proteins are degraded by variety of proteases into dipeptides and amino acids.
   - (iii) Fats are degraded by 'lipases' and esterases into monoglycerides and free fatty acids.
3. ***Absorption.*** During absorption, nutrients, water and electrolytes are transported from the GIT (mainly from the small intestine) to the circulation.
4. ***Egestion.*** During egestion, the undigested food, along with various secretions and sloughed-off epithelial cells from the GIT, pass into rectum and constitute the

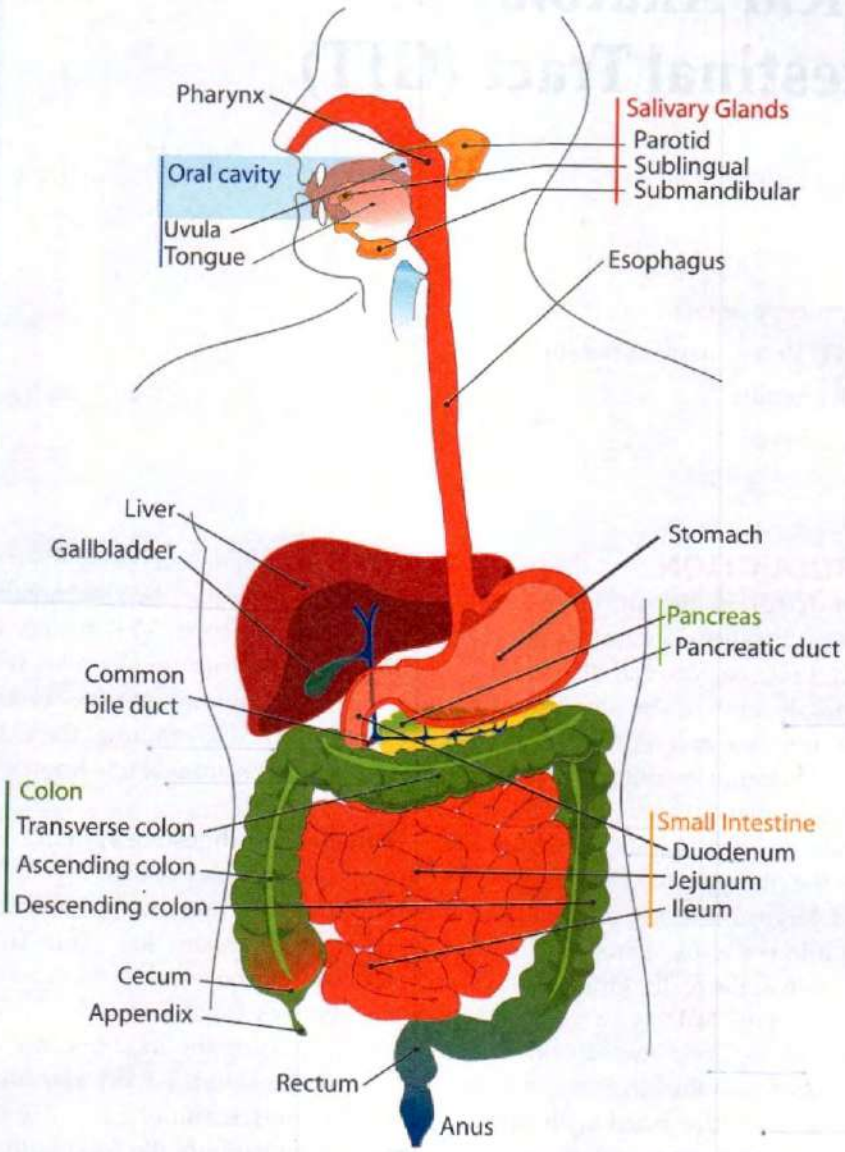


Fig. 24.1 The digestive system

faeces which are voided through the anus at periodic intervals.

ORGANISATION OF STRUCTURE OF GIT

In general, organisation of wall of 'GIT' from posterior pharynx to the anus has the following layers from outside inwards: **(Fig. 24.2)**

1. The *serous layer (serosa)*. This layer helps in the attachment of gut to the surrounding structures.
2. The *longitudinal smooth muscle layer*. Its contraction causes decrease **in length** of that segment of the GIT.
3. The *circular smooth muscle layer*. This layer is thicker at the sphincter. Its contraction causes a decrease in diameter of the lumen of that segment of the GIT. The longitudinal and circular smooth muscle layers help in both local mixing and forward propulsion of the contents of the gut.
4. The *submucous layer*. It consists of loose connective tissue, blood vessels and lymphatics.
5. The *mucous layer*. This is lined by epithelium and consists of a stroma containing:
   (i) glands (for secretion or absorption)
   (ii) 'muscularis mucosae' of smooth muscle, (contraction causes a change in the surface area for secretion or absorption); and
   (iii) loose connective tissue rich in lymphocytes.

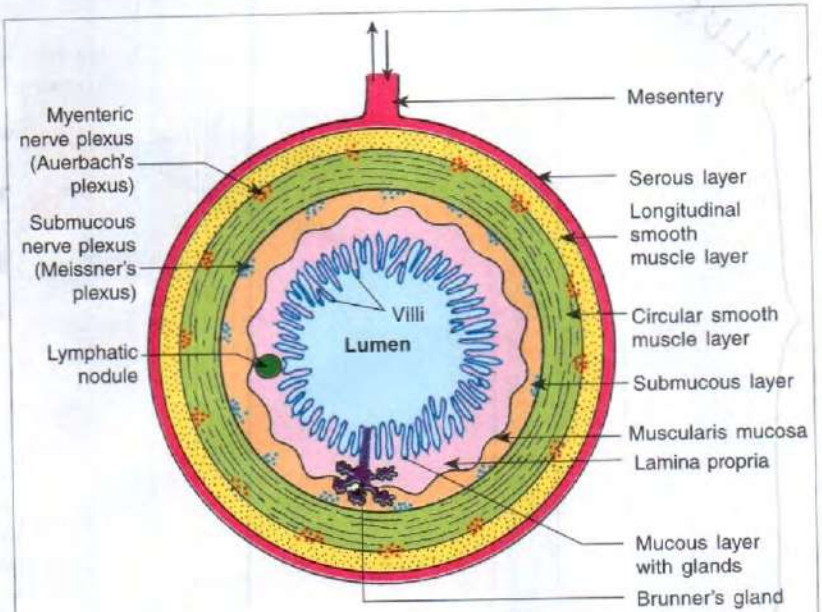


Fig. 24.2 Cross-section of GIT, showing the different layers of its wall

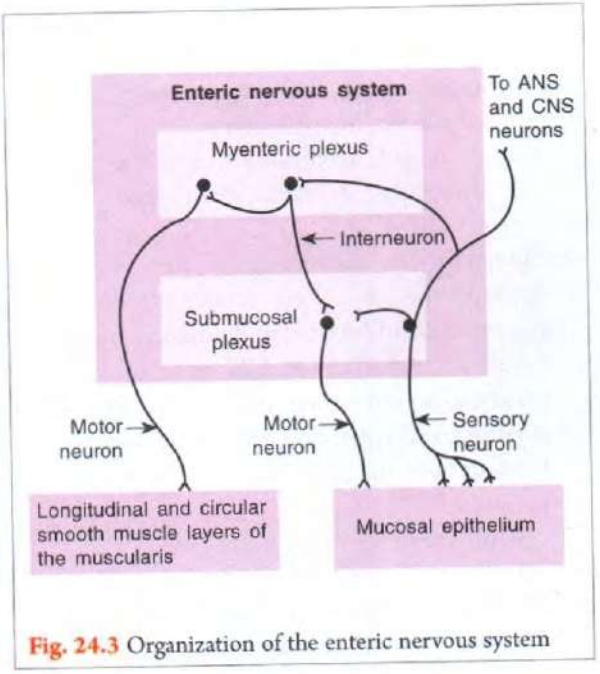


Fig. 24.3 Organization of the enteric nervous system

INNERVATION OF GIT: ENTERIC NERVOUS SYSTEM

There are two major networks of nerve fibers that innervate the GIT: 'intrinsic' and 'extrinsic', *together they form the* ***Enteric nervous system*****–the Little Brain. (Fig. 24.3)**

A. Intrinsic innervation

It is further divided into two: Myenteric and Meissner's plexuses.

1. ***Myenteric plexus (Auerbach's plexus).*** It lies between longitudinal and circular smooth muscle layers and innervates both the layers. It is mainly ***motor*** in function. Its stimulation increases the activity of gut by:
   (i) increasing tone of gut wall
   (ii) increasing intensity of rhythmic contraction
   (iii) increasing rate of rhythmic contractions, and
   (iv) increasing velocity of conduction of excitatory waves along the gut wall.
   Thus, it is concerned with control of peristaltic activity of the GIT (page 247).
2. ***Submucous plexus (Meissner's plexus).*** It lies between the submucous layer and inner circular smooth muscle layer. It is mainly ***sensory*** in function and is concerned with control of exocrine and endocrine secretions by the cells in the GIT. It also innervate the submucosal blood vessels to control local blood flow.

Both the plexuses are interconnected and are under the extrinsic autonomic nerves control, by both parasympathetic and sympathetic nerve fibers.

Important Note

The enteric nervous system contains > 100 million neurons as many as are found in the whole spinal cord. Thus, it is also called as *Little Brain*.

B. Extrinsic innervation

This is under the autonomic nervous control *i.e. parasympathetic and sympathetic nerve fibers.*

1. ***Parasympathetic (cholinergic)*** nerves, release acetylcholine (A-ch) at their endings and by depolarization of smooth muscle membrane produce contraction of GIT musculature. The salient anatomical features of the parasympathetic supply to GIT are given in **Table 24.1.**

Table 24.1: Parasympathetic nerves to GIT

Cranial nerve	Site of connector cells	Site of ganglion cells	Structures supplied
1. X: Vagus	Dorsal nucleus of Xth nerve (vagus)	• Myenteric plexus • Meissner's plexus	Gastric and intestinal glands and smooth muscle of most of the GIT upto the junction between proximal 2/3rd and distal 1/3rd of transverse colon.
2. $S_{2,3,4}$: Sacral	Segment 2, 3 and 4 of sacral nerves (*nervi erigentes*)	Hypogastric ganglia	Rest of the large intestine.

Stimulation of parasympathetic nerves to GIT produces:

(i) increase in motility and tone
(ii) relaxation of sphincters
(iii) increased secretions from the stomach (specially of enzymes) and of the intestine.

2. ***Sympathetic*** (adrenergic) *nerves*, release epinephrine at their endings which by hyperpolarization of smooth muscle membrane result in relaxation of GIT musculature. The salient anatomical features of the sympathetic supply to GIT are given in **Table 24.2**.

Stimulation of sympathetic nerves to GIT produces:

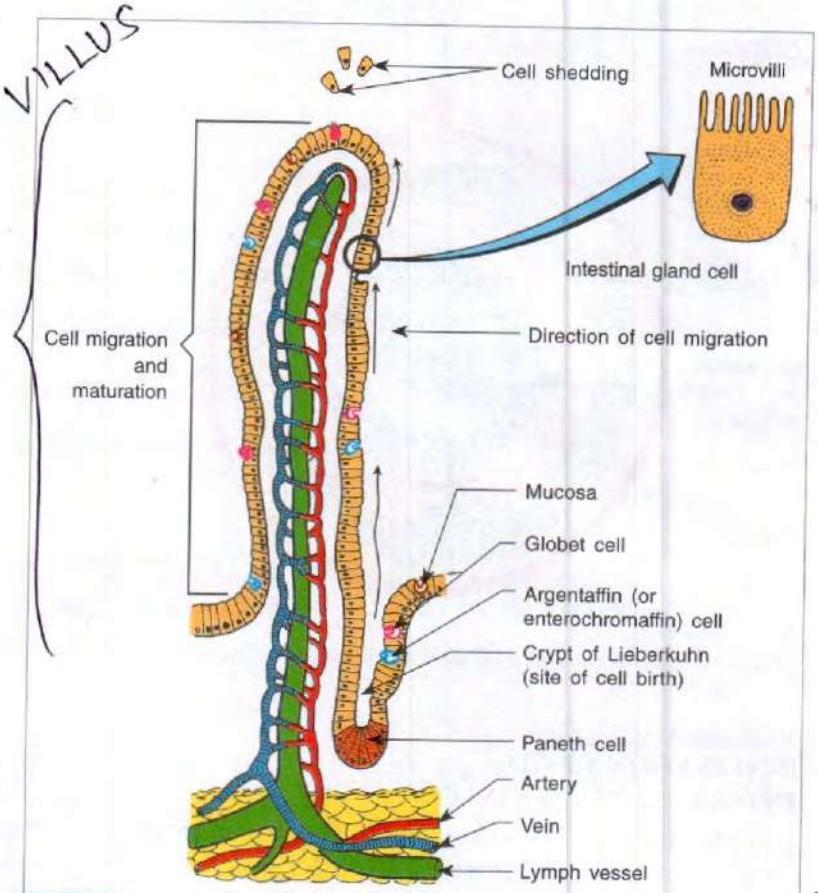
(i) decrease in motility and tone
(ii) contraction of sphincters
(iii) inhibition of secretions from the stomach and probably also inhibit intestinal secretions.

STRUCTURE OF SMALL INTESTINE

Mucosal surface of duodenum and small intestine is adapted to provide a huge area for absorption. Mucosa shows finger like projections of approx. 1 mm height called ***villi***. There are 20-40 villi per mm^2 of mucosa. These are covered by a layer of columnar cells which possess a 'brush border' consisting of ***microvilli*** (1 µm length and 0.1 µm width). Finally, the microvilli increases the absorptive surface area of small intestine to about 2 million cm^2. Each villus in its core contains: **(Fig. 24.4)**.

(i) a lymph vessel (***lacteal***) continuous with lymphatic plexus of submucosa;
(ii) smooth muscle fibers continuous with muscularis mucosa;
(iii) an arteriole and venule with their relevant capillary plexus;
(iv) a nerve net which has connections with submucosal and myenteric plexuses.

The *brush border* is lined on its luminal side by an amorphous layer called the ***glycocalyx***, which is rich in neutral and amino-sugars and may serve a protective function.



Note

GIT epithelium is one of the most rapidly dividing tissue in the body.

Fig. 24.4 Arrangement of vascular supply in small intestine

Between villi are the *intestinal glands* which are known as ***Crypts of Lieberkuhn***. These are simple tubular glands and they do not penetrate muscularis mucosa. They are lined by low columnar epithelium. They show 'active mitosis' and replace the cells shed from the tip of villi. Every three days the lining of small intestine is replaced by this rapid turnover. The 'secretion' of proteins into the lumen due to cell sloughing has been calculated to be upto 30 gm/day. The crypts of Lieberkuhn contain:

(i) ***Goblet cells*** which secrete '*mucus*'. It protects the surface of the intestinal mucosa; helps lubricate the food and holds immunoglobulins in place.

Table 24.2: Sympathetic nerves to GIT

Organs supplied	Site of connector cells	Site of ganglion cells	Route of post-ganglionic fibers
1. Viscera of abdomen proper	T_6–L_2 (chiefly)	Upper abdominal ganglia (superior mesenteric and coeliac etc.)	Along the blood vessels
2. Pelvic viscera	L_1–L_2 (chiefly)	Hypogastric ganglia	Along the blood vessels and in hypogastric nerves

(ii) *Argentaffin* (or *enterochromaffin*) *cells* which synthesize 'secretin'; and 5 hydroxytryptamine (5 HT), a powerful stimulant of intestinal motility.

(iii) Large endocrine acidophilic *paneth cells*, that secrete *defensins*, a naturally occurring peptide antibiotics. They also secrete ***guanylin***, a polypeptide that binds to guanylyl cyclase (page 23) and regulate secretion of Cl^- into the intestine lumen.

Epithelial and paneth cells are *zymogenic i.e.* they produce a great variety of enzymes capable of digesting proteins, carbohydrates, fats and nucleic acids. They also produce ***enterokinase*** which activates trypsinogen forming trypsin. The crypts of Lieberkuhn are also the site of cAMP-mediated secretion of water and electrolytes.

During digestion and absorption, the villi contract quickly with an irregular rhythm and relax slowly. Their muscular fibers serve to pump the lymph from core of villus towards the submucosal lacteals.

Duodenum

In addition to general features of small intestine it possesses:

(i) Special submucosal mucous glands which resemble gastric pyloric glands known as ***Brunner's gland***. They are tortuous, long and penetrate the muscularis mucosa. Their ducts empty into the crypts of Lieberkuhn. They are numerous in first part of duodenum (duodenal cap or bulb) and are less below the common opening of bile and pancreatic ducts.

(ii) At rest, *Brunner's glands* show small basal secretion. Ingestion of fatty food or 'secretin' injection produces large volume of thick alkaline mucous secretion which probably helps to protect the duodenal mucosa from the gastric acid.

Jejunum

Jejunum is a latin word, and it means 'empty' because it was found to be empty at postmortem. Upper 40% of small intestine is called jejunum; at ligament of Treitz, the duodenum becomes jejunum. *Duodeno-jejunal junction* is located at left side of L_2 vertebrae.

Features

(i) It shows a progressive increase in the number of *goblet cells* and lymphoid tissue.

(ii) The lymph node lies immediately below the mucosa.

(iii) Its mucosa shows maximal folding, folds being known as ***plicae circulares***.

Ileum

Ileum means roll or coil. The lower 60% of the small intestine is called the ileum. Its main features are:

1. The *goblet cells* of the mucosa and lymphoid tissues reach their maximal density.
2. It contains aggregated lymphatic follicles, known as ***Peyer's patches***.

Note

GIT has a very well developed local immune system - both natural and acquired

STRUCTURE OF LARGE INTESTINE (COLON)

Characteristic features

1. Mucosal surface of colon is smooth *i.e.* there are no villi on the mucosa.
2. There are **no** *plica circulares*.
3. Simple tubular glands are abundant and are formed of simple columnar epithelium with large number of 'goblet cells' which secrete mucus.
4. Solitary lymph nodes are found in ascending colon, specially in the caecum and appendix.
5. Circular smooth muscle is distributed as usual in the gut.
6. Wall of the colon is usually folded into sacs by contraction of circular smooth muscle thus producing ***sacculations***. The mucous membrane is thrown into folds opposite to the constrictions between the sacculations.
7. The longitudinal smooth muscle layer is not equally distributed throughout the gut wall but is collected into the three distinct bands, called ***teniae coli***, which can be seen through the serous layer.
8. When these longitudinal bands which form puckering (or out pouchings) of wall (***haustra***) between the taeniae are cut, mucosal folds can be smoothed out.
9. Little fatty tags project from the colonic serosa; they are peritoneal bags of fat called ***appendices epiploicae***.
10. 'Anal sphincters' (page 254).

Study Questions

1. **Write short notes on:**
   (i) Crypts of Lieberkuhn
   (ii) Brunner's gland
   (iii) Intestinal glands
   (iv) Structure and function of the enteric nervous system
   (v) Glycocalyx
   (vi) Peyer's patches

2. Name the structures contained in the core of a villus of the small intestine.
3. Draw and label:
   (i) cross-section of GIT, showing (ii) different layers of its wall (iii) structure of a villus
4. Describe briefly "Enteric nervous system". Justify, it is also called a "little brain".
5. What will happen and why, if nerve supply to GIT get distruppted.
6. Give an account of structure and functions of small intestine.

MCQs

1. Deglutition refers to:
   (a) Chewing the food into smaller particles
   (b) Swallowing the food
   (c) Degradation of food by digestive enzymes
   (d) Transport of nutrients from small intestine into the circulation
2. Ingested food is degraded by digestive enzymes into:
   (a) Monosaccharides
   (b) Amino acids
   (c) Free fatty acids
   (d) Usable nutrients
3. Which is *not* seen with activation of Myenteric plexus in GIT?
   (a) Increased tone of gut wall
   (b) Increased secretions
   (c) Increased peristalsis
   (d) Increased rhythmic contraction of gut wall
4. *Not true* about submucous (meissner's) plexus:
   (a) Lies between submucous and inner circular muscle layer
   (b) Mainly sensory in function
   (c) Controls exocrine secretions
   (d) Controls tone of the gut wall
5. Stimulation of parasympathetic nerve to GIT produces:
   (a) Contraction of sphincters
   (b) Inhibition of secretion from the stomach
   (c) Inhibition of intestinal secretions
   (d) Increase in motility and tone
6. The life span of lining of small intestine is ..... days:
   (a) 3 (b) 7 (c) 10 (d) 15
7. Which of the following intestinal glands *do not* secrete mucus?
   (a) Paneth cells (b) Crypts of lieberkuhn (c) Goblet cells (d) Brunner's glands
8. In the GIT, the various layers are organized from outside inwards as:
   (a) Serosa – Muscle layer – Submucosa – Mucosa
   (b) Mucosa – Submucosa – Muscle layer – Serosa
   (c) Mucosa – Submucosa – Serosa – Muscle layer
   (d) Muscle layer – Serosa – Submucosa – Mucosa
9. Mucous layer stroma in the GIT contains all of the following *except*:
   (a) Loose connective tissue
   (b) Glands
   (c) Muscular mucosae
   (d) Nerve plexus
10. The myenteric plexus receives which type of extrinsic innervation?
    (a) Preganglionic parasympathetic
    (b) Postganglionic parasympathetic
    (c) Preganglionic sympathetic
    (d) Postganglionic sympathetic
11. Secretions of proteins into the lumen of small intestine due to cell shedding is upto ..... mg/day:
    (a) 1 (b) 15 (c) 30 (d) 60
12. *Not* a characteristic of the colon:
    (a) Mucosa shows plica circularies
    (b) Simple tubular glands are abundant
    (c) Its walls shows sacculations and haustra
    (d) Presence of appendices epiploicae

Answers

1. (b) 2. (d) 3. (b) 4. (d) 5. (d) 6. (a) 7. (a) 8. (a) 9. (d) 10. (a) 11. (d) 12. (b)

Chapter 25

Physiology of Salivary Secretion

I. Salivary Glands: Types, Histology; Innervation
II. Composition of Saliva
III. Functions of Saliva
IV. Mechanism of Salivary Secretion
V. Control of Salivary Secretion
VI. Applied: Aptyalism; Sialorrhoea

SALIVARY GLANDS

Types

There are 3 pairs of salivary glands – *parotid, submandibular (or submaxillary) and sublingual salivary gland*. The chief differentiating features of these glands are given in Fig. 25.1 and Table 25.1.

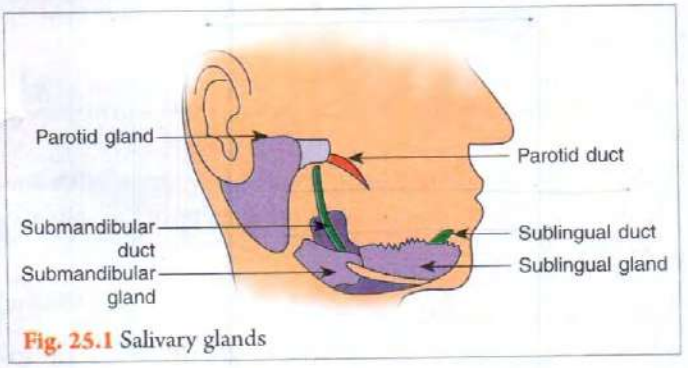


Fig. 25.1 Salivary glands

Histology

Salivary glands contain chiefly two types of cells: *'mucous' cells* and *'serous' cells* **(refer Table 25.2)**.

Both sets of glands contain their secretory cells in acini where the cells are arranged around a central lumen which leads into a duct. These ducts join to form *intralobular* and *interlobar ducts* which lead into the main duct. **(Fig. 25.2)**

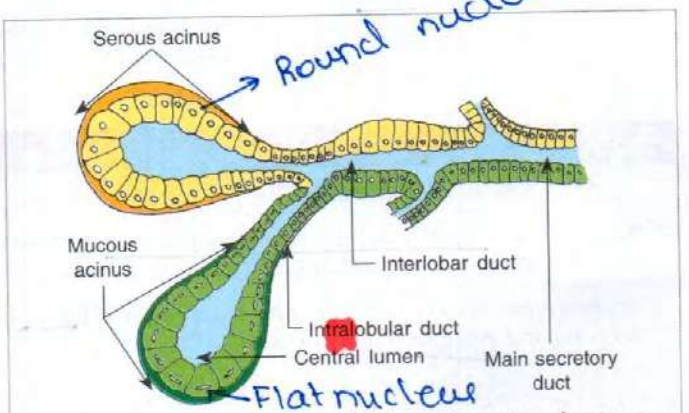


Fig. 25.2 Structure of submandibular (submaxillary) salivary gland

Innervation

All 3 pairs of salivary glands are supplied by 'efferent' and 'afferent' nerves. **(Fig. 25.3)**

A. Efferent nerve supply

It is by both parasympathetic and sympathetic division of autonomic nervous system.

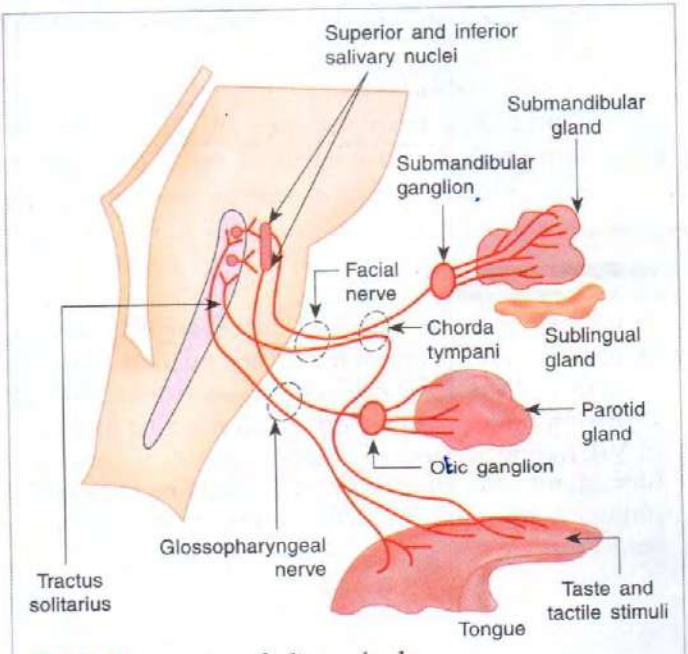


Fig. 25.3 Innervation of salivary glands

[LSH PP]

Table 25.1: Salivary glands – main differentiating features

Gland type and weight	Location	Route of the secretory duct	Histology	Percentage of total salivary secretion (1500 mL/day)	Source of parasympathetic nerve supply
1. *Parotid;* 20-30 gm each	In front of the ears	Its secretion passes via *"Stensen's"* duct which opens opposite to the second molar tooth in the mouth.	Contains purely *"serous"* cells	25%	IX nerve
2. ***Submandibular or submaxillary;*** 8-10 gm each	Medial to the mandible in submaxillary triangle	Its duct *i.e. "Wharton's"* duct opens into floor of the mouth along the side of the frenulum linguae.	*"Mixed" i.e.* contain both "serous" and "mucous" cells in the ratio of 4:1.	70%	VII nerve
3. ***Sublingual*** 2-3 gm each	Subjacent to mucosa of floor of the mouth.	Its secretions are discharged by 5-15 small ducts (ducts of *"Rivinus"*) into the sublingual part of the mouth.	*"Mixed"*: mainly "mucous" cells. (Serous and mucous cells in the ratio of 1:4)	5%	VII nerve

Table 25.2: Two types of cells in the salivary gland compared

Mucous cells	Serous cells
1. They contain large translucent ***mucinogen*** granules consisting of a precursor of ***mucin*** and appear pale or translucent.	They contain opaque small ***zymogen*** granules consisting of a precursor of ***ptyalin***.
2. It forms a viscous secretion containing *mucin*, a useful lubricant for food and protecting the oral mucosa.	It forms a thin watery secretion containing *ptyalin* (also called *salivary α-amylase*) which initiates digestion of starch to maltose.

1. *Parasympathetic nerve supply*

(i) ***To parotid glands***

It originates from the cells in *inferior salivary nucleus i.e.* dorsal nucleus of glossopharyngeal (IX) nerve.

(ii) ***To submandibular and sublingual glands***

It originates from the cells of *superior salivary nucleus i.e.* dorsal nucleus of facial (VII) nerve. *Parasympathetic nerves are secretomotor to the salivary glands.*

Important Note

As taste fibers from posterior 1/3rd of tongue pass via IX nerve to end in inferior salivary nucleus (dorsal nucleus of IX nerve); and taste fibers from anterior 2/3rd of tongue pass via nervus intermedius (branch of VII nerve) to end in superior salivary nucleus (dorsal nucleus of VII nerve); therefore, afferent impulses from mouth reflexly excite the salivary secretion.

2. *Sympathetic nerve supply*

It originates from the lateral horn cells of $T_{1,2}$ segments of the spinal cord; axons via ventral roots enter paravertebral sympathetic chain to synapse with the cells in superior cervical ganglion to supply the salivary glands.

(motor)

B. Afferent nerve supply

Afferent nerve fibers from salivary glands are found in chorda tympani (branch of VII nerve) and IX nerves. These fibers carry pain impulses from salivary glands.

COMPOSITION OF SALIVA

Daily secretion: 1500 mL/day.

Digestive enzymes

(i) ptyalin or salivary α-amylase

(ii) lysozymes (bactericidal)

(iii) kallikrein, a proteolytic enzyme (Serine protease)

(iv) lipase, a lipolytic enzyme (secreted by glands on the tongue, therefore, also called ***lingual lipase***).

Mucin (glycoprotein)

IgA – first immunological defence against bacteria and viruses

Cations:

		plasma level
Na^+	15–20 mEq/L	(145 mEq/L)
K^+	20–25 mEq/L	(5 mEq/L)
Ca^{2+}	traces	

Anions:		*plasma level*
Cl^-	15–20 mEq/L	(110 mEq/L)
HCO^{3-}	10–15 mEq/L	(27 mEq/L)
phosphate	traces	
bromide	traces	

Organic contents: urea, uric acid, creatinine, and free amino-acids

pH: slightly below 7.0 (under resting state); 8.0 (during active secretion)

FUNCTIONS OF SALIVA

1. ***Ptyalin (salivary α-amylase)*** *Aids digestion of starch* to 1 : 4α linkages producing *α-limiting dextrins* and *maltose* (to some extent). It can only digest starch after the natural plant granules have been burst *e.g.* by cooking. It acts in a neutral or faintly acidic medium (optimally at pH 6.5). Given time, it can digest starch to maltose. Such digestion continues in the interior of the bolus of food formed by chewing and mixing with the saliva even when this bolus has reached the stomach. Amylase digestion can thus continue in the stomach for approx. half an hour, until it is arrested by the excessive acidity of the gastric contents. Amylase is readily inactivated at pH ≤ 4.0.
2. ***Mucin*** *functions*
   (i) it lubricates the food, thus assists mastication and facilitates swallowing
   (ii) it protects the oral mucosa
   (iii) it aids speech by facilitating movements of lips and tongue.
3. It keeps the mouth moist and serves as a solvent for the molecules that stimulate the taste buds.
4. It minimizes risk of buccal infection and dental caries as it contains:
   (i) *lysozymes* which kill the bacteria (bactericidal)
   (ii) *IgA* which provides immunological defence against bacteria and viruses (Also refer to page 126)
   (iii) *lactoferrin* which binds iron and arrests the bacterial multiplication (bacteriostatic)
5. Buffers and ***'proline' rich proteins*** in saliva help to bind toxic tannins and maintain the oral pH at 7.0. At this pH saliva is saturated with calcium, therefore teeth do not lose calcium to oral fluids. Thus it *protects tooth enamel*. Acidic oral pH causes calcium loss from the teeth.
   Buffers in saliva also help to neutralize gastric acid and relieve heart burn when gastric juice is regurgitated into the oesophagus.
6. It is a *vehicle for the excretion* of certain drugs, *e.g.* alcohol and morphine; and of certain inorganic ions *e.g.* K^+, Ca_2^+, HCO_3^-, iodine and thiocyanate (SCN^-).

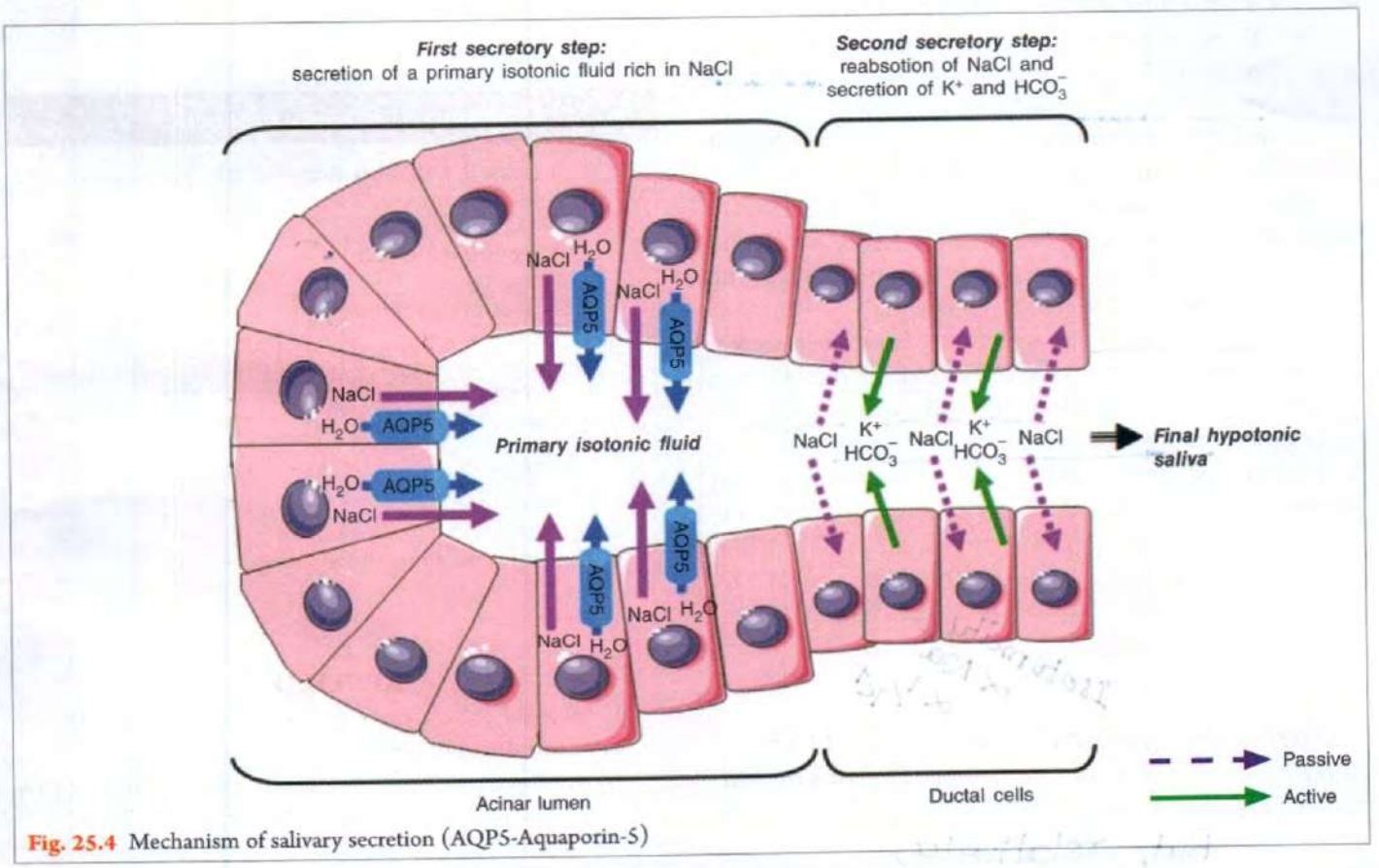


Fig. 25.4 Mechanism of salivary secretion (AQP5-Aquaporin-5)

MECHANISM OF SALIVARY SECRETION

1. The acinar cells secrete K^+ and HCO_3^- by active process into the acinar lumen, accompanied by sufficient Cl^- to preserve electrical neutrality; simultaneously, passage of water into the acinar lumen makes this ***primary secretion – isotonic***. **(Fig. 25.4)**
   The salivary duct cells which drain the acini have a rich blood supply, therefore, they actively reabsorb Na^+ and an accompanying anion (Cl^-) and transfer some K^+ and HCO_3^- into the saliva. The duct cells being relatively impermeable to water make the ***final salivary secretion – hypotonic***. Following secretion, the number of granules in the protoplasm of secreting cells falls sharply.
2. Saliva is a hypo-osmolar (hypotonic) secretion of salivary glands, therefore, metabolic activity *i.e.* O_2 consumption of the gland is increased by 5 folds during secretory activity as compared to that at rest.
3. *At rest*, saliva contains more of K^+ and less of Na^+, Cl^- and HCO_3^- compared to their plasma concentrations. *As salivary flow increases*, there is less time for ions exchange in the ducts, as a result saliva becomes less hypotonic (almost isotonic *i.e.* resembles the primary secretion). Na^+, Cl^- and HCO_3^- concentration increases and K^+ concentration decreases. **(Fig. 25.5)**
   (i) Na^+ concentration increases to a plateau concentration of 80-90 mEq/L, and
   (ii) Cl^- concentration increases to 50 mEq/ L. Na^+ and Cl^- concentrations of saliva are always lower than that in the plasma.
   (iii) HCO_3^- concentration increases and exceeds that in the plasma, and
   (iv) K^+ concentration decreases to 15-20 mEq/L.
   As a result of this, pH of saliva which is < 7.0 at low secretory rates increases to approx. 8.0 as the rate of salivary secretion increases.
4. Iodide and thiocyanate, which are excreted in saliva, are also actively transported from plasma directly into the lumen of the ducts by the cells of the duct wall. They are not transported by the acinar cells.
5. Aldosterone increases the K^+ concentration and decreases Na^+ concentration of saliva. (Aldosterone acts on the ductal cells, an action similar to its action on the kidneys page 718). Thus, a high salivary Na^+/K^+ ratio is seen when aldosterone is deficient (*Addison's disease*) (page 727).

Isotonicity ∝ Na^+ ∝ $1/K^+$

CONTROL OF SALIVARY SECRETION

Saliva production is unique in that it is increased by both parasympathetic and sympathetic activity (former is more important). But, relatively,

PNRA ⇒ ↑↑↑ salivation

1. Stimulation of parasympathetic nerves, liberates a proteolytic enzyme kallikrein from the gland cells which acts on plasma α_2-globulins in interstitial fluid to form bradykinin. The effect is mediated via release of A-ch. In addition, it also causes local release of VIP (vasoactive intestinal peptide) (page 272). This produces:
   (i) vasodilatation of blood vessels of salivary glands, and
   (ii) stimulates secretion from acini
   Therefore, it causes profuse secretion of watery saliva with a relatively low content of organic material and protein. Atropine reduces the salivary secretion.
2. Stimulation of sympathetic nerve supply causes secretion of small amounts of saliva rich in organic constituents and mucus from submandibular and sublingual salivary glands. Because probably sympathetic fibers innervate the serous cells of these glands and have no effect on parotid gland.
3. Salivary secretion ***increases*** either by:
   (i) taste of food within 20-30 seconds (*Inborn reflex*); or
   (ii) by sight, smell or thought of food (*Psychic or conditioned reflex*).
4. Dry food, particularly if finely ground, is a powerful stimulus for the mouth receptors, and causes secretion of greater amounts of saliva than does moist food.

Summary: Salivary production

1. **is increased** via activation of the parasympathetic nervous system by:
   (i) food in the mouth
   (ii) smells
   (iii) conditioned reflexes
   (iv) nausea
2. **is decreased** via inhibition of the parasympathetic nervous system by:
   (i) sleep
   (ii) dehydration
   (iii) fear; and
   (iv) anticholinergic drugs (atropine)

APPLIED

1. ***Aptyalism*** (*xerostomia*) *i.e.* suppression of salivary secretion. It is seen in:
   (i) Anxiety, fear, fever or dehydration. All these cause temporary suppression of salivary secretion.

(ii) Duct obstruction due to calculus causes permanent suppression of salivary secretion.
(iii) Irradiation therapy in the area of salivary glands.

Important Note

Aptyalism patients have higher than normal incidences of dental caries.

2. Hypersecretion *i.e.* ***Sialorrhoea*** is seen in:
   (i) pregnancy
   (ii) neoplasm (tumour)
   (iii) Parkinsonism (page 997)
   (iv) schizophrenia
   (v) chorda tympani nerve damage.

Study Questions

1. **Give physiological significance of:**
   (i) Types of salivary glands
   (ii) Primary and final salivary secretion
2. **Write short notes on:**
   (i) Composition and functions of saliva
   (ii) Mechanism and control of salivary secretion.
   (iii) Innervation of salivary glands
   (iv) Abnormalities of salivary secretion
3. **What will happen and why?**
   (i) If pH of saliva becomes acidic.
   (ii) to salivary secretion following ingestion of dry food.
   (iii) If food is not cooked properly
4. **Draw labelled diagram to show:**
   (i) Effect of secretory rate on the electrolyte composition of saliva
   (ii) Mechanism of salivary secretion
5. **Give physiological basis of:**
   (i) Increased salivary secretion in infants
   (ii) Decrease in salivary secretion during vica-voce examination

MCQs

1. **Maximum contribution to total daily salivary secretion is by:**
   (a) Parotid glands
   (b) Submandibular glands
   (c) Sublingual glands
   (d) Equal secretion by all of the three above
2. **Amount of saliva secreted per day is:**
   (a) 500 mL (b) 1000 mL (c) 1500 mL (d) 2000 mL
3. **A proteolytic enzyme present in saliva is:**
   (a) Ptyalin (b) Lysozyme (c) Kallikrein (d) Lipase
4. **If one loses a large quantity of saliva externally, which of the following ions would be lost in the greatest amount in relation to its concentration in plasma?**
   (a) Sodium (b) Chloride (c) Potassium (d) Magnesium
5. **Salivary α-amylase has an optimal activity around a pH:**
   (a) 1.0-2.0 (b) 2.0-4.0 (c) 4.0-6.0 (d) 6.5-7.0
6. **Which is *not* a function of mucin in the saliva?**
   (a) Assists mastication
   (b) Facilitates swallowing
   (c) Maintains the oral pH at 7.0
   (d) Aids speech
7. **Secretion of saliva increases in all, *except*:**
   (a) When sympathetic nerves to the glands are stimulated
   (b) When touch receptors in mouth are stimulated
   (c) Just before vomiting
   (d) More by sweet than by bitter food
8. **In 'water brash', fluid brought into mouth in one or two gushes is mainly:**
   (a) Saliva (b) Oesophageal secretion (c) Acid (d) Bile

9. **Suppression of salivary secretion is seen in:**
   (a) Pregnancy (b) Vomiting (c) Dry food (d) Anxiety
10. **One of the following is *not* a constituent of saliva:**
   (a) Glucose (b) Bicarbonate (c) Lysozyme (d) Phosphate
11. **Salivary α-amylase readily inactivated at pH:**
   (a) 7.0 (b) 6.5 (c) 5.5 (d) Below 4.0
12. **Saliva differs from plasma in all *except* that saliva is:**
   (a) Hypotonic (b) Low HCO_3^- (c) High Na^+ (d) High K^+

Answers

1. (b) 2. (c) 3. (c) 4. (c) 5. (d) 6. (c) 7. (d) 8. (a) 9. (d) 10. (a)
11. (d) 12. (c)

Chapter

26

Mouth and Oesophagus

I. Mastication
II. Swallowing (Deglutition).
Stages of swallowing
Upper and lower oesophageal sphincters
III. Applied: Disorders of Swallowing
Abolition of deglutition reflex
Aerophagia
Achalasia cardia

In the mouth food is mixed with saliva and propelled into oesophagus. *Peristaltic waves* in oesophagus move the food into the stomach.

MASTICATION

Features

1. Mastication (chewing) is the mechanical process which breaks up larger food particles into smaller pieces which:
   (i) makes it easier for the food to be swallowed;
   (ii) mixes the food with the secretions of the salivary glands to soften it; and
   (iii) increases the surface area of the food particles, thus helping in subsequent digestion of the food.

Important Note

Larger food particles can be digested, but they cause strong and often painful contractions of the oesophageal musculature.

2. Chewing is specially important for most fruits and raw vegetables, because these have undigestible *cellulose* membrane around their nutrient portions which must be broken before the food can be acted upon by enzymes.
3. Chewing can be carried out voluntarily, but for most part it is a 'reflex act'.
4. ***Muscles of mastication:*** Chewing action of the teeth *i.e.* the movements of lower jaw against the upper jaw is brought about by the muscles of mastication **(Table 26.1 and Fig. 26.1: A)**.

SWALLOWING (DEGLUTITION)

It is a *reflex response* controlled via vagus (X) nerve and its centre is located in the medulla oblongata.

It occurs in 3 stages **(Fig. 26.1: B)**:

Stage I	: Oral stage	–	Voluntary stage
Stage II	: Pharyngeal stage	–	Involuntary (reflex) stage
Stage III	: Oesophageal stage	–	Involuntary (reflex) stage

Table 26.1: Muscles of mastication

Muscle	Innervation	Principal action
1. Masseter	Mandibular division of trigeminal (V) nerve	Raises the mandible; clenches the teeth and helps to protract (prolong) the mandible.
2. Temporalis	-do-	Raises the mandible; retracts the mandible after protraction.
3. Internal and external pterygoid	-do-	Protudes the mandible; depresses the chin, therefore, helps in opening the mouth. When right and left muscles are acting alternately, it produces grinding movement.
4. Buccinator	Facial (VII) nerve	Accessory muscle of mastication, prevents accumulation of food between the cheek and teeth.

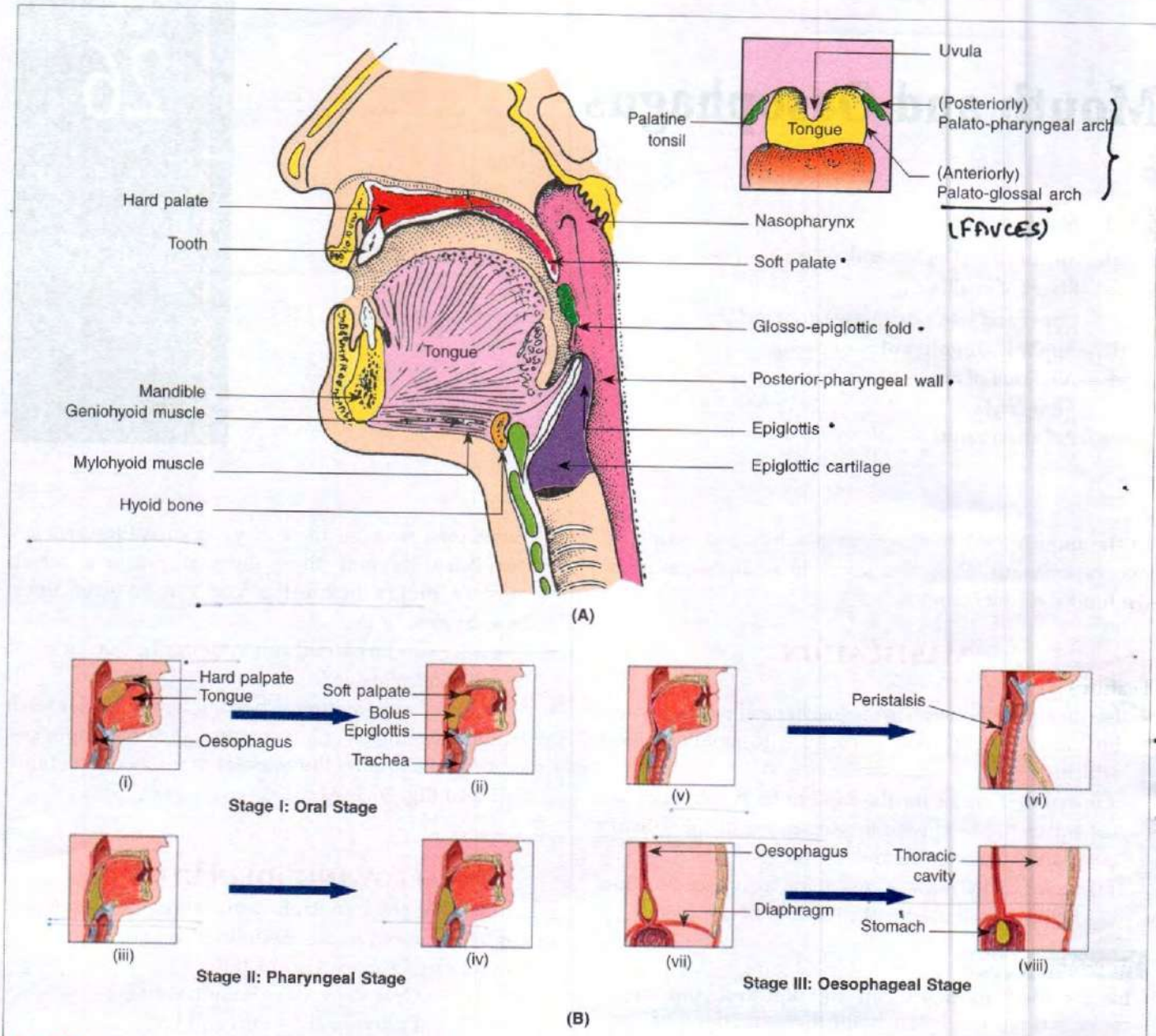


Fig. 26.1 (A) Paramedian section through nose, mouth, pharynx and larynx and (B) Stages of swallowing (deglutition)

Stage I – Oral Stage (Voluntary)

Features

1. After mastication chewed food, moistened and lubricated by saliva, is rolled into a bolus; by movements of the cheeks and tongue the bolus comes to lie in the curve of the tongue.
2. Contraction of the front part of the tongue presses the bolus against hard palate, while a series of movements of the middle part push it to the back, forcing it past the anterior pillar of the *fauces* (palato-glossal folds) to the root of the tongue. **(Fig. 26.1)**
3. Swallowing commences by closure of the mouth and voluntary contraction of mylohyoid muscle, which throws the bolus back between the anterior and posterior pillars of *fauces* (*i.e.* palato-glossal and palato-pharyngeal folds) on to the posterior pharyngeal wall.
4. ***Swallowing (Deglutition) reflex***

   It is triggered by *afferent* impulses arising from:
   (i) mucous membrane covering anterior and posterior pillars of fauces, and tonsils
   (ii) posterior pharyngeal wall
   (iii) soft palate; and
   (iv) epiglottis

   These impulses travel in trigeminal (V), glossopharyngeal (IX) and vagus (X) nerves to stimulate a group of nerve cells *(deglutition centre)* located in the floor of IVth ventricle near the respiratory centre in the medulla.

The *efferent* fibers pass through motor fibers of V, VII (facial), IX, X and XII (hypoglossal) nerves to the pharyngeal musculature and the tongue. This initiates involuntary pharyngeal stage.

Stage II – Pharyngeal Stage

This stage shows involuntary, complex, closely coordinated movements in the pharynx that pushes the 'bolus' into the oesophagus. It lasts for 1-2 seconds.

Features

1. Soft palate is elevated and thrown against posterior pharyngeal wall to close off the nasal cavity. This prevents the food from entering the nasal cavity.
2. Larynx rises with elevation of hyoid bone, vocal cords are approximated and breathing is momentarily inhibited *(deglutition apnoea)*. Epiglottis guards the laryngeal opening until 'bolus' reaches the oesophagus. Aspiration of food into larynx is also prevented by associated reflex apnoea.
3. Posterior pillars of 'fauces' *i.e.* palatopharyngeal folds approximate to shut off the mouth cavity.
4. Cricopharyngeal muscle briefly relaxes and 'bolus' enters the upper oesophagus.
5. Then cricopharyngeal muscle contracts and vocal cords open to allow resumption of rhythmic breathing.

Stage III – Oesophageal Stage

Structure of oesophagus: The oesophagus is approx. 25 cm in length. It is separated from the oral cavity by the 'upper oesophageal sphincter' and from the stomach by the 'lower oesophageal sphincter'. At rest it is relaxed and may contain air.

Upper oesophageal sphincter

1. It is formed by cricopharyngeal muscle, approx. 3 cm in length, covering upper end of oesophagus. It possesses high resting tone and is completely under the control of vagus (X) nerve.
2. It is normally shut off from the pharynx, thereby preventing swallowing of air during respiration.
3. It opens reflexly upon swallowing for 0.2-0.3 second after the beginning of a swallow and remains open for 0.5-1 second. Thus permitting the 'bolus' to enter the body of oesophagus.

Lower oesophageal sphincter or Cardiac sphincter

1. The last 2.5 cm of oesophagus is sphincteric in action (*not* a true anatomical sphincter). At rest, it is in a state of tone and its walls are tightly in apposition. Its activity is regulated by X nerve fibers originating within the dorsal motor nucleus.
2. Swallowing or distension of oesophagus with food causes reflex relaxation of the sphincter within 1.5-2.5 seconds. The reflex is mediated via X nerve and myenteric plexus that *secrete nitric oxide (NO)* and 'vasoactive intestinal peptide' (VIP) (page 272).
3. When peristaltic contractions reach this region the sphincter closes and may undergo a strong and prolonged after-contraction, thereby preventing regurgitation of food, gastric juice and air.
4. During gastric digestion, 'gastrin' (page 216) released by gastric mucosa, increases the tone of sphincter and keeps it close. 'Secretin' (page 232) decreases the tone and produces relaxation.

Types of oesophageal peristalsis – These are of 2 types: primary and secondary **(Table 26.2)**.

Swallowing or local stimulation of oesophagus at any level causes development of ***peristalsis***. It consists of a lumen-obliterating contractions, 4-8cm in length which moves down at a speed of approx. 2-4 cm/sec. Thus, after reaching the oesophagus, food is propelled into the stomach by peristalsis. The strength of peristaltic contractions is proportional to the size of the 'bolus' entering the oesophagus. Gravity plays little role in this process, as its rate of progress along oesophagus is as rapid in supine as in the erect position.

The oesophageal stage for liquid food lasts for 1-2 seconds and for dry food it lasts for 10 seconds.

Important Note

There is a complicated relationship between the 'deglutition' centre and other centres (respiratory and cardiac) located in the medulla. Therefore, each act of swallowing is associated with arrest of respiration *(deglutition apnoea)* and an increase in heart rate.

Table 26.2: Main differentiating features between primary and secondary oesophageal peristalsis

Primary oesophageal peristalsis	Secondary oesophageal peristalsis
1. It is initiated by swallowing and begins when food passes into the oesophagus from the pharyngeal cavity.	1. It is initiated by the presence of food within the oesophagus after primary peristalsis is completed and is due to stimulation of mechanical or irritant receptors.
2. It is coordinated by vagal fibers originating from the swallowing centre within the medulla.	2. It is coordinated by intrinsic nervous system of the oesophagus.

APPLIED: DISORDERS OF SWALLOWING

I. *Deglutition reflex.* If *abolished*, it causes regurgitation of food into nose or aspiration of food into larynx. It is seen by;
1. lesions involving medulla, IX or X nerves;
2. anaesthetizing pharynx with cocaine (temporary abolition of the deglutition reflex).

II. *Aerophagia.* In nervous individuals the upper oesophageal sphincter resting tone is low, therefore, air is unavoidably swallowed in the process of eating and drinking. The swallowed air:
1. gets regurgitated (*belching*) partly;
2. some of the gases it contains get absorbed; and
3. majority of it passes on to the colon and is then expelled as *flatus* (page 255).

III. *Achalasia Cardia* (Fig. 26.2). This condition is characterised by failure of lower oesophageal sphincter to relax completely on swallowing due to destruction of local nerve plexuses. Therefore, the number of 'VIP' and nitric oxide containing neurons in the lower oesophagus are decreased and sphincter becomes hyperresponsive to circulating gastrin. Constriction of sphincter causes food accumulation in the oesophagus and proximal oesophagus thus dilates.

IV. *Incompetence of lower oesophageal sphincter* causes reflux of acid gastric contents into oesophagus producing heartburn and oesophagitis (*gastroesophageal reflux disease-GRD*). This can lead to ulceration and stricture due to scarring.

V. *Dysphagia.* It means difficulty in swallowing. It may occur due to disorders involving any of the stages of swallowing.

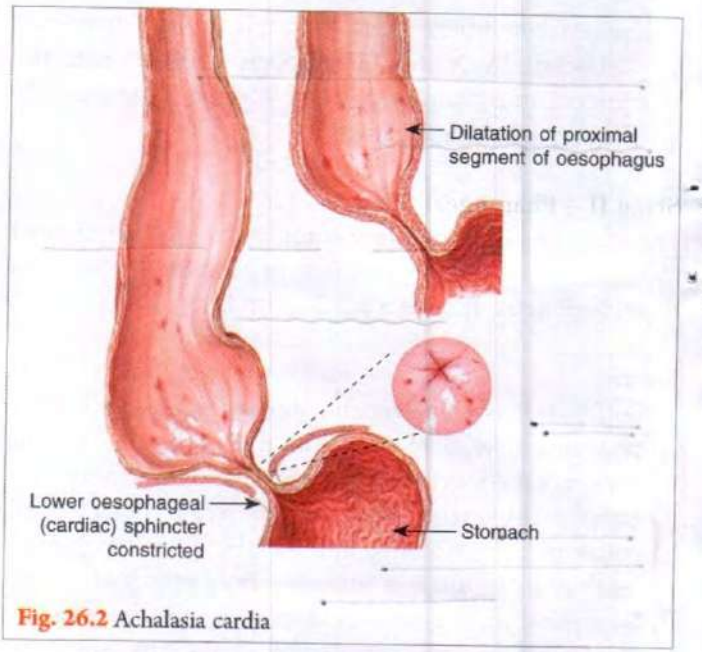


Fig. 26.2 Achalasia cardia

Study Questions

1. Write short notes on:
   (i) Principal action of muscles of mastication
   (ii) Deglutition reflex and Deglutition apnoea
   (iii) Cardiac sphincter
   (iv) Disorders of swallowing
   (v) Achalasia cardia
   (vi) Oesophageal peristalsis
   (vii) Upper and lower oesophageal sphincter.
2. Does gravity play any significant role during deglutition? Justify.
3. What will happen and why?
   (i) If pharynx is anaesthetised with cocaine
   (ii) If deglutition reflex is abolished.
   (iii) If lower oesophageal sphincter fails to relax properly
   (iv) If we do not chew the food properly before swallowing
4. Give physiological basis of:
   (i) Gastroesophageal reflax disease (GRD)
   (ii) Stoppage of breathing during swallowing
   (iii) Belching
   (iv) Difficulty in swallowing

MCQs

1. Which phase of the swallowing process is involuntary?
   (a) Oral and oesophageal
   (b) Pharyngeal and oesophageal
   (c) Only oesophageal
   (d) Oral and pharyngeal
2. To generate the swallowing reflex integrity of chain involves all *except*:
   (a) Medulla
   (b) Trigeminal nerve
   (c) IX and X cranial nerve
   (d) Hypothalamus

3. *Not true* statement with reference to deglutition apnoea is:
   (a) Refers to momentary inhibition of breathing during swallowing
   (b) Lasts until 'bolus' reaches the oesophagus
   (c) Prevents aspiration of food into larynx
   (d) A voluntary reflex
4. Dysphagia means:
   (a) Loss of deglution reflex
   (b) Difficulty in breathing
   (c) Inhibition of breathing
   (d) Difficulty in swallowing
5. The principal function of the lower oesophageal sphincter is:
   (a) To allow stomach acid into the oesophagus
   (b) To maintain food in the oesophagus for digestion
   (c) To prevent reflux of stomach contents
   (d) Non-existent
6. Deglutition reflex, if abolished, causes:
   (a) Bradycardia
   (b) Hypernoea
   (c) Regurgitation of food into nose
   (d) None of the above
7. Achalasia cardia is characterized by:
   (a) Accumulation of food in the oesophagus
   (b) Relaxation of cardiac sphincter
   (c) Decreased response of lower oesophageal sphincter to circulating gastrin
   (d) Increase in number of nitric oxide neurons in the lower oesophagus
8. Deglutition (swallowing) centre is situated in:
   (a) Midbrain
   (b) Pons
   (c) Medulla
   (d) Cerebellum
9. The process of swallowing involves all of the following *except*:
   (a) Closure of the glottis
   (b) Involuntary relaxation of the upper oesophangeal sphincter
   (c) Involuntary movements of the tongue against the palate
   (d) Oesophageal peristalsis

Answers

1. (b) 2. (d) 3. (d) 4. (d) 5. (c) 6. (c) 7. (a) 8. (c) 9. (c)

Chapter 27

The Stomach

I. Physiological Anatomy of the Stomach: Structure; Innervation; Functions
II. Composition and Functions of Gastric Juice
III. Mechanism of Secretion of Gastric Juice
IV. Regulation of Secretion of Gastric Juice → #: factors & Regulation are DIFFERENT
V. Regulation of Gastric Motility and Emptying
VI. Applied Aspects

Total gastrectomy Pathophysiology of peptic ulcer Physiology of vomiting

PHYSIOLOGICAL ANATOMY OF THE STOMACH

Gross structure of the stomach

The stomach is a hollow muscular organ consisting of the following parts: (Fig. 27.1)

1. *Fundus.* It is the upper part of the stomach above the level of the cardiac orifice.
2. *Body.* It is the main part of the stomach to the left of the angular notch, the incisura angularis in the lesser curvature.
3. *Pyloric part.* It is the lower part of the stomach and is divided into:
   (i) *pyloric antrum* or *vestibule*, and
   (ii) *pyloric canal.*

The pyloric antrum and pyloric canal are anatomically continuous and respond to nervous control as a *unit*. Functionally, first part of the duodenum is associated with the pyloric part of the stomach.

Arrangement of musculature

1. As in the general organization of the wall of the GIT (page 198), stomach has an outer longitudinal and an inner circular smooth muscle layer. Between the mucous membrane and circular layer there is an additional smooth muscle layer whose fibers run obliquely to fuse finally with the circular smooth muscle layer. They maintain the normal length of the lesser curvature.
2. The stomach and duodenum are divided by a thickened circular smooth muscle layer called ***pylorus*** or ***pyloric sphincter.***
3. Each muscle layer in the stomach forms a functional syncytium and, therefore, acts as a unit.
4. In the fundus, where the layers are relatively thin, strength of contraction is weak; in the antrum, where the muscle layers are thick, strength of contraction is greater.
5. When stomach is empty its walls are firmly in apposition. When food enters the stomach, inner muscle fibers get elongated to enlarge the size of the cavity uniformly in order to accommodate the new contents, without much change in internal pressure.

pits in Rugae = Foveolae.

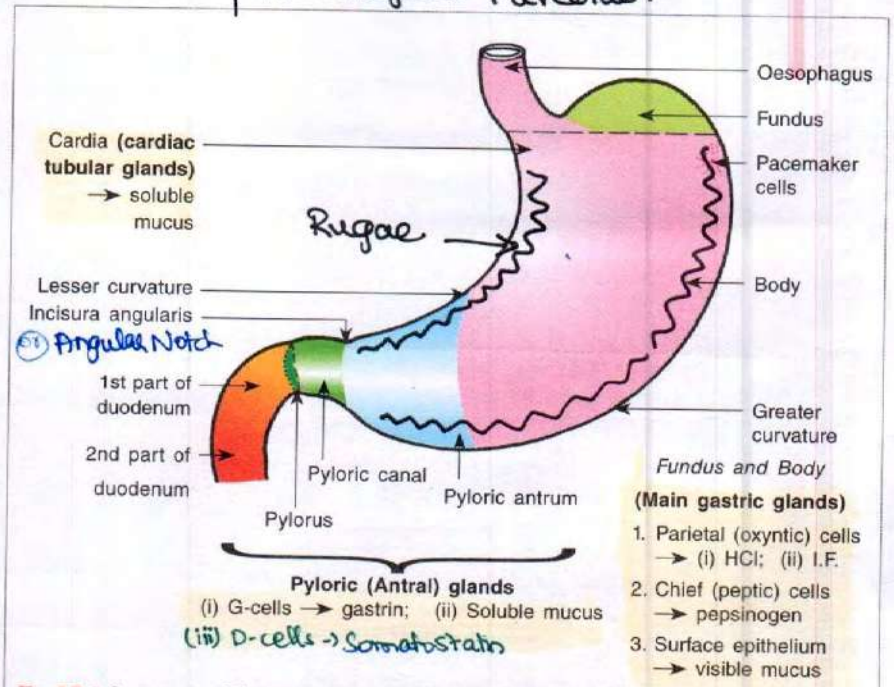


Fig. 27.1 Structure of the stomach and function of types of glands in it

innermost = Oblique

Gastric mucous membrane

Gastric mucous membrane contains three types of gastric glands: *(A) main gastric glands; (B) cardiac tubular glands and (C) pyloric or antral glands.*

(A) MAIN GASTRIC GLANDS

These glands are *maximum* in number compared to other gastric glands and are found in the mucosa of body and fundus of the stomach **(Fig. 27.2)**. The glands possess short ducts and long alveoli *i.e.* body. The alveoli contains:

1. Chief or peptic or zymogen cells which secrete *pepsinogen*
2. parietal or oxyntic cells which secrete *HCl*.

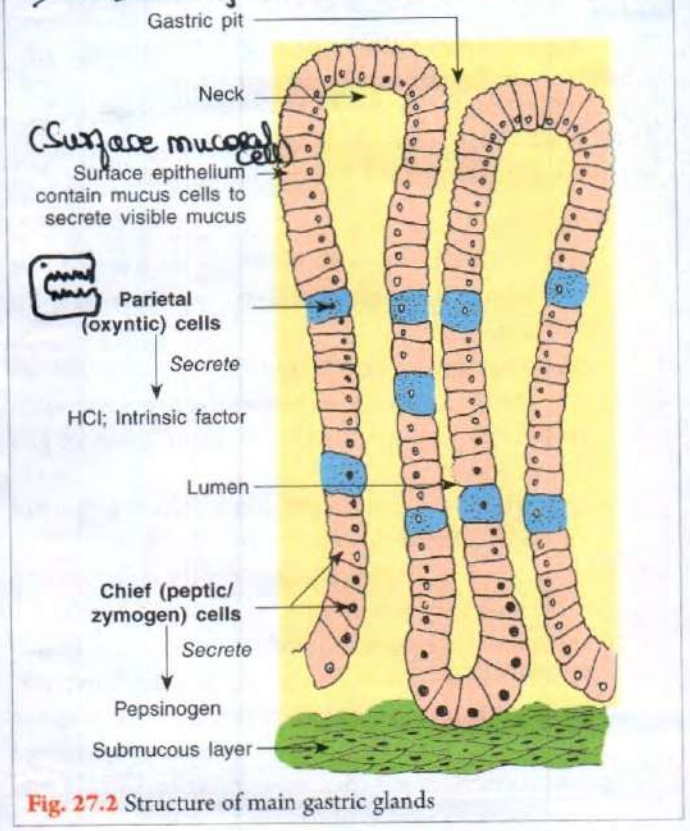


Fig. 27.2 Structure of main gastric glands

Pepsinogen

It is secreted as an inactive protein (MW 42500) which is converted by acid HCl (optimally at pH = 2) to active proteolytic enzyme ***pepsin*** (MW 35000). Also a small amount of pepsin can cause activation of the remaining pepsinogen, called *autocatalytic action of pepsin*. Atropine abolishes pepsinogen secretion.

pepsinogen (inactive) —(HCl, only when pH of gastric secretion is < 6.0)→ *pepsin* (active)

Functions

1. It digests proteins to polypeptides (proteoses and peptones), by hydrolyzing peptide bonds between phenylalanine and other amino acids. Optimal activity is seen at pH < 4.0 and gets inactivated at pH > 5.0
2. ***Curdles milk*** – pepsin acts on water soluble *caseinogen* (milk protein) to form soluble 'casein'. This combines with calcium salts to form insoluble calcium caseinate, which gets readily digested enzymatically. (In other animals, this function is carried out by ***Rennin***, found in gastric juice of these animals, in humans this function is performed by pepsin.)

Parietal (oxyntic) cells

These are found chiefly in the body of the stomach. Pure parietal cell secretion is a clear colourless fluid which contains H^+ concentration equivalent to 0.17 N 'HCl' with pH as low as 0.87. However, body of the stomach is less acidic (pH = 1.0) than this, because of its neutralization or dilution with non-acid secretions. Approx. 1000×10^6 parietal cells can produce maximum output of 20 mEq H^+/hour in response to histamine stimulation.

Functions of HCl

1. It provides H^+ concentration necessary for optimum pepsin activity.
2. Activates 'pepsinogen' to 'pepsin' (page 217), and 'prosecretin' to 'secretin' (page 232).
3. Helps in killing ingested bacteria (bacteriocidal effect).
4. Helps in iron absorption by converting ferric (Fe^{3+}) to ferrous (Fe^{2+}) form of iron, the form in which iron can be absorbed.
5. Stimulates bile and pancreatic juice secretion.

The parietal (oxyntic) cells also secrete a heat labile glycoprotein, the ***Intrinsic Factor*** *(I.F.)* which combines very firmly with dietary vitamin B_{12} and helps in its subsequent absorption from the small intestine. Gastric mucosa atrophy causes deficiency of 'I.F.' with subsequent failure to absorb vitamin B_{12} and eventually *pernicious anaemia* (page 71) develops.

The *surface epithelium* of gastric mucosa consists of columnar or 'mucus' cells which secrete ***mucus*** and HCO_3^-. Mucus is made up of glycoproteins (mucins) which is *'visible'* (as compared to 'soluble' mucus secreted by pyloric and cardiac tubular glands). ***Visible mucus*** is a gel like substance, alkaline in nature; it lubricates the food and coats the mucosa. This mucus along with HCO_3^- plus tight junction between the mucosal cells ***(Mucosal-Bicarbonate Barrier)*** serve a protective function preventing damage to the mucosa of stomach (and duodenum also) by acid or peptic digestion **(Fig. 27.3)**. Any mechanical stimulation of surface mucosa and prostaglandins increases its secretion and also that of HCO_3^-.

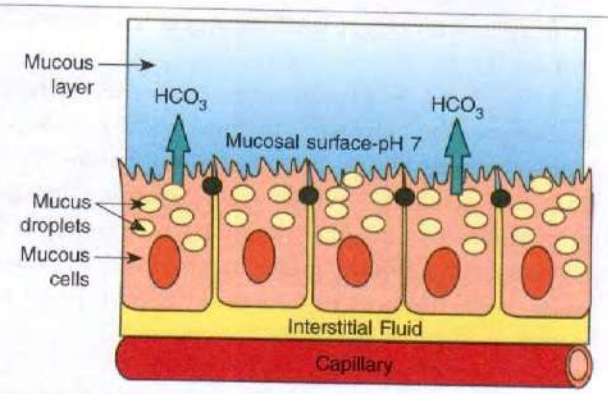


Fig. 27.3 Mucosal bicarbonate barrier

The surface of gastric mucosa is pitted by opening of gastric glands called ***gastric pits;*** there are approx. 100 pits/mm^2 of gastric mucosa.

(B) CARDIAC TUBULAR GLANDS

These glands are found in the cuff of gastric mucosa which immediately surrounds the oesophagus. It secrets *soluble mucus.*

(C) PYLORIC (ANTRAL) GLANDS

1. These glands are found in the mucosa between the level of incisura angularis on lesser curvature and pylorus, and to a lesser extent on the greater curvature.
2. They have a long duct and a short alveoli (body) and secrete ***soluble mucus*** and a rich alkaline viscid juice which is poor in enzyme content. Mucus helps to lubricate the surface over which a large volume of 'chyme' moves back and forth during digestion.
3. The rate of secretion by these glands is 0.5-5 mL/hour and is unaffected by feeding or by vagal stimulation.
4. Deeper portion of pyloric gland have ***G-cells*** or ***Gastrin cells*** which secrete a hormone *gastrin.*

GASTRIN

1. It is secreted by ***G-cells*** or ***gastrin cells*** from deeper portion of pyloric (antral) glands in the gastric mucosa. G-cells are flask-shaped, containing many gastrin granules which disappear after feeding, leaving vacuoles.
2. Gastrin is also found in the pituitary gland (anterior and intermediate lobes), hypothalamus, medulla oblongata, vagus and sciatic nerves.
3. It is secreted as *progastrin* (inactive) which get converted to *gastrin* (active) by HCl or products of digestion.
4. G-cells appear to be neural in origin, because they take up amine-precursors and decarboxylate them. Thus called ***APUD cells*** *i.e.* cells responsible for amine precursor uptake and decarboxylation.
5. It occurs in 3 forms: G34, G17 and G14, containing 34, 17 and 14 amino-acids respectively. ***G-17*** (MW 2000) is the *principal form with respect to gastric acid secretion.* It has half life of 2-3 minutes in the circulation and is inactivated mainly in the kidney and small intestine.
6. *Actions*
   (i) Stimulates the gastric acid (mainly) and pepsin secretion. Its blood level increases specially after ingestion of proteins *i.e.* by amino-acids and peptides. Receptors mediating gastrin response to change in gastric contents are present in the microvilli.

Note

Gastrin also increases the acid secretion by stimulating secretion of histamine from *enterochromaffin like cells* (ECL) present in the body of the stomach, page 219). These cells get hypertrophy when gastric acid secretion is inhibited for prolonged periods.

   (ii) Stimulates the growth of mucosa of the stomach, small and large intestine ***(trophic action).***
   (iii) Stimulates gastric motility. It also causes contraction of lower oesophageal sphincter.
   (iv) It has feeble influence on contraction of gall bladder.
   (v) Stimulates insulin and glucagon secretion but only after a protein meal.
7. *Penta-gastrin* is a synthetic gastrin used for testing gastric secretory function.
8. ***Gastrinoma*** are gastrin producing tumours which can occur in stomach or duodenum or pancreatic tumour of δ-cells (*Zollinger-Ellison syndrome*) (page 225). These tumours secrete large amounts of gastrin which causes excessive secretion of HCl and predisposes to ***peptic ulcer.***

Factors Affecting Gastrin Secretion

*A. Factors that **increase** gastrin secretion*

(1) Luminal factors

(i) Products of protein digestion: peptides and amino-acids. (specially phenylalanine and tryptophan)

(ii) Distention of pyloric antrum: This response is *not* dependent on extrinsic nerve supply as distension of denervated antral pouch (***Heidenhain pouch***) increases gastrin release. Distension of pyloric antrum stimulates stretch receptors, impulses enter the Meissner's plexus to stimulate post-ganglionic parasympathetic

neurons which end in parietal cells and finally increase acid secretion.

Important Note

A small pouch of stomach with intact nerve and blood supply is called *Pavlov's pouch (Pavlov, I.P.).*

(2) Neural factors (Not ATROPINE-independent)

Increased vagal discharge, (mediated via gastrin releasing peptide - GRP or Bombesin). Atropine does *not* inhibit the gastrin response to a test meal because the transmitter secreted by the post-ganglionic vagal fibers that innervate the G-cells is 'GRP' (and not A-ch).

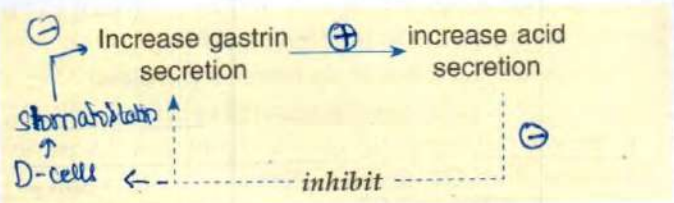
(3) Blood borne factors: Calcium and epinephrine.

Note

In pernicious anaemia patients - page 71, gastrin secretion is chronically elevated due to atrophy of gastric mucous membrane.

*B. Factors that **inhibit** gastrin release*

(1) *Luminal factor* – Acid in the antrum inhibits gastrin secretion by a direct action on G-cells and by release of somatostatin. It is the basis of a negative feedback loop regulating gastrin secretion. How?



(2) *Blood borne factors* – GIT and other hormones, for example:

(i) Secretin (Secretin → acidity + Calcitonin)
(ii) Gastric inhibitory peptide (GIP)
(iii) Vasoactive intestinal peptide (VIP)
(iv) Glucagon
(v) Calcitonin

Innervation of the stomach

(page 199).

Functions of the stomach

1. *Storage* – Stomach serves as a reservoir for the food ingested. Food remains in the stomach for several hours (page 222) and passes gradually into the intestine.
2. *Digestive*-Nutritive substances undergo chemical changes by its secretory activity which provides the enzymes; and HCl is required for the initial digestion of proteins.
3. HCl kills many of the ingested bacteria (also to see page 215).
4. *Intrinsic factor* is necessary for absorption of vitamin B_{12} from the small intestine. Decreased absorption of vitamin B_{12} produces pernicious anaemia (page 69).
5. Food is released at a controlled steady rate into the duodenum to provide proper time for digestion and absorption by small intestine.

COMPOSITION AND FUNCTIONS OF GASTRIC JUICE

Daily secretion : 2.5–3 litres/day; *"isotonic"* with plasma.
pH : 1–2 (0.7–4) (alters with pro-ein content ∝)
Reaction : Acidic due to HCl

99.5% = Water; Other = Org. & Inorg.

Electrolytes

Cations : Na^+; K^+; H^+; Mg^{2+}. At low secretory rates, Na^+ con-centration is high and H^+ concentration is low; but as acid secretion increases, Na^+ concentration falls. (Na^+ ↑ H^+ ↓; 20–150 mEq/L; Ca^{++})

Anions : Cl^-; HCO_3^-; HPO_4^{2-}; SO_4^{2-}

(INORGANIC)

Enzymes (ORGANIC)

1. *Pepsinogen* (inactive) —HCl, optimal pH = 2→ *pepsin* (active)

helps to digest proteins. (Also refer to page 224)

2. *Rennin* – (in infants and in other animals); curdles milk (page 215) *i.e.* causes coagulation of milk thus prevents rapid passage of milk from the stomach.
3. *Gelatinase* – Liquifies gelatin, a protein contained in connective tissues.
4. *Gastric lipase* – weak fat splitting enzyme from stomach; optimal pH is 4–4.5; inactivated at pH 2.5.
5. *Lysozymes* – bacteriocidal enzymes.
6. *Urease* – hydrolyse urea to produce ammonia.
7. *Carbonic anhydrase* – present in small amounts.

• Gastric amylase

Mucus – It is of two types:

1. *'Soluble' mucus* secreted by pyloric and cardiac tubular glands.
2. *'Visible' mucus* secreted by surface epithelium of gastric mucosa. (page 215)

Intrinsic factors (IF) – helps in absorption of vitamin B_{12}.

Water → 99.5%

* Post-prandial Alkaline Tide
↳ Due to excess prod-⇒ ↑↑ HCl
⇒ ↑ HCO_3^- in blood. ⇒ Alk ⓤ urine

MECHANISM OF SECRETION OF GASTRIC JUICE

Mechanism of pepsinogen secretion

(page 219, see under regulation of secretion of gastric juice).

Mechanism of secretion of HCl

(Davenport, *H.W. 1971*) (Fig. 27.4)

1. The Pure parietal cell secretion contain 0.17 N HCl with pH 0.87 (page 215). Plasma concentration of H^+ is 0.00004 mEq/ L (40 ng / L) and that of Cl^- is 100 mEq/L; whereas in the gastric juice H^+ concentration is equal to the Cl^- concentration of 150 mEq/ L *i.e.*

	Plasma	*Gastric Juice*
H^+ Concentration (mEq/L)	0.00004	150
Cl^- concentration (mEq/L)	100	150

by parietal cell

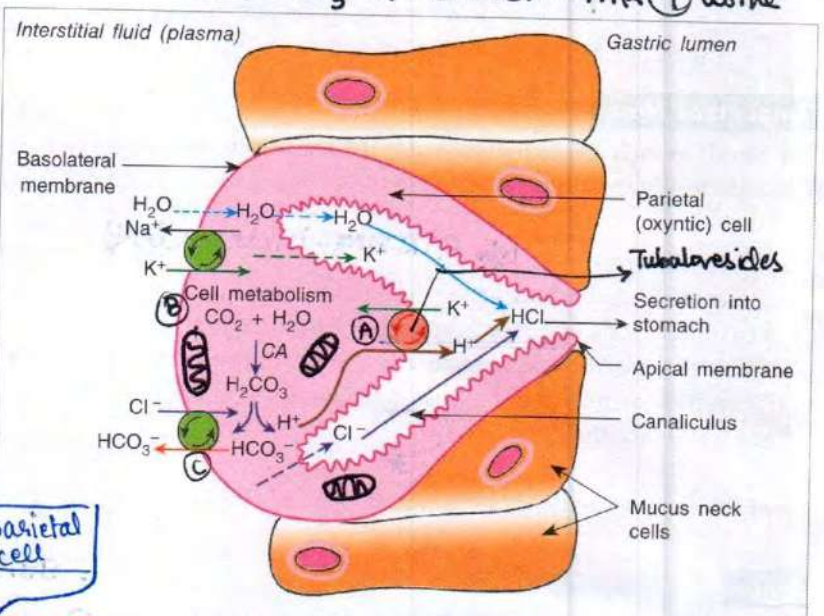


Fig. 27.4 HCl secretion by parietal cells in the stomach. (H^+ is secreted into the lumen of the canaliculi in exchange for K^+ by $H^+ - K^+$ ATPase, i.e. *proton pump*)

Since, gastric juice is isotonic with plasma, therefore, mechanism in the parietal cells that can *actively* transport H^+ against a concentration gradient of this magnitude is **$H^+ - K^+$ ATPase** (or *proton pump*) in the apical membrane of the cells which faces the lumen of the gastric glands.

2. The resting parietal cells have intracellular canaliculi, which open on the apical membrane of the cell; and many vesicles in the cytoplasm which contain **'$H^+ - K^+$ ATPase' in inactive form.**
3. When the parietal cells are stimulated, the vesicles fuse with the cell membrane and microvilli project into the canaliculi, so the cell membrane in contact with gastric lumen is greatly increased, and $H^+ - K^+$ exchange begins. H^+ is secreted into the lumen of the canaliculi in exchange for K^+ by *'$H^+ - K^+$ ATPase'*, the energy for this is provided by hydrolysis of ATP. The ATP is generated by mitochondria found in very high concentration within the parietal cells. Inhibition of ATP generation prevents H^+ secretion.
4. Cl^- is also secreted down its electro-chemical gradient either by pump or through a channel that is activated by cAMP in the apical membrane (the concentration gradient is directed 'inward', but the electrical gradient directed 'outward' is much greater). The flow of Cl^- creates a negative potential inside the canaliculi, causing K^+ to flow passively into the canaliculi.
5. Water enters the canaliculi down the osmotic gradient created by the movement of HCl into the canaliculi and gastric juice is 'isotonic' with plasma.
6. The H^+ that is secreted into the lumen comes from H_2CO_3 which in turn is formed by hydration of CO_2 in the presence of enzyme 'carbonic anhydrase'. The parietal cells are particularly rich in this enzyme.
7. The HCO_3^- formed by dissociation of H_2CO_3 is discharged into the interstitial fluid (blood) by an 'antiport' in the basolateral membrane of the parietal cell that exchange HCO_3^- mainly for Cl^- (since Cl^- is most abundant anion in interstitial fluid).
8. Thus, when gastric acid secretion is increased after a meal, sufficient H^+ may be secreted to raise the HCO_3^- in the gastric venous blood, which contributes a greater amount of HCO_3^- to the systemic circulation and pH of systemic blood rises. This is called ***post-prandial alkaline tide***, which is characterised by:
   (i) high pH of urine (alkaline urine)
   (ii) breathing slightly depressed and alveolar pCO_2 rises. (Biryani khaake "mast")

Regulation of HCl secretion **(Fig. 27.5)**

A. Increased by: **(Table 27.1)**

B. Decreased by Prostaglandins E_2 (PGE_2). It acts via G_1 receptors to decrease adenylate cyclase activity and intracellular cAMP **(Fig. 27.5)**. Somatostatin also decreases H^+ secretion by inhibiting release of histamine and gastrin directly. (↓)

Note

All the factors which alter (increase or decrease) HCl secretion, they do so by causing release of these agents like histamine, A-ch, gastrin or PGE_2. The actions of these agents are summarized in Fig. 27.6.

Applied: ↑ HCl secretion ↑ Acetazolamide

REGULATION OF SECRETION OF GASTRIC JUICE

The regulation of secretion of gastric juice is broadly brought about by two mechanisms: (A) *nervous* and (B) *humoral mechanism.*

A. NERVOUS REGULATION

The neural mechanisms are:

(1) local autonomic reflexes involving cholinergic neurons; and

(2) impulses from the CNS by way of the vagus nerve.

The vagus (X) nerve is secretomotor nerve to the stomach. It causes ***direct*** stimulation of the secretion of gastric juice. The juice is rich in HCl and rich in pepsin ***(vagal juice)***; and begins after a latency of 5-7 minutes, the volume reaches its peak within 1 hour and response lasts for 3 hours. This is abolished by atropinization or vagotomy.

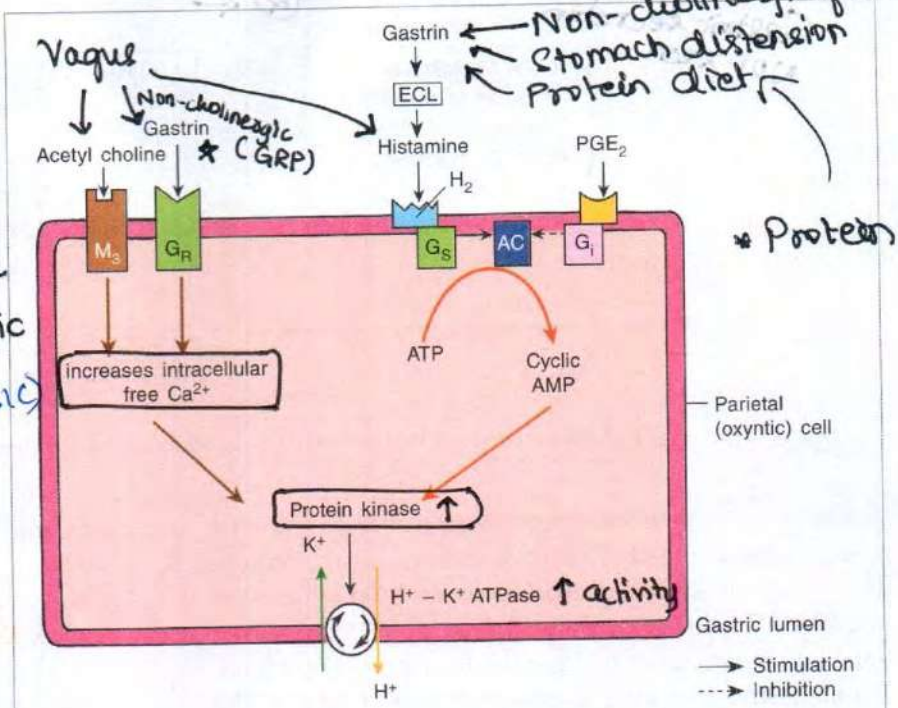


Fig. 27.5 Regulation of gastric acid secretion by the parietal cell (M_3 : M_3 muscarinic receptor; G_R : gastrin receptor; AC : adenylate cyclase; ECL : Enterochromaffin like cells; G_s and G_i protien: The subscripts 's' and 'i' denotes stimulatory and inhibitory respectively)

Vagal stimulation increases gastric juice secretion by:

(i) Release of ***gastrin releasing peptide (GRP)*** which increases gastrin secretion. This, in turn, increases acid secretion mainly (page 217);

(ii) Release of ***acetylcholine (A-ch)*** which acts directly on gland cells located in the body and the fundus of the stomach to increase acid and pepsinogen secretion.

B. HUMORAL REGULATION: Role of gastrin

The humoral mechanism is mediated by release of hormone *gastrin*.

Psychic stimuli, food and its digestive products in the stomach reflexely increase the gastric juice secretion. *Mechanism* – the impulses travel in vagus (X) nerve to reach antral mucosa where they cause release of an excitatory

Table 27.1: Agents that increase HCl secretion in the stomach

	Agent	Sources	Mechanism of action
1.	Histamine	(i) Cells in gastric mucosa that resemble mast cells called, ***enterochromaffin-like cell (ECL)***, page 216); gastric mucosa also has high histamine content. (ii) foodstuffs *e.g.* meat, cabbage, etc.	Histamine binds to H_2 receptors and via G_S increases adenylate cyclase activity and intracellular cAMP.
2.	Acetyl-choline (A-ch)	Endings of post-ganglionic cholinergic neurons innervating the parietal cells.	Via M_3 muscarinic receptors and increases intracellular free Ca^{2+}.
3.	Gastrin	G-cells of pyloric (antral) glands; reach the parietal cells via the circulation (also refer to page 216).	Via gastrin receptors which increase intracellular free Ca^{2+}.

cAMP and Ca^{2+} act via protein kinase to increase the transport of H^+ into the gastric lumen by '$H^+ – K^+$ ATPase'. The intracellular events interact, therefore, activation of one receptor type potentiates the response of another to stimulation.

Important Note

Potentiation means simultaneous stimulation of two stimulants is greater than the sum of response to either agent. Potentiation of gastric H^+ secretion occurs because each agent has a different mechanism of action on the parietal cell.

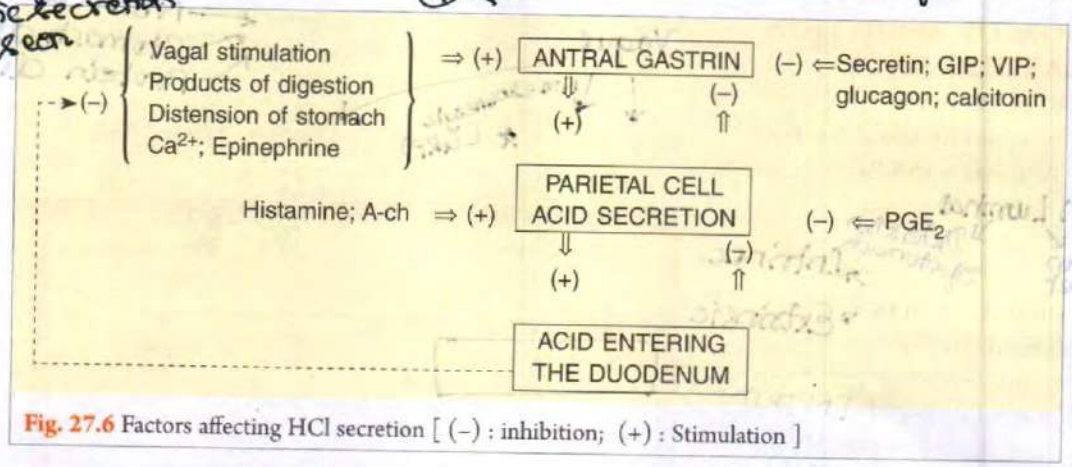


Fig. 27.6 Factors affecting HCl secretion [(–) : inhibition; (+) : Stimulation]

hormone, *gastrin* from pyloric antral mucosa into the gastric venous blood. This gastrin then passes through the portal and systemic circulation and then returns via the arterial blood to stimulate the parietal (oxyntic) cells of the body of the stomach. It excites the liberation of highly acidic gastric juice after a latency of 30–60 minutes. The response lasts for 2 hours.

Therefore, vagal activity causes:

(1) direct stimulation of secretion of gastric juice by the parietal cells; and

(2) by liberation of gastrin from the 'gastrin cells' of pyloric antrum (Also refer to the factors affecting gastrin secretion: page 216).

Phases of Gastric Juice Secretion

The physiological regulation of gastric juice is usually discussed in 3 phases: *cephalic*, *gastric* and *intestinal* phase. The main characteristic features of each phase are given in Table 27.2 and Fig. 27.7.

(a) Cephalic Influences

Reflex stimulation of vagus occurs in response to 'psychic' stimulation which increases gastric juice secretion, called *Appetite Juice*. It occurs by:

1. Taste of the food, reflexly increases gastric secretion; and
2. Sight, smell or thought of food *(Sham Feeding)*, increases gastric secretion by *conditioned reflexes i.e. inborn reflexes* that are established early in life.

Neural pathway

Neurogenic signals originate in the cerebral cortex or in the *appetite centre* of the hypothalamus or amygdala. They are transmitted to the dorsal motor nuclei of the vagi. The efferent fibers travel in vagus (X) nerve to reach stomach where they stimulate the gastric glands to cause increased secretion of pepsin and HCl.

(b) Gastric Influences

Food in the stomach further augments the increase in gastric juice secretion produced by cephalic influences. How?

(1) *Local reflex pathway i.e.* reflex arc is totally within the wall of the stomach.

(i) Food in the stomach by stretching the receptors in the wall of the stomach (*mechanical stimulus*); and

(ii) products of digestion in the stomach, mainly amino-acids stimulate gastric mucosa (*chemical stimulus*).

The fibers from the receptors enter Meissner's plexus (page 199) where the cell bodies of the receptor neurons are located. They synapse on post ganglionic parasympathetic neurons that end on parietal cells and stimulate acid secretion.

Important Note

The post-ganglionic neurons in the local reflex are the same ones innervated by the descending vagal pre-ganglionic neurons from the brain that mediate cephalic phase of secretion.

(2) **The products of protein digestion**, increase '*gastrin*' secretion and this increases secretion of gastric acid mainly.

(c) Intestinal Influences

The intestinal phase of secretion starts when food (chyme, page 221) begins to enter the duodenum from the stomach. Its influence on gastric juice secretion is small **(Table 27.2)**. However, fats, carbohydrates and acid in the duodenum inhibit gastric acid and pepsin secretion via GIP (gastric inhibitory peptide), secretin and other GIT hormones.

Proof: Gastric acid secretion is directly proportional in degree to the amount of intestine removed. This may be due to removal of the source of hormones *(peptide YY)* that inhibit acid secretion.

Table 27.2: Characteristic features of 3 phases of gastric juice secretion (Fig. 27.7)

Cephalic Phase	Gastric Phase	Intestinal Phase
(1) It occurs even before food enters the stomach *i.e. psychic* stimulation of gastric juice in response to sight, smell, thought or taste of the food.	It occurs once food enters the stomach.	It occurs when food enters the duodenum.
(2) Its influences on gastric juice secretion are *vagally* mediated responses induced by activity in the CNS.	Its influences on gastric juice secretion are primarily *local reflex* responses and response to *gastrin*.	Its influences on gastric juice secretion are the *reflex* and *hormonal feedback* effects initiated from the mucosa of the small intestine.
(3) It accounts for approx. 30-50% of the total gastric juice secretion normally.	It accounts for upto 50-60% of the total gastric juice secretion.	Its contribution to gastric juice secretion is much less as compared to cephalic and gastric phase.

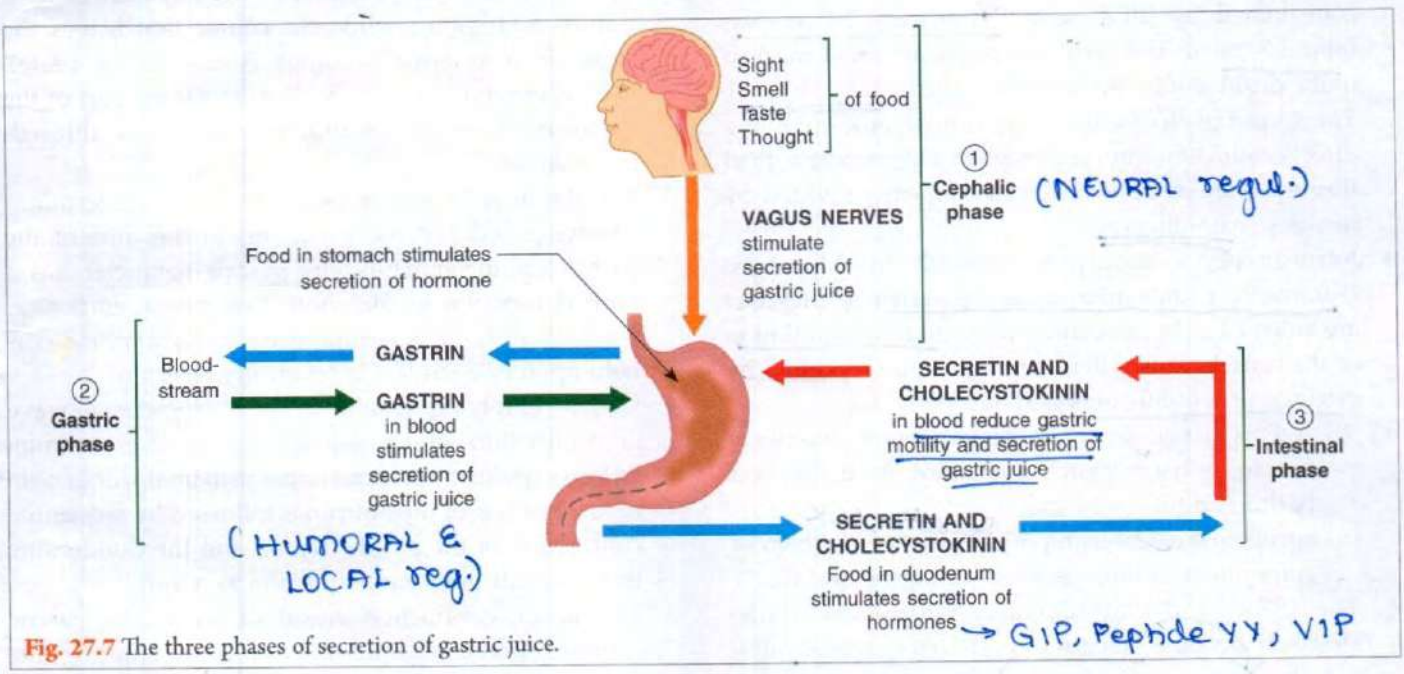


Fig. 27.7 The three phases of secretion of gastric juice.

Important Note

The nervous and humoral stimulation of gastric juice secretion interact. Both these mechanisms are not merely additive but are *markedly synergistic*. How? **(Fig. 27.8)**

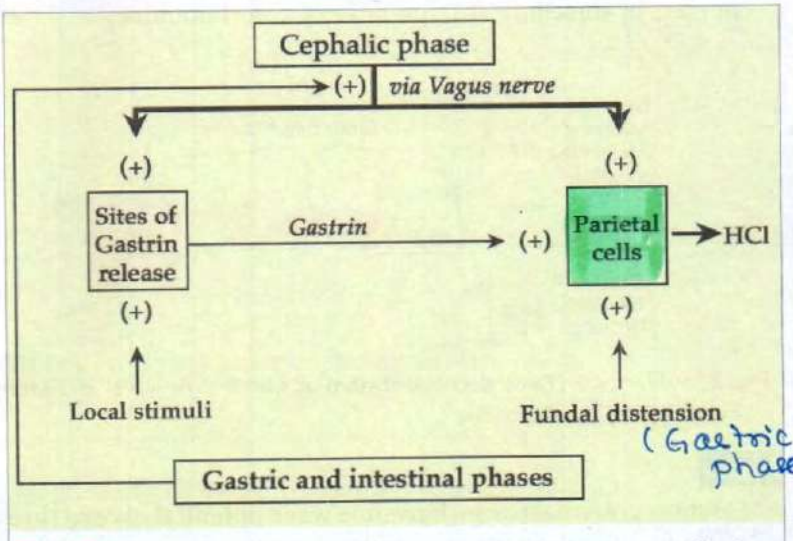


Fig. 27.8 Interaction of nervous and humoral mechanisms responsible for gastric juice secretion. {Size type arrows; (+) : stimulation}

(d) Other Influences

1. *Hypoglycemia* increases gastric juice secretion (both acid and pepsin), it acts via brain and vagal efferents.
2. *Alcohol* and *caffeine* both act directly on the gastric mucosa to increase acid and pepsin secretion.
3. *Emotional effects* (vagally mediated). Anger, excitement increase the gastric juice secretion whereas fear and depression decrease it.

REGULATION OF GASTRIC MOTILITY AND EMPTYING

GASTRIC MOTILITY

1. Stomach shows a ***basic electrical rhythm (BER) or gastric slow wave*** *i.e.* a wave of depolarization of smooth muscle cells proceeding from the circular muscles of the fundus of the stomach to the pylorus (pyloric sphincter) **(Fig. 27.9)** (Also refer to page 214).
2. *BER* is initiated by *pacemaker cells* (called ***interstitial cells of Cajal***) situated near the fundus on the greater curvature (in the circular muscle layer) which propagates initially slowly – less than 1 cm/sec but faster as the contraction wave approaches the antrum at a speed of 4 cm/sec.
3. In man, ***peristaltic contractions*** in the stomach are coordinated by 'BER' and occur every 20 seconds (about 3/min). The *peristaltic waves* are most marked in the distal half of the stomach (called ***antral systole***). The spread of electrical activity is myogenic involving direct conduction from cell to cell by electrotonic spread through the low resistance cell contacts provided by nexuses (gap junctions).
4. Intrinsic nerve supply to stomach by Myenteric (Auerbach's) and submucous (Meissner's) plexuses are believed to be concerned with efferent stimulation of the muscle cells. Their activity can be modified by extrinsic autonomic nerves (page 199).
5. (i) Agents that *initiate* contraction of smooth muscles of the stomach are: gastrin, histamine, A-ch, nicotine, barium and K^+; whereas
   (ii) agents that *inhibit* it are: enterogastrone (see below), epinephrine, norepinephrine, atropine and Ca^{2+}.
6. The 'BER' and consequently 'peristalsis' in the stomach become irregular and disorganised after vagotomy (due to unopposed action of sympathetic nerve); or injection of large dose of catecholamine, or by transection of the stomach wall. Therefore, the gastric slow wave (BER) plays a major role in the control of gastric emptying.

GASTRIC EMPTYING

1. Normally the food stays in the stomach for $2^1/_2$–3 hours on a mixed diet. When food enters the stomach, it relaxes (primarily the fundus) by a reflex process to easily accommodate 1-2 litres of food, called ***receptive relaxation***. Stretch receptors in the stomach detect the presence of food and initiate ***vaso-vagal reflex*** producing receptive relaxation without much increase in the pressure. Thus serve to mix the food and to control its delivery to duodenum.
2. Food that enters the stomach is usually a mixture of liquids and solids, while the ***chyme*** that leaves the stomach is essentially liquid (semisolid or paste). Gastric emptying begins as soon as a large part of the gastric contents become fluid enough to pass through the pylorus.
3. It is the force of gastric peristalsis which determines emptying and not the variations in the tone of the pyloric sphincter. Thus, the pyloric sphincter has a limited function in the control of gastric emptying. Gastric emptying is normal if the pyloric sphincter is held open or even if it is surgically resected.
4. Gastric emptying results from a progressive wave of contraction which sequentially involves antrum, pylorus (pyloric sphincter) and proximal duodenum *i.e.* contraction of the antrum is followed by sequential contraction of the pyloric region and the duodenum; therefore, all these three function as a ***unit***.
5. In antrum, contraction ahead of advancing gastric contents prevents solid masses from entering the duodenum, therefore, gastric contents are ejected in a bit at a time into the small intestine.

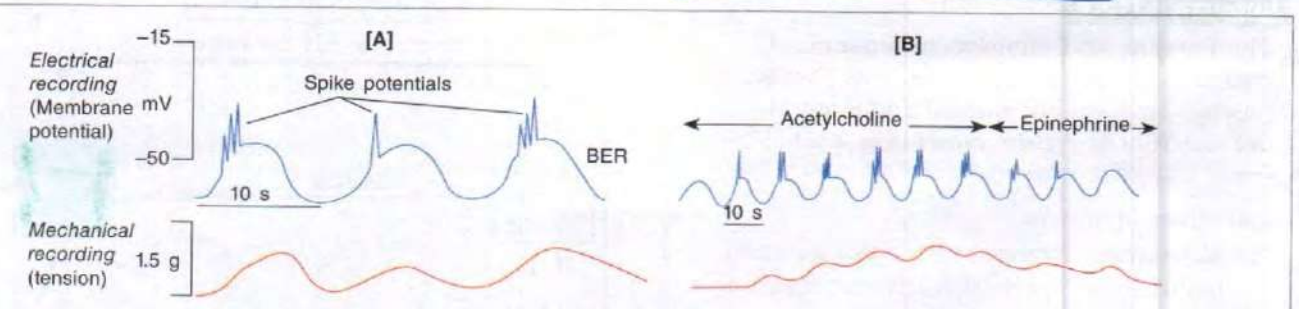


Fig. 27.9 Electrical (Basic electrical rhythm or gastric slow wave) and Mechanical response of GIT smooth muscle (A); and effect of chemical agents on them (B).

Notes

1. Action potential fires when slow wave potential exceed threshold.
2. The force and duration of muscle contraction are directly related to the amplitude and frequency of action potential.

6. *Regurgitation from the duodenum is normally prevented:*
   (i) because the contraction of the pyloric segment tends to persist slightly longer than that of the duodenum; and
   (ii) due to stimulating action of cholecytokinin ('CCK') and 'secretin' on the pyloric sphincter.
7. *Rate of gastric emptying* depends on:
   (i) Directly on the square root of volume of liquid meal remaining in the stomach. According to ***Laplace Law*** (page 318), tension (T) on the wall of an organ is a direct function of its radius (R), therefore, tension in the stomach wall acts as the adequate stimulus for peristalsis by stimulating gastric receptors in the vagi.
   (ii) Products of protein digestion and acid in duodenum act via stimulating receptors in the duodenal mucosa and '*decrease*' gastric emptying. This is a neural mediated reflex called, ***enterogastric reflex***.
   (iii) Size of duodenal ***osmoreceptors***: Rate of gastric emtpying is controlled by the size of 'osmoreceptors' present in the duodenum. Thus, *hypo-osmolar* chyme in duodenum causes distension of 'osmoreceptors', thereby mildly inhibit the rate of gastric emptying; while *hyperosmolar* chyme in duodenum by causing shrinkage of 'osmoreceptors' markedly inhibit the gastric emptying. These effects are neural in origin.

Note

The rate of gastric emptying is *fastest* when stomach contents are *isotonic* and *slowest* if the stomach contents are *hypertonic*.

(iv) Type of food ingested:

Type of food	Gastric emptying	
(a) carbohydrate rich food	rapid, within a few hours	(1)
(b) protein rich food	slow	(3)
(c) fats rich food	slowest	(2)

Important Note

Alcohol intoxication can be avoided if it is consumed after ingestion of a drink rich in fats, e.g. milk, cream etc.

(v) Miscellaneous factors: Gastric emptying is:
   (a) *Inhibited by:* GIP (gastric inhibitory peptide); 'CCK'; secretin; (CCK and secretin collectively called ***enterogastrones***, released from the duodenum); vagotomy; fear and *peptide YY*.
   (b) *Stimulated by*: Gastrin, excitement.

Types of Gastric movem.

Hunger Contractions

Soon after stomach is emptied, mild peristaltic contractions begin which are associated with *migrating motor complex* (MMC) (see below). They gradually increase in intensity over a period of hours. The more intense contractions can be felt and may even be mildly painful. These hunger contractions are associated with sensation of hunger.

Migrating Motor Complexes (MMC)

These are the modified pattern of electrical and motor activity in the smooth muscles of the GIT. This motor activity (MMC) is initiated by *motilin* and migrates from the stomach to the distal ileum at a regular rate (5 cm/min) during fasting. Each cycle has *three* phases **(Fig. 27.10)**:

Phase I : No electrical (spike potential) or mechanical (contraction) activity (Quiescent phase)
Phase II : Irregular electrical and mechanical activity
Phase III : Regular electrical and mechanical activity

They are completely inhibited by a meal and resumed 90-120 minutes after a meal. Each MMC is associated with increased secretion of bile (from liver), gastric juice and pancreatic juice. They help clear the GIT (specially stomach and small intestine) and prepare it for the next meal.

"Broomstick of GIT"

Retroperistalsis:

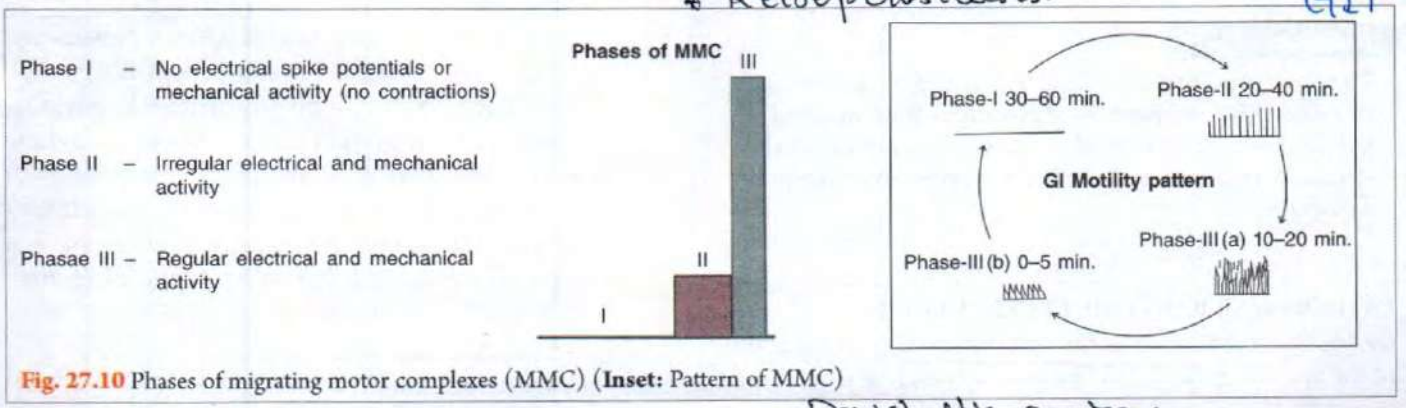


Fig. 27.10 Phases of migrating motor complexes (MMC) (**Inset:** Pattern of MMC)

Gastric emptying movements → Peristaltic contrac, Antral contrac, Retropulsion

APPLIED ASPECT

TOTAL GASTRECTOMY

It means removal of the whole stomach. It exhibits the following features:

1. Deficiency of intrinsic factor produces *pernicious anaemia* (page 71).
2. Protein digestion is normal in the absence of pepsin and nutrition can be maintained.
3. Predisposes to the development of *iron deficiency anaemia* (page 73). As conversion of iron from ferric (Fe^{3+}) to ferrous (Fe^{2+}) form requires HCl (form in which iron is absorbed). However, no more than a trace of iron is absorbed in the stomach.
4. Because of rapid absorption of glucose from the intestine, the resultant hyperglycemia causes abrupt rise in insulin secretion. This leads to hypoglycemia approx. 2 hours after meals.
5. *Dumping Syndrome* (Fig. 27.11). A condition characterized by development of weakness, dizziness and sweating after meals. It is partly due to:
   (i) hypoglycemia (see above); and
   (ii) rapid entry of hypertonic meal into the intestine which causes rapid movement of water into the gut from plasma, thus producing hypovolemia and hypotension.

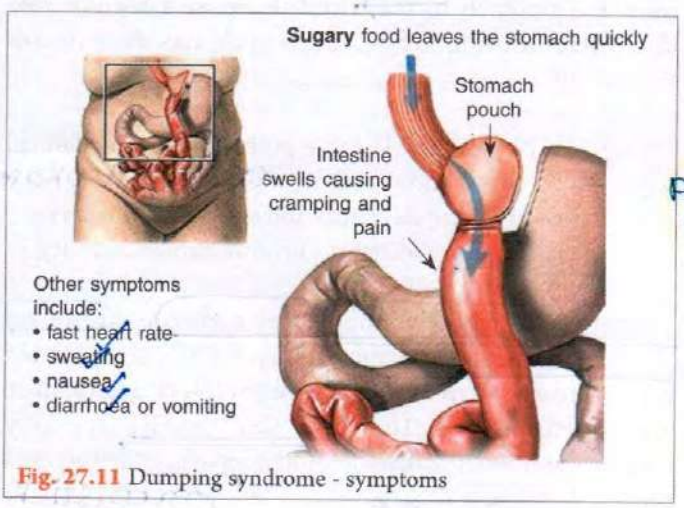


Fig. 27.11 Dumping syndrome - symptoms

Important Note

The obese patients often undergo a surgical procedure in which the stomach is stitched so that most of it is bypassed; therefore, the reservoir function of the stomach is lost resulting in a distressing *Dumping Syndrome*.

PATHOPHYSIOLOGY OF PEPTIC ULCER

Definition: *Peptic ulcer* is the term referred to breakdown of the *mucosal-epithelium* of the stomach and/or duodenum (Fig. 27.12).

Etiology (cause) and pathogenesis

1. The *mucosal-bicarbonate barrier disruption* (page 215). This barrier normally prevents irritation and autodigestion of the mucosa by the gastric secretion. The substances/agents which tend to distrupt the 'barrier' and cause irritation includes:

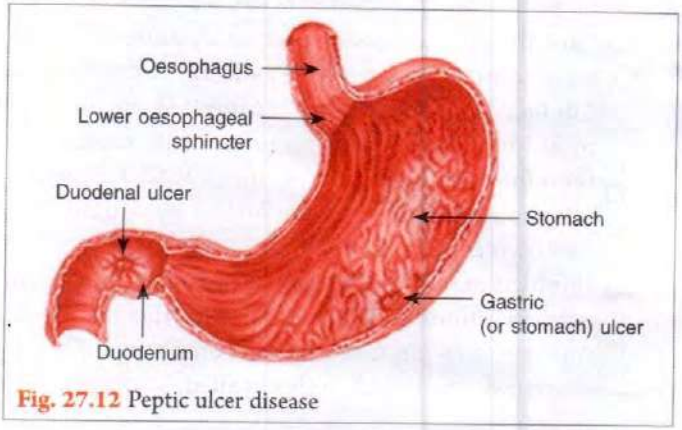


Fig. 27.12 Peptic ulcer disease

   (i) infection with a bacterium, ***Helicobacter pylori*** (*A major cause of gastric ulcer*). H. pylori inhibits somatostatin secretion (Thus stimulating gastric H^+ secretion) and inhibits intestinal HCO_3^- secretion.
   (ii) ethanol (ethyl alcohol)
   (iii) vinegar
   (iv) bile salts
   (v) non-steroidal anti-inflammatory drugs (NSAID), for example, aspirin, brufen, voveran etc. These drugs inhibit prostaglandin synthesis which stimulates 'mucus' secretion and inhibits acid secretion by activating G_i receptors (page 219). This is the reason, why the incidence of peptic ulcer (specially gastric ulcer) increases in persons taking anti-inflammatory drugs.
2. *Hypersecretion of gastric acid.* This leads to ulcers of the duodenum and pre-pyloric portion of the stomach. Gastric acid secretion is often elevated in patients who present with ulcer in this region. Hypersecretion of gastric acid may be due to:
   (i) ***Pepsinogen I.*** Human gastric mucosa contains two immuno-histochemically distinct pepsinogen groups; pepsinogen I and pepsinogen II. Pepsinogen I is found only in acid secreting region, whereas pepsinogen II is also found in pyloric region. Maximal acid secretion correlates with pepsinogen I levels; and patients with congenitally elevated circulating pepsinogen I levels have a five fold greater incidences of peptic ulcer than individuals with normal levels.
   (ii) *Role of Gastrin*
      (a) In most patients with gastric and duodenal ulcers resting gastrin levels are normal,

but their gastrin responses to feeding are greater than normal and their *parietal cells are hyperresponsive* to gastrin.

In conditions such as pernicious anaemia, the acid secreting parietal cells in the stomach are damaged, as a result, gastrin secretion is chronically elevated. This may explain in part the increased incidences of ulcers of the duodenum and pre-pyloric portion of the stomach in patients of pernicious anaemia.

(b) *Zollinger-Ellison syndrome* – This syndrome is seen in patients with gastrinomas *i.e.* tumours that secrete gastrin (page 216). These tumours can occur in the stomach and duodenum, but most of them are found in the pancreas.

PHYSIOLOGY OF VOMITING

Definition: Vomiting or emesis is the forceful expulsion of the food from the stomach and/or intestine.

Sequence of Events **(Fig. 27.13)**

1. 'Nausea' is first experienced, and then salivary secretion is increased and breathing becomes deep, rapid and irregular.
2. 'Retching' may occur which consists of:
   (i) incoordinated spasmodic contraction of respiratory muscles, and
   (ii) descent of diaphragm when expiratory muscles contract.
3. Glottis closes and remains shut till the expulsion of vomited material is effected. This increases intra-pulmonary pressure causing compression of oesophagus. This also prevents aspiration of vomitus into the trachea.
4. Pyloric part contracts firmly and at the same time body of stomach relaxes so that gastric contents are forced into it; *anti-peristalsis* may take place in stomach.
5. Flaccid stomach is compressed by the raised intra-abdominal pressure resulting from descent of diaphragm and contraction of abdominal wall.
6. The cardiac sphincter and oesophagus relaxes and gastric contents are, therefore, driven into the dilated oesophagus.
7. Oesophagus contracts by:
   (i) increased intra-pulmonary pressure,
   (ii) active contraction throughout its length, or
   (iii) a wave of *anti-peristalsis* may pass over it.
   As a result, oesophageal contents are emptied into the mouth.
8. Soft palate is raised and shuts off the nasal cavity from the throat.
9. Towards the end of act of vomiting:
   (i) diaphragm relaxes *i.e.* it ascends; and
   (ii) expiratory muscles and abdominal wall contracts.

Initiation of Vomiting

All these complex series of movements occurring during vomiting are controlled through a *vomiting centre* located in dorsal portion of lateral reticular formation in medulla. "Afferent" impulses may arise from:

1. irritation of mucosa in the stomach and other parts of GIT

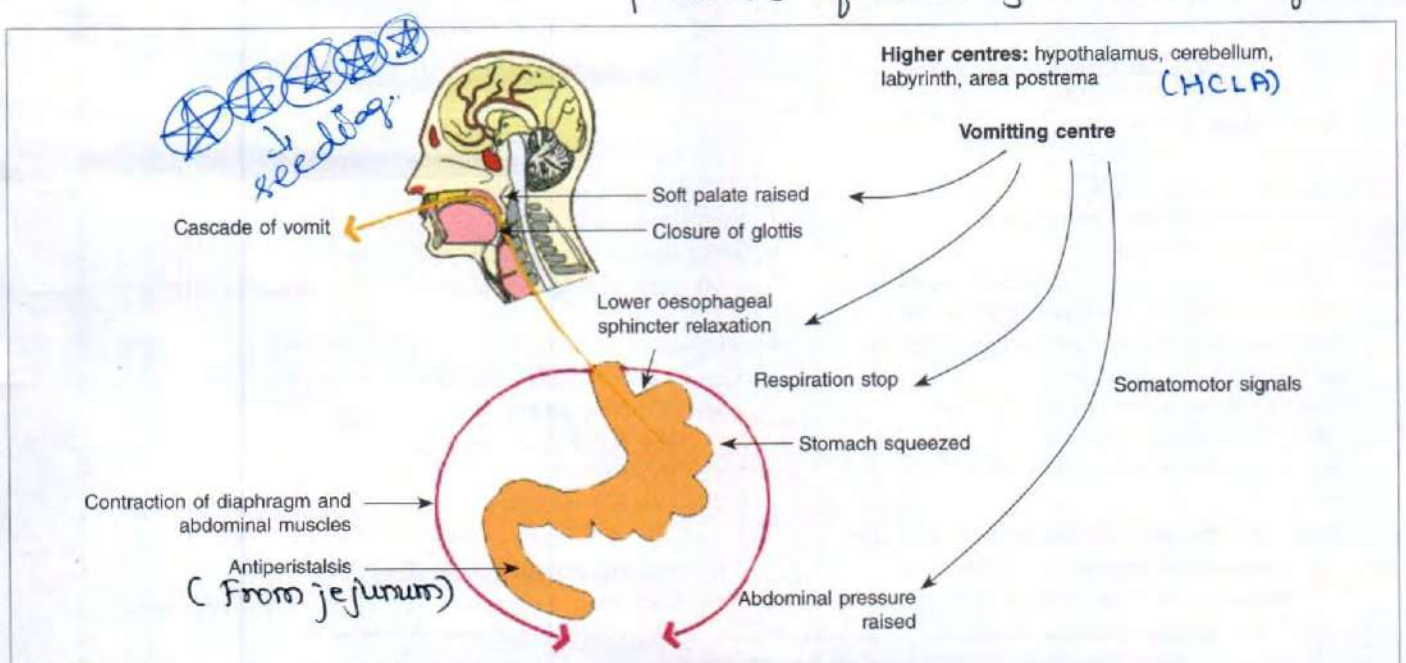


Fig. 27.13 Sequence of events in vomiting

2. vestibular apparatus (*e.g.* in motion sickness)
3. heart and other organs.

Impulses are relayed from the *vomiting centre* over visceral afferent pathways in the sympathetic nerves and vagi. Other afferent impulses from the limbic cortex, which may originate, in response to 'emotions' such as 'nauseating smells' and 'sickening sights' may also reach the vomiting centre. (Fig. 27.14)

True vomiting centre is the ***chemoreceptor trigger zone*** (CTZ) located in or near 'area postrema' on medullary surface (page 373). The 'CTZ' contains 'chemoreceptor' cells which initiate vomiting when they are stimulated by certain circulating toxins (chemical agents) released in many clinical disorders. Drugs like apomorphine, digitalis are called ***central emetics*** since they act by stimulating 'CTZ'.

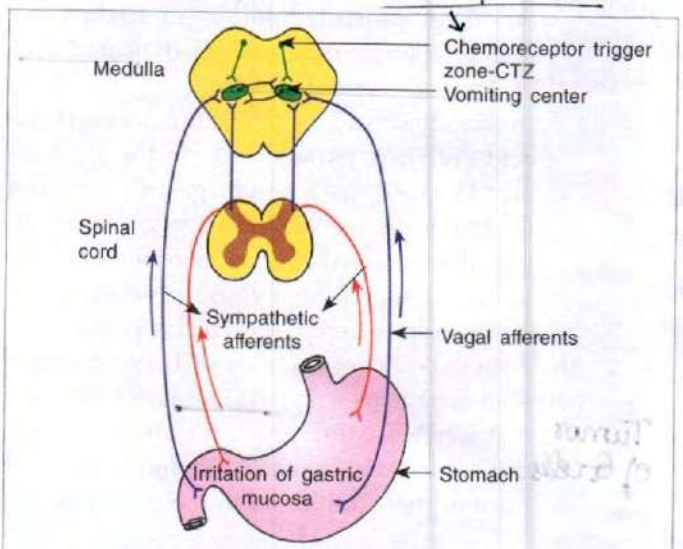


Fig. 27.14 Afferent pathways for vomiting reflex

Study Questions

1. **Give physiological basis of:**
   - (i) Gastrin release though vagally mediated is not blocked by atropine.
   - (ii) Protection of gastric mucosal surface from damage by gastric acid.
   - (iii) Post prandial alkaline tide.
   - (iv) How regurgitation from duodenum is normally prevented?
   - (v) Alcohol intoxication can be avoided if it is consumed after ingestion of a drink rich in fat.
   - (vi) Duodenal ulcer can be treated by vagotomy.
   - (vii) Gastric mucosa is resistant to auto-digestion.
   - (viii) Appetite juice
   - (ix) Sham feeding
   - (x) Receptive relaxation
   - (xi) Antiperistalsis
2. **What will happen and why?**
   - (i) If gastric mucosa gets atrophied.
   - (ii) If gastrin is released in large amounts.
   - (iii) If total gastrectomy is done.
   - (iv) to urine pH and breathing following a meal.
   - (v) to BER after vagotomy
3. **Write briefly about:**
   - (i) Rennin and Gastrin
   - (ii) Gastric mucous membrane.
   - (iii) Main gastric glands.
   - (iv) Functions of HCl in the stomach.
   - (v) Zollinger-Ellison syndrome.
   - (vi) Peptic ulcer.
   - (vii) Functions of stomach.
   - (viii) Actions of gastrin and factors affecting it.
   - (ix) Mechanism of secretion of HCl and its regulation.
   - (x) Composition and function of gastric juice.
   - (xi) Role of vagus in secretion of gastric juice.
   - (xiii) Interaction between nervous and humoral mechanisms regulating gastric juice secretion.
   - (xiii) Basic electrical rhythm.
   - (xiv) Gastric motility and gastric emptying
   - (xv) Factors influencing gastric motility.
   - (xvi) Dumping syndrome
   - (xvii) Mucosal-bicarbonate barrier
   - (xviii) True vomiting centre.
   - (xix) Pavlov and Heidenhain pouch
   - (xx) Antral systole
   - (xxi) Chyme
   - (xxii) Enterogastric reflex
4. **Show with the help of well labelled diagram:**
   - (i) Physiological anatomy of the stomach.
   - (ii) Structure of main gastric glands.
   - (iii) Mechanism of HCl secretion in the stomach.
   - (iv) Regulation of gastric acid secretion by the parietal cells.
   - (v) Electrical and mechanical activity of GIT smooth muscle and effect of A-ch and Epinephrine on it.
   - (vi) Phases of Motor Migrating Complexes

MCQs

1. **The gas present in the fundus of the stomach is mostly due to:**
   (a) Fermentation (b) Putrefaction (c) Swallowing (d) Diffusion
2. **Which is *not* a function/action of pepsin?**
   (a) Activates pepsinogen to pepsin (b) A proteolytic enzyme
   (c) Curdles milk (d) Bactericidal effect
3. **Pepsin optimal activity is seen at pH:**
   (a) Below 4.0 (b) 4.5 (c) 5.0 (d) 6.0
4. **Atrophy of gastric mucosa produces:**
   (a) Indigestion (b) Hyperacidity (c) Pernicious anaemia (d) Belching
5. **Visible mucus in the stomach differs from the soluble mucus in all of the following *except*:**
   (a) Secreted by main gastric glands (b) Alkaline pH
   (c) Lubricates the food (d) Prevents damage of gastric mucosa by acid-pepsin digestion
6. **Mucus secretion in the stomach is increased by:**
   (a) Gastric distension (b) Products of digestion
   (c) Any mechanical stimulus (d) Vagal stimulation
7. ***True* about gastrin:**
   (a) Increases secretion of pepsinogen (b) Decreases gastric motility
   (c) Acid stimulates gastrin secretion (d) Its secretion increases specially after protein meal
8. **Raised gastrin levels without associated increase in gastric acid secretion is seen in:**
   (a) Gastrinoma (b) G-cell hyperplasia (c) Pernicious anaemia (d) Carcinoma stomach
9. **Which of the following is *not* a function of stomach?**
   (a) Reservoir for the food ingested (b) Provide HCl for initial digestion of proteins
   (c) Release of food at a controlled rate into the duodenum (d) Absorbs vitamin B_{12}
10. ***Not true* of gastric juice is:**
    (a) It is isotonic with plasma (b) Pure juice pH is 0.87
    (c) Contains the enzyme rennin in infants only (d) Digests 20% of ingested proteins
11. **Parietal cells in the stomach are characterized by all of the following *except*:**
    (a) High H^+–K^+ ATPase
    (b) Rich in mitochondria
    (c) Secrete H^+ into the lumen of canaliculi in exchange for K^+
    (d) Synthesize and secrete Cl^- into the lumen of canaliculi
12. **Gastric acid secretion by pyloric chief cells ceases when gastric contents have a pH of about:**
    (a) 2.0 (b) 3.0 (c) 4.0 (d) 5.0
13. **Post-prandial alkaline tide is caused by:**
    (a) Rise in HCO_3^- in systemic blood pH following a meal (b) Loss of HCO_3^- in urine
    (c) Depressed breathing (d) Rise in alveolar pCO_2
14. **The mechanism which causes maximum gastric acid secretion is:**
    (a) Cholinergic receptor stimulation (b) H_2 receptor stimulation
    (c) Neurogenic (d) Na^+–K^+ ATPase pump
15. ***True* about cephalic phase of gastric secretion:**
    (a) Mediated by vagus (b) Mucous has no role to play
    (c) Leads to 100% of gastric secretions (d) Starts immediately after food enters the mouth
16. **Gastric emptying is promoted by:**
    (a) Hyperosmolarity of duodenal chyme (b) Presence of protein in the duodenum
    (c) Distension of duodenum (d) Decreased secretion of cholecystokinin
17. ***False* about migrating motor complexes:**
    (a) Modified electrical activity in the smooth muscles of the GIT
    (b) Migrate from the stomach to distal ileum @ 5 cm/min
    (c) Seen during fasting and are associated with sensation of hunger
    (d) Associated with decreased bile secretion
18. **Blood sugar level which produces vagal stimulation is:**
    (a) 100 mg/dL (b) 80 mg/dL (c) 65 mg/dL (d) 45 mg/dL
19. **Total gastrectomy may lead to all, *except*:**
    (a) Protein indigestion (b) Severe anaemia
    (c) Giddiness (d) Dizziness, pallor and sweating after meals
20. **Commonest cause of peptic ulcer is:**
    (a) Infection with Helicobacter pylori (b) Hypersecretion of gastric acid
    (c) Disruption of mucosal-bicarbonate barrier (d) Intake of anti-inflammatory drugs

21. **The stomach *does not* digest itself because the:**
(a) Gastric mucosa is protected by thick layer of mucus
(b) Gastric mucosal cells are not digestible
(c) Gastric mucosal cells transport hydrogen ions out of the gastric mucosa
(d) Hydrogen ions are completely neutralized by food

22. **Drugs proved to be very effective in the treatment of peptic ulcer are:**
(a) H_1 receptor blockers
(b) H_2 receptor blockers
(c) M_1-muscarinic receptor blockers
(d) Gastrin receptor blockers

23. **True vomiting centre is:**
(a) Lateral reticular formation
(b) Located in the stomach
(c) Vestibular apparatus
(d) Chemoreceptor trigger zone

24. **Pepsinogen I is secreted from:**
(a) Parietal (oxyntic) cells (b) Chief (or peptic) cells (c) G-cells (d) Mucous cells

25. **pH of pure gastric HCl is:**
(a) 0.20 (b) 0.87 (c) 1.20 (d) 1.80

26. **Bicarbonate ions from the parietal cells are discharged into the plasma in exchange for which of the following anion?**
(a) Phosphate (b) Protein (c) Chloride (d) Sulphate

27. ***True* about gastric slow wave:**
(a) A wave of depolarization
(b) Initiated by distension of stomach
(c) Conducted @ 4-6 cm/sec
(d) Coordinated contraction of the stomach which occurs about 3/min

28. **The major stimulus for receptive relaxation of the stomach is:**
(a) Food in the stomach (b) Food in the intestine (c) Cholecystokinin (d) Secretin

29. **Alcohol intoxication can be avoided if it is consumed after ingestion of a drink rich in:**
(a) Carbohydrates (b) Proteins (c) Fats (d) Mixed nutrients

30. **Hypersecretion of gastric acid leads to ulcer of the:**
(a) Body of the stomach (b) Fundus of the stomach (c) Oesophagus (d) Duodenum

Answers

1. (c)	2. (d)	3. (a)	4. (c)	5. (d)	6. (c)	7. (d)	8. (c)	9. (d)	10. (d)
11. (d)	12. (a)	13. (a)	14. (b)	15. (a)	16. (d)	17. (d)	18. (d)	19. (a)	20. (c)
21. (a)	22. (b)	23. (d)	24. (b)	25. (b)	26. (c)	27. (a)	28. (a)	29. (c)	30. (d)

Chapter 28

Pancreas

I. Physiological Anatomy of Pancreas: Structure; Nerve supply
II. Composition and functions of Pancreatic Juice
III. Regulation of the Secretion of Pancreatic Juice
IV. Pancreatic Exocrine Function Tests
V. Applied : Total removal of pancreas

PHYSIOLOGICAL ANATOMY OF PANCREAS

Structure

The pancreas is a double function organ containing both *'exocrine'* as well as *'endocrine'* cells.

(A) The portion of the pancreas which subserves *exocrine* function comprises ***compound alveolar tissue***, *i.e.* secretory acini and duct cells that secrete pancreatic juice. *Zymogen granules* containing the digestive enzymes are concentrated at the apices of the acinar cells. The pancreatic juice is discharged from apices of the cells into the lumen of the pancreatic ducts. **(Fig. 28.1)**

The pancreatic juice passes via 'intercalated' and 'excretory' ducts to be collected by two ducts: duct of 'Wirsung' and duct of 'Santorini'. **(Fig. 28.2)**

1. *Duct of Wirsung* – It is the single major pancreatic duct formed by joining of small intercalated and excretory ducts. The duct joins the common bile duct to form the ***ampulla of Vater*** which opens through the 'duodenal papilla' into the second part of the duodenum. 'Ampulla of Vater' is guarded by the *"sphincter of Oddi"*.
2. *Duct of Santorini* – It is the accessory pancreatic duct which also opens into the duodenum some 2 mm higher than the ampulla of Vater.

(B) The portion of the gland which subserves ***endocrine*** function comprises of the *Islets of Langerhans*, which forms internal secretions (hormones); insulin, glucagon and somatostatin (for details, refer to page 741).

Nerve Supply

Pancreatic acini are innervated by vagi. Pre-ganglionic vagal fibers synapse with ganglionic cells embedded in the pancreatic tissue; the post-ganglionic fibers innervate both the acinar cells and smooth muscle of the ducts.

Vagal stimulation increases pancreatic juice secretion.

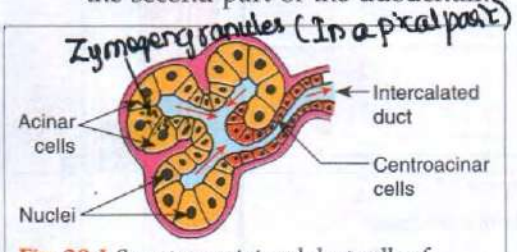


Fig. 28.1 Secretory acini and duct cells of pancreas

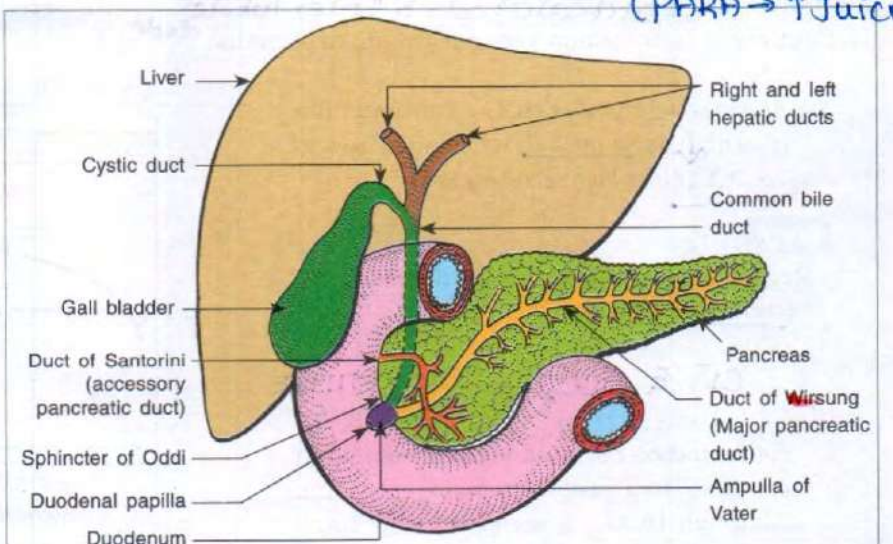


Fig. 28.2 Pancreas and its structure

#: Except bile juice, all GI juices are around (1.5L/day) (HCO_3^- ∝ Rate of flow)

COMPOSITION AND FUNCTIONS OF PANCREATIC JUICE (SM)

Daily secretion: 1200-1500 mL; transparent; colourless fluid; *isotonic* with plasma. #: Gastric juice = 2.5L-3L

Specific Gravity: 1010-1018

pH: 7.8 to 8.4, *markedly alkaline* due to high HCO_3^- concentration; approx. 120 mEq/L; 4-5 times that of plasma HCO_3^- concentration. Thus pancreatic juice along with alkaline bile neutralizes the gastric acid and helps in raising the pH of the duodenal contents. Failure to neutralize the acid as it enters the duodenum will result in *duodenal ulcer.*

Water - 98% ; 2% = Solids

Electrolytes (major)

Cations – Na^+, K^+, Ca^{2+}, Mg^{2+}, Zn^{2+}

Anions – HCO_3^-, Cl^- and traces of SO_4^{2-}, HPO_4^{2-}.

Enzymes (secreted by acinar cells) (CPS)

1. Pancreatic α-amylase (Carbohydrate lytic)
2. Pancreatic lipase (& Lipolytic)
3. Pancreatic esterase
4. Pancreatic pro-phospholipase A_2 (Proteolytic)
5. Pancreatic *proteolytic enzymes* – These are powerful protein splitting enzymes in the pancreatic juice and are secreted as *inactive proenzymes:*
   - (i) trypsinogen } for pool
   - (ii) chymotrypsinogen
   - (iii) pro-carboxypeptidase A and B } for aa
   - (iv) ribonuclease } Nucleic acid
   - (v) deoxy - ribonuclease
   - (vi) pro-elastase
6. *Trypsin inhibitor* – It is secreted by the same acinar cells and at the same time as pancreatic pro-enzymes. It protects the pancreas from 'autodigestion'.

MECHANISM :

Electrolytes – Intercalated & Intercalated ducts (SM)

1. Electrolytic composition varies with rate of secretion. **(Fig. 28.3)**
   - (i) *At low secretory rates* HCO_3^- concentration is as high as 80 mEq/L which increases to 120 mEq/L *at high secretory rates.*

Note

Regardless of the flow rates, pancreatic secretion are *isotonic.*

Cl^- & HCO_3^- → COMPETITIVE

   - (ii) Cl^- concentration falls as HCO_3^- concentration rises and total concentration of these two anions remains constant. Although HCO_3^- is secreted in the small ducts, it is reabsorbed in the large ducts in exchange for Cl^-. The magnitude of the exchange is inversely proportional to the rate of flow.
   - (iii) Na^+ and K^+ concentrations are almost identical with those of plasma and are constant on changing rate of secretion. (Indep. of RATE)
2. Pancreatic secretion involves chemical work and is associated with an increased O_2 consumption. The duct cells of the pancreas contain carbonic anhydrase (CA) and form the HCO_3^- which is secreted into the duct lumen. HCO_3^- are formed via the same reaction that occurs in the parietal cells of the stomach (page 213). Therefore, acetazolamide by inhibiting 'CA' and alloxan by causing cellular vacuolization of duct cells, decreases HCO_3^- secretion.

Acinar cells

Enzymes

1. **Pancreatic α-amylase** [ALEPPE]
   It is stable in pH range of 4-11; MW 45000. It splits α-1-4 glycosidic bond of starch and digests starch (boiled and unboiled) to maltose. Starch (multi α-glu subunits) → Dextrins → Maltose
2. **Pancreatic Lipase**
   - (i) Hydrolyses neutral fats to glycerol esters and fatty acids. Neutral fat → Glycerol esters & FA
   - (ii) Its pH range of activity is from 7 to 9.

Note

Another pancreatic lipase that is activated by bile salts (*Bile salt activated lipase*) catalyzes the hydrolysis of cholesterol esters, phospholipids and triglycerides (page 262). (activator)

3. **Pancreatic Esterase** – converts cholesterol esters to cholesterol.
4. **Pancreatic pro-phospholipase A_2**
   It is secreted in inactive form and gets converted to phospholipase A_2 (active) by trypsin. This splits a fatty

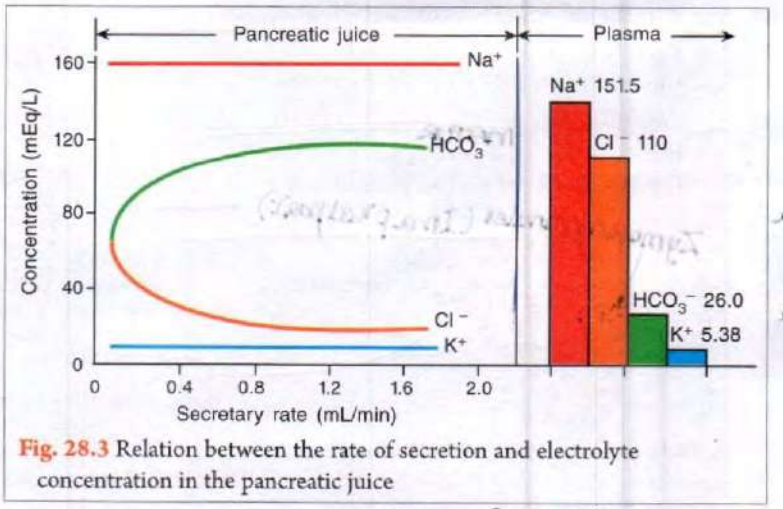


Fig. 28.3 Relation between the rate of secretion and electrolyte concentration in the pancreatic juice

$CO_2 \rightarrow H_2CO_3^-$ (Mech. of secretion)

Enzyme secretion → RER → SER → Golgi apppa.

Release from

acid off lecithin (*i.e.* phospholipid, a normal constituent of bile) forming lysolecithin, which damages cell membrane.

Lecithin → Lysolecithin
↑
panc PLPA2

Important Note

In acute pancreatitis, phospholipase A_2 gets activated in the pancreatic ducts causing disruption of pancreatic tissue and necrosis of surrounding fat which may be fatal. This disease is invariably associated with high serum amylase and lipase levels.

5. **Pancreatic proteolytic enzymes**

trypsinogen (inactive) —[Enterokinase (enteropeptidase) secreted by duodenal mucosa and trypsin (autocatalyst)]→ *trypsin* (active)

(i) **Trypsinogen**

Trypsin is the *most powerful* proteolytic enzyme of the pancreatic juice. It hydrolyses proteins by splitting bonds in the protein molecule which contain L-lysine and L-arginine in which ε-amino or guanidino groups are free. Therefore, proteins are digested mainly to *small polypeptides*. Some amino-acids are also formed, because trypsin can hydrolyse some dipeptides.

(ii) **Chymotrypsinogen**

Chymotrypsinogen (inactive) —[trypsin]→ *Chymotrypsin* (active)

It digests protein to small polypeptides.

(iii) **Pro-carboxypeptidase A and B**

pro-carboxypeptidase A and B (inactive) —[Enterokinase / trypsin]→ *Carboxypeptidase A and B*

It splits peptide chain by the stepwise removal of amino acid residues from the free carboxyl group at the end of the chain.

(iv) **Ribonuclease**

(v) **Deoxyribonuclease**: They split nucleic acids of ribose and deoxyribose type into nucleotides.

(vi) **Pro-elastase**

Pro-elastase (inactive) —[trypsin]→ *Elastase* (active)

It converts elastin and some other proteins into simplified substances.

Elastin → Other simple proteins

REGULATION OF THE SECRETION OF PANCREATIC JUICE

The secretion of pancreatic juice is controlled in part by a reflex mechanism (*nervous regulation*) and in part by the GIT hormones (*humoral regulation*).

Parasym: Vagus Sympath. → Coeliac & Superior mesenteric plexus

NERVOUS REGULATION

1. **Stimulation of vagus (X) nerve** produces:
   (i) *Enzyme rich* pancreatic juice secretion with *consistency of glycerine.*
   (ii) Causes disappearance of zymogen granules from the gland cells which are reformed during subsequent rest.

   Vagal effect is mediated by release of acetylcholine (A-ch), which in turn activates phospholipase C to cause increased secretion of pancreatic juice from the acinar cells. A similar effect on pancreatic juice secretion is seen with cholecystokinin (CCK) which also exerts its effect by activating phospholipase C.
2. Within few minutes of ingestion of food (if vagi are intact), there is increase in pancreatic juice secretion, which lasts for 6-14 hours, depending on the composition of food and peak secretion occurs during 2nd to 3rd hour.
   Enzyme composition of juice alters with the character of food. Therefore, amount of 'lipase' increases with a diet rich in fats; the quantity of 'amylase' with a carbohydrate rich diet and amount of 'trypsin' with a protein rich diet.
3. After administration of atropine, stimulation of vagus causes no increase in pancreatic juice secretion, proving that the response is mediated via release of A-ch at vagal nerve endings.
4. ***Reflex stimulation*** of vagus nerve causes increase in pancreatic juice secretion. This is seen with
   (i) *conditioned reflexes e.g.* sight and smell of food; and
   (ii) *unconditioned reflexes e.g.* chewing and swallowing of food.

Vagal stimulation = CCK (Hormonal regulation)

HUMORAL REGULATION

Two GIT hormones, *Secretin* and *Cholecystokinin-pancreozymin* (CCK-PZ) on liberation into portal venous blood via systemic circulation reach pancreatic tissue to stimulate the secretion of pancreatic juice. **(Fig. 28.4)**

ROLE OF SECRETIN

1. It was the *1st hormone to be discovered* (*Bayliss* and *Starling 1902*), a polypeptide, MW: 5000, consists of 27 amino-acids, structure resembling that of glucagon, GLI, VIP and GIP (page 272)

↓
Glucagon like (Secretin family)
Immunoreactive

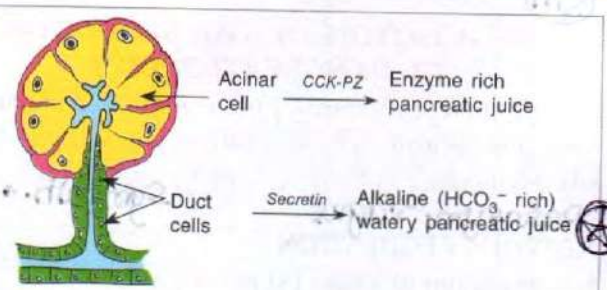
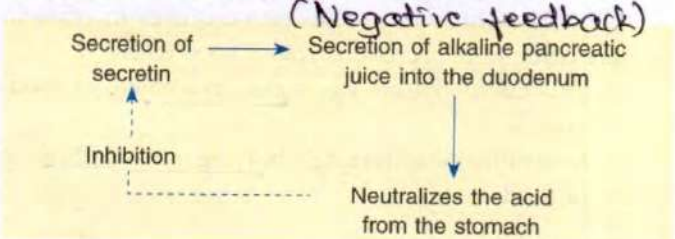


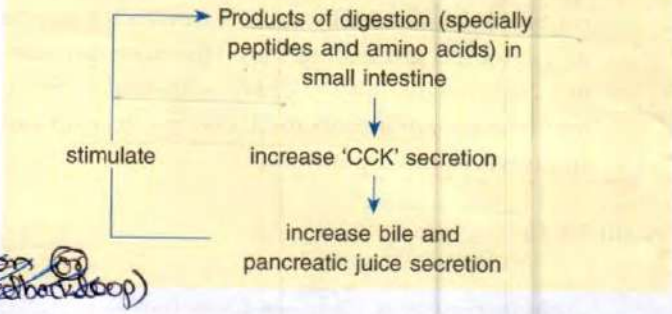
Fig. 28.4 Sites of action and effects of secretin and cholecytokinin-pancreozymin (CCK-PZ) on pancreatic juice secretion

2. It is produced by *argentaffin* (or *S*) *cells* in the crypts of the mucosa of upper part of small intestine, duodenum and jejunum. It is secreted as *prosecretin* (inactive), which gets converted by gastric HCl and salts of fatty acids (soaps) into *secretin* (active).
3. It acts on the duct cells of pancreas to produce a flow of *alkaline watery pancreatic juice* poor in enzymes. The effect is mediated by increase in intracellular cAMP.
4. The volume of flow of juice is directly proportional to the secretin dose injected I.V. As volume of pancreatic secretion increases, its Cl^- concentration falls and HCO_3^- concentration rises. Although HCO_3^- are secreted in small ducts, it is reabsorbed in the large ducts in exchange for Cl^-. The magnitude of the exchange is inversely proportional to the rate of flow.
5. It also stimulates bile secretion and potentiates the effect of "CCK-PZ" on the pancreas.
6. It along with "CCK-PZ" causes contraction of pyloric sphincter and delays gastric emptying. Thus, preventing the reflux of duodenal contents into the stomach.
7. *Feedback control of secretin secretion*



ROLE OF CHOLECYSTOKININ-PANCREOZYMIN (CCK-PZ)

1. Previously it was thought that two separate hormones are released from the duodenal mucosa, one 'CCK' which causes contraction of the gall bladder to release bile; and second 'PZ' which stimulates the secretion of pancreatic juice. However, now it has been found that a single hormone is released from the duodenal mucosa to carry out both the above functions, hence the name 'CCK-PZ'.
2. It is a polypeptide containing 33 amino-acids with 5 terminal amino-acids as those of gastrin, which account for some stimulant properties that they have in common.
3. It is produced by granular mucosal cells of upper portion of small intestine, the duodenum and jejunum.
4. It causes contraction of gall bladder to release bile and also causes secretion of pancreatic juice *rich in enzymes* by causing discharge of zymogen granules from the pancreatic acinar cells. The effect is mediated by activation of phospholipase C (page 22).
5. It also increases the secretion of *enterokinase* (enteropeptidase) from the duodenum and may also increase the motility of small and large intestine.
6. It produces *trophic* effect (*i.e.* increased growth) on pancreas.
7. It is also found in neurons in the brain (specially in the cerebral cortex) where it is involved in the regulation of food intake and is related to the production of anxiety and analgesia.
8. CCK and gastrin both stimulate glucagon secretion.
9. *Positive feedback control*



This positive feedback control of CCK-PZ is terminated when the product of digestion enters the ileum.

Important Notes

1. 'Secretin' amplifies the stimulation of enzyme and electrolyte rich pancreatic juice by a given dose of 'CCK-PZ'. Similarly, 'CCK-PZ' amplifies the secretory response of pancreas to 'secretin' produced as a result of acidifying the contents of the intestinal lumen. Therefore, each hormone 'potentiates' the action of other.
2. Both 'secretin' and 'CCK-PZ' delay gastric emptying (Also refer to pages 223) and stimulate secretion of 'succus entericus' (intestinal juice).

Factors which increase 'Secretin' and 'CCK-PZ' release

1. Acid in the duodenum causes 'more of secretin' liberation and feeble stimulation of 'CCK-PZ'.
2. Products of carbohydrates, fats and protein digestion in small intestine (specially peptides, amino acids are major stimuli), cause more of 'CCK-PZ' release.

Interaction of Nervous and Humoral Regulation

Pancreatic secretion is initiated by direct vagal stimulation of the exocrine secretory cells. Vagal activity also releases 'gastrin' from the pyloric antrum, which causes powerful excitation of acid secretion from the gastric parietal (oxyntic) cells. Acid entering the duodenum increases secretion of 'secretin' and 'CCK-PZ'.

PANCREATIC EXOCRINE FUNCTION TESTS

1. *Estimation of serum-amylase levels:* Its levels are markedly increased in acute pancreatitis. Normal value: 50-120 units/L.
2. *Faecal fat excretion test:* Fat is present in food as neutral fat or triglyceride. It is split by lipases, (mainly from pancreas) into glycerol and fatty acids. Some of fatty acids, if unabsorbed combine with bases to form soaps. Fats may, therefore, be found in faeces as neutral fat, fatty acids and soaps.
   For estimation of the fats in the stools, the person may be placed on diet containing 100 gm of fat per day. The stools are collected over 3-5 days and then estimated for fat contents. Normal fat excretion is 5-6 gm/day. In patients with pancreatic exocrine insufficiency it may increase to 40-50 gm/day.
3. *Lundh test:* This test relies on an assessment of trypsin activity in pancreatic juice collected following duodenal intubation. Indirect stimulation of pancreas is done by ingestion of a meal. The mean trypsin activity of < 6 IU / L, indicates presence of pancreatic exocrine insufficiency.
4. *Secretin and 'CCK-PZ' stimulation test*

   Collection of pure pancreatic juice: A special double lumen tube is swallowed and then screened into position in the duodenal loop. It has a weighted bulbous end and contains 2 sets of holes, one for duodenal and other for gastric aspiration. Therefore, pure (uncontaminated) pancreatic juice can be collected from the duodenum. Now-a-days, fiber optic catheter can be introduced under direct vision into the pancreatic duct for collecting the pure pancreatic juice.

   Procedure

   After a short period is allowed for stabilization, 'secretin' is given I.V. and duodenal aspirate collected at 10 minutes intervals over the next 30-80 minutes; 'CCK-PZ' is then given and the whole process repeated. The aspirated volume, pH, HCO_3^- and concentration of enzymes for each sample are then measured and calculated.

   Conclusions

   (i) With normal pancreatic function, there is a rapid increase in flow rate of pancreatic juice following the injection (more so with secretin), with maximum flow rate usually being reached in 20 minutes (Fig. 28.5). Secretin causes mainly increase in HCO_3^- concentration with flow rate and may increase as high as 120 mEq/L; whereas CCK-PZ causes increased flow of pancreatic juice rich in enzymes (see above).

   (ii) Obstruction of pancreatic duct due to carcinoma or calculus produce secretion of pancreatic juice of low volume and low enzyme levels.

   (iii) Pancreatitis causes secretion of pancreatic juice low in volume with low levels of HCO_3^- and normal or low enzyme levels. In this disease HCO_3^- concentration is markedly decreased to 90 mEq/L.
5. *Cytological examination* – Fresh, uncontaminated duodenal aspirate is collected and cytological examination performed for cancer cells. This helps in the diagnosis of pancreatic carcinoma.

APPLIED

Total Removal of the Pancreas

Total removal of the pancreas in man is indicated in carcinoma of pancreas. It will result in:

1. *Diabetes mellitus* due to pancreatic endocrine deficiency of insulin (for details, refer to page 751).
2. Development of digestive disturbances which include:
   (i) Increase in faecal fat contents upto 40-50 gm/day due to imperfect digestion of fats which leads to poor fat absorption. As a result stools are bulky, foul smelling, pale and greasy (*Steatorrhoea*).

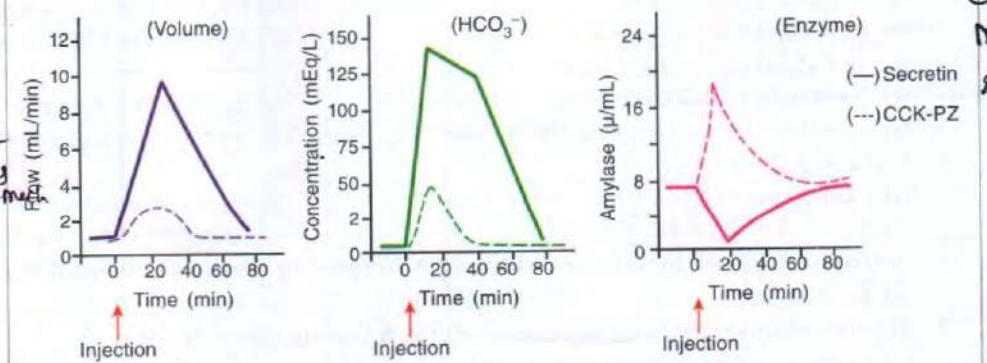


Fig. 28.5 Effect of administration of secretin and CCK-PZ on the composition and volume of the pancreatic juice in humans

(ii) Faecal nitrogen content increases to 4-8 gm/day (normal: 1 gm/day) due to incomplete proteolysis.
(iii) Usually no abnormality of carbohydrate digestion and absorption, due to other important carbohydrates splitting enzymes are present in intestinal juice.

Important Note

Digestive disturbance due to pancreatic insufficiency causes loss of approx. 30% of calorific value of the food ingested.

* ACUTE pancreatitis → Cardinal diagnosis: ↑ AMYLASE & ↑ $PLPA_2$
→ Excess Alc. consump
→ Stones in panc. duct (Blockage)
⟹ AUTO-Digestion of pancreas

Study Questions

1. Give composition and functions of pancreatic juice.
2. Write briefly about:
   (i) Enterokinase
   (ii) Trypsin inhibitor
   (iii) Role of vagus in regulation of secretion of pancreatic juice
   (iv) Bile salt activated lipase
   (v) Interaction of nervous and humoral mechanism regulating pancreatic juice secretion
   (vi) Pancreatic exocrine function tests
   (vii) Steatorrhoea.
3. What will happen and why, if:
   (i) Acid chyme entering the duodenum fails to get neutralized.
   (ii) Autodigestion of pancreas occurs.
   (iii) Phospholipase A_2 gets activated in the pancreatic ducts.
   (iv) Pancreas is removed in toto.
4. List proteolytic enzymes in the pancreatic juice. Give their actions.
5. List signs of pancreatic insufficiency.
6. Draw labelled diagram to show:
   (i) Relation between rate of secretion and electrolyte concentration in pancreatic juice
   (ii) Effect of administration of secretin and CCK-PZ on pancreatic juice secretion

* Chronic pancreatitis

* Cystic fibrosis
⇓
Secretions are thick ⇒ Blocks the ducts

MCQs

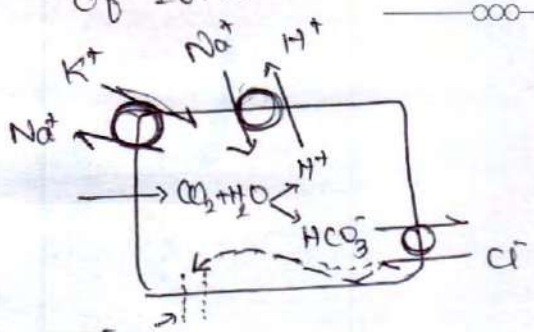
1. *True* about trypsin inhibitor:
   (a) Secreted as inactive proenzyme
   (b) Powerful protein splitting enzyme
   (c) Protects the pancreas from auto-digestion
   (d) Helps raising pH of duodenal contents
2. Which carbohydrate is *not* hydrolysed by pancreatic amylase?
   (a) Starch (b) Glycogen (c) Cellulose (d) Dextrin
3. Pancreatic α-amylase differs from salivary α-amylase in that:
   (a) It digests starch to maltose
   (b) It cannot digest boiled starch
   (c) It is stable in pH range of 4-11
   (d) It hydrolyses 1:4 α linkages
4. *Not true* about pancreatic lipase:
   (a) Hydrolyses neutral fats to glycerol and fatty acids
   (b) Acts best in pH range of 7 to 9
   (c) Its activity is greatly decreased by bile salts
   (d) Secreted by pancreatic acinar cells
5. Secretin *does not* cause:
   (a) Bicarbonate secretion
   (b) Augments the action of CCK
   (c) Contraction of pyloric sphincter
   (d) Increase in gastric secretion
6. Pancreatic juice rich in water and electrolytes but poor in enzymes is secreted in response to:
   (a) Pancreaozymin (b) Cholecystokinin (c) Secretin (d) Gastrin
7. The duodenum secretes a hormone which has following effects *except:*
   (a) Causes copious pancreatic juice rich in bicarbonate
   (b) Increases gastric motility
   (c) Causes gall bladder to contract and sphincter of Oddi to relax
   (d) Volume of pancreatic juice secretion increases directly with the hormone dose injected

8. **Best stimuli for cholecystokinin secretion is:**
   (a) Acid in duodenum (b) Protein digestion products
   (c) Bile (d) Secretin
9. **Pancreatic exocrine function is best assessed by:**
   (a) Faecal fat estimation (b) Trypsin activity in pancreatic juice
   (c) Cytological examination of pancreas (d) Measurement of HCO_3^- in pancreatic juice
10. **In patients with pancreatic exocrine insufficiency faecal fat excretion increases to:**
    (a) 5-6 gm/day (b) 10-15 gm/day (c) 20-30 gm/day (d) 40-50 gm/day
11. **A large greasy smelly pale stool usually indicates failure of digestion of:**
    (a) Carbohydrates (b) Fats (c) Proteins (d) Cellulose
12. **In digestive disturbance due to pancreatic insufficiency, calorific value of the food ingested is loss to approximately:**
    (a) 10% (b) 20% (c) 30% (d) 40%
13. **pH of pancreatic juice is:**
    (a) Slightly alkaline (b) Markedly alkaline (c) Light acidic (d) Neutral
14. ***False* about pancreatic secretion is:**
    (a) Daily secretion: 1.5 L/day
    (b) Isotonic with plasma
    (c) Rich in HCO_3^-
    (d) Pancreozymin-enzyme rich
15. **The hormone generally considered to be the major stimulus for enzyme secretion by the pancreas is:**
    (a) Cholecystokinin (b) Secretin (c) Trypsin (d) Gastrin
16. **The most important stimulus for secretion of secretin is:**
    (a) Vagal stimulation (b) Mechanical duodenal distension
    (c) Acidic chyme entering duodenum (d) Fat reaching the duodenum

Answers

1. (c) 2. (c) 3. (c) 4. (c) 5. (d) 6. (c) 7. (b) 8. (b) 9. (d) 10. (d)
11. (b) 12. (c) 13. (b) 14. (d) 15. (a) 16. (c)

Mechanism of secretion of IONS



Phases (REGULATION)

→ Cephalic - Neural:
- vagovagal reflex
- Sight, smell, taste

→ Gastric → Food in stomach ↓ panc. juice (By Gastrin stimulation)

→ Intestinal → Hormone
Neural
- Secretin
- CCK-PZ

Voluminous, Highly alk. (↑↑ HCO_3^-) rich

Enzyme rich

Chapter 29

Liver and Gall Bladder

I. Physiological Anatomy of the Liver
Structure and special features Hepatic blood flow and oxygen consumption
II. Functions of the Liver and Signs of Liver Insufficiency
III. The Bile
Composition of liver bile Functions of bile Control of bile secretion
IV. Bilirubin Metabolism, Excretion and Jaundice
V. Functions of Gall Bladder
VI. Applied: Cholecystectomy; Complete biliary obstruction; Gall stones

PHYSIOLOGICAL ANATOMY OF THE LIVER

Structure and special features (Fig. 29.1)

1. Liver is the ***largest gland*** in the body, weighing about 1.5 kg in an adult. It consists of 'lobes' which are subdivided into *lobules* which is the basic functional unit of the liver.
2. The **liver lobules** are made up of columns of hepatic cells whose outlines are indistinct forming a syncytium.
3. *Portal vein* divides into branches, the 'interlobular veins', which surround the lobules; from these vessels blood passes between the hepatic cells in *sinusoids* (vascular capillaries) to reach the centre of the lobule *i.e.* the 'central vein', which drains into the 'hepatic vein' via its 'intralobular' branches and thence into the 'vena cava'.
4. Similarly, *hepatic artery* divides into branches which accompany those of the portal veins between the lobules; ultimately hepatic artery blood also enters the 'sinusoids', where it mixes with the blood from the portal vein.
5. The endothelium of sinusoids has large fenestrations thereby forming an intimate contact between the blood and hepatic cells. This helps the liver to transform or modify many of the constituents of blood.
6. ***Kupffer cells*** are part of macrophages (R.E. System) which lie along the endothelium of the sinusoids at regular intervals.
7. ***Bile*** is formed in tiny vacuoles in the interior of *hepatic cells (hepatocytes)* and is discharged through fine *'canaliculi'* into the 'intralobular' bile ducts which join via 'interlobular' bile ducts to form the right and left 'hepatic ducts'. These ducts join outside the liver to form the *common hepatic duct*.

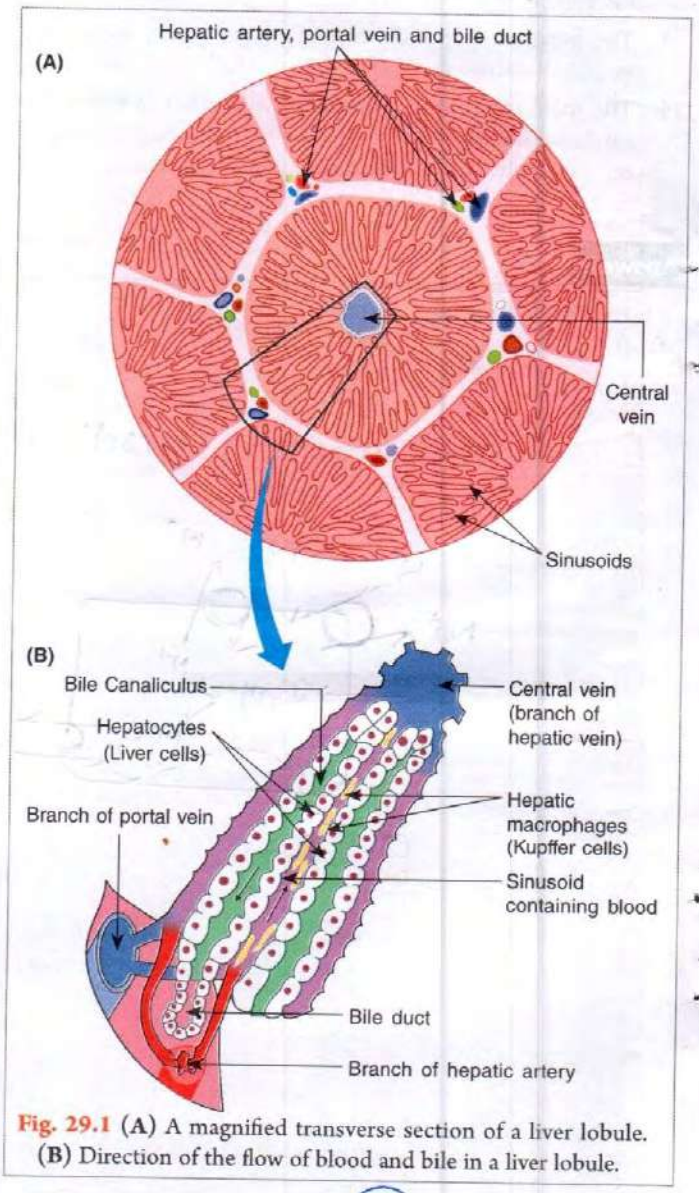


Fig. 29.1 **(A)** A magnified transverse section of a liver lobule. **(B)** Direction of the flow of blood and bile in a liver lobule.

8. *Physiological reserve of liver*
   (i) 80% of liver tissue can be removed without bile salts and bile pigments being retained in the blood or excreted in urine.
   (ii) Even if 90% of bile ducts are ligated, the volume of bile secreted remains normal.
   (i) and (ii) indicate physiological reserve of the liver.
9. *Regeneration power of liver* – Removal of 3/4th of liver causes, within 6-8 weeks, restoration of original liver mass by proliferation of remaining tissue as a result of active mitotic divisions of the cells. This can be repeated many times and is always followed by regeneration.
10. Liver resists many forms of stress at its best when its stores of carbohydrates and proteins are ample; its efficiency is impaired when it is laden with fats.

Hepatic blood flow and oxygen consumption (Also refer to page 381)

1. *Hepatic blood flow* is 1500 mL/min:
   (i) 20% (300 mL/min) is delivered by hepatic artery; and
   (ii) 80% (1200 mL/min) from portal venous supply.

2. *Hepatic O_2 consumption*
   (i) Hepatic artery blood O_2 content: 19 mL/dL (95% saturated with O_2).
   (ii) Hepatic vein blood O_2 content: 13.4 mL/dL.
   (iii) Portal vein blood O_2 content: 17 mL/dL (85% saturated with O_2).
   (iv) Hepatic artery *minus* hepatic vein O_2 difference is (19 – 13.4) *i.e.* 5.6 mL/ dL. Therefore, 300 mL of hepatic artery blood supply (300 × 5.6/100) = 16.8 mL O_2/min ... (a)
   (v) Portal vein – hepatic vein O_2 difference is (17 – 13.4) *i.e.* 3.6 mL/dL. Therefore, 1200 mL of portal vein blood supply (1200 × 3.6/100) = 43.2 mL O_2/min. ... (b)

   Thus, total O_2 consumption of liver (a+b) is, 16.8 + 43.2 = 60 mL O_2/min (70% of this is supplied by portal vein and the remaining 30% by hepatic artery). Therefore, *portal venous blood is more desaturated during digestion*. However, there is marked increase of portal blood flow during digestion, so that although the portal O_2 content per 100 mL of blood is reduced, the total O_2 flow is increased due to hyperaemia.

3. *Mean pressures in hepatic and portal system*
   (i) mean hepatic arterial pressure : 100 mmHg
   (ii) mean portal vein pressure : 7 mmHg
   (iii) mean hepatic vein pressure : 5 mmHg

Therefore, *resistance of blood flow through the liver is low.*

FUNCTIONS OF THE LIVER AND SIGNS OF LIVER INSUFFICIENCY

The major functions of the liver and corresponding signs of liver insufficiency (or liver damage) are given in the following **Table 29.1.**

THE BILE

Bile is secreted continuously by the hepatic cells into the bile capillaries, from where it is collected by the hepatic ducts which joins with cystic duct to form the common bile duct. Between the periods of digestion, the tone of *sphincter of Oddi* is high, therefore, bile is diverted via the cystic duct into the 'gall bladder', where it is concentrated and stored. When food enters the mouth, the 'sphincter of Oddi' relaxes; and when food enters the upper part of small intestine, release of 'secretin' and 'CCK-PZ' from duodenum causes the gall bladder to contract.

Composition of Liver Bile

Daily secretion	:	500-1000 mL; transparent alkaline fluid; light (golden) yellow in colour.
pH	:	7.8 – 8.6; *isosmotic* with plasma
Water	:	97%
Bile salts	:	0.7% (120-180 mg/dL). These are sodium and potassium salts of bile acids.
Bile pigments	:	0.2%. These include *biliverdin* and *bilirubin* and its derivatives.
Lecithin (a phospholipid)	:	0.1% (140-810 mg/dL). It is present in inactive form; when converted to *lysolecithin* (active), it damages the cell membrane.
Fats	:	0.1%
Fatty acids	:	0.15%
Cholesterol	:	0.06% (60-170 mg/dL).
Enzyme alkaline phosphatase	:	It helps in converting organic phosphate to free phosphate.
Electrolytes	:	0.7%
(i) Cations	:	Na^+ 180-220 mEq/L
		K^+ 6-8 mEq/L
		Ca^{2+} 2.5-4.8 mEq/L
(ii) Anions	:	Cl^- 60-70 mEq/L
		HCO_3^- 60-70 mEq/L

HCO_3^- concentration increases with an increase in rate of bile secretion. I.V. administration of acetazolamide (in high dose), decreases HCO_3^- concentration of bile, thereby decreases pH from 8.6 to 7.4.

Bile Salts

1. These are the sodium and potassium salts of bile acids, half life: 3 days. The two principal

Table 29.1: Functions of the liver and signs of liver insufficiency

Functions of the Liver	Signs of liver insufficiency or liver damage
I. Synthetic: Liver synthesizes;	
1. Most of the '***plasma proteins***' specially albumin; it *does not synthesize* immunoglobulins, which are synthesized in R-E system.	*Hypoproteinemia*, resulting in ***oedema***.
2. *Some* ***clotting factors***, *e.g.* fibrinogen (I), prothrombin (II) and factors V, VII, IX and X.	Haemorrhagic disorders.
3. ***Enzymes***, *e.g.* (i) Alkaline phosphatase (ii) SGOT (serum glutamic oxaloacetic transaminase) (iii) SGPT (serum glutamic pyruvic transaminase) (iv) SICD (serum iso citrate dehydrogenase)	(i) Increase in concentration of plasma alkaline phosphatase (normal 5-13 KÅ unit) (ii) increase in concentration of SGOT; SGPT and SICD (normal: 5-40 units % or 5-25 IU/L) because these enzymes are released from damaged hepatic cells.
4. ***Urea*** – Liver removes ammonia from the body to synthesize urea.	(i) Blood urea decreases (*normal*: 20-40 mg/dL) (ii) Blood ammonia rises (*normal*: 20-80 µg/dL) (iii) Urine ammonia rises (*normal*: 350-1200 µg/dL) (All these changes occur, provided kidneys are normal).
5. ***Cholesterol*** – it is synthesized from active acetate.	S. cholesterol decreases; however, in obstructive jaundice it increases (*normal*: 120-200 mg/dL).
II. Metabolic: Liver is the central organ of metabolism and participates in all three major metabolisms of the body.	
1. ***On carbohydrate metabolism***: Liver helps in synthesis, storage and release of glucose by the following processes: (i) *Glycogenesis* – glycogen is formed from glucose and stored in the liver. (ii) *Glycogenolysis* – liver glycogen is broken down to glucose. (iii) *Gluconeogenesis* – formation of glucose from non-carbohydrate sources. (ii) and (iii) help regulation of blood glucose level by the liver (*Glucose buffer function*).	(i) Decrease in liver glycogen producing more damage to the liver. (ii) Decrease in blood glucose produces: muscle weakness, personality changes, tremors, slurred speech, convulsions, coma and finally death. These all are the features of ***pre-hepatic coma***. *Normal* fasting blood glucose level: 70-90 mg/dL.
2. ***On protein metabolism***: Liver synthesizes plasma proteins, blood clotting factors, enzymes, urea and lipoprotein from amino-acids.	Increase in amino-acid concentration in the blood producing ***aminoaciduria***. Normal blood amino-acid level: 30-65 mg/dL.
3. ***On Fat Metabolism***: Liver is the site of: (i) β-oxidation, a process which occurs within the mitochondria which oxidise the fatty acids to form active acetate *i.e.* aceto-acetic acid. (ii) Non-esterified fatty acids (NEFA) *i.e.* fatty acids with < 14 'C' atom which reach the liver via blood are esterified to form triglycerides in the liver. (iii) Synthesis of lipoproteins (*e.g.* HDL, LDL, VLDL, chylomicrons etc.) which are vehicles of fat. (iv) Synthesis of saturated fatty acids from the active acetate via Kreb's cycle within the mitochondria. (v) Synthesis of cholesterol and phospholipids (*e.g.* lecithin, sphingomyelin, cephalin etc.) for cell membrane.	(i) Lack of β-oxidation causes accumulation of fatty acids in the liver; and (ii) Decrease in lipoproteins, increases stores of liver fat. Therefore, the neutral fats (triglycerides) content of the liver is increased. Fat laden liver arising from these conditions is called a ***Fatty Liver***, its features include: – Liver is grossly enlarged containing massive deposition of neutral fats. – Precipitate hepatitis → fibrosis → ***cirrhosis of liver*** → increases portal pressure (***portal hypertension***) → (a) transudation of fluid from abdominal visceral capillaries → ***ascites***; (b) establishment of collateral between portal and systemic circulation → haemorrhage from dilated venules at the junction of oesophagus and stomach (***oesophageal varices***) and from mucosa of anus (***haemorrhoids***). (iii) Decrease in serum cholesterol.

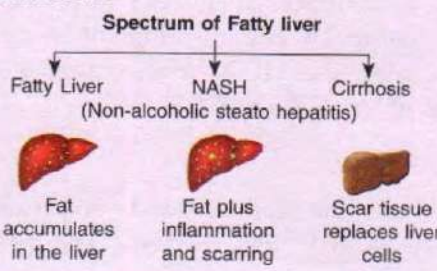


Table 29.1: Functions of the liver and signs of liver insufficiency

Functions of the Liver	Signs of liver insufficiency or liver damage
III. Bile Secretion: Liver is the site for:	
1. Synthesis of bile salts and bile acids from cholesterol, which helps in (i) activation of lipase; and (ii) emulsification (breakdown) of fats.	Defective fat digestion and absorption producing ***steatorrhoea*** (bulky, pale, greasy, foul smelling stools) (also refer to page 229).
2. Conjugation of free bilirubin, bile pigments with UDPGA (uridine diphosphate glucuronic acid) in the presence of glucuronyl transferase-forming water soluble *bilirubin glucuronides*.	***Hepatic jaundice***, therefore, s.bilirubin (free) increases. (*Normal* s.bilirubin 0.2-0.8 mg/dL).
IV. Detoxicating and Protective Function Liver exerts its detoxicating and protective function:	
1. By complete destruction of drugs *e.g.* nicotine and short acting barbiturates.	Standard dose of drugs specially tranquilizers; barbiturates leads to symptoms of overdose.
2. By activity of its R-E system (Kupffers cells) helps in immune mechanism.	***Foetid breath*** (like the smell of a dead body) due to failure to detoxicate nitrogenous materials, specially ammonia absorbed from the GIT. (Also refet to page 621)
3. By conjugation *i.e.* by combining the unwanted substances with another molecule or chemical group (*e.g.* sulphate, glycine, glucuronic acid, acetic acid), the resulting compound is then excreted in urine.	
V. Miscellaneous Functions	
1. ***Storage Organ*** – Liver stores glycogen, fat, protein, vitamins (A and B_{12}), substances concerned in blood formation and regeneration of blood.	Deficiency of these substances produces corresponding deficiency symptoms.
2. *Hormone Inactivation* – Liver is the site of inactivation of many hormones specially cortisol, aldosterone, insulin, glucagon, testosterone and thyroxine.	Blood level of these hormones increases, resulting in corresponding symptoms of hormonal excess.
3. *Antibacterial action* – Liver being a part of R-E system removes and destroys the bacteria.	Bacterial invasion of the body predisposing to infection.
4. Site of formation and destruction of RBCs.	***Anaemia***.

primary bile acids formed in the liver from cholesterol are: *cholic acid* and *chenodeoxycholic acid*. These get converted by colon bacteria to *Deoxycholic acid* and *Lithocholic acid* respectively, called ***secondary bile acids***.

2. Conjugation of bile acids occurs in the liver with 'taurine' or 'glycine'. Thus, cholic acid:
   (i) when conjugates with taurine form *Taurocholic acid;* and
   (ii) when conjugates with glycine form *Glycocholic acid*.

   The conjugates, tauro and glycocholic acids, form sodium and potassium salts in the alkaline bile, called ***Bile Salts*** (Fig. 29.2A). Their concentration is 120-180 mg/dL or 10-20 mEq/L.
3. At pH of bile 7.8–8.6, bile salts exist as anions and do not contribute to the osmotic pressure of the bile. *Osmotic pressure of bile* is identical with plasma, 290 mosm/ L.
4. Bile salts together with some cations form aggregates which are osmotically inactive.

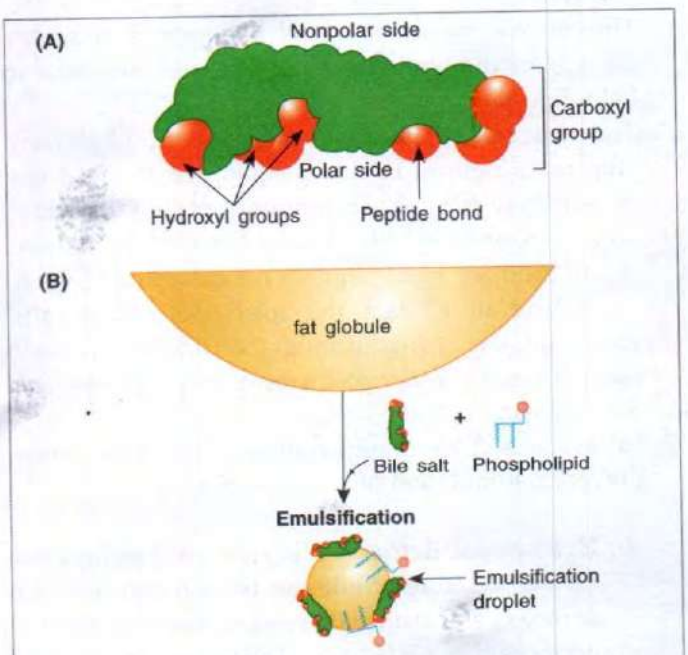


Fig. 29.2 Structure of bile salt (**A**) and Emulsification of fat by bile salts and phospholipids (**B**).

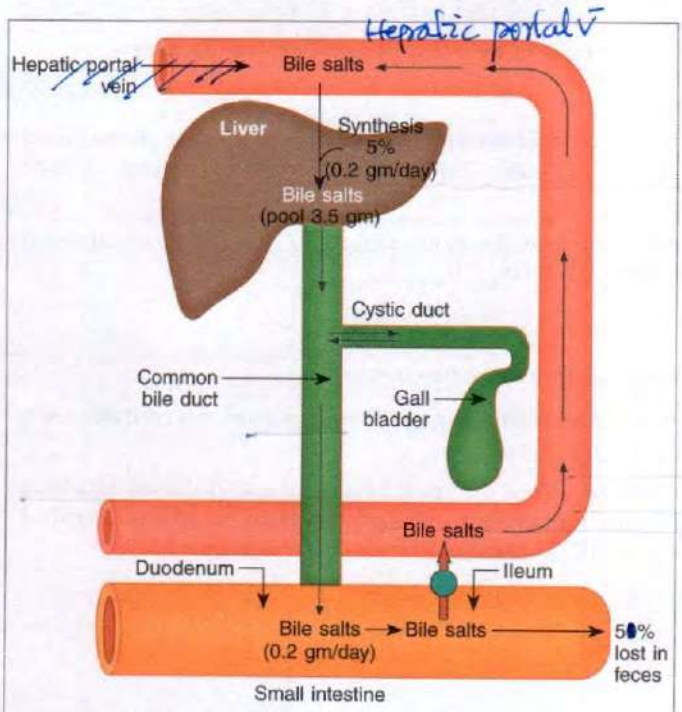


Fig. 29.3 Entero-hepatic circulation of bile salts

5. Of the total bile salts which enter the duodenum, 90-95% are reabsorbed actively from the terminal ileum in the portal vein and return to the liver, to be excreted again, the so called *Entero-Hepatic Circulation* **(Fig. 29.3)**. The remaining 5-10% enter the colon and are converted to the salts of 'deoxycholic acid' and 'lithocholic acid'. 'Lithocholate' is relatively insoluble and is mostly excreted in stools, but 'deoxycholate' is absorbed.
6. The *enterohepatic circulation* of bile salts is necessary because of the limited pool of bile salts available to help digestion and absorption of fats. How?
   The normal rate of bile salts synthesis is 0.2-0.4 gm/day. Approx. 3.5 gm of bile salts comprises the *total pool of circulating bile salts i.e.* amount of bile salts which recycle repeatedly via 'entero-hepatic circulation'. As an ordinary meal requires 6-8 gm of bile salts to digest and absorb fats, the 'total pool of bile salts' must circulate 'twice' during the digestion of each meal. Thus, *the entire pool recycles twice per meal and 6-8 times/day.*
7. Intravenous (I.V.) administraiton of bile salts causes powerful stimulation of biliary secretion.
8. *Actions*
   (i) ***Hydrotropic Action*** – Bile salts are ***amphipathic*** *i.e.* the structure of bile salt is such that one side of molecule is *hydrophilic* (water-attracting) and the opposite side is *hydrophobic* (water repelling or lipid attracting). This is responsible for characteristic property of bile salts *i.e.* lowering of surface tension of aqueous solution, therefore, allowing the formation of stable solutions or *emulsions* (breaking) of many fatty materials. Thus,
   (a) bile salts combine with lipids to form cylindrical discs called ***Micelles*** *i.e.* water soluble complexes from which the lipids can be more easily absorbed (Refer to page 262);
   (b) bile salts by decreasing the surface tension, in conjugation with phospholipids and monoglycerides are responsible for the ***emulsification*** of fats preparatory to its digestion and absorption in small intestine. **(Fig. 29.2B)**
   (ii) Bile salts also play a role in activating lipases in the intestine. [*Bile salts activated lipase* (page 230)]

Important Note

Diseases of small intestine *e.g.* sprue (page 247) or resection of ileum leads to:
(a) increased excretion of bile salts in faeces, this results in watery diarrhoea since bile salts inhibit water and sodium absorption from the colon;
(b) increased excretion of fats in the stools because when the entero-hepatic circulation is interrupted, liver cannot increase the rate of bile salt production to a sufficient degree to compensate for the loss.

Bile Pigments

1. Bile pigments are *bilirubin, biliverdin* and its derivatives. These are formed from haem portion of haemoglobin after the destruction of old RBCs in R-E system. 1 gm of haemoglobin produces 40 mg of bilirubin.
2. They are responsible for golden yellow colour of liver bile.
3. They are only excretory products and have *no digestive function*.

Cholesterol

1. Normal blood cholesterol is 120-200 mg/ dL (5 mEq/L); whereas biliary content of cholesterol is 60-170 mg/dL.
2. Concentration of bile which occurs in gall bladder further increases concentration of cholesterol and thus its crystallization. In hypercholesterolemia cholesterol crystals lead to formation of nuclei for crystallization of bile pigments and calcium salts *i.e.* ***Gall Stones*** (page 242).

Functions of Bile

1. *Bile salts functions:*
   (i) help in digestion and absorption of fats (see above)
   (ii) help in absorption of fat soluble vitamins.

2. *Neutralization of acid* – bile is the reservoir of alkali which helps to neutralize the acid 'chyme' from the stomach.
3. *Excretion:* It removes many drugs, adrenocortical and other steroid hormones, toxins, bile pigments and various inorganic substances *e.g.* copper, zinc and mercury.
4. *Solubility of cholesterol:* Large quantities of cholesterol present in the bile are solubilized in 'micelles', allowing cholesterol to be transported without precipitation in bile via biliary tract to the intestine.

Control of Bile Secretion

The secretion of bile is brought about by two major regulatory mechanisms: *nervous* and *humoral*.

1. Nervous Regulation

Vagal stimulation increases bile secretion by:

(i) contraction of gall bladder; and
(ii) relaxation of 'sphincter of Oddi'.

This vagal effect on bile secretion is abolished by atropine.

2. Humoral Regulation

Acid; products of carbohydrates, fats and proteins digestion in the stomach and small intestine increase the release of hormones, *Secretin* and *CCK-PZ* from the duodenum. Most potent stimuli for liberation of 'CCK-PZ' are fats and products of protein digestion, whereas acid in the duodenum is the major stimulus for 'Secretin' release.

These hormones, 'Secretin' and 'CCK-PZ', increase biliary secretion by contraction of the gall bladder. Therefore, following a meal, biliary flow increases within 30 minutes, which reaches the peak in 3-5 hours. The effect is initiated by interaction of both nervous and humoral mechanisms, which increase the water and bicarbonate content of bile.

As digestion proceeds to its completion; tone of 'sphincter of Oddi' increases and bile is prevented from entering the intestine. However, entero-hepatic circulation of bile salts causes continuous secretion of bile from the liver during the rest of the digestive phase.

Choleretic substances are the substances which increase biliary secretion from the liver *e.g. bile salts and bile acids.*

Cholagogues are the substances that cause contraction of gall bladder *e.g.* fatty acids; acid in the small intestine; products of protein digestion, Ca^{2+} etc. These substances produce contraction of the gall bladder by release of 'CCK-PZ' from the duodenum.

BILIRUBIN METABOLISM, EXCRETION AND JAUNDICE

(Refer to page 78)

FUNCTIONS OF GALL BLADDER

1. *Storage of bile.* Gall bladder is a thin walled organ with storage capacity of 60 mL. The mucous membrane is extensively folded, this increases its surface area and gives the interior of gall bladder a honey comb appearance. Its mucosa can 'actively' absorb fluid and electrolytes and concentrate them 5-6 times. That is why its average water content is 89% as compared to liver bile whose water content is 97%. Gall bladder bile is almost black in colour, thicker than liver bile and rich in solid substances due to:
   (i) secretion of mucus by gall bladder mucous membrane; and
   (ii) absorption of water.

   The differences between the liver bile and gall bladder bile are given in **Table 29.2.**
2. *Control of flow of bile following a meal:* (see above).
3. *Reduces the alkalinity of the stored bile:* Rapid absorption of HCO_3^- (mainly); Na^+ and Cl^-, decreases the bile pH from 8.6 to 7.4.
4. *Regulates equalization of pressure in biliary system*

   When the bile duct and cystic duct are clamped, the pressure in the biliary system rises to above 30 cm of bile in 30 minutes, and bile secretion stops. However, when the bile duct is clamped alone, water is reabsorbed in the gall bladder, and the pressure in the biliary system rises to only 10 cm of bile in several hours. Therefore, during fasting:
   (i) rate of secretion of bile is low and pressure in the biliary system is correspondingly small; and
   (ii) 'sphincter of Oddi' is contracted and it can resist pressure of approx. 30 cm of bile.

   When the pressure of bile in common bile duct is above 7 cm of bile, bile passes without appreciable resistance along the cystic duct into the gall bladder.
5. Gall bladder secretes ***mucin*** (mucus) which makes the bile thick (viscous).

APPLIED

1. **Cholecystectomy** *i.e.* removal of gall bladder.

   Disadvantages:
   (i) After cholecystectomy, bile from the liver empties slowly but continuously into the intestine, allows sufficient digestion of fats to maintain good nutrition. However, diet rich in fats leads to fats indigestion.

Table 29.2: Liver bile and gall bladder bile compared

		Liver bile	Gall bladder bile
1.	Colour	Light golden yellow	Almost black
2.	Consistency	Almost like water	Thicker than liver bile due to (i) presence of mucus and (ii) absorption of water by the gall bladder mucous membrane.
3.	pH	7.8–8.6	7.0–7.4
4.	Water	97%	89%
5.	Percentage of solids	2–4%	*more*; 10–12%
6.	Bile salts/bile acid (mg/dL)	120–180	*more*; 5–6 times the liver bile
7.	Bile pigments (mg/dL)	50	-do-
8.	Cholesterol (mg/dL)	60–170	-do-
9.	Lecithin (mg/dL)	140–810	-do-
10.	Electrolytes (mEq/L)		
	(i) Na^+	180–220	*increases* by 2 times
	(ii) K^+	6–8	-do-
	(iii) Cl^-	60–70	*decreases* by 5–6 times
	(iv) HCO_3^-	60–70	-do-

(ii) The bile ducts become dilated to accommodate some of the bile which is continuously secreted by liver, therefore,
   (a) if tone of 'sphincter of Oddi' is high, it causes gradual rise of pressure in biliary passage; when this pressure exceeds secretory pressure of liver cells, it interferes with bile secretion;
   (b) if tone of 'sphincter of Oddi' is low, it causes dribbling of bile in intestine when it is not needed and results in wastage of bile.

2. Complete Biliary Obstruction

The results of complete biliary obstruction are:

(i) Absence of bile from intestine producing
   (a) impaired fat digestion and absorption, producing ***steatorrhoea*** (page 233);
   (b) decreased absorption of fat soluble vitamins A, D, E and K leads to corresponding deficiency symptoms;
   (c) defective haemoglobin formation and anaemia (cause not known).

(ii) Retention of bile in blood and tissue fluids leading to:
   (a) jaundice
   (b) itching
   (c) loss of appetite; and
   (d) increased blood levels of all organic bile constituents (*e.g.* bile pigments, bile salts, cholesterol) and their appearance in urine.
   (e) increased serum alkaline phosphatase (page 79)

(iii) Injury to liver due to back pressure producing:
   (a) hepatomegaly (liver enlarges), stained with bile pigments, smooth and firm
   (b) impairment of liver functions.

3. Gall Stones *i.e.* presence of stones in the gall bladder or bile ducts. The condition is also called ***Cholelithiasis*** **(Fig. 29.4)**.

Causes

(i) when a substance that is not normally present appears in the bile; or
(ii) when the relative composition of the bile changes, this causes precipitation of normal constituents.

Types – The gall stones are mainly of 2 types: *cholesterol stones and calcium bilirubinate stones.*

(i) Almost 85% of gall stones are ***'cholesterol' stones***. They are found when concentration of cholesterol, lecithin, bile salts in bile gets altered. Normally ratio of bile cholesterol to bile salts is 1:20 or 1:30; when it falls below 1:13 it leads to precipitation of cholesterol. Cholesterol is normally present in bile in 'micelles' made up of lecithin and bile

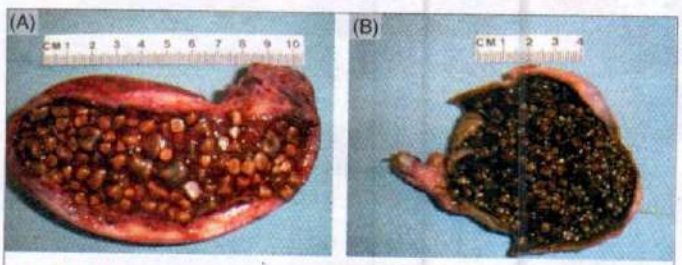


Fig. 29.4 Gall stones (A) Cholesterol and (B) pigment stones

salts. Relatively slight change in bile composition causes crystal formation which gradually joins to form cholesterol stones (page 240). These stones are ***radiolucent*** *i.e.* cannot be visualised on plain X-ray.

(ii) Remaining 15% are ***'pigment' stones*** mainly of calcium bilirubinate. These are formed when the conjugated bilirubin in the bile is deconjugated by the action of β-glucuronidase found in certain bacteria. The free bilirubin combines with Ca^{2+} to form calcium bilirubinate which is highly insoluble in bile. These stones are ***radiopaque***.

Applied → Part of Biliary obstruction ⇒ (JAUNDICE) (A)
(Where NO enterohep. circulation of bile)

Study Questions

1. **Write short notes on:**
   (i) Liver and gall bladder bile
   (ii) Functions of gall bladder
   (iii) Enterohepatic circulation of bile salts
   (iv) Control of bile secretion
   (v) Signs of liver insufficiency
   (vi) Functions of liver
   (vii) Physiological reserve of liver
   (viii) Bile salts and bile pigments
   (ix) Choleretic and cholagogues
   (x) Neural and humoral regulation of bile secretion
   (xi) Types of gall stones
2. **Draw well labelled diagram to show enterohepatic circulation of bile salts.**
3. **Give physiological basis of:**
   (i) Why resistance of blood flow through liver is low?
   (ii) Anaemia associated with liver disorder.
   (iii) What will happen if tone of sphincter of Oddi is decreased?
   (iv) Consequences of cholecystectomy.
   (v) Fatty liver
   (vi) Portal hypertension
   (vii) Bile salts and bile pigments
   (viii) Micelles
   (ix) Steatorrhoea
   (x) Foetid breath in liver failure
4. **How gall stones are formed?**
5. **What will happen and why?**
   (i) If ¾ of the liver is removed (ii) If metabolic function of liver fails (iii) If a person suffers from liver insufficiency

MCQs

1. **What percentage of liver can be removed without producing ill effects on the body?**
   (a) 20% (b) 40% (c) 60% (d) 80%
2. **Efficiency of liver decreases when it is loaded with:**
   (a) Carbohydrates (b) Proteins (c) Fats (d) Glycogen
3. **The normal mean portal venous pressure is ..... mmHg:**
   (a) 7 (b) 10 (c) 15 (d) 20
4. **Loss of liver function tends to cause all, *except*:**
   (a) A fall in free bilirubin level in blood
   (b) A rise in level of unconjugated bilirubin in blood
   (c) Increase in blood ammonia
   (d) A fall in the level of alpha and beta but not gamma globulins
5. **Portal hypertension is characterized by all of the following *except*:**
   (a) Ascites
   (b) Portal vein pressure increases to 5-7 mmHg
   (c) Haemorrhage from oesophagus
   (d) Haemorrhoids
6. **Foetid breath in liver failure is due to:**
   (a) Increased blood urea levels
   (b) Defective fat metabolism
   (c) Failure to detoxicate nitrogenous materials
   (d) Decrease in amino acid concentration in blood
7. **Bile salts:**
   (a) Are conjugated sodium and potassium salts of bile acids
   (b) Are derivatives of biliverdin and bilirubin
   (c) Concentration in liver bile is 140-810 mg/dL
   (d) Provide alkaline reaction to the bile

8. **The entire circulating pool of bile salts recycle ..... times/day:**
   (a) 2-3 (b) 3-4 (c) 4-5 (d) 6-8
9. **Failure to absorb bile salts in the distal ileum causes:**
   (a) Constipation (b) Watery diarrhoea (c) Dysentry (d) No effect
10. **Which is *not* a function of bile?**
   (a) Helps in absorption of fat soluble vitamins (b) Helps to neutralize the acidic chyme
   (c) Solubilizes the cholesterol in micelles (d) Acts as a choleretic substance
11. **Most potent stimulus for bile secretion from the liver is:**
   (a) Cholecystokinin (b) Secretin (c) Bile pigments (d) Bile salts
12. **Choleretics are the substances which causes**
   (a) Contraction of the gall bladder (b) Increase biliary secretion from the liver
   (c) Neutralization of acid from the stomach (d) Solubility of fats in micelles
13. **Cholagogues are the substances which cause:**
   (a) Contraction of the gall bladder (b) Increase concentration of bile
   (c) Increase secretion of bile (d) Favour acidification of bile
14. **A major percentage of gall stones are:**
   (a) Cholesterol stones (b) Pigment stones
   (c) Mixed stones (d) Radiopaque
15. **Cholelithiasis means:**
   (a) Substances that cause contraction of gall bladder (b) Substances which increase biliary secretion from the liver
   (c) Stones in the gall bladder (d) Bile salts and bile pigments
16. **Which of the following is *not* synthesized in the liver?**
   (a) Albumin (b) Immunoglobulins (c) Urea (d) Cholesterol
17. **Which is *not* a feature of fatty liver?**
   (a) Hepatomegaly (b) Hepatitis (c) Cirrhosis of liver (d) Increase in blood urea
18. **pH of liver bile is:**
   (a) 1–2 (b) 4–6 (c) 6.4–7.2 (d) 7.8–8.6
19. **Which of the following bile acid has minimum concentration in human bile?**
   (a) Cholic acid (b) Lithocholic acid (c) Deoxycholic acid (d) Chenodeoxycholic acid
20. **Amount of bile salts required to digest and absorb fat on an ordinary meal is ..... gm:**
   (a) 1-2 (b) 2-4 (c) 4-6 (d) 6-8
21. ***Not* characteristic of bile pigments:**
   (a) They are bilirubin and biliverdin (b) Formed from haem portion of haemoglobin
   (c) Responsible for golden yellow colour of bile (d) Have no digestive function

Answers

1. (d)	2. (c)	3. (a)	4. (a)	5. (b)	6. (c)	7. (a)	8. (d)	9. (b)	10. (d)
11. (d)	12. (b)	13. (a)	14. (a)	15. (c)	16. (b)	17. (d)	18. (d)	19. (b)	20. (d)
21. (b)									

Chapter 30

Small Intestine

I. Introduction
II. Structure of Small Intestine
III. Intestinal Juice (Succus Entericus): Composition, functions and control of secretion
IV. Digestion in the Small Intestine
Applied: Malabsorption Syndrome
V. Movements of Small Intestine
Applied: Adynamic (paralytic) ileus
Mechanical obstruction of small intestine
Ileo-caecal Valve and Gastro-ileal Reflex

INTRODUCTION

1. The small intestine is the major site of *digestion* and *absorption* of carbohydrates, proteins and fats. Here, the intestinal contents are mixed with *intestinal juice* (secretions of the mucosal cells) and with pancreatic juice and bile.
2. Digestion which begins in the mouth and stomach is completed in the lumen and mucosal cells of small intestine. The nutrients and fluid that are not absorbed in the small intestine are passed on to the colon.
3. The small intestine is presented with approximately 9 litres of fluid/day, of this 2L comes from dietary sources and 7L from GIT secretions; however, only 1-2L passes into the colon.

GIT 7L + Diet 2L → 9 → 9 − 7 = 2 → To colon (Absorbed)

STRUCTURE OF SMALL INTESTINE

(page 200)

INTESTINAL JUICE – SUCCUS ENTERICUS

A. COMPOSITION AND FUNCTIONS

Crypts of Lieberkuhn (page 200), which characterize the whole of small intestine, continuously form the cells which migrate up the villus and secrete the *intestinal juice*, also called *succus entericus*. (Enterocyte)

1.5–2 ltrs (Generally)

Daily secretion : 3 litres; colourless fluid, slightly cloudy due to admixture of mucus, epithelial cells and cholesterol.

S.G. : 1010; *'isotonic'* with plasma

pH : 7.6 (Slightly alk.)

Water : 98.5%

Solids : 1.5%

(i) Inorganic – 0.7%;
Cations : Na^+, K^+, Ca^{2+}, Mg^{2+}
Anions : Cl^-, HCO_3^-, PO_4^{3-}

(ii) Organic – 0.8%; Enzymes

Enzymes are located in the luminal (brush) border of the epithelial cells. These are: (ENTEROCYTES)

1. Enterokinase (enteropeptidase) – it activates trypsinogen.
2. Proteolytic enzymes
   (i) **Erepsin**, a mixture of several different specific peptidases *e.g.* aminopeptidases and dipeptidases.
   (ii) Nuclease and related enzymes *e.g.* nucleotidase, nucleosidase.
3. Enzymes for splitting disaccharides into monosaccharides *e.g.* invertase (sucrase); maltase; lactase and α-limiting dextrinase, Isomaltase
4. Intestinal lipase.
5. Cholesterol esterase, converts cholesterol esters to free cholesterol.
6. Lecithinase, converts phospholipids (lecithin and lysolecithin) to simpler phospholipids.
7. Alkaline phosphatase, converts organic phosphate to free phosphate.

B. CONTROL OF SECRETION OF INTESTINAL JUICE

Control of secretion of intestinal juice is neither nervous nor humoral; but it is affected by local, mechanical and chemical stimuli on intestinal mucosa in the presence of 'chyme' and food particles which it contains.

ONLY SALIVARY SECRETION IS HYPOTONIC

Brunner's glands → In duodenum, Protects mucosa from acidic chyme

Chyme & Intestinal juice

1. Ingestion of meal causes flow of intestinal juice, which is most obvious at the upper end of the gut. The flow of juice is slight during the 1st two hours but it is markedly increased in the 3rd hour.
2. Mechanical stimulation *e.g.* distension of intestinal mucous membrane via local myenteric reflex, increases the volume and total enzymes output of the small intestine, that is why, greater is the 'chyme', the greater is the secretion of the intestinal juice.
   Similar effects are produced during colicky intestinal contractions and with ingestion of water, glucose, egg, milk etc.
3. Local irritants increase the volume of the intestinal juice rich in 'mucus' content (Function of mucus: Refer to page 200). → Lubrication, Protects intes mucosa

Note

VIP, a GIT hormone (page 272) stimulates the secretion of intestinal juice. Vagal stimulation increases the secretion of Brunner's glands (page 201).

(III^r to pyloric glands)

(300)

DIGESTION IN THE SMALL INTESTINE

1. *Carbohydrate Digestion:* Intestinal juice contains disaccharidases *i.e.* enzymes for splitting disaccharides into monosaccharides, therefore,

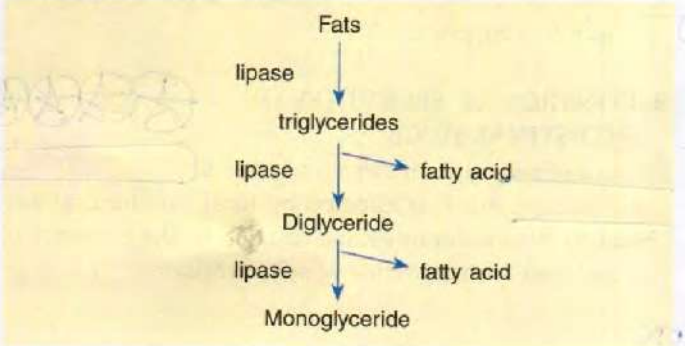
(i) *Sucrose* $\xrightarrow[\text{pH 5–7}]{\text{invertase (sucrase)}}$ *Glucose and fructose*

(ii) *Maltose* $\xrightarrow[\text{pH 5.8–6.2}]{\text{Maltase}}$ Two molecules of *glucose*

(iii) *Lactose* $\xrightarrow[\text{pH 5.4–6.0}]{\text{Lactase}}$ *Glucose and galactose*

(iv) *α-limiting dextrins* $\xrightarrow{\text{α-limiting dextrinase}}$ *Glucose*

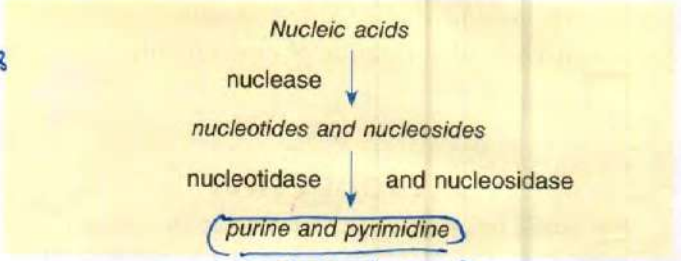
2. *Fat Digestion:* Intestinal lipase is particularly concerned with the hydrolysis of the primary ester linkages. Triglyceride digestion begins first by removing the terminal fatty acid to produce an 'α-β-diglyceride' (DAG); the other terminal fatty acid is then removed leaving a 'β-monoglyceride', the main end products of fat digestion. (MAG)



3. *Protein Digestion*
   (i) 'Erepsin', a mixture of several specific enzymes, acts primarily and rapidly on peptones and polypeptides, converting them into amino-acids.

Peptones and polypeptides $\xrightarrow{\text{Erepsin}}$ *Amino acids*

   (ii) 'Erepsin' can also break down 'caseinogen' and other proteins slowly.
   (iii) Nuclease' and related enzymes hydrolyse the nucleic acids to liberate purine and pyrimidine bases.



(N_2-bases)

APPLIED – MALABSORPTION SYNDROME

Some of the common causes of malabsorption syndrome are:

1. Resection of the small intestine
2. Gastro-colic fistula
3. Sprue
4. Coeliac disease

1. Resection of the small intestine

Excision of the small intestine must be very extensive (more than 50%) to interfere with digestion or absorption. For example, resection of the whole small intestine leaving only duodenum, jejunum and small length of ileum shows:

(i) 99% of ingested carbohydrates are absorbed.
(ii) 70% of ingested proteins are absorbed.
(iii) Fat absorption is markedly decreased which produces:
   (a) diarrhoea due to deficient absorption of bile salts.
   (b) fatty infiltration of liver and cirrhosis of liver (page 238)
   (c) increases fat excretion in faeces mainly as fatty acids resulting in ***steatorrhoea*** (page 233)
   (d) deficiency symptoms due to deficiency of fat soluble vitamins (vitamin A, D, E and K).
(iv) Marked decrease in calcium absorption due to formation of insoluble calcium soaps results in:
   (a) hypocalcemia (decrease in S. Ca^{2+}) which produces ***tetany***
   (b) hypercalciuria, this precipitates *renal stones*.
(v) Others: arthritis and hyperuricemia

gall stones

2. Gastro-colic Fistula

Here food completely short circuits the small intestine by-passing directly from the stomach into the transverse colon. It manifests the change described above plus:

(i) 'pernicious' anaemia due to failure of absorption of vitamin B_{12} (page 71);
(ii) multiple vitamin deficiency symptoms;
(iii) defective absorption of amino acids produces marked muscular weakness with wasting, hypoproteinaemia which results in oedema.

3. Sprue

Here absorption defect is in part due to lack of digestive enzymes and in part due to folic acid deficiency.

4. Coeliac Disease or Gluten Enteropathy

It is characterized by congenital absence of the enzymes *gluten hydrolase* from the intestinal mucosal cells. Thus gluten escapes digestion and gets deaminated in the brush border of small intestine. This results in formation of a toxic polypeptide *'Gliadin'* from ***Gluten***, a protein in wheat, rye, barley and oats. 'Gliadin' causes intestinal T cells to produce an inflammatory allergic response that flattens and disrupts the formation of microvilli. The disease is treated by omission of these grains from the diet.

Important Notes

1. Distal small bowel resection (*ileal resection*) produces greater degree of malabsorption than removal of complete length of proximal small intestine (jejunum) because resection of ileum prevents absorption of bile salts causing decreased fat absorption and unabsorbed bile salts to enter the colon. This inhibits Na^+ and water reabsorption producing diarrhoea.
2. The capacity of jejunum to adapt is less than that of ileum *i.e.* removal of short segment of small intestine (jejunum or ileum) leads to compensatory hypertrophy and hyperplasia of the remaining mucosa with gradual return of the absorption function towards normal, called *intestinal adaptation*. This adaptation is due to:
   (i) a direct effect of nutrients in the intestinal lumen on the mucosa; and
   (ii) GIT hormones.

MOVEMENTS OF SMALL INTESTINE

Three types of contractions are seen in the small intestine: **(Fig. 30.1)**

A. Rhythmic segmental contractions or pendular movements; and
B. Peristalsis
C. Tonic Contractions

A. RHYTHMIC SEGMENTAL CONTRACTIONS or PENDULAR MOVEMENTS

These are ring like contractions that appear at fairly regular intervals (rhythmic) along the gut involving a localised 'segment' of 1-2 cm by increase in Ca^{2+} influx. They are of 2 types: *eccentric* and *concentric contractions* **(refer Table 30.1)**.

Therefore, by 'rhythmic segmental contractions', the food is divided (segmented) and mixed together with digestive juices thoroughly (again and again) and finally ***chyme*** is formed.

Control of rhythmic segmental contractions

1. These contractions are initiated by *pacemaker cells* which are located in 2nd part of duodenum near the entry of common bile duct. A *basic electrical rhythm* (page 222) of 'slow wave' can be demonstrated which is conducted caudally by the longitudinal muscle layer; coordination of this rhythm is achieved by Myenteric plexus.
2. The '*frequency*' of their contractions is directly related to the frequency of the 'slow waves' initiated by *pacemaker cells* within the wall of the small intestine. It is not influenced by either neural activity or circulating hormones.
3. The '*strength*' of the contractions is proportional to the frequency of the spike generated by the 'slow waves'. This frequency is controlled by the amplitude of the 'slow wave'. Thus, the greater the 'slow wave' amplitude, the greater the frequency of spike generated, and the greater the strength of the contraction.
   'Slow wave' amplitude is increased by GIT hormones released during digestion *e.g.* gasrtin, 'CCK-PZ' and motilin; whereas secretin and glucagon decrease the slow wave amplitude.
4. 5-HT released during contractions make the smooth muscle *sensitive to distension*. Distension of a short segment causes proximal segment contraction and distal segment relaxation.

B. PERISTALSIS

Characteristic features

1. When intestinal wall is stretched or distended by food (chyme), a circular constriction forms above it due to contraction of circular muscle layer while the lumen below it is dilated due to contraction of longitudinal muscle layer (*receptive relaxation*, page 222). Therefore, intestinal contents move towards the dilated part; then contraction of circular muscles spread to this part which in turn is constricted, while the segment below it is dilated by contraction of longitudinal muscle layer. Several of these wave-like

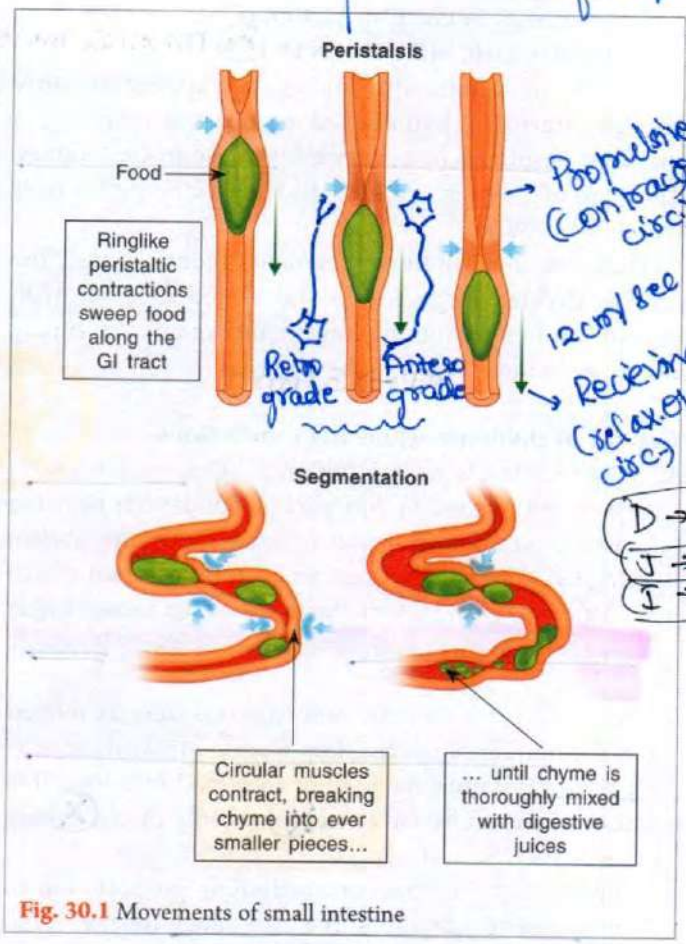


Fig. 30.1 Movements of small intestine

contractions occur simultaneously along the length of the intestine, so that its movement is *vermiform* (like a worm), therefore, they are called ***vermicular or peristaltic movements.***

2. 'Peristalsis' is a coordinated reaction in which a wave of contraction preceded by a wave of relaxation passes down a hollow viscus. The contents of viscus as they are propelled along would always enter a segment which had actively relaxed and enlarged to receive them.
3. It can occur in either direction from a stimulated point but it normally dies out rapidly in oral direction, while continuing for considerable distance towards the anus. This is called ***Polarity of the intestine or Law of the Gut.*** *Proof*: Removal and resuturing of a segment of intestine in its original position does not block progression and waves will cross a small gap where the intestine has been replaced by a plastic tube. However, if an intestinal segment is reversed and sewed back in place, 'peristalsis' stops at the reversed segment.
4. *Why peristalsis always proceeds from the proximal to the distal end of the GIT?* This is because:
   (i) the frequency of BER (page 222) decreases progressively from the duodenum (12/min) to the ileum (9/min); and
   (ii) of receptive relaxation which appears only distally (see above).
5. *Function:* These waves propel the intestinal contents towards ileo-caecal valve. Each peristaltic wave lasts for 1-2 sec and propels the 'chyme' a few cms.
6. The usual stimulus for peristalsis is distension, as a result peristaltic waves pass along the intestine towards the rectum at rates varying from 2 to 25 cm/sec. The local stretch releases serotonin, which activates sensory neurons that stimulate the Myenteric plexus (page 199). This response to stretch is called ***Myenteric reflex.***

Important Note

Activity in the Myenteric plexus from a stimulus point travels in either direction to activate neurons that releases:

(i) *A-ch* and *substance P* above the point of stimulus, producing a circular constriction; and

(ii) *Nitric oxide, VIP* and *ATP* below the point of stimulus, producing relaxation.

Table 30.1: Rhythmic segmental contractions ('Eccentric' and 'Concentric' contractions)

Eccentric contractions	Concentric contractions
1. They are mainly due to contraction of outer longitudinal smooth muscle layer.	They are mainly due to contraction of inner circular smooth muscle layer.
2. They initially produce a segment shortening and consequently dilates it.	These contractions cause narrowing of lumen and push the contents away from the constricted segment. They also cause local rise of intra-luminal pressure.
3. They are mainly concerned with *mixing function i.e.* move the 'chyme' to and fro to increase its exposure to mucosal surface. Therefore, food is mixed thoroughly with digestive juices again and again.	These contractions are mainly concerned with ***churning action*** *i.e.* food is divided (segmented) and is propelled towards the large intestine.
4. These are *localised* contractions which occur in several regions at once or sequentially.	These contractions occur over a segment longer than eccentric contraction segment and frequency of these contractions during digestion is highest in the duodenum (12/min) and lowest in the ileum (9/min).

7. It is an inherent property of any syncytial smooth muscle hollow viscus *e.g.* GIT, bile ducts, ureters, glandular ducts etc.
8. *Weak peristalsis* are sometimes seen in the colon.
9. Peristaltic contractions are usually superimposed upon rhythmic segmental contractions. Both of these contractions can occur in the absence of extrinsic innervation but are dependent upon the integrity of myenteric nerve plexus. Therefore,
   (i) strong emotions by activating parasympathetic component via vagus nerve, increase muscular contraction and increase the tone of the small intestine; and
   (ii) anger, fear and pain by activating sympathetic component via splanchnic nerve, decrease muscular contraction and decrease the tone of the small intestine.

C. TONIC CONTRACTIONS

These are relatively prolonged contractions that isolate one segment of the intestine from another. Along with segmental contraction, permits longer contact of the chyme with the enterocytes and promotes absorption.

APPLIED

1. Adynamic Ileus or Paralytic Ileus

It is a *painless* condition *produced by*:

(i) ***Handling*** of intestine during abdominal operations or trauma to intestine. This causes direct inhibition of smooth muscles.

(ii) ***Irritation*** of peritoneum causes reflex inhibition of smooth muscles due to increased discharge of noradrenergic fibres in splanchnic nerves.

(i) and (ii) decrease the intestinal motility to cause *adynamic* (*paralytic*) *ileus*. Therefore, intestinal contents are not propelled into the colon causing retention of contents in the small intestine. This produces irregular distension of the small intestine by pockets of gas and fluid. The condition usually get relieved by rest to the bowels, intestinal peristalsis returns in 6-8 hours.

2. Mechanical Obstruction of Small Intestine

This condition is associated with production of severe pain. How? Localised mechanical obstruction of small intestine initiates very intense peristaltic waves called ***peristaltic rush***. This causes severe cramping pain (*intestinal colic*). The segment of intestine proximal to obstruction dilates and becomes filled with gas and fluid. This increases the pressure in the segment causing:

(i) compression of blood vessels in its wall producing local ischaemia, and

(ii) stimulate visceral afferent nerve fibers to cause sweating, hypotension and severe vomiting. If the obstruction is not relieved it may prove fatal due to resultant metabolic alkalosis and dehydration.

ILEO-CAECAL VALVE

Ileo-caecal junction serves as a valve which offers low resistance to the passage of ileal contents into the caecum but it strongly opposes reflux into opposite direction. Ileal opening is 2-3 mm in diameter. Rhythmic contraction of circular muscle occurs and in the interval between these, small jets of fluid, approx. 15 mL, escape into the caecum. Closure may be prolonged if no food has been ingested, but in a few minutes after a meal, rhythmic opening and closing every 30 sec permits the escape of ileal fluid. 'Gastrin' produces relaxation, whereas 'Secretin' causes contraction.

Note

These agents show opposite effects on cardiac sphincter.

GASTRO-ILEAL REFLEX

When food leaves the stomach, the caecum relaxes and passage of chyme through the ileocaecal valve increases, called ***gastro-ileal reflex***. This is a vagally mediated reflex. Sympathetic stimulation increases the contraction of the valve.

Study Questions

1. Give physiological basis of:
   (i) Sprue
   (ii) Coeliac disease
   (iii) Peristaltic rush
   (iv) Pendular movements
   (v) Peristalsis always proceeds from proximal to distal end
2. Write short notes on:
   (i) Composition and function of succus entericus
   (ii) Control of intestinal juice secretion
   (iii) Enterokinase
   (iv) Intestinal adaptation
   (v) Gastro-colic fistula
   (vi) Law of gut

(vii) Myenteric reflex
(viii) Adynamic ileus
(ix) Movements of small intestine
(x) Electrical basis of rhythmic segmental contraction
(xi) Gastro-ileal reflex

3. **Removal of which part of small intestine will produce greater degree of malabsorption and why?**
4. **What determines the strength of contraction of pendular movements? Name the agents affecting it.**
5. **What will happen and why?**
   (i) To intestinal movements after denervation
   (ii) If small intestine is resected
6. **How digestion of carbohydrates is brought about in small intestine?**
7. **Draw labelled diagram to show movements of small intestine.**

MCQs

1. **Intestinal Juice contains:**
   (a) Enzymes released in response to vagal activity
   (b) Saccharidases
   (c) Potassium equal in concentraiton to E.C.F.
   (d) A substance which activates trypsinogen
2. **All of the following *except* one can cause increased secretion of intestinal juice:**
   (a) Ingestion of a meal
   (b) Distension of small intestine
   (c) Vagal nerve stimulation
   (d) Irritation of small intestinal mucosa
3. **Surgical removal of about 90% of the ileum and jejunum tends to cause all, *except*:**
   (a) Steatorrhoea
   (b) Osteomalacia
   (c) A fall in extracellular fluid volume
   (d) Anaemia
4. **Coeliac disease is characterized by:**
   (a) Congenital absence of enzyme gluten hydrolase from small intestine
   (b) Food bypassing directly from the stomach into the transverse colon
   (c) Fat indigestion
   (d) Fatty infiltration of liver
5. ***Not true* of intestinal adaptation which is seen following resection of short segment of small intestine:**
   (a) Capacity of ileum to adapt is less than that of jejunum
   (b) Compensatory hypertrophy of remaining mucosa
   (c) Causes gradual return of absorption function
   (d) GIT hormones are responsible for it
6. **The rhythmic contraction in GIT is highest in:**
   (a) Stomach (b) Duodenum (c) Ileum (d) Colon
7. **Peristalsis movements of small intestine are characterized by all *except*:**
   (a) Also called vermicular movements
   (b) Can occur in either direction from a stimulated point
   (c) Propel the intestinal contents towards ileo-caecal valve
   (d) Pass along the intestine at a rate of 1-2 cm/sec
8. **The usual stimulus of peristallsis is:**
   (a) Distension (b) Sympathetic stimulation (c) Acid chyme (d) Alkaline chyme
9. **Severe irritation of the mucosal lining of small intestine may cause:**
   (a) A stop in the propulsion of chyme
   (b) Intestinal obstruction
   (c) The chyme to move more slowly to reduce the irritation
   (d) A "peristaltic rush" to quickly move the chyme into the colon
10. ***Not true* of gastro-ileal reflex:**
    (a) When food leaves the stomach, reflex relaxation of ileum occurs
    (b) Passage of chyme through ileo-caecal valve increases
    (c) A vagally mediated reflex
    (d) sympathetic stimulation inhibits the reflex
11. **Following are the movements of small intestine *except*:**
    (a) Peristalsis (b) Segmentation (c) Pendular (d) Mass peristalsis
12. **Rhythmic segmental contraction – *not true* is:**
    (a) Involves a localised segment of 1-2 cm
    (b) Causes food to be divided and mixed together with digestive juices
    (c) Initiated in 2nd part of duodenum
    (d) Frequency of these contractions is not related to the slow-waves spike
13. **Peristalsis may be initiated by all of the following, *except*:**
    (a) Inhibitors of striated muscle contraction
    (b) Distension of the gut wall
    (c) Irritation of the mucosa
    (d) The composition of the chyme

14. **Small intestinal peristalsis is dependent upon the integrity of:**
    (a) Myenteric nerve plexus (b) Meissner's nerve plexus
    (c) Sympathetic nervous system (d) Parasympathetic nervous system
15. **Irregular distension of small intestine with retention of contents most likely seen in:**
    (a) Coeliac disease (b) Gastro-colic fistula
    (c) Paralytic ileus (d) Obstruction of small intestine

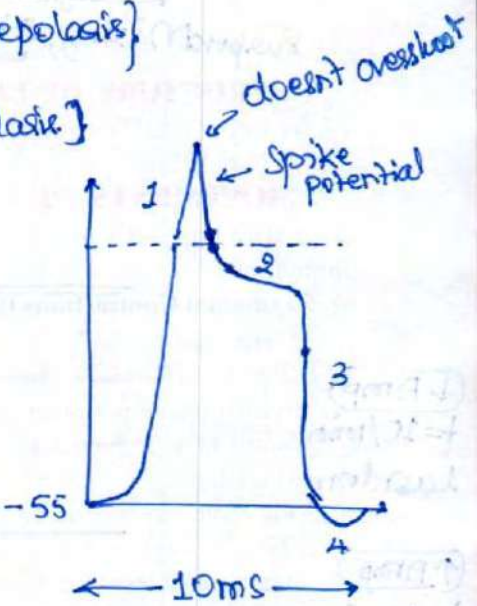
Answers

1. (d)	2. (c)	3. (c)	4. (a)	5. (a)	6. (b)	7. (d)	8. (a)	9. (d)	10. (a)
11. (d)	12. (d)	13. (a)	14. (a)	15. (c)					

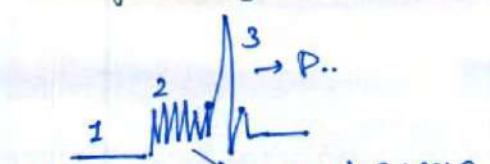
BASIC ELECTRICAL RHYTHM

P-cells → pacemaker cells = Interstitial cells of Cajal (Basal electrical Rhythm). (SLOW waves)

3 phases:
→ I phase: Influx of Ca^{++}, Na^{+} {Depolasis}
→ II phase: Plateau
→ III phase: K^{+} efflux. {~~Hyppse~~ Repolasis}



* migratory motor complex: 90min ;
→ in interdigestive period
→



* Role of sphincters
  - (Anatomical) → Lower oesophageal sph
    - → Gastro duodenal sph. (Pylorus)
    - → Ileo-caecal
  - (Physiological) → Upper oesophageal sph.
    - ↳ acts as sphincter
    - ↳ But, no modific. seen histologically

Chapter 31

Large Intestine (Colon)

I. Structure of Large Intestine
II. Movements of Large Intestine
Transit time
Disorders of large intestine motility
(Hirschsprung's disease; constipation; diarrhoea)
III. Defecation
IV. Absorption and Secretion in Large Intestine
V. Faeces-Dietary fibers

STRUCTURE OF LARGE INTESTINE

(Refer to page 201)

MOVEMENTS OF LARGE INTESTINE

Two main types of waves of contractions are seen in the large intestine: *segmental* and *peristaltic* contractions.

A. **Segmental Contractions (or Haustrations)** – They are of two types:

1. *Type I contractions* – These are small amplitude waves which cause pressure rise upto 5 cm saline. They occur at a frequency of 10-12/min and are of 5 sec duration.
Function: aid mixing of the contents of the colon.
2. *Type II contractions* – These are larger pressure waves as compared to type I contractions; occur at a frequency of 1-2/min and last for about 30 sec.
Functions:
(i) aid mixing of the contents of the colon; and
(ii) facilitate absorption by exposing more of the contents to the mucosa.

Note

The combined contractions of the circular and longitudinal muscles cause the unstimulated portion of the large intestine to bulge outwards into baglike sacs, called Haustrations (Also refer to page 201).

B. **Peristaltic Contractions** – They are also of two types:

1. *Type III contractions*. These are very small pressure waves of prolonged duration.
Function: They propel the contents towards the rectum.
2. *Type IV contractions. Mass Peristalsis* or ***Mass Action Contractions***. These are simultaneous contractions of the smooth muscle occurring at the same time over a large portion of the colon. These contractions occur more in the descending and sigmoid colon. They last for 3-4 minutes with pressure rising steeply to a peak upto 100 cm saline, and then decline slowly.
Function: These contractions propel contents from the caecal region towards the rectum *i.e.* serve to empty the colon rapidly. They are the predominant contraction force during *defecation*.
Both segmental and peristaltic contractions occur infrequently but are particularly evident following a meal. Thus, distension of the stomach by the food initiates contractions of the rectum and frequently a desire to defecate, called *Gastrocolic Reflex*. Because of the response, *defecation after meals is the rule* in children. There are also some evidences that this is due to action of 'gastrin' on the colon and is not mediated via nerves.

Note

The movement of the colon are conducted by the B.E.R. of the colon. The frequency of these waves increases along the colon from about 2/min. at the ileo-cecal valve to 6/min. at the sigmoid colon.

TRANSIT TIME IN THE SMALL INTESTINE AND COLON (Fig. 31.1)

After barium meal (ingestion of barium), the first part of barium meal reaches caecum in 4-5 hours, the hepatic flexure in 6 hours, the splenic flexure in 9 hours, the pelvic colon in 12-18 hours.

From pelvic colon to the anus, transport is much slower. When small coloured beads are fed with a meal,

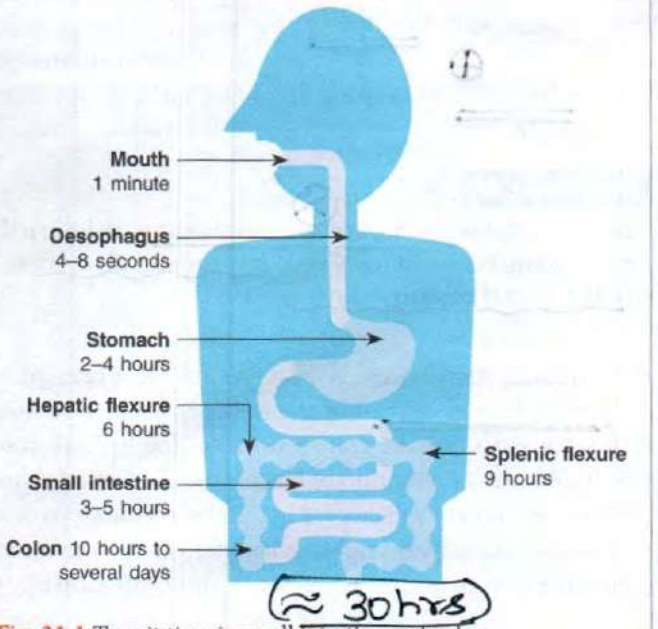


Fig. 31.1 Transit time in small intestine and colon

an average of 70% of them are recovered in the stool in 72 hours; but total recovery requires more than one week.

DISORDERS OF LARGE INTESTINE MOTILITY

1. Hirschsprung's Disease or Aganglionic Megacolon

(i) The disease is commonly seen in children and is *due* to congenital absence of the ganglionic cells in both the plexuses (Myenteric and submucous) or due to degeneration of Myenteric plexus. There appears to be a mutation in the *endothelin B receptors* that are necessary for normal cranial to caudal migration of neuroblast (neural crest cells) during development.

(ii) The ***site*** of involvement is usually the *distal colon* and the *'pelvic-rectal' junction*. This leads to blockage of both the peritalsis and mass contractions, therefore, faeces pass the aganglionic segment with difficulty and accumulate in the large intestine **(Fig. 31.2)**.

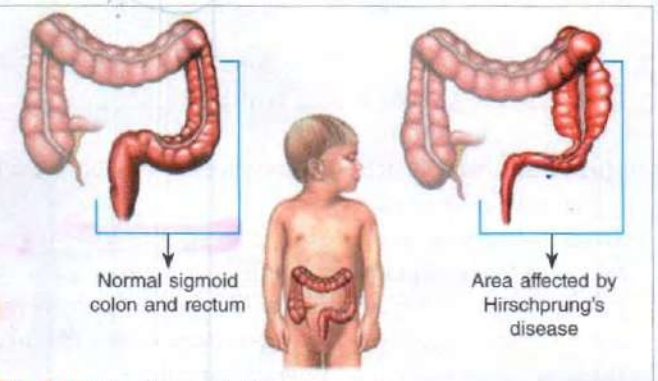


Fig. 31.2 Hirschprung's disease

(iii) Children with this disease may defecate only once in every 3 weeks.

(iv) The disease can be treated by cutting the aganglionic portion of pelvic-rectal junction and anastomosing the cut ends.

(v) In some individuals, the disease is *associated with* dilated urinary bladder and ureters, indicating some deficiency in parasympathetic supply to the urinary tract in these individuals. In addition, there are no 'VIP' containing nerve cells or fibers and the involved colon is hyper-responsive to exogenous VIP. The substance P is also low.

2. Constipation

Constipation is the result of neglected calls to defecate. It is due to adaptation of sensory mechanism and its reflex effects. The symptoms caused by constipation include: anorexia, abdominal discomfort, distension, furred tongue and foul breath etc.; other symptoms such as headache, restlessness, irritability are due to anxiety. These symptoms are not due to absorption of toxic substances because they are quickly relieved with evacuating the rectum.

3. Diarrhoea

Increase in frequency of passage of stools is called *diarrhoea* or ***loose motions***. It has many causes *e.g.* due to infection, typhoid, gastro-enteritis (food poisoning), ulcerative colitis etc.

Severe diarrhoea is debilitating and can be fatal specially in infants. Loss of large amount of electrolytes (Na^+ and K^+) and water in the diarrhoeal stool causes dehydration, hypovolemia, electrolyte imbalance, shock, cardiovascular collapse and finally death.

Note

Emotional factors strongly influence large intestinal motility via ANS. *Irritable bowel syndrome* may occur during period of stress and may result in either constipation (increased segmental contraction) or diarrhoea (decreased segmental contraction).

DEFECATION

1. ***Anal Sphincters*** – Two types: *Internal* and *External*.
   (i) *Internal or involuntary anal sphincter* – It consists of thickening of circular smooth muscles at pelvic-rectal flexure and is innervated by:
      (a) parasympathetic pelvic splanchnic nerves are inhibitory;
      (b) sympathetic nerves are excitatory.
   This sphincter relaxes when the rectum is distended.
   (ii) *External or voluntary anal sphincter* – It consists of somatic skeletal muscle. It is innervated by

pudendal nerves and is maintained in a state of tonic contraction. Mild to moderate distension of rectum increases the force of its contraction, whereas moderately severe distension of rectum relaxes it.

2. Rectum is normally empty. Distension of rectum with faeces (to 25% of its capacity) initiates reflex contraction of its musculature, and the desire to defecate. The mass peristalsis waves along with the *gastrocolic reflex* (page 252) induced after a meal, drives the faeces into the rectum and rectal pressure increases.
3. The first urge to defecate occurs when rectal pressure increases to about 20 mmHg by stimulation of receptors in the rectal wall. When the rectal pressure increases to about 55 mmHg, both the anal sphincters (internal and external) relax and the contents of the rectum are expelled.
4. ***Pathway for defecation reflex*** **(Fig. 31.3)**: The afferent impulses travel in pelvic nerves and induce reflex parasympathetic discharge (mainly from S_2) over the pelvic splanchnic nerves to cause:
   (i) inhibition (*i.e.* relaxation) of internal anal sphincter; and
   (ii) inhibition of discharge in somatic pudendal fibers, this relaxes the external anal sphincter.
5. ***Voluntary defecation:*** Before the pressure that relaxes the external anal sphincter is reached, voluntary defecation can be initiated by voluntarily relaxing the external anal sphincter and contracting the abdominal muscles (*straining at stools*). This plus reduction in the angle between the anus and the rectum to 15° (normal 90°) thus aiding the reflex emptying of the distended rectum.

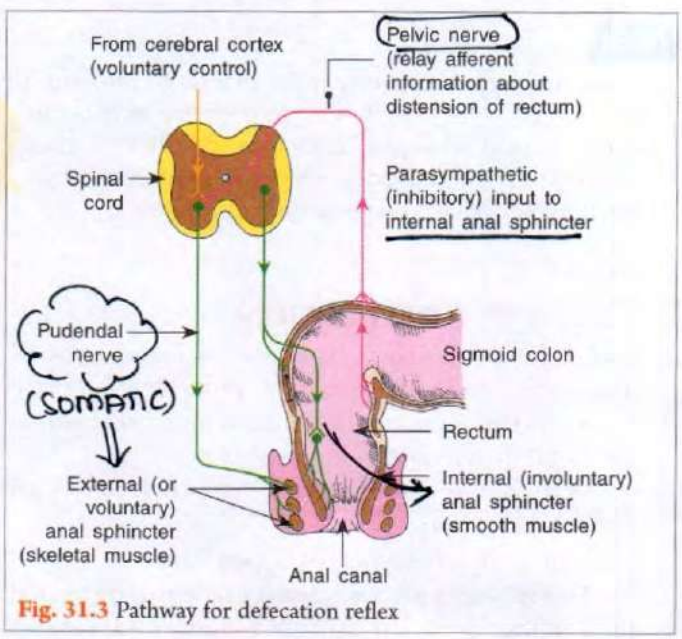


Fig. 31.3 Pathway for defecation reflex

Defecation reflex is, therefore, a ***spinal reflex*** that can be voluntarily 'facilitated' by relaxing the external anal sphincter and contracting the abdominal muscles or 'inhibited' by keeping the external anal sphincter contracted.

Important Note

Here excitatory sympathetic innervation of internal anal sphincter is not involved, only the sacral segment of the spinal cord are concerned.

6. In infants, defecation is a *simple spinal reflex*; social training brings control of the reflex by higher centres.
7. **Applied:** Complete transection of the spinal cord, initially causes retention of faeces, the defecation reflex, however, returns quickly (although no voluntary control is ever regained) and reflex evacuation of rectum occurs when rectal pressure increases to about 55 mmHg.

ABSORPTION AND SECRETION IN LARGE INTESTINE

1. The main function of the colon is absorption of water. *Main sites of water absorption* are caecum and ascending colon.
   (i) Approx. 1-2 L of isotonic 'chyme' daily enters the colon from the ileum and only 100-150 mL of water reaches the rectum per day *i.e.* colon can remove approx. 90% of the fluids.
   (ii) Large intestine can absorb Na^+, K^+, Cl^-, glucose and certain vitamins with net secretion of K^+ and HCO_3^-, but it *cannot* absorb protein, fat or Ca^{2+}. Na^+ is actively absorbed from the colon, and water follows along the osmotic gradient thus generated.

Important Note

Many compounds, such as anaesthetics, tranquilizers, sedatives and steroids, when administered per rectally, get absorbed rapidly into the circulation. This makes *rectal instillation of drugs a clinical practical route, specially in children.*

2. No digestive enzymes are secreted in the colon.
3. 'Goblet' cells secrete mucus which helps:
   (i) lubricate the intestinal mass, and
   (ii) neutralize the acid which is formed by bacteria in the large intestine.
4. At birth, the colon is sterile but the ***colonic bacterial*** flora becomes established early in life. The micro-organisms present in the colon include bacilli such as *Escheria coli, Enterobacter aerogenes* and gas gangrene bacilli. Some of these are beneficial and others harmful.

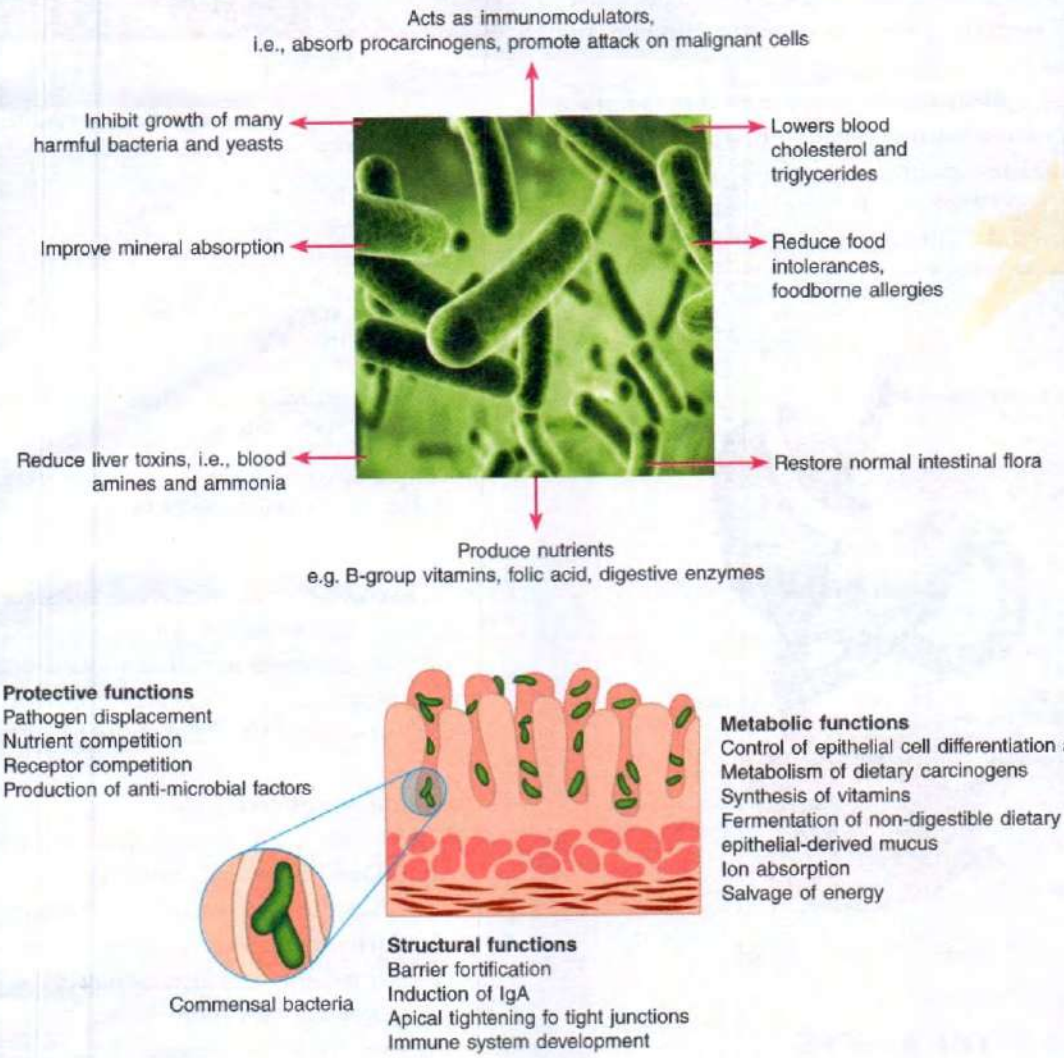


Fig. 31.4 Beneficial effects of clonic bacteria

The *beneficial effects of colonic bacterial flora* are (Fig. 31.4):

(i) Synthesis of some of the vitamins *e.g.* vitamin K, number of B-complex vitamins and folic acid.

(ii) Production of carbon-dioxide (CO_2), hydrogen sulphide (H_2S), hydrogen and methane which contribute to the ***flatus***. Most of the flatus passed through rectum, is however, nitrogen derived from swallowing of air. The smell is largely due to sulphides. The volume of gas normally found in the human GIT is approx. 200 mL, and the daily production is 500-1500 mL. In some individuals, gas in the intestine causes cramps, ***borborygmi*** (rumbling noises), and abdominal discomfort.

(iii) Organic acids formed from carbohydrates by bacteria are responsible for the slightly acidic reaction of the stools (pH 5 to 7).

(iv) A number of *amines* are formed in the colon by bacterial enzymes that decarboxylate amino-acids. These amines are:

(a) Histamine and tyramine, which are potentially toxic substances; and

(b) Indole and skatole, responsible for the *odour* of the faeces.

These substances are capable of causing intoxication if absorbed into the blood. Therefore, they are detoxified by the liver.

(v) *Pigments* formed from the bile pigments by the intestinal bacteria are responsible for the brown colour of the stools.

(vi) Play a role in cholesterol metabolism by decreasing plasma cholesterol and LDL levels.

Applied: BLIND LOOP SYNDROME

This condition develops in patients with surgically created blind loops of small intestine or when there is stasis of

the contents of the small intestine (Fig. 31.5). This causes overgrowth of bacteria within the intestinal lumen and is characterized by:

1. Pernicious anaemia (page 71); this occurs due to uptake of ingested cyanocobalamin (vitamin B_{12}) by the bacteria producing malabsorption of vitamin B_{12}.
2. *Steatorrhoea* (page 233); it is due to excessive hydrolysis of conjugated bile salts by the bacteria resulting in improper fat digestion.

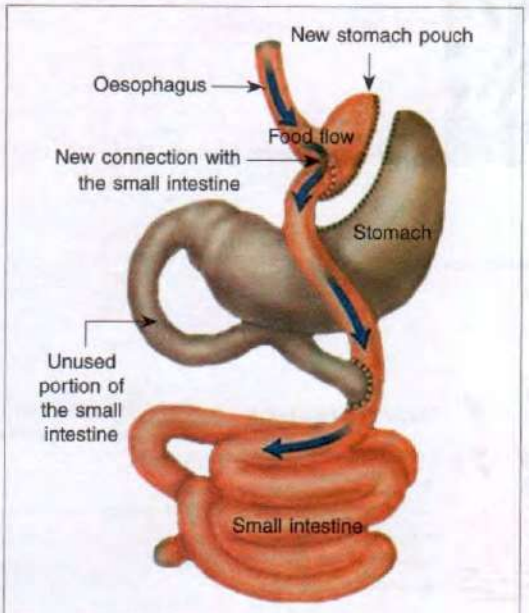


Fig. 31.5 Blind loop syndrome-technique

THE FAECES

1. The faeces are derived:
   (i) mainly from the intestinal secretions; and
   (ii) partly from the ingested food.

   Proof: Faeces in starving animals are decreased in bulk but differ comparatively little in composition from those of normally fed animals.
2. If vegetables and coarsely ground cereals are excluded from the diet, the faeces have fairly constant composition (Table 31.1).
3. The *brown colour* of stools is due to pigment formed from the bile pigments by the intestinal bacteria. When bile fails to enter the intestine, stools become white ***(acholic stools)***, as seen in obstructive jaundice.
4. On an average fat intake of 100 gm/day, 5-6 gm per day is normally lost in faeces; whereas on an average protein intake of 100 gm/day, 1.5 gm/day nitrogen is normally lost in faeces (this corresponds to 10 gm of proteins).
5. ***DIETARY FIBERS.*** In humans there is no appreciable digestion of *dietary fibers e.g.* cellulose, hemicellulose and lignin etc. due to absence of certain micro-organisms in GIT which break down these substances. Therefore, ingested cellulose passes out unchanged and substances which are enclosed in cellulose wall escape digestion and absorption. Various algal polysaccharides, and pectic substances also contribute to dietary fibers.

Table 31.1: Composition of faeces on an average diet

(i) Water : 75% of total faecal matter weight	
(ii) Solids : 25% of total faecal matter weight	
	percent of total solids
(a) Cellulose and other indigestible fibers	variable
(b) Bacteria	30%
(c) ***Ash*** *i.e.* inorganic material, mostly compounds of Ca^{2+}, PO_4^{3-}, iron, Mg^{2+}	15%
(d) Fat and fat derivatives (fatty acids, neutral fats, lecithin, cholic acids, etc.)	5%
(iii) Desquamated mucosal cells, mucus and small amounts of digestive enzymes (most of which are dead).	

Physiological significance

(i) The increased 'bulk' of this undigested residue stimulates intestinal peristalsis which, in turn, increases the passage of food through the intestine. Similarly, increased cellulose content of food (by coarser milling of flour), increases the bulk of faeces-*i.e.* faeces contain more water, solids and more of ingested food and nitrogen. Thus *high fiber diet* plays an important role in prevention and treatment of constipation.

(ii) It *reduces the efficacy of absorption* of digested foodstuffs by forming a mechanical barrier between the nutrients and absorptive surface. Thus high fiber diet helps reducing the sudden increase in blood glucose level after a meal (***postprandial hyperglycemia***). This also reduces the peak requirements of insulin during postprandial phase. This is why a high fiber diet is of great help in prevention and treatment of an individual suffering from diabetes mellitus.

(iii) It *reduces the blood cholesterol level* by binding the bile salts. How? Binding of bile salts (BS) by dietary fibers → ↑ excretion of BS in faeces → ↓ amount of BS available for entero-hepatic circulation → ↑ synthesis of fresh BS in the liver → ↑ cholesterol utilization → ↓ blood cholesterol level. Thus high fiber diet is useful for controlling all metabolic disorders associated with over-nutrition such as obesity, atherosclerosis, hyper-cholesterolaemia and diabetes mellitus.

(iv) Dietary fiber *decreases the incidence of cancer* of the colon by:
(a) dilution of carcinogens by the water held by the dietary fibers;
(b) reducing the duration of contact between carcinogen and mucous membrane of colon (as fibers increase the colon motility); and
(c) binding of carcinogen to dietary fiber.

Important Note

The individuals with low dietary fiber intake in meals have a greater than normal capacity to break down cellulose and related products, thus reducing the residue in their colons. It has been claimed that there are higher incidences of constipation, cancer of the colon, diverticulitis, diabetes mellitus and coronary artery disease in such individuals.

1. Taenia coli, 2. Haustrations ✓

Large intestine movements → Segmentation movem. (or) Haustrational contrac.
↓ Peristalsis → MMC

Study Questions

1. Give physiological basis of:
   (i) Mass action peristalsis
   (ii) Flatus and Borborygmi
   (iii) Acholic stools
   (iv) Defecation after meals in children
   (v) Characteristic odour and colour of faeces
   (vi) If a child passes stools only once in 3 weeks
2. Write briefly about:
   (i) Gastrocolic reflex
   (ii) Hirschsprungs disease
   (iii) Colonic bacteria
   (iv) Blind loop syndrome
   (v) Role of dietary fiber in food
   (vi) Defecation reflex
   (vii) Composition of faeces
   (viii) Movements in large intestine
   (ix) Anal sphincter
3. Describe the functional consequences of having bacteria in the GIT.
4. Draw labelled diagram to show transit time in small intestine and colon.

Reflexes:
A) Colonic colon reflex
B) Gastro colic reflex

Type IV → MASS peristalsis → Due to scratching of ant. thigh, strong peristaltic movem. → Emptying of rectum → Defaecation

MCQs

1. Mass action contractions are commonly seen in which part of GIT?
   (a) Oesophagus (b) Stomach (c) Small intestine (d) Colon
2. In infants, defecation often follows a meal. Most likely cause will be:
   (a) Increased circulatory levels of CCK (b) The gastro-ileal reflex
   (c) The gastro-colic reflex (d) The entero-gastric reflex
3. The first part of test meal reaches the caecum in about ..... hours, the hepatic flexure in ..... hours, the splenic flexure in ..... hours and sigmoid colon in ..... hours:
   (a) 4, 6, 9, 12 (b) 6, 9, 12, 16 (c) 2, 4, 6, 8 (d) 2, 6, 8, 12
4. The usual cause of aganglionic megacolon (Hirschsprung's diseases):
   (a) Infection in the colon
   (b) Excessive parasympathetic stimulation
   (c) Excessive sympathetic stimulation
   (d) Congenital absence of the Myenteric plexuse in distal colon
5. Defecation is severely impaired if:
   (a) Meissner's plexus is destroyed
   (b) Myenteric plexus is destroyed
   (c) Both the above plexuses need to be destroyed together
   (d) A person is dehydrated
6. A patient suffering from severe diarrhoea characteristically has all, *except*:
   (a) A decrease in the potassium content of the body (b) A decrease in the sodium content of the body
   (c) Decreased E.C.F.V. (d) An alkalosis rather than acidosis
7. Complete transection of spinal cord causes:
   (a) Retention of faeces
   (b) Defecation reflex persists
   (c) Voluntary defecation never possible
   (d) Reflex evacuation of rectum occurs when rectal pressure increases to 20 mmHg

TRANSIT TIME:
4 → Gastrum + Duo.
1 → Jejuno small inter
8 → ...
12 → ...
24-46 → Rectum.

8. The large intestine absorbs all *except*:
   (a) Water (b) Minerals (c) Glucose (d) Protein
9. Removal of the entire colon would be expected to cause:
   (a) Death (b) Electrolyte imbalance (c) Megaloblastic anaemia (d) Severe malnutrition
10. The term borborygmi refers to:
   (a) Gas in the intestine (b) Rumbling noises in intestine
   (c) Abdominal discomfort (d) Flatus
11. Smell in the flatus is largely due to:
   (a) Nitrogen (b) Carbon dioxide (c) Methane (d) Hydrogen sulphide
12. Faeces are mainly derived from:
   (a) Ingested food (b) Intestinal secretion
   (c) Dead colonic bacterial flora (d) Undigested food products
13. High fiber diet helps reducing in all of the following *except*:
   (a) Postprandial hyperglycemia (b) Blood cholesterol
   (c) Intrestinal motility (d) Cancer of colon incidences
14. In individual with low dietary fiber intake, capacity to break down cellulose:
   (a) Increases (b) Decreases
   (c) Either increases or decreases (d) No change from the normal
15. Which of the following contractions are *not* seen in the colon?
   (a) Segmental (b) Peristaltic (c) Mass action (d) Eccentric
16. Following a test meal, total recovery of the substance in stool requires:
   (a) 1 day (b) 3 days (c) 5 days (d) More than 7 days
17. The first urge to defecate occurs when rectal pressure increases to:
   (a) 10 mmHg (b) 20 mmHg (c) 40 mmHg (d) 80 mmHg
18. It is *not* possible to suppress defecation reflex voluntarily once rectal pressure increases beyond:
   (a) 25 mmHg (b) 35 mmHg (c) 45 mmHg (d) 55 mmHg
19. *True* about defecation reflex is:
   (a) Purely a voluntary reflex (b) An involuntary reflex
   (c) Partly a voluntary and partly an involuntary reflex (d) A spinal reflex
20. Man is unable to digest dietary:
   (a) Glycogen (b) Dextrin (c) Saccharose (d) Cellulose

Answers

1. (d)	2. (c)	3. (a)	4. (d)	5. (b)	6. (d)	7. (c)	8. (d)	9. (b)	10. (b)
11. (d)	12. (b)	13. (c)	14. (a)	15. (d)	16. (d)	17. (b)	18. (d)	19. (d)	20. (d)

Swallowing Reflex
or Deglutition.
→ oral or Buccal (volun.)
→ Pharyngeal phase (involunt)
→ Oesophageal phase
Afferent: V, IX, X
Centre: NTS, NA
Efferent: V, IX, X, XII
↳ causes primary peristalsis
↳ If food remains
secondary peristalsis
(Eg: In space, [invested])

Deglutition apnea
→ Due to overspilling of impulses from NTS → Respiratory centre
⇓
Temporary apnea
→ It can occur in any phase of Respiration.

Chapter 32

Digestion and Absorption in the GIT

I. Introduction

II. Digestion and Absorption of Carbohydrates, Fats and Proteins

III. Absorption of Water and Electrolytes (Na^+, K^+, Cl^-, HCO_3^-)

IV. Absorption of Vitamins and Minerals (calcium, iron)

V. Summary of Digestive Processes

INTRODUCTION

1. Digestion of the major foodstuffs is an orderly process involving the action of a large number of digestive enzymes. The action of the enzymes is helped by the HCl secreted by the stomach and the bile secreted by the liver.
2. Substances pass from the lumen of the GIT to the ECF and thence to the lymph and blood by various transport processes, similar to those that are responsible for transport of substances across the cell membrane (page 13).

DIGESTION AND ABSORPTION OF CARBOHYDRATES

CARBOHYDRATES OF FOOD

Daily intake of carbohydrates represent approx. 50-60% of the diet. The principal carbohydrates of food are:

1. Polysaccharides

(i) Starch (rich in potatoes)

(ii) Cellulose and pectin, these cannot be digested by the enzymes in the human GIT.

(iii) Glycogen, here glucose molecules are mostly in long chain (1:4 α linkage).

2. Disaccharides

(i) Sucrose (cane or beet sugar)

(ii) Lactose (milk sugar)

(iii) Maltose

3. Monosaccharides

(i) Hexoses *e.g.*

(a) glucose (dextrose)

(b) fructose (in fruits and vegetables)

(c) galactose, it is not ingested as such but is split off from lactose.

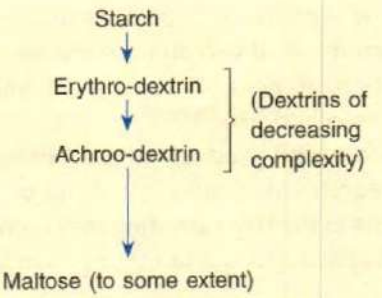
(ii) Pentoses, they do not occur in free form but found in

(a) nucleic acids; and

(b) in certain polysaccharides, *e.g.* pentosans of fruits and gums.

DIGESTION OF CARBOHYDRATES

1. ***Mouth*** – salivary α-amylase (ptyalin) aids digestion of starch to 1 : 4α linkages producing α-limiting dextrins and maltose (to some extent) (page 205).



Note

Both the salivary and the pancreatic α-amylase hydrolyse 1:4 α linkages.

2. ***Stomach*** – The 'HCl' of the gastric juice may hydrolyse some sucrose.
3. ***Duodenum*** – α-amylase of pancreatic juice rapidly converts all form of starch and dextrins completely into maltose. It acts in an alkaline medium and its digestive activity is increased by presence of bile salts.

Starch and Dextrin —(Pancreatic α-amylase; Cl^-; pH 7.1)→ *Maltose* (completely)

4. ***Small Intestine*** – Succus entericus contains disaccharidases *i.e.* enzymes for splitting disaccharides into monosaccharides. For example: sucrase (invertase), maltase, lactase and α-limiting dextrinase. Therefore,

Sucrose —(sucrase (invertase); pH 5-7; Cl^-; pH 7.1)→ *Glucose and fructose*

Maltose —(maltase; pH 5.8-6.2)→ *2 Glucose*

Lactose —(lactase; pH 5.4-6.0)→ *Glucose and Galactose*

α-limiting dextrins —(α-limiting dextrinase)→ *Glucose*

Important features

1. Digestion by these disaccharidases occurs in the luminal part (brush border of the epithelial cells) and their activity is maximum in jejunum and proximal ileum.
2. Hereditary defects of lactase or sucrase (invertase) produces malabsorption of lactose or sucrose respectively which results in:
   (i) ***fermentive diarrhoea*** due to increased number of osmotically active disaccharides;
   (ii) bloating (bulging) and flatulence due to production of CO_2 and H_2.
3. Low lactase levels are associated with intolerance to milk, called *Lactose Intolerance*. However, curd is better tolerated than milk in intolerant individuals because it contains its own bacterial lactase.
4. End products of digestion of nucleic acids and partial digestion of pentosans cause liberation of pentoses (monosaccharides).
5. Therefore, end products of carbohydrate digestion are monosaccharides, most important of which is glucose. Bacteria in the large intestine may convert some glucose into methane, CO_2 and other products.

ABSORPTION OF CARBOHYDRATES

1. Monosaccharides which are formed either in the gut lumen or in the brush border of the epithelial cells are absorbed *from the jejunum and upper ileum*; negligible absorption also occurs in stomach and colon. In general, carbohydrates are completely absorbed at 100 cm from the duodenum *i.e.* before the remains of a meal reach the terminal ileum.
2. ***Process of absorption.*** It includes monosaccharide movements across the cell into the blood capillaries by (i) *simple diffusion* and (ii) *active transport*. **(Fig. 32.1)** (They do not normally pass in the opposite direction because of one way permeability of the epithelium).

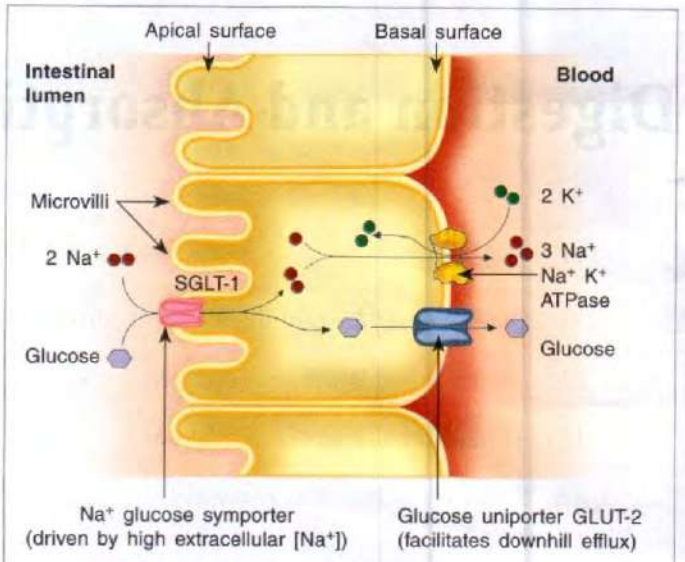


Fig. 32.1 Mechanism of glucose absorption across intestinal epithelium (SGLT-1: Sodium dependent glucose transporter-1; GLUT-2: Glucose transporter-2)

   (i) ***Simple diffusion*** occurs when concentration of sugar in the gut exceeds that in the blood.
   (ii) *Active transport* – this causes absorption of sugars against their concentration gradient into the mesenteric blood, which requires energy. Energy required for the process is supplied from cellular metabolism.

Mechanism of transport – carrier hypothesis

Glucose transport occurs in 2 steps, both are sodium dependent (an example of secondary active transport, page 19).

Step 1

Glucose combines with a 'carrier', a mobile component of cell membrane to form *glucose-carrier complex*, which moves the glucose across the lipid barrier of cell membrane and releases the glucose inside the cell. The glucose and Na^+ have the same carrier (cotransporter or symport) molecule called ***sodium-dependent glucose transporter-1 (SGLT-1)***. This step *requires no energy* (as it occurs by way of simple diffusion).

Step 2

To concentrate the glucose within the cell, the 'carrier' (*i.e. Na^+-glucose transporter, SGLT*) is coupled to a source of energy, which is provided indirectly by the active transport of Na^+ out of the cell into intercellular spaces. Na^+ affects the supply of energy from ATP by activating ATPase in the cell membrane. The glucose is transported by ***glucose transporter-2 (GLUT-2)*** into the interstitial space and thence to the blood capillaries.

Important Note

When 'Na⁺-glucose carrier' (cotransporter – SGLT-1) is absent or defective, glucose malabsorption occurs, which produces severe diarrhoea.

Active transport mechanism is dependent on:

(i) Presence of Na^+. *Proof*: 'Ouabain' which prevents active Na^+ reabsorption, also decreases glucose absorption.

(ii) Electrical activity of the cell. Proof: 'Phlorhizin', depresses the glucose stimulated transmural potential, thereby causing marked decrease in glucose absorption.

3. Maximum *rate of glucose absorption* from the intestine is about 120 gm/hour.

Glycosuria (glucose appears in urine, page 751) after ingestion of a high carbohydrate diet, called ***alimentary glycosuria***. This may occur in normal persons or those actually having mild diabetes mellitus.

4. Rate of absorption of monosaccharides is variable; it is fastest with glucose and galactose, intermediate with fructose and slowest with mannose or pentoses.

Rate of absorption:					
Galactose	*Glucose*	*Fructose*	*Mannose*	*Xylose*	*Arabinose*
1.1	1	0.4	0.2	0.15	0.1

Factors affecting rate of glucose absorption from the intestine:

(i) State of mucous membrane and length of time during which the carbohydrate is in contact with it. Therefore, in diarrhoea, excision of small intestine, gastro-colic fistula; because of intestinal hurry, absorption decreases. Similarly enteritis, coeliac disease, due to abnormal mucous membrane, decreases the absorption.

(ii) Role of endocrines

(a) *Thyroid* – Thyroxine acting directly on intestinal mucosa, increases the absorption, therefore, in myxoedema absorption decreases and thyrotoxicosis increases the absorption.

(b) *Anterior pituitary* – It affects glucose absorption by influencing the thyroid. Therefore, hyperpituitarism by causing over-activity of thyroid, increases the absorption; opposite is seen in hypopituitarism.

(c) *Adrenal cortex deficiency*, it decreases Na^+ concentration, thereby decreases glucose absorption.

(d) *Insulin*, it has **no** effect on absorption of glucose from the GIT.

5. Some disaccharides are absorbed as such into the epithelial cells, there to be hydrolyzed by maltase and sucrase present in the brush border of the villi.

6. ***Galactose absorption*** from the GIT occurs by the same mechanism which transports glucose; whereas *'fructose'* utilizes a different carrier; it is transported by 'facilitated diffusion'. Some fructose is converted to glucose in the mucosal cells. *'Pentoses'* are absorbed by 'simple diffusion'.

FATE OF GLUCOSE IN THE BODY

Of the total glucose ingested:

1. 5%, stored as glycogen in the liver and muscles.
2. 50-60%, catabolised in all tissues to produce energy (1 gm of glucose when completely oxidised to CO_2 and H_2O, produces approx. 4 kcal of energy) and transamination of some intermediary products of glucose break down, to form amino-acids.
3. 30-40%, converted to fat, and stored in fat depots.

DIGESTION AND ABSORPTION OF FATS

CLASSIFICATION OF FATS (LIPIDS)

1. ***Simple Fats or Neutral Fats.*** (major constituent in food of animal origin) These are triglycerides which are formed from one molecule of glycerol and 3 molecules of fatty acids. The common known fatty acids are:

(i) Palmitic acid, an 16C saturated acid;

(ii) Stearic acid, an 18C saturated acid, and

(iii) Oleic acid, an 18C unsaturated acid.

Waxes are esters of fatty acids with long chain monohydric alcohols.

2. ***Compound Fats*** – These are complex compounds formed from fatty acids, glycerol (or related substances), and various nitrogen containing bases, and often containing phosphate groups. They are integral parts of the general cell structure and are present in large amounts in nervous tissue; they are employed in fat transport. The main compound fats are:

(i) *Phospholipids (phosphatides)* – they contain glycerol, 2 molecules of fatty acids (generally unsaturated), phosphate and nitrogen base. Examples:

(a) *Lecithins* – nitrogen containing base is 'choline'.

(b) *Cephalin* – nitrogen containing base is 'cholamine' (ethanolamine).

(ii) *Sphingomyelins* – they contain fatty acids, phosphate, choline and a complex base (sphingosine), but no glycerol.

(iii) *Galactolipids* (cerebrosides) – they contain galactose, fatty acid and sphingosine, but no phosphate or glycerol.

3. ***Associated Fats*** – These are of two main types:
   (i) those components (the 'split' fat) that are obtained by the hydrolysis of lipids (*i.e.* glycerol, fatty acids, soaps); ***soaps*** are salts of fatty acids and are obtained by hydrolysis of fats in alkali (*saponification*);
   (ii) those components that are associated with the lipids in tissue extracts simply because they are dissolved by the fat solvents. Examples:
      (a) steroids *i.e.* hormones of ovary, testis, adrenal cortex, cholesterol;
      (b) fat soluble vitamins *i.e.* vitamins soluble in fat and in fat solvents.

Important Note

Fat of food consists mainly of neutral (simple) fats, together with small amounts of free fatty acids, lecithin and cholesterol esters.

DIGESTION OF FATS

Some hydrolysis of neutral fats take place during cooking.

1. Mouth

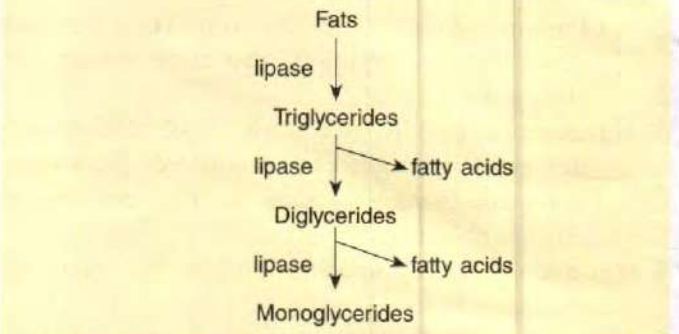
None; however, salivary lipase is active in the stomach and can digest as much as 30% of dietary triglycerides.

2. Stomach

(i) Gastric lipase is a weak fat splitting enzyme; it acts at an optimal pH 4–4.5 (gastric juice pH 1-2) and inactivated at pH 2.5.
(ii) Fat digestion (lipolysis) in stomach occurs only in exceptional circumstances. For example:
   (a) when pancreatic lipase may regurgitate into the stomach from the duodenum;
   (b) in achlorhydria;
   (c) in young suckling animals which ingest large quantities of milk; the fat of milk is present in an emulsified and digested form, and inhibits the secretion of gastric juice.

3. Small Intestine

(i) **Pancreatic lipase** and bile salts enter the 2nd part of duodenum at duodenal papilla and begin the fat digestion.
(ii) Pancreatic juice is markedly alkaline (pH 7.8 to 8.4) converts and adjusts the highly acidic chyme (pH approx. 6.0) to the chyme at pH slightly above 7.0, which is optimal pH for lipase action.
(iii) **Bile salts** play a role in activating lipase and exert a 'detergent' action *i.e.* by its *'hydrotropic' action* (page 246) lowers the surface tension, thus promotes ***'emulsification' of fats*** (emulsification or breaking of many fatty materials), thereby providing a greater surface area for the lipase to digest.
(iv) Lipase cannot act on fat droplets, but it acts at the interface between the fat particles and water (*i.e.* emulsified fats) and successively hydrolyzes the 1- and 3- bonds of the triglycerides (triacylglycerol) into diglycerides and monoglycerides with release of the associated fatty acids.



(v) Most of the dietary cholesterol is in the form of cholesterol esters; pancreatic *bile salt activated lipase* (page 230) and cholesterol esterase of succus entericus hydrolyzes these esters in the intestinal lumen to cholesterol.

ABSORPTION OF FATS

1. Pancreatic electrolytes, monoglycerides, fatty acids and bile salts interact spontaneously to form polymolecular aggregates, called *micelles*.

   'Micelles' features:
   (i) They are *water soluble* complexes, 3-10 nm in diameter with variable lipid concentration.
   (ii) They can *dissolve* hydrophobic compounds in their interior and such constituents as cholesterol are, therefore, made water soluble. Thus, micellar formations further solubilize the lipids and provide a mechanism for their transport into the intestinal cells.
2. Micelles themselves are *"passively" absorbed* along their concentration gradient into the luminal brush border, where the bile salts are detached to return to the intestinal lumen, finally to be *actively reabsorbed* more distally *in the ileum*.

Important Note

Steatorrhoea (page 233) **which is due to the lipase deficiency is of severe intensity and most commonly seen in patients with diseases that destroy the exocrine portion of the pancreas; because in addition, in the absence of bicarbonate (that is secreted from the pancreas), the relative acidic medium in the duodenum precipitates some bile salts.**
Thus hypersecretion of gastric acid can cause steatorrhoea. It can also be caused by defective absorption of bile salts in the distal ileum.

3. *Fat content* of micelles consists of monoglycerides and fatty acids. Once inside the intestinal wall, they are dealt with in 2 ways: **(Fig. 32.2)**
   (i) Monoglycerides and fatty acids with>14C atoms are re-esterified to triglycerides in the mucosal cell, which are then coated with a layer of β-lipoprotein, cholesterol and phospholipids (all synthesized by rough endoplasmic reticulum of cell), forming ***chylomicrons*** of approx. 1 μm diameter. 'Chylomicrons' contain 0.5% protein, therefore, called ***Esterified fatty acids***. Chylomicrons enter the lymphatics and via thoracic duct gain access into the blood stream.
   (ii) Short chain fatty acids with <12-14C atoms pass directly from the mucosal cell into the villus blood capillaries and are transported as ***free fatty acids*** (*FFA*), also called ***Non-esterified fatty acids*** (*NEFA*) or 'unesterified' fatty acids. They are bound to albumin in the blood stream.
4. Fat absorption is *greatest* in the upper part of the small intestine but appreciable amounts are also absorbed from the ileum.
5. Movements of villi, compress the lacteals and villus capillaries, this increase the mobilization of lipids towards the thoracic duct and portal vein respectively.
6. Cholesterol, like the short chain fatty acids, is absorbed directly into the lymphatics and reconverted (esterified) therefrom as cholesterol esters. Its absorption requires presence of bile, fatty acids and pancreatic juice. It is mainly absorbed from the distal small intestine. Non-absorbable plant 'sterols' such as those found in soyabeans reduce the absorption of cholesterol, by combining with cholesterol for esterification with fatty acids.
7. On a moderate fat intake, more than 95% of the ingested fat is absorbed. Only 5-6% is excreted in stools, much of this faecal fat is derived from cellular debris and microorganisms.
8. The process involved in fat absorption is not mature at birth, therefore, infants faecal fat content is 10-15% of the ingested fat.

Important Note

Fatty acids with 2 to 5C atoms **are formed in the colon by action of colonic bacteria on complex carbohydrates, resistant starches and components of dietary fibers. These fatty acids exerts a trophic effect on the colonic epithelium, reduces inflammation and helps to maintain acid-base balance by exchange of H^+.**

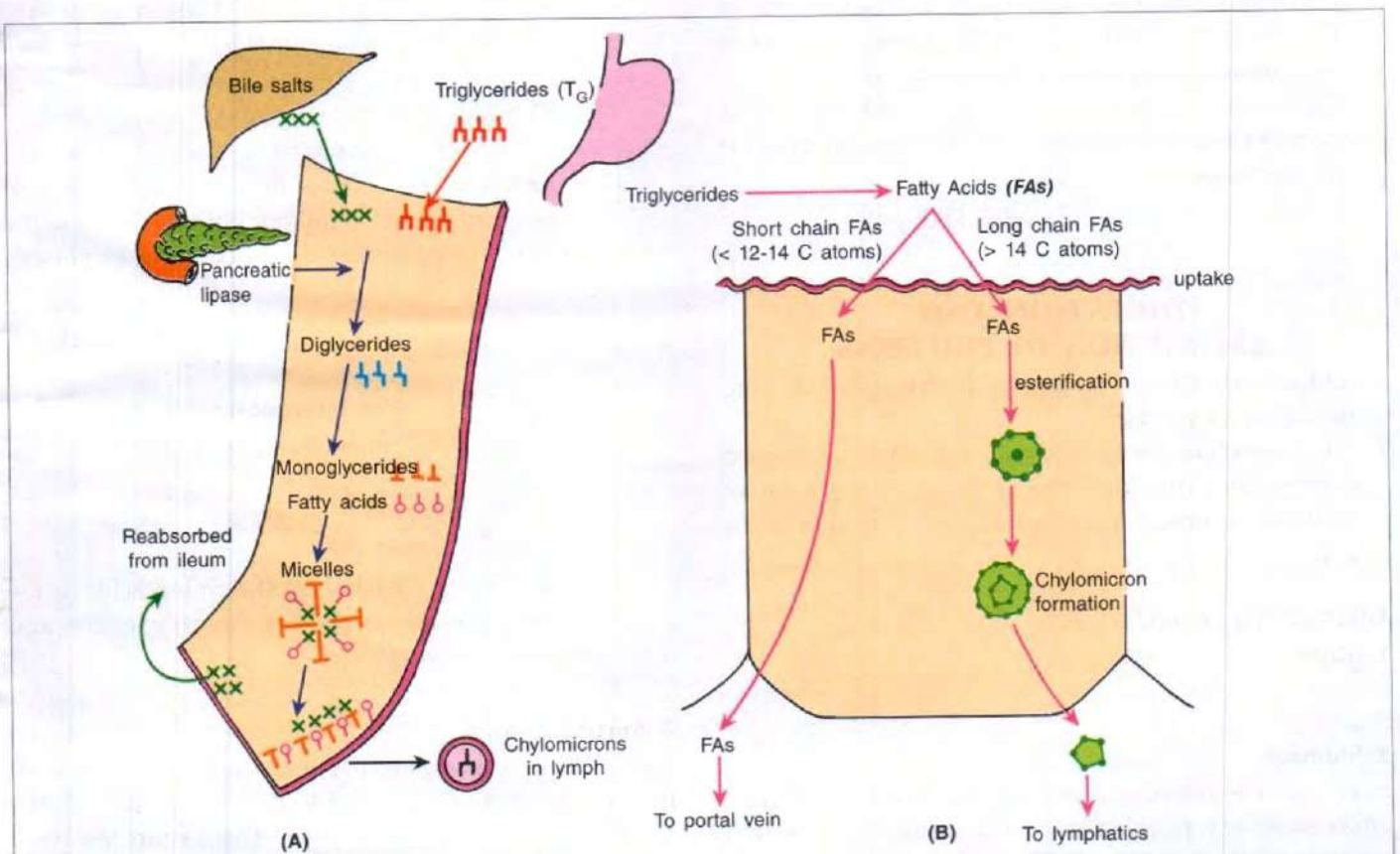


Fig. 32.2 Intraluminal events during fat digestion and absorption (A); absorption of fat by intestinal mucosal cells (B)

Blood Fat

Plasma lipids (triglycerides, free fatty acids, phospholipids and steroids) are bound to plasma proteins to form ***lipoprotein complexes*** which vary in density according to their lipid content (also see page 53).

As fat is lighter than water (because its density is less), therefore, lipoproteins complexes can be separated by centrifugation into:

1. *Very low density lipoprotein* (VLDL; density < 1.006).
2. *Low density lipoprotein* (LDL).
3. *High density lipoprotein* (HDL; density 1.060 – 1.200).

Normal Values

(i) Serum triglyceride concentration (bound to lipoprotein): 30-150 mg/dL

(ii) Serum cholesterol: 120-200 mg/dL (72% of which is esterified)

(iii) Serum phospholipids (lecithin and cephalins): 150-300 mg/dL, they are bound to α-lipoproteins and form HDL.

(iv) Serum FFA *i.e.* NEFA: 10-30 mg/dL.

Fat Stores

Fat is stored in adipose cells of fat depots as triglycerides. Body adipose tissue are of 2 types: white and brown.

1. ***White adipose tissue*** – it represents the biggest store of energy in the body and helps in maintenance of FFA (NEFA) concentration in the blood. Its oxygen consumption is approx. 8 mL/100 gm/min.
2. ***Brown adipose tissue*** – it is found only in infants and has a higher metabolic rate as compared to white adipose tissue.

(Details under Metabolism Unit, page 610).

DIGESTION AND ABSORPTION OF PROTEINS

1. Amino acids linked together in chain by peptide bond are called *polypeptides*.
2. All proteolytic enzymes *i.e.* enzymes concerned with protein digestion are secreted in the form of inactive precursors (proenzymes) and activated in the GIT.

DIGESTION OF PROTEINS

1. Mouth

None

2. Stomach

Here pepsins hydrolyze the bonds between aromatic amino-acids (*e.g.* phenylalanine or tyrosine) and a second amino-acid, therefore, the product of peptic digestion are polypeptides of variable sizes.

(i) *Protein*

↓ pepsin-HCl mixture; optimal activity at pH 1.6-3.2; inactivated at pH > 5.0

Proteoses and peptones
(large polypeptides)

Approx. 10% of ingested protein is broken down completely in the stomach.

(ii) *Caseinogen* (milk protein water soluble)

↓ Rennin (in other mammals) Pepsin (in humans)

Soluble casein

↓ Calcium salts

Coagulum of calcium casein
(it gets readily digested enzymatially)

3. Duodenum

(i) *Proteoses and peptones*

↓ Trypsin and chymo-trypsin in alkaline pH 8.0

Peptides of various chain
(i.e. small polypeptides)
and few free amino-acids

(These enzymes act at interior peptide bonds in the peptide molecules and are called ***endopeptidases***.)

(ii) *Polypeptides*

↓ 'carboxypeptidases' in pancreatic juice at pH 8.0

lower peptides and
free amino-acids

(These enzymes hydrolyze the amino acids at the carboxyl and amino ends of the polypeptide and are called ***exopeptidases***.)

4. Small Intestine

Here protein digestion *occurs at 3 places:* in the intestinal lumen; the wall (brush border) of intestine and the cytoplasm of the mucosal cells. This occurs as some 'peptides' are actively transported into the intestinal cells and hydrolyzed by intracellular 'peptidases'.

Peptides

↓ Succus entericus containing 'Erepsin' *i.e.* a set of mixed peptidases, each of which specifically hydrolyzes terminal peptide bonds; pH 7.6

Free Amino-acids

DIGESTION OF NUCLEO PROTEIN

The cellular foodstuffs which are rich in nuclei (liver, kidney, pancreas, yeast etc.) are likewise rich in nucleic acid and nucleo-proteins.

Nucleoprotein

↓ HCl in stomach

Removes protein portion which is digested together with other food proteins

Free Nucleic Acid (RNA and DNA)

↓ Ribonuclease and Deoxyribonuclease from pancreatic juice in duodenum, pH 8.0

Nucleotides and Nucleosides

↓ nucleases nucleotidases nucleosidases in small intestine, pH 7.6

Pentoses purine and pyrimidines

ABSORPTION OF AMINO ACIDS

1. D-amino acids are absorbed solely by 'passive' diffusion only, while L-amino acids are 'actively' transported. *L-amino-acids are absorbed more rapidly than D-isomers.*
2. Absorption of both D and L isomers is coupled to Na^+ transport like cotransport of glucose and Na^+ (page 260). The transported amino-acids accumulate in the mucosal cell and from these cells they enter the intestinal capillaries 'passively' by simple diffusion and facilitated diffusion; then via portal vein they reach the liver and general circulation. Therefore, after ingestion of a protein meal, there occurs a sharp transient rise in the free amino-acids content of the portal blood, which provides the whole body requirements of protein.
3. There are 3 *separate transport systems* for amino-acids:
   (i) A 'neutral' amino acid carrier system in the brush border that transports neutral amino-acids. It is *Na^+ dependent.*
   (ii) A 'basic' carrier amino-acid system also in the brush border that transports basic amino acids. It is *Na^+-independent.*
   (iii) A separate carrier system in the basolateral membrane of intestinal cells that transports proline, hydroxyproline and few other compounds.
4. Absorption of amino-acids *is rapid in the duodenum* and jejunum but slow in the ileum.
5. Of the total protein digested, approx. 50% comes from ingested food; 25% from proteins in digested juices and 25% from desquamated mucosal cells.
6. Only 2-5% of the proteins in the small intestine escapes digestion and absorption. This is so because:
   (i) diet which consists of proteins of animal origin, 95-99% of proteins is digested and absorbed; but
   (ii) diet containing proteins of plant origin, only 75-80% of proteins is digested and absorbed.
7. Some of the ingested proteins enter the colon and are finally digested by bacterial action.
8. The proteins in stool are not of dietary origin, but come from bacterial and cellular debris.
9. Protein absorption *decreases with age.* In infants, moderate amounts of undigested proteins are also absorbed.
10. The protein antibodies (IgA) in maternal colostrum that contribute to 'passive' immunity against infections enter the circulation from the intestine, although this transfer of antibodies is relatively small.
11. *Absorption of foreign proteins* from the intestine into circulation provokes the formation of antibodies and allergic symptoms may develop after eating certain foods, specially cow milk and egg.
12. *Absorption of protein antigens* (bacterial and viral proteins) takes place in the large ***Microfold (or M) cells*** *i.e.* specialized intestinal epithelial cells overlying Peyer's patches. M-cells pass the antigen to the lymphoid cells; activated lymphoblast enter the circulation and later return to the intestinal mucosa where they secrete IgA in response to subsequent exposure to the same antigen (page 126). This ***secretory immunity*** plays an important role in localized protection of the intestinal mucosa.

ABSORPTION OF WATER AND ELECTROLYTES

A. WATER ABSORPTION

Intestines are presented each day with approximately 2000 mL of ingested fluid and 7000 mL of secretions from the mucosa of GIT and associated glands; more than 90-95% of this fluid is reabsorbed with a daily fluid loss of only < 5% *i.e.* 200 mL in the stools. **(Table 32.1)** and **(Fig. 32.3)**

Table 32.1: The overall water balance in the GIT

Total Input (9000 mL/day)	Reabsorbed (8800 mL/day)
1. Ingested – 2000 mL	1. Jejunum > 60% : 5500 mL
2. Endogenous secretions – 7000 mL	2. Ileum 20-25% : 2000 mL
(i) Salivary glands : 1500 mL	3. Colon 10-15% : 1300 mL
(ii) Stomach : 2500 mL	
(iii) Bile : 750 mL	
(iv) Pancreas : 750 mL	
(v) Intestine : 1500 mL	
Balance in stools, < 5% : 200 mL/day.	

Important Note

Only small amounts of water move across the gastric mucosa, but water moves in both directions across the mucosa of small intestine and colon in response to osmotic gradient.

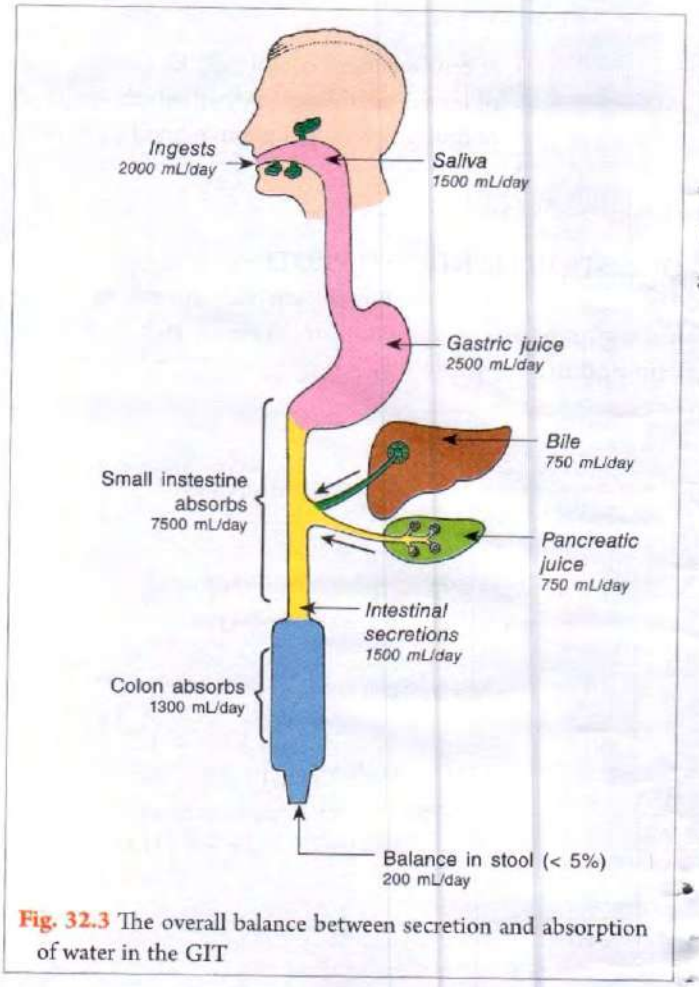


Fig. 32.3 The overall balance between secretion and absorption of water in the GIT

B. SODIUM ABSORPTION

1. Some Na^+ diffuses into or out of small intestine 'passively' depending on concentration gradient. In addition, Na^+ is actively transported out of the lumen into the small intestine and colon by $Na^+ - K^+$ pumps.
2. *In the ileum and jejunum* Na^+ transport from intestine to blood is facilitated by aldosterone.
3. In small intestine, active transport of Na^+ *helps in absorption of glucose,* amino-acids and other substances. Conversely, the presence of glucose in intestinal lumen, facilitates the reabsorption of Na^+. This is why for treatment of Na^+ and water loss in diarrhoea, oral administration of solutions containing NaCl and glucose or cereals containing carbohydrates are helpful. **(Fig. 32.4)**

C. POTASSIUM ABSORPTION

1. Movement of K^+ across the GIT mucosa is mainly due to passive diffusion down its electrochemical gradient. There is also some secretion of K^+ into the intestinal lumen, specially as a component of mucus.
2. Net movement of K^+ is directly proportional to the potential difference between blood and intestinal lumen; in jejunum this potential difference is approx.

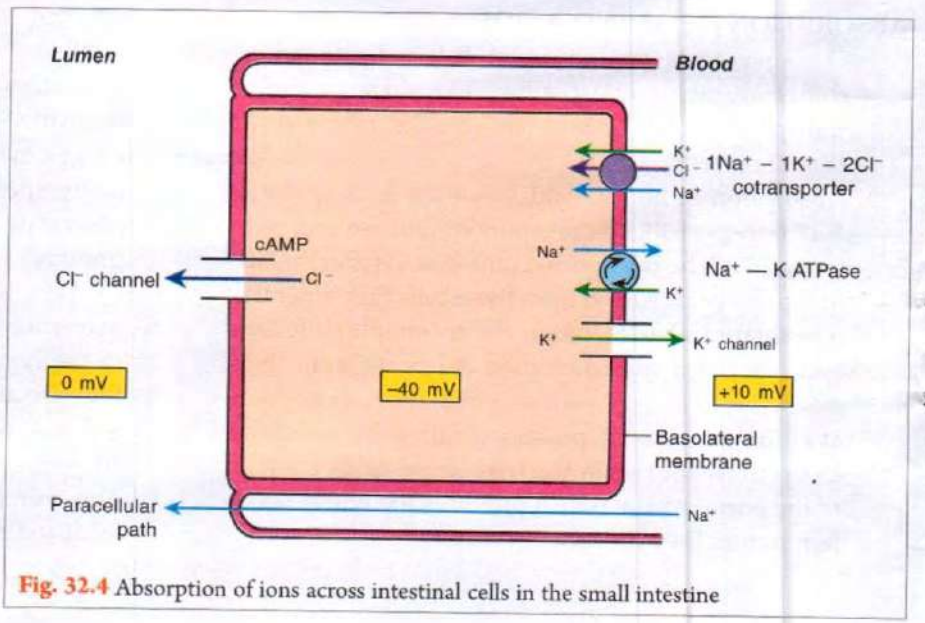


Fig. 32.4 Absorption of ions across intestinal cells in the small intestine

5 mV, in ileum is 25 mV and in colon 50 mV. That is why concentration of K^+ is approx. 6 mEq/L in jejunum; approx. 13 mEq/L in ileum and 30 mEq/L in colon. As a result, *ileal or colonic fluid loss in chronic diarrhoea results in severe hypokalemia.*

D. CHLORIDE AND BICARBONATE ABSORPTION

1. Cl^- is normally secreted into the lumen of the small intestine by Cl^- channels that are activated by cAMP. Intestinal cells also take up Na^+, K^+ and Cl^- by a 1 Na^+ – 1 K^+ – 2 Cl^- cotransporter in their basolateral membranes.
2. In the ileum and the colon, it appears that Cl^- is actively reabsorbed in a one-for-one exchange for HCO_3^-, thus make the intestinal content more alkaline.

ABSORPTION OF VITAMINS AND MINERALS

A. ABSORPTION OF VITAMINS

1. Water soluble vitamins are absorbed rapidly.
2. Fat soluble vitamin (A, D, E and K) absorption decreases if fat absorption decreases due to pancreatic enzyme or bile deficiency.
3. Most vitamins are absorbed in the upper small intestine but vitamin B_{12} binds to *intrinsic factor (I.F.)* secreted by the stomach mucous membrane and the *vitamin B_{12}–I.F. complex* is absorbed in the ileum.

Note

Vitamin B_{12} and folic acid absorption occurs independent of Na^+ absorption

B. CALCIUM ABSORPTION

1. 30-80% of ingested Ca^{2+} (normal intake 1 gm/day) is absorbed by 'active' transport primarily in upper small intestine and some of it is also absorbed by 'passive' diffusion.
2. Factors affecting active transport of Ca^{2+}.
   (i) **Facilitated by:**
      (a) 1,25 dihydroxycholecalciferol (DHCC), the metabolite of vitamin D, that is produced in the kidneys. 1,25 DHCC rate of production is inversely proportional to serum calcium levels.
      (b) Deficiency of Ca^{2+}
      (c) Lactase, and
      (d) Proteins
   (ii) **Inhibited by**
      (a) phosphate and oxalates. These anions form insoluble salts with Ca^{2+} in the intestine.
      (b) Excess of Ca^{2+}.

C. IRON ABSORPTION

1. In males, the amount of iron lost from the body is approx. 0.6–0.75 mg/day; whereas in females, loss is approx. 1.2–1.5 mg/day due to extra iron lost in blood shed during menstruation.
2. Average iron intake is approx. 20-25 mg/day, but the amount absorbed is equal only to the losses because if iron absorption exceeds its excretion, iron 'overload' will develop. Therefore, amount of iron absorbed is approx. 3-6% of the amount ingested.
3. Iron is readily absorbed in ferrous (Fe^{2+}) state but most of dietary iron is in ferric (Fe^{3+}) form.

$$Fe^{3+} \xrightarrow[\text{and ferric reductase in the brush border of enterocytes}]{\text{reduced by HCl and Ascorbic Acid}} Fe^{2+}$$

That is why partial gastrectomy and achlorhydria are often associated with iron deficiency anaemia.

4. Phytic acid (found in cereals), phosphate, oxalates, pancreatic juice react with iron to form insoluble compounds in the intestine and thereby decrease iron absorption.
5. Iron absorption is an 'active' process. Only traces of iron is absorbed from stomach, otherwise it is actively absorbed in upper small intestine (duodenum and jejunum).
6. Transport of Fe^{2+} into the enterocytes occurs via iron transporter **divalent metal transporter –1 (DMT – 1).** **(Fig. 32.5)**

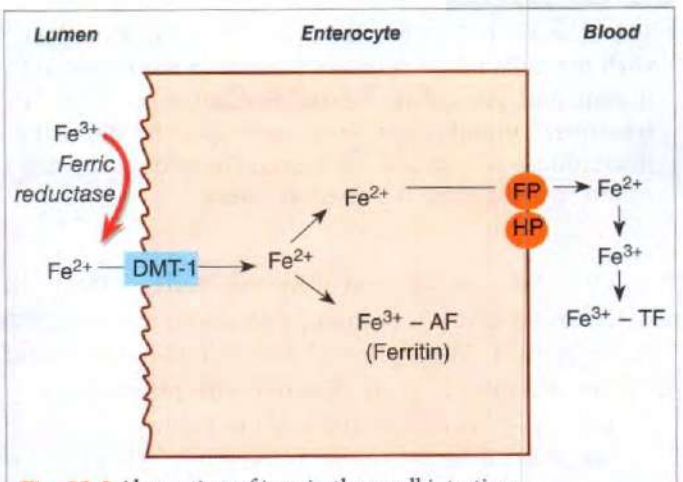


Fig. 32.5 Absorption of iron in the small intestine
(DMT : Divalent metal transporter; FP : Ferroportin–1; HP : Haphaestin; AF : Apoferritin; TF : Transferrin)

   (i) Some of the intracellular Fe^{2+} is converted to Fe^{3+} and iron is bound to an iron binding protein ***Apoferritin*** to form ***Ferritin***, which is the principal storage form of iron in the intestine and many tissues.

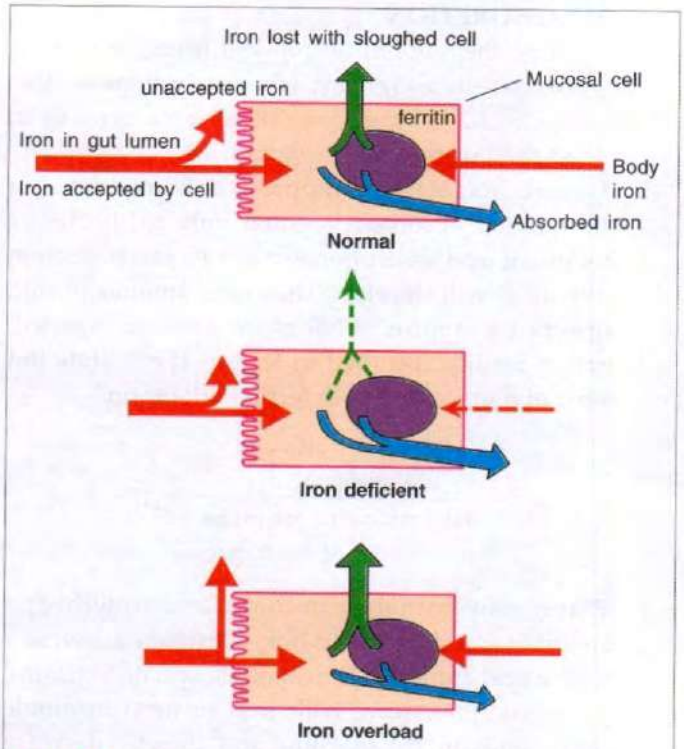


Fig. 32.6 Control of iron absorption by the intestinal mucosa (size type arrows)

(ii) The remainder is transported out of the enterocyte by a transporter ***Ferroportin–1*** with the help of a protein called *Hephaestin* **(Fig. 32.6)**.

Important Note

The iron bound to *ferritin* in intestinal cells is lost with the cells when they are shed into the intestinal lumen and passed in faeces. Ferritin molecules in lysosomal membranes may aggregate in deposits that contain as much as 50% iron. These deposits are called *Hemosiderin*; it also stores iron.

7. Of total iron (4–5 gms) in the body, 70% is in haemoglobin; 3% in myoglobin and remaining 27% in 'ferritin'. Ferritin is also found in the plasma and 'ferritin-iron' is in equilibrium with plasma iron.
8. In the plasma Fe^{2+} is converted to Fe^{3+} and bound to the protein. *Transferrin* or siderophilin, a β_1-globulin binds the iron in the lumen of GIT and most of the iron is transported bound to this globulin across the mucosal brush border. It is 35% saturated with iron. Mucosal cells pass part of the iron directly into blood stream but much of it is bound to *apoferritin*.
9. Normal plasma iron level is 60–160 µgm/dL
10. *Control of iron absorption by the intestinal mucosal cells* **(Fig. 32.6)**.

Iron absorption into blood stream is increased:

(i) when body iron stores are depleted; and

(ii) when erythropoiesis is increased.

Decreased iron absorption occurs under opposite conditions. How?

(a) *In iron deficiency:* the amount of circulating transferrin is increased, and its percent saturation with iron is decreased; therefore, more iron moves from the intracellular iron carrier to 'transferrin' and less binds to 'apoferritin'.

(b) *In iron overload,* opposite of (a) occurs. Therefore, factors which regulate iron uptake from the GIT are:

(i) Amount of iron in the mucosal cells; and

(ii) the ability of the mucosa to prevent excess ingested iron being absorbed (***mucosal block***).

12. **Applied** – When iron absorption exceeds its excretion, iron overload occurs. This results in *hemosiderin* (insoluble form of iron) accumulation in the tissues producing ***hemosiderosis***. Large amounts of hemosiderin damages the tissues producing ***hemochromatosis*** which is characterized by:

(i) skin pigmentation

(ii) pancreatic damage with diabetes *(bronze diabetes)*

(iii) cirrhosis of liver

(iv) hepatic carcinoma, and

(v) gonadal atrophy

Common causes of *hemochromatosis* are:

(i) Excessive iron uptake, and

(ii) Idiopathic or congenital. Here 'mucosal block' mechanism fails.

SUMMARY OF DIGESTIVE PROCESSES

Refer to Table 32.2.

Table 32.2: Summary of digestive processes

Source of Secretion and Stimulus for secretion	Enzyme (*Inactive form within bracket*)	Method of Activation and optimal condition for activity	Substrate	Action and End products
SALIVARY GLANDS - Secrete saliva at pH 7.0 resting state; (reaches 8.0 during active secretion) (i) in reflex response to presence of food in mouth (ii) Psychic or conditioned reflex in response to thought or sight of food	(1) Salivary α-amylase (Ptyalin), a starch splitting nzyme	Cl^-; pH 6.6–6.8 (average 6.5); inactivated at pH ≤4.0	Starch (polysaccharides or Complex sugar) Glycogen	Hydrolyses 1:4α linkages, producing α-limiting dextrins and maltose (to some extent)
	(2) Lingual Lipase	–	Triglycerides	Hydrolyses 1- and 3-bonds of the triglycerides forming fatty acids plus 1, 2-diacyl glycerols.
STOMACH – Secrete gastric juice at pH 1–2 (pepsinogen by chief cells and HCl by parietal cells) in response to: (i) reflex stimulus by Psychic stimulation via vagus (ii) chemical action of 'Gastrin'	(1) Gastric Lipase	pH 4–4.5 (inactivated at pH 2)	fats (butter, cream etc.)	hydrolysis of triglycerides (to some extent)
	(2) Pepsin (Pepsinogen)	HCl and pepsin itself; optimal pH 1.6–3.2; inactivated at pH > 5.0	(i) proteins; (ii) Caseinogen (milk protein)	proteoses and peptones (large polypeptides) coagulates milk (soluble casein)
	(3) Rennin (in infants)	Ca^{2+}; pH 4.0	Caseinogen (mil protein)	coagulates milk (soluble casein)
PANCREAS – presence of acidic 'Chyme' and products of digestion (fat, carbohydrates, protein) from the stomach activates duodenum to cause release of two hormones: (i) *Secretin* causes flow of alkaline, watery pancreatic juice rich in $HCO3^-$ (ii) *CCK–PZ:* causes flow of pancreatic juice rich in enzymes	(1) Trypsin (trypsinogen)	Enterokinase; trypsin; pH 8.0	protein; proteoses and peptones	Peptides of various chains *i.e.* small polypeptides and few free amino-acids
	(2) Chymotrypsin (chymotrypsinogen)	Trypsin; pH 8.0	protein, proteoses and peptones	-do-
	(3) Carboxypeptidases (procarboxy peptidases)	Trypsin; pH 8.0	polypeptides	Smaller peptides and free aminoacids
	(4) Ribonuclease	–	RNA	Nucleotides
	(5) Deoxy ribonuclease	–	DNA	Nucleosides
	(6) pancreatic α-amylase	Cl^- ; pH 7.1	Starch, Dextrin	Maltose (completely)
	(7) Pancreatic Lipase	Bile salts; pH 7.0	Triglycerides	Monoglycerides and fatty acids
	(8) Phospholipase (prophospholipase)	Trypsin; Ca^{2+}	Phospholipids	Fatty acids
LIVER AND GALLBLADDER – CCK-PZ; Secretin; gastrin stimulate gall bladder to cause secretion of bile from the liver and gall bladder	Bile contains no Enzymes (bile salts and alkali)	–	Fats, unemulsified fats *i.e.* large fat droplets; also neutralizes acidic chyme	'Fatty acids–bile salts' conjugates and finely emulsified 'neutral fats' – bile salts 'micelles' are formed.

Table 32.2: Summary of digestive processes

Source of Secretion and Stimulus for secretion	Enzyme (*Inactive form within bracket*)	Method of Activation and optimal condition for activity	Substrate	Action and End products
SMALL INTESTINE – Mechanical stimulus (Distension) of intestinal mucous membrane to cause secretion of intestinal juice (Succus entericus) from crypts of Lieberkuhn.	(1) Enteropeptidase (enterokinase)	–	Trypsinogen	Trypsin
	(2) Erepsin	–	Peptides	Free aminoacids
	(3) Nucleotidase and Nucleosidase	–	Nucleostides and Nucleotides respectively	Pentoses, purine and pyrimidines
	(4) Sucrase (invertase)	pH 5–7	Sucrose *i.e.* cane sugar	Glucose and fructose
	(5) Maltase	pH 5.8–6.2	Maltose *i.e.* malt sugar	2 Glucose
	(6) Lactase	pH 5.4–6.0	Lactose *i.e.* milk sugar	Glucose and Galactose
	(7) α-limiting Dextrinase	–	α-limiting dextrins	Glucose
	(8) Alkaline phosphatase	pH 8.6	Organic phosphates	Free phosphate
	(9) Intestinal Lipase	–	Triglycerides	Monoglycerides and fatty acid

Study Questions

1. **Write short notes on:**
   (i) Digestion of foodstuffs from the small intestine
   (ii) Micelles.
   (iii) Lipoprotein complexes
   (iv) Absorption of amino acids from small intestine
   (v) Absorption of hexoses from the intestine
   (vi) Absorption of fatty acids from small intestine
2. **Give physiological basis of:**
   (i) Lactose intolerance.
   (ii) Steatorrhoea of pancreatic lipase deficiency is of more severe intensity as compared to other causes.
   (iii) Faecal fat content in an infant is more than that of an adult.
   (iv) Loss of fluid from the colon in chronic diarrhoea results in severe hypokalemia.
   (v) Achlorhydria is associated with iron deficiency anaemia.
   (vi) Bloating and flatulence
   (vii) Fermentive diarrhoea
   (viii) Mucosal block
3. **Draw labelled diagram to show:**
   (i) Mechanism of glucose and fats absorption across intestinal epithelium.
   (ii) Absorption of iron in the small intestine
4. **Describe briefly how the GIT and its associated glands function to aid digestive and absorptive processes.**
5. **What is secondary active transport and why is it important in the GIT?**

MCQs

1. **Malabsorption of sucrose results in:**
   (a) Milk intolerance (b) Intolerance to curd (c) Fermentive diarrhoea (d) Steatorrhoea
2. **A Na^+-dependent active transport system is necessary for the intestinal absorption of:**
   (a) Bile salts (b) Glucose (c) Vitamin C (d) Fatty acid

3. **Maximum rate of glucose absorption from the GIT is approximately:**
   (a) 80 gm/hour (b) 100 gm/hour (c) 120 gm/hour (d) 140 gm/hour
4. **Rate of glucose absorption from the GIT is increased by all *except*:**
   (a) Insulin (b) Thyroxine (c) Glucocorticoids (d) Growth hormone
5. **Lipase *cannot* act on:**
   (a) Fat droplets (b) Emulsified fats (c) Trigycerides (d) Diglycerides
6. **Steatorrhoea is of severe intensity if it is due to:**
   (a) Liver insufficiency (b) Biliary obstruction
   (c) Destruction of exocrine portion of the pancreas (d) Malabsorption syndrome
7. **Chylomicron are composed of protein around a core of:**
   (a) Triglycerides only (b) Triglycerides, phospholipids and cholesterol
   (c) Phospholipids (d) Triglycerides and cholesterol
8. **On a moderate fat intake, a normal healthy individual is expected to excrete ..... % of fat per day in faeces:**
   (a) 1-2 (b) 3-4 (c) 5-6 (d) 7-8
9. **What percentage of ingested protein is broken down completely in the stomach?**
   (a) 10% (b) 30% (c) 50% (d) 100%
10. ***Not true* of protein digestion and absorption:**
    (a) Of the total protein digested, 50% come from digestive juices
    (b) Upto 2-5% of the proteins in small intestine escapes digestion and absorption
    (c) Some of percentage of proteins also gets digested in the colon by bacterial action
    (d) Proteins in stools mainly comes from bacterial and cellular debris
11. **Colonic fluid loss in chronic diarrhoea results in:**
    (a) Pernicious anaemia (b) Dehydration (c) Hypotension (d) Severe hypokalemia
12. **Iron absorption from GIT is decreased by all of the following *except*:**
    (a) Phytates (b) Vitamin C (c) Phosphate (d) Taurine
13. **Which of the following has the highest pH?**
    (a) Bile (b) Saliva (c) The intestinal juice (d) Pancreatic juice
14. **Range of optimal pH for activity of disaccharidases is approximately:**
    (a) 1-2 (b) 3-4 (c) 5-6 (d) 7-8
15. **Digestion of carbohydrates is maximum in:**
    (a) Stomach (b) Duodenum
    (c) Jejunum and proximal ileum (d) Distal ileum
16. **Alimentary glycosuria:**
    (a) Appears after ingestion of high carbohydrate diet (b) Occurs in normal persons
    (c) Occurs in individuals with mild diabetes mellitus (d) All of the above
17. **Optimal pH for pancreatic lipase action:**
    (a) 5.0 (b) 6.0 (c) 7.0 (d) 8.0
18. **Fat concentration in micelles is:**
    (a) 5 % (b) 10 % (c) 20 % (d) Variable
19. **Fat absorption is greatest in:**
    (a) Duodenum (b) Proximal portion of small intestine
    (c) Distal ileum (d) Equal in all parts of the small intestine
20. **Iron is stored in the body in the following *except*:**
    (a) Tissue macrophages (b) Spleen
    (c) Gall bladder (d) Bone marrow

Answers

1. (c)	2. (b)	3. (c)	4. (a)	5. (a)	6. (c)	7. (b)	8. (c)	9. (a)	10. (a)
11. (d)	12. (b)	13. (d)	14. (c)	15. (c)	16. (d)	17. (c)	18. (d)	19. (b)	20. (c)

Chapter

33

GIT Hormones

- Gastric Inhibiting Polypeptide
- Gastrin Releasing Peptide
- Ghrelin
- Glucagon-like Immunoreactivity
- Ghrelin

I. Introduction

II. Gastrin Family of Hormones

III. Secretin Family of Hormones

IV. Other GIT Hormones

INTRODUCTION

BASICS

GIT has biologically active polypeptides secreted in the mucosa, called ***GIT hormones***. They play important role in the regulation of GIT secretion and motility. In high doses their actions overlap but in physiological amounts, actions are discrete.

Based on physio-anatomical similarities GIT hormones are broadly classified into 3 types: *Gastrin* family, *Secretin* family and *other* GIT hormones.

GASTRIN FAMILY OF HORMONES

1. Gastrin (details page 216).
2. CCK-PZ (details page 232).

SECRETIN FAMILY OF HORMONES

1. Secretin
2. GIP: Gastric Inhibitory Polypeptide
3. VIP: Vasoactive Intestinal Peptide
4. Glucagon (page 742)
5. GLI: Glucagon like immuno reactivity (Glicentin)

SECRETIN

(Details page 231)

GIP: GASTRIC INHIBITORY POLYPEPTIDE

(OR GLUCOSE DEPENDENT INSULINOTROPIC POLYPEP.

(i) It is produced by cells in the mucosa of the duodenum and jejunum in response to presence of glucose and fat. It contains 42 amino-acids.

(ii) Actions

(a) in high doses, inhibits gastric juice secretion and its motility, that is why called GIP;

(b) stimulates β-cells of pancreas to increase insulin secretion, therefore, also called ***glucose-dependent insulinotropic polypeptide***.

VIP: VASOACTIVE INTESTINAL PEPTIDE (VIP)

(i) VIP contains 28 amino-acids. It is found in nerves in the GIT, blood, brain and autonomic nerves. It is released from jejunum in response to fatty meals.

(ii) Actions = GIP + Dilator + Secretor (2)

(a) It markedly increases intestinal secretions of electrolytes and water,

(b) relaxes intestinal smooth muscle including the sphincters,

(c) inhibits gastric acid secretion,

(d) increases action of A-ch on salivary glands, and

(e) dilates peripheral blood vessels.

GLUCAGON

(i) It is secreted by cells in the mucosa of the stomach and duodenum and by α-cells in the pancreatic islets.

(ii) It plays important role in hyperglycemia of diabetes.

GLI: GLUCAGON LIKE IMMUNOREACTIVITY (GLICENTIN)

It is secreted along with glucagon by α-cells in the pancreatic islets.

OTHER GIT HORMONES

1. MOTILIN = MMC + Smooth m.

(i) It is a polypeptide containing 22 amino-acids and is secreted by M_0 cells in the stomach, duodenal and colon mucosa; some of these cells are enterochromaffin cells (page 216).

(ii) Actions

(a) It causes contraction of intestinal smooth muscles, and

(b) regulates intestinal motility during inter-digestive phase and prepares the intestine for next meal.

(c) It is a major regulator of the migrating motor complexes (page 223).

2. NEUROTENSIN = Anti-motilin

(i) It is produced by mucosal cells of the ileum in response to fatty acids.

(ii) Actions

(a) It inhibits GIT motility, and

(b) increases ileal blood flow.

3. SUBSTANCE P = motilin

(i) It is found in the endocrine cells and nerve cells in the GIT.

(ii) Increases motility of the small intestine.

4. GRP: GASTRIN RELEASING PEPTIDE

(i) It is present in the vagal nerve endings that terminate on 'G' cells (page 216). (Non-Ach)

(ii) Acts as a neurofransmitter at vagal nerve endings to cause increase in gastrin secretion (page 217).

5. SOMATOSTATIN (Also refer to page 661)

(i) It is a *growth hormone inhibiting hormone* (*GHIH*) produced by the δ-cells in the pancreatic islets and in the GIT mucosa. It occurs in two forms: SS14 and SS28 - latter form is more active.

(ii) Actions

(a) It inhibits the secretion of gastrin, VIP, GIP, secretin and motilin,

(b) inhibits pancreatic exocrine secretion, as well as endocrine secretion of insulin; glucagon and pancreatic polypeptide.

(c) inhibits gastric acid secretion and motility, and eventually producing dyspepsia and slows gastric emptying.

(d) inhibits gall bladder contraction, and may precipitate gall stones.

(e) inhibits the absorption of glucose, amino-acids and triglycerides; and

(iii) Its secretion is increased by 'acid' in the lumen of stomach and stimuli that increase insulin secretion (page 750).

6. GHRELIN = Pro-Growth (H)

Ghre-(European root for the word growth)

(i) secreted by the stomach.

(ii) involved in control of food intake as its secretions increased by fasting and decreased after ingestion of food (for details refer to page 662).

(iii) Also stimulates growth hormones secretion by acting directly or receptors in the pituitary.

7. PEPTIDE YY = GIP

Also refer to pages 220 and 223

(i) Secreted by the jejunum following a diet rich in fats.

(ii) Inhibits gastric acid secretion and motility.

8. GUANYLIN → Binds to Guanylyl cyclase, → Regulates Cl^- secretion

Refer to page 201.

Anti-secretin family, Anti-Gastrum, Intestine, Gallb., Pancreas (10th) (RS2)

Summary: Major GIT Hormones

Hormones	Site of Secretion	Control of Secretion	Major actions
1. GASTRIN	G-cells of gastric antrum	**Stimulated by** i. Small peptides and amino acids (chem.) ii. Distention of stomach (mech.) iii. Vagus mediated via GRP (Nervous) **Inhibited by** i. H^+ in the stomach (Chem.) ii. Somatostatin (Hormonal)	i. Increases gastric H^+ secretion by gastric parietal cells. ii. Stimulates growth of gastric mucosa iii. Stimulates gastric motility iv. Stimulates insulin and glucogon secretion Insulinotropic & Glucagonotropic motility mucosa H^+, (H)
2. CHOLECYSTOKIN-PANCREOZYMIN (CCK-PZ)	I-Cells of duodenum and jejunum (Granular cells)	**Stimulated by** i. Small peptides and amino acids (Prot.) ii. Fatty acid (Fat) iii. Monoglycerides	i. Stimulates contraction of gall bladder and relaxes sphicter of Oddi → Bile secretion (Cholagogue) ii. Increases pancreatic enzymes and HCO_3^- ↓ secretion iii. Increases growth of exocrine pancreas (Tropic) iv. Inhibits gastric emptying
3. Secretin	S-Cells of duedenum (Enterochromaffin cells)	i. H^+ in duodenum (acid) ii. Fatty acids in duodenum (Fat)	i. Increases pancreatic HCO_3^- secretion ii. Increases biliary HCO_3^- secretion

Summary: Major GIT Hormones

Hormones	Site of Secretion	Control of Secretion	Major actions
			iii. Decreases gastric H^+ secretion by gastric parietal cells. iv. Delays gastric emptying. (Synergy with CCK)
4. Gastric inhibitory peptide (GIP)	Duodenum and jejunum	**Stimulated by** i. Fatty acids ii. Amino acids iii. Oral glucose (Nutrient)	i. Increases insulin secretion ii. Decrease gastric H^+ secretion and motility.
5. Somatostatin (Growth hormone inhibiting hormone) (GHIH)	Throughout the GIT	**Stimulated by** H^+ in the lumen **Inhibited by** Vagal stimulation	i. Inhibits the release of all GIT hormones and GH. ii. Inhibits both exocrine and endocrine pancreatic secretion iii. Inhibits gastric H^+ secretion and motility
6. Vasoactive intstinal peptide (VIP)	From neurons in the mucosa and smooth muscle of the GIT	**Stimulated by** Fatty meals (Cover from back)	i. Relaxes GIT smooth muscles including sphinters. ii. Increases action of A-ch on salivary glands iii. Stimulates pancreatic HCO_3^- secretion iv. Inhibits gastric H^+ secretion v. Dilates peripheral blood vessels

Study Questions

1. Describe briefs the aactions of GIP and VIP on the GIT.
2. Write short notes on:
   (i) somatostatin (ii) gastrin releasing peptide.
3. Name the gastrin and secretin family of GIT hormones. Describe any one of them in detail.
4. List the principal GIT hormones and describe the main physiological function of each of these hormones.

MCQs

1. Which is *not* an action of gastric inhibitory peptide?
   (a) Relaxes gastric sphincters (b) Inhibits gastric juice secretion
   (c) Increases insulin secretion (d) Also called glucose-dependent insulinotropic polypeptide
2. Which is *not* a function of vasoactive intestinal peptide?
   (a) Inhibits gastric acid secretion (b) Inhibits the motility of the intestine
   (c) Stimulates intestinal secretions (d) Constricts peripheral blood vessels
3. *Not true* of somatostatin:
   (a) It is a growth hormone inhibiting hormone (b) Inhibits gastric acid secretion
   (c) Stimulates GIT motility (d) Its secretion is increased by acid in the stomach
4. Which is *not* an action of gastric inhibitory peptide?
   (a) Relaxes gastric sphincters (b) Inhibits gastric juice secretion
   (c) Increases insulin secretion (d) Also called glucose-dependent insulinotropic polypeptide
5. Motilin:
   (a) Produced by the ileum (b) Inhibits GIT motility
   (c) Increases ileal blood flow (d) Regulates intestinal motility during inter-digestive phase

Answers

1. (a) 2. (d) 3. (c) 4. (a) 5. (d)

Unit V

THE CARDIO-VASCULAR SYSTEM (CVS)

Chapter 34: Physiological Anatomy of the Heart

Cardiac chambers : General; Valves in the heart; Heart sounds, Pacemaker tissue of the heart

Chapter 35: Properties of the Cardiac Muscle

Morphological; Electrical; Mechanical and Metabolic properties.

Chapter 36: The Cardiac Cycle

Definition; Events; 'JVP' record; 'ECG' changes; Heart Sounds

Chapter 37: The Electrocardiogram (E.C.G.)

Normal ECG; electrocardiograph; cardiac vector (cardiac axis)

Abnormal ECG: Heart block; Extra systoles; Arrhythmias; WPW syndrome; Myocardial infarction (MI); Effect due to changes in the ionic composition of blood

Chapter 38: General Principles of the Circulation

Introduction: functions, pressure changes in the vascular system, organisation and functions of the vascular system (vasculogenesis, angiogenesis)

Dynamics of Blood flow: Biophysical considerations-Flow-pressure-resistance relationships; Law of Laplace.

Chapter 39: Cardio-Vascular Regulatory Mechanisms

Local : Basal myogenic tone (BMT); Role of endothelial cells (nitric oxide, endothelins)

Systemic: (a) Chemical; (b) Neural: autonomic and medullary ('VMC'; 'CVC') – baroreceptors and chemoreceptors

Chapter 40: The Heart Rate (HR)

Factors affecting HR and its control

Chapter 41: The Cardiac Output

Definition; Distribution; Control (Heterometric and Homometric regulation), Methods of measurements

Chapter 42: The Arterial Blood Pressure

Definition; Factors affecting; Determinants of BP; Regulation of arterial BP.

Chapter 43: The Regional Circulation

Capillary, Coronary, Cerebral including cerebrospinal fluid (CSF); Cutaneous (skin); Muscle; Splanchnic (intestinal and hepatic) and Pulmonary circulation.

Chapter 44: Cardio-vascular Homeostasis in Health and Disease

Regulation of Blood Volume; Compensation for gravitational effects; Shock and Syncope; Heart failure; High BP (Hypertension)

Chapter 34

Physiological Anatomy of the Heart

I. Cardiac Chambers: General; Valves in the heart; Heart sounds

II. Pacemaker tissue of the heart

CARDIAC CHAMBERS

GENERAL

(1) The human heart weighs approximately 300 gm and contains 4 chambers: (Fig. 34.1)
   (i) Two thin walled *atria* separated from each other by an interatrial septum; and
   (ii) Two thick walled *ventricles* separated from each other by an interventricular septum.

(2) *Atria*
   (i) Serve a capacity function as well as that of contraction.
   (ii) Right atrium (RA) receives blood from the systemic circulation via superior and inferior vena cavae, while left atrium (LA) receives blood from the lungs via pulmonary veins.

(3) *Ventricles*
   (i) Serve as the pumps. They consist of 2 separate pumps:
      (a) Right ventricle (RV) supplies the lung circuit via pulmonary artery. Because of intrathoracic location of pulmonary blood vessels, pulmonary circuit offers less resistance to blood flow.
      (b) Left ventricle (LV) supplies the systemic circuit via aorta.
   (ii) As the systemic arteries offer greater resistance to blood flow, 'LV' has to do larger amount of work compared to 'RV'. Therefore, 'LV' wall becomes thicker than the 'RV'.
   (Thickness – LV wall : RV wall :: 3 : 1) *(physiological hypertrophy of LV)*.

Important Note

Although the 'RV' and 'LV' are connected in series, but the systemic organs receive blood through a parallel arrangement of vessels. Therefore, systemic organs have the same arterial composition of pO_2; pCO_2; pH; glucose etc.

(4) The cavities of the cardiac chamber are lined by endothelial lining, called ***endocardium***, whereas the muscles of the heart including pacemaking and conducting system structures are called ***myocardium***.

(5) The entire heart is enclosed by a double layered structure, called ***pericardium***. In between its two layers is a cavity called ***pericardial cavity***. It normally contains 5-30 mL of clear fluid, called ***pericardial fluid*** which lubricates the heart; permits it to contract with minimal friction and protects the heart from external injury.

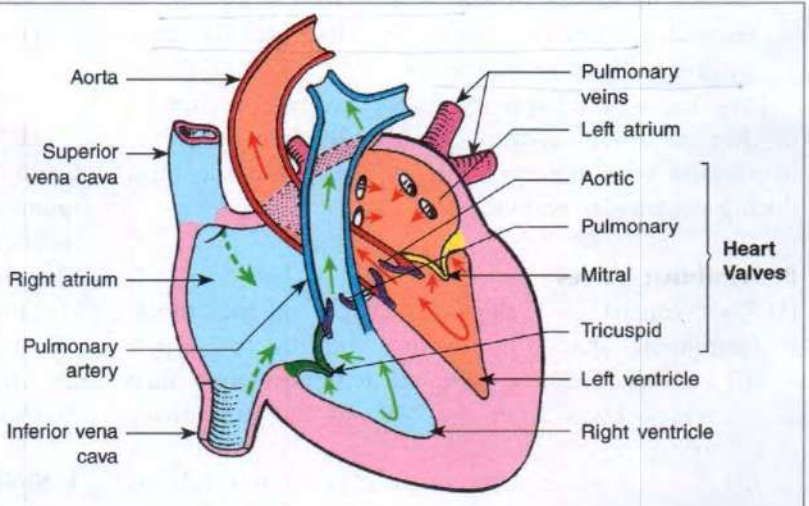


Fig. 34.1 Cardiac chambers (→ direction of blood flow)

VALVES IN THE HEART

A. Atrio-ventricular Valve (A-V Valve)

(1) Atria and ventricles are connected by a fibrous A-V ring; on the right side by the *Tricuspid* valve, and on the left by *Mitral* (Bicuspid) valve. These A-V valves:
   (i) prevent the backward flow of blood from the ventricles to the atria during ventricular systole; and
   (ii) close and open passively with the pressure gradient forces.

(2) The 'tricuspid' and 'mitral' valves consist of ***flaps (or cusps)*** which are attached at the periphery of the valve ring. ***Chordae Tendinae,*** the cord like structures originate from ***papillary muscles*** arising from the inner border of the ventricle, are attached to the free edges of the valve flaps. **(Fig. 34.2)**

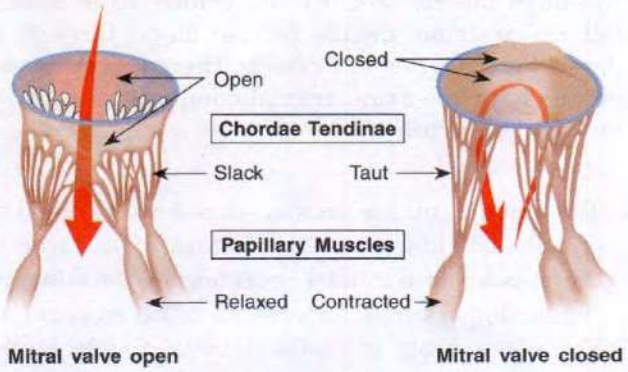


Fig. 34.2 Arrangement of papillary muscles and chordae tendinae to the cusps of the mitral valve

The papillary muscles contract when the ventricular walls contract, but they do not help the valves to close; instead prevent the bulging of valve into the atria during ventricular contraction.

{**Applied aspect:** Papillary muscle paralysis or rupture of chordae tendinae causes 'A-V valves' to bulge far backwards with regurgitation of blood into the atria during ventricular systole.}

B. Semilunar Valves

(1) They consist of 3 flaps (or cusps) of half moon (semilunar) shaped appearance, and are of 2 types:
   (i) *Pulmonary Valve:* situated at pulmonary orifice which leads from the 'RV' to the pulmonary artery.
   (ii) *Aortic Valve:* situated at aortic orifice which leads from the 'LV' to the aorta.

(2) These valves also open and close with passive gradient forces. Therefore, the valves open at the onset of ventricular ejection and close when the relevant arterial pressure exceeds that of the corresponding ventricle when it begins to relax.

(3) These valves are well adapted to withstand extra physical trauma of:
   (i) high pressure in arteries at the end of systole; and
   (ii) high velocity of blood ejection through them.

PV.

Note

Except the mitral valve, which is also called the bicuspid valve, all the remaining heart valves consists of 3 flaps, hence tricuspid. (Fig. 34.3)

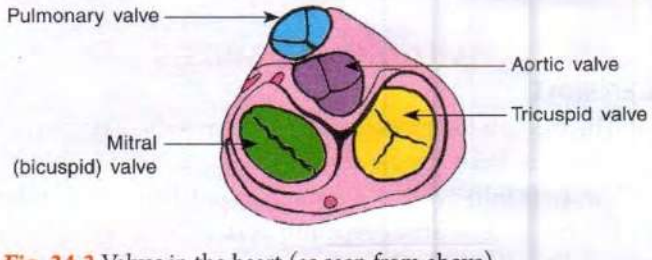


Fig. 34.3 Valves in the heart (as seen from above)

HEART SOUNDS

Opening of valves is a slowly developing process and does not produce any noise; while closure of valves is a sudden process causing surrounding fluid to vibrate producing noise. Thus,

(1) closure of *A-V valves* causes the ***First Heart Sound;*** and
(2) closure of *Semilunar valves* causes the ***Second Heart Sound.***

(For further details refer page 287)

PACEMAKER TISSUE OF THE HEART

Certain tissues in the heart, concerned with the initiation (generation of impulse) and propagation (conduction) of the heart beat, are called ***pacemaker tissues*** **(Fig. 34.4).** They include:

(1) Sinu or Sino Atrial Node (SAN),
(2) Atrio-Ventricular Node (AVN),
(3) Atrio-Ventricular Bundle or Bundle of His, and
(4) Purkinje fibers *i.e.* subdivision of Bundle of His.

1. SINU-ATRIAL NODE (SAN)

(i) *Location:* on the posterior aspect of heart at the junction of the superior vena cava (SVC) with right atrium (RA).

(ii) *Dimensions:* Length: 15 mm; Width: 2 mm and Thickness: 1 mm.

(iii) *Structure:* more embryonal in character *i.e.* cell outline ill defined; highly vascular; consists of thin, elongated muscle fibers (approx. 1/3rd the size of heart muscle fibers); rich in glycogen and mitochondria, fusiform in shape with longitudinal striations. These are called ***P-cells*** or ***pacemaker cells***. These fibers normally can generate and discharge impulses more rapidly than any other pacemaker tissue and their rate of discharge determines the rate at which the heart beats. That is why SAN is called the ***Cardiac Pacemaker***.

(iv) *Innervation:* It develops from structures on the right side of the embryo. That is why, in adults, SAN is innervated by right vagus nerve. It also receives sympathetic nerve fibers predominantely of right side from the cervical sympathetic ganglia via the cardiac nerves (page 324).

2. ATRIO VENTRICULAR NODE (AVN)

(i) *Location:* posteriorly on right side of the interatrial septum near the opening of coronary sinus.

(ii) *Structure:* same as that of 'SAN'.

(iii) *Innervation:* It is a left sided structure of the embryo. Therefore, in adults, it is innervated by left vagus nerve; also receives sympathetic nerve supply primarily from left side.

How the cardiac impulse generated at 'SAN' reaches the 'AVN'?

Modified atrial muscle fibers from the region of coronary sinus collect fanwise and unite with the 'AVN', called ***internodal atrial pathways***. They conduct impulses from 'SAN' to the 'AVN' by arranging themselves in 3 bundles:

(i) Anterior internodal tract of ***Bachmann***. It can also conduct impulses from 'SAN' directly to 'LA';

(ii) Middle internodal tract of ***Wenckebach***; and

(iii) Posterior internodal tract of ***Thorel***.

3. ATRIO-VENTRICULAR BUNDLE or THE BUNDLE OF HIS

(i) It takes origin from AVN and then runs upwards to the posterior margin of the membranous interventricular septum and then forwards below it, ensheathed and isolated in a canal. At the anterior part of the membranous interventricular septum the bundle divides into a right and a left branch.

(ii) The left branch pierces the membrane and then lies on the upper border of the muscular septum to divide into an ***anterior fascicle and a posterior fascicle***. The right branch passes down the right side of the septum.

(iii) Both branches divide repeatedly to form a network of fibers lying subendocardially in the ventricles.

4. PURKINJE FIBERS

(i) Takes origin from terminal divisions of right and left branch of the Bundle of His to penetrate the ventricular wall.

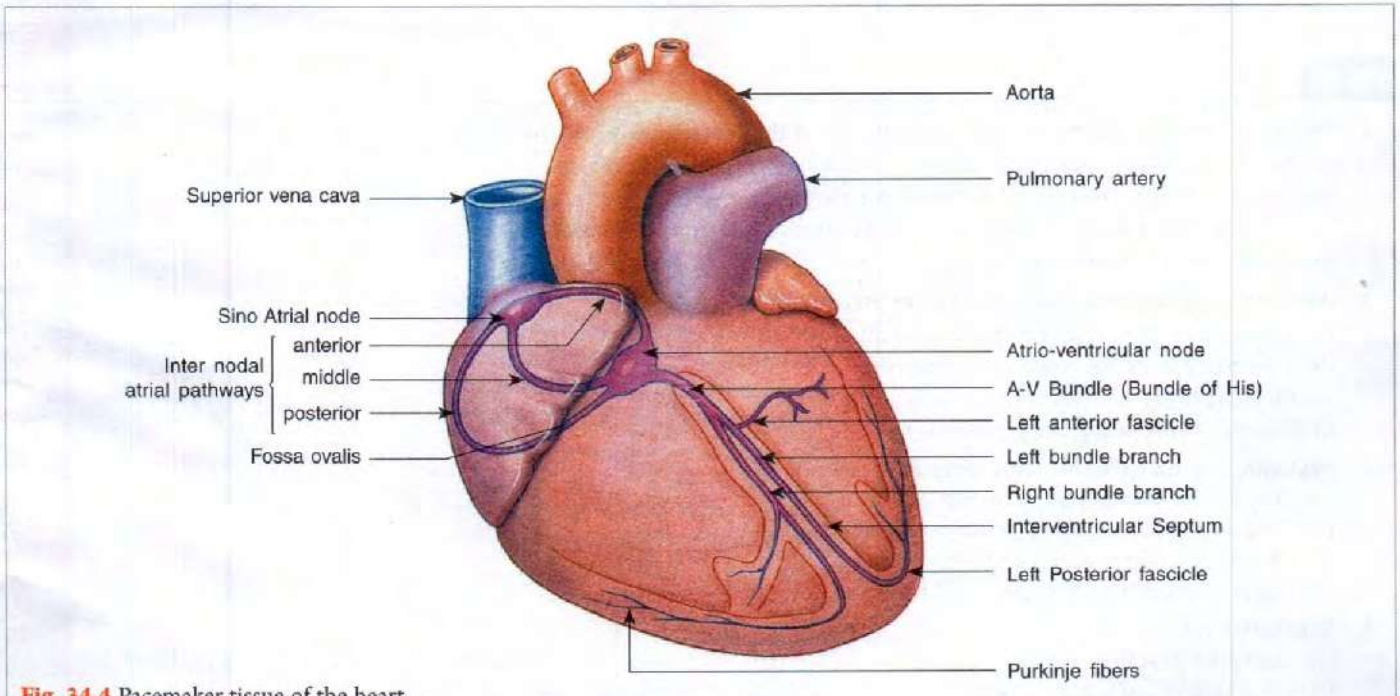


Fig. 34.4 Pacemaker tissue of the heart

(ii) These fibers are somewhat larger and thicker than the SAN fibers; length: 10-46 µm and diameter 70-80 µm.

(iii) Because of large diameter and very high level of permeability of the gap junction at intercalated discs, they transmit the impulse at a fast velocity of 4 mts/sec as compared to other conducting tissue. This allows almost immediate transmission of the cardiac impulse throughout the entire ventricular system. (Table 34.1)

Table 34.1: Conduction speed in cardiac tissue

Cardiac tissue	Conduction speed (mts/sec)
(i) Sinu atrial node	0.05
(ii) Atrial pathways	1.0
(iii) Atrio-ventricular node	0.05
(iv) Bundle of His	1.0
(v) Purkinje system	4.0
(vi) Ventricular muscles	1.0
(vii) Atrial muscles	1.0

Note

The slow conduction in SAN and AVN is caused by decreased numbers of gap junction between the successive cells in the conducting pathways.

Study Questions

1. **Write short notes on:**
   (i) Pacemaker tissue of heart (ii) A-V valves (iii) Semilunar valves
2. **Give physiological basis of:**
   (i) Physiological hypertrophy of the left ventricle (ii) All systemic organs have same arterial composition
   (iii) Opening and closing of valves in the heart (iv) Production of heart sounds
3. **What will happen if papillary muscle gets paralysed?**
4. **How is the cardiac impulse generated at SAN reaches AVN? What is apex beat?**
5. **Give the conduction speed in various parts of cardiac tissues.**
6. **Draw labelled diagram to show arrangement of mitral valve.**

MCQs

1. **Left ventricle (LV) has more mass compared to right ventricle (RV) because:**
   (a) Left coronary artery supplying the left ventricle has a greater flow
   (b) LV does more work to propel the blood compared to the RV
   (c) LV consists of 4 groups of fibers whereas RV consists of 2 groups of fibers only
   (d) By the nature of hereditary
2. **All statements are *true* about A-V valves *except*:**
   (a) These valves close and open passively with the pressure gradient forces
   (b) They prevent the backward flow of blood from ventricles to atria during systole
   (c) Opening of these valves is responsible for the first heart sound
   (d) Chordae tendinae are attached to the free edges of the valve flaps
3. **Systemic organs have the same arterial composition of pO_2, pCO_2, pH etc. because:**
   (a) They receive blood through parallel arrangement of blood vessels
   (b) They receive blood through series arrangement of blood vessels
   (c) Of phenomenon of autoregulation
   (d) Perfusion pressure is the same in all these organs
4. **Semilunar valves:**
   (a) Consists of two flaps/cusps (b) Are so called because of half moon shaped appearance
   (c) Are tricuspid and mitral valves (d) Close and open actively

5. **Fibres of internodal atrial pathways are:**
   (a) Modified atrial muscle fibres (b) Modified nerve fibres
   (c) Highly contractile (d) Conduct impulses rapidly
6. **In a normal healthy adult individual left ventricle to right ventricle wall thickness is:**
   (a) 1 : 3 (b) 3 : 1 (c) 2 : 3 (d) 3 : 2
7. **Which is a *false* statement regarding the pericardial fluid?**
   (a) It is normally 150-300 mL of clear fluid contained in the pericardial cavity
   (b) It lubricates the heart and provides nutrition to the myocardium
   (c) It permits the heart to contract with minimal friction
   (d) It protects the heart from external injury
8. **Rupture of chordae tendinae:**
   (a) Help the A-V valve to close
   (b) Prevent the bulging of A-V valve into the atria during ventricular contraction
   (c) Causes regurgitation of blood into the atria during ventricular systole
   (d) Has no effect on haemodynamics of circulation
9. **Sino atrial node (SAN) is called cardiac pacemaker because:**
   (a) It allows almost immediate transmission of cardiac impulse throughout the entire ventricular system
   (b) It is capable of generation of impulse and conduction of the heart beat
   (c) It determines the rate at which the heart beats
   (d) The speed of conduction of impulse is fastest in SAN
10. **The component of cardiac tissue having the highest propagation velocity is:**
    (a) Purkinje fibers (b) AV node (c) Atrial muscle (d) Ventricular muscle

Answers

1. (b) 2. (c) 3. (a) 4. (b) 5. (a) 6. (b) 7. (a) 8. (c) 9. (c) 10. (a)

Chapter
35

Properties of the Cardiac Muscle

I. Morphological Properties

II. Electrical Properties

III. Mechanical Properties; and

IV. Metabolic Properties

{Refer to Unit III, page 175-183}.

Chapter 36

The Cardiac Cycle

I. Definition
II. Events in the Cardiac cycle
(A) Atrial Systole (B) Ventricular Systole
(C) Ventricular Diastole (D) Atrial diastole
III. Atrial pressure changes during cardiac cycle - JVP Record
IV. ECG changes during cardiac cycle
V. Heart Sounds

DEFINITION

The sequence of changes in the pressure and flow in the heart chambers and blood vessels in between the two subsequent cardiac contractions is known as ***Cardiac Cycle***. Normal duration: 0.8 sec at heart rate of 75 per min (60/0.8).

Ventricular systole: 0.3 sec; *ventricular diastole:* 0.5 sec; *atrial systole:* 0.1 sec and *atrial diastole:* 0.7 sec. (Fig. 36.1)

EVENTS IN THE CARDIAC CYCLE

At the beginning of cardiac cycle, ***characteristic features*** during diastole are:

1. All 4 cardiac chambers *i.e.* both atria and ventricles are relaxed and filled with blood due to venous return.

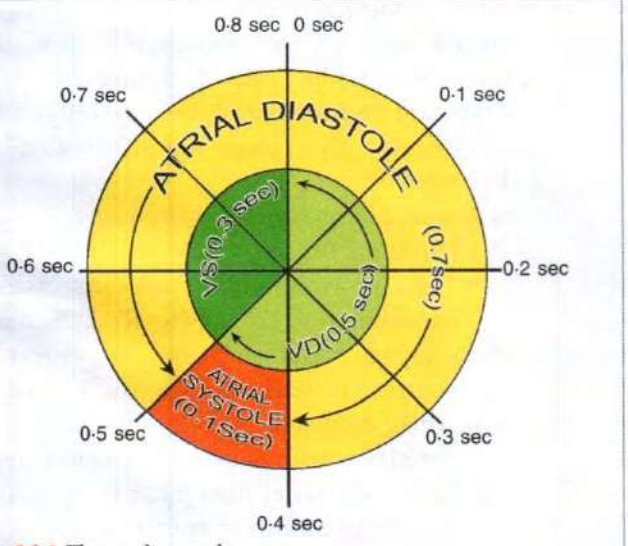


Fig. 36.1 The cardiac cycle
(Normal duration: 0.8 sec at heart rate of 75 per min)
VS and VD: Ventricular systole and diastole respectively.

2. As 'A-V valves' are open, therefore, atrium and ventricle of each side are in continuity and the pressure in each cavity is almost identical.
The parts of the heart normally beat in an orderly sequence: *atrial systole, atrial diastole, ventricular systole,* and *ventricular diastole* (Fig. 36.1).

Note

There are two separate functional syncytium in the heart (the artrial and ventricular) and their sequence of events overlap during the cardiac cycle.

A. ATRIAL SYSTOLE: ATRIAL CONTRACTION PHASE

Duration 0.1 sec. It is seen following the impulse generation in the *SAN.*

Characteristic Features

1. Atrial muscle contracts and atrial pressure (P_A) rises with ventricular pressure (P_V) following it. Right atrial pressure rises 4 to 6 mmHg, whereas left atrial pressure rises approx. 7 to 8 mmHg. (Fig. 36.2)
2. It propels approx. 30% additional blood into the ventricles.
3. It narrows the orifices of inferior and superior vena cavae (IVC and SVC) and pulmonary orifice but some regurgitation of blood occurs into the great veins as no valves are present between the atria and the great veins.
4. Narrowing of orifices of 'IVC', 'SVC' and pulmonary veins decreases venous return to the heart.

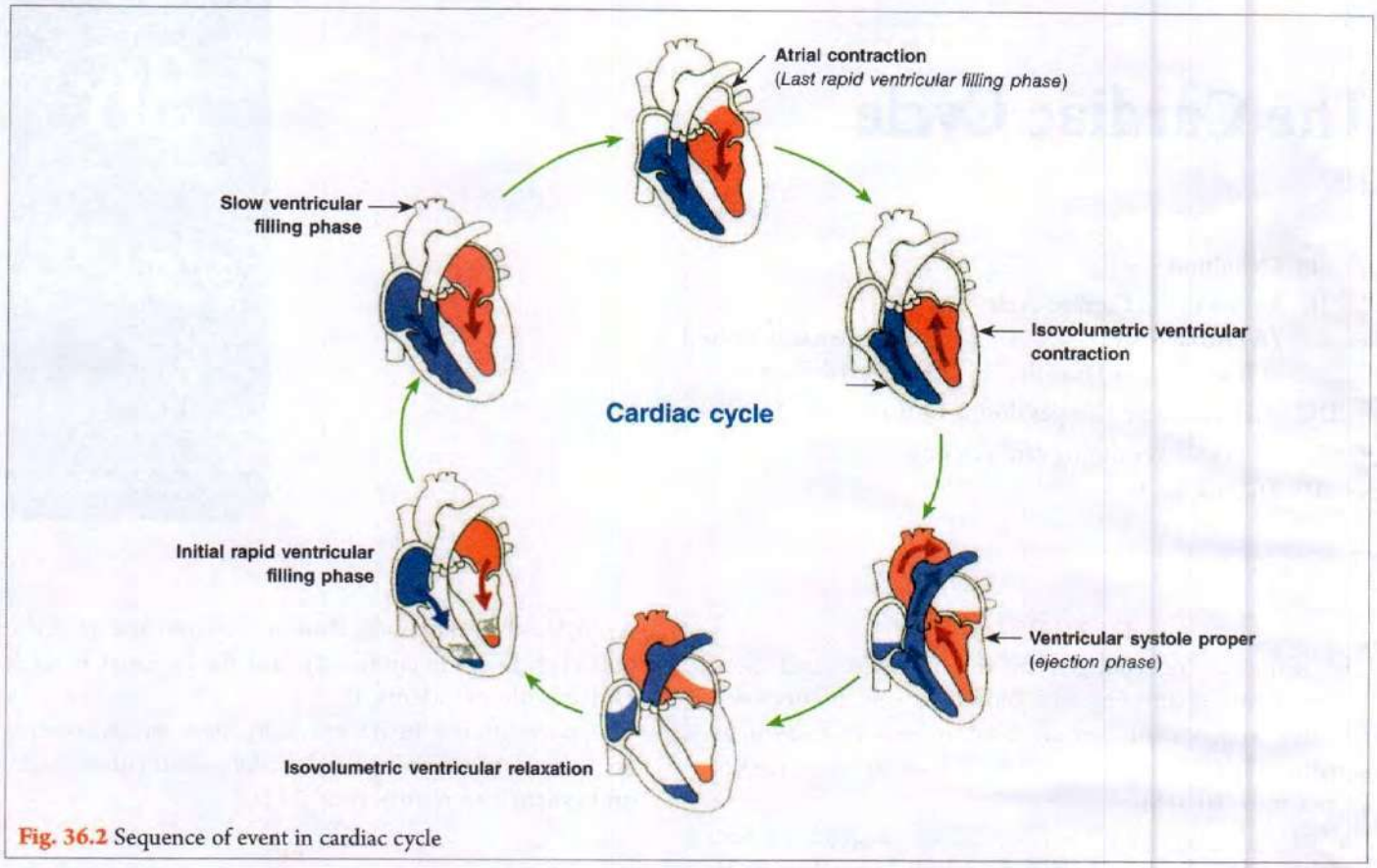


Fig. 36.2 Sequence of event in cardiac cycle

B. VENTRICULAR SYSTOLE

Total duration: 0.3 sec; it has two major phases:

1. *isovolumetric ventricular contraction:* 0.05 sec.
2. *ventricular systole proper:* 0.25 sec.

1. *Isovolumetric (Isometric) Ventricular Contraction:*
Duration 0.05 sec.

Characteristic Features

(i) As the atrial contraction phase passes off, the pressure in both atria and ventricles falls and in the meantime the ventricles get invaded by the excitation process. Ventricular contraction begins and ventricular pressure exceeds atrial pressure very rapidly causing closure of the AV valves with production of *First Heart Sound* (HS_1). **(Fig. 36.3)**

(ii) The ventricles are now a closed chamber, and the pressure rises promptly during ***isometric*** phase as the myocardium presses on the blood in the ventricles.

(iii) Onset of ventricular systole causes bulging of 'A-V valve' into the atrium; this results in small but sharp rise in atrial pressure.

(iv) During this phase, although contraction is occurring in the ventricles but there is no emptying; therefore, this phase is called *isovolumetric (isometric) ventricular contraction.*

2. *Ventricular Systole Proper:* *Duration* 0.25 sec. It is associated with ejection of blood out of ventricles.

Characteristic Features

(i) When the pressure in the 'LV' exceeds the pressure in the aorta (80 mmHg); and the pressure in the 'RV' exceeds the pressure in the pulmonary artery (10 to 12 mmHg), opening of 'semilunar' (aortic and pulmonary) valves occurs. **(Fig. 36.3)**

(ii) With the opening of semilunar valves there follows the 'ejection phase'; during this phase, arterial and ventricular pressures follow each other closely. This phase is subdivided into 3 divisions:

(a) ***Initial Phase:*** *Rapid Ejection Phase*

- Duration: 0.1 sec.
- The intraventricular pressure rises to maximum (LV: 120 mmHg; RV < 25 mmHg) causing rapid increase in output of ventricular volume. Approx. 2/3 of the ***stroke volume*** is ejected in this phase.
- Arterial pressure also rises, as blood enters the vessels faster than it can escape via the peripheral arteriolar branches.
- Opening of semilunar valves causes 'A-V valves' to come back to their original position, causing sharp fall in atrial pressure.

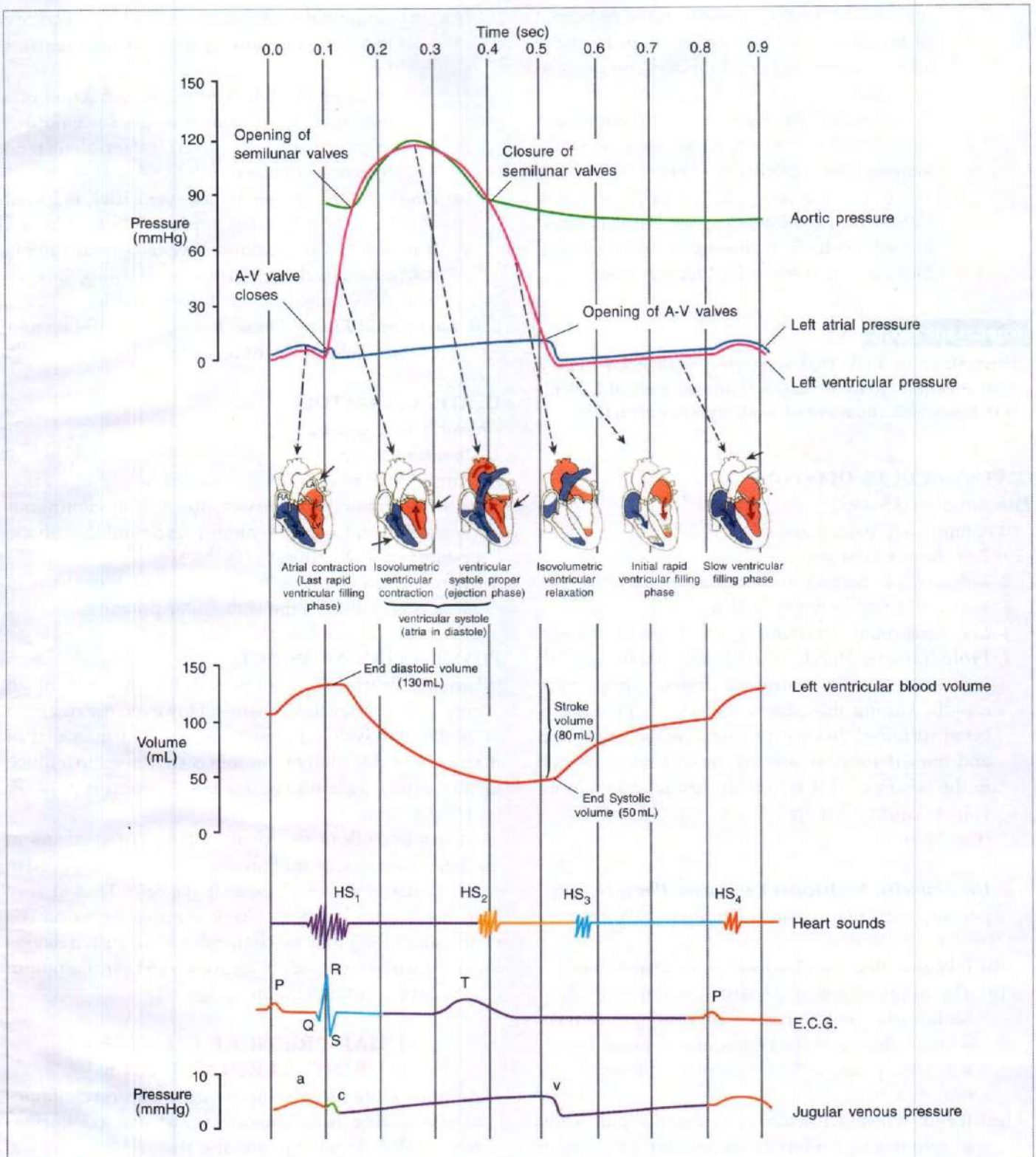


Fig. 36.3 Corelation of events in the cardiac cycle (Abbreviations as given in the text)

(b) The ***Summit*** (*peak*) of the ventricular pressure curve is reached when aortic or pulmonary artery pressure actually exceeds the ventricular pressure; but for a short period momentum keeps the blood moving forward.

(c) ***Final Phase: Slow Ejection Phase***

- Duration: 0.15 sec.
- The ventricular pressure declines as the ventricular contraction begins to subside with slow ejection of blood from the ventricles

into the arteries (*i.e.* slow increase in output of ventricular volume); while flow from the artery to its peripheral branches continues to be high.

The amount of blood ejected by each ventricle per stroke at rest is 70-80 mL. This is called as ***Stroke Volume***. This is approx. 65% of the *End diastolic ventricular blood volume (EDV)* (normal: 120-140 mL) and leaves approx. 50 mL of blood in each ventricle at the end of systole, called ***End-systolic ventricular blood volume***.

Important Note

Percentage of EDV that is ejected with each heart beat is called *Ejection Fraction* (Normal: 65% of EDV). It is a valuable measure of ventricular contractility.

C. VENTRICULAR DIASTOLE

Total duration: 0.5 sec.

It comprises 4 major phases:

1. *Protodiastole:* 0.04 sec.
2. *Isovolumetric ventricular relaxation phase:* 0.08 sec.
3. *Ventricular diastole proper*: 0.28 sec.
4. *Last rapid filling phase due to atrial systole:* 0.1 sec.

1. Protodiastole: Duration: 0.04 sec.: At the end of ventricular systole, ventricular pressure drops more rapidly. During this phase, the arterial pressure is better sustained due to elastic recoil of the vessel wall and immediately the arterial pressure exceeds that in the ventricle. This results in closure of semilunar valves, causing sharp *second heart sound (HS_2)*. **(Fig. 36.3)**

2. Isovolumetric Ventricular Relaxation Phase *i.e.* initial part of ventricular diastole. Duration: 0.08 sec.

Characteristic Features

(i) It begins after the closure of semilunar valves.

(ii) The intraventricular pressure continues to drop rapidly, the ventricular muscle continues to relax without change in the ventricular volume. That is why, this phase is called *isovolumetric ventricular relaxation phase*.

(iii) It ends when ventricular pressure falls (practically to zero mmHg) below atrial pressure resulting in opening of 'A-V valves'.

3. Ventricular Diastole Proper: Approx. 70% of the ventricular filling occurs passively during this phase. It has 2 divisions:

(i) ***Initially, rapid filling*** of the ventricles, characterized by:

(a) the opening of 'A-V valves' and it occurs for 0.1-0.12 sec.;

(b) continued relaxation of the ventricles, therefore, pressure in the ventricles remains low;

(c) atrial pressure falls to within a fraction of a mmHg of ventricular pressure since normal opening of 'A-V valves' offers almost no resistance to blood flow.

(ii) ***Finally, slow filling*** of the ventricles is called *Diastasis*. It occurs for approx. 0.18 to 0.20 sec. It is due to the continuous venous return filling both atrium and ventricle and readjusting the end-diastolic volume (EDV) of the ventricle.

4. Last Rapid Filling Phase: It is due to atrial systole. This terminates the cardiac cycle.

D. ATRIAL DIASTOLE

Characteristic features

1. *Duration* 0.7 sec.
2. During this phase, atrial muscles relax and atrial pressure gradually increases due to the continuous venous return to drop to almost zero mmHg with the opening of 'A-V valves'. **(Fig. 36.3)**
3. Then the pressure rises again during the phase of *diastasis* and follows the ventricular pressure.

PHYSIO-CLINICAL ASPECT

When the heart rate is increased the duration of all events of cardiac cycle decreases. However, the duration of ventricular systole is much more fixed than that of diastole. The duration of diastole is shortened to a much greater extent from 0.5 sec. (at HR 72 bpm) to 0.14 sec (at HR 200 bpm).

At rest as bulk of ventricular filling (70%) occurs in diastole, moreover, blood flows to subendocardial portion of left ventricle only in diastole (page 360). Thus at very high heart rates (such as heavy exercise, arrhythmias) ventricular filling may be compromised to such a degree that ventricular contractility decreases and cardiac output falls resulting in heart failure (page 391).

ATRIAL PRESSURE CHANGES DURING CARDIAC CYCLE

1. Atrial pressure rises during atrial systole and continues to rise during isovolumetric ventricular contraction when A-V valves bulge into the atria.
2. With the onset of ventricular systole proper, 'A-V valves' are pulled downwards, atrial pressure falls rapidly and then rises as blood flows into the atria (via continuous venous return) until the 'A-V valves' open early in ventricular diastole.
3. As the atrial blood passes into the relaxing ventricles, atrial pressure falls to rise again during the phase of ***diastasis***.

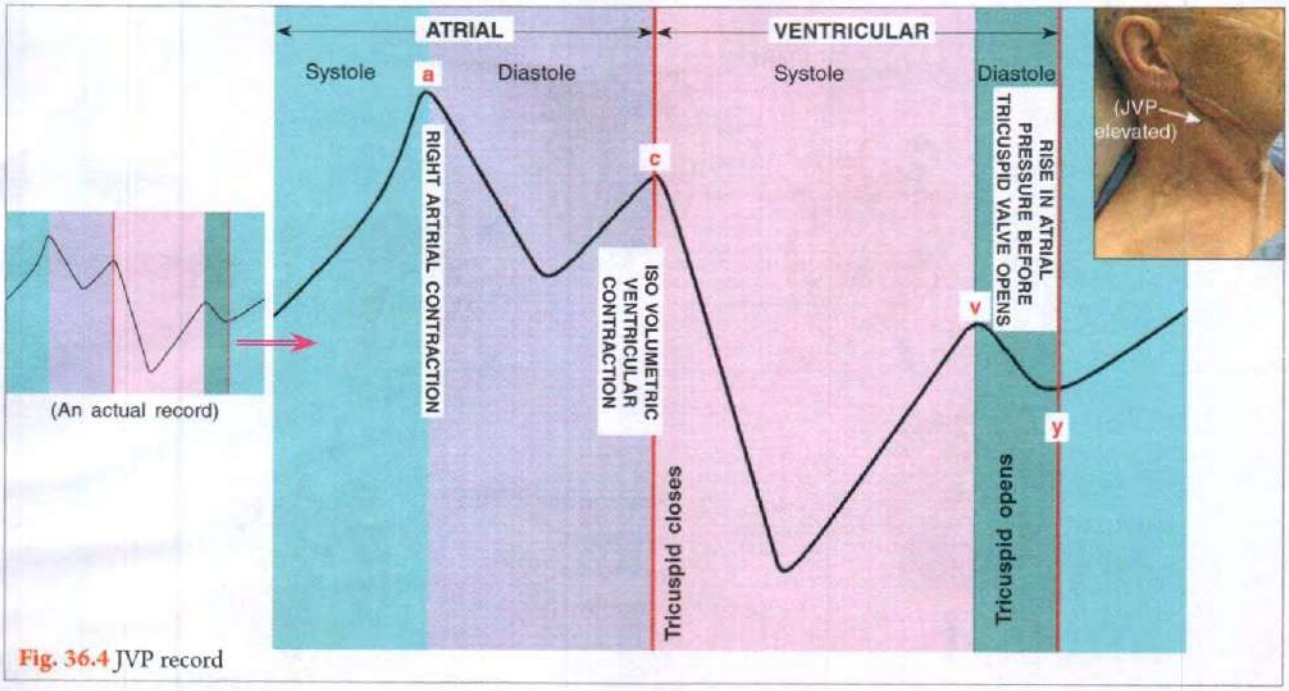


Fig. 36.4 JVP record

JUGULAR VENOUS PRESSURE (JVP) RECORD

There is no valve at the junction of superior vena cava (SVC) and right atrium (RA), therefore right atrial pressure changes are transmitted to the jugular vein in the neck, producing '3' characteristic waves **(Fig. 36.4)**.

1. The **'a wave'**: It is due to atrial systole. Some blood regurgitates into the great veins when atria contracts, even though the orifices of 'IVC' and 'SVC' are constricted. In addition, venous inflow stops, causing rise in venous pressure, contributing to the 'a wave'.
2. The **'c wave'**: It is the transmitted manifestation of the rise in atrial pressure produced by the bulging of the tricuspid valve into the right atrium during isovolumetric ventricular contraction phase.
3. The **'v wave'**: It is due to the rise in atrial pressure before the tricuspid valve opens during diastole.

E.C.G. CHANGES DURING CARDIAC CYCLE

E.C.G. (*Electrocardiogram*) is the record of electrical changes generated with each heart beat. The waves associated with electrical activity of the heart during each cardiac cycle are represented by letter P, Q, R, S and T. **(Fig. 36.5)**

(i) ***'P' wave*** is due to atrial depolarization and precedes atrial systole.

(ii) 'Q', 'R' and 'S' waves together constitute the ***QRS complex*** and are due to ventricular depolarization. It precedes ventricular systole.

(iii) ***'T' wave*** is due to ventricular repolarization. It coincides with closure of semilunar valves.

(For details, refer to page 291.)

HEART SOUNDS

Differences between first and second heart sound are given in **Table 36.1**.

Table 36.1: Differences between first and second heart sound

First Heart Sound (HS_1)	Second Heart Sound (HS_2)
1. ***Cause***: It is due to closure of 'AV valves' and marks the onset of ventricular systole.	1. ***Cause***: It is due to closure of semilunar valves and marks the onset of ventricular diastole.
2. ***Character***: Low pitch; frequency: 30-80/sec, producing *loud sound*. It sounds like the syllable ***L-U-B-B***.	2. ***Character***: High pitch; frequency: 150-200/sec, producing *sharp sound*. It sounds like the syllable, D-U-P.
3. ***Duration***: Longer: 0.1 to 0.17 sec.	3. ***Duration***: Shorter: 0.1-0.14 sec.
4. ***Site***: It can be heard all over the chest but best heard over mitral and tricuspid areas. (***Mitral Area*** is located in left 5th intercostal space internal to mid clavicular line; ***tricuspid area*** is located in left 5th intercostal space near the sternal border.) **(Fig. 36.6)**	4. ***Site***: It can be heard all over the chest but best heard over pulmonary and aortic areas. (***Pulmonary area*** is located in left 2nd intercostal space just outside the sternum; ***aortic area*** is located in right 2nd intercostal space just outside the sternum).
5. ***Correlation***: It coincides with the 'R-wave' of E.C.G.	5. ***Correlation***: It coincides with end of 'T-wave' of E.C.G.

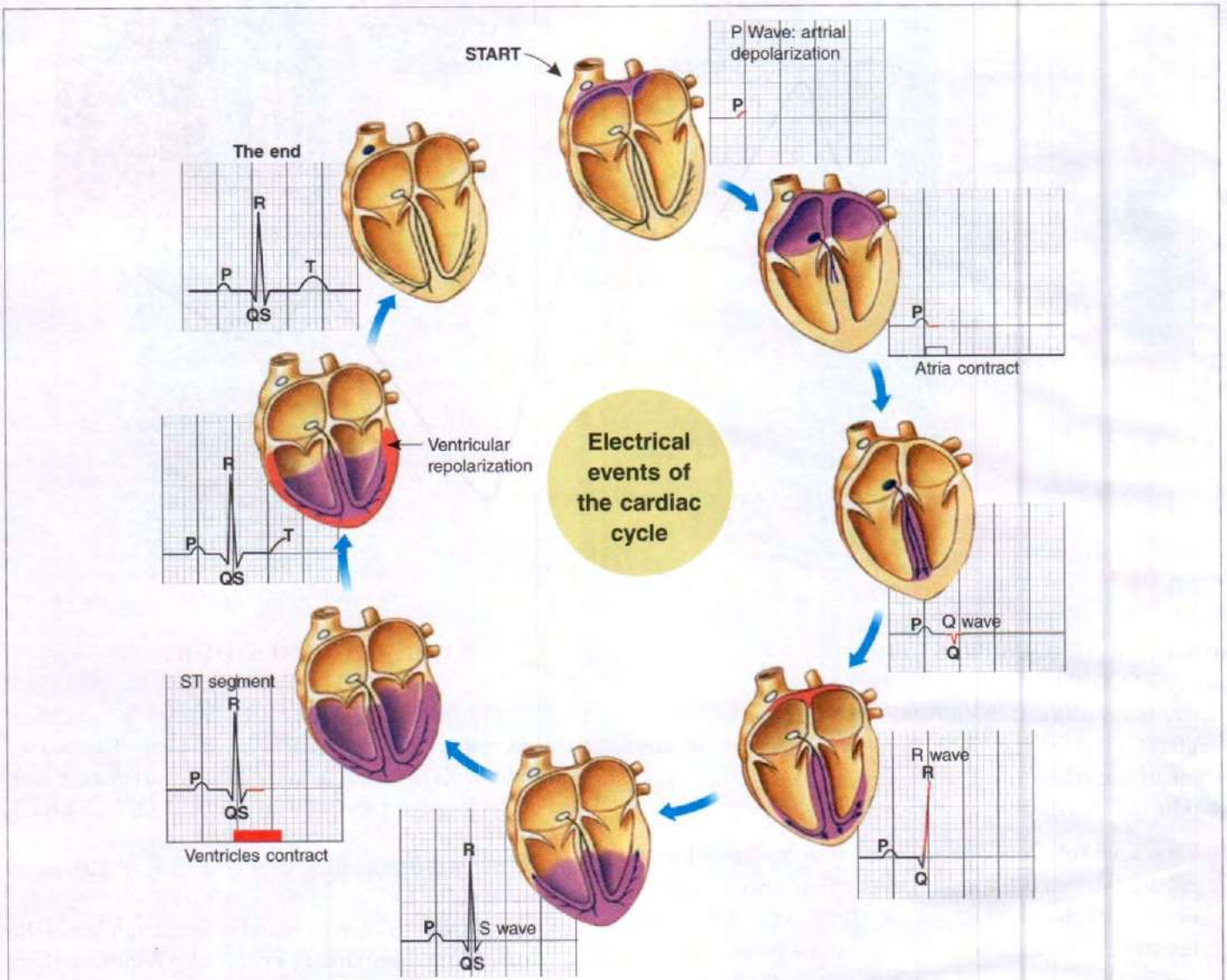


Fig. 36.5 ECG changes during cardiac cycle

Third Heart Sound (HS_3): It is due to vibrations of the cardiac walls produced by the rapid filling phase of the ventricles during ventricular diastole proper. It is low pitched, has low intensity and of 0.1 sec duration.

Fourth Heart Sound (HS_4): It is due to atrial systole, characterised by low frequency (20 cps) and low amplitude.

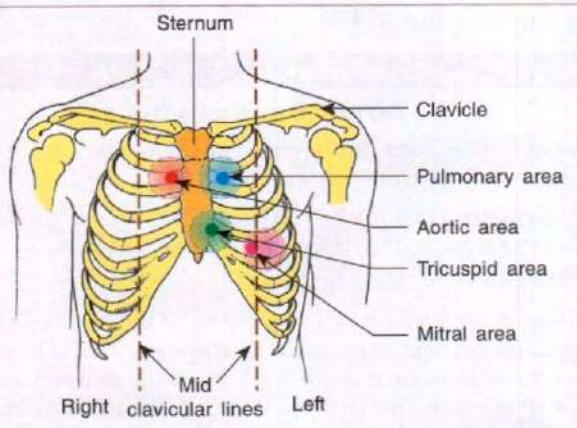


Fig. 36.6 Location of 'heart areas' over the chest

Note

HS_3 and HS_4 are less important, being normally inaudible.

Important Note

A number of different adventitious sounds or heart *murmurs*, may also be detected. These abnormal sounds are usually associated with turbulence of blood generated as blood passes through the leaking valves or through a valve that has a narrowed orifice resulting from a disease.

Study Questions

1. **Give physiological significance of:**
   (i) End diastolic volume
   (ii) Heart sounds
2. **Write short notes on:**
   (i) Atrial systole
   (ii) Isovolumetric ventricular contraction phase
   (iii) Isovolumetric ventricular relaxation phase
   (iv) Ventricular systole proper
   (v) Protodiastole
3. **Draw well labelled diagram to show:**
   (i) Pressure changes in left side of the heart during cardiac cycle
   (ii) Left ventricle blood volume changes during cardiac cycle
   (iii) JVP record
   (iv) Heart sounds and ECG changes during cardiac cycle
   (v) Location of heart areas over the chest
4. **Define murmur. How are they produced? What do they signify?**
5. **What is ejection fraction? Give its physio-clinical significance.**
6. **Define cardiac cycle. List its components with duration.**

MCQs

1. **The contribution of atrial systole to ventricular filling is ..... %:**
   (a) 25-30 (b) 50-55 (c) 75-80 (d) 100
2. **Isovolumetric venticular contraction is characterized by:**
   (a) Production of second heart sound
   (b) Contraction of ventricles but there is no emptying
   (c) Ventricles as a closed chamber and pressure within them remaining low
   (d) Fall in atrial pressure
3. **Semilunar valves opens when:**
   (a) Pressure in great vessels exceeds the pressure in the corresponding ventricles
   (b) Pressure in ventricles exceeds the pressure in the corresponding great vessels
   (c) Pressure in left atrium is more than the pressure in the left ventricle
   (d) Pressure in right atrium is more than the pressure in the right ventricle
4. **The maximally achievable systolic pressure by left ventricle is ..... mmHg:**
   (a) 120 (b) 150 (c) 200 (d) 300
5. **In a healthy individual, ... % of end-diastolic volume to the stroke volume is:**
   (a) 45 (b) 55 (c) 65 (d) 75
6. **Ejection fraction of the ventricle refers to the ratio of:**
   (a) Amount of blood received/amount of blood ejected (b) Stroke volume/end-diastolic volume
   (c) End-systolic volume/end-diastolic volume (d) Stroke volume/end-systolic volume
7. **Maximum filling of the ventricles takes place during which phase of the cardiac cycle?**
   (a) Protodiastole (b) Isovolumetric ventricular relaxation phase
   (c) Ventricular diastole proper (d) Last rapid filling phase due to atrial systole
8. **'a' wave of jugular venous pulse is caused by:**
   (a) Atrial systole (b) Ventricular systole (c) Atrial diastole (d) Ventricular diastole
9. **'c' wave of the jugular venous pressure record is due to the:**
   (a) Atrial systole
   (b) Bulging of tricuspid valve into the atria during isovolumetric ventricular contraction
   (c) Rise in atrial pressure before the tricuspid valve opens during diastole
   (d) Pulling down of A-V valves with onset of ventricular systole proper

10. First heart sound (HS_1):
(a) Marks the onset of ventricular systole
(b) Caused by the closure of semilunar valves
(c) Best heard over aortic and pulmonary areas
(d) Characterised by high pitch and sharp sound

11. Heart sound indicating condition of myocardium is:
(a) HS_1 (b) HS_2 (c) HS_3 (d) HS_4

12. Systolic murmurs are common in anaemia, because:
(a) Turbulence occurs more frequently as viscosity of blood is lower
(b) Turbulence occurs more frequently as diameter of vessel wall is increased
(c) Anaemia is more frequently associated with aortic stenosis
(d) Turbulence occurs more frequently as velocity of blood flow increases

13. During isovolumetric ventricular contraction phase of cardiac cycle:
(a) Intraventricular pressure rises to its maximum value
(b) A small but short fall in the atrial pressure occurs
(c) Sudden opening of semilunar valves occurs
(d) It is of 0.05 sec duration

14. Opening of aortic valves is initiated when:
(a) Ventricular pressure exceeds aortic pressure
(b) Ventricles contract
(c) Ventricles relax
(d) Atria contracts

15. Closure of the semilunar valves occurs during:
(a) Isovolumetric ventricular contraction phase
(b) Rapid ejection phase
(c) Protodiastole
(d) Isovolumetric ventricular relaxation phase

16. *True* statement regarding diastasis is:
(a) It occurs during the emptying phase of ventricles
(b) Slow filling of ventricles occur in this period
(c) Third heart sound coincides with this period
(d) A-V valve opens at the beginning of this period

17. The first heart sound (HS_1) occurs due to closure of AV valves during:
(a) Isotonic ventricular relaxation
(b) Isotonic ventricular contraction
(c) Isovolumetric ventricular contraction
(d) Isovolumetric ventricular relaxation

18. The second heart sound differs from first heart sound in that:
(a) Is occasionally split
(b) Has higher frequency
(c) Duration greater than first sound
(d) Due partly to turbulence set up by valve closure

Answers

1. (a)	2. (b)	3. (b)	4. (d)	5. (c)	6. (b)	7. (c)	8. (a)	9. (b)	10. (a)
11. (c)	12. (a)	13. (d)	14. (a)	15. (c)	16. (b)	17. (c)	18. (b)		

Chapter 37

The Electrocardiogram (E.C.G.)

I. Normal ECG
II. Electrocardiography
(A) Unipolar recording; precordial leads; Augmented limb leads; (B) Bipolar Recording
III. Cardiac Vector or Cardiac axis
IV. Abnormal ECG
(A) Heart block; (B) New Rhythm Centre: Extrasystoles; Arrhythmias; WPW Syndrome; (C) Myocardial Infarction (MI); (D) Effect due to changes in ionic composition of blood

INTRODUCTION

Body is a ***volume conductor*** *i.e.* body fluids are good conductor of electricity because it contains large quantities of electrolytes; therefore, electrical changes occurring in the heart with each heart beat are conducted all over the body and can be picked up from the body surface. The record of these electrical fluctuations during cardiac cycle is called ***Electrocardiogram (ECG)***. Thus, the ECG recorded at the surface of the body represents the resultant activity in the individual myocardial fiber.

NORMAL ECG

Recording conventions

1. ECG is recorded on a mm square graph paper, moving at a speed of 25 mm/sec;
'X-axis' represents the time, therefore, 1 mm = 0.04 sec (along the X-axis)
'Y-axis' represents the voltage, and,1 mm = 0.1 mV (along the Y-axis).
2. Any deflection of the record above the baseline is regarded as ***positive deflection***. It occurs when the active electrode becomes positive relative to the indifferent electrode. Any deflection below the baseline is regarded as ***negative deflection***. It is seen when active electrode becomes negative. No deflection from the baseline means the ***isoelectric line*** or ***isoelectric segment***.
3. Spread of the ***excitation wave*** *i.e.* depolarization process *towards* a positive electrode gives an upward deflection (positive deflection); and spread of excitation wave *away* from it causes a downward (negative) deflection. A depolarization wave moving *perpendicular* to an electrode will produce a partly positive and a partly negative deflection ***(Biphasic wave)*** **(Fig. 37.1)**
4. **Effect of repolarization** on the ECG are similar to those of depolarization except that the changes are reversed. A wave of repolarization moving *towards* a positive electrode produces *negative* deflection on the ECG, and a repolarization wave moving *away* from it causes a *positive* deflection. A *perpendicular* wave produces deflection of the *biphasic* wave; however, the negative deflection of the *biphasic* wave now precedes the positive deflection.

Position of the heart in the chest

Heart is placed obliquely in the chest in most of the individuals. In this position, the atria are located posteriorly in the chest; the ventricles form the base and the anterior surface of the heart, and RV lies anterior and medial to the LV.

Waves associated with ECG

The waves associated with the electrical activity of the various parts of the heart tissue during each cardiac cycle are represented by letters **P**, **Q**, **R**, **S**, **T** and **U**. **(Fig. 37.2)**

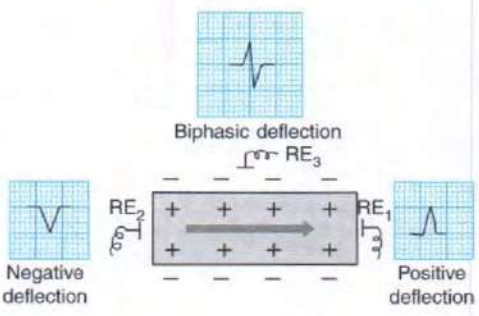


Fig. 37.1 Wave pattern during recording
(RE: recording electrode; →: Direction of excitation wave)

Note

A *segment* is a straight line connecting two waves, whereas an *interval* comprises at least one wave plus the connecting straight line. (segment)

'P' wave

(i) 1st wave of ECG of duration 0.1 sec; directed upwards, rounded or pointed;
(ii) it is due to ***atrial depolarization*** and represents the spread of impulse from 'SA Node' to atrial muscles;
(iii) its peak represents invasion of AV Node' by excitation process;
(iv) its height is up to 0.5 mV, which represents the functional activity of atrial muscles;

P-R Segment

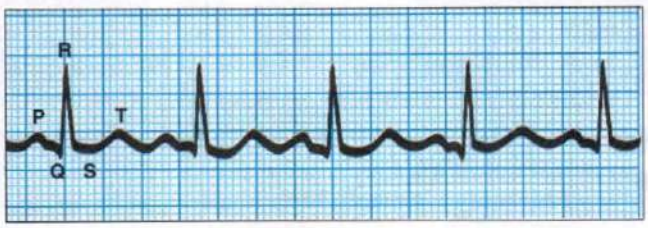
Following the 'P' wave there is a brief iso-electric period of 0.04 sec, called *P-R segment*. This is a period of conduction pause at AV Node.

QRS Complex

(i) it is due to the ***ventricular depolarization***;
(ii) it is completed just before the opening of semilunar valves;
(iii) Atrial repolarization activity merges with the QRS complex.

'Q' wave

(i) it is a small negative deflection of height less than 0.2 mV and duration less than 0.04 sec;



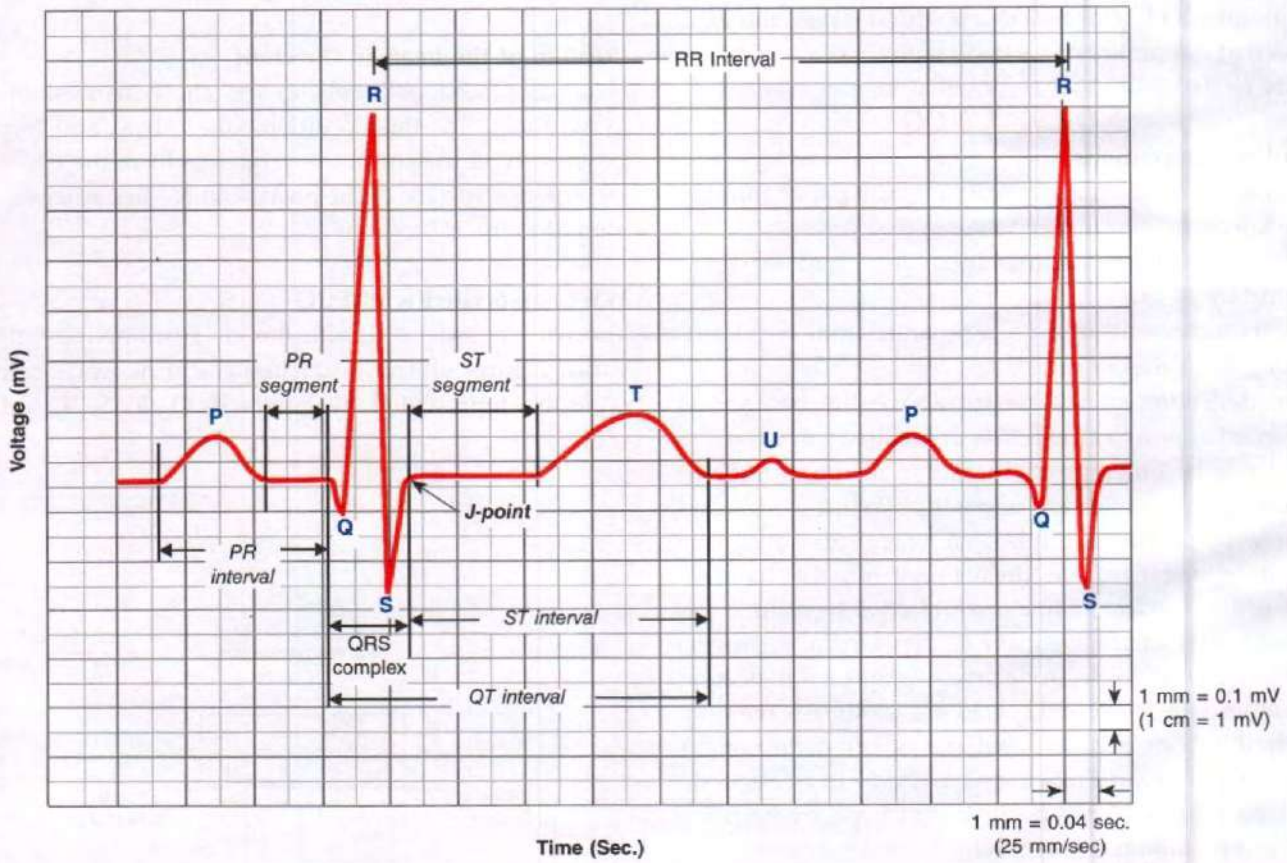


Fig. 37.2 Normal Electrocardiogram (ECG) (**Inset:** An actual record)

(ii) beginning of 'Q' wave represents invasion of mid-portion of the interventricular septum by excitation process.

Note

Q wave may *not* always be visible in the ECG.

'R' wave

(i) prominent, pointed positive wave;
(ii) its upstroke coincides with the onset of ventricular systole;
(iii) it represents excitation process suddenly invading both ventricles *i.e.* interventricular apex and major portion of both ventricles;
(iv) its height is directly proportional to the functional activity of ventricles and does not exceed 2.5 mV (25 mm) in normal individuals.

'S' wave

(i) negative pointed deflection which follows the 'R' wave;
(ii) it represents excitation of more basal parts of ventricles.

Thus, the QRS complex extends from the beginning of 'Q' wave to the end of 'S' wave with 0.08 to 0.12 sec duration and height 1.5 to 2 mV.

If its duration is more than 0.12 sec, it indicates *heart block* (page 297) *i.e.* conduction block in both or one of the branches of bundle of His.

S-T segment

Following QRS complex there is a long isoelectric period which extends from the end of 'S' wave to the beginning of 'T' wave, called *S-T segment*. Its duration is 0.04 to 0.08 sec. It measures the time from the end of ventricular depolarization to the start of ventricular repolarization.

'T' wave

(i) rounded positive deflection of duration 0.27 sec and 0.5 mV height;
(ii) it represents ***ventricular repolarization***;
(iii) end of T-wave coincides with the closure of semilunar valves.

Iso-electric period

Following T-wave is a brief iso-electric period of 0.04 sec.

'U' wave

(i) rarely seen, as positive small round wave of 0.08 sec duration and 0.2 mV height;
(ii) it is due *to slow repolarization of papillary muscles.*

PR interval

(i) interval from the beginning of 'P' wave to the beginning of Q or R wave (if Q wave is absent);
(ii) it represents atrial depolarization plus conduction time of bundle of His;
(iii) Normal duration 0.12 to 0.16 sec at a heart rate (HR) of 72/min; duration decreases with increase in HR.
(iv) if duration is more than 0.2 sec, indicates delayed conduction in bundle of His;
(v) duration of less than 0.12 sec indicates impulse has probably arisen in the AVN.

QT interval

(i) interval from the beginning of 'Q' wave to the end of T-wave; normal duration 0.40 to 0.43 sec;
(ii) it represents ventricular depolarization and repolarization.

ST interval

(i) (QT minus QRS complex) *i.e.* end of 'S' wave to end of 'T' wave; normal duration 0.32 sec.
(ii) it represents ventricular repolarization.

TP segment

(i) period from the end of 'T' wave to the beginning of 'P' wave of next cardiac cycle;
(ii) it represents polarized state of whole heart;
(iii) its duration is inversely related to H.R. Normal is 0.2 sec @ H.R. 75/min.

J point

(i) It is a point at the end of 'S' wave and satrt of the ST segment;
(ii) it is a point of 'no' electrical activity and is important in assessing ST segment elevation or depression.

ELECTROCARDIOGRAPHY: PROCEDURE OF RECORDING OF ECG

ECG can be recorded with the help of ECG machine *i.e.* ***Electrocardiograph*** (a galvanometer with a voltage amplifier recorder system). It gives a permanent record of ECG on a 'mm' graph paper recorded usually at a speed of 25 mm/sec (page 291).

Two methods are commonly employed for recording ECG are:

A. *'Unipolar'* method in which ECG is recorded using ***one*** 'active' (exploring) electrode.
B. *'Bipolar'* method in which ECG is recorded using ***two*** 'active' (exploring) electrodes.

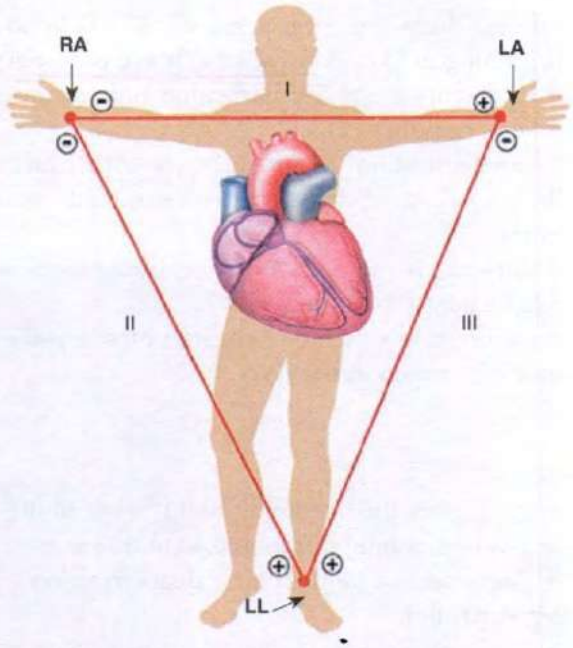


Fig. 37.3 Einthoven's triangle superimposed on chest (LA: *Left arm*; RA: *Right arm*; LL: *Left leg*)
Note: A lead consists of two electrodes, one positive and one negative

A. UNIPOLAR RECORDING

Here two electrodes are used for recording purposes: one is the ***active (exploring) electrode***, placed on the area of the body surface; and the other is the ***indifferent electrode***, which is kept at 'zero' potential by connecting electrodes placed respectively on right arm (RA), left arm (LA) and left foot (LF) to a central terminal through 5000 ohms resistance.

How zero potential is achieved?

In a volume conductor, the sum of potentials at the end points of an equilateral triangle with a current source in the centre is zero at all times. A similar triangle can be approximated in our body by placing electrodes on RA, LA and LF with current source as the heart at its centre. This is called ***Einthoven Triangle*** (Leyden physiologist, *Wilhelm Einthoven*, 1860-1927) **(Fig. 37.3)**. For graphical representation the points chosen are two acromian processes and pubic symphysis. Arms and legs only act as conducting cables, therefore, the current from 3 limbs neutralizes one another, hence electrodes can be placed on limbs and, therefore, this ***indifferent electrode*** undergoes no significant change during the cardiac cycle.

Thus in unipolar record, ECG records potential changes which affect the active electode only. A record obtained in this method is labelled by letter 'V'. All unipolar leads are termed 'V' leads.

In unipolar recording the following unipolar leads are used **(Fig. 37.4)**:

Name of unipolar lead	*Position of exploring electrode*
(i) Precordial Leads of Wilson	
1. V_1	4th intercostal space to right of sternum
2. V_2	4th intercostal space to left of sternum
3. V_3	mid-way between V_2 and V_4
4. V_4	5th left intercostal space in mid-clavicular line
5. V_5	5th left intercostal space in anterior axillary line
6. V_6	5th left intercostal space in mid-axillary line
(ii) Unipolar Limb Leads	
1. V_L	Left arm
2. V_F	Left foot
3. V_R	Right arm

Characteristic features of unipolar precordial leads

1. These are influenced by electrical activity throughout the heart, but specially by that part of the heart which lies nearest to the electrode. Therefore,

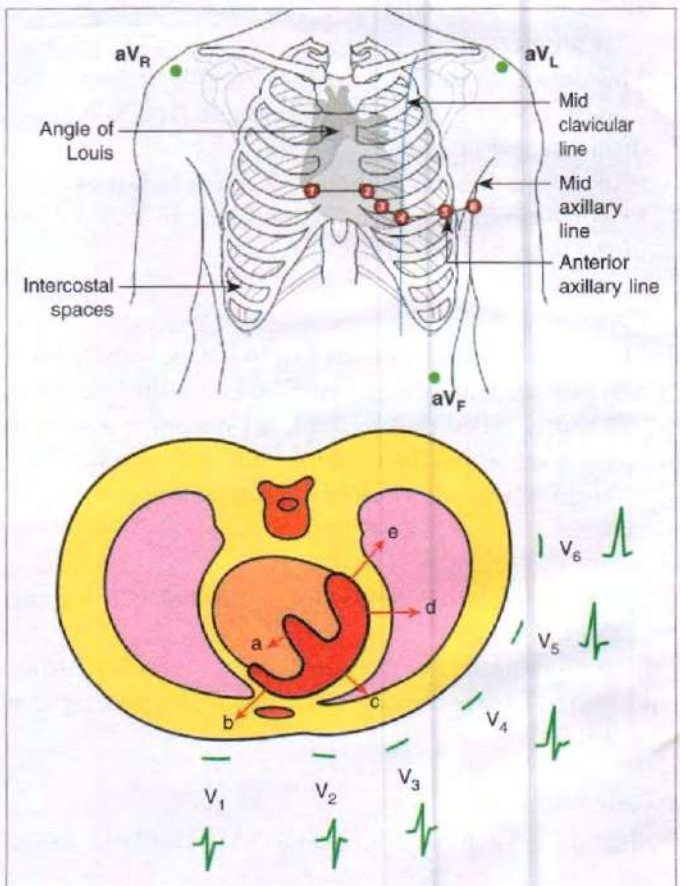


Fig. 37.4 "Cross-section through chest showing precordial leads and their relation to heart"
(a, b, c, d and e shows the order in which the electrical impulse spreads through the ventricles)

(i) V_1 and V_2 reflect right ventricular activity and the initial portion of the QRS complex is a small upward deflection followed by a large 'S' wave *i.e.* main QRS deflection in V_1 and V_2 is ***negative***, whereas

(ii) V_3 and V_4 reflect activity of both the ventricles including interventricular septum activity and there may be an initial small 'Q' wave followed by moderate 'R' and 'S' wave *i.e.* QRS deflection in V_3 and V_4 is ***biphasic***; and

(iii) V_5 and V_6 reflect left ventricular activity mainly; initially, there is a small 'Q' wave followed by a large 'R' wave *i.e.* the main QRS deflection in V_5 and V_6 is ***positive***.

2. Since the atria are located posteriorly in the chest, therefore, when excitation wave moves from 'SAN' to 'AVN' *i.e.* from posterior to anterior, it produces ***positive*** 'P' wave in all the precordial leads.
3. T-wave follows the main direction of QRS complex. Important *rule of the thumb* is that **(Fig. 37.5)**:
   (1) As we pass across the chest (V_1 to V_6 leads), 'R' wave gradually increases in size and 'S' wave becomes smaller. In lead V_3 'R' wave is equal to the 'S' wave (site of interventricular septum).
   (2) 'R' wave in V_6 and 'S' wave in V_1 represent 'LV' activity whereas 'R' wave in V_1 and 'S' wave in V_6 represent 'RV' activity.

Characteristic features of unipolar limb leads

These reflect the electrical activity of that part of the heart which faces the electrode **(Fig. 37.6)**, therefore

1. V_F – reflects the electrical activity of the inferior surface of the heart; this may be formed by both, right and left ventricles; therefore, *'QRS' deflection* in V_F will be the same as seen in leads V_3 or V_4 *i.e.* ***predominantly 'biphasic'***.
2. V_L – reflects the electrical activity of the left outer side of the heart therefore, *QRS deflection* in V_L will be same as seen in lead V_6 *i.e.* ***predominantly 'positive'***.
3. V_R – reflects the electrical activity of the cavity of the ventricles, whatever may be the position of the heart. Thus, *the* ***'P' wave, QRS complex and 'T' wave all are negative deflection***.

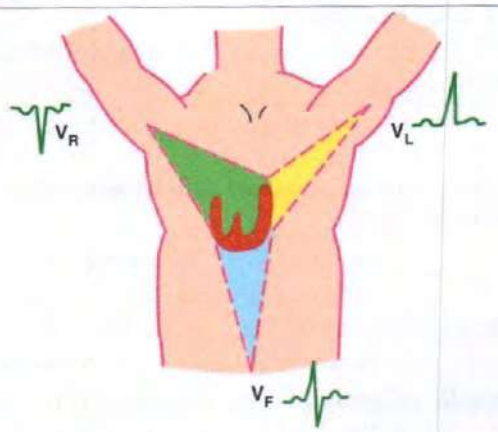


Fig. 37.6 Patterns of ECG waves in the unipolar limb leads with oblique position of heart.

Augmented Limb Leads of Goldberger

These are unipolar limb leads with slight modification in the recording technique. The augmented limb leads are the recording between one limb connected to the positive terminal of ECG machine and the other two limbs connected through electrical resistance to the negative terminal of the ECG machine. This arrangement increases the size of potentials by 50% without any change in configuration (pattern) from the non-augmented record; therefore,

Vector of augmented limb lead = 3/2 vector of unaugmented limb lead

Augmented limb leads are designated by letter 'a' prefixed to the name of the limb lead, therefore, these are aV_R, aV_L and aV_F. Thus,

PROOF:

$$aV_R = V_R - \left(\frac{V_L + V_F}{2}\right)$$

$$2\,aV_R = 2V_R - (V_L + V_F) \quad \ldots (i)$$

Since

$$V_R + V_L + V_F = \text{Zero (Einthoven triangle)}$$

$$V_R = -(V_L + V_F) \quad \ldots (ii)$$

From (i) and (ii)

$$2\,aV_R = 2V_R + V_R$$

$$aV_R = 3/2\,V_R$$

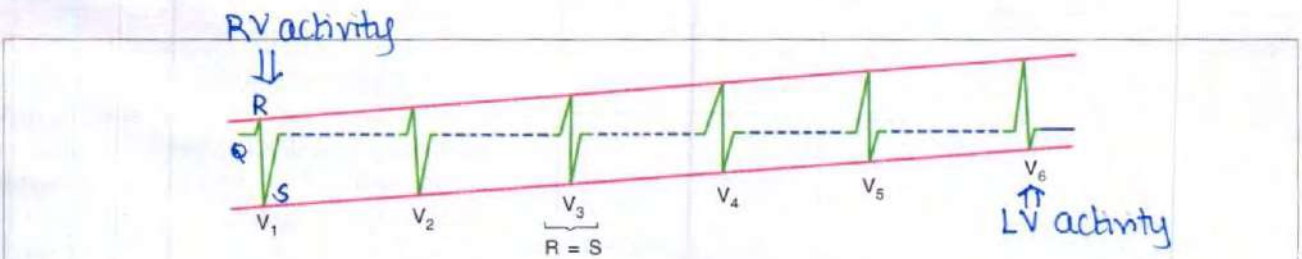


Fig. 37.5 Pattern of ventricular complexes in chest leads (V_1 to V_6)

B. BIPOLAR RECORDING

Here ECG is recorded using two active electrodes; one as a positive and the other as a negative. Therefore, the deflection recorded here at any moment represents the algebaric sum of the potentials of two constituent leads.

Bipolar leads or classical (standard) limb leads of Einthoven are:

1. *Lead I* : records the difference in potential between RA and LA ('LA' positive)
2. *Lead II* : records the difference in potential between RA and LF ('LF' positive)
3. *Leads III* : records the difference in potential between LA and LF ('LF' positive)

(RA, LA, LF: Right arm, left arm and left leg respectively)

Thus,

Lead I, records $(V_L - V_R)$, therefore, main QRS deflection is same as in aV_L

Lead II, records $(V_F - V_R)$, therefore, main QRS deflection is same as in aV_F

Lead III, records $(V_F - V_L)$, therefore, main QRS deflection is same as in aV_F.

Important Note

Standard (or classical) limb lead II is often used for cardiac monitoring as positioning of the electrodes most commonly resemble the pathway of current flow in normal atrial and ventricular depolarization. Moreover, on analysis it can be shown that at any given instant (moment) the sum of potentials in Lead I and Lead III equals the potential in Lead II (Einthoven's Law), *i.e.*

PROOF:

$$\text{Lead II} = \text{Lead I} + \text{Lead III}$$

$$V_F - V_R = (V_L - V_R) + (V_F - V_L)$$

$$= V_F - V_R$$

Why is it necessary to have a 12 lead E.C.G.?

It is necessary to have a 12 lead ECG becasue:

1. The ***six limb leads*** (I, II, III, aV_R, aV_L and aV_F) reflect depolarization of the heart in a ***vertical (frontal) plane*** *i.e.* electrical activity moving up and down, and left and right across the heart.
   (i) *Leads I and aV_L* are called ***Left Lateral Leads*** as they view the left lateral wall of the heart, predominantly formed by the left ventricle.
   (ii) *Leads II, III and aV_F* are called ***Inferior Leads*** as they view the inferior surface of the heart, formed by the right and left ventricles. *(Lead aV_R is oriented to the cavities of the heart whatever may be the position of the heart).*
2. The ***precordial leads (V_1 to V_6)*** reflect electrical activation of the heart in the ***Horizontal plane*** i.e. view the electrical activity moving anteriorly and posteriorly.
   (i) *Leads V_1 through V_4* are referred as the ***anterior leads***, this may be formed by the right and left ventricles.
   (ii) *Leads V_5 and V_6* are referred as ***Left lateral*** leads (see above)

CARDIAC VECTOR or CARDIAC AXIS

Since the classical (standard) limb leads I, II and III are records of the potential difference between two points, therefore, deflection in each lead at any moment indicates the magnitude and direction in the axis of the electromotive force (EMF) generated in the heart. This is called as ***cardiac vector or cardiac axis***.

CALCULATION OF CARDIAC AXIS

Cardiac axis at any given moment can be calculated from any two classical limb leads. How?

In an Einthoven triangle, if perpendiculars are dropped from the mid-points of the sides of the equilateral triangle, the lines will intersect at the centre of the electrical activity **(Fig. 37.7A)**. Thus, the Einthoven triangle can be modified in such a way, so that the leads intersect each other at a common central point without altering the mathematical relationship of the leads. The lead potentials are seen to be placed as in the diagram **(Fig. 37.7B)**.

The electrical axis of the heart is plotted using the average QRS deflection in any two classical limb leads. This is called as ***mean QRS vector***. The average QRS deflection is the distance equal to the height of 'R' wave minus the height of the largest negative deflection in the QRS complex. **(Fig. 37.8)**

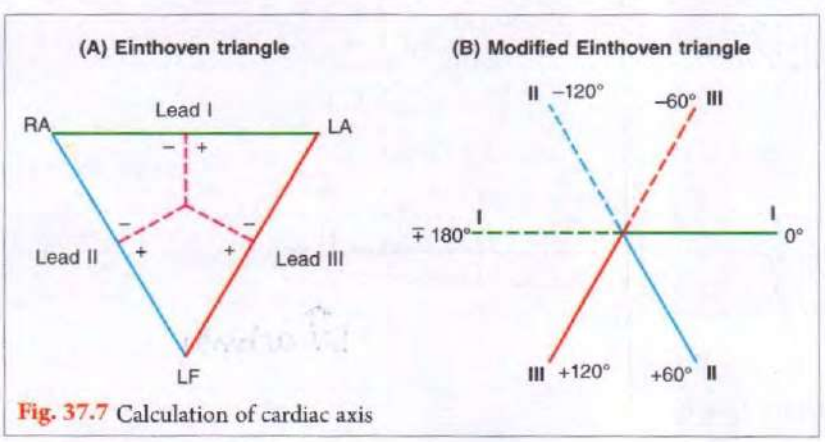


Fig. 37.7 Calculation of cardiac axis

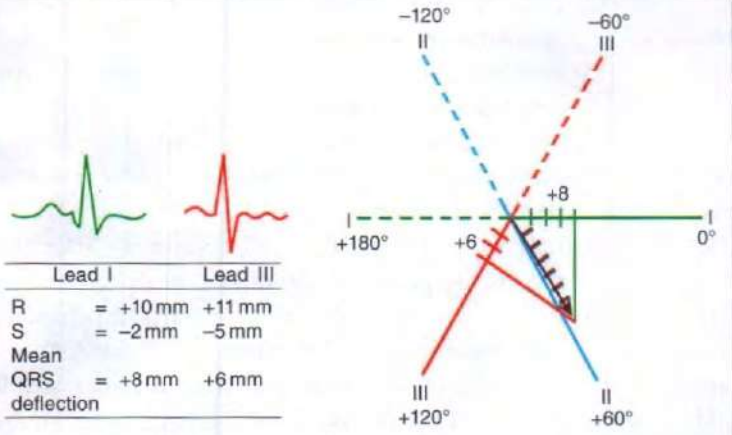


Fig. 37.8 Calculation of mean QRS vector (12 mm; +55°)

The distance equal to the average QRS deflection is measured off from the mid-point of the sides of the triangle representing that lead; perpendiculars are extended from the distance measured off. An arrow is drawn from the centre of the electrical activity to the point of intersection of perpendiculars. This represents the magnitude and direction of the mean QRS vector.

The normal direction of the mean QRS vector is −30° to +110°. (Fig. 37.9)

(1) −30° to +30° is normal ***left axis deviation*** (**LAD**). It represents horizontal position of the heart.

(2) +75° to +110° is normal ***right axis deviation*** (**RAD**). It represents vertical position of the heart.

(3) +30° to +75° represents oblique position of the heart (page 291).

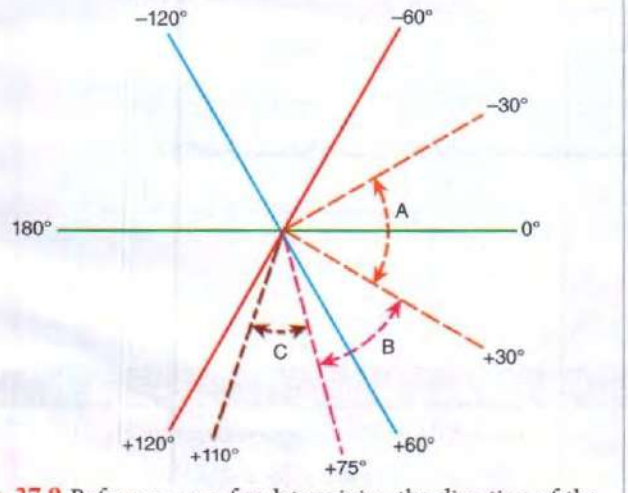


Fig. 37.9 Reference axes for determining the direction of the vector: Normal left axis deviation – horizontal heart (A); oblique heart (B) and normal right axis deviation – vertical heart (C)

If the calculated axis falls to the left of −30° or to the right of +110°, then ***abnormal*** left or right axis deviation is said to be present. Conditions in which cardiac axis becomes abnormal are:

Abnormal RAD

1. Right bundle branch block.
2. Right ventricular hypertrophy.
3. Posterior (or inferior) myocardial infarction (MI).

Abnormal LAD

1. Left bundle branch block.
2. Left ventricular hypertrophy.
3. Antero-lateral MI.

Note

In general, when the sum of the voltage of all the QRS complexes of the three standard limb lead is greater than 4 mV (40 mm), the individual is considered to have an abnormal axis deviation.

ABNORMAL E.C.G.

Classification: Abnormalities in the ECG are broadly of 4 types:

I. Heart Block
II. New Rhythm Centre
III. Myocardial Infarction (MI).
IV. Effects due to change in the ionic composition of blood.

I. HEART BLOCK

Definition: It is disturbance in the normal transmission of impulses generated in 'SAN'.

Cause: Coronary artery disease (*i.e.* decrease blood supply to the myocardium).

Types:

(A) 'Sino-atrial' nodal block:
Level of block: at SAN *i.e.* block within the substance of SAN

(B) 'Atrio-ventric ular' nodal block:
Level of block: at AVN *i.e.* block within the substance of AVN

(C) Bundle branch block (BBB):
Level of block: at bundle of His *i.e.* block of one of the branches of bundle of His

A. Sino-Atrial Nodal Block

Initially whole heart beat is lost *i.e.* neither atrial nor ventricular contractions occur. After an interval of approx. two cardiac cycle, the heart resumes its normal action as some new pacemaker other than SAN takes over. **(Fig. 37.10)**

B. Atrio-Ventricular Nodal Block

This produces disturbance of conduction between atria and ventricles. It is of two types:

(1) *Incomplete (partial) heart block;* and

(2) *Complete heart block.*

1. *'Incomplete' heart block* – It is due to partial disturbance of conduction between atria and ventricles. It is of two types: First and second degree incomplete heart block. **(Table 37.1)**
2. *'Complete (or III degree) heart block* – It is due to complete interruption of conduction between atria and ventricles. Therefore, ventricles beat with a slower rate (45 bpm) and independent of rhythm of 'SAN', called as ***Idio-ventricular rhythm.*** **(Fig. 37.11B)**

If complete heart block develops suddenly, there occurs a delay before ventricles start beating at their own rate. During this period the systemic blood pressure falls to a very low level and blood supply to brain becomes inadequate. If ventricular standstill lasts for few seconds, it causes dizziness and faintness, called ***Stokes Adams syndrome;*** or if it is more prolonged (exceeds 1 minute) it leads to loss of consciousness, convulsions and death.

C. Bundle Branch Block (BBB)

That is, block of one of the branches of bundle of His (right or left), therefore, excitation process passes normally down to bundle on the intact side and then sweeps back through the muscles to activate the ventricle on the blocked side.

It is ***characterised by*** **(Fig. 37.12):**

(1) normal heart rate

(2) prolongation of QRS complex (>0.12 sec) with abnormal appearance

(3) abnormal ST segment and T-wave (because repolarization is also affected)

(4) Split 2nd heart sound (HS_2). How?

Normally, right ventricle (RV) contracts after left ventricle (LV), therefore,

(i) in *Right BBB* (RBBB): 'RV' contracts after 'LV', producing prolongation of HS_2 *as* A_2P_2

(ii) in *Left BBB* (LBBB): 'RV' contracts much before 'LV', producing prolongation of HS_2 *as* P_2A_2

Block can also occur in the anterior or posterior fascicles of the left bundle branch producing ***Hemiblock*** or ***Fascicular Block.***

Left anterior hemiblock produces abnormal left axis deviation; and *left posterior* hemiblock produces abnormal right axis deviation in the ECG.

II. NEW RHYTHM CENTRES

General

1. Only pacemaker tissues are capable of initiating and maintaining the heart beat over prolonged periods. However, under abnormal conditions *spontaneous beat* may arise due to irritable focus within the substance of the heart.
2. These spontaneous beats are known as *Premature beats* or *Extrasystole.* It is of 2 types:
   (i) Atrial Extrasystole,
   (ii) Ventricular Extrasystole.

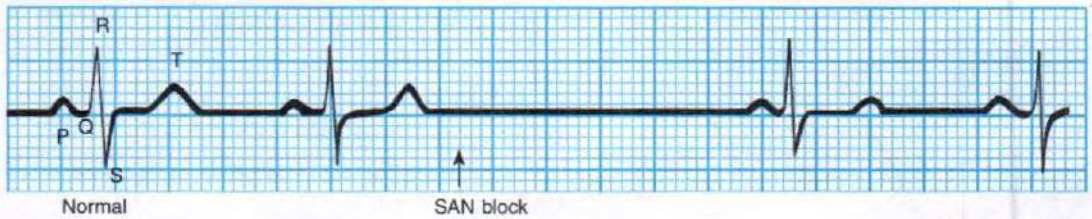


Fig. 37.10 Sino-atrial nodal (SAN) block

Table 37.1: Types of incomplete heart blocks compared

'First degree' incomplete heart block	'Second degree' incomplete heart block
When all atrial impulses reach the ventricles but PR interval is abnormally long (> 0.2 sec); however, atrial rate : ventricular rate : : 1 : 1. **(Fig. 37.11Aa)**	When all atrial impulses are not conducted to the ventricles producing: (i) 2:1 or 3:1 heart block (atrial : ventricular rate :: 2 : 1 and 3 : 1 respectively) or (ii) *'Wenckebach' phenomenon i.e.* gradual lengthening of 'PR interval' until one ventricular beat is dropped and the cycle being repeated indefinitely. **(Fig. 37.11Ab)**

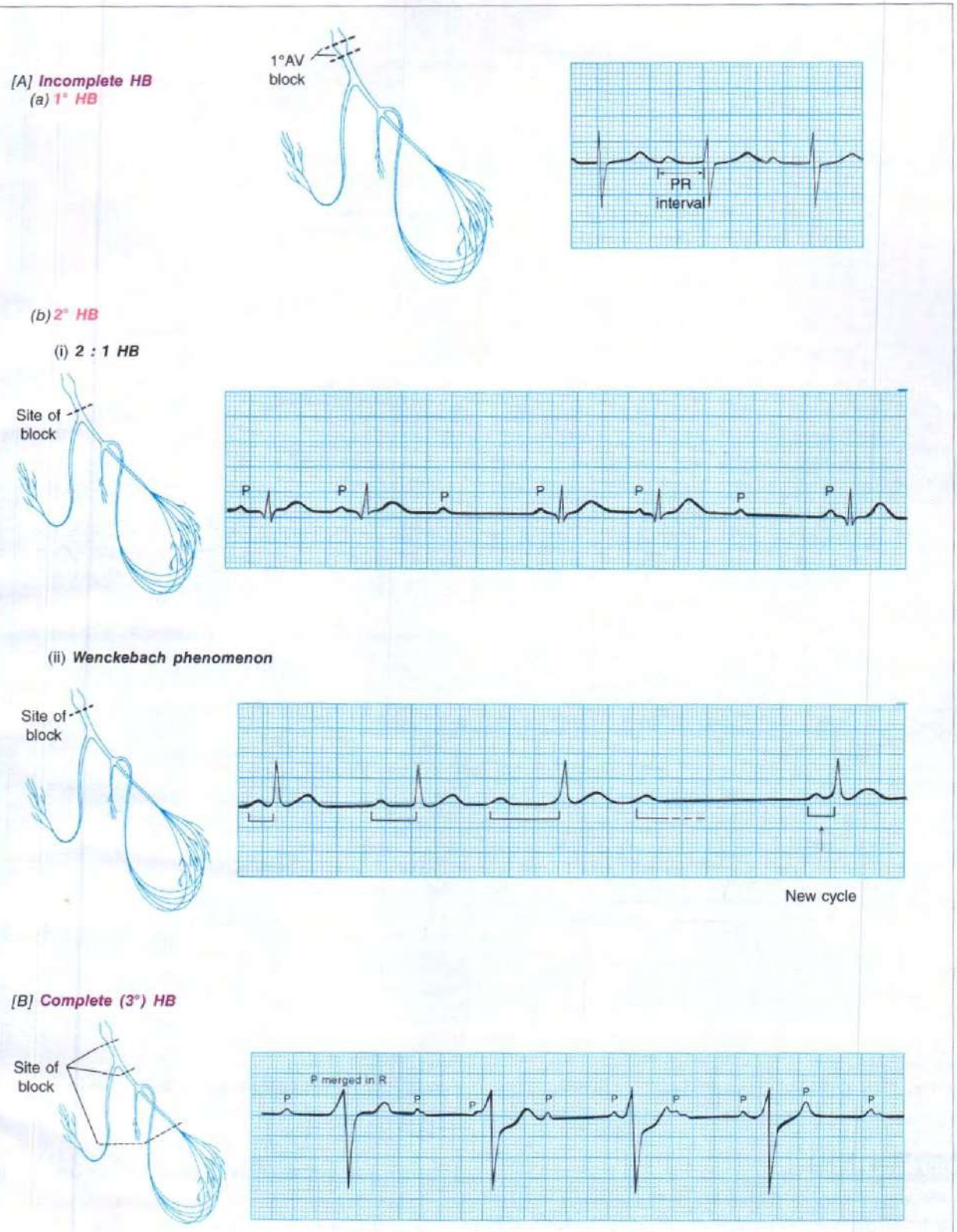


Fig. 37.11 Atrio ventricular nodal (AVN) block –
[A] Incomplete/partial HB (a) 1° HB; (b) 2° HB: (i) 2:1 HB; (ii) **wenckebach phenomenon**, and [B] complete (3°) HB

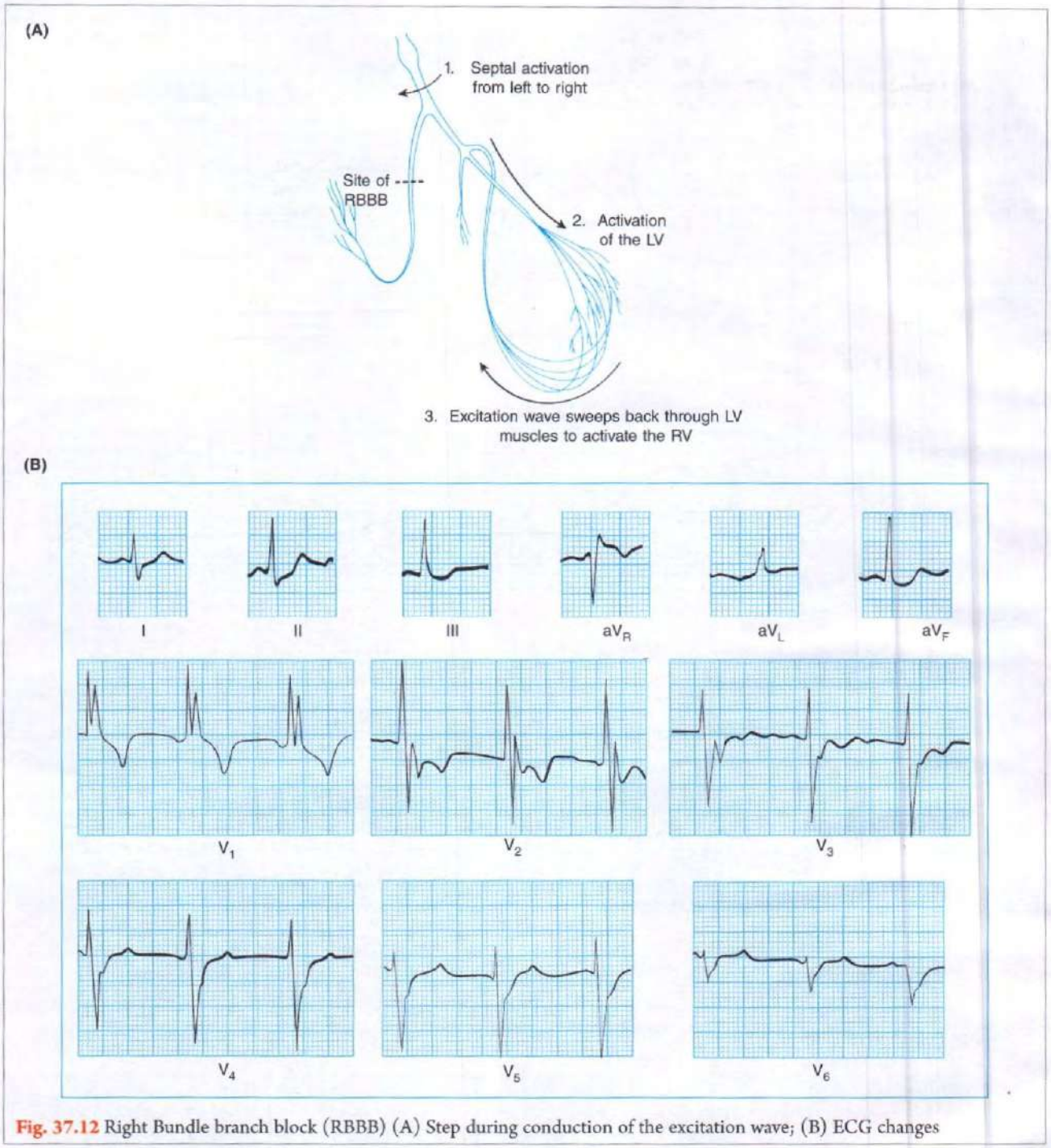


Fig. 37.12 Right Bundle branch block (RBBB) (A) Step during conduction of the excitation wave; (B) ECG changes

Important Note

Two to four extrasystoles/min are regarded as normal. This may result from such factors as smoking, lack of sleep, ingestion of too much coffee or alcohol.

3. When any *irritable focus* is capable of discharging repetitively at a rate more rapid than that of 'SAN', it can initiate and maintain heart beat for several seconds to few hours. This is called as *Ectopic cardiac beat* or *Ectopic cardiac rhythm* or *cardiac arrhythmias*. It is of 2 types:
   (i) Atrial arrhythmias; and
   (ii) Ventricular arrhythmias.

   Thus, *New Rhythm Centres* can be classified as
   (1) Extrasystoles; } Atrial; or
   and (2) Cardiac arrhythmias } ventricular

A. Extrasystoles

1. ***Atrial Extrasystole:*** It is *characterized by* **(Fig. 37.13:A)**
   (i) Abnormal 'P' wave followed by long PR interval, because extrasystolic excitation impulse takes abnormal sinus path to reach AVN.
   (ii) Normal QRS complex.
   (iii) There is *pause* between extrasystole and the next normal beat, therefore 'TP interval' is more than its normal value of 0.2 sec.
2. ***Ventricular Extrasystole:*** It is *characterized by* **(Fig. 37.13:B)**
   (i) There is **no** 'P' wave preceding QRS complex.
   (ii) QRS complex is prolonged and abnormal in appearance due to abnormal route of spread of excitation wave in the ventricles.
   (iii) The 'P' wave of next cardiac cycle is generally burried within this abnormal ventricular complex. (QRS)

B. Cardiac Arrhythmias

1. *Atrial Arrhythmias:* It is further divided into (i) Atrial *tachycardia*, (ii) Atrial *flutter*, and (iii) Atrial *fibrillation* **(details, Table 37.2 and Fig. 37.14).**
2. *Ventricular Arrhythmias:* Like the atrial arrhythmias these are also divided into: *tachycardia, flutter and fibrillations* **(details, Table 37.3 and Fig. 37.15)**
3. *Paroxysmal Tachycardia*
   If tachycardia appears and declines abruptly it is called as *paroxysmal tachycardia*. The attack may last from seconds or minutes to many days. It may be having focus either in the atria

Table 37.2: Atrial Arrhythmias compared (Also refer to **Fig. 37.14**)

	Atrial Tachycardia	Atrial Flutter	Atrial Fibrillation
1. Rate of discharge of atrial impulses	upto 200 bpm	between 200-350 bpm	350 to 500 bpm
2. Type of atrial contractions	Atria respond regularly with contractions of uniform size.	There occurs a ***partial refractory state*** in atria. This produces alternate large and small atrial contractions.	The length of refractory period of atrial muscle fibers increases, thus, atria show an irregular ***oscillations*** of the surface, called ***fibrillations***.
3. Atrial (A) to ventricular (V) contraction ratio.	All atrial impulses travel to ventricles producing regular uniform ventricular contractions A : V : : 1 : 1	As 'AVN' cannot transmit more than 180 to 200 impulses per minute, therefore, A : V : : 2 : 1	The excitation wave takes an irregular sinus path producing irregular discharge from 'AVN' causing irregular ventricular contractions. Therefore, A : V : : 2 or 3 : 1
4. ECG findings	(i) all time intervals, such as PR interval, TP interval shorten. (ii) T wave merges with P wave of next cardiac cycle.	(i) features as seen in tachycardia; (ii) second degree type of heart block, specially 'wenckebach' phenomenon and 2:1 heart block;	(i) absence of P wave; (ii) appearance of ***fibrillation*** (f) ***waves***, which show a constant change in height and width. (iii) irregular QRS complexes (iv) *No* T wave.

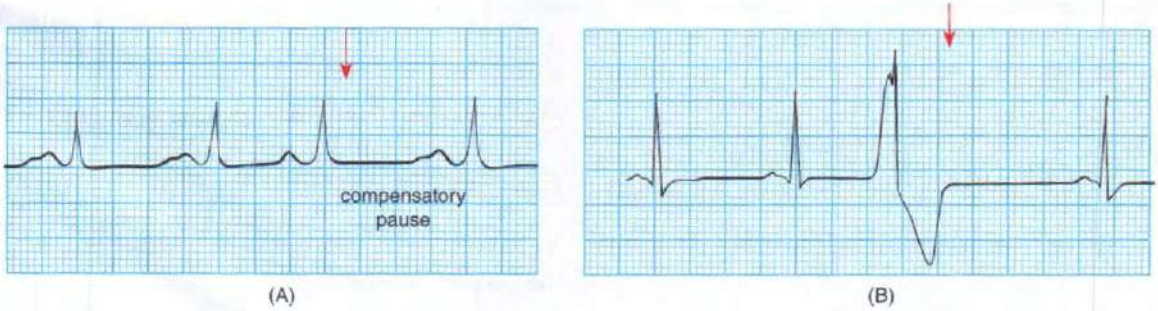


Fig. 37.13 Extrasystoles (*Arrow*): Atrial (A) and Venticular (B)

Table 37.3: Ventricular Arrhythmias compared (Also refer Fig. 37.15)

	Ventricular Tachycardia	Ventricular Flutter	Ventricular Fibrillation
1. Rate of ventricular contractions	Upto 200 bpm	Between 200-350 bpm	350 to 500 bpm
2. Characteristic features in ECG	(i) The QRS complexes are highly *polymorphic*, analogous to the ventricular extra systoles. (ii) undisturbed atrial activity is interspersed (scattered) with P waves without any relation to QRS complexes.	ECG shows large oscillations ***(Hair Pin Curves)*** where main and terminal deflections can no longer be differentiated.	ECG shows irregular, extremely fast, small potential fluctuations in rate, rhythm, amplitude and appearance. (AR)²

Important Note

It is a fatal condition, because fibrillating ventricles cannot pump blood effectively and circulation of blood stops causing sudden death.

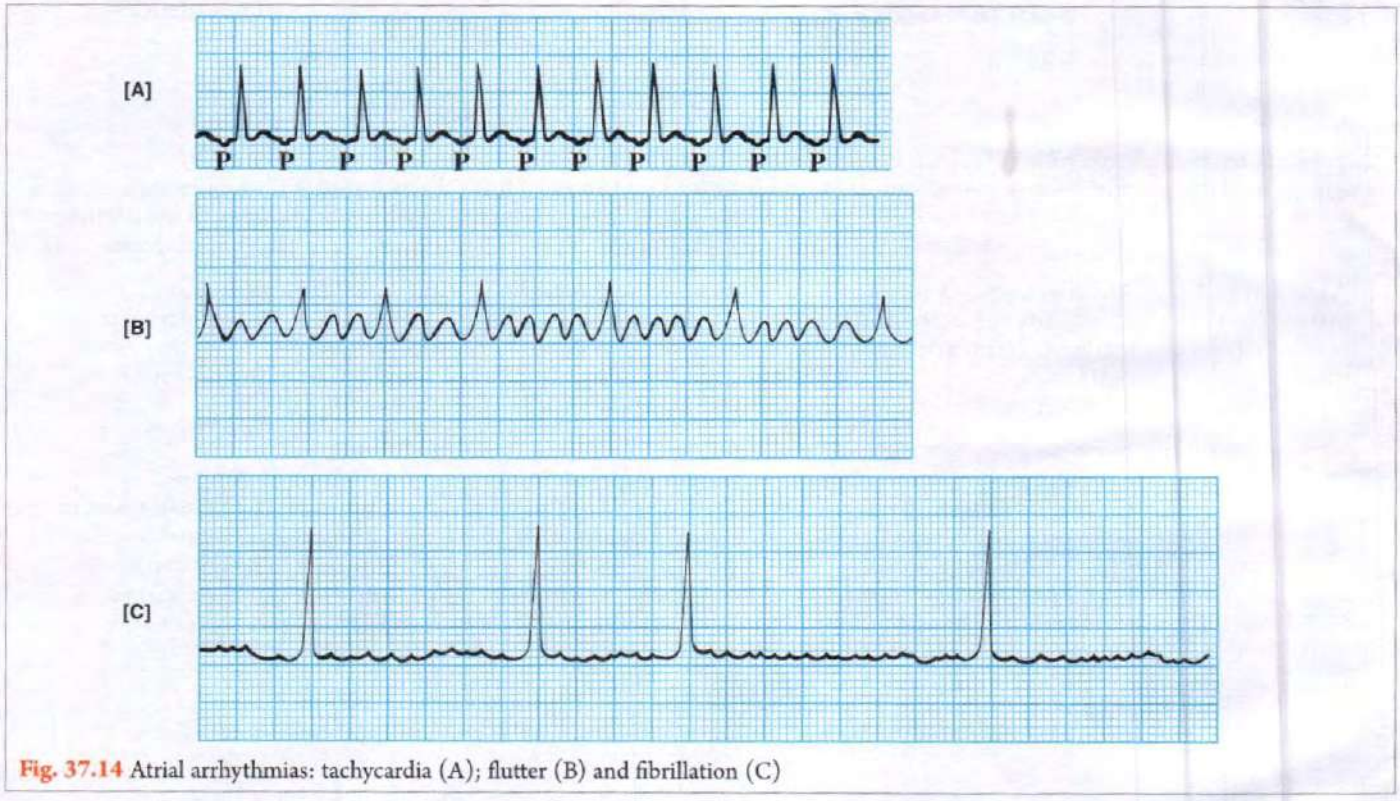


Fig. 37.14 Atrial arrhythmias: tachycardia (A); flutter (B) and fibrillation (C)

i.e. ***paroxysmal atrial tachycardia (PAT)*** or in ventricles *i.e.* **paroxysmal ventricular tachycardia (PVT)**.

Ventricular tachycardia in which irritable ectopic focus appears above the ventricles is called *Supra Ventricular Tachycardia*.

Causes of Flutter and Fibrillations

These are due to the formation of ***Circus Movements*** *i.e.* formation of circuit around a ring of myocardial fibers by the excitation process.

Sites

(1) the tissues joining the opening of inferior and superior vena cavae; and
(2) around the AV valves.

Mechanism of development of 'circus movements'

Two mechanisms:

(1) Depolarization of a ring of cardiac muscle (common cause), and
(2) Retrograde conduction due to transient block in bundle of His

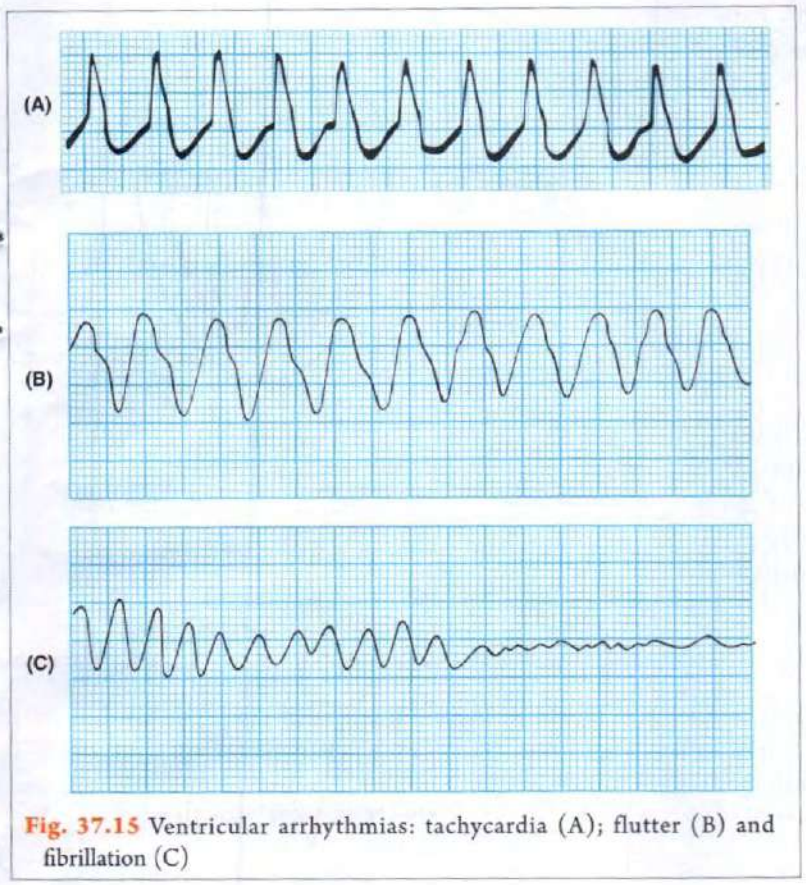


Fig. 37.15 Ventricular arrhythmias: tachycardia (A); flutter (B) and fibrillation (C)

(1) *Depolarization of a ring of cardiac muscle:* Normally the excitation impulse spreads in both directions in the ring and the two impulses cancel when they meet on opposite side. If there is a transient block on one side due to slowed conduction, the impulse reaching here earlier via a shorter route will find this area refractory and die off. However, the impulse which goes around the ring and reaches here late, finds this area no longer refractory. It will pass this area and continue to circle indefinitely producing circus movements. **(Fig. 37.16:A)**

Note

A frequent cause of atrial fibrillation is atrial enlargement. The dilated atrial walls provide ideal conditions for a long conductive pathway, as well as slow conduction, both of which predisposes to atrial fibrillation.

(2) *Retrograde conduction due to transient block in bundle of His:* Transient block due to slowed conduction in parts of conduction pathway prevents 'anterograde' conduction in a portion of bundle of His and then this area is invaded from below. The area above the block has had time to recover and is no longer refractory producing *Re-entry i.e. Retrograde conduction* causing atrial depolarization resulting in atrial beat called as ***Atrial Echo Beat.*** **(Fig. 37.16:B)**

Wolff-Parkinson-White Syndrome (WPW Syndrome)

Normally the only conducting pathway between the atria and ventricles is the AVN. Individuals with WPW syndrome have additional nodal connecting tissue

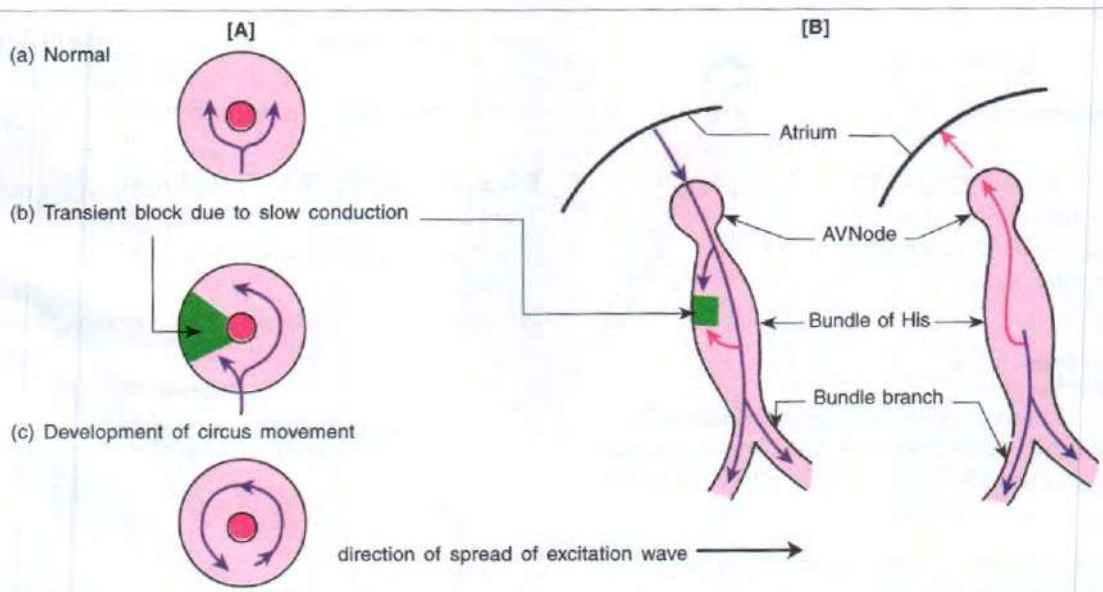


Fig. 37.16 Mechanism of development of circus movements: Depolarization of a ring of cardiac muscle (A); and Retrograde conduction in bundle of His (B)

between atria and ventricles called as ***Bundle of Kent***. This conducts more rapidly than AVN, therefore, one ventricle is excited early. **(Fig. 37.17)**

ECG findings

(1) Short PR interval.
(2) Prolonged QRS deflection with slurred upstroke, because manifestation of early activation of ventricles merges with normal QRS pattern.
(3) Normal 'PJ' interval.

Complication

Paroxysmal atrial tachycardia (PAT) (page 302) because impulses which produce ventricular activation via AV node spread on to the *bundle of Kent* and find it no longer refractory and the impulses are transmitted in retrograde fashion to the atrium. A circus movement is thus established.

Important Note

Atrial tachycardia can be converted to sinus rhythm by stimulating reflex vagal discharge by pressing on the eye ball (*Oculocardiac Reflex*). A-ch liberated at vagal endings depresses conduction in the atrial muscle fibers and AV node.

III. MYOCARDIAL INFARCTION (MI)

Introduction

Occlusion of coronary artery (either by thrombus or embolism)

↓ *leads to*

Myocardial ischaemia (myocardial hypoxia)

↓ *leads to*

Accumulation of 'P' factor within myocardial tissue

↓

Substernal pain radiating to inner border of left arm, called ***Angina Pectoris***. Thus, *angina is the cry of the dying myocardial fibers.*

Important Note

Anginal pain is increased by cold or after meals. The pain is frequently described as hot, pressing and constricting and usually lasts for only a few minutes

If myocardial ischaemia is prolonged or severe enough to cause death of myocardial cells (*i.e. aseptic necrosis of the myocardium*), it is called as ***Myocardial Infarction***. In this condition there occurs the irreversible damage to the myocardium. E.C.G. is done to diagnose and localise the area of infarction, and to find the duration of infarct.

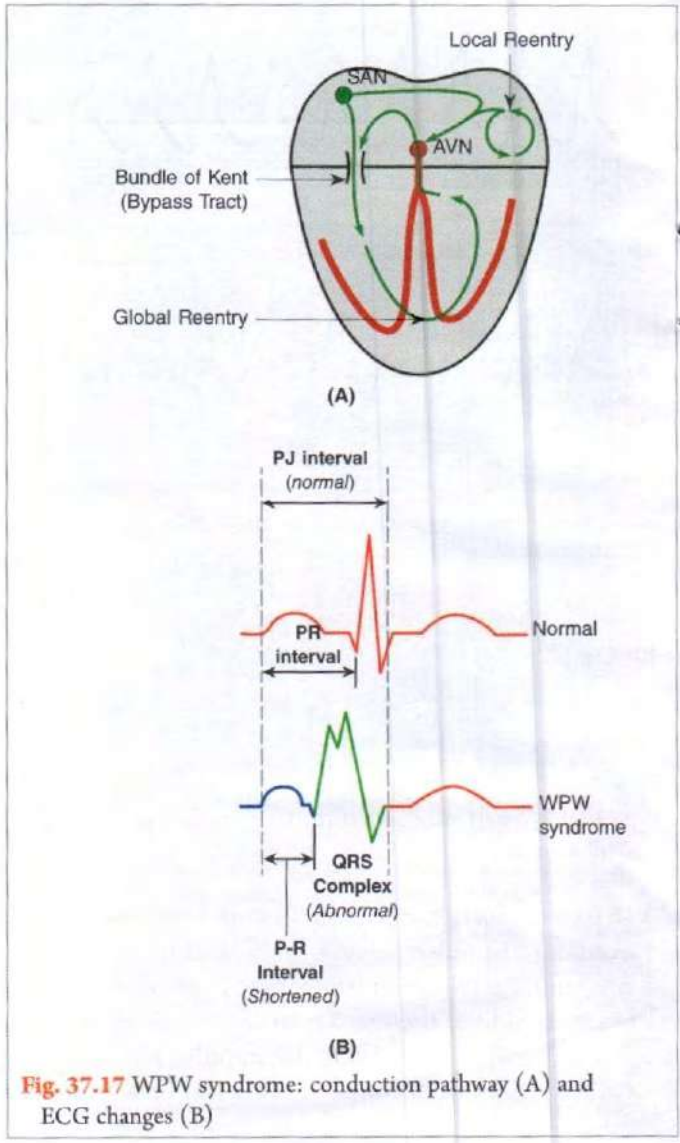


Fig. 37.17 WPW syndrome: conduction pathway (A) and ECG changes (B)

Diagnosis of M.I. (E.C.G. findings)

The ***hallmark*** of 'Acute MI' is **(Fig. 37.18)**

(1) *elevation of ST segment in the leads overlying the area of infarct*, and
(2) *depression of ST segment in the reciprocal leads.*

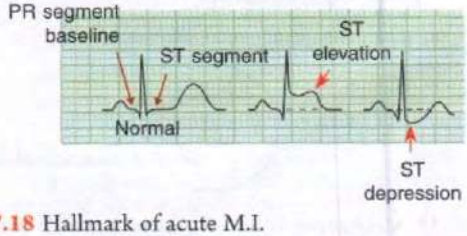


Fig. 37.18 Hallmark of acute M.I.

Location of MI (Also refer to Fig. 37.20)

Type of infarct	Leads overlying area of M.I.	Changes in reciprocal leads
1. Anterior MI	I, aV_L, V_{3-5}	II, III, aV_F
2. Posterior (inferior) MI	II, III, aV_F	I, aV_R, aV_L, V_{1-6}
3. Lateral MI	I, aV_L, V_6	II, III, aV_F, V_1
4. Septal MI	V_{1-3}	–

Mechanism of development of E.C.G. changes (Fig. 37.19)

1. *Normal myocardial area at rest* is in polarized state *i.e.* inside is negative with reference to outside and resting membrane potential (RMP) is approx. –90 mV.

2. *Infarcted area at rest*
Decrease in RMP of infarcted cells during diastole (*i.e.* at rest) due to breakdown of membrane. This produces: (i) increase K^+ efflux and (ii) increase Na^+ influx. Therefore, inside of infarcted cells becomes less negative (shown as positive in figure) compared to surrounding normal area. As a result, the resultant extracellular current flows into the infarct.

3. *During depolarization*
The infarcted area gets *slowly depolarized* compared

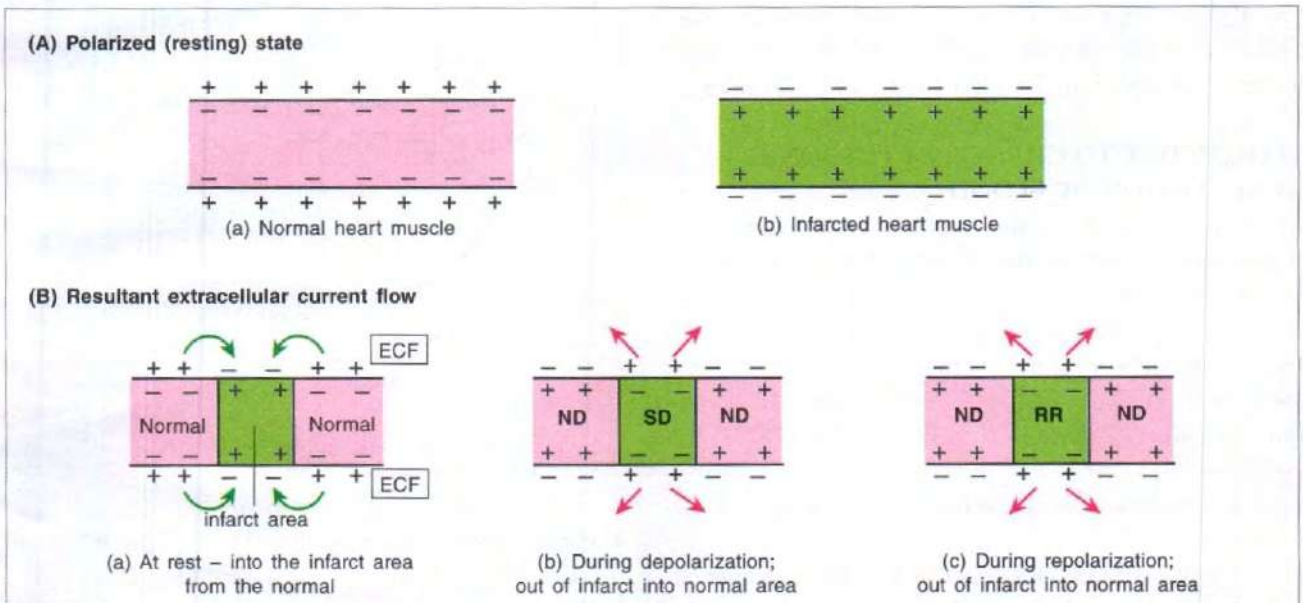


Fig. 37.19 Mechanism of development of ECG changes in Myocradial infarction (M.I.) (ND and SD: Normal and slow depolarization; RR: Rapid repolarization)

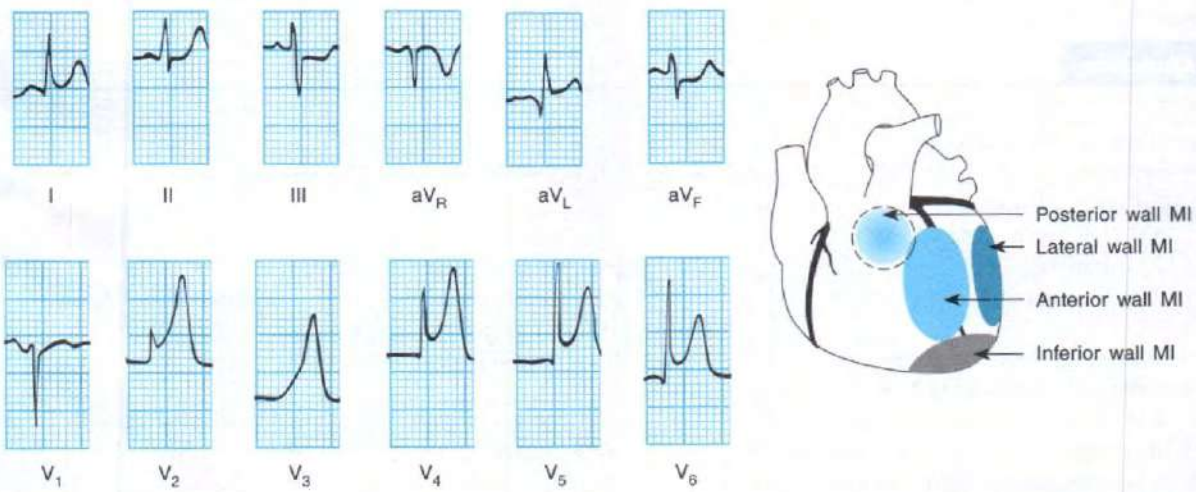


Fig. 37.20 E.C.G. change following anterior wall M.I. (Myocardial Infarction) (**Inset:** Location of MI)

to normal surrounding area. Because of delayed depolarization of infarcted area, the inside of infarcted cells attains positivity later than the normal cells *i.e.* it remains negative compared to adjacent positive area, so the resultant extracellular current flows out of the infarct.

4. *During repolarization*

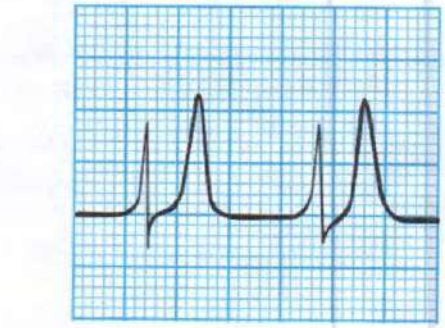
The infarcted cells get *rapidly repolarized i.e.* (inside becomes negative) due to accelerated opening of K^+ channels causing increase K^+ efflux resulting in rise of membrane potential, whereas the surrounding normal cells are still in depolarized state.

Thus the resultant extracellular current flows out of infarct during depolarization and repolarization. The flow of the current towards the recording electrode over the infarcted area causes increased positivity between 'S' and 'T' waves which is recorded as ST elevation.

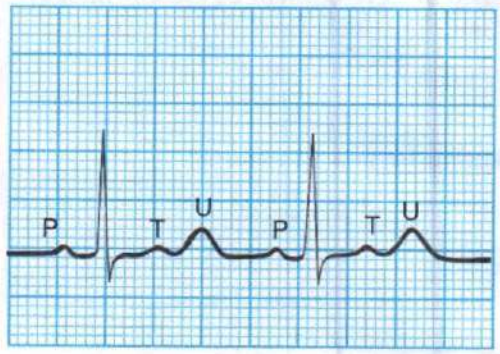
IV. EFFECTS DUE TO CHANGE IN THE IONIC COMPOSITION OF BLOOD

The electrical activity of the heart depends upon the distribution of ions across the myocardial cell membranes. Therefore, alteration in ECF ionic concentration is associated with abnormalities in the ECG. **(Fig. 37.21)**

1. ***Decrease in Na^+*** concentration in ECF is associated with low voltage ECG.
2. ***Hyperkalemia:*** increase $[K^+]$ in ECF (page 178) produces:
   (i) QRS complex is prolonged and abnormal in appearance.
   (ii) T-wave is tall and peaked due to altered repolarization of myocardial cells.
3. ***Hypokalemia:*** decrease $[K^+]$ in ECF produces:
   (i) PR interval prolongation
   (ii) ST segment depression
   (iii) T-wave inversion
   (iv) Prominent U wave.
4. ***Hypercalcemia:*** increase $[Ca^{2+}]$ in ECF produces ***calcium rigor*** (page 178).
5. ***Hypocalcemia:*** decrease $[Ca^{2+}]$ in ECF causes prolongation of ST segment, as a result QT interval also increases.



(A)



(B)

Fig. 37.21 ECG changes during hyper (A); and hypokalemia (B)

Study Questions

1. **Give physiological basis of:**
   (i) Excitation of ventricles during bundle branch block?
   (ii) New rhythm centres originating within the heart.
   (iii) Flutter and fibrillation.
   (iv) Atrial echo beat.
   (v) ECG changes in acute MI.
   (vi) Volume conductor
   (vii) Electrocardiogram
   (viii) Cardiac vector
   (ix) Idioventricular rhythm
   (x) Extrasystole
   (xi) Arrhythmia
   (xii) Augmented limb leads
   (xiii) Modified Einthoven triangle
   (xiv) Angina pectoris
2. **Draw labelled diagram to show:**
   (i) Normal ECG
   (ii) Pattern of ventricular complexes in chest leads
   (iii) The normal direction of mean QRS vector
   (iv) ECG changes in WPW syndrome
   (v) ECG changes during hyper and hypo-kalemia.
   (vi) SAN and AVN block
   (vii) Ventricular extrasystole
   (viii) Atrial arrhythmias
   (ix) Mechanism of developmen of circus movements
   (x) Acute myocardial infarction

3. **What will happen and why if?**
   (i) 3° heart block sets in suddenly? (ii) Prolonged myocardial ischaemia occurs?
   (iii) RBBB developes (iv) Development of irritable focus within the substance of heart
   (v) Additional conducting bundle develop between atrial and ventricles
4. **Write short notes on:**
   (i) PR interval (ii) QRS complex and its significance
   (iii) Complete heart block (iv) Wencheback phenomenon
   (v) Stokes-Adams syndrome (vi) Myocardial infarction
   (vii) WPW syndrome (viii) Atrial echo beat
   (ix) Extra systole and compensatory pause
5. **Explain/Justify:**
   (i) $aV_R = 3/2\,V_R$ (ii) Lead II = Lead I + Lead III
6. **Why is there a need to record ECG using 12 leads?**
7. **How many extrasystoles per minute are regarded as normal? Give its common causes.**
8. **Which is more fatal and why?:**
   (i) flutter or fibrillation (ii) atrial fibrillation or ventricular fibrillation.
9. **Mention the role of ECG in MI.**
10. **Give characeristic features of ECG record in unipolar leads.**
11. **Give physiological significance of:**
    (i) J-point (ii) Augmented limb leads
    (iii) ECG in lead II (iv) Abnormal axis deviation
    (v) Oculocardiac reflex

MCQs

1. **An ECG gives information about all of the following functions *except*:**
   (a) Contractility (b) Conductivity (c) Electrical activity (d) Site of pacemaker
2. **Which of the following is *not* recorded in ECG?**
   (a) Atrial depolarization (b) Atrial repolarization (c) Ventricular depolarization (d) Ventricular repolarization
3. **ST segment:**
   (a) Extends from end of 'S' wave to end of 'T' wave (b) Extends from the end of 'S' wave to the beginning of 'T' wave
   (c) Represents ventricular depolarization (d) Normal duration is 0.32 sec.
4. **The PR interval in an E.C.G. is measured by finding the interval between the:**
   (a) Beginning of the P wave and the beginning of the R wave
   (b) Beginning of the P wave and the beginning of the QRS complex
   (c) End of the P wave and the beginning of the QRS complex
   (d) End of the P wave and the end of the QRS complex
5. **A decrease in the velocity of impulse conduction through the A-V node will usually cause:**
   (a) The PR interval to increase (b) The PR interval to decrease
   (c) Disappearance of the T-wave (d) Increased heart rate
6. **If duration of PR interval is less than 0.13 sec, most probable diagnosis is:**
   (a) Heart block (b) It is regarded as normal
   (c) Cardiac impulse has arisen in the SA node (d) Cardiac impulse has arisen in the AV node
7. **Which of the following description of electrocardiographic leads V_1 through V_6 is *true*?**
   (a) They are unipolar leads measuring electric potential in the frontal plane
   (b) They are unipolar leads measuring electric potential in the horizontal plane
   (c) They are bipolar leads measuring electric potential in the frontal plane
   (d) They are bipolar leads measuring electric potential in the horizontal plane
8. **Changes in the lateral wall of the left ventricle are usually detected by examining which of the following ECG leads?**
   (a) V_1, V_2, V_3 (b) V_4, V_5, V_6 (c) I, II (d) II, aV_F
9. **Which of the following statements about the second-degree heart block is *correct*?**
   (a) The PR interval is shortened (b) Sino-atrial nodal conduction is slower
   (c) AV nodal conduction is completely interrupted (d) Not all atrial impulses reach the ventricles

10. Stokes Adams syndrome is loss of consciousness following:
(a) Head injury (b) Complete heart block (c) Ventricular tachycardia (d) Ventricular fibrillation

11. How many extrasystoles per minute are regarded as normal?
(a) 2-4 (b) 4-6 (c) 6-8 (d) 8-10

12. An irregular, rapid heart rate with abnormal QRS complexes and no P waves suggests:
(a) Sinus arrhythmia (b) Second degree heart block (c) Paroxysmal tachycardia (d) Atrial fibrillation

13. Which of the following is a very serious and emergent condition?
(a) Paroxysmal atrial tachycardia (b) Paroxysmal ventricular tachycardia
(c) Atrial fibrillation (d) Ventricular fibrillation

14. The hallmark finding in the ECG of acute myocardial infarction is:
(a) Depression of the ST segment in the leads overlying the area of infarct
(b) Elevation of the ST segment in the leads overlying the area of infarct
(c) Appearance of Q wave in the leads in which normally it is absent
(d) The absence of Q wave in the leads in which normally it is present

15. Myocardial infarction differs from angina pectoris, the former is:
(a) Cry of dying myocardial fibers
(b) Reversible damage to the myocardium
(c) Produced by transient occlusion of coronaries supplying the myocardium
(d) Aseptic necrosis of the myocardium

16. The Q wave is due to:
(a) Atrial depolarization (b) Depolarization of interventricular septum
(c) Depolarization of major portion of both the ventricles (d) Depolarization of base of ventricles

17. Normal QRS complex is approx.:
(a) 0.02 sec (b) 0.04–0.06 sec (c) 0.08–0.12 sec (d) 0.1–0.15 sec

18. Prolonged P-R interval indicates increased:
(a) Sympathetic tone to SA node (b) Parasympathetic tone to SA node
(c) Parasympathetic tone to AV node (d) Sympathetic tone to AV node

19. Characteristic feature of unipolar precardial leads is:
(a) R wave in V_1 and S wave in V_6 represent left ventricle activity
(b) R wave in V_6 and S wave in V_1 represent right ventricle activity
(c) R wave in V_6 and S wave in V_1 represent left ventricle activity
(d) In lead V_3, R wave is equal to S wave and reflects the electrical activity of the inferior surface of the heart

20. 1° heart block differs from 2° heart block in that, in 1° heart block:
(a) All the atrial impulses are not conducted to the ventricles
(b) Atrial:Ventricular rate :: 2:1 or 3:1
(c) Gradual lengthening of PR interval until one ventricular beat is dropped
(d) PR interval is abnormally long with atrial:ventricular rate :: 1:1

21. What is *not true* for ventricular extrasystole?
(a) Fails to produce radial pulse (b) Hints at serious heart ailment
(c) Associated with abnormal QRS complex (d) Tendency to be followed by a compensatory pause

22. Fibrillating ventricles is a serious emergent condition because:
(a) Ventricles contract at a very high rate (b) Work of ventricle increases markedly
(c) Ventricles cannot pump blood effectively (d) Ventricles show incoordiniated contraction

23. Circus movements in the heart can be caused by all of the following *except*:
(a) Increased myocardial conduction velocity (b) Increased myocardial refractory period
(c) Damage to the purkinje system (d) During sinus bradycardia

24. Wolff-Parkinson-White Syndrome is characterised by:
(a) Prolongation of PR interval (b) QRS deflection shortens with slurred up stroke
(c) Presence of irritable ectopic focus in the ventricles (d) Normal PJ interval

Answers

1. (a) 2. (b) 3. (b) 4. (b) 5. (a) 6. (d) 7. (b) 8. (b) 9. (d) 10. (b) 11. (a) 12. (d) 13. (d) 14. (b) 15. (d)
16. (b) 17. (c) 18. (c) 19. (c) 20. (d) 21. (b) 22. (c) 23. (a) 24. (d)

Chapter 38

General Principles of the Circulation

I. Introduction
   (A) Functions of the circulation
   (B) Functions of the heart
   (C) Pressure changes in the vascular system
   (D) Structure of the blood vessels: vasculogenesis, angiogenesis
II. Organisation and Functions of the Vascular System
III. Dynamics of Blood Flow; Biophysical consideration

INTRODUCTION

A. FUNCTIONS OF THE CIRCULATION

1. Supplies the tissues of the various organs with nutrient substances, for example: O_2, carbohydrates, amino acids, fats, hormones and immunological agents.
2. Removes waste products of tissue metabolism.
3. Controls blood flow to the skin and limbs to regulate heat loss to the environment.
4. Aids in body's defence mechanisms by delivering antibodies, platelets and leucocytes to affected areas of the body.

Note

The normal average circulation time (arm to tongue) is 15 secs.

B. FUNCTIONS OF THE HEART

1. Heart possesses 4 chambers (2 atria and 2 ventricles). Blood enters the left atrium (LA) and left ventricle (LV) at a pressure near zero. The 'LV' pumps the blood into ***systemic circulation*** via the main distributing artery, the *aorta*. Aorta divides into arteries which undergo successive divisions finally forming *arterioles* (100-500 µm diameter). These in turn subdivide into ***metarterioles*** and lead via *pre-capillary sphincter* to *capillaries*. Capillaries distribute the blood finally to 'venules' and thence to the 'veins' which return the blood to the right atrium (RA) and right ventricle (RV) via great veins (inferior and superior vena cavae: IVC and SVC).
2. 'RA' and 'RV' receive blood which has returned from the tissues via the veins and which reaches the 'RA' at a pressure only slightly above 'zero'. The 'RV' pumps the blood into ***pulmonary circulation*** via pulmonary artery which like aorta divides into arterioles, metarterioles and finally leads via pre-capillary sphincter into pulmonary capillaries. Pulmonary capillaries are only one cell thick and facilitate exchange of gases across them. The oxygenated blood is then collected from the pulmonary capillaries by pulmonary venules and veins and is transported to the 'LA' and 'LV'.
3. Heart provides the pressure for the circulation. How? Blood flows from the heart to the periphery and thence via the veins back to the heart only because of pressure gradient.

C. PRESSURE CHANGES IN THE VASCULAR SYSTEM

Pressure changes in the systemic and pulmonary circulation are given in Table 38.1.

Blood enters the LA or RA at a pressure near 'zero' which rises to 7-8 mmHg in LA and 4-6 mmHg in RA during systole. Pressure changes in the arterial system (ventricles, aorta, pulmonary artery and their large branches) is ***pulsatile*** in nature *i.e.* pressure rises to a peak value during systole and decreases to minimum during diastole. However, the pressure thereafter at the periphery is ***steady*** pressure. This is due to the elastic recoil of the arterial system and resistance to blood outflow offered by the arterioles. (Fig. 38.1)

Important Note

Based on the pressure changes, the whole of the vascular system is broadly divided into *high pressure* (> 25 mm Hg) and *low pressure systems* (upto 25 mm Hg) (Fig. 38.1). High pressure system is responsible for the control of systemic arterial pressure and distribution of blood flow; whereas low pressure system is responsible for the control of blood volume and venous return.

Table 38.1: Pressure changes in the vascular system in mmHg

Systemic circulation		Pulmonary circulation	
1. Left atrium	7-8/zero	1. Right atrium	4-6/zero
2. Left ventricle	120/zero	2. Right ventricle	25/zero
3. Aorta and its larger branches	120/70	3. Pulmonary artery and its larger branches	25/8
4. Arterioles	60	4. Arterioles	10
5. Metarterioles	40	5. Metarterioles	8
6. Capillaries	25	6. Capillaries	6-8
7. Venules and larger veins	10	7. Venules and larger branches	5
8. Vena cava (superior and inferior)	2	8. Pulmonary veins	2

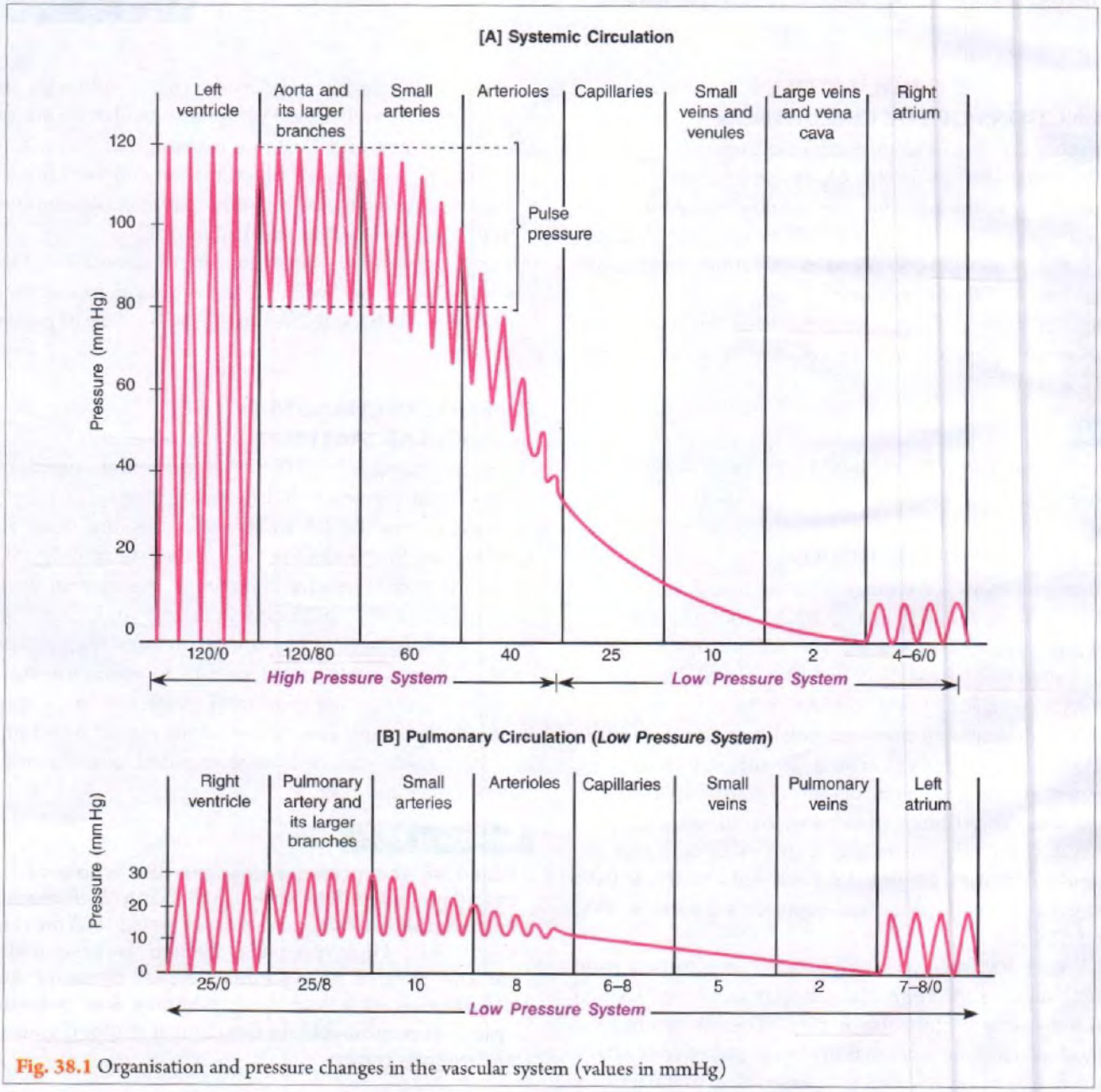


Fig. 38.1 Organisation and pressure changes in the vascular system (values in mmHg)

D. STRUCTURE OF THE BLOOD VESSELS

In general, all blood vessel walls, except the capillaries, have 3 coats: (Fig. 38.2)

1. *Tunica intima:* It is the innermost coat and consists of a single continuous layer of endothelial cells. This is separated from the tunica media by the acellular internal elastic lamina.
2. *Tunica media:* It is the middle coat and consists of smooth muscle cells which are innervated by sympathetic nerve fibers. It produces the mechanical strength of the blood vessel.
3. *Tunica adventitia:* It is the outermost coat and is made up of collagen and fibroblasts.

Vasculogenesis and Angiogenesis

1. During embryonic development, some blood vessels are formed by *angioblast (vasculogenesis)* and some are formed by budding from existing blood vessels *(angiogenesis)*. The angiogenesis is the major form of formation of new blood vessels in adults.
2. The formation of new blood vessels is important for:
   (i) normal body growth,
   (ii) wound healing,
   (iii) formation of new endometrium after mensturation, and
   (iv) formation of corpus luteum after ovulation (page 802).
3. Factors affecting angiogenesis
   (i) *Stimulators*
      (a) Growth factors (GF)
      (b) Platelet-derived endothelial growth factor (PDGF) (pages 92 and 127)
      (c) Vascular endothelial growth factor. (VEGF)
      (d) Interleukins (page 127) (IL)
      (e) Tumour necrosis factor (page 127). (TNF)
   (ii) *Inhibitors*—mainly platelet factor 4 (page 91).

ORGANISATION AND FUNCTIONS OF THE VASCULAR SYSTEM

The above mentioned functions of the circulation are achieved because the vascular system comprises the following types of the blood vessels:

(A) Windkessel (distensible) vessels
(B) Resistance vessels
(C) Exchange vessels
(D) Capacitance vessels, and
(E) Shunt (thoroughfare) vessels.

A. WINDKESSEL VESSELS or DISTENSIBLE VESSELS

These are the vessels which are highly elastic (*windkessel means elastic reservoir*). For example, *aorta, pulmonary artery and their larger branches.*

As heart contracts intermittently at a rate of 70-80 beats per minute (bpm), therefore, pressure and blood flow in the arteries and their large branches from the heart is *pulsatile.* This pulsatile ejection of the heart must be converted into *steady* outflow through the capillaries to get the maximum exchange between blood and tissues. This is achieved by the:

(1) Elastic recoil of the arterial system *i.e.* the stretch produced during cardiac contraction on the walls of the elastic tissues of the aorta and its branches comes back to regain its original position during the diastolic phase (***Windkessel effect***) (Fig. 38.3).

(2) Resistance to outflow offered by the peripheral arterioles.

Important Note

The degenerative changes which set in the tunica media of these vessels with aging cause loss of arterial wall elasticity and *Windkessel effect* decreases. Thus, with aging, (1) systolic blood pressure (SBP) increases, and (2) diastolic blood pressure (DBP) decreases, as extra blood leaves the aorta very rapidly. This results in increase pulse pressure (SBP minus DBP) resulting in defective perfusion at the periphery.

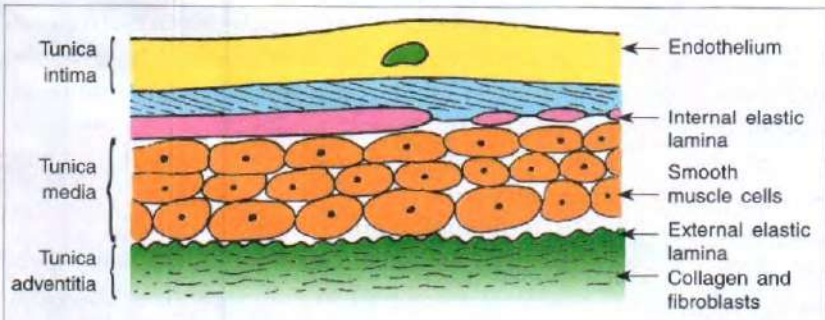


Fig. 38.2 Cross-section of a blood vessel wall

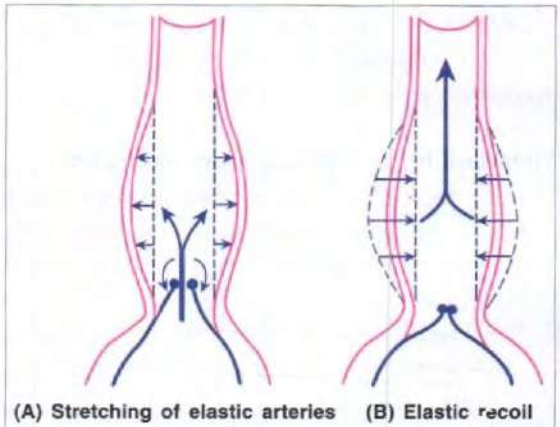


Fig. 38.3 Windkessel effect

B. RESISTANCE VESSELS

These are the vessels which offer resistance to blood flow towards the capillaries, therefore, also called as *pre-capillary resistance vessels*. For example: ***arterioles, metarterioles and pre-capillary sphincters.***

Arterioles

1. They offer maximum resistance to blood flow towards the capillaries and thus are the *main site of* ***peripheral resistance.***
2. They decrease the hydrostatic pressure within the capillaries, therefore, when arterioles get damaged, marked transudation of fluid occurs across the capillary wall causing decrease in blood volume.
3. Resistance to blood flow (R) is inversely proportional to the fourth power of the radius (r) of arterioles, (*i.e.* $R \propto 1/r^4$). Therefore,
   (i) if radius is decreased to half, resistance to blood flow increases by 16 times; or
   (ii) if radius is increased to 2 times, resistance to blood flow decreases by 16 times *i.e.* 6% of its previous value.
   Physiological significance: That is why, blood flow to the different body organs can be so effectively regulated by small changes in the caliber of the arterioles; and variations in arteriolar diameter have such a pronounced effect on the systemic blood pressure.
4. They are less elastic but highly muscular and show an efficient local myogenic control of their own vascular radius. Superimposed on myogenic tone is an extrinsic neural control effected by sympathetic constrictor nerves. These nerves normally discharge @ 1 impulse/sec. This rate of impulse discharge increases to 10-16 impulse/ sec in *maximum vasoconstriction* (*e.g.* in haemorrhage); and decreases to 'zero' in *maximum vasodilatation* (*e.g.* during heat stress).
5. Resistance to blood flow by different organs of the body varies.

The skin and skeletal muscle blood vessels represent by far the ***most important site of peripheral resistance*** *and offer maximum resistance to blood flow.*

Metarterioles and pre-capillary sphincters

1. They determine the size of the capillary exchange area at one particular moment in the tissue. For example, increase in sphincter patency, increases number of open capillaries.
2. Their radius is controlled both by:
   (i) Neurogenic factors *i.e.* sympathetic constrictor nerves; and
   (ii) Local concentration of tissue metabolites, *i.e.*
      (a) *Local constrictors:* serotonin (5-HT); fall in temperature;
      (b) *Local dilators:* hypoxia (oxygen lack); hypercapnia (accumulation of CO_2); acidemia (fall in blood pH); increase in body temperature; K^+; lactic acid and adenosine etc.

C. EXCHANGE VESSELS

These are the vessels which allow easy exchange of gases and nutritive substances across them. For example, *capillaries.*

1. They consist of only single layer of endothelial cells.
2. Each ideal capillary is 500 μm in length; 1 μm thick and 6 μm in diameter with cross-sectional area of 30 μm^2.
3. Total number of systemic capillaries is approx. 50,000 millions. However, at rest only 25% of capillaries are patent (open).
4. Total cross-sectional area of capillary bed if fully patent is 4000 times that of aorta.
5. Vital organs like heart, brain, kidneys, liver, lungs etc. possess a dense capillary network (400-500 capillaries/μL). Rest of the body tissues show much smaller density than this.
6. *True Capillaries* do not have smooth muscles. Thus they are **not** controlled by either nervous or metabolic factors. It is the alteration in the tone of pre-capillary sphincters which determines the number of capillaries patent and hence the surface area available for exchange between blood and interstitial fluid.
7. Electron microscopic studies classified the capillaries into 3 ***types***: **(Fig. 38.4)**
   (i) ***Continuous or Non-fenestrated capillaries***
   Here single layer of endothelial cells is almost continuous except at the intercellular regions which is 10 nm wide. Some of the intercellular spaces are traversed by channels approx. 4 nm wide. These capillaries allow exchange of fluid and lipid soluble substances within interstitial fluid by simple diffusion (page 14). Most of body capillaries are of this type.
   (ii) ***Fenestrated capillaries***
   Here, fenestrations or pores (20–100 nm in diameter) seen are intracellular openings which serve the function of rapid and large transudation of fluid. *Sites:* Renal glomeruli, vasa recta of renal medulla, endocrine and exocrine glands, choroid plexuses, intestinal villi.
   (iii) ***Discontinuous capillaries or Sinusoids***
   These capillaries possess a thin endothelial layer with large gaps between individual cells. Therefore, allow easy exchange of large protein molecules and RBCs. *Sites:* bone marrow, liver and spleen.

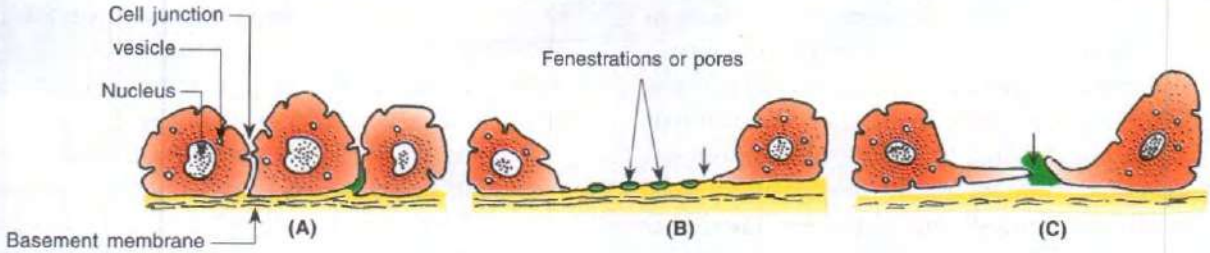


Fig. 38.4 Classification of capillaries: Continuous or non-fenestrated (A); Fenestrated (B) and Discontinuous or sinusoids (C)

D. CAPACITANCE VESSELS

These are the vessels which simply by change in their luminal shape can accommodate large volume of blood per unit length without much increase in the transmural pressure (pressure across the wall). For example, ***venules and venous compartments.***

1. They are collapsible and assume an oval profile (shape) when the transmural pressure falls below 6 cm H_2O. By simply change in their luminal shape from oval to circular cross-sectional profile, they can accommodate large volume of blood per unit length without much increase in the transmural pressure **(Fig. 38.5)**. Therefore, *they are very important site of change in the capacity of the vascular system.* That is why veins are called ***capacitance vessels.***

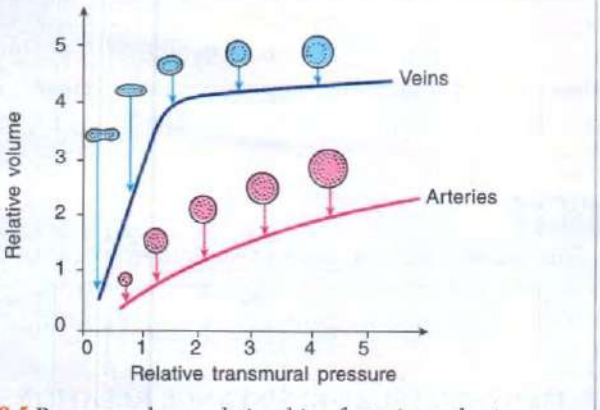


Fig. 38.5 Pressure-volume relationship of arteries and veins

Important Note

At rest, 54% of the circulating blood volume is in systemic veins, 12% in cardiac chambers, 18% in pulmonary circulation, 2% in aorta, 8% in the arteries; 1% in the arterioles and 5% in the capillaries. (Fig. 38.6)

2. Like arterioles, ***myogenic tone*** of veins induced by sympathetic constrictor nerves helps to adjust the capacity of the vascular system. This is specially useful in postural changes (page 385).

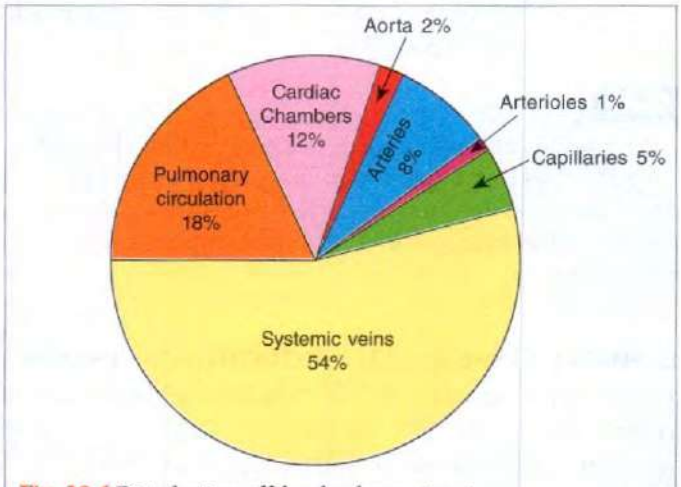


Fig. 38.6 Distribution of blood volume at rest

3. ***Veins***
   (i) *Veins* contain little smooth muscle and are thin walled structure which begin at a diameter of 500 µm. At this junction the endothelial lining first shows internal folds called ***venous valves*** which permit flow of blood only towards the heart.
   (ii) Venous valves are prominent particularly in veins of legs and arms.
   Limb veins are divided into two types:
      (a) *Superficial veins* run in the subcutaneous tissue and possess numerous valves; they are connected to deep veins by 'communicating' veins containing many valves and permit blood flow only from superficial to deep veins during rhythmic contraction of limb muscles.
      (b) *Deep veins* are located within the skeletal muscles and deep body structures.
   (iii) The venae cavae (IVC and SVC), portal veins and cerebral veins do not possess functional valves.
   (iv) *Venous pressure:* It is of 2 types:
      (a) *Central venous pressure (CVP)* = RA pressure
      Blood from all systemic veins flows into right atrium (RA), therefore, RA pressure is called CVP. Normally, it is 4-6 mmHg.

(b) *Peripheral venous pressure (PVP)*

Large veins offer considerable resistance to blood flow because they remain compressed at many points by the surrounding tissues. For example, abdominal veins by different abdominal organs and intra-abdominal pressure; arm veins by first rib and neck veins by atmospheric pressure. Therefore, pressure in these peripheral veins is referred as *peripheral venous pressure*. Normally it is about 10 mmHg. It is affected by the gravity in the similar fashion like the arterial pressure. (for venous pressure in the legs and head, refer to page 348).

Note

Peripheral venous pressure, to a great extent, depends on the level of central venous pressure, therefore, any factor which increases 'RA' pressure *i.e.* central venous pressure will increase the peripheral venous pressure.

E. SHUNT VESSELS or THOROUGHFARE VESSELS

These are the vessels which bypass the capillaries. These vessels directly connect the metarterioles with venules, therefore, also called as ***Arterio-Venous shunts(A-V shunts) or 'A-V anastomoses'*** **(Fig. 38.7)**.

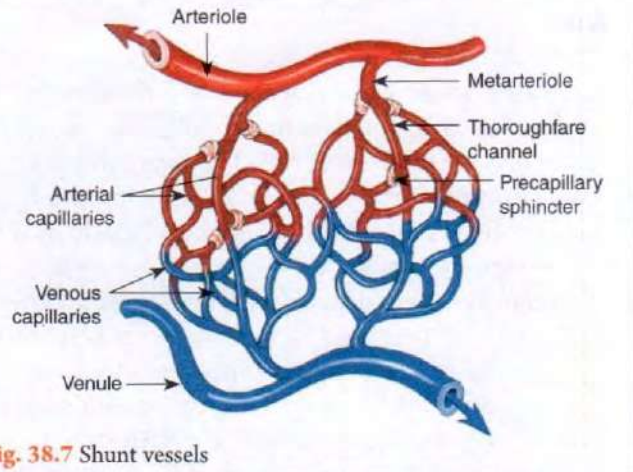


Fig. 38.7 Shunt vessels

1. When patent, these vessels permit a rapid flow of blood which serves no nutritive purpose to tissues concerned.
2. These vessels occur only in few tissues – most notably in the skin. **Important sites** are the areas exposed to maximal cooling. For example, skin, toes, legs, fingers, palms, ear lobes, lips, etc.
3. The walls of these vessels have strong muscular coats innervated by sympathetic constrictor nerves. When fully constricted, they decrease blood flow into veins or when maximally dilated they cause extremely rapid flow of warm blood into the veins.
4. At normal body temperature, sympathetic discharge helps these A-V anastomoses almost closed.
   (i) exposure to heat dilates these vessels, increases blood flow to veins thus promotes heat loss from the body;
   (ii) conversely, exposure to cold constricts these vessels and decreases heat loss from the body.

Thus, these vessels are particularly *helpful during thermal stress*.

DYNAMICS OF BLOOD FLOW (BIOPHYSICAL CONSIDERATIONS)

A. FLOW–CROSS SECTIONAL AREA RELATIONSHIP

It is the relationship between *average velocity of blood flow* to the total *cross-sectional area* of the vascular circuit. **(Table 38.2 and Fig. 38.8)**

It is clear that increase in total cross-sectional area of blood vessels decreases the average velocity of blood flow.

Physiological significance

It is a common experience that if the outlet of a tube through which water is flowing be partially occluded, keeping the flow constant, then velocity of water coming out of the tube increases considerably. Similarly, if the diameter of the blood vessel through which blood is flowing gets narrowed, keeping the rate of flow constant (*i.e.* amount of flow/unit time), the velocity of flow increases.

Note

The average velocity of fluid movement at any point in a system of tubes in parallel is inversely proportional to the total cross-sectional area at that point.

B. FLOW–PRESSURE-RESISTANCE RELATIONSHIP

The relationship among *blood flow* (per unit time), *pressure* and *resistance* to the blood flow in the blood vessel is similar to the relationship between the current (I) (amount of charges flowing per unit time), electromotive force or voltage (E) and resistance (R) in an electrical circuit as given by ***ohm's law*** *i.e.* $I = E/R$.

The same law can be applied to systemic circulation, as 'I' is represented by amount of blood flow per unit time (Q); and 'E' is represented by pressure head at the two ends of the blood vessel (P_1–P_2). Therefore,

$$Q\ (mL/sec) = \frac{P_1–P_2\ (mmHg)}{R} \qquad \ldots (1)$$

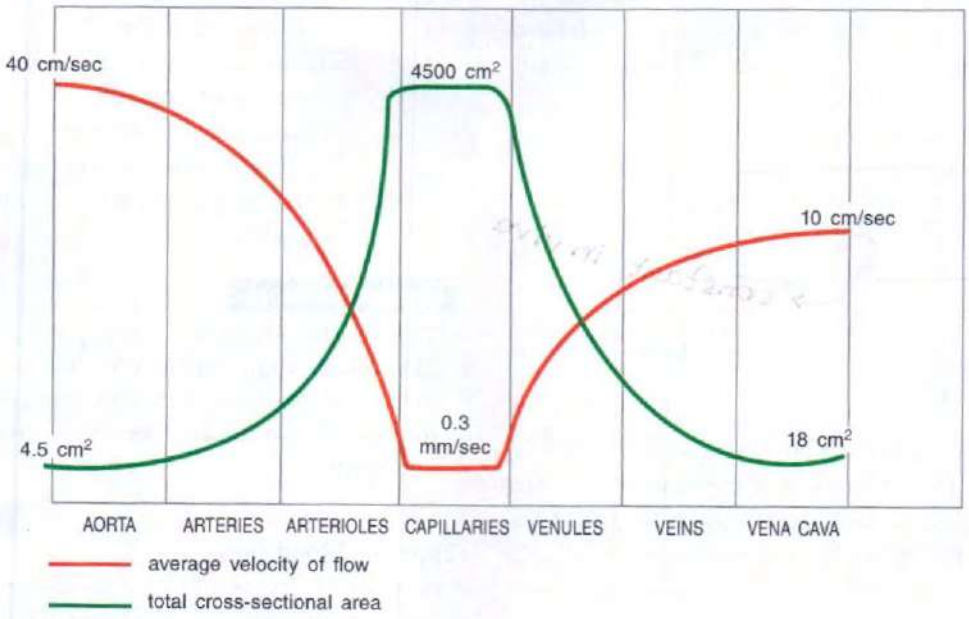


Fig. 38.8 Velocity of flow and cross-sectional area relationship through the systemic circulation (in lying position—when all blood vessels are at heart level)

Table 38.2: Total cross-sectional area and average velocity of blood flow of the vascular system

	Total cross-sectional area	Average velocity of blood flow
1. Aorta	4.5 cm^2	40 cm/sec
2. Small arteries (of diameter 1 mm; approx. number 8000)	72 cm^2 (16 times that of aorta)	1.4 cm/sec
3. Arterioles	400 cm^2	0.5 mm/sec
4. Capillaries (at rest when only 25% of capillaries are patent)	4500 cm^2 (1000 times of aorta)	0.3 mm/sec
5. Venules	4000 cm^2	1-2 cm/sec
6. Small veins	80 cm^2	1-2 cm/sec
7. IVC and SVC	18 cm^2	7-10 cm/sec

Thus, the amount of blood flow per unit time in any portion of the vascular system is determined by the ***effective perfusion pressure*** (*i.e.* mean intraluminal pressure at the arterial end minus mean pressure at the venous end) and the 'resistance' offered to the blood flow.

$$\text{Also, } R = \frac{P_1-P_2 \text{ (mmHg)}}{Q \text{ (mL/sec)}}$$

Therefore, the units of ***peripheral resistance*** (PR) in CVS are: mmHg/mL/sec; also called *peripheral resistance units* (PRU) or ***R-units***.

At rest, pressure difference from systemic arteries to systemic veins is 100 mmHg and rate of blood flow through circulatory system is 100 mL/sec. Thus, 'resistance' of entire systemic circulation, called ***total PR*** is:

$$\frac{100 \text{ mmHg}}{100 \text{ mL/sec}} = 1 \text{ P.R.U.}$$

During *maximum vasoconstriction*, it may increase to 4 PRU and with *maximum vasodilatation*, it may decrease to 0.2 PRU. According to **Poiseuille-Hagen** formula, in vitro, the volume of the fluid flowing per unit time (Q) through rigid tubes of capillary diameter is given by the following equation:

$$Q = \frac{\pi}{8} \cdot \frac{(P_1-P_2)r^4}{L\eta} \quad \ldots (2)$$

where,

P_1-P_2 = pressure difference at the two ends of the tube
r = radius of tube
L = length of tube
η (eta) = viscosity of fluid
$\pi/8$ = derived from the mathematical deduction of the volume passing per unit time

Thus, flow of fluid is directly proportional to the pressure head (P_1–P_2); the 4th power of the radius (r) and inversely related to the length of tube (L) and viscosity of fluid (η).

From (1) and (2) it follows:

$$Q = \frac{P_1-P_2}{R} = \frac{\pi}{8} \cdot \frac{(P_1-P_2)r^4}{L\eta}$$

→ constant in vivo

Therefore,

$$R = \frac{8L\eta}{\pi r^4} \quad \ldots (3)$$

It is evident, from (1), blood flow is an inverse function of resistance; and from (3), the resistance to blood flow depends on L, η and r. However, in vivo, length of the vascular circuit remains constant, therefore, resistance to blood flow is primarily determined by the 'radius' and 'viscosity of blood'.

Resistance to blood flow

Resistance to blood flow is determined by two main factors:

(1) *Radius of resistance vessels* (page 312); and

(2) *Viscosity of blood.*

Viscosity means lack of slipperiness in between the adjacent laminae. The unit of viscosity is ***poise*** (after poiseuille)

Viscosity of water at 21°C
= 0.01 poise (or 1 centipoise: cp)

Viscosity of water at normal body temperature of 37°C
= 0.695 cp, which is taken as 1

Viscosity of plasma at 37°C
= 1.2 cp *i.e. relative viscosity* of 1.7 as compared to water

Viscosity of whole blood (plasma plus cells) at 37°C
= 2.8–3 cp *i.e. relative viscosity* of 4–5

Factors affecting viscosity of blood

(i) Viscosity of blood is ***inversely proportional*** to the:
  (a) *Temperature* within limits; and
  (b) *Flow rate* – at high flow rate, cells occupy the central axis of blood vessel moving with their axes parallel to the direction of flow leaving cell free zone of plasma at the periphery, called *plasma skimming*. This causes least friction between the cells and the plasma and viscosity decreases.

(ii) Viscosity of blood is ***directly proportional*** to the:
  (a) *Haematocrit i.e.* packed cell volume (PCV);
  (b) *Concentration of plasma proteins*, specially increase in high molecular weight proteins *e.g.* Ig.;
  (c) *Diameter of vessel wall:* Relative viscosity of blood decreases when vessel wall diameter falls below 300 μm (arterioles, capillaries and venules). This is because of phenomenon of ***plasma skimming*** in the capillaries. Moreover, capillaries secrete a substance (probably mucopolysaccharide) which further decreases the viscosity of blood in the capillaries.

Important Note

This is why (i) the PCV of capillary blood is approx. 25% lower than the whole blood haematocrit and (ii) haematocrit changes have relatively little effect on the 'peripheral resistance' except when the changes are large.

Types of blood flow

There are two types of blood flow – *laminar (or streamline) and turbulent blood flow.* The main differences between the two are given in **Table 38.3** and **Fig. 38.9**.

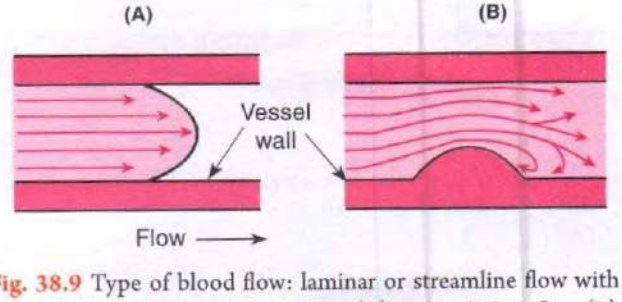


Fig. 38.9 Type of blood flow: laminar or streamline flow with ***parabolic*** distribution of velocities (A) and turbulent flow (B)

Resistance to blood flow is parallel and series vascular circuit **(Fig. 38.10)** and **Table 38.4**.

C. PRESSURE FLOW RELATIONSHIP

The relationship between *mean intraluminal pressure* in the blood vessel to the blood flow.

In rigid tubes there is linear relationship between pressure and flow of homogenous fluid *i.e.* as pressure increases flow increases **(Fig 38.11)** However, in blood vessels, this relationship is not linear but somewhat 'curved' because blood vessels are distensible elastic tubes and show an efficient local myogenic control of their own vascular radius. Furthermore, blood is not a perfect fluid but a system of liquid and cells. Thus, they serve to stabilize the blood flow over a wide range of pressure *e.g.* 80-200 mmHg showing ***autoregulation***. Moreover, in small blood vessels when pressure is reduced, a point is reached at which no flow of blood occurs even though the pressure is not zero. This is so because:

(1) it takes some pressure to force RBCs through capillaries which have diameter less than the RBC, and

Table 38.3: Main differences between Laminar and Turbulent blood flow (Fig. 38.9)

Laminar or streamline blood flow	Turbulent blood flow
1. Here blood flows in large number of layers at a steady rate with each layer remaining at the same distance from the vessel wall, also the central portion stays in the centre of the vessel. It shows *parabolic distribution* of velocities *i.e.* velocity of flow being greater in the centre of the stream.	1. Here blood flows in all directions in the vessel and continuously mixing within the vessel. Thus, it causes a greater energy loss as compared to laminar flow.
2. It is characteristic of most part of the vascular system.	2. Ventricles and aorta are the normal sites of turbulence. None of the small resistance vessels of vascular system show turbulence.
3. It is ***silent*** in nature.	3. It is ***noisy*** flow.
4. Within limits, it shows a linear relationship with pressure.	4. ***Turbulence*** is given by ***Reynold's number (Re)*** = $V\rho d/\eta$, where V = velocity of flow in cm/sec ρ(Rho) = density of blood equal to 1 d = diameter of vessel (in cm) η (eta) = viscosity of blood in poise

Note

Turbulence develops when 'Re' exceeds 2000. This is usually seen following constriction of an artery which increases the velocity of blood flow or in anaemia where viscosity of blood decreases. (If the radius is used in the formula than Re would be 1000.)

Table 38.4 : Resistance to blood flow in parallel and series vascular circuit

Parallel Resistance	Series Resistance
1. *Arrangement of blood vessel:* Each systemic organ is supplied by an artery that branches off the aorta. This permits each organ to regulate its own blood flow independent of flow to other tissues.	Each organ is supplied by a large artery, smaller arteries, arterioles and capillaries. This represent arrangement of blood vessels within a given organ.
2. Total resistance is given by: $\frac{1}{R_{total}} = \frac{1}{R_1} + \frac{1}{R_2} \ldots\ldots\ldots\ldots \frac{1}{R_n}$ 1, 2, ...n are the resistance of the renal, hepatic and other arteries respectively. Thus total resistance is less than the resistance of any single blood vessel.	$R_{total} = R_{artery} + R_{arteriole} + R_{capillaries}$ The total resistance is the sum of the individual resistance

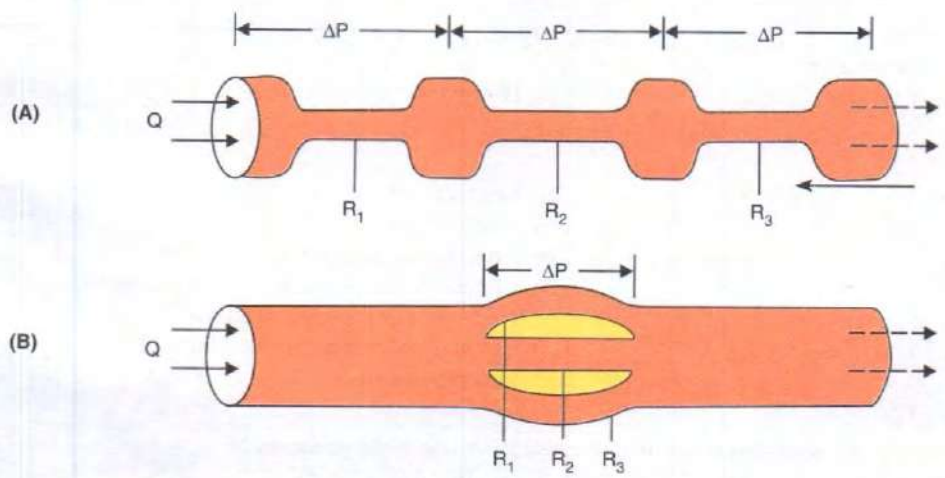


Fig. 38.10 Vascular resistance in series (A) and in parallel (B) (Q: Amount of blood flow per unit time; R_1, R_2, R_3: Resistance(s); ΔP: Change in pressure

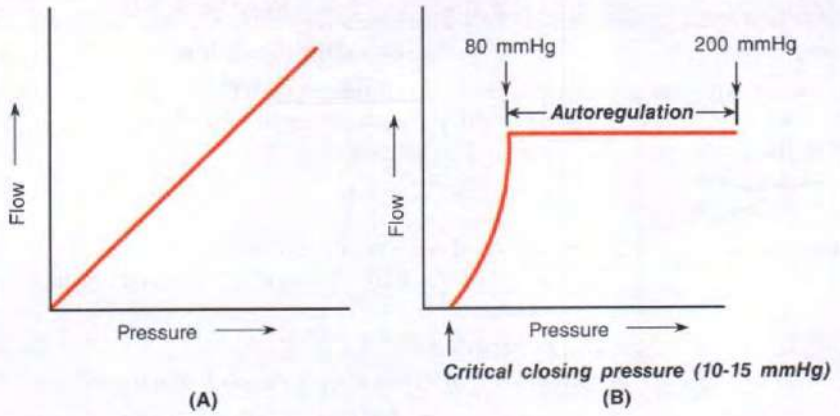


Fig. 38.11 Relation of pressure to flow: in a rigid walled system (A) and in the vascular system (B)

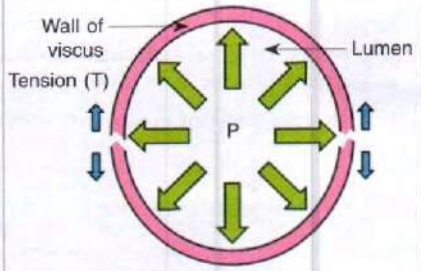


Fig. 38.12 Relation between distending pressure (P) and wall tension (T) in a hollow viscus

(2) extravascular tissues (vessels surrounded by tissues) exert a small but definite pressure on vessels, and when the intraluminal pressure falls below this extravascular pressure, the vessel collapses. The pressure at which flow ceases is called the *critical closing pressure*. Normally it is 10-15 mmHg.

D. LAW OF LAPLACE

It is the relationship between *distending pressure* and tension on the wall in a hollow viscus **(Fig. 38.12)**. It states that the distending pressure (P) in a distensible hollow viscus is equal at equilibrium to the tension in the wall (T) divided by two principal radii of curvature of the viscus (R_1 and R_2). Therefore,

$$P = (T/R_1 + T/R_2)$$

i.e. $$P = T\,(1/R_1 + 1/R_2)$$

In a sphere, such as lung alveoli, $R_1 = R_2$; therefore, $P = 2T/R$. **(Fig. 38.12)**

In a cylinder, such as blood vessel, as one radius is infinite, therefore, $P = T/R$.

Physiological significance

1. Smaller the radius of the blood vessel, lesser the tension in the wall necessary to balance the distending pressure. This is why *the thin walled and delicate capillaries are less prone to rupture.*
2. Dilated heart has to do more work than a non-dilated heart, because when the radius of a cardiac chamber is increased, a greater tension must be developed in the myocardium to produce any given pressure.

(Also refer to pages 574, 223 and 414)

Study Questions

1. **Give physiological significance of:**
   (i) Precapillary resistance vessels
   (ii) Effective perfusion pressure
   (iii) Plasma skimming
2. **Write briefly about:**
   (i) Windkessel vessels
   (ii) Resistance vessel
   (iii) Capacitance vessels
   (iv) Shunt vessels
   (v) Windkessel effect
   (vi) Arterio-venous shunt
   (vii) Reynold's number
   (viii) Critical closing pressure
   (ix) Significance of law of Laplace to CVS
   (x) High and low pressure system.
   (xi) Central and peripheral venous pressure.
   (xii) Laminar and turbulent blood flow.
   (xiii) Venous pressure
   (xiv) Reynold's number
3. **Give physiological basis of:**
   (i) How pulsatile ejection of the heart gets converted into steady outflow at the periphery?
   (ii) How different body organs can precisely autoregulate their own blood flow?
   (iii) Variation in arteriolar diameter has marked effect on the systemic blood pressure.
   (iv) PCV of capillary blood is lower as compared to whole blood.

(v) Mild to moderate variation in PCV has little effect on the peripheral resistance.
(vi) The thin walled and delicate capillaries are less prone to rupture.
(vii) Dilated heart has to do more work than a non-dilated heart.

4. **Name the major vessels in the vascular system and give one main function of each type of vessel.**
5. **Mention most important sites of peripheral resistance. Why are they so called?**
6. **Name the factors influencing the diameter of metarterioles and pre-capillary sphincters.**
7. **What are the types of capillaries in the body? Give the function of each type.**
8. **Depict diagrammatically:**
   (i) Velocity of flow and cross-sectional area relationship through the systemic circulation
   (ii) Pressure volume relationship of arteries and veins
   (iii) Relation of pressure to blood flow in vascular system
   (iv) Relation between distending pressured and wall tension is a hollow viscus
9. **What will happen and why when:**
   (i) Windkessel effect decreases
   (ii) Peripheral resistance increases
   (iii) Blood flow become turbulent

MCQs

1. **Blood enters the atria at a pressure:**
   (a) Near 'zero' mmHg (b) 7-8 mmHg (c) 8-10 mmHg (d) 10-12 mmHg
2. **Pulmonary capillary pressure ranges between:**
   (a) 3-5 mmHg (b) 6-8 mmHg (c) 9-11 mmHg (d) 12-15 mmHg
3. **The pulsatile ejection of the heart which gets converted into steady outflow through the capillaries is achieved by all of the following *except*:**
   (a) Windkessel effect (b) Arteriolar resistance
   (c) Smaller diameter of the capillaries (d) Elastic recoil of the aorta
4. **The most important organ of peripheral vascular resistance is:**
   (a) Skeletal muscles (b) Brain (c) Heart (d) Liver
5. **Total cross-sectional area of capillary bed when fully patent is:**
   (a) 1000 times that of aorta (b) 2000 times that of aorta
   (c) 3000 times that of aorta (d) 4000 times that of aorta
6. **The volume of blood in pulmonary circulation at any given time is:**
   (a) 2% (b) 8% (c) 18% (d) 50%
7. **Sudden standing can cause loss of circulating blood volume upto:**
   (a) 10% (b) 20% (c) 30% (d) 40%
8. **Shunt vessels are particularly helpful:**
   (a) Site of change in the capacity of vascular system
   (b) To allow easy exchange of nutritive substances across them
   (c) During thermal stress
   (d) In controlling systemic arterial B.P.
9. **Assuming all other factors are constant, the ratio of flow in a vessel 1.0 cm in diameter to the flow in a vessel 0.1 cm diameter is:**
   (a) 10 : 1 (b) 100 : 1 (c) 1000 : 1 (d) 10000 : 1
10. **Total peripheral vascular resistance of entire systemic circulation at rest is:**
    (a) 1 PRU (b) 2 PRU (c) 3 PRU (d) 4 PRU
11. **As a function of vessel size, the resistance to fluid flow in a vessel is:**
    (a) Directly proportional to the square of the radius (b) Directly proportional to the fourth power of the radius
    (c) Inversely proportional to the fourth power of the radius (d) Inversely proportional to the square of the radius
12. **Effect of drinking 1 L of normal saline on peripheral resistance will be:**
    (a) No change (b) Increases
    (c) Decreases (d) First increases then decreases

13. **Changes in PCV have no effect on blood viscosity in the capillaries because:**
    (a) It is difficult to get the blood from the true capillaries
    (b) Haematocrit of capillary blood is approx. 25% lower compared to whole blood haematocrit
    (c) Capillaries have small diameter (less than 6 μm)
    (d) Capillaries consist of only single layer of endothelial cell
14. **Critical closing pressure is defined as:**
    (a) The pressure in the ventricles at which A-V valve closes
    (b) The pressure in the ventricles at which semilunar valve closes
    (c) The pressure in small blood vessels at which flow ceases
    (d) It is the pressure within the alveoli at which small airway closes
15. **The thin walled and delicate capillaries are less prone to rupture because:**
    (a) Extravascular tissues exert a small but definite pressure on them
    (b) The pressure within the capillaries is almost zero
    (c) The pre-capillary sphincters offer a great resistance to blood flowing towards the capillaries
    (d) Capillaries being smaller in diameter, less tension on the wall is necessary to balance the distending pressure.
16. **The pressure (mmHg) in left ventricle in diastole is:**
    (a) 0 (b) 25 (c) 80 (d) 120
17. **High pressure system is responsible for:**
    (a) Control of blood volume
    (b) Control of venous return to the heart
    (c) Control of distribution of blood flow
    (d) Conversion of pulsatile ejection of heart into steady flow through the capillaries
18. **Angiogenesis:**
    (a) Is the major form of formation of new blood vessels in adults
    (b) Refers to formation of some blood vessels by angioblast
    (c) Refers to formation of new blood ves-sels during embryonic development
    (d) Inhibited by interleukins
19. **The resistance of a blood vessel is 16 PRU. Doubling the vessel diameters would change the resistance to:**
    (a) 1 PRU (b) 2 PRU (c) 8 PRU (d) 12 PRU
20. **Caliber of the arterioles is increased by all of the following *except*:**
    (a) Circulating angiotensin II (b) Kinins
    (c) Increased pCO_2 (d) Decreased pH
21. **Capillary diameter is precisely controlled by:**
    (a) Alteration in the tone of pre-capillary sphincters
    (b) Efficient local myogenic tone
    (c) Extrinsic sympathetic constrictor nerves superimposed on myogenic tone
    (d) Local concentration of tissue meta-bolites
22. **Most of the body capillaries are:**
    (a) Non-fenestrated type (b) Fenestrated type (c) Discontinuous type (d) Sinusoids
23. **Central venous pressure is the value of blood pressure found in the:**
    (a) Inferior vena cava (b) Superior vena cava (c) Pulmonary artery (d) Right atrium
24. **According to Poiseuille Hagen formula, the relationship is calculated among the following *except*:**
    (a) Flow in a long narrow tube (b) Viscosity of fluid
    (c) Radius of tube (d) pH of blood

Answers

1. (a)	2. (b)	3. (c)	4. (a)	5. (d)	6. (c)	7. (b)	8. (c)	9. (d)	10. (a)
11. (c)	12. (a)	13. (b)	14. (c)	15. (d)	16. (a)	17. (c)	18. (a)	19. (a)	20. (a)
21. (a)	22. (a)	23. (d)	24. (d)						

Chapter 39

Cardio-Vascular Regulatory Mechanisms

I. Local autoregulatory mechanisms
Basal Myogenic Tone; Role of endothelial cells (Nitric oxide, endothelins)
II. Systemic regulatory mechanisms
(A) Chemical
(B) Neural
Autonomic (Cardiac and Peripheral vascular innervation)
Medullary (VMC and cardiac Vagal centre)
• Baroreceptors • Chemoreceptors • Corticohypothalamic descending pathways

INTRODUCTION

1. *Main functions of cardiovascular regulatory mechanisms* are:
   (i) increase in blood supply to active tissues;
   (ii) increase or decrease in heat loss from the body by redistributing the blood;
   (iii) in emergencies *e.g.* haemorrhage etc. maintain the blood flow to vital organs at the expense of the circulation to the rest of the body.
2. These mechanisms bring out circulatory adjustments primarily by:
   (i) altering the diameter of the arterioles and thus change the hydrostatic pressure in the capillaries;
   (ii) altering blood storage in venous reservoirs, and
   (iii) altering the rate and stroke volume of the heart.
3. The mechanisms are broadly divided into ***two types***:
   (i) *Local 'autoregulatory' mechanisms*. They operate to maintain blood flow through an organ at relatively constant level despite fluctuations in perfusion pressure.
   (ii) *Systemic regulatory mechanisms (chemical and neural)*. These mechanisms operate along with the local mechanisms to adjust vascular responses throughout the body.
4. The terms ***vasoconstriction*** and ***vasodilatation*** refer to constriction and dilatation of the resistance vessels; whereas changes in the diameter of the veins are referred to specifically as ***venoconstriction*** or ***venodilatation***.

LOCAL AUTOREGULATORY MECHANISMS

Definition: The capacity of tissues to regulate their own blood flow is called ***autoregulation***.

Sites: In all organs of the body including the mesentery and skeletal muscles, but specially well developed in all the vital organs of the body.

Cause: The blood vessels of these organs possess *basal (resting) myogenic tone – BMT i.e.* an intrinsic capacity to compensate for moderate changes in perfusion pressure by changes in vascular resistance. Therefore, blood flow remains relatively constant.

BMT of vessel wall is produced by *inherent myogenic activity of* ***pace-maker cells*** in the smooth muscle of blood vessels and increases with stretch *(myogenic theory of autoregulation)*. It is responsible for tone of small vessels.

Regulation of BMT

BMT is regulated by sympathetic constrictor nerves which at rest discharge @ 1 impulse per second. Blood vessels which show BMT along with their myogenic activity and sympathetic innervation are given in **Table 39.1**.

The variability in the density of sympathetic innervation in these vessels determines the state of tone of the resistance vessels and pre-capillary to post-capillary resistance ratio at rest. *Normal* ***pre-capillary to post-capillary resistance ratio is 4-5 : 1.*** With increase in sympathetic activity, the ratio increases and resistance to blood flow increases. This decreases the mean capillary hydrostatic pressure causing filtration to decrease.

Factors affecting basal myogenic tone of vessel wall

1. Local vasodilator metabolites

Metabolites released locally with increased tissue activity, suppress the pacemaker cell activity of pre-capillary

Table 39.1: Comparison of myogenic activity and sympathetic innervation of various types of blood vessels

Blood Vessel	Myogenic activity	Sympathetic innervation
1. Arterioles	Well synchronized	Dense
2. Metarterioles	Asynchronized	Less dense
3. Pre-capillary sphincters	Asynchronized	Less dense
4. Venules	Poorly synchronized	Feeble

sphincters only (post-capillary resistance vessels are not susceptible to these tissue metabolites). As a result, the pre to post-capillary resistance ratio decreases, thus producing 'vasodilatation' *(metabolic theory of autoregulation)*.

The metabolic changes that produce vasodilatation include:

(i) decreased arterial pO_2 *(hypoxia)*
(ii) decreased blood pH *(acidemia)*
(iii) increased arterial pCO_2 (***hypercapnia***), its direct dilator action is most pronounced in the skin and brain
(iv) increased blood osmolality
(v) increased body temperature
(vi) K^+, specifically plays a role in the dilatation that occurs in skeletal muscles
(vii) lactic acid
(viii) adenosine - plays a vasodilator role in cardiac muscle.

2. *Local vasoconstrictors*

They produce vasoconstriction by increasing the pacemaker cell activity. These are:

(i) serotonin (or 5-HT) released from platelets secondary to injury
(ii) decrease in body temperature.

Important Note

The membranes of the vascular smooth muscle cells contain vairous types of K^+, Ca^{2+}, and Cl^- channels. The molecular mechanism that appear to be involved in contraction and relaxation is the way high and low intracellular Ca^{2+} can have different and even opposite effects (page 22).

ROLE OF ENDOTHELIAL CELLS

The endothelial cells *i.e.* endothelium of blood vessels secretes a variety of substances in response to: stretch, increase in blood flow and inflammatory mediators. The substances include:

1. Various growth factors which help in formation of new blood vessels (page 311).
2. Vasoactive substances like prostaglandins and thromboxane-A_2 (page 93), nitric oxide and endothelins.

Nitric Oxide (NO)

1. It is released by the endothelium of blood vessels to produce *vasodilatation*, therefore, also called ***endothelium derived relaxing factor (EDRF)***.
2. ***Mechanism of vasodilatation.*** After its formation, it diffuses rapidly into the vascular smooth muscle cells and binds to guanylyl cyclase that converts (guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP), which in turn mediates relaxation of smooth muscles. **(Fig. 39.1)** (Also see to page 23)
3. ***Synthesis.*** 'NO' is synthesized from arginine in a reaction catalyzed by nitric oxide synthase (NOS), an endothelium enzyme activated by increased intracellular Ca^{2+} concentration.

Important Note

A-ch, bradykinin, VIP, substance P or shear stress causes relaxation of vascular smooth muscles by increasing intracellular Ca^{2+} concentration.

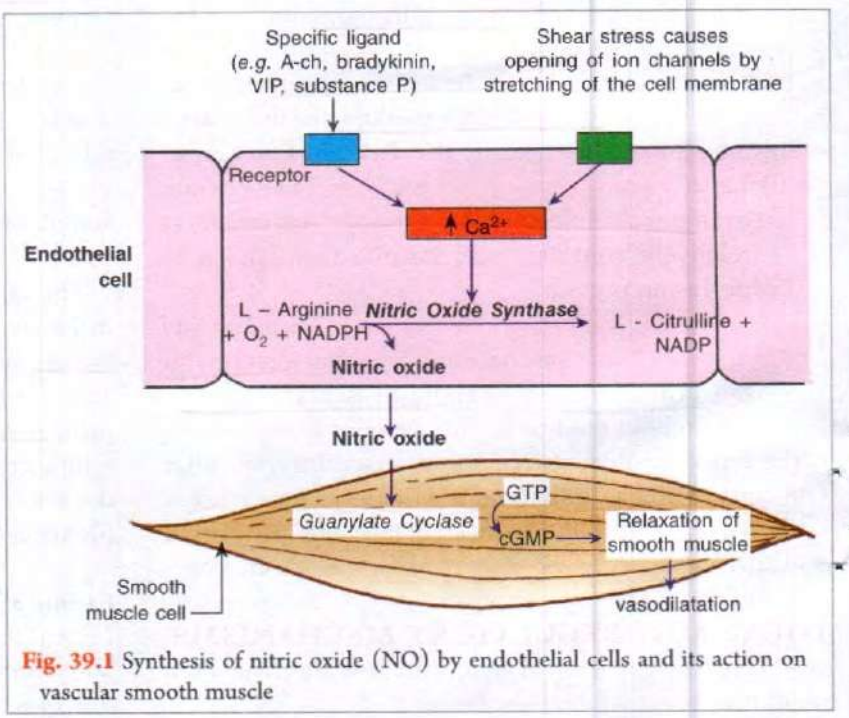


Fig. 39.1 Synthesis of nitric oxide (NO) by endothelial cells and its action on vascular smooth muscle

4. Nitric oxide is necessary for maintenance of normal blood pressure (*Proof:* 'NO' deficiency is associated with hypertension).
5. *Actions*
   (i) It plays an important role in the relaxation of vascular smooth muscle (see above) and in brain functions (page 1050).
   (ii) It is necessary for cytotoxic activity of macrophages and their ability to kill cancer cells.
   (iii) In the GIT, it produces smooth muscle relaxation.
   (iv) It causes vasodilation and engorgement of spongy tissue of the corpora cavernosa to bring out penile erection.
   (v) It is bacteriostatic and helps prevent infection.

Endothelins

1. Endothelial cells form three similar polypeptides, *endothelin I, II and III.* The ***endothelin I (ET-1)*** being one of the most *potent vasoconstrictor* agent which acts via ET_A receptors. coupled to G-protein to activate phospholipase (page 23) It also produces vasoconstriction in many body tissues.

2. *Factors affecting* endothelin-1 secretion:
   (i) *Stimulators:* angiotensin II, catecholamines, growth factors, hypoxia, insulin, stress etc.
   (ii) *Inhibitors:* nitric oxide, atrial natriuretic peptide (ANP), prostaglandin E, prostacyclin.

3. *Actions*
   (i) *On CVS*
      (a) contracts vascular smooth muscles (veins more than the arteries),
      (b) increases HR and force of contraction of myocardium.
   (ii) *On CNS:* regulates transport across the blood brain barrier and across the synapses.
   (iii) *On kidneys:* decreases GFR and renal blood flow, and regulates Na^+ reabsorption.
   (iv) *On endocrines:* increases plasma level of renin, aldosterone, catecholamines and ANP.
   (v) *On respiration:* produces bronchoconstriction.
   (vi) *On GIT:* regulates GIT blood flow.

SYSTEMIC REGULATORY MECHANISMS

A. CHEMICAL REGULATORY MECHANISMS

1. Circulating vasodilators

The circulating vasodilator substances include: kinins, VIP (page 272) and atrial natriuretic peptide (page 559).

Kinins: These are the peptides which bring about the dilatation of blood vessels throughout the body. For example:

(i) Bradykinin (Nonapeptide)	:	found in plasma
(ii) Lysyl bradykinin or kalidin (decapeptide)	:	found in body tissues
(iii) Methionyllysyl bradykinin	:	found in urine

Kinins are formed from the circulating α_2-globulin, *kininogens* by the action of proteolytic enzymes, *kallikreins*. Kinins release is inhibited by glucocorticoids. **(Fig. 39.2)**

Actions of kinins

Kinins actions resemble those of histamine.

(i) Cause contraction of most types of smooth muscles *e.g.* ileum, uterus, bronchiole *but* cause vasodilatation of vascular smooth muscles via nitric oxide, thus producing:
   (a) decrease in systemic BP;
   (b) increase blood flow in actively secreting glands specially salivary, sweat and exocrine pancreas.
(ii) Increase vascular permeability by an action on venules which causes escape of plasma proteins.
(iii) Excite sensory nerve endings producing pain (which is enhanced by 5-HT).
(iv) In high concentration promote migration of leucocytes from blood to tissues.

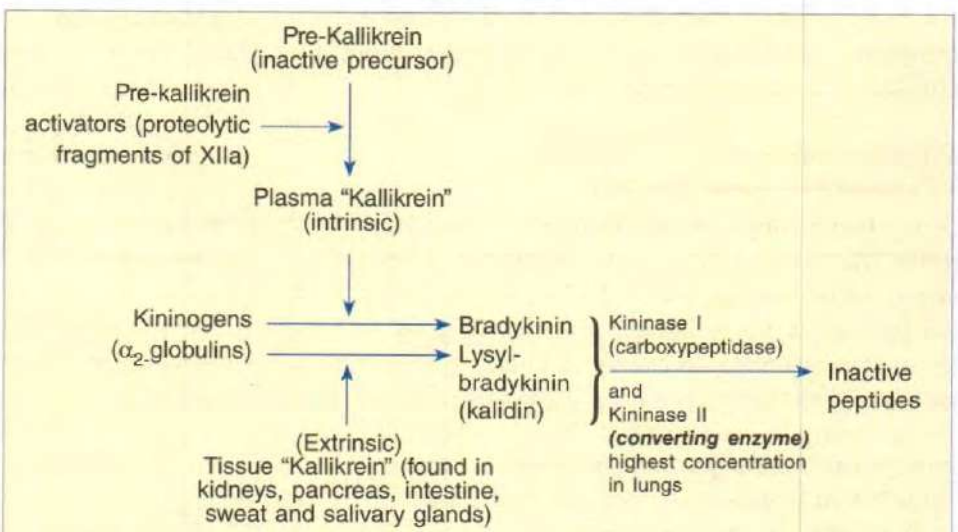


Fig. 39.2 Formation and metabolism of 'kinins'

The actions (ii), (iii) and (iv) are characteristically seen during inflammation, therefore kinins are responsible for ***inflammation.***

(v) They are formed by antigen-antibody interactions and may thus help to produce bronchoconstriction in bronchial asthma.

(vi) Appear to be responsible for some episodes of vasodilatation in patients with carcinoid tumours.

(vii) Probably, play a role in thermo-regulatory vascular adjustments.

2. Circulating vasoconstrictors

(i) **Catecholamines**

(a) Epinephrine: via α-adrenergic receptors produces vasoconstriction in skin and splanchnic area; and via β-adrenergic receptors, dilates the vessels in skeletal muscle, coronaries and liver. Latter action being dominant, net effect is fall in peripheral resistance producing fall in systemic BP.

(b) Nor-epinephrine (for details, refer to page 738).

(ii) **Angiotensin II** (page 506).

(iii) **Vasopressin** (in high dose, page 673)

(i)b; (ii) and (iii), these agents help to maintain systemic B.P. by producing generalised vasoconstriction.

B. NEURAL REGULATORY MECHANISMS

These mechanisms bring about the circulatory adjustments throughout the body primarily by 2 ways: ***Autonomic*** regulation and ***medullary*** regulation.

Autonomic Regulation

The two divisions of autonomic nervous system, sympathetic and parasympathetic are distributed to the heart *(cardiac innervation)* and to the blood vessels *(peripheral vascular innervation)*, thus bring about the circulatory adjustment in the body.

1. Cardiac innervation

(i) Sympathetic supply **(Fig. 39.3)**

Sympathetic supply to the heart is by the sympathetic nerve cells which lie in the intermediolateral horn of the spinal cord extending from T_1 to T_5 spinal segments. Their pre-ganglionic fibers (small, myelinated) pass into the sympathetic trunk to superior, middle and inferior 'cardiac ganglia'. Here they synapse and the post-ganglionic fibers (long, unmyelinated) pass via the superior, middle and inferior ***cardiac sympathetic nerves*** to supply:

(a) Nodal tissues – SA node, AV node; and

(b) muscles of atria and ventricles

Sympathetic fibers to the heart are ***epicardial*** and their stimulation produces the following effects:

(a) Increases heart rate by increasing rhythmicity of SAN, called positive ***chronotropic*** action;

(b) Increases speed and force of contraction of myocardium, called positive ***inotropic*** action;

(c) Increases conductivity in conduction tissue, positive ***dromotropic*** action; and

(d) Increases excitability of the heart, positive ***bathmotropic*** action.

(ii) Parasympathetic supply

Parasympathetic supply to the heart is via two vagus nerves with their cell bodies located in the medulla in the *nucleus ambiguus* (NA) **(Fig. 39.3)**. Pre-ganglionic fibers (long, myelinated) travel in the vagi to synapse with ganglionic cells which are located near the SA node, AV node and in the atria. From here post-ganglionic fibers (small, unmyelinated) arise and are distributed to the SA node, AV node and muscles of atria. *No vagal motor fibres are distributed to the ventricles.* Parasympathetic fibers to the heart are *endocardial* and their stimulation produces the following effects:

(a) Decreases rate of impulse generation by SAN causing heart rate to decrease; negative *chronotropic* action. It may cause stoppage of the heart and thus account for the indirect effect of vagal stimulation on ventricles.

(b) Decreases conductivity in conduction tissue; negative *dromotropic* action.

(c) Decreases speed and force of contraction of atria only: negative *inotropic* action.

(d) Does not influence contraction of ventricular myocardium directly.

In adults, at rest, the heart beat @ 70-80 beats/ min under the influence of vagus, called ***vagal tone***. Cutting the vagus (*vagotomy*) or atropine administration which abolishes the transmission of the vagal effects upon the SA node causes heart rate to increase to 160-180 beats/min. The difference between the resting heart rate before and after vagotomy or atropinization gives a measure of the ***degree of vagal tone***. In humans in whom both sympathetic and parasympathetic nerves to heart are blocked, the heart rate is approx. 100 beats/min.

2. Peripheral Vascular Innervation

It broadly produces two major effects on the vascular system:

(i) vasoconstriction (mainly), and

(ii) vasodilatation

(i) **Vasoconstriction** of the parts of vascular system is predominantly achieved by sympathetic vasoconstrictor nerves. These nerves arise from the cell bodies in

the intermediolateral horns of spinal cord extending from T_1 to L_2 spinal segments. This *thoracolumbar outflow* exerts a tonic influence on almost all of the vascular system with the *exception* of the:

(a) cerebral regional circulation; and

(b) true capillaries of the system as a whole.

Although main arteries and veins do receive sympathetic constrictor innervation, the *main density* of such innervation occurs in the arterioles, metarterioles, pre capillary sphincters and post-capillary venules. Therefore, this system has profound influence on peripheral resistance and on the *pre/post-capillary resistance ratio* (page 321).

Important Note

Spinal cord section in lower cervical region decreases mean B.P. from a normal resting value of 100 mmHg to 40 mmHg. As the spinal cord transection moves distally towards L_2, less profound will be the fall in systemic B.P.

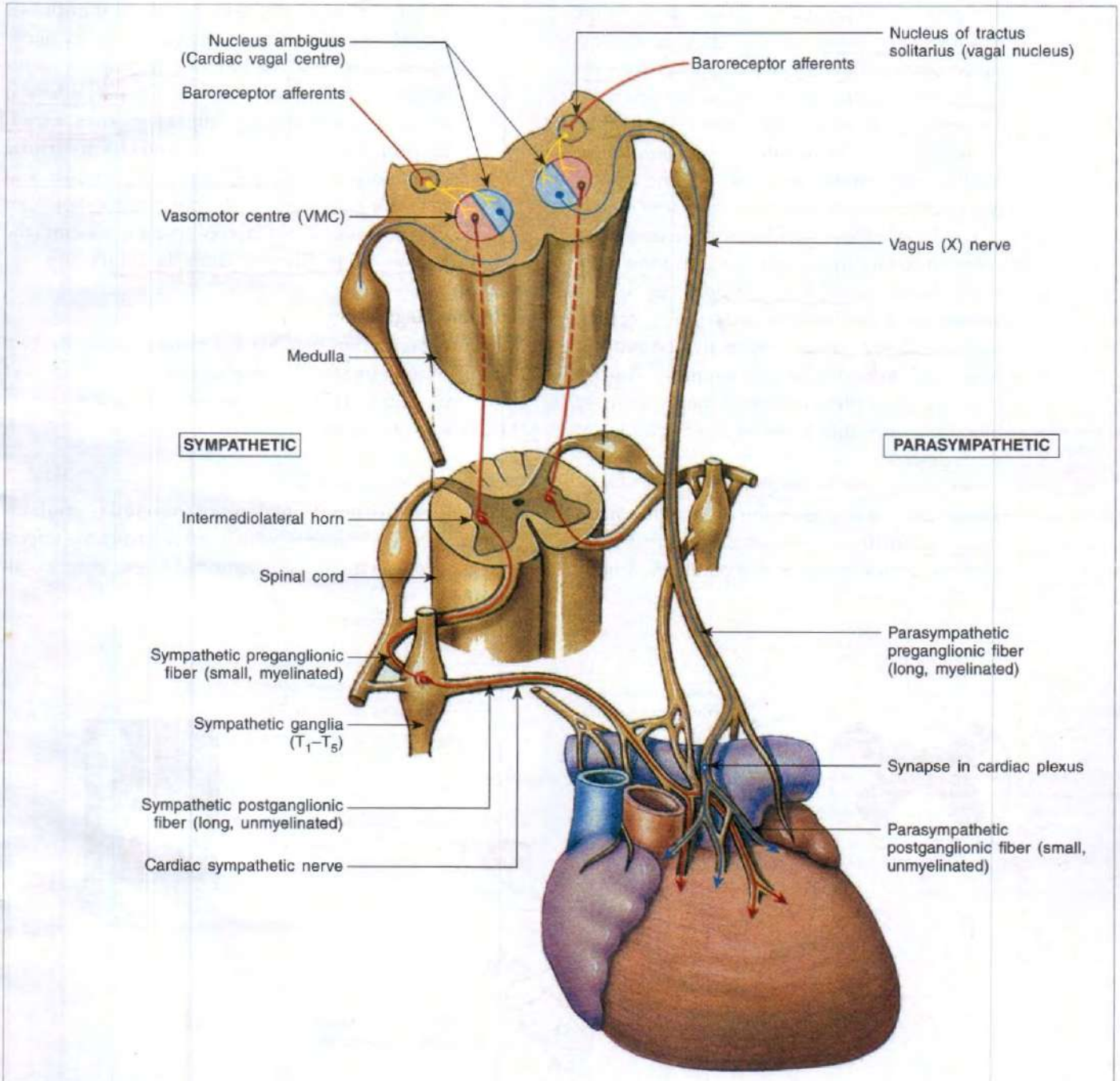


Fig. 39.3 Pathway for autonomic regulation of the heart. (**Note:** No vagal motor fibers are distributed to the ventricles)

(ii) **Vasodilatation** of parts of the vascular system can be achieved by 3 mechanisms:

(a) ***Reduction of Sympathetic Constrictor Tone***: This occurs in the following conditions, for example: flushed skin of heat stress via hypothalamus; and vasodilatation of the skeletal muscle, viscera and skin blood vessels through 'baroreceptor' stimulation (page 328).

(b) ***Specific Activation of Vasodilator Nerves***: These nerves are further divided into:

– *Sympathetic cholinergic vasodilators*

Sympathetic nerve endings where epinephrine or nor-epinephrine is the transmitter are called *adrenergic nerves*; whereas where acetylcholine is transmitter are called ***sympathetic cholinergic nerves*** and their activation produces vasodilatation (***sympathetic cholinergic system***, page 332). Some of the body organs like skeletal muscles, heart, lungs, liver, kidneys and uterus receive both types of sympathetic innervation. However, sympathetic constrictor supply to blood vessels of these organs is tonically active; the vasodilator supply gets activated only in biological emergencies, for example, during exercise, child birth etc., thus, helps increase blood flow through these organs in biological emergencies.

– *Parasympathetic vasodilator nerves*

These are best represented by the *nervi erigentes* which supplies sexual erectile tissue. Other example includes the parasympathetic vasodilator nerve supply to the salivary glands.

Activation of such nerves contributes to pleasure and fulfilling important biological functions. It plays little role in the overall economy of the circulation.

(c) ***Dorsal root vasodilatation*** **(Axon Reflex)**. Normally afferent impulses in sensory nerve from skin are conducted in *orthodromic* fashion to reach the spinal cord, called *orthodromic conduction* **(Fig. 39.4)** However, if a firm stroke is applied across the skin, the afferent impulses are relayed down the branches of sensory nerve (dorsal nerve root) to the endings near cutaneous arterioles, called *antidromic conduction* where it releases a vasodilator substance, ***substance P*** to produce long lasting cutaneous arteriolar dilatation. It is a local neural mechanism and does not involve the CNS connections, therefore, called *Axon Reflex*. It is responsible for the redness spreading out from the injury site.

Medullary Regulation

Medullary regulation of CVS is brought about by two different centres located in the medulla:

(1) *Vasomotor centre (VMC)* or *vasomotor area*; and

(2) *Cardiac vagal centre*.

1. Vasomotor Centre (VMC)

(i) The ventrolateral region of medulla contains *glutaminergic neurons* which exert *excitatory* effects on spinal sympathetic neurons. These neurons in

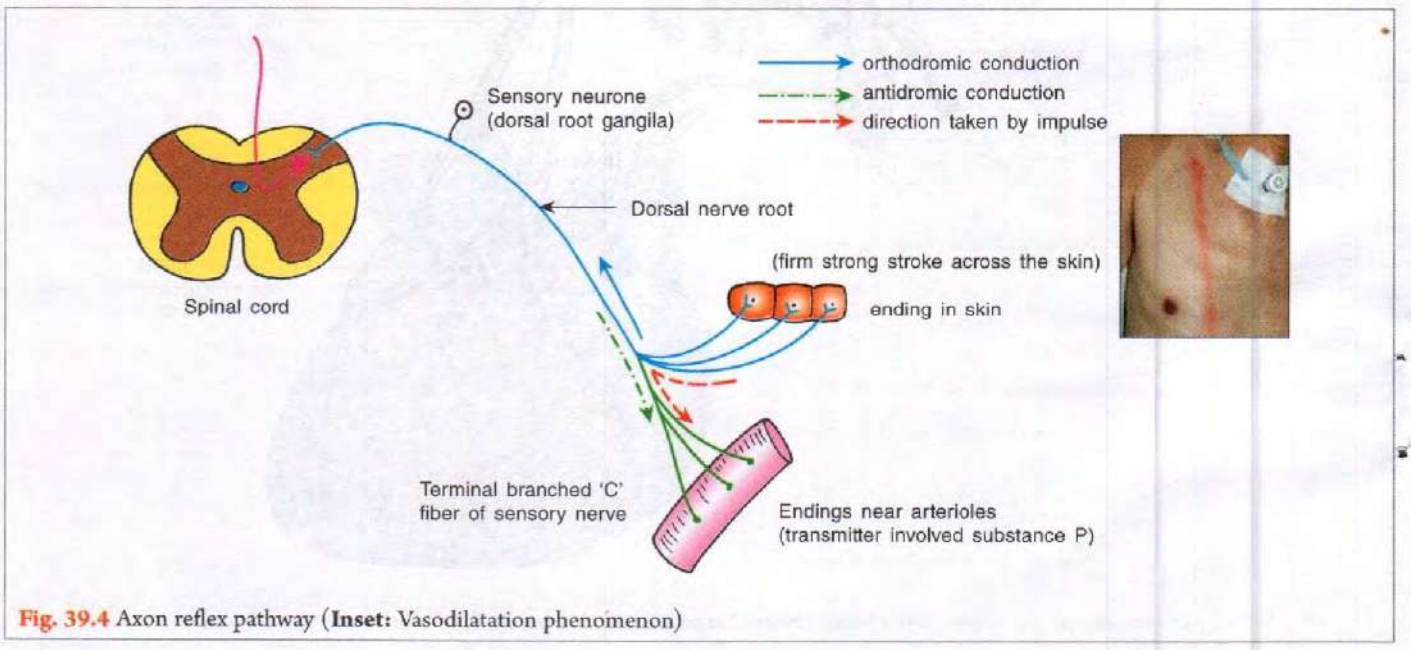


Fig. 39.4 Axon reflex pathway (**Inset:** Vasodilatation phenomenon)

medulla show *inherent tonic activity i.e.* they discharge rhythmically in a tonic fashion to excite sympathetic pre-ganglionic neurons in the intermediolateral grey column of the spinal cord. Thus, sympathetic activity increases producing ***pressor effects*** on the CVS which includes:

(a) increase in heart rate,
(b) increase in stroke volume,
(c) increase in systemic BP due to increase arteriolar constriction, and
(d) venoconstriction which decreases blood stored in venous reservoir and increases venous return.

Therefore, the area in the ventrolateral region of medulla where these neurons are located is called ***Pressor Area.*** **(Fig. 39.5)**

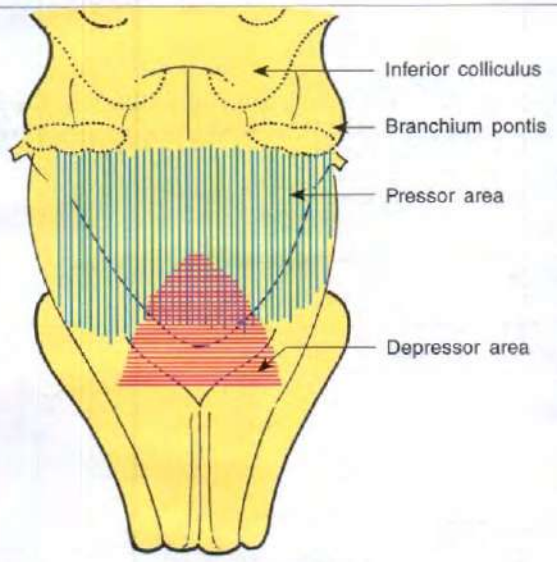


Fig. 39.5 The Vasomotor Centre (VMC) showing 'pressor' and 'depressor' areas

(ii) Near medial and caudal parts of fourth ventricle in medulla certain groups of neurons are located. Stimulation of these neurons decreases sympathetic activity by inhibiting the tonically discharging impulses of the 'pressor' area neurons producing ***depressor effects*** on the CVS. The effects include:
(a) decrease in heart rate,
(b) decrease in stroke volume,
(c) decrease in systemic blood pressure due to vasodilatation, and
(d) venodilatation which increases storage of blood in venous reservoirs and decreases venous return.

Thus the area in the medulla where these neurons are located is called ***Depressor Area.***

(iii) In the intermediate region of the upper medulla, the 'depressor' and 'pressor' areas overlap *i.e.* there is no clear anatomical separation between two areas. Thus, the two areas together constitute ***medullary cardio-vascular centre***. However, these neurons exert predominantly excitatory effect on thoraco lumbar sympathetic neurons in the spinal cord. Therefore, the term ***vasomotor centre (VMC)*** is more commonly used.

Summary: VMC can thus produce either:
(i) increase in sympathetic activity due to increase activity of *pressor area*; or
(ii) decrease in sympathetic activity due to increase activity of *depressor area* which in turn inhibits the pressor area.

2. Cardiac Vagal Centre

(i) Inhibitory pathways in the form of 'vagal' fibers descend from the medulla to converge on:
(a) sympathetic pre-ganglionic neurons of the spinal cord to decrease sympathetic activity; and
(b) heart to decrease the heart rate and force of cardiac contraction.

(ii) Vagal fibers arise from the neurons of three different nuclei located in the medulla which are:
(a) The *dorsal motor nucleus of the vagus*, previously it was thought to send inhibitory impulses to heart and so was called, ***cardio-inhibitory centre***. Now it has been found to control the smooth muscle activity of bronchi and GIT.
(b) *The Nucleus of tractus solitarius (NTS).* It receives afferents from most of the baroreceptors (see below) and its fibers are projected to the nucleus ambiguus and dorsal motor nucleus of vagus.
(c) The *Nucleus ambiguus (NA).* It lies lateral to the medullary reticular neurons; receives afferents via NTS and in turn sends impulses to the heart. Thus, called as ***Cardiac Vagal Centre***. The neurons located in this centre are *not tonically active.*

Factors influencing vasomotor centre (VMC) and cardiac vagal centre (CVC)

The vasomotor centre (VMC) and cardiac vagal centre get influenced by the discharge in afferent fibers which converge on them. The various afferents and effects of their discharge on these centres are given in **Table 39.2** and **Fig. 39.6**.

BARORECEPTORS

These are receptors sensitive to stretch, therefore also called ***mechanoreceptors***.

Table 39.2: Factors affecting Vasomotor Centre (VMC) and Cardiac Vagal Centre (CVC)

Afferent	Effect on 'VMC'	Effect on 'CVC'— nucleus ambiguus
1. *Baroreceptors* (receptors sensitive to stretch. For example: (i) Arterial baroreceptors: carotid sinus and arch of aorta. (ii) Cardiac baroreceptors: (page 331).	Inibition	Stimulation
2. *Chemoreceptors* (receptors sensitive to change in blood chemistry). For example: carotid and aortic bodies.	(a) In eupnoea (normal respiration): no or little stimulation. (b) During hypoxia or asphyxia: stimulation (direct effect)	Stimulation Inhibition
3. *Cortico-hypothalamic descending pathways i.e.* descending tract to medullary centres from the cerebral cortex, particularly limbic cortex that relays in the hypothalamus.	Stimulation or inhibition	Stimulation *or* Inhibition

Important Note

When VMC is stimulated there is usually an associated decrease in the tonic activity of vagal fibers to the heart. Some stimuli such as hypoxia, CO_2 and pain inputs, directly stimulate the VMC. However, prolonged serere pain may produce vasodilation resulting in fall in B.P. and fainting.

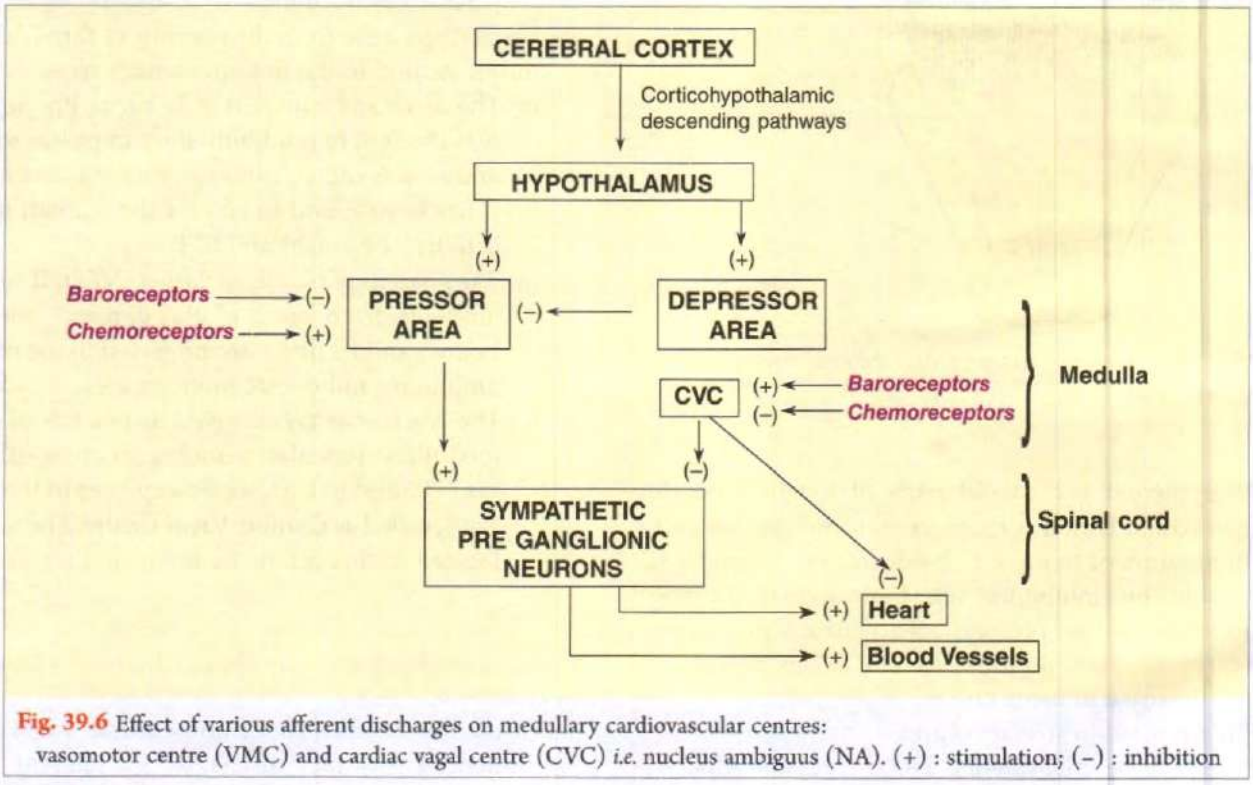


Fig. 39.6 Effect of various afferent discharges on medullary cardiovascular centres: vasomotor centre (VMC) and cardiac vagal centre (CVC) *i.e.* nucleus ambiguus (NA). (+) : stimulation; (–) : inhibition

Important Note

If the stretch is prevented by surrounding the wall with a closely applied rigid plaster of Paris cast, the receptors do not respond when the intraluminal pressure is raised. *Clinical significance:* Baroreceptor Resetting, page 394.

Classification and location – Refer Table 39.3

Innervation

Except the carotid sinus which is supplied by carotid sinus nerve (branch of glossopharyngeal nerve), all the other baroreceptors are supplied by vagus nerve. (Fig. 39.7)

Table 39.3: Classification and Location of Baroreceptors

ARTERIAL
(located in the walls of blood vessels, distributed mainly in adventitial layer)

SYSTEMIC

Examples :

1. *Carotid sinus i.e.* dilated initial part of internal carotid artery.
2. *Aortic arch i.e.* transverse part of arch of aorta.
3. Root of right subclavian artery.
4. Junction of thyroid artery with common carotid artery.

PULMONARY
pulmonary trunk and its division into right and left pulmonary artery

CARDIAC
(located in the walls of the heart, 'subendocardial' distribution)

Examples:

1. *Atrio-caval receptors* – located at junction of right atrium with inferior and superior vena cavae.
2. *Pulmonary veno-atrial receptors* – located at junction of pulmonary vein with left atrium.
3. *Atrial receptors* – scattered throughout the atria and inter-atrial septum.
4. *Ventricular receptors* – scattered throughout the left ventricle and interventricula septum.

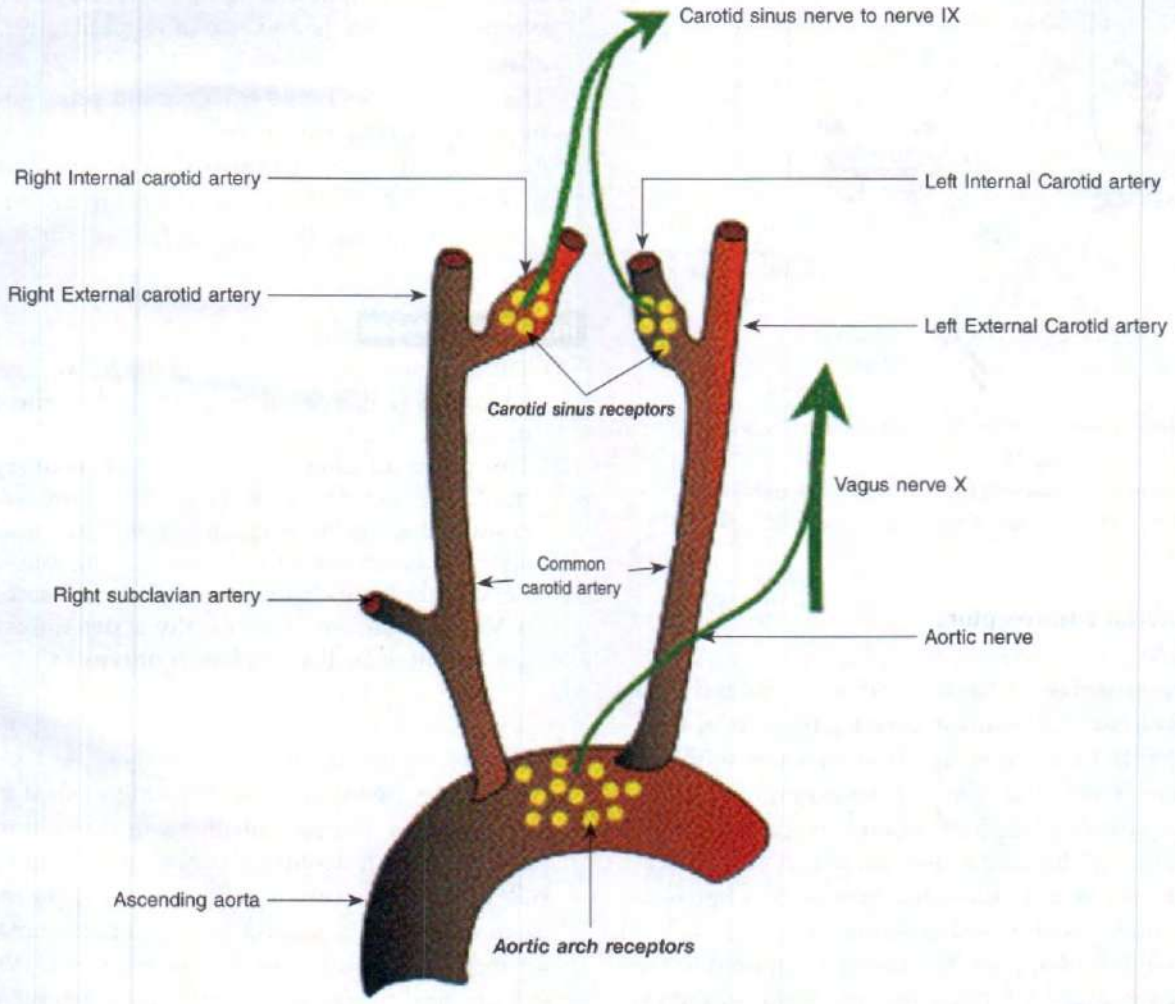


Fig. 39.7 Systemic arterial baroreceptors (location and innervation)

The afferents from most of the baroreceptors end in the nucleus of tractus solitarius (NTS), where they secrete an ***excitatory*** neurotransmitter, glutamic acid **(Fig. 39.8)**. The excitatory ***glutaminergic projections*** from the NTS terminate on to the:

(i) 'pressor' area of the VMC, where they stimulate GABA-secreting inhibitory neurons; and

(ii) nucleus ambiguus and dorsal motor nucleus of the vagus, to activate their neurons.

Therefore, increased baroreceptor discharge *inhibits* the tonic discharge of pressor area of the VMC and *excites* the cardiac vagal centre (CVC).

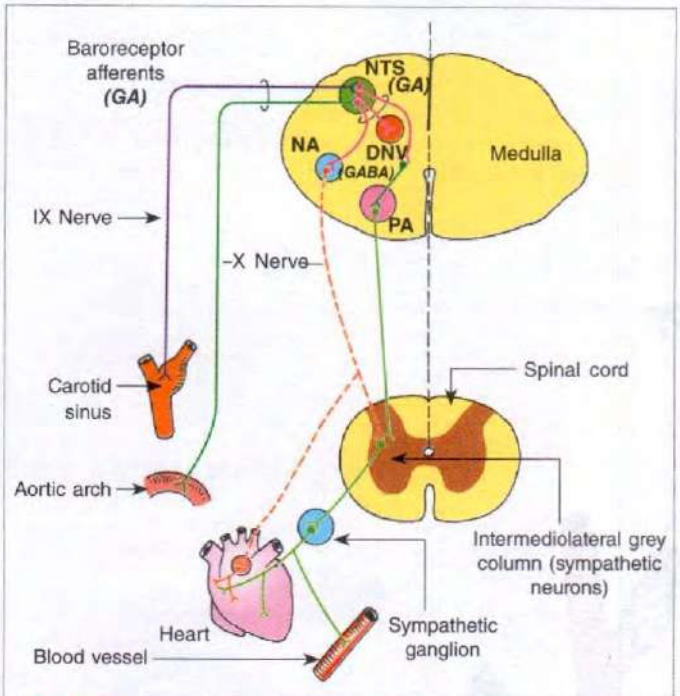


Fig. 39.8 Pathway for medullary regulation of cardiovascular system—also see to **Fig. 39.3**
(NTS: nucleus of tractus solitarius; NA: nucleus ambiguus; DNV: dorsal motor nucleus of vagus; GA: glutamic acid; PA: pressor area of VMC)

Systemic arterial baroreceptors

Salient features

1. They are sensitive to stretch and their discharge is caused by a rise of pressure expanding the arterial wall.
2. The afferents from these receptors synapse with the neurons of 'VMC' and 'CVC'. The normal tonic effect of these stretch receptors discharge causes reflex inhibition of 'VMC' and reflex stimulation of 'CVC'.
3. Range of operation of these baroreceptors is between 60-200 mmHg mean blood pressure, *i.e.*
   (i) a mean B.P. of approx. 60 mmHg is required in the systemic arterial B.P. before any reflex inhibition of 'VMC' and reflex stimulation of 'CVC' is seen. This is called *Threshold of Baroreceptor Reflex*.
   (ii) At mean B.P. of 200 mmHg, rate of baroreceptor discharge being maximum, the degree of inhibition of 'VMC' and stimulation of 'CVC' are maximal.

Note

Baroreceptors respond much more rapidly to *pulsatile* (*i.e.* changing) pressure than to a stationary pressure.

4. At normal mean B.P. of 95-100 mmHg, baroreceptors discharge at a slow rate and are responsible for reflex vagal tone (page 324).
5. When mean B.P. decreases, baroreceptor discharge decreases, causing *less* inhibition of 'VMC' and *less* stimulation of 'CVC'. This results in reflex increase in sympathetic activity and reflex decrease in vagal activity. The net effect is, prevents any fall in B.P.
6. Conversely, when mean B.P. rises, baroreceptor discharge increases, causing more inhibition of 'VMC' and more stimulation of 'CVC'. This results in fall in sympathetic activity and rise in vagal actvity. The net effect is, prevent any rise in B.P.
   The carotid sinus nerve from carotid sinus and aortic nerve (branch of vagus) from arch of aorta together known as ***sino-aortic nerves*** prevent any rise or fall in systemic B.P. Sino-aortic nerves, thus, act as B.P. regulators and are, therefore, called as *Buffer Nerves*.

Important Notes

1. The activity of the neurons in 'VMC' is *inherently tonic* and is increased by cutting the sino-aortic nerves.
2. The different afferent fibers which converge on the 'CVC' can increase its tonic activity and are responsible for the *reflex vagal tone*. Thus maintain a slower heart rate at rest. Cutting the sino-aortic nerves abolishes 'vagal tone'. This indicates that 'CVC' neurons are *not* tonically active unless they get activated by these afferent nerves.

7. Integrity of the *baroreceptor reflex can be tested* with the valsalva maneuver (*i.e.* expiring against a closed glottis). This causes an increase in intrathoracic pressure, which decreases venous return and arterial B.P. If the baroreceptor reflex is intact, the decrease in arterial B.P. is sensed by the baroreceptors and an increase in heart rate would be noted. When the person stops the maneuver, there is a rebound increase in venous return and arterial B.P., resulting in reflex decrease in heart rate.

Important Note

The marked increase in systemic B.P. due to nerve involvement is called *neurogenic hypertension.*

Cardiac Baroreceptors

Classification and location (page 329)

Innervation

All cardiac baroreceptors are innervated by vagus nerve *i.e.* Xth cranial nerve.

1. ***Atrio-caval and pulmonary veno-atrial receptors*** (discovered by *A.S. Paintal*, an Indian Scientist in 1953). *These are of 3 types:*

(i) ***'A' type receptors*** – These receptors discharge during atrial systole only and their impulse activity occurs in the 'PR interval' of the E.C.G. **(Fig. 39.9)**

(ii) ***'B' type receptors*** –

(a) These receptors discharge late in diastole, at the time of peak atrial filling *i.e.* discharge just before the onset of atrial contraction and reach peak after the T-wave of E.C.G. **(Fig. 39.9)**

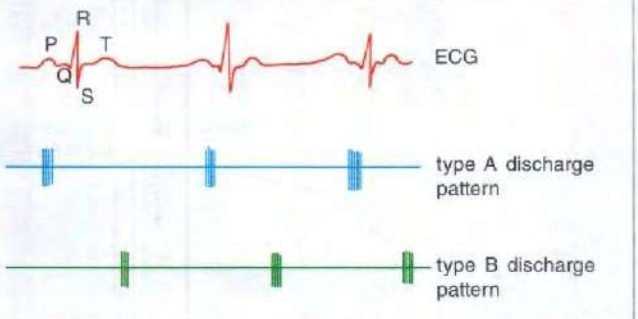


Fig. 39.9 Pattern of right atrium receptor discharge as recorded from vagus nerve and its correlation with ECG

(b) Rate of discharge of receptors is directly proportional to the venous return (intensity of atrial filling). This shows that these receptors respond primarily to distension of atrial walls, and are called as ***volume receptors***. Thus, they provide information about the circulating blood volume *i.e.* bigger the venous return, greater will be the discharge from the receptor fibers.

(iii) ***Intermediate type of receptors***

(a) These receptors discharge both during atrial systole and diastole. Their discharge is characterised by an 'A' type receptors discharge followed by 'B' type receptors discharge.

(b) Their stimulation produces tachycardia due to stretching of SA nodal fibers, hypotension and can also modify the output of ADH; because increased distension of left atrium produces moderate diuresis (probably due to reflex secretion of a diuretic hormone from the hypothalamus or due to decrease ADH secretion) (Also see to page 672).

Physiological Significance

Bainbridge Reflex (*Bainbridge*, 1918)

Rapid perfusion of blood or saline in anaesthetized animals produces a rise in heart rate if the initial heart rate is low. Atrial receptors of both sides of the heart may be responsible for the *Bainbridge Reflex*, because distension of pulmonary veno-atrial orifice or distension of a pouch of the LA or distension of superior venacaval-right atrial junction produces reflex tachycardia.

2. ***Atrial receptors***

These receptors are innervated by non-myelinated C-fibers of vagus nerve, therefore also called as *Non-Myelinated Atrial Afferents*. The receptors are scattered throughout the atria and the inter-atrial septum; their discharge is sparse (1 impulse/sec) and is irregular.

Function: Increase in atrial pressure increases their impulse activity resulting into reflex vasodilatation, specially in renal vascular circuit.

3. ***Ventricular receptors***

These receptors are located in the left ventricle and inter-ventricular septum. They discharge irregularly @ 1 impulse/sec. They get stimulated either by chemicals *e.g.* injection of veratridine, serotonin or nicotine into the coronary arteries (supplying left ventricle) or pulmonary artery or by partial occlusion of aorta or coronary sinus or by substances released from the damaged myocardium.

Reflex effects include: *apnoea, profound bradycardia* and *hypotension*. The fall in B.P. may be due to reflex inhibition of sympathetic discharge, specially in renal circuit called ***Bezold-Jarisch reflex or coronary-chemoreflex or pulmonary chemoreflex.***

Clinical significance

Strong emotions *(vaso-vagal syncope)* or sudden heart attack (acute myocardial infarction) may be associated with profound bradycardia, hypotension and apnoea due to reflex aroused by activation of these ventricular receptors.

CHEMORECEPTORS

These receptors are sensitive to change in blood chemistry. Their main function is to keep alveolar pCO_2 at normal level of 40 mmHg and also to maintain arterial pO_2, pCO_2 and pH. The important chemoreceptors are (**Fig. 48.5**, page 444):

1. ***Carotid bodies*** – These are located near common carotid artery bifurcation and are innervated by carotid sinus nerve, branch of glossopharyngeal nerve.

2. *Aortic bodies* – These are scattered around aortic arch and are innervated by aortic nerve, branch of vagus nerve.
   Afferent fibers from these chemoreceptors ascend to relay in nucleus of tractus solitarius (NTS) of medulla. These bodies get stimulated by chemical changes in the blood. For example:
   (i) hypoxia (O_2 lack)
   (ii) hypercapnia (accumulation of CO_2)
   (iii) asphyxia (hypoxia plus hypercapnia), and
   (iv) acidemia (fall in blood pH).
   At rest chemoreceptor activity contributes little to cardiovascular features because they operate between 40-100 mmHg mean B.P. When these receptors get stimulated, they stimulate the 'VMC' and respiratory neurons in the medulla producing 'pressor' effects with increase in rate and depth of respiration.

CORTICO HYPOTHALAMIC DESCENDING PATHWAYS

This pathway is the descending tract to medullary centres ('VMC' and 'CVC') from cerebral cortex particularly the *limbic cortex* (page 1024) that relays in hypothalamus. These fibers discharge sporadically (scattered manner) in response to emotions producing emotional effects on CVS. For example:

1. Sexual or other type of excitement, fear, anger, rage etc. increase sympathetic activity producing 'pressor effects' *i.e.* increase in heart rate, blood pressure, etc.
2. Conversely, sudden shock, grief, apprehension etc. decrease sympathetic activity causing fall in heart rate and blood pressure.
   These effects are induced by stimulation of *limbic system* *i.e.* cortical and sub-cortical structures which form a ring around the brain stem ('*limbus*' means a ring).

Important Note

The cholinergic sympathetic vasodilator fibers of *sympathetic vasodilator system* (page 326) also discharge in response to emotions. This system originates in the cerebral cortex, relays in the hypothalamus and midbrain, and passes through the medulla without relay to the sympathetic neurons located in the intermediolateral grey column of the spinal cord. Their pre-ganglionic fibers pass into the sympathetic trunk to activate post-ganglionic fibers that secrete A-ch at their endings Fig. 39.10. (Also refer to page 378)

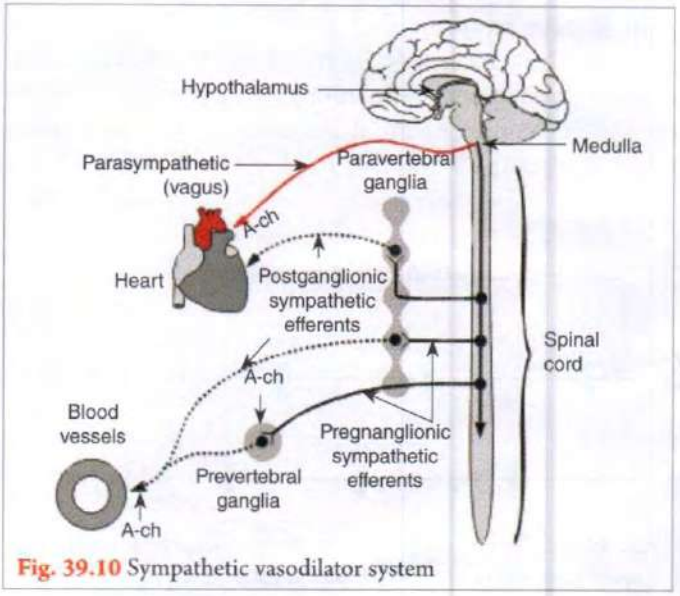


Fig. 39.10 Sympathetic vasodilator system

SUMMARY

CARDIO-VASCULAR REGULATORY MECHANISMS

- **LOCAL**
  - 'Autoregulatory' Mechanisms (by basal myogenic tone)
  - – Role of endothelial cells (nitric oxide; endothelins)
- **SYSTEMIC**
  - **CHEMICAL**
    1. Vasodilator peptides : Kinins-bradykinin
    2. Vasoconstrictors: Catecholamines; angiotensin II; ADH in high dose
  - **NEURAL**
    - **AUTONOMIC REGULATION**
      - Cardiac innervation
        1. Sympathetic (T_1 to T_5)
        2. Parasympathetic (Vagus)
      - Peripheral Vascular innervation
        1. Sympathetic Vasoconstriction (T_1 to L_2)
        2. Vasodilatation
           (i) Reduction of Sympathetic tone
           (ii) Vasodilator nerves activation
           (iii) Dorsal root Vasodilatation
    - **MEDULLARY REGULATION**
      - Vasomotor Centre (VMC) Cardiac Vagal Centre
      - influenced by
        1. Baroreceptors
        2. Chemoreceptors
        3. Corticohypothalamic descending pathways

Study Questions

1. **Write short notes on:**
   (i) Autoregulation of blood flow
   (ii) Sympathetic vasodilator nerves
   (iii) Axon reflex
   (iv) Medullary cardiovascular centre
   (v) Factors influencing VMC and cardiac vagal centre
   (vi) Neurogenic hypertension
   (vii) Volume receptors
   (viii) Systemic baroreceptors
   (ix) Bainbridge reflex
   (x) Bezold Jarisch reflex
   (xi) Emotional effects on CVS
   (xii) Factors affecting basal myogenic tone
   (xiii) Buffer nerves
2. **How can vasodilatation in parts of the vascular system be achieved?**
3. **Is the term VMC appropriate? Explain.**
4. **Classify baroreceptors and give their innervation.**
5. **What determines the reflex vagal tone to the heart?**
6. **How do kinins produce inflammation?**
7. **What will happen if:**
   (i) If nitric oxide deficiency occurs in the blood vessels.
   (ii) Both sympathetic and parasympathetic nerves to heart are cut.
   (iii) Plastercine is put around the baroreceptors and pressure within them is raised.
   (iv) Bilateral occlusion of common carotid artery is performed in an animal with vagi cut.
   (v) Nucleus of tractus solitarius is bilaterally destroyed.
   (vi) If respiratory chemoreceptors get activated.
   (vii) If spinal cord is transected in the lower cervical segment.
8. **Draw well labelled diagram to show:**
   (i) Autonomic regulation of the heart
   (ii) Axon reflex
   (iii) Medullary regulation of CVS
   (iv) Sympathetic vasodilator system
   (v) Location and innervation of systemic arterial baroreceptors

MCQs

1. **Autoregulation phenomenon:**
   (a) Refers to the capacity of tissues regulate their own blood flow despite fluctuations in perfusion pressure
   (b) Is seen in all vital organs of the body
   (c) Is brought about by the presence of basal myogenic tone in the blood vessels of body organs
   (d) All of the above
2. **All of the followings are the action of endothelins *except*:**
   (a) Contracts vascular smooth muscles
   (b) Increases heart rate and force of contraction of myocardium
   (c) Produces bronchoconstriction
   (d) Increases GFR
3. **Most potent vasopressor is:**
   (a) Angiotensin II (b) Renin (c) Aldosterone (d) Cortisol
4. **A stronger than normal heart might be observed during:**
   (a) Sympathetic stimulation (b) Myocardial ischemia (c) Stokes-Adams syndrome (d) Atrial fibrillation
5. **Parasympathetic system affects CVS mainly by altering:**
   (a) Vascular resistance
   (b) Vascular compliance
   (c) Force of contraction of heart
   (d) Heart rate
6. **When spinal cord is cut at the level of T_1:**
   (a) The arterioles are constricted
   (b) The blood pressure falls
   (c) The sympathetic vasoconstrictors are stimulated
   (d) Quadriplegia occurs
7. **In axon reflex, neurotransmitter responsible to produce long lasting cutaneous arteriolar dilatation is:**
   (a) Serotonin (b) Substance P (c) Acetyl choline (d) K^+ released from the damaged cells
8. **Axon reflex is so called because:**
   (a) It produces long lasting cutaneous arteriolar vasodilatation
   (b) Afferent impulse from skin are conducted via axons in orthodromic fashion to reach the spinal cord
   (c) It is a local neural response and does not involve CNS connections
   (d) It is a superficial reflex and mediated via nerve fibers

9. **Increased pressure in the carotid sinus causes all of the following, *except*:**
   (a) Reflex slowing of the heart rate (b) Increased vagal stimulation of the heart
   (c) Reflex dilatation of the peripheral blood vessels (d) Increased sympathetic stimulation of the heart
10. **Increased pressure within carotid sinus produces:**
   (a) Reflex increase in venous pressure (b) Reflex hyperpnoea
   (c) Reflex bradycardia (d) Increase in heart rate
11. **Threshold of baroreceptor reflex is seen at mean blood pressure of:**
   (a) 15 mmHg (b) 40 mmHg (c) 60 mmHg (d) 80-200 mmHg
12. **Cutting the sino-aortic nerves:**
   (a) Increases the activity of neurons in vasomotor centre (b) Decreases the activity of neurons in vasomotor centre
   (c) Increases the activity of cardiac vagal centre (d) Bradycardia occurs
13. **Volume receptors:**
   (a) Discharge with onset of atrial contraction (b) Discharge both during atrial systole and diastole
   (c) Provide information about circulating blood volume (d) Help in regulation of systemic arterial BP
14. **Sudden death may occur in an individual following a massive heart attack due to activation of:**
   (a) Bainbridge reflex (b) Cushing reflex (c) Bezold Jarisch reflex (d) Hering-Breurer reflex
15. **Emotional effects on CVS are result of activation of:**
   (a) Baroreceptors (b) Chemoreceptors
   (c) Corticohypothalamic descending pathways (d) Cerebral cortex.
16. **Circulatory adjustments in the body under different situations is primarily brought about by all of the following *except*:**
   (a) Alteration in the arteriolar diameter (b) Alteration in the capacity of the venous reservoirs
   (c) Alteration in the output of the heart (d) Alteration in the tone of the shunt vessels
17. **Nitric Oxide:**
   (a) Also called endothelium derived relaxing factor (EDRF) (b) Deficiency is associated with hypotension
   (c) Produces smooth muscle relaxation throughout the body (d) Helps in formation of new blood vessels
18. **The portion of the mammalian heart which is *not* innervated by vagus nerve is:**
   (a) SA node (b) AV node (c) Muscles of the atria (d) Muscles of the ventricles
19. **Which one of the following effects in the heart would *not* be a result of increased vagal nerve activity?**
   (a) Acetylcholine release at the nerve endings (b) Decreased S-T interval
   (c) Bradycardia (d) Hyperpolarization of the S-A node
20. **The difference between the resting heart rate before and after vagotomy:**
   (a) 75 beats per minute (b) 120 beats per minute
   (c) 180 beats per minute (d) Gives a measure of the degee of vagal tone
21. **Vasomotor centre:**
   (a) Produces only increase in sympathetic activity
   (b) Produces only decrease in sympathetic activity
   (c) Can produce either increase or decrease in sympathetic activity
   (d) The only centre in the medulla to bring about regulation of CVS
22. **The main inhibitary centre of heart is:**
   (a) Nucleus tractus solitarius (b) Nucleus ambiguus
   (c) Nucleus parabrachialis (d) Dorsal nucleus of vagus
23. **Range of operation of baroreceptors is between:**
   (a) 0-60 mmHg (b) 0-200 mmHg (c) 60-200 mmHg (d) 150-200 mmHg
24. **Sino-aortic nerves are called as buffer nerves because:**
   (a) They originate from vagus nerve (b) They innervate both baroreceptors and chemoreceptors
   (c) They buffer any alteration in blood pH (d) They act as BP regulators

Answers

1. (d) 2. (d) 3. (a) 4. (a) 5. (d) 6. (b) 7. (b) 8. (c) 9. (d) 10. (c) 11. (c) 12. (a) 13. (c) 14. (c) 15. (c)
16. (d) 17. (a) 18. (d) 19. (b) 20. (d) 21. (c) 22. (b) 23. (c) 24. (d)

Chapter 40

The Heart Rate

I. Factors Affecting Heart Rate

II. Control of Heart Rate

Normal resting heart rate (HR) in adults is 70-80 beats per minute (bpm).

FACTORS AFFECTING HEART RATE

1. Age

After birth, as age increases, *vagal tone* increases and HR decreases, but in old age HR is slightly higher due to fall in vagal tone.

foetal HR	:	140-150 bpm
at birth	:	130-140 bpm
at 12 years	:	upto 100 bpm
adults	:	70-80 bpm
old age	:	upto 100 bpm

2. Sex

HR is slightly higher in females as compared to males due to:

♀ > ♂

(i) lower systemic B.P.; and

(ii) resting sympathetic tone is more.

3. Body temperature

(i) For each 1°F rise in body temperature, HR increases by about 10 bpm due to its direct effect on SA node. It also produces cutaneous vasodilatation causing fall in systemic B.P.

(ii) Conversely, fall in body temperature decreases the HR. It also produces vasoconstriction causing B.P. to rise.

Thus, HR is inversely related to the systemic B.P., referred to as *Marey's Law*.

Important Note

In general, stimuli that increase the HR also increase the B.P., whereas those that decrease the HR lower the BP. However, there are *exceptions* such as during exercise, fever, Cushing reflex (see below), haemorrhagic shock (page 387) etc.

[SERF]

4. Drugs

(i) **Epinephrine** increases HR due to its direct action on the heart.

(ii) **Nor-epinephrine** (NE) increases HR due to its direct effect on the heart, but its 'pressor' action stimulates the baroreceptors causing reflex increase in vagal tone to overcome direct effect and produces bradycardia.

(iii) Bainbridge reflex (page 331).

5. Diseases

(i) **Increase in intracranial tension** above 33 mmHg (normal 0-7 mmHg) decreases blood supply to medulla producing local hypoxia and hypercapnia. This directly stimulates the medullary centre (VMC) which tends to restore normal blood supply to medulla by increasing systemic B.P. Increase in B.P. via baroreceptors mechanism stimulates vagal outflow to cause decrease in HR and respiration. Thus increased intracranial tension is associated with bradycardia, a reflex called *Cushing reflex*.

(ii) **Thyrotoxicosis** (hypersecretion of thyroid hormone) is associated with high resting HR as thyroxine produces:

(a) direct positive chronotropic effect on the heart; and (↑ HR)

(b) potentiates the action of circulating catecholamines.

(iii) **Hypoxia** via stimulation of chemoreceptors increases the HR and systemic B.P.

6. Emotional stimuli

Emotions such as excitement, fear, anger etc. are associated

with tachycardia and hypertension whereas sudden shock, grief, apprehension are associated with bradycardia and hypotension. The emotional effects on CVS are mediated through corticohypothalamic descending pathways (page 332).

7. Exercise

HR increases in linearity with the severity of exercise. The heart rate increases during exercise due to:

(i) increase in sympathetic activity
(ii) decrease in vagal tone
(iii) increase in body temperature
(iv) release of catecholamines and thyroxine into the circulation
(v) change in blood chemistry *e.g.* hypoxia, hypercapnia and acidemia.

8. Painful stimuli

(i) Superficial pain is carried by unmyelinated 'C' fibers to synapse with pressor area of 'VMC'. Therefore, their activation causes sympathetic stimulation producing tachycardia and rise in B.P.
(ii) Pain arising from deep body tissues is carried by thin, myelinated A_δ fibers to synapse with depressor area of 'VMC'. Therefore, their stimulation causes overall sympathetic inhibition producing bradycardia and fall in systemic B.P.

9. Respiration

HR increases with inspiration and decreases during expiration, a phenomenon called ***sinus arrhythmia*** (*mechanism*, see below).

CONTROL OF HEART RATE

Control of HR is primarily determined by two mechanisms: cardiac innervation and medullary cardiovascular centres.

A. ROLE OF CARDIAC INNERVATION

It consists of the innervation of the heart by two divisions of ANS viz. sympathetic and parasympathetic (page 324).

B. ROLE OF MEDULLARY CARDIOVASCULAR CENTRES

Medullary cardiovascular centres include 'VMC' and cardiac vagal centre (nucleus ambiguus). Both these centres in turn get influenced by afferents converging on them from baroreceptors, chemoreceptors and corticohypothalamic descending pathways. (page 328).

Cardiac vagal impulse activity is reflex in origin and gets modified by impulses from the systemic arterial baroreceptors (carotid sinus and aortic arch). Impulses from these baroreceptors travel in sino-aortic nerves into the nucleus of tractus solitarius (NTS) in the medulla where they relay, second order neurons then excite the cell bodies of the *nucleus ambiguus (NA)*. These are the cell bodies of the vagal motor neurons which course down the vagus to innervate the heart. Therefore, 'NA' is also called as ***Cardiac Vagal Centre (CVC)***.

Impulse activity of the motor neurons in the cardiac vagal centre (CVC) *i.e.* nucleus ambiguus depends mainly on baroreceptor input which in turn depends on:

(1) the height of mean arterial BP; and
(2) the pulse pressure.

Interaction of heart rate and respiration

Normally baroreceptor input is phasically interrupted by the activity of the 'inspiratory centre'. How? **(Fig. 40.1)**

Neuronal activity of inspiratory neurons in the medulla, besides initiating inspiration also discharges to nucleus of tractus solitarius (NTS) and nucleus ambiguus (NA) and inhibits both the relays of the *baroreceptors–NTS-NA pathway;* thus, inhibiting the cardiac vagal motor discharge. This is why HR increases with inspiration and decreases during expiration, a phenomenon called ***Sinus Arrhythmia***.

Important Note

Cell bodies in 'NA' also discharge via recurrent laryngeal nerve to intrinsic (abductor) muscles of the larynx, therefore, as inspiration occurs, impulses from 'NA' cause widening of laryngeal opening.

A. Role of Baroreceptors

These are solely responsible for ***resting vagal tone*** in the normally breathing individual, because:

(1) Their reflex pathway via *Sino-Aortic nerves* includes, synapses in the 'NTS'; axons from 'NTS' pass by monosynaptic or polysynaptic relays to the NA and then from NA via the vagi to the heart.
(2) Collateral branches of *Sino-Aortic Nerves* cause mild inhibition of neurons of the inspiratory center and, therefore, produce negligible influence on respiration.

B. Role of Chemoreceptors

They also relay in the NTS and cause marked stimulation of neurons of inspiratory center. In *Eupnoea* (normal respiration), chemoreceptor input is of less significance, but in hypoxia, produces marked *hyperpnoea* (increase in frequency of respiration). How?

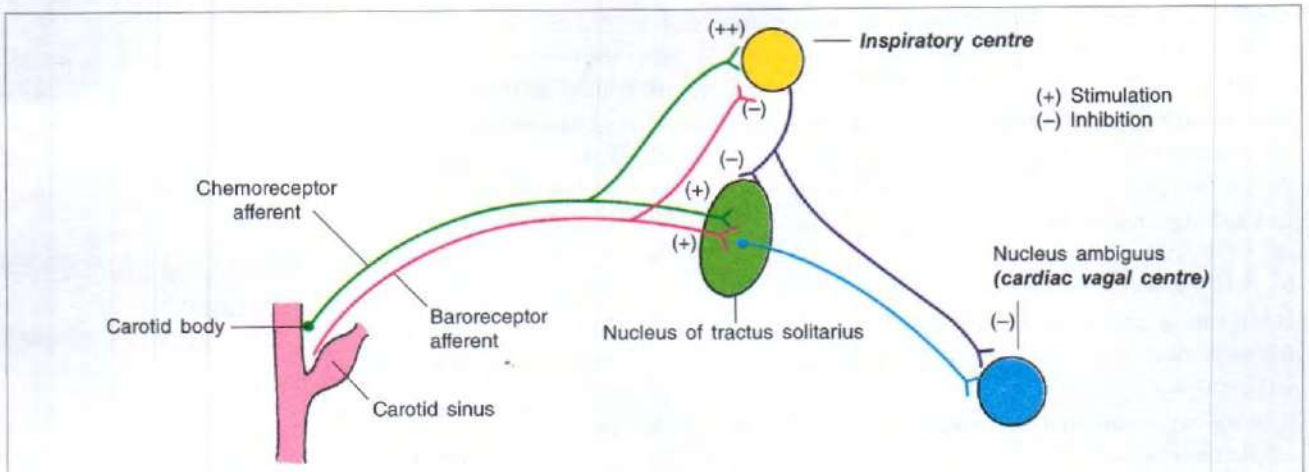


Fig. 40.1 Pathways relating interaction of cardiac and respiratory reflexes

In eupnoea, inspiratory center neuronal discharge normally ***Gates*** (controls) the baroreceptor inputs relay to the 'NA' (gating may be at the 'NTS' or 'NA' or both). The effect is to prevent cardiac vagal discharge during inspiration.

However, in hypoxia, chemoreceptor stimulation causes reflex marked stimulation of inspiratory center neurons which overrides chemoreceptors reflex vagal bradycardia producing marked hyperpnoea.

Study Questions

1. **Give physiological significance of:**
   - (i) Marey's law
   - (ii) Tachycardia
   - (iii) Bradycardia
   - (iv) Cushing reflex
   - (v) Bainbridge reflex
2. **Give physiological basis of:**
   - (i) Emotional stimuli can either produce tachycardia or bradycardia in a person
   - (ii) Sinus arrhythmia
   - (iii) Increase HR during exercise
3. **Write short notes on:**
   - (i) Effect of painful stimuli on HR
   - (ii) Control of HR and factors affecting it
   - (iii) Interaction between HR and respiration

MCQs

1. **Marey's law denotes relationship between heart rate and:**
   - (a) Conductivity
   - (b) Force of contraction
   - (c) Blood volume
   - (d) Blood pressure
2. **Cushing's reflex helps in maintaining:**
   - (a) Coronary blood flow
   - (b) Cerebral blood flow
   - (c) Hepatic blood flow
   - (d) Skin blood flow
3. **Heart rate is slowed by the following *except*:**
   - (a) Expiration
   - (b) Fear
   - (c) Increased intra-cranial pressure
   - (d) Hypoxia

4. **Heart rate increases with inspiration, a phenomenon called:**
   (a) Marey's Law
   (b) Cushing reflex
   (c) Sinus arrhythmia
   (d) Bainbridge reflex
5. **Impulse activity of the motor neurons in the nucleus ambiguus depends mainly on:**
   (a) Baroreceptor input
   (b) Chemoreceptor input
   (c) Corticohypothalamic descending pathway input
   (d) All of the above
6. **In Cushing's reflex, there is:**
   (a) ↑ HR, ↑ BP
   (b) ↓ HR, ↑ BP
   (c) ↑ HR, ↓ BP
   (d) ↓ HR, ↓ BP
7. **Heart rate is accelerated by the following *except*:**
   (a) Inspiration
   (b) Grief
   (c) Exercise
   (d) Anger
8. **Resting vagal tone in a normally breathing individual is due to:**
   (a) Baroreceptor activity
   (b) Chemoreceptor activity
   (c) Cerebral cortex activity
   (d) Inspiratory centre activity

Answers

1. (d) 2. (b) 3. (d) 4. (c) 5. (a) 6. (b) 7. (b) 8. (a)

Chapter 41

The Cardiac Output

I. Definition
II. Distribution
III. Control of Cardiac Output
(A) Control of heart rate (Extrinsic Autoregulation)
(B) Control of stroke volume (Intrinsic Autoregulation)
1. Heterometric regulation - factors affecting venous return 2. Homometric regulation
IV. Methods of measurement of Cardiac output

DEFINITION

The amount of blood pumped out by each ventricle into the circulation per minute is called *cardiac output (CO).* The amount of blood pumped by each ventricle per beat is called *Stroke Volume (SV)* – *Normal*: 80 mL.

Therefore, cardiac output can be calculated as: heart rate × stroke volume. Normally it is 5-6 L/min (average: 5.5 L/min). The output of the two ventricles is *exactly* the same. Thus, each ventricle pumps 5-6L of blood into the circulation per minute. This is made possible since the two ventricles are connected in series.

Cardiac Index

As the 'CO' changes with height and weight of the individual, therefore, the 'CO' is frequently stated in terms of *Cardiac Index*.It is the 'CO' expressed as a function of body surface area. *Normal value*: 3.2L/m^2/min.

DISTRIBUTION

Body organ	Amount flow of blood (mL/min)		Percentage of total 'CO'	
Liver	1500		25%	
Kidneys	1300		approx. 25%	
Brain	750			75%
Lungs	500	1500		
Heart	250		25%	
Skeletal muscle and other body organs	1000			
		1500		
Skin	500		25%	

Important Note

Of the total 'CO', 75% is distributed to the vital organs of the body.

CONTROL OF CARDIAC OUTPUT

Why important?

The two pumps right and left ventricles (RV and LV) pump exactly the same quantity of blood per unit time. The accuracy with which this adjustment is made can be understood by considering what would happen if the 'RV' output exceeds the 'LV' output by as little as 0.1 mL/beat, equivalent to 7 mL/min. Then in a period of 3 hours, the pulmonary blood volume would have increased by more than 1 litre (7 × 60 × 3 = 1260 mL). This will prevent the optimal exchange of gases across the lungs and result in severe pulmonary insufficiency.

As cardiac output is a product of function of heart rate (HR) and stroke volume (SV), variations in cardiac output can be produced by changes in 'HR' or 'SV' or both. Therefore, cardiac output is controlled by two main regulatory process:

A. Control of 'HR' or *Extrinsic autoregulation;* and
B. Control of 'SV' or *Intrinsic autoregulation.*

Important Note

Factors affecting cardiac output: **All the condition that affect either the heart rate (page 335) or stroke volume (Figs. 41.1 and 41.3) or both will produce variations in the cardiac output.**

A. CONTROL OF HEART RATE or EXTRINSIC AUTOREGULATION

It is primarily governed by *'Cardiac innervation'* and cardiovascular centres located in the medulla

(vasomotor centre, VMC and Cardiac Vagal Centre, CVC) (page 323).

B. CONTROL OF STROKE VOLUME or INTRINSIC AUTOREGULATION

It is governed by 'Two' mechanisms:

1. *Heterometric Regulation*; and
2. *Homometric Regulation*.

For differentiating features between the two, refer **Table 41.1**.)

1. Heterometric Regulation

The force of contraction of the myocardium is dependent upon its *pre-load* and *after load*. The ***pre-load*** is the degree to which the myocardium is stretched before it contracts; and the ***after load*** is the resistance against which the ventricles pump the blood (page 180).

The relationship between the extent of pre-load (*i.e.* initial length of muscle fiber) and the total tension developed in the cardiac muscle is given by *Length-Tension Relationship* in cardiac muscle as given by ***Frank-Starling Law*** of the heart (page 182).

In the heart, extent of 'pre-load' is directly proportional to the *end-diastolic volume (EDV) i.e.* amount of blood remaining in the ventricles at the end of diastole. Any factor which increases *venous return (VR) i.e.* volume of blood returning to heart will increase 'EDV'. Therefore, more is 'EDV', more will be stretching of the myocardium, *i.e.* initial length of cardiac muscle fiber; and more will be the force of contraction of the myocardium. **(Fig. 41.1)**

Table 41.1: The main differentiating features between Heterometric and Homometric regulatory mechanisms

Heterometric Regulation	Homometric Regulation
1. *Hetero* means different; *metric* means measure. Therefore, here change in myocardial contractility varies with the initial (resting) length of cardiac muscle fibers. Thus within physiological limits, force of ventricular contraction is directly proportional to the initial length of muscle fibers *(Frank-Starling law of the heart)* (page 182).	1. *Homo* means same; therefore, here change in myocardial contractility is *independent* of the resting length of cardiac muscle fibers.
2. This effect is *independent* of cardiac innervation.	2. This effect is *dependent* on cardiac innervation. For example, (i) Sympathetic stimulation increases, and (ii) parasympathetic stimulation decreases myocardial contractility.

Factors affecting venous return

1. *Thoracic Pump or Respiratory pump*

The normal intra-thoracic pressure (*i.e.* pressure within the chest, but outside the lungs) at the end of expiration

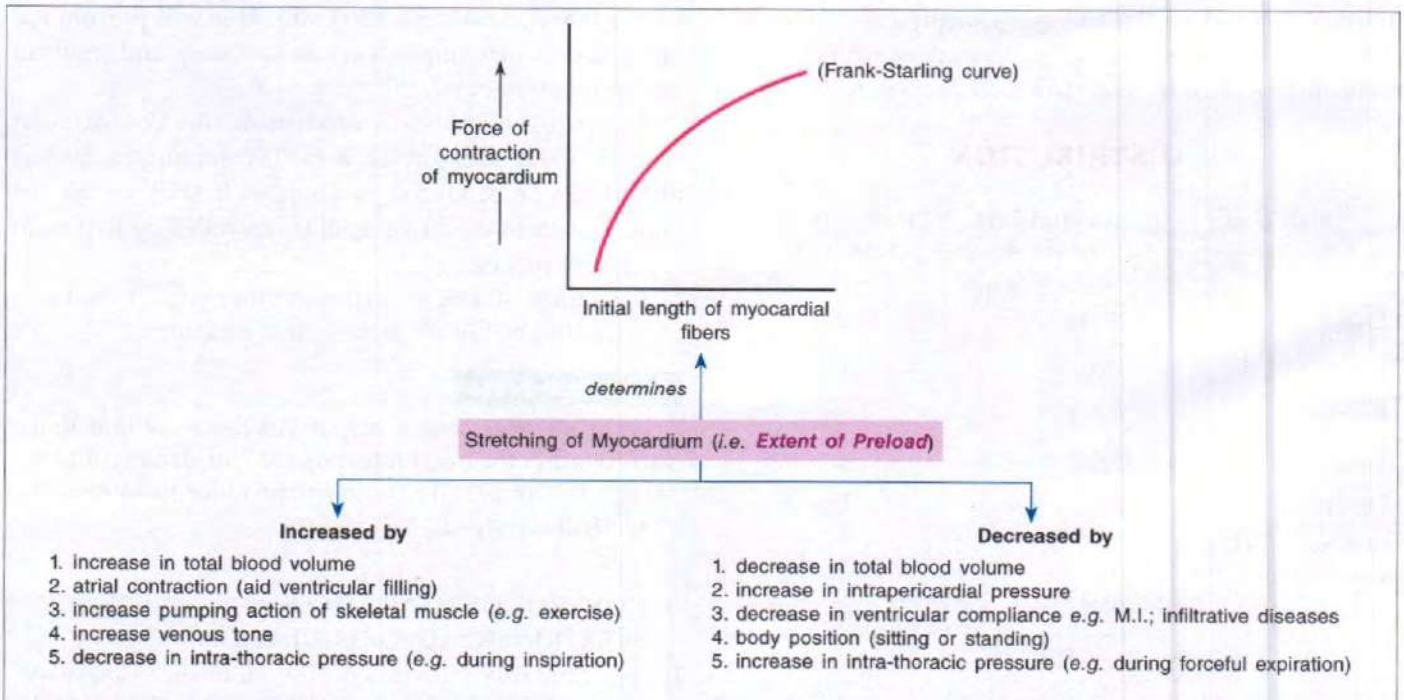


Fig. 41.1 Heterometric regulation of cardiac output: length-tension relationship (**Frank-Starling Curve**) with major factors affecting it

is sub-atmospheric, (–2 mmHg). During inspiration:

(i) *Intra-thoracic pressure* decreases to –5 mmHg causing less pressure over larger veins and arteries; and

(ii) *descent of diaphragm,* increases intra-abdominal pressure to squeeze blood out of the abdomen.

These two factors combine to favour venous return to the heart during inspiration.

2. *Cardiac Pump*

(i) ***Vis A Tergo*** *i.e. force from behind* which drives the blood forward.

'VR' is mostly dependent on the forward push from behind (*vis a tergo*) *i.e.* the propelling force which is imparted by: (a) the contraction of the heart to the blood during its passage through the heart. This is assisted by the (b) elastic recoil of arterial wall (Windkessel effect, page 311), therefore, the blood enters the venules with an appreciable pressure higher than that of right atrium (RA). The flow of blood in the veins is, therefore, towards the heart.

(ii) ***Vis A Fronte*** *i.e. force acting from the front* to attract blood in the veins towards the heart. It is exerted by the contraction of the ventricles and has 2 components:

(a) *Ventricular systolic suction:* Ventricular systole causes descent of A-V junction and enlarges great venous reservoirs *i.e.* atria and venae cavae (SVC and IVC) and atrial pressure drops. Thus by its piston like downward movement attracts blood from the greater veins into the atria.

(b) *Ventricular diastolic suction:* Ventricular diastole causes sudden decrease in atrial pressure, increases venous blood flow into the atrium.

3. *Muscle Pump*

With the rhythmic contraction of the skeletal muscles, the venous segments are squeezed and the rise of pressure forces blood towards the heart out of each successive segment; the venous valves prevent back flow **(Fig. 41.2)**. As soon as the muscles relax the depleted segments are promptly refilled from the more peripheral venous channels and also from the superficial veins via their valved communicating channels. The more frequent and powerful such rhythmic movements are, the more efficient is the *muscle pumping*.

4. *Total Blood Volume*

Increase in total blood volume increases, while decrease in total volume decreases the 'VR'.

5. *Capacity of the Venous System*

Veins are capacitance vessels. Increase sympathetic activity, increases venous tone, thus increase 'VR'.

6. *Body Position*

In standing posture, peripheral pooling of blood occurs and 'VR' decreases.

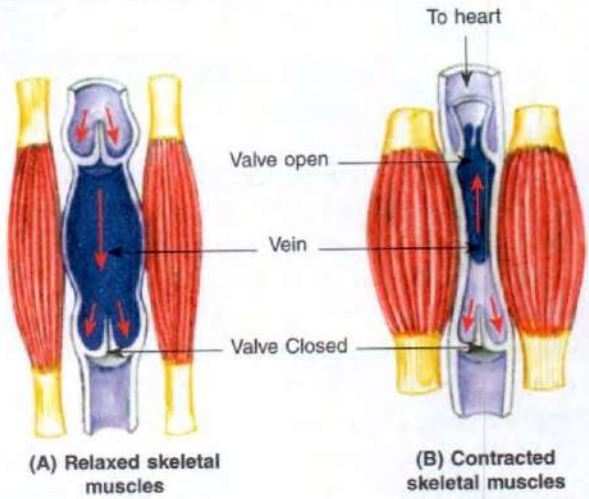


Fig. 41.2 Role of skeletal muscle contractions and venous valves in the venous return

7. *Ventricular Compliance (distensibility)*

Following myocardial infarction (heart attack) the damaged myocardium becomes fibrotic and non-functional and ventricular compliance decreases. This decreases the 'VR'. Similarly, 'VR' decreases with infiltrative diseases of the heart and an increase in intra-pericardial pressure.

2. Homometric Regulation

Here *myocardial contractility* increases without an increase in initial length of cardiac muscle fibers. The myocardial contractility differs from force of contraction of the myocardium.

Increased myocardial contractility means:

1. Ventricles are able to do more work per stroke at a given ventricular end diastolic pressure (EDP).
2. The ventricles develop tension more rapidly.
3. The myocardial fiber shortens more quickly.
4. Ejection of blood is faster.
5. The duration of myocardial contraction is brief.
6. Rate of ventricular pressure fall or relaxation, is faster, even when the diastolic ventricular volume and stroke volume are not appreciably altered.

This is possible since cardiac muscle can alter its work and power (*i.e.* rate of working) at any one load and muscle length by nature of its changing *force-velocity relationships* in different chemical environments (page 181).

Increase in force of contraction of the myocardium

It is due to Frank-Starling mechanism which is always accompanied by an increase in end diastolic volume of the ventricle and increase in ventricular end diastolic pressure.

Thus, with increased myocardial contractility, more of the blood that remains in the ventricles is expelled, and the end systolic ventricular volume falls.

Factors affecting myocardial contractility **(Fig. 41.3)**

Increased by

1. **Catecholamines.** The effect is mediated via β_1-adrenergic receptors through cAMP. How?
   cAMP;
   (i) increases Ca^{2+} influx from the ECF, making more Ca^{2+} available to bind to troponin 'C', and
   (ii) via protein kinase, increases active transport of Ca^{2+} to the sarcoplasmic reticulum.
2. **Stimulation of sympathetic nerves** to heart has the same effect as of catecholamines.
3. Changes in Heart Rate and Rhythm
   (Force-Frequency Relation)
   (i) Myocardial contractility increases as HR increases within limits. How? (Exact mechanism not known).
   (ii) *Staircase phenomenon* observed following 'compensatory pause' after extrasystole (page 182) is also known as ***Post Extrasystolic Potentiation.*** It is probably due to increased availability of intracellular Ca^{2+}.
4. **Drugs**
   (i) Xanthine *e.g.* caffeine and theophylline, inhibits the breakdown of cAMP producing *positive inotropic effect.*
   (ii) Glucagon (a) increases formation of cAMP, and (b) also increases A-V conduction.
   (iii) Digitalis, inhibits $Na^+ - K^+$ ATPase in the myocardium to increase intracellular Na^+; thus decreases Ca^{2+} efflux and finally increases Ca^{2+} availability in the cell.

Decreased by

1. ***Stimulation of vagus nerve*** to heart produces "negative inotropic effect" on the atrial muscle. Since vagi do not innervate the ventricles, decreased atrial contractility indirectly causes mild 'negative inotropic' effect on ventricles.
2. ***Intrinsic myocardial depression*** due to heart failure decreases catecholamine content of myocardium to produce negative inotropic effect on myocardium.
3. ***Myocardial infarction*** causes part of myocardium to become fibrotic and non-functional.
4. ***Hypercapnia, hypoxia and acidosis,*** decrease cAMP formation thus produce negative inotropic effect on the heart.
5. ***Pharmacological depressants;*** for example, quinidine, procainamide and barbiturates have direct 'negative inotropic' effect on the myocardium.

METHODS OF MEASUREMENT OF CARDIAC OUTPUT

1. DIRECT FICK METHOD

Fick Principle *(Fick, A. 1855):* It states that the amount of a substance taken up by an organ (or by the whole body) per unit of time is equal to the arterial level of the substance minus the venous level (A-V difference) times the blood flow, *i.e.* amount of substance taken per min = (A–V) difference of the substance × blood flow/min.

$$\text{Then blood flow/min} = \frac{\text{amount of substance taken/min}}{\text{(A–V) difference of the substance}}$$

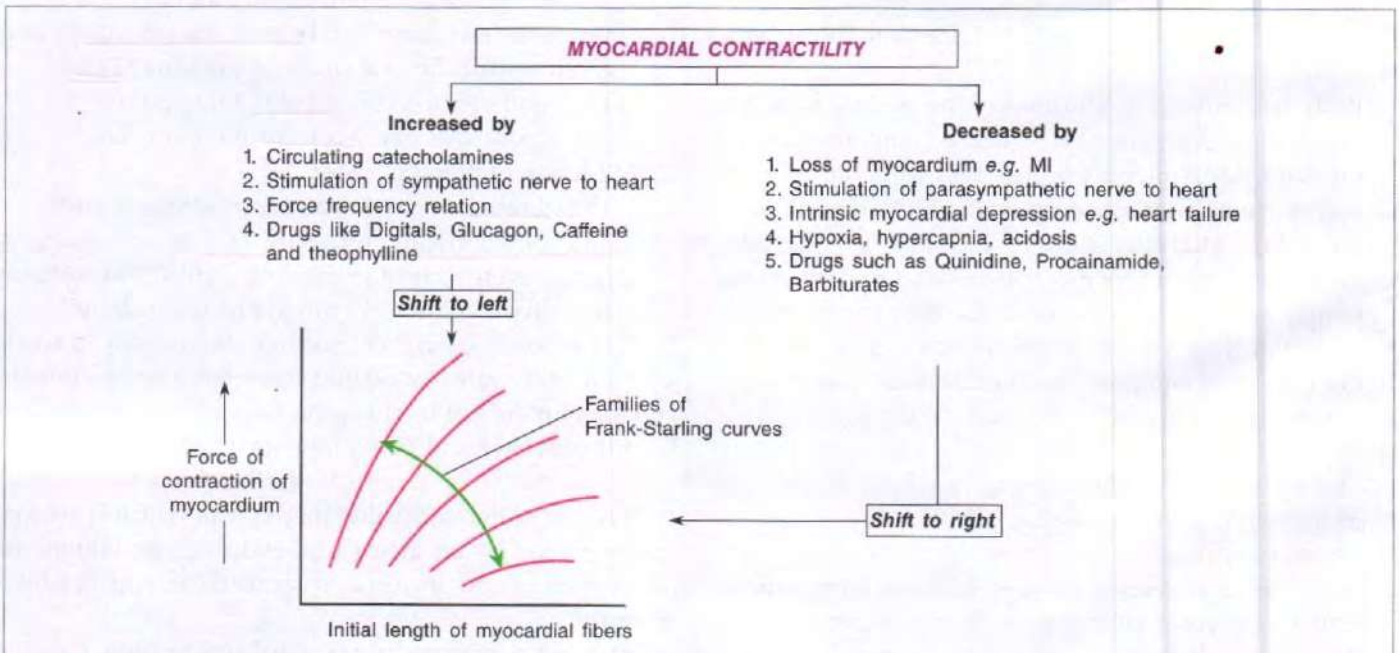


Fig. 41.3 Homometric regulation of cardiac output: Effect of changes in myocardial contractility on the '**Frank-Starling Curve**' with factors affecting myocardial contractility

This principle can be used to determine 'CO'; Therefore,

$$\text{'LV' output} = \frac{\text{the amount of } O_2 \text{ consumed by the whole body per unit time}}{\text{(A–V) } O_2 \text{ difference across the lungs}}$$

Since arterial blood has the same oxygen content in all parts of the body, the arterial O_2 content can be measured in a sample obtained from any convenient artery. A sample of venous blood in pulmonary artery is obtained by means of a cardiac catheter inserted through a forearm vein and to guide its tip into the 'RA' with the aid of fluoroscope, thence through the 'RV' into the pulmonary artery. O_2 consumption of whole body is determined by means of close circuit spirometry.

The calculation of 'CO' using a typical set of values is as follows:

$$\text{'LV' output} = \frac{\text{Resting } O_2 \text{ consumption of the body (mL/min)}}{[A_{O2}] - [V_{O2}]}$$

$$= \frac{250 \text{ mL/min}}{\begin{matrix}19 \text{ mL/dL arterial blood } \textit{minus} \\ 14 \text{ mL/dL venous blood in} \\ \text{pulmonary artery}\end{matrix}}$$

$$= \frac{250 \text{ mL/min}}{5 \text{ mL/dL}}$$

$$= \frac{250 \times 100}{5}$$

$$= 5000 \text{ mL/min or } 5 \text{ L/min}$$

Disadvantages of direct Fick method

1. The subject is exposed to all risks of invasive procedures *e.g.* haemorrhage, infection etc.
2. The subject becomes conscious of whole technique and the 'CO' may, therefore, be somewhat higher than normal.
3. This method when employed for measuring cardiac output during heavy exercise, an indwelling atrial or ventricular catheter may precipitate ventricular fibrillation which is fatal.

2. INDICATOR (or DYE) DILUTION METHOD

Principle: A known amount of the dye is injected into an arm vein. It will first pass through the heart, then the pulmonary circulation and finally will be evenly distributed in the blood stream. Its mean concentration during its 1st passage through an artery can be determined from successive samples of blood taken from the artery. The blood flow (F) is given by the formula:

$$F = \frac{I}{c.t}$$

where,

I = total amount of dye injected
c = mean concentration of the dye
t = duration in sec of the 1st passage of the dye through the artery

Criteria of the indicator (dye) used

(i) It should stay in the circulation during the test.
(ii) It should not have any harmful effects on the body.
(iii) Its concentration can easily be measured in the body.
(iv) It should not alter the 'CO' or hemodynamics of blood flow.

Such an indicator or dye commonly used to determine cardiac output in humans is *Evan's blue (T-1824)* or *Radioactive isotopes.*

Procedure

(i) Before the injection of the dye, few mL of venous blood is withdrawn from any convenient vein, to this is added sufficient dye (say 5 mg).
(ii) This solution containing 5 mg of dye is then injected rapidly into the vein.
(iii) Immediately after the injection, samples of arterial blood are taken serially at intervals of 0.5 – 2.00 sec into a series of tubes, and each analysed for concentration of the dye.
(iv) The concentration of successive samples are plotted on semi-logarithmic paper. **(Fig. 41.4)**
(v) The curve shows that the dye concentration reaches a peak and then steadily declines only to rise again owing to recirculation of the blood containing the dye.
(vi) However, if the early descent from the initial peak is extrapolated to cut on the X-axis, the point on the

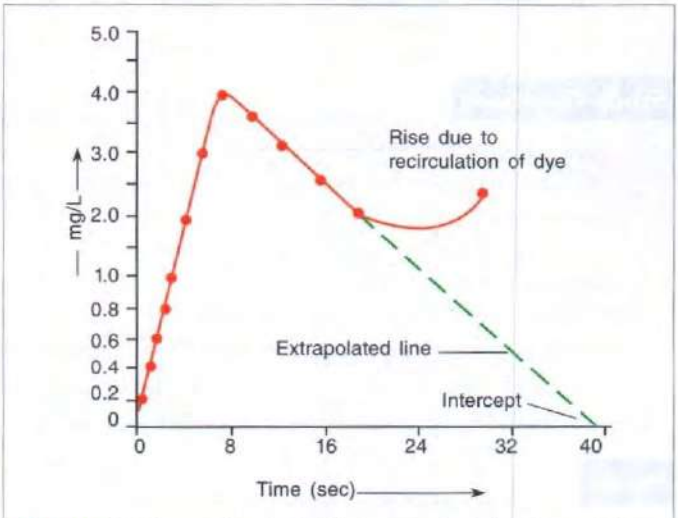


Fig. 41.4 Indicator (or dye) dilution method for determining cardiac output (log concentration scale)

time scale at which this occurs gives the duration of the 1st passage of dye through the artery.

(vii) From the resulting curve the mean concentration of the dye during the first passage through the artery can be calculated. Let it be 1.6 mg/L during the time 't' of 39 sec. Therefore, in 39 sec, 5 mg of dye must have been diluted by 5/1.6 = 3.1 litres of blood. In one minute the flow *i.e.* 'CO' would have been = 3.1 × 60/39 = 4.77 L/min.

Important Note

Thermodilution, a recently introduced indicator dilution technique is also used clinically. Here the indicator used is 'cold saline', which is injected through a catheter inserted into an arm vein and adavanced to the right atrium. The change in blood temperature is measured by a second thermostat passed retrogradely from a peripheral artery to the base of aorta. The temperature change is inversely related to the amount of blood flowing through the aorta (*i.e.* extent to which cold saline is diluted by blood). The shape and time-course of the change in aorta blood temperature is similar to that of the dye concentration curve following dye injection. (Note: The blood need not be withdrawn for sampling). This is almost a harmless technique and repeated determination of cardiac output can be made (even in infants and children).

3. ECHOCARDIOGRAPHY

A ***non-invasive*** technique that does not involve injection or insertion of a catheter **(Fig. 41.5)**.

In echo-cardiography, pulses of ultrasonic waves, at a frequency of 2.25 mega-hertz (MHz), are emitted from a transducer that also functions as a receiver to detect waves reflected back from various parts of the heart. Reflections occur wherever acoustic impedence changes. Reflected wave *i.e.* echoes are displaced against time on an oscilloscope to obtain a record ***(echocardiogram)***. This provides a record of the movements of the ventricular wall and its septum; heart valves during the cardiac cycle. It is particularly useful in evaluating:

(i) End-diastolic volume (EDV);
(ii) End-systolic volume (ESV);
(iii) Cardiac output and
(iv) Valvular defects.

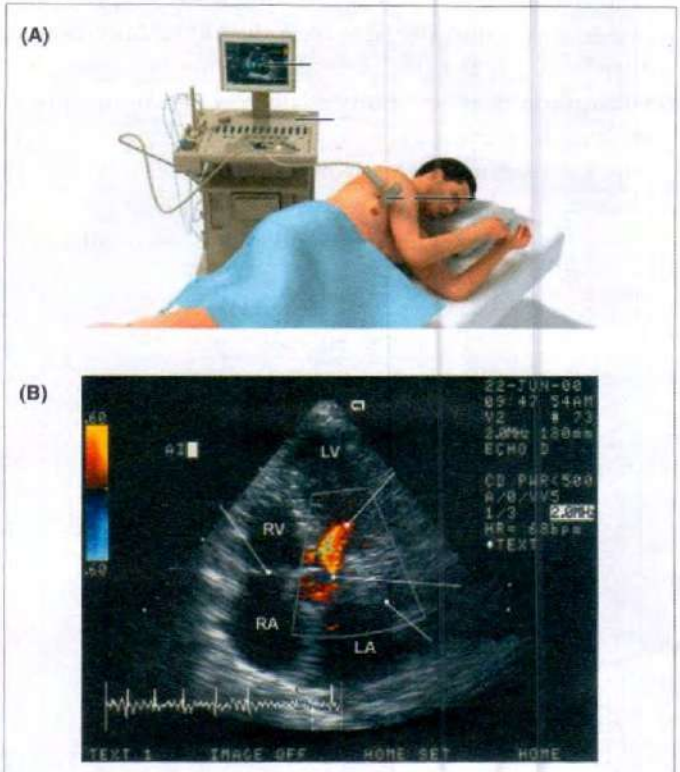


Fig. 41.5 Echocardiography (A) procedure and (B) an actual record

Study Questions

1. Give physiological significance of:
   (i) Vis-a-tergo and vis-a-fronte (ii) Fick principle (iii) Echocardiography
2. Write short notes on:
   (i) Factors affecting venous return. (ii) Factors affecting myocardial contractility.
   (iii) Factors affecting cardiac output (iv) Factors determining end diastolic volume.
   (v) Factors affecting Frank-Starling curve (vi) Homometric regulation of cardiac output
3. Give the significance and causes of shift of Frank-Starling curve to right and left.
4. What will happen if RV output exceeds the LV output as little as 0.1 ml/beat?
5. Lists methods for measurement of cardiac output. Describe non-invasive technique for its determination.

MCQs

1. Which of the following about cardiac output is *correct*?
   (a) Not necessarily increases with heart rate (b) Increases to 50L/min in exercise
   (c) Proportional to blood pressure (d) Proportionally falls with increase in heart rate

2. **What percentage of total cardiac output is distributed to the vital organs?**
   (a) 25% (b) 50% (c) 75% (d) 100%
3. **Increased venous return leads to increased cardiac output by way of the Frank-Starling mechanism. Which one of the following would *not* happen?**
   (a) Increased end diastolic sarcomere length (b) Increased myocardial tension during systole
   (c) Increased stroke volume (d) Decreased end diastolic volume
4. **Pre-load of the heart is determined by:**
   (a) End diastolic volume (b) Ejection systolic volume (c) End systolic volume (d) Systolic vascular resistance
5. **Homometric regulation of cardiac output is:**
   (a) Independent of cardiac innervation
   (b) Primarily governed by heart rate
   (c) Independent of the resting length of cardiac muscle fibers
   (d) Governed by Frank-Starling Law of the heart
6. **Heterometric regulation of cardiac output is:**
   (a) Not dependent on the resting length of cardiac muscle fibers
   (b) Dependent on parasympathetic innervation of the heart
   (c) Dependent on sympathetic innervation of the heart
   (d) Dependent on Frank-Starling Law of the heart
7. **The term 'vis-a-tergo' refers to:**
   (a) Increased venous return to the heart due to decrease in intrathoracic pressure
   (b) Force from the front to attract blood in the veins towards the heart
   (c) Propelling force on blood which drives the blood forward
   (d) Rhythmic contraction of skeletal muscles that squeezes blood out of veins towards the heart
8. **Intrinsic myocardial depressant decreases the myocardial contractility by:**
   (a) Causing part of myocardium to become fibrotic (b) Decreasing cAMP formation in myocardium
   (c) Increasing breakdown of cAMP in myocardium (d) Decreasing catecholamine content of myocardium
9. **Digitalis increases myocardial contractility by:**
   (a) Inhibiting Na^+–K^+ ATPase in the myocardium (b) Decreasing breakdown of cAMP
   (c) Increasing formation of cAMP (d) Activating proteinkinase
10. **Direct Fick method of measuring cardiac output requires estimation of all *except:***
    (a) O_2 content of arterial blood (b) O_2 consumption per unit time
    (c) O_2 content of blood from right ventricle (d) O_2 content in inferior vena cava
11. **In echocardiography, pulses of ultrasonic waves are emitted at a frequency of:**
    (a) 1 mega hertz (b) 2 mega hertz (c) 20 hertz (d) 2000 hertz
12. **Heart receives about:**
    (a) 1% of cardiac output (b) 2% of cardiac output (c) 5% of cardiac output (d) 10% of cardiac output
13. **Intrinsic autoregulation of cardiac output is governed by:**
    (a) Mean arterial pressure (b) Control of stroke volume (c) Sympathetic system (d) Right atrial pressure
14. **With normal cardiac function, a 10 mmHg change in which of the following pressures would have the greatest effect on cardiac output?**
    (a) Pressure in the carotid arteries (b) Pressure in the pulmonary artery
    (c) Aortic pressure (d) Right atrial pressure
15. **The following decrease the cardiac output *except*:**
    (a) Sitting or standing from lying position (b) Rapid arrhythmias
    (c) Heart disease (d) High environmental temperature
16. **Cardiac output is maximally increased in:**
    (a) Anxiety (b) After meals (c) Exercise (d) Late pregnancy

Answers

1. (a) 2. (c) 3. (d) 4. (a) 5. (c) 6. (d) 7. (c) 8. (d) 9. (a) 10. (d)
11. (b) 12. (c) 13. (b) 14. (d) 15. (d) 16. (c)

Chapter 42

The Arterial Blood Pressure

I. Introduction
II. Factors affecting arterial BP
III. Determinants of arterial BP
IV. Regulation of arterial BP
(A) Rapidly acting regulatory mechanisms (B) Intermediate acting regulatory mechanisms
(C) Long-term acting regulatory mechanisms (D) Miscellaneous mechanisms

INTRODUCTION

Definition

The arterial blood pressure (B.P.) is the pressure of the column of blood in the arterial system.

Components

Salient features of different components of systemic arterial BP with normal values in the brachial artery in young adults in the sitting or lying position at rest are given in **Table 42.1**. For resting measurements the subject should be quiet for at least five minutes before the measurements are taken.

Functions

1. To maintain a sufficient pressure head to keep the blood flowing through the blood vessels.
2. To provide the motive force of filtration at the capillary bed; thus assuring nutrition to the tissue cells, formation of urine, lymph etc.

FACTORS AFFECTING ARTERIAL B.P.

Since Arterial B.P. is a function of product of *cardiac output* (CO) and *peripheral resistance* (PR), therefore, it is affected by conditions that affect either or both of these factors. In general, increase in 'CO' increases SBP (systolic BP), whereas increase in 'PR' increases DBP (Diastolic BP).

1. Age

Both SBP and DBP increase with age; SBP increases more than DBP due to decreased distensibility of arteries as their walls become increasingly more rigid following arteriosclerosis with advancing age. However, DBP decreases after 50–60 years of age.

Roughly normal SBP in adults is (100 + age in years).

Newborn : SBP 20-60 mmHg average: 40 mmHg
At 15 days : SBP 70 mmHg
30 days : SBP 80 mmHg
12 years : SBP 105 mmHg; DBP 50 mmHg
17 years : SBP 120 mmHg DBP 60 mmHg
60 years : SBP 140 mmHg; DBP 90 mmHg

2. Sex

In females, before menopause SBP is 4-5 mmHg less than that in males of same age. After menopause SBP is 4-5 mmHg more than males of same age. This effect may be due to oestrogen which prevents atherosclerosis.

3. Body Built

In obese individuals, brachial arterial pressure gives high readings, because there is more tissue between the 'cuff' and the artery. Some of the cuff pressure is dissipated, therefore, pressure obtained using the standard arm cuff are falsely high. For the similar reasons the BP recorded even in recumbent position from thigh in popliteal artery is higher than in the brachial artery.

In such conditions, accurate pressure can be obtained by using a cuff that is wider than the standard arm cuff.

4. Climate

(i) *Exposure to cold* via hypothalamus produces vasoconstriction and increases both SBP and DBP.
(ii) *Exposure to warmth* via hypothalamus produces vasodilatation and decreases both SBP and DBP.

Table 42.1: Components of systemic arterial B.P.

Component	Definition	Characteristic features	Normal Values
1. Systolic blood pressure (SBP)	It is the maximum pressure exerted during systole	(i) It undergoes considerable fluctuations: (a) *increased by:* excitement, exercise, meals etc. (b) *decreased by:* sleep, rest. (ii) The height of SBP indicates: (a) extent of work done by the heart or the force with which the heart is working, and (b) the degree of pressure which the arterial walls have to withstand.	Range: 100-130 mmHg Average: 120 mmHg
2. Diastolic blood pressure (DBP)	It is the minimum pressure exerted during diastole.	(i) It undergoes much less fluctuations. (ii) It is the measure of total *peripheral resistance.* (iii) It indicates the constant load against which heart has to work.	Range: 70-85 mmHg Average: 80 mmHg
3. Pulse pressure (PP)	It is the difference of SBP and DBP *i.e.* PP = SBP minus DBP.	(i) It determines the pulse volume. (ii) High 'PP' is indicative of *systolic hypertension* and indirectly determines decrease in elasticity of blood vessels.	Average: 40 mmHg
4. Mean blood pressure (MBP)	It is the average pressure throughout the cardiac cycle. It can be computed as: MBP = DBP + 1/3 PP.	(i) It is same for each organ and determines the pressure head *i.e.* regional blood flow through an organ depends on it. (ii) *All cardiovascular reflexes are sensitive to changes in MBP.*	Range: 95-100 mmHg

{***Clinically,*** *blood pressure is expressed as SBP/DBP; Normal average: 120/80 mmHg*}.

Important Note

Currently recommendation from WHO refer to: < 120/< 80 mmHg as optimal BP

5. Diurnal Variation

Diurnal variation of 5-10 mmHg is common in SBP; peak values are observed during the afternoon and lowest during early hours of morning. Reverse rhythm is observed in night workers.

6. Exercise

Table 42.2: Effect of exercise on systemic arterial B.P.

Type of Individual	Intensity of Exercise					
	Mild		Moderate		Severe	
	SBP	DBP	SBP	DBP	SBP	DBP
Trained individuals	–	–	–	–	+ ↑	–
Untrained individuals	+ ↑	–/+ ↓	++ ↑	+ ↑	+++ ↑	+ to ++ ↑

(–) : no change; +, ++, +++ : mild, moderate, severe; ↑ : increase; ↓ : decrease

B.P. comes back to normal within 5 min after stoppage of exercise due to sudden relaxation of muscles which produces vasodilatation. Initial increase is due to sympathetic vasoconstriction (for details, refer to page 478).

7. Emotions

Excitement, fear, worry increase SBP due to increase in 'CO' secondary to increased sympathetic activity. (Also refer to page 332)

8. Gravity

The pressure in any vessel below heart level is increased and that in any vessel above heart level is decreased by the effect of gravity **(Fig. 42.1)**.

The magnitude of the gravitational effect at the normal density of the blood is 0.77 mmHg for each cm vertical distance above or below the heart.

Therefore, in the upright position, when MBP at the heart level in all large arteries is 100 mmHg, the MBP in a large artery in the head (50 cm above heart) will be:

= 100 – (0.77 × 50) = 62 mmHg; and the mean pressure in a large artery in the foot (100 cm below heart) will be:

= 100 + (0.77 × 100) = 177 mmHg

The effect of gravity on *'venous pressure'* is similar.

9. Hereditary

Familial tendencies of hypotension or hypertension with SBP are common.

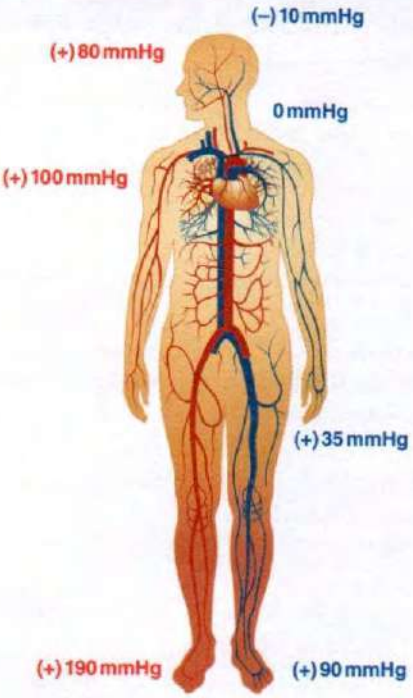


Fig. 42.1 Effect of gravity on arterial (red) and venous (blue) blood pressure

10. Meals

Changes in systemic BP are seen due to:

(i) pressure over heart due to distended abdomen increases 'HR', and

(ii) increase epinephrine release from adernal medulla (higher centres influence).

Therefore, SBP increases by 5-6 mmHg upto 1 hour after meals. DBP remains same or slightly decreases due to vasodilatation in digestive organs.

11. Sleep

SBP falls in early hours of sleep by 15-30 mmHg, because of general vasodilatation of vessels in complete relaxed state. However, disturbed sleep increases SBP due to increased sympathetic discharge secondary to skeletal muscle tensioning.

12. Posture

Changes in DBP are more with change of body posture. In standing posture DBP increases; in sitting remains normal and decreases in lying posture.

Mechanism

Upon standing:

(i) peripheral pooling of blood in dependent parts → ↓ VR → ↓ CO → ↓ SBP → *(by 6-23 mmHg)* ↓ baroreceptor discharge $\xrightarrow{\text{via 'VMC'}}$ ↑DBP

(ii) ↑ 'PR' → ↑ DBP

Therefore, *sudden standing* increases DBP, if recorded within 30-60 sec of change in posture after that it comes back to normal via the operation of baroreceptor reflexes (page 330).

Important Note

During *prolonged standing*, any fall in BP incraeses the sympathetic discharge via baroreceptor reflexes so as to maintain venous return; as a result DBP remain elevated throughout the period of standing.

DETERMINANTS OF ARTERIAL B.P. (FACTORS CONTROLLING B.P.)

Arterial B.P. is a function of *product of 'CO' and total 'PR'* *i.e.* BP = 'CO' × 'PR'.

Therefore, any condition that will alter either the 'CO' or 'PR' (other factors remaining unchanged) will cause a change in arterial B.P. Both these factors are thus often manipulated in the control of arterial B.P.

A. ROLE OF CARDIAC OUTPUT (CO)

Pumping action of the heart is the main factor for controlling 'CO'; because in each effective contraction of the ventricle certain amount of blood is ejected into the aorta.

Therefore, alteration of 'CO' will alter arterial B.P.

As 'CO' is a function of product of 'HR' and stroke volume (SV) *i.e.* CO = HR × SV

Thus, increase in "CO" due to either increase in HR or SV or both will increase the systemic arterial B.P.

1. If increase in 'CO' is due to increase in HR, it increases DBP (because with increased HR diastole duration decreases and tendency of BP to fall during diastole decreases).
2. If increase in 'CO' is due to increase in SV, it increases SBP.
3. If increase in "CO" is due to both, it increases systolic as well as diastolic BP.

B. ROLE OF PERIPHERAL RESISTANCE (page 312)

It is the resistance which blood has to overcome while passing through the periphery. The chief site of peripheral resistance (PR) is the *'arterioles'*. Total 'PR' depends on the following factors:

1. Velocity of Blood

The total 'PR' is inversely related to the velocity of blood flow.

Since average velocity of blood flow is inversely related to total cross sectional area (TSA) of the vessel wall (page 314), that is why average velocity of blood is rapid in the aorta, declines steadily in smaller vessels and is slowest in the capillaries.

which is more for small vessels.

According to ***Bernoulli's Principle***, in a tube or blood vessel, the sum of the kinetic energy of flow and the pressure energy is constant (For details, refer to Fig. 42.2).

Therefore, when fluid flows through the narrow portion of tube, *kinetic energy* of flow increases as the velocity increases and the *potential energy* is reduced. Thus, the greater the velocity of flow in a vessel, the lesser will be the *lateral pressure* distending its walls.

Important Note

Bernoulli's principle application: When a vessel wall is narrowed (physiologically or pathologically due to arteriosclerosis) the velocity of flow in the narrowed portion increases and the distending pressure decreases.

2. Viscosity of Blood

It determines the resistance to flow. Total 'PR' is a direct function of the viscosity of blood. Alteration of blood viscosity will affect the DBP by its effect on total 'PR'. *Factors affecting viscosity of blood* (page 316).

3. Total Quantity of Blood in Arterial System

Increase in blood volume increases both SBP and DBP due to

(i) increased quantity of blood in arterial system, and
(ii) greater stretching of the arterial walls.

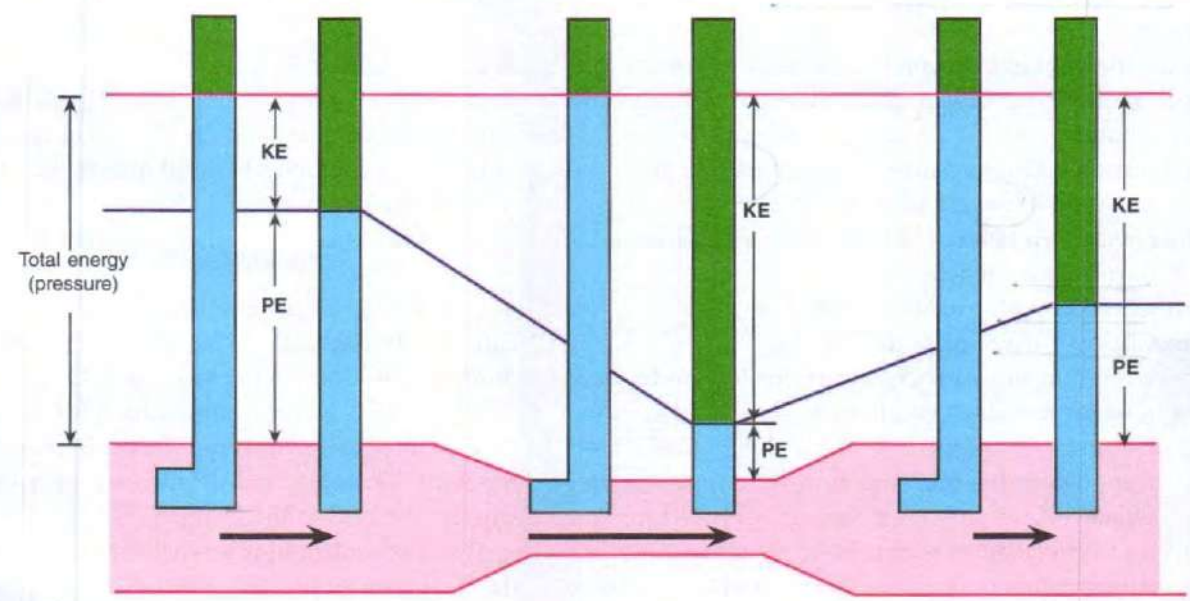
4. Elasticity of the Vessel Wall

Total peripheral resistance (PR) is inversely related to the elasticity of vessel wall.

It is due to elastic properties, the arteries can dilate and accommodate considerable amount of blood with relative rise of BP. In normal subjects, during diastole, elastic recoil produces slight stretch on vessel wall. Thus DBP is maintained at 70-80 mmHg. However, during systole with overstretching of vessel wall there is a tendency to increase in pressure.

If BP decreases below 30-40 mmHg, blood flows in the vessel without stretching its wall and blood vessel behaves like a rigid tube.

In advancing age, due to loss of elasticity of blood vessels, stretching decreases which results in increased pressure during systole with normal DBP ***(systolic hypertension)***.



: tube faces upstream direction of flow, therefore, records total energy (KE + PE) and measures *total pressure* (also called ***end pressure*** *or* ***perfusion pressure***)

: tube inserted sideways, records PE only (measures ***lateral pressure***)

At high velocity of blood flow (in the narrowed section, a high resistance); P.E. (lateral pressure) is reduced and KE of flow increases as the velocity increases (due to conversion of PE to KE). However, total energy (KE and PE) *i.e.* perfusion pressure remains the same

Fig. 42.2 Bernoulli's principle: Effect of velocity of blood flow on potential energy (PE) and total energy (kinetic energy; KE + PE). The arrows length (⟶) represents relative velocities in different segments of the system and direction of flow.

It is characterized by high pulse pressure (SBP minus DBP). If rigidity of small vessels also occurs it leads to increase in DBP also.

REGULATION OF ARTERIAL B.P.

The various mechanisms exist within the body to regulate the systemic arterial B.P. These mechanisms are well interconnected and their *main aim* is ***to maintain the normal MBP within a narrow range***, between 95 to 100 mmHg. The different mechanisms available are:

A. Rapidly acting regulatory mechanisms;
B. Intermediate acting regulatory mechan-isms;
C. Long-term acting regulatory mechanisms; and
D. Miscellaneous mechanisms.

A. RAPIDLY ACTING ARTERIAL B.P. REGULATORY MECHANISMS or NERVOUS REGULATORY MECHANISMS

Salient Features

1. They begin to act within seconds to minutes after the arterial B.P. becomes abnormal.
2. Most of these mechanisms lose their capability for pressure control after a few hours or a few days.
3. *None* of these mechanisms ever succeed in bringing the arterial B.P. all the way back to normal.
4. These are *primarily the circulatory reflexes* which begin to act within seconds and help to control B.P. from rising extremely high or falling extremely low. For example,
   (i) during sudden change in body posture; and
   (ii) profusely bleeding persons.
5. The ***circulatory reflexes*** of these mechanisms include:
   (i) Baroreceptor reflexes,
   (ii) Chemoreceptor reflexes; and
   (iii) CNS ischaemic response.

   These mechanisms can act approx. for few hours or at the most for few days because of:
   (a) *Baroreceptor adaptation i.e.* they lose their responsiveness; this is seen in 1-2 days no matter whatever pressure level they are exposed to;
   (b) *CVS adaptation* to sympathetic response; and
   (c) *Autoregulation i.e.* long-term arterial pressure regulatory mechanism comes into play and nullifies the effect of short-term system.

   Therefore, insignificant in long-term regulation of B.P.

[BCI]

1. *Baroreceptor Reflexes* (page 330)

Fall in arterial B.P. decreases inhibitory discharge from baroreceptors to cause:
(i) less inhibition of vasomotor centre (VMC); and
(ii) less stimulation of cardiac vagal centre (CVC).

This results in increased sympathetic and decreased parasympathetic discharge to restore blood pressure back to normal. Conversely, high arterial B.P. has opposite effects, reflexely causing the arterial B.P. to fall back to normal level.

Features

(i) it operates between 60-200 mmHg range of MBP beyond which no discharge from baroreceptor occurs;
(ii) it corrects 2/3rd fall in B.P. *i.e.* if B.P. tends to fall from 100 to 40 mmHg, a fall of 60 mmHg pressure, it will instead fall only 80 mmHg (2/3rd of 60 mmHg = 40 mmHg fall gets corrected by this mechanism).

2. *Chemoreceptor Reflex*

Features

(i) It operates between 40-100 mmHg range of MBP.
(ii) It can correct approx. 2/3rd of the further fall in B.P.

Mechanism of operation

Fall in arterial B.P., specially <80 mmHg, decreases blood flow to tissues (tissue ischaemia), this decreases pO_2 and increases pCO_2 to carotid and aortic bodies and causes stimulation of 'VMC', cardiac vagal centre (CVC) and respiratory centre. The final effect is increase in 'HR', BP and increase in rate and depth of respiration.

3. *CNS Ischaemic Response*

Features

(i) It operates between 15-50 mmHg range of MBP;
(ii) It does not operate untill arterial B.P. falls to 50 mmHg;
(iii) It can correct 11/12th of a further fall in B.P.

Mechanism of operation

Fall in arterial B.P. < 50 mmHg, specially to 20-30 mmHg, → CNS ischaemia → CO_2 accumulation in "VMC" which directly stimulates VMC, *Pressor area* → tremendous powerful sympathetic discharge throughout the body. Thus HR and BP increase to maintain normal supply of blood to the brain.

This mechanism thus acts as ***last ditch stand*** to prevent the final demise (death) of a person.

B. INTERMEDIATE ACTING ARTERIAL BLOOD PRESSURE REGULATORY MECHANISMS or INTRINSIC PHYSICAL REGULATORY MECHANISMS

Salient features

1. They begin to act within a few minutes and reach full function within a few hours.
2. These mechanisms remain functional from few days to a month only.

3. They primarily correct any alteration in B.P. by altering the blood volume.

The mechanisms includes:

1. Capillary fluid shift mechanism; and
2. Stress relaxation and reverse stress relaxation mechanism.

1. Capillary Fluid Shift Mechanism

Since mean capillary pressure is directly proportional to arterial B.P., therefore, rise in arterial B.P., increases hydrostatic pressure at arterial end and fluid shifts out of capillaries to the interstitial fluid compartments. Thus blood volume decreases to restore the B.P. **(Fig. 42.3)**

This mechanism is two times more effective than baroreceptor reflex mechanism in returning the B.P. back to normal but it begins to act much more slowly than baroreceptor mechanism.

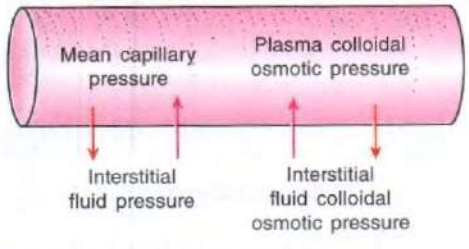


Fig. 42.3 Capillary fluid shift mechanism. Arrows show direction of movement of fluid

2. Stress Relaxation and Reverse Stress Relaxation Mechanisms

Rise in arterial B.P. *e.g.* following massive slow intravenous (I.V.) transfusion, increases perfusion pressure in blood storage organs such as veins, liver, spleen, lungs etc. This causes relaxation of blood vessels simply by 'local vascular tone' adjustment. Therefore, 'VR' and 'CO' decreases and B.P. returns to normal ***(stress relaxation mechanism)***.

Conversely, fall in arterial B.P. *e.g.* following prolonged slow bleeding, decreases perfusion pressure in blood storage organs causing tightening of blood vessels around the amount of blood that is left in these organs to restore the B.P. back to normal ***(Reverse stress relaxation mechanism)***.

These mechanisms have very definite **limits of operation**. Therefore, acute changes in blood volume in the range of +30% to –15% (*i.e.* 30% above and 15% below the normal blood volume) can be corrected by these mechanisms.

C. LONG-TERM ARTERIAL B.P. REGULATORY MECHANISMS OR "AUTOREGULATION" OF B.P. BY KIDNEYS

Salient features

1. These mechanisms, almost invariably, are slow to begin acting. Generally it takes 3-10 days for these mechanisms to come to complete equilibrium.
2. Their function is to control the arterial B.P. over a period of days to years.
3. The effectiveness of most of these mechanisms becomes steadily greater with time.
4. These mechanisms have the ability to bring the arterial B.P. all the way back to normal. These mechanisms broadly operate in two ways **(Fig. 42.4)**:
   (i) *Direct mechanisms*
   These mechanisms involve control of blood volume by kidneys directly, therefore, also called *renal fluid mechanism* or *ECFV mechanism*.
   (ii) *Indirect mechanism*
   These mechanisms involve control of kidney functions indirectly via *hormonal system* viz.,
   (a) *Aldosterone system*; and
   (b) *Renin-angiotensin system*.

Important Note

The rapidly acting and intermediately acting regulatory mechanisms play a major role in *controlling* arterial B.P., whereas long-term regulatory mechanisms help in *regulating* the B.P. Thus, systemic arterial B.P. is preserved relatively constant within a narrow range under several conditions. (For differences between *control* and *regulation*, see to page 657)

D. MISCELLANEOUS MECHANISMS

1. Role of Sympathetic Nerves

The kidneys are strongly supplied by sympathetic nerves and degree of sympathetic stimulation can alter renal functions tremendously. Therefore, nervous signals can alter the long-term regulation of B.P. For example: when sympathetic nerves to kidneys are stimulated for several weeks continuously, renal retention of fluid occurs to cause chronically elevated B.P. as long as the sympathetic stimulation continues. Therefore, it is possible for nervous regulation of kidneys to cause chronic elevation of arterial B.P.

Important Note

One of the probable cause of essential hypertension (page 393) is increased sensitivity of kidneys to sympathetic discharge.

2. Role of ADH

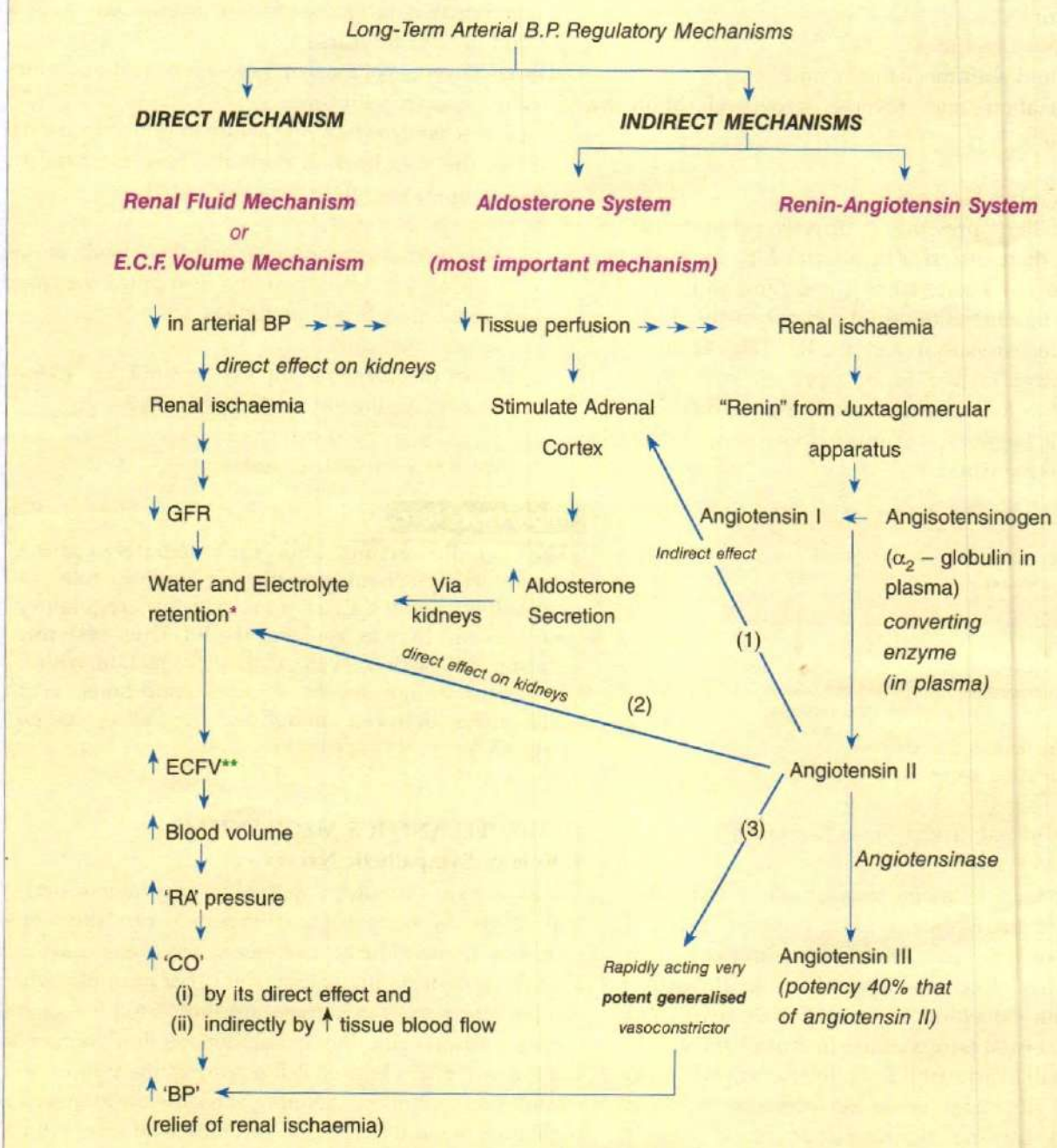
(i) Direct effect

In large dosage ADH by its direct effect produces:

(a) arteriolar constriction to increase peripheral resistance, and

(b) venular constriction to increase venous return.

(a) and (b) finally increase arterial B.P.



* At normal MBP of 100 mmHg the urinary output, water and electrolyte is normal. (Fig. 42.5)
At MBP of 50 mmHg the urinary output, water and electrolyte is essentially 'zero'.
At MBP of 200 mmHg the urinary output, water and electrolyte is 6-8 times that of normal.

** In fact, as small as 3 to 5% change in ECFV that lasts for more than a few days, can alter arterial B.P. as much as 20-40 mmHg.

Fig. 42.4 Schematic representation of long-term arterial B.P. regulatory mechanisms

(ii) Indirect effect

Fall in B.P. decreases right atrial (RA) pressure; atrio-caval and pulmonary veno-atrial discharge decreases; this promotes ADH secretion from posterior pituitary.

This acts by retaining fluids by kidneys to increase the B.P.

Conversely, rise in B.P. increases 'RA' pressure and ADH secretion gets inhibited resulting in fall of B.P.

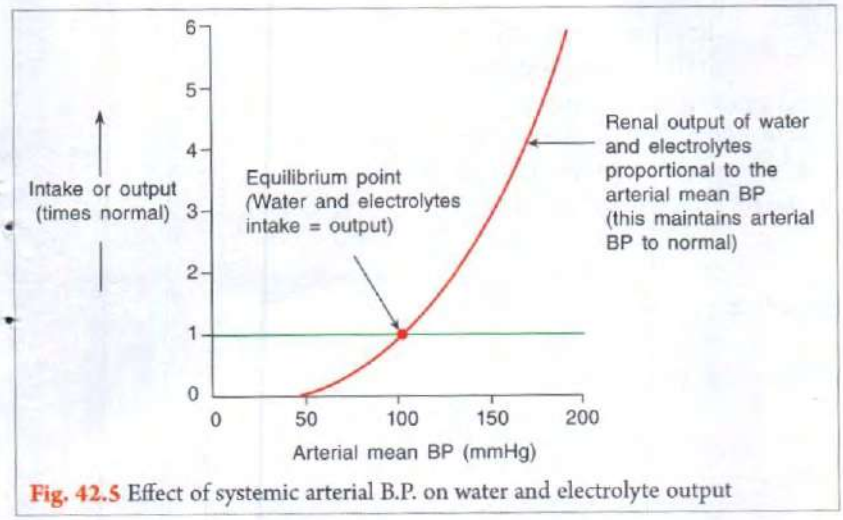


Fig. 42.5 Effect of systemic arterial B.P. on water and electrolyte output

Therefore, ADH system plays an important role in acute as well as long-term regulation of arterial B.P., viz.,

(i) It is responsible for correcting 75% fall in arterial B.P. within a few minutes after acute haemorrhage by its 'direct' vasoconstrictor action on blood vessels.

(ii) It also plays an 'indirect' role in the long-term regulation of arterial B.P. through its effect on the kidneys in causing decrease excretion of water.

(Also see to page 668)

Study Questions

1. Define BP and its various components. Give the characteristic features of each component of B.P.
2. Mention effects of graded exercise on systemic arterial B.P.
3. Give correlation between ECFV and alteration of systemic B.P.
4. Write short notes on:
   (i) Factors affecting and determining arterial B.P.
   (ii) Significance of mean B.P.
   (iii) Rapidly acting B.P. regulatory mechanisms
   (iv) Last ditch stand
   (v) Role of kidney in regulation of B.P.
   (vi) Bernoulli's principle
   (vii) Stress and reverse stress relaxation mechanism.
   (viii) Role of ADH in regulation of blood pressure.
5. Draw labelled/line diagram:
   (i) Long term arterial BP regulatory mechanisms
   (ii) Effect of systemic arterial BP on water and electrolyte output.

MCQs

1. Which component of systemic arterial BP undergoes much less fluctuations?
   (a) Systolic BP (b) Diastolic BP (c) Mean BP (d) Pulse pressure
2. All cardiovascular reflexes are sensitive to changes in the:
   (a) Systolic blood pressure (b) Diastolic blood pressure
   (c) Mean blood pressure (d) Pulse pressure
3. Magnitude of gravitational effect on mean BP is ..... mmHg for each cm vertical distance from heart is:
   (a) 0.66 (b) 0.77 (c) 0.88 (d) 0.99
4. According to Bernoulli's principle:
   (a) When blood flows through a narrowed section of vessel, kinetic energy decreases
   (b) Sum of the kinetic energy of flow and pressure energy is always constant in a blood vessel
   (c) At high velocity of blood flow, pressure energy increases and kinetic energy is reduced
   (d) Kinetic energy of flow increases as the blood flow increases
5. Baroreceptor reflexes:
   (a) Correct only fall in BP
   (b) Help to control BP from rising extremely high or falling extremely low
   (c) Operate between 60-150 mmHg range of mean BP
   (d) Can correct 11/12th fall in BP
6. Chemoreceptors operate between pressure range of:
   (a) Below 90 mmHg (b) 40-100 mmHg (c) 70-150 mmHg (d) 70-220 mmHg

7. **The most powerful stimulus to initiate CNS ischaemic response is:**
   (a) CO_2 accumulation in the medulla (b) Fall in arterial pO_2
   (c) Rise in arterial pCO_2 (d) Fall in the blood pH
8. **Limit of operation of intrinsic physical BP regulatory mechanisms is:**
   (a) 30% below and 15% above the normal blood volume (b) 15% below and 30% above the normal blood volume
   (c) Acute changes in blood volume in the range of ±20% (d) They remain functional for a day only
9. **Which mechanism is most effective in returning the BP precisely back to normal:**
   (a) Baroreceptor reflexes (b) Chemoreceptor reflexes
   (c) Capillary fluid shift mechanism (d) Long-term BP regulatory mechanism
10. **3-5% change in ECF volume lasting for few days can alter systemic arterial BP by:**
    (a) 10-20 mmHg (b) 20-40 mmHg (c) 40-60 mmHg (d) 60-80 mmHg
11. **All the statements are *true* for the actions of angiotensin-II *except*:**
    (a) It is generalised powerful vasoconstrictor
    (b) By its direct action on kidneys it can cause retention of water and electrolytes
    (c) Increases ADH secretion from the posterior pituitary
    (d) Indirectly can stimulate adrenal cortex to cause increase aldosterone secretion
12. **At systemic arterial mean BP of 50 mmHg, urine output is:**
    (a) Nil (b) 0.5 mL/min (c) 1 mL/min (d) 2 mL/min
13. **Which of the following plays an important role in acute as well as long-term regulation of arterial BP?**
    (a) Renin-angiotensin system (b) Capillary fluid shift mechanism
    (c) ADH system (d) Aldosterone system
14. **Diastolic B.P. is mainly detremined by:**
    (a) Capillary permeability (b) Venous capacitance (c) Venous elasticity (d) Peripheral resistance
15. **Pulse pressure varies as blood flows to the extremities, due to:**
    (a) Cross-sectional area (b) Change in vessel wall elasticity
    (c) Change in viscosity (d) Change in velocity
16. **Disturbed sleep increases systolic BP:**
    (a) Secondary to skeletal muscle tensioning (b) Due to increase epinephrine release from adrenal medulla
    (c) Secondary to CNS ischaemia (d) Due to alteration in velocity of blood flow
17. **Changes in diastolic BP upon standing comes back to normal within:**
    (a) 15-30 sec. (b) 30-60 sec. (c) 60-90 sec. (d) 90-120 sec.
18. **Main aim of various BP regulatory mechanisms is to maintain the normal:**
    (a) Systolic BP (b) Diastolic BP (c) Mean BP (d) Pulse pressure
19. **Baroreceptor reflexes:**
    (a) Correct only fall in BP
    (b) Help to control BP from rising extremely high or falling extremely low
    (c) Operate between 60-150 mmHg range of mean BP
    (d) Can correct 11/12th fall in BP
20. **All the following statements are *true* for intermediate acting B.P. regulatory mechanisms except that:**
    (a) They begin to act within a few minutes after arterial BP becomes abnormal
    (b) These mechanisms primarily correct any alteration in the BP due to changes in the blood volume
    (c) These mechanisms lose their capability for BP control after a month
    (d) They have the ability to bring BP all the way back to normal within the narrow range
21. **The renal-body fluid volume mechanism for regulating arterial pressure is important for:**
    (a) Raising the pressure when a person stands suddenly after having been in a lying position
    (b) Minimising a decrease in arterial pressure following severe hemorrhage
    (c) Increasing arterial pressure during strenuous physical exercise
    (d) Maintaining arterial pressure at a normal level over a period of weeks, months or years
22. **At arterial mean BP of 50 mmHg, urine output is:**
    (a) Nil (b) 0.5 mL/min (c) 1 mL/min (d) 2 mL/min

Answers

1. (b) 2. (c) 3. (b) 4. (b) 5. (b) 6. (b) 7. (a) 8. (b) 9. (d) 10. (b) 11. (c)
12. (a) 13. (c) 14. (d) 15. (b) 16. (a) 17. (b) 18. (c) 19. (b) 20. (d) 21. (d) 22. (a)

Chapter 43

The Regional Circulation

I. Capillary Circulation •
II. Coronary Circulation •
III. Cerebral Circulation including cerebrospinal fluid (CSF) •
IV. Cutaneous (Skin) circulation •
V. Muscle Circulation
VI. Splanchnic Circulation (intestinal and hepatic)
VII. Pulmonary Circulation

CAPILLARY CIRCULATION

INTRODUCTION

At a time, only 5% of the circulating blood is in the capillaries, which allows the continuous exchange of O_2, CO_2, nutrients and waste products between blood and the interstitial fluid. The exchange across the capillary wall is essential for the survival of the tissues, called ***transcapillary exchange***. It is of *three types*:

A. *Filtration-absorption:* It maintains blood volume constant.
B. *Diffusion:* It is responsible for metabolic changes in the tissues, for example:
   1. Supply of nutrients to the tissues and removal of waste from them, and
   2. Continuous exchange of gases (O_2 and CO_2) across the capillary wall.
C. *Micropinocytosis:* It provides an active transport route for macromolecules (*e.g.* γ-globulin, large proteins) against concentration gradient across the capillary wall.

A. FILTRATION-ABSORPTION

The rate of filtration at any point along a capillary depends upon the balance of forces, called ***Starling Forces*** (*E.H. Starling*, 1896) (page 55).

Mechanism of adjustment of capillary pressure (P_{cap})

The capillary pressure is not constant in any one tissue **(Table 43.1)** and depends on the state of tone of the resistance vessels.

Capillary pressure is determined by the pre-capillary to post-capillary resistance ratio (page 321). In the systemic capillaries, this ratio is 4-5 : 1, which maintains the ***Mean Capillary Pressure*** to approx. 25 mmHg, thus balancing the osmotic pressure within the capillaries. As a result, ideal systemic capillaries will show *no net loss or gain* of fluid thereby maintaining the blood volume constant. However:

(1) At the arteriolar end of the capillary the hydrostatic pressure (HP) is higher (approx. 37 mmHg) than the mean P_{cap} 25 mmHg and, therefore, some fluid is forced out of the capillary bed into the interstitium. This filtration force being 37 – 25 = 12 mmHg; and
(2) At the venous end of the capillary the 'HP' is lower (15 mmHg) than the mean P_{cap} and some fluid will

Table 43.1: Capillary pressure of various body tissues at rest

Tissue		Capillary pressure (mmHg)
Glomeruli	70	
Liver	6	(low, because liver sinusoids receive bulk of their blood supply from portal vein whose pressure is 7-10 mmHg.
Lungs	8	(low because of intrathoracic location)
Feet	100	(high to prevent back flow of blood into dorsal vein of foot where pressure is 80-90 mmHg.

be absorbed (pulled back) by the osmotic forces. This absorption force is 25 – 15 = 10 mmHg.

The change in P_{cap} can be assessed by measuring *Capillary Filtration Coefficient* (CFC). 'CFC' is the fluid filtered per mmHg rise of pressure per 100 gm tissue per minute.

The range of CFC values for different tissues is a wide one with 0.006 mL/100 g/min (in muscle capillaries) to 1.2 mL/100 g/min (in renal glomeruli)(page 517).

It has been calculated that in a day, approx. 24 litres of fluid escapes from the capillaries; of this volume approx. 20-22 litres is reabsorbed by the capillaries and the remaining approx. 2L/day is absorbed by lymphatics and returned back to the systemic veins.

The extravascular circulation resulting from the capillary filtration and absorption is very important for the regulation of blood volume. During the process of filtration the plasma proteins are not filtered to a great extent and thereby exert an effective osmotic pressure which restricts the fluid bulk filtered. The effective osmotic pressure results from plasma proteins which constitute 7 gm/dL of plasma and exert an osmotic pressure of 25 mmHg.

Factors Affecting Filtration-Absorption Process

1. *Increase in sympathetic activity* increases pre/post-capillary resistance ratio; this decreases mean P_{cap} and decreases filtration.
2. *Sympathetic blockade* causes:
   (i) decrease pre/post-capillary resistance ratio, and
   (ii) venodilatation of the venous capacity vessels.
   (i) and (ii) increases mean P_{cap} to increase filtration.

Important Note

Stimulation of sympathetic vasodilatation nerves (page 332) causes relaxation of the arterioles but not of pre-capillary sphincters, as a result blood flow increases via thoroughfare channels from arterioles to venules whereas mean P_{cap} remains same.

3. *Exercise* or increase tissue activity causes:
   (i) increase in sympathetic activity raising pre/post-capillary resistance ratio;
   (ii) increase in local metabolites which:
      (a) relax pre-capillary sphincters only;
      (b) increase osmolality of the interstitial fluid which further favours fluid transudation into the interstitium; and
      (c) decrease basal myogenic tone.

   The sum effect of (i) and (ii) is fall in pre/post-capillary resistance; increase in mean P_{cap} and causing fluid transudation into the interstitium (*i.e.* increased filtration).
4. *Left ventricular failure (LVF)* increases pulmonary P_{cap}; when it exceeds 20 mmHg, pulmonary oedema occurs. (Also see to page 392)
5. *Hypoproteinaemia* *i.e.* decrease in plasma protein concentration decreases plasma osmotic pressure. Thus, absorption of interstitial fluid decreases and ***oedema*** occurs *i.e.* collection of fluid in interstitial spaces (page 55).
6. *Tissue injury* causes protein release from the damaged cells which are rapidly broken down by enzymes released from the injured lysosomes. Thus marked increase in osmotic pressure of the interstitial fluid produces ***inflammatory oedema***.

B. DIFFUSION

The metabolic changes *i.e.* exchange of nutrients and waste materials between blood and the tissues occurs by diffusion mechanism (page 14).

Factors affecting diffusion across capillary wall

1. Lipid solubility

(i) Lipid soluble substances (O_2 and CO_2) can utilize the entire surface area of the capillary wall and diffuse rapidly with ease across it.
(ii) Lipid insoluble molecules can only diffuse through intercellular pores which provide aqueous channels for such diffusion.

2. Size of a substance

The permeability of the capillary wall to a substance falls rapidly with increase in MW in the range between 10,000 to 60,000.

3. Capillary wall surface area

The capillary wall surface area available for diffusion is determined by the tone of the pre-capillary sphincters.

4. Velocity of flow of the blood

(i) If it decreases, diffusion decreases (because rate of substance delivered by the vessel to interstitial fluid decreases).
(ii) If it increases, diffusion decreases (because the time of transit is insufficient for complete equilibration across the capillary wall).

C. MICROPINOCYTOSIS

It is a very slow process and provides an active transport route for macromolecules (*e.g.* γ-globulin, immunological proteins etc.) against concentration gradient from the blood to the tissues. Even proteins from the interstitial

space can be transported to the blood in this fashion through intercellular pores.

The pore area constitutes only a fraction, approximately 1/1000th of the total capillary surface area, and there are about 1000×10^6 pores/cm^2 of capillary membrane. Two types of pore system exist in the capillary wall: 'small pore' and 'large pore' system (Table 43.2).

Table 43.2: Comparison of small and large pore system in the capillary wall

	Small pore system	Large pore system
1. Pore diameter	3–4.5 nm	More than 4.5 nm.
2. Total capillary wall surface area	It represents less than 0.1% of total surface area.	It represents less than 0.001% of total surface area.
3. Size of substance transported	It permits transport of substance upto MW 60000 by way of *diffusion* process.	It accounts for process of *micropinocytosis*.

Applied Aspect: OEDEMA

Definition: It is the accumulation of fluid in interstitial spaces in abnormally large amounts.

Mechanism: The amount of fluid in interstitial spaces depends on the *transcapillary exchange* mechanism. (page 355).

Causes

1. *Increased filtration pressure* due to
   (i) arteriolar dilatation,
   (ii) venular constriction, and
   (iii) increased venous pressure due to heart failure; incompetent valves; venous obstruction; increased total ECFV, prolonged standing.
2. *Decreased osmotic pressure gradient* across capillary due to:
   (i) decreased plasma protein level (*hypoproteinaemia page 55*) and
   (ii) accumulation of osmotically active substances in interstitial space, seen after exercise or tissue injury.
3. *Increased capillary permeability* due to histamine and related substance, kinins etc.
4. *Inadequate lymph flow (called* ***Lymphoedema****):* In filariasis, parasitic worms migrate into the lymphatics and obstruct them; fluid accumulation and tissue reaction produces marked swelling, usually of legs and scrotum ***(elephantiasis)***.

Study Questions

1. **Give physiological significance of:**
   (i) Transcapillary exchange and Oedema
   (ii) Micropinocytosis
   (iii) Capillary filtraiton coefficient.
2. **Write briefly:**
   (i) Factors affecting filtration-absorption process
   (ii) Factors affecting diffusion
   (iii) Elephantiasis
3. **How does hypoproteinaemia produce oedema?**

MCQs

1. **At any given time what percentage of total blood volume is present in the capillary circulation?**
   (a) 1% (b) 5% (c) 10% (d) 20%
2. **Rate of filtration at any point along a capillary is determined by:**
   (a) Mean capillary pressure (b) Starling forces
   (c) Colloidal osmotic pressure (d) Capillary filtration coefficient
3. **Mean capillary pressure is maintained by:**
   (a) Hydrostatic pressure across capillary wall (b) Colloidal osmotic pressure
   (c) Pre to post-capillary resistance ratio (d) Interstitial fluid pressure

4. **Oedema can occur as a result of all of the following factors *except*:**
   (a) Increased vasomotion with persistent tonic contraction of pre-capillary resistance vessels
   (b) Increase in mean capillary pressure
   (c) Increase in negativity of pressure normally found in interstitial fluid space
   (d) Decrease in albumin concentration in circulating plasma
5. **All of the following statements concerning capillary circulations are true, *except* that capillaries:**
   (a) Together account for the largest surface area of all vascular compartments
   (b) Sustain the minimal tangential tension encountered in all vessels
   (c) Do not respond by active constriction of local epinephrine
   (d) Together contain more blood than that contained in the veins
6. **Capillary filtration coefficient:**
   (a) Is the fluid filtered per mmHg rise of pressure per 100 gm tissue per minute
   (b) Its value is same for different tissues in the body
   (c) Can be assessed by capillary pressure
   (d) None of the above
7. **Increase in sympathetic activity causes:**
   (a) Decrease in mean capillary pressure
   (b) Increase in mean capillary pressure
   (c) No change in mean capillary pressure
   (d) Increases filtration across the capillary wall
8. **Which of the following changes would tend to cause accumulation of fluid (oedema) in the tissues?**
   (a) Increased precapillary vascular resistance
   (b) Decreased postcapillary vascular resistance
   (c) Increased plasma colloid osmotic pressure
   (d) Increased venous pressure

Answers

1. (b) 2. (b) 3. (c) 4. (a) 5. (d) 6. (a) 7. (a) 8. (d)

CORONARY CIRCULATION

I. PHYSIOLOGICAL ANATOMY

A. Arterial Supply to the Heart

The heart is supplied by two coronary arteries, right and left, arising from the root of the ascending aorta. **(Fig. 43.1)**

1. Right coronary artery

It traverses in the right A-V sulcus to the back of the heart and gives out several *descending branches* to both the ventricles. It terminates by anastomosing with left coronary artery. The heart areas supplied by this artery include:

(i) whole of the right atrium
(ii) greater part of right ventricle
(iii) a small part of left ventricle near posterior interventricular groove
(iv) posterior part of interventricular septum, and
(v) major portion of the conducting system of the heart, including SAN.

2. Left coronary artery

It gives off two main branches:

(i) *Anterior descending branch* or anterior interventricular branch which runs in the interventricular groove to reach the apex of the heart and gives out *septal branches.*
(ii) *Left circumflex branch* which runs in A-V groove to the left and proceeds downwards as posterior descending branch.

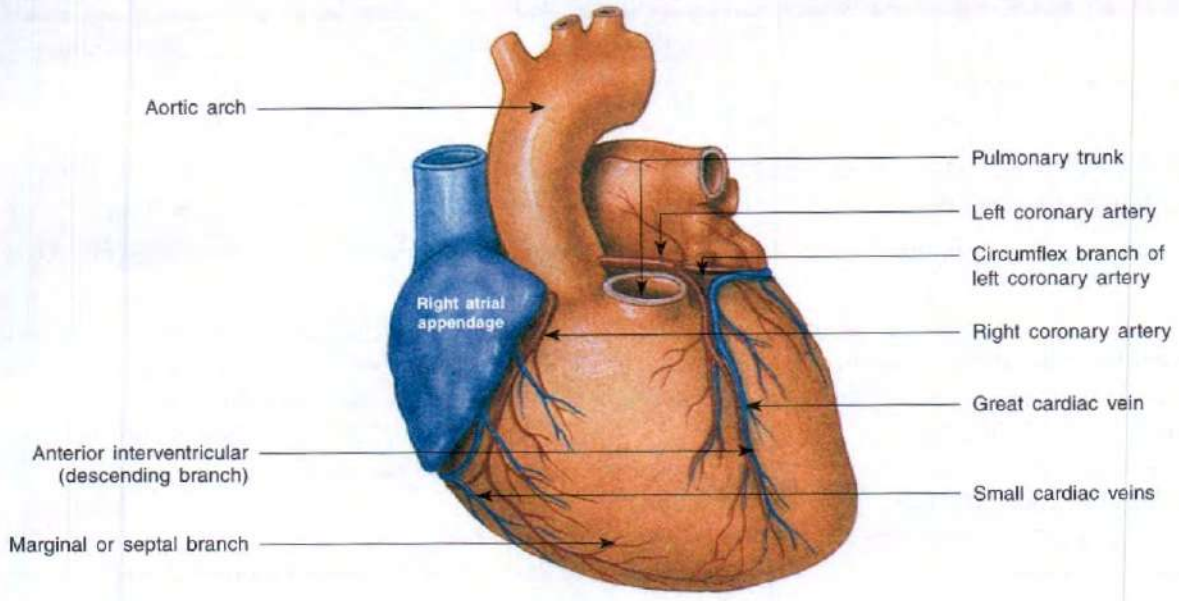
Left coronary artery supplies the following areas of the heart:

(a) whole of the left atrium
(b) greater part of left ventricle
(c) a small part of right ventricle near anterior interventricular septum
(d) anterior part of interventricular septum, and
(e) a part of the left branch of bundle of His (A-V bundle).

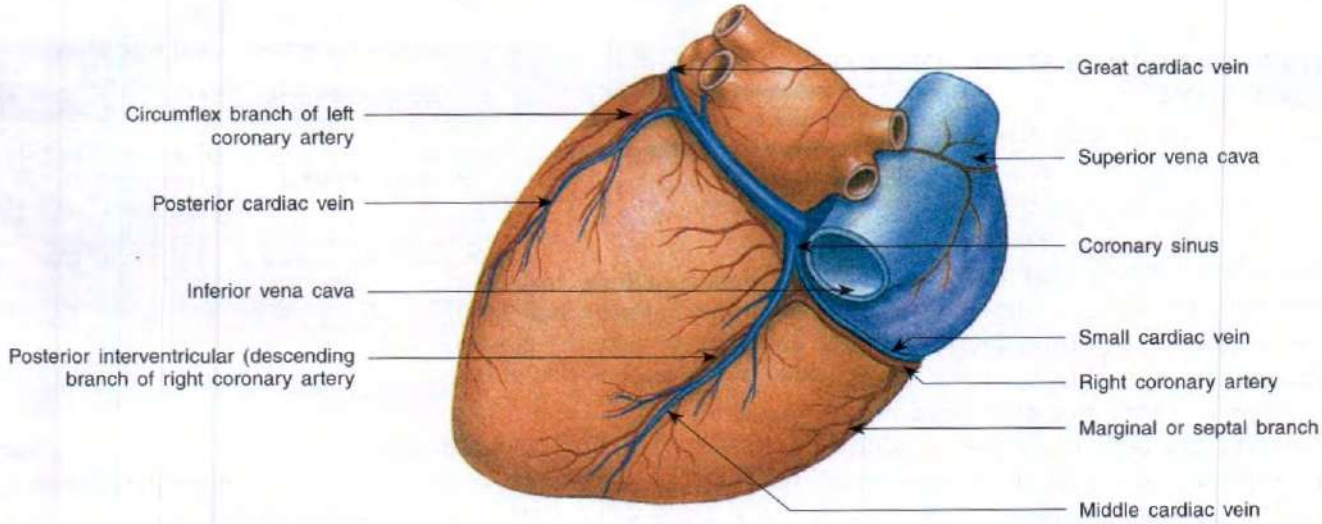
In 20% individuals myocardium is predominantly supplied by left coronary artery; in 50% individuals predominant supply is by right coronary artery; and in the remaining 30% individuals it is the balanced supply *i.e.* equal supply by the two coronary arteries.

Normally, the coronary arteries are ***end arteries*** *i.e.* a given area of heart muscle is supplied by a single artery and there is no overlap. However, the ***functional anastomoses*** are present and become active under abnormal conditions like ischaemic heart disease. These anastomoses are of two types: *cardiac and extra-cardiac.*

1. *Cardiac anastomosis i.e.* anastomosis between:
   (i) branches of one coronary artery with that of the other; and



(A) ANTERIOR VIEW



(B) POSTERIOR VIEW

Fig. 43.1 Location and distribution of principal coronary vessels

(ii) branches of coronary arteries and branches of deep system of veins.

2. *Extra cardiac anastomosis* *i.e.* anastomosis between coronary arteries and vessels lying outside the heart; for example:
   (i) vasa-vasora of aorta *i.e.* other arteries of aorta
   (ii) vasa-vasora of pulmonary arteries *i.e.* branches of pulmonary arteries
   (iii) intra thoracic arteries
   (iv) bronchial arteries; and
   (v) phrenic arteries.

B. Venous Drainage of the Heart

It is divided into two systems:

1. *Superficial system* (lies beneath the epicardium), it ends in:
   (i) *Coronary Sinus:* It is the largest vein of the heart and mainly drains the blood coming from the myocardium supplied by the left coronary artery; and partly by right coronary artery. It ends in posterior wall of right atrium.
   (ii) *Great Cardiac Vein:* It drains blood from left heart and ends in the coronary sinus.

Table 43.3: Oxygen supply and its utilization by myocardium and rest of body tissue compared

	Rest of body tissues	Myocardium
(i) O_2 content (a) arterial	19 mL/dL	19 mL/dL
(b) venous	14 mL/dL	6-7 mL/dL
(ii) A-V O_2 difference	19 – 14 = 5 mL/dL	19–6 = 13 mL/dL *(greater)*
(iii) Coefficient of O_2 utilization	5/19 × 100 = 26%	13/19 × 100 = 70%
(iv) O_2-saturation of venous blood	14/19 × 100 = 70% with pO_2 40 mmHg	6/19 × 100 = 35% with pO_2 < 20 mmHg

(iii) *Anterior Cardiac Vein:* It receives blood mainly from the myocardium supplied by right coronary artery and then opens directly in the anterior wall of the right atrium.

2. ***Deep system*** arises from within the substance of myocardium from the fine branches of coronary arteries. It opens directly into the cardiac chambers via 3 sets of vessels:
   (i) *Arterio sinusoidal vessels*
   (ii) *Arterio luminal vessels, and*
   (iii) *Thebesian vessels.*

II. CHARACTERISTIC FEATURES OF CORONARY BLOOD FLOW

1. Normal coronary blood flow of both the arteries is 60-80 mL/100 gm/min or 250 mL/ min.
2. O_2 consumption (V_{O_2}) of the myocardium is very high (Table 43.3). How? Resting V_{O_2} of whole body is 250 mL/min; whereas that of left ventricle (LV) alone is 8-10 mL per 100 gm/ min or 25 mL/min *i.e.* 10% of whole body V_{O_2}. Three-fourth of V_{O_2} by 'LV' is achieved during systole.
3. O_2 supply and utilization of myocardium as compared with rest of the body tissue (see to Table 43.3).
4. Coronary blood flow (CBF) fluctuates with each cardiac cycle. This is specially true with LV where 80% 'CBF' occurs during diastole, and the remaining 20% 'CBF' occurs during systole. Therefore, 'CBF' increases during diastole and decreases during systole, called *Phasic Coronary Flow.*

Variations in coronary blood flow during different phases of cardiac cycle

Why variations in 'CBF' occurs?

Blood flow through any organ is determined by two factors:

(1) by *pressure head* (*i.e.* mean BP, page 347), and
(2) by *extravascular pressure* which provides resistance to blood flow.

In the heart, ventricular action thus affects the coronary circulation as under:

(i) by altering the aortic pressure which determines the 'pressure head'; and
(ii) by altering the 'extra-vascular pressure' which varies with systole, exerting a variable degree of compression on the coronary vessels.

Therefore, 'CBF' shows strong phasic variations with reference to cardiac cycle.

Blood flow through coronary vessels during cardiac cycle

Blood flow through coronary vessels during different phases of cardiac cycle can be understood by studying the pressure gradient between ventricles and aorta as shown in Table 43.4.

Table 43.4 : Pressure in aorta and two ventricles in systole and diastole (in mmHg)

Phase of cardiac cycle	Pressure (mmHg) in			Pressure difference (mmHg) between Aorta and	
	Aorta	LV	RV	LV	RV
Systole	120	121	25	–1	95
Diastole	80	zero	zero	80	80

Since pressure difference between aorta and 'LV' is very small during systole, therefore, *blood flows to subendocardial portion of 'LV' only in diastole.* However, pressure difference is more in superficial portion of 'LV' to permit some flow in this region throughout the cardiac cycle; while blood flow to 'RV' and atria occurs both during systole and diastole. Blood flow through coronary arteries during the cardiac cycle is given in Table 43.5 and Fig. 43.2.

Blood flow through coronary sinus

In the coronary sinus the outflow of blood gradually rises from the 'isovolumetric ventricular contraction' phase and reaches its peak during 'protodiastole' phase and then gradually falls.

Clinical importance

1. Variation in CBF with heart rate. The duration of diastole is shortened to a much greater extent from 0.5 sec (at HR 72 bpm) to 0.14 sec at (HR 200 bpm). Therefore CBF also decreases. Refer to page 363.

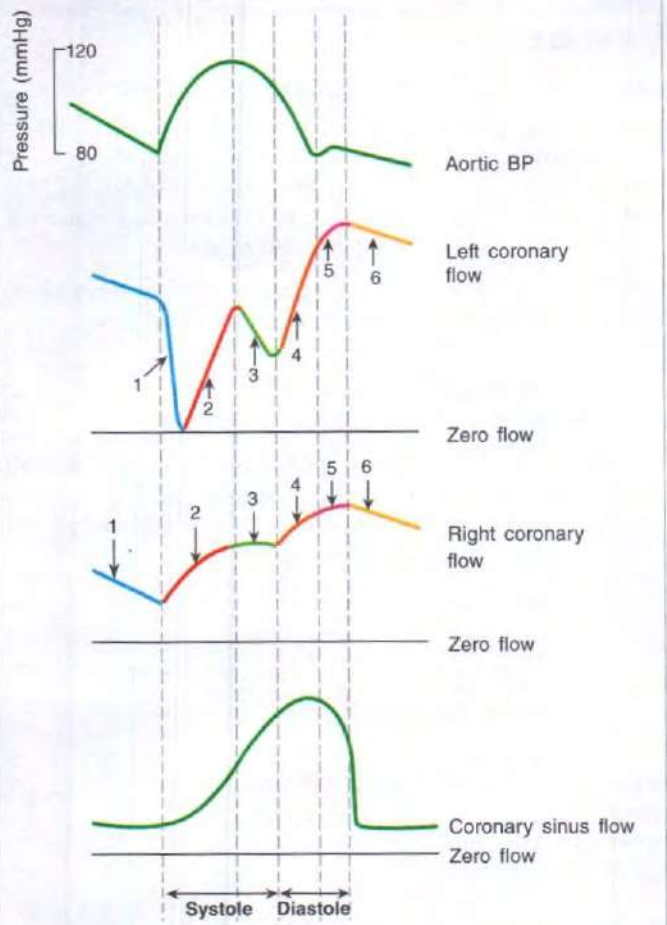


Fig. 43.2 Blood flow through coronary vessels during different phases of cardiac cycle.
1. Isovolumetric ventricular contraction;
2. Rapid ejection phase;
3. Slow ejection phase;
4. Protodiastole and isovolumetric ventricular relaxation;
5. Rapid filling phase and
6. Slow filling phase and last rapid filling phase.

2. Because there is no blood flow during systole in subendocardial portion of 'LV', therefore, this portion of 'LV' is more prone to 'MI' (Myocardial infarction).
3. *In Aortic Stenosis:* 'LV' pressure is further increased because the pressure in the 'LV' must be much greater than that in the aorta to eject blood, therefore, coronaries supplying the 'LV' are severely compressed during systole, hence leads to MI due to:
   (i) Compression of coronaries, and
   (ii) 'LV' has to do more work to expel the blood through the stenosed valves; this increases oxygen consumption of the 'LV'.
4. **In congestive cardiac failure (CCF)**, increase in venous pressure decreases aortic diastolic pressure. Thus effective coronary perfusion pressure falls and 'CBF' decreases.

Compensatory mechanism in the subendocardial portion of 'LV' where incidences of 'MI' are higher, is shown in **Table 43.6**.

However, the inner myocardium (subendocardial portion) of LV is still more prone to 'MI' because:
(i) No blood flows to this portion during systole as explained above.
(ii) Ratio of lactic acid to pyruvic acid goes on increasing from outer to inner layers showing that anaerobic respiration (metabolism) goes on in the inner layers which increases further under conditions of stress.

III. REGULATION OF CORONARY BLOOD FLOW

Myocardium has high oxygen consumption at rest. This can be increased significantly by increasing 'CBF'. Therefore, blood flow increases when the metabolism of myocardium increases. Like other vital organs of the body, coronary circulation shows well developed phenomenon of ***Autoregulation*** (page 318). It is influenced by two factors: *Chemical* and *Neural*.

A. Chemical Factors

1. Myocardial ischaemia either due to generalised hypoxia or increased myocardial metabolism converts intracellular myocardial *adenine nucleotides* to form *adenosine*. This comes out into the ECF. The ECF 'adenosine', a potent coronary vasodilator, increases 'CBF' and myocardial hypoxia gets relieved **(Fig. 43.3)**. Moreover, further increase in 'CBF' is regulated as ECF adenosine gets washed out.

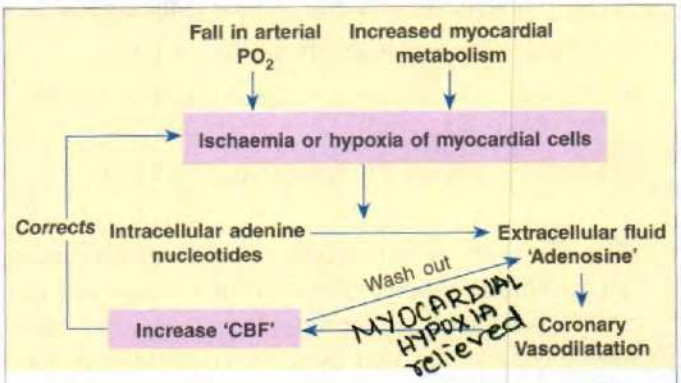


Fig. 43.3 Chemical regulation of coronary blood flow (CBF)

2. Increase in concentration of vasodilator metabolites locally such as CO_2; H^+; K^+; lactic acid; prostaglandins; adenosine and adenine nucleotides produces coronary vasodilatation, as a result 'CBF' increases.

Mechanism of metabolic regulation of 'CBF': Berne Hypothesis (Robert Berne 1950)

1. 'ATP' and 'ADP' are very potent vasodilator substances, being 4 times as active as 'AMP' and adenosine itself.

Table 43.5: Blood flow through left and right coronary artery during cardiac cycle

Phase of cardiac cycle with duration	Blood Flow Through	
	Left coronary artery	Right coronary artery
1. Isovolumetric ventricular contraction (0.05 sec.)	Ventricular pressure rises rapidly and approaches aortic pressure. This compresses intramyocardial vessels and decreases the perfusion pressure. As a result 'CBF' declines sharply to achieve almost 'zero' flow level.	'CBF' falls sharply but not to 'zero' flow level because pressure gradient is more (aortic pressure 80 mmHg and RV pressure 25 mmHg), thus maintains the high perfusion pressure.
2. Rapid ejection phase (0.1 sec.)	Sudden rise in aortic pressure causes rapid increase in perfusion pressure within coronaries. This results in rapid increase in 'CBF'.	Same changes are observed here as seen in the left coronary blood flow.
3. Slow ejection phase (0.15 sec.)	Gradual fall in aortic pressure decreases perfusion pressure within coronaries as a result 'CBF' decreases.	-do-
4. (i) Protodiastole (0.04 sec.) (ii) Isovolumetric ventricular relaxation (0.08 sec.)	Ventricular pressure decreases rapidly to 'zero' while aortic pressure decreases only to 80 mmHg *i.e.* it remains fairly high. Therefore, intra myocardial compression of blood vessels is minimal and perfusion pressure is maintained fairly high. As a result, 'CBF' rises sharply. *Maximum 'CBF' occurs during this phase.*	'CBF' rises but not so steeply like the left coronary blood flow because rise in pressure gradient is not so sharp.
5. Rapid filling phase (0.1 sec)	Relaxation of cardiac musculature continues and vessels open up further, causing slow continuous rise in 'CBF'.	-do-
6. Slow filling phase (0.18 sec) and last rapid filling phase due to atrial systole (0.1 sec.)	Pressure is gradually built up within the ventricles due to continuous venous return and aortic pressure decreases gradually; therefore, perfusion pressure within coronaries gradually falls and 'CBF' diminishes slowly.	Same changes are observed here as seen in the left coronary blood flow.

Table 43.6: Compensatory (protective) mechanism in subendocardial portion of 'LV' where incidences of 'MI' are higher

Deep layers (subendocardial portion) of the myocardium in LV	Superficial layers of the myocardium in LV
(i) Capillary density: More; 1100 capillaries/mm^2	(i) Less; 750 capillaries/mm^2
(ii) Minimum diffusion distance between capillary and myocardial cells: 16.5 μm *i.e.* 20% shorter	(ii) 20.5 μm
(iii) Myoglobin content (O_2 storage pigment): Higher	(iii) Lower

However, they are not capable of crossing myocardial cell membrane; whereas the nucleotide ***Adenosine*** can easily cross the cell membrane. Therefore, when these substances are liberated by the myocardial cell, they gain access to the resistance vessels, including the pre-capillary sphincters of the coronary system producing coronary vasodilatation.

2. Normally extracellular adenosine either gets deaminated to form inosine and hypoxanthine or re-enters the myocardial cell to form ATP again.
3. Myocardial hypoxia causes formation of adenosine (a vasodilator metabolite) which is not deaminated (to form inosine) before it escapes from the myocardial cells to cause increased CBF. *Evidences* in this regard are:
   (i) CBF increase is probably due to liberation of vasodilator substance (adenosine) in the hypoxic tissue. A similar increase in 'CBF' is produced in the area supplied by a coronary artery if the artery is occluded and then released, a phenomenon called ***Reactive Hyperaemia***.
   (ii) Asphyxia, hypoxia and intracoronary injection of cyanide, all increase CBF by 2-3 times in denervated as well as intact hearts.

B. Neural Factors

The coronary arterioles contain α-adrenergic receptors, which mediate vasoconstriction, and β-adrenergic receptors which mediate vasodilatation.

1. **Injection of epinephrine**, via β-adrenergic receptors, causes coronary vasodilatation to increase 'CBF' (direct effect).
2. **Nor-adrenergic nerve stimulation** (nerve ending where nor-epinephrine is chemical transmitter) or injection of nor-epinephrine:
   (i) via stimulation of α-receptors, decreases 'CBF'.
   (ii) via stimulation of β-receptors, increases 'HR' and force of myocardial contraction. Thus, increased activity of heart helps conversion of ATP to ADP which by producing coronary vasodilatation, increases 'CBF'. The net effect is increase in the 'CBF'.
3. When the inotropic and chronotropic effect of Nor-adrenergic discharge are blocked by a β-adrenergic blocking drug, stimulation of the Nor-adrenergic nerves or *Nor-epinephrine* injection, decreases CBF due to its direct effect on α-receptors. Therefore, the *'direct' effect* of Nor-adrenergic stimulation is constriction rather than dilatation of coronary vessels, whereas due to its *'indirect' effect* it increases CBF.
4. The **vagi** are not proven to supply the coronary vessels but stimulation of vagus nerve sufficient to cause cardiac arrest, increases CBF due to:
   (i) decreased intramural tissue pressure; and
   (ii) decrease in extravascular resistance caused by asystole.

Important Note

Coronary circulation is controlled almost entirely by local metabolic factors (hypoxia and adenosine; sympathetic nerves play a minor role).

Factors Affecting Coronary Blood Flow (C.B.F.)

1. *Muscular Exercise.* 'CBF' increases from its resting value of 60-80 mL/100 g/min to 300-400 mL/100 g/min during maximum exercise (*i.e.* by 4 times).

Mechanism

Exercise increases sympathetic activity and 'CBF' increases due to:
(i) increased activity of the heart,
(ii) increase in 'cardiac output' (≥ 5 folds) and
(iii) increase in mean arterial B.P.

During rest, systolic/diastolic flow ratio is 20:80 (0.25). During exercise, this ratio becomes 50:50 (unity). Increased CBF during systole is distributed:
(i) to more superficial layer of myocardial muscle; and
(ii) to the atrial regions and 'RV'; some to the 'LV', because extravascular tissue pressure in atria and RV is much lower and, therefore, offers less resistance to blood flow.

2. *Emotional excitement e.g.* fright, olfactory and auditory stimuli increase sympathetic discharge, so HR and ventricular contractility increases, thus 'CBF' increases secondary to increase in myocardial oxygen consumption and metabolism.
3. *Hypotension* causes reflex increase in nor-adrenergic discharge producing coronary vasodilatation to increase 'CBF'. This effect is observed secondary to the metabolic changes in the myocardium at a time when the cutaneous, renal and splanchnic vessels are constricted.
4. *Heart Rate:* When HR is increased, stroke volume decreases, therefore, phasic CBF and O_2 consumption per beat decreases. (also refer to page 360)
5. *Hormones*
   (i) *Thyroid:* Thyrotoxicosis increases myocardial metabolism to increase 'CBF'.
   (ii) *Epinephrine and Nor-epinephrine* act via β adrenergic receptors and result in increased 'CBF'.
   (iii) *Nicotine* also increases 'CBF' through the liberation of Nor-epinephrine.
   (iv) *Pitressin*, by increasing coronary resistance, decreases 'CBF'.
   (v) *Acetylcholine* increases 'CBF' (mechanism same as seen with stimulation of vagus nerve to the heart).
6. *Temperature*
   (i) *Hyperthermia* increases body metabolism and 'CBF' increases. This helps to maintain normal O_2 requirement.
   (ii) *Hypothermia*, markedly decreases body metabolic rate, O_2 requirement decreases and CBF decreases.
7. *Anaemia* increases 'CBF'.

Mechanisms:

(i) It decreases O_2 carrying capacity of blood (hypoxia), which causes release of adenosine;
(ii) A compensatory increase in 'HR' produces metabolic hypoxia.

Physio-clinical significance: *Myocardial Infarction (MI)*

1. The most common cause of MI (page 304) is rupture of an atherosclerotic plaque or haemorrhage into the coronary arteries. A common site for development of atheromatic plaque is in the first few centimeters of the coronary artery.
2. There is a strong positive correlation between *atherosclerosis* (page 615) and circulating levels of *homocysteine*. This substance damage endothelial cells. It is converted to non-toxic methionine in the presence of folate and vitamin B_{12}. Thus both of these vitamins lower the incidences of coronary artery disease.
3. Atherosclerosis has an important inflammatory component (page 615) and there is a positive

correlation between increased levels of C-Reactive protein and MI.

4. When myocardial cells die, they leak enzymes into the circulation and measuring the rises in the serum enzymes plays an important role in the diagnosis of MI. The enzymes most commonly measured are:
   (i) *MB isomer of creatine kinase* (CK-MB)
   (ii) *Troponin T and I;* and
   (iii) *Lipoprotein* (a) - Lp (a)

Study Questions

1. **Give physiological significance of:**
   (i) End arteries (ii) Phasic coronary flow (iii) Reactive hyperaemia
2. **Write short notes on:**
   (i) Arterial supply to the heart
   (ii) Characteristic features of coronary blood flow
   (iii) Protective mechanisms in the left ventricle where incidences of MI are high
   (iv) Regulation of coronary blood flow
   (v) Factors affecting coronary blood flow
3. **Draw well labelled diagram to show blood flow through coronary vessels during various phases of cardiac cycle.**
4. **Give physiological basis of:**
   (i) Decreased blood flow in inner portion of left ventricle wall during systole
   (ii) Subendocardial portion of left ventricle is more prone to MI
   (iii) Maximum coronary blood flow occurs during protodiastole and iso-volumetric ventricular relaxation phase of cardiac cycle.
5. **Describe briefly variation in coronary blood flow with heart rate.**
6. **Give the correlation between atherosclerosis and myocardial infarction (MI). Mention the bio-chemical tests done to diagnose MI.**
7. **Which portion of the myocardium is most susceptible to ischaemia and why?**

MCQs

1. **Coronary arteries are end arteries because:**
   (a) A given area of the myocardium is supplied by a single artery
   (b) There occurs overlapping of arteries supplying an area of the myocardium
   (c) Anastomosis is seen between the branches of coronary arteries and branches of deep system of veins
   (d) Anastomosis is seen between coronaries and vessels lying outside the heart
2. **Oxygen usage by the myocardium at rest is:**
   (a) 8-10 mL/100 gm/min (b) 20-40 mL/min
   (c) 60-80 mL/100 gm/min (d) 250 mL/min
3. **Oxygen saturation of coronary sinus blood is:**
   (a) 70% with pO_2 40 mmHg (b) 70% with pO_2 20 mmHg
   (c) 35% with pO_2 20 mmHg (d) 35% with pO_2 40 mmHg
4. **Phasic coronary blood flow (CBF) refers to:**
   (a) Almost zero flow level through coronaries (b) Maximum blood flow through coronaries
   (c) Incrase in CBF during diastole (d) Variation in CBF with reference to cardiac cycle
5. **Subendocardial portion of left ventricle (LV) is most common site of myocardial infarction because:**
   (a) Left coronary artery supplying the LV has a greater flow
   (b) LV does more work to propel the blood compared to the right ventricle
   (c) LV has more muscle mass compared to right ventricle
   (d) There is no blood flow during systole in the subendocardial portion of LV
6. **Maximum blood flow to coronaries occurs during:**
   (a) Early part of systole (b) Systole proper (c) Early part of diastole (d) Diastole proper

7. **Which of the following factors probably affects myocardial blood flow to the greatest extent under normal conditions:**
   (a) Degree of parasympathetic stimulation
   (b) Rate of release of adenosine from the myocardium
   (c) Degree of sympathetic stimulation of coronary vessels
   (d) Myocardial carbon dioxide concentration
8. **Blood flow through both the coronary arteries at rest is:**
   (a) 3-5 mL/100 gm/min (b) 40-55 mL/100 gm/min (c) 60-80 mL/100 gm/min (d) 300-400 mL/100 gm/min
9. **Normal A-V O_2 difference of myocardium at rest is:**
   (a) 2.0 mL/dL (b) 5.0 mL/dL (c) 10.0 mL/dL (d) 13 mL/dL
10. **Pressure difference between aorta and left ventricle during systole is:**
    (a) +1 mmHg (b) –1 mmHg (c) 80 mmHg (d) 95 mmHg
11. **All statements are *true* for protective compensatory mechanism in subendo-cardial portion of LV where incidences of myocardial infarctions are higher except that:**
    (a) Capillary density is more
    (b) Minimal diffusion between capillaries and myocardial cell is shorter
    (c) Myoglobin content is higher
    (d) Ratio of lactic acid to pyruvic acid is more
12. **Coronary blood flow is increased by all of the following *except*:**
    (a) β-adrenergic blockade (b) A decrease in arterial pO_2
    (c) An increase in arterial pCO_2 (d) Vagal stimulation

Answers

1. (a) 2. (a) 3. (c) 4. (d) 5. (d) 6. (c) 7. (b) 8. (c) 9. (d) 10. (b)
11. (d) 12. (a)

CEREBRAL CIRCULATION

I. PHYSIOLOGICAL ANATOMY

1. ***Arterial Supply:*** The brain is supplied by two arteries:
   (i) two internal carotid arteries, and
   (ii) two vertebal arteries, which unite to form *Basilar Artery.*

Basilar Artery along with two internal carotid arteries forms ***Circle of Willis*** which gives origin to 6 large vessels and provides entire blood supply to the brain. In addition, *basilar artery supplies:* occipital lobes, cerebellum, pons, medulla; and *internal carotid artery supplies:* upper brain stem and remaining part of cerebral hemispheres.

2. ***Venous Drainage:*** Venous drainage from the brain is carried out by two systems: *superficial and deep veins.* Veins from these two systems anastomose and open into the 'Venous Sinuses' (which lie between the dura mater and bone). These veins have no valves and are kept open by the structures of the dura around their orifices. Venous drainage from the brain is mainly:
   (i) via internal jugular vein,
   (ii) by channels which join the vertebral venous plexus, and
   (iii) by anastomoses with the orbital and pterygoid plexuses.
3. ***Innervation*** of cerebral blood vessels.
   (i) *Sympathetic supply*
      (a) Pial arteries and arterioles are supplied by sympathetic nor-adrenergic fibers which come from the cervical ganglia of the sympathetic ganglion chain.
      (b) Brain blood vessels are supplied by intracerebral nor-adrenergic neurons that have their cell bodies in the brain stem.

   Activation of sympathetic neurons mediate vasoconstriction via the release of nor-epinephrine or neuropeptide-Y at their endings.
   (ii) *Parasympathetic supply* comes from facial nerve via the greater superficial petrosal nerve. The post ganglionic neurons contain A-ch, VIP or substance-P and cause vasodilation.
4. ***General features***
   (i) Adult brain weighs 1400 gms; 60% (840 gm) is comprised of white matter (WM), and remaining 40% (560 gm) is grey matter (GM).
   (ii) **Cerebral blood flow** is 750 mL/min or 50-60 mL/min/100 gm. ***Critical flow level*** is approx. 18 mL/min/100 gm as flow less than this produces unconsciousness.
   (iii) **Cerebral O_2 consumption** is 3.3 mL/100 gm/min or 45 mL/min *i.e.* 20% of the whole body at rest.
   (iv) Rapid oxygen consumption of the brain is

essentially the function of 'GM', since 'WM' consists of axons and their oxygen consumption is only 0.3 mL/100 g/min. Therefore, 'GM' uses the rest of O_2, *i.e.* 3.3–0.3 = 3 mL/100 gm/min.

Important Note

Thus, the 'GM', less than 1% of the body weight uses nearly 20% of the O_2 consumed by the whole body at rest. This is possible since the 'GM' has a capillary density of approx. 4000 capillaries/mm^2.

(v) The brain is **extremely sensitive to hypoxia** and occlusion of its blood supply within 10 sec produces unconsciousness. The vegetative structure in the *brain stem* are more resistant to hypoxia than the cerebral cortex. For example, conditions causing fairly prolonged hypoxia show normal vegetative functions but severe permanent intellectual deficiencies due to hypoxic damage to: cerebral cortex, basal ganglia, thalamus and inferior colliculus.

(vi) ***Energy sources:*** Under normal conditions, glucose is the main source of energy for the brain. Some utilization of amino-acids from the circulation may also take place (specially during prolonged starvation or convulsions).

Important Note

Glucose enters the brain via GLUT-1 in cerebral capillaries and insulin is *not* required for cerebral cells to utilize glucose.

II. REGULATION OF CEREBRAL CIRCULATION

1. *Cerebral blood flow depends on:*
   (i) perfusion pressure *i.e.* mean systemic arterial BP minus cerebral venous pressure, which is effectively the ***intracranial pressure***.
   (ii) resistance *i.e.* the viscosity of the blood; and
   (iii) degree of active constriction or dilatation of the cerebral arteries.

The alteration in cerebral blood flow under different conditions is shown in **Table 43.7**.

The table clearly indicates *Autoregulation* of cerebral blood flow between 65 to 140 mmHg mean BP. This autoregulation of cerebral blood flow is seen only when the arterial pCO_2 and pO_2 are maintained at or near normal values. **(Fig. 43.4)**

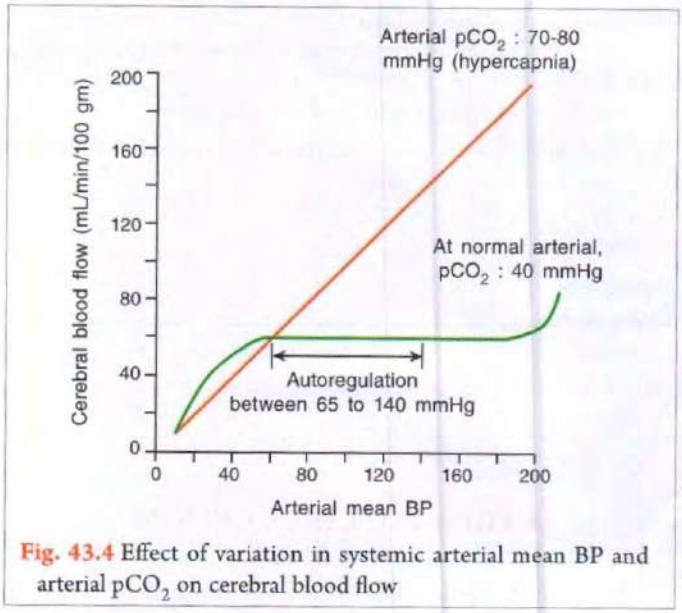


Fig. 43.4 Effect of variation in systemic arterial mean BP and arterial pCO_2 on cerebral blood flow

2. *Effect of brain metabolism* on cerebral vessels
   (i) Slight fall in arterial pO_2 by producing cerebral vasodilatation, increases 'cerebral blood flow';

Table 43.7: Factors affecting cerebral blood flow

Condition	Mean cerebral arterial pressure (as determined by systemic mean BP)	Cerebral venous pressure (as reflected by internal jugular pressure)	Cerebral blood flow
1. Recumbent position	100 mmHg	Less than 10 mmHg	Normal
2. Upright position	Falls to 20-30 mmHg	Falls similarly due to gravitational forces	No change (because fall in both the pressures keeps the perfusion pressure fairly constant).
3. Coughing or straining at stools	Normal	Increases	No change (because of simul-taneous increase in intracranial CSF pressure which minimizes the change of pressure in transmural venules and capillaries)
4. Repeated haemorrhage	Decreases	Normal	Cerebral blood flow remains steady until mean cerebral arterial pressure falls below 65 mmHg; after that cerebral blood flow decreases markedly.
5. In hypertensive patients	When increases upto 140 mmHg	Normal	Cerebral blood flow is reasonably constant; if mean BP increases above 140 mmHg, Cerebral blood flow increases accordingly.

whereas, moderate to severe decrease in arterial pO_2, decreases 'cerebral blood flow' secondary to vasoconstriciton.

(ii) 'Cerebral blood flow' increases in linearity with rise in arterial pCO_2.

Increase in arterial pCO_2 or fall in arterial pH, produces arteriolar dilatation in brain to increase 'cerebral blood flow' (*direct effect*).

There are evidences that the effect of CO_2 are mediated via changes in pH. How?

(a) A maintained rise of arterial pCO_2 of 1 mmHg above its normal range, increases cerebral blood flow by 3 mL/100 gm/min.

(b) A maintained fall of arterial pCO_2 of 1 mmHg below its normal range, decreases cerebral blood flow by 1.5 mL/100 gm/min. This is an important factor in production of cerebral symptoms during hyperventilation.

Limitations

(a) Arterial pCO_2 when increases above 80 mmHg, no further increase in 'cerebral blood flow' occurs due to maximal dilatation of cerebral vessels.

(b) Arterial pCO_2 below 20 mmHg causes no further decrease in cerebral blood flow due to cerebral vasoconstriction. Cerebral vasoconstriction occurs following cerebral hypoxia with production of lactic acid.

Important Note

Lactic acid produces cerebral vasodilatation only when it diffuses into cerebral interstitium.

(iii) Inhalation of hyperbaric O_2 causes cerebral vasoconstriction and thus cerebral blood flow decreases.

High cerebral pO_2 disrupts neuronal metabolism producing convulsions, coma and death (page 461).

3. *Anaesthetic agents decrease cerebral* blood flow and depress metabolism by influencing cerebral vascular sensitivity.

4. *Effect of intracranial pressure changes*

Any change in intracranial pressure causes a similar change in venous pressure promptly and vice versa. For example, increased intracranial pressure compresses cerebral vessels, venous pressure decreases. As a result, effective perfusion pressure decreases to decrease cerebral blood flow. This relationship helps to compensate for changes in arterial BP at the level of the heart, for example:

(i) If the body is accelerated upwards *(positive 'g')*, blood moves towards the feet and arterial pressure at the level of the head decreases; however, venous pressure also falls and intracranial pressure falls, so that the pressure on the vessels decreases and cerebral blood flow remains fairly constant.

(ii) Conversely, during acceleration downwards, force acting towards the head *(negative 'g')* increases arterial pressure at head level, but intracranial pressure also rises, so that the cerebral vessels are supported and do not rupture.

Important Note

Cerebral circulation is controlled almost entirely by local metabolic factors. The most important local vasodilator for the cerebral circulation is CO_2. Vasoactive substances in the systemic circulation have little or no effects because such substances cannot cross the blood brain barrier easily.

Mechanism of Cerebral Autoregulation

Three mechanisms are responsible for autoregulation of 'cerebral blood flow'.

1. *Role of Metabolism:* This is the main factor responsible for achieving autoregulation of 'cerebral blood flow'. How?

(i) Mild to moderate decrease in perfusion pressure decreases driving forces for flow, resulting in CO_2 accumulation which by release of H^+ produces cerebral vasodilatation.

(ii) Marked decrease in perfusion pressure causes cerebral hypoxia; release of pyruvic and lactic acid, which by diffusing into the interstitial fluid causes further increase in its $[H^+]$.

2. *Role of basal myogenic tone of blood vessels:* Due to myogenic response of the small pre-capillary blood vessels these vessels contract in response to distending pressure. ***(Bayliss-Folkow hypothesis - 1902)***, therefore, a sudden rise in transmural pressure causes transient increase in 'cerebral blood flow' which returns to normal within 1-2 secs.

3. *Role of vasomotor nerves:* Even though cerebral vessels are innervated by nor-adrenergic vasoconstrictor fibers and cholinergic vasodilator fibers; vasomotor reflexes play little, role in the regulation of 'cerebral blood flow' in humans. However, cholinergic sympathetic vasodilator nerves are responsible for autoregulation of 'cerebral blood flow' when mean arterial blood pressure decreases.

Effect of Intracranial Pressure Changes

1. On systemic arterial BP

Normally, cerebrospinal fluid ('CSF') occupies

approx. 10% of the intracranial volume with a pressure between 'zero' to 7 mmHg. When 'CSF' volume increases:

(i) CSF gets displaced into the spinal canal due to compression of the epidural venous plexuses there. This mechanism provides approx. 65% of the compensatory capacity of the rigid skull.

(ii) Beyond this even small increase in CSF volume causes marked increase in intracranial pressure which may correspondingly approach the mean arterial pressure.

(iii) *Cushing Reflex:* to maintain cerebral blood flow (page 335).

2. On Cerebral Blood Flow

In lying down position, normal jugular venous pressure is 7 mmHg.

Kety's Experiment:

(i) Increase of CSF pressure from 7 to 33 mmHg causes linear rise of arterial B.P. Thus helps to maintain normal cerebral blood flow in spite of increase in extravascular pressure;

(ii) When CSF pressure exceeds 33 mmHg, intracranial pressure increases above the arterial BP; this decreases 'cerebral blood flow' with fall in arterial B.P. progressively, finally producing coma.

This shows increase of CSF pressure from its normal level initially leading to ***Bulbar Asphyxia*** which stimulates vasomotor centre producing extracerebral vasoconstriction and increases arterial mean B.P.

However, marked increase in CSF pressure produces direct depression of vasomotor centre and compensatory mechanism fails.

Study Questions

1. **Write short notes on:**
   (i) Technique for measurement of cerebral blood flow
   (ii) Regulation of cerebral circulation
   (iii) Effects of altering of arterial pCO_2 and pO_2 on cerebral blood flow
   (iv) Effect of positive and negative 'g' on cerebral circulation
   (v) Bayliss-Folkow hypothesis
2. **Define intracranial pressure. Mention the effects of its changes on cerebral circulation.**
3. **Which part of the brain is resistant to hypoxia? Give its clinical significance.**
4. **Give physiological basis of:**
   (i) Critical blood flow level to brain
   (ii) Brain is extremely sensitive to hypoxia
5. **Depict diagrammatically the effect of variation in systemic arterial mean BP and arterial pCO_2 on cerebral blood flow.**

MCQs

1. **Which part of the brain is more resistant to hypoxia?**
   (a) Cerebral cortex (b) Basal ganglia (c) Thalamus (d) Brain stem
2. **Autoregulation of cerebral blood flow is seen between ..... mmHg mean BP:**
   (a) 40-100 mmHg (b) 65-140 mmHg (c) 65-200 mmHg (d) 15-50 mmHg
3. **The most important factor for regulating cerebral blood flow under normal conditions is the:**
   (a) Rate of cerebral carbon dioxide formation
   (b) Rate of cerebral oxygen consumption
   (c) Degree of sympathetic stimulation of peripheral vasculature
   (d) Rate of release of adenosine from the cerebrum
4. **Oxygen consumption of whole human brain in mL/min is about:**
   (a) 25 (b) 35 (c) 45 (d) 55
5. **Slight decrease in cerebral arterial pO_2 will lead to:**
   (a) No change in rate of arterial circulation
   (b) Vasoconstriction of cerebral arterioles
   (c) Vasodilatation and increase in rate of cerebral circulation
   (d) Significant increase in neuronal excitazbility

Answers

1. (d) 2. (b) 3. (a) 4. (c) 5. (c)

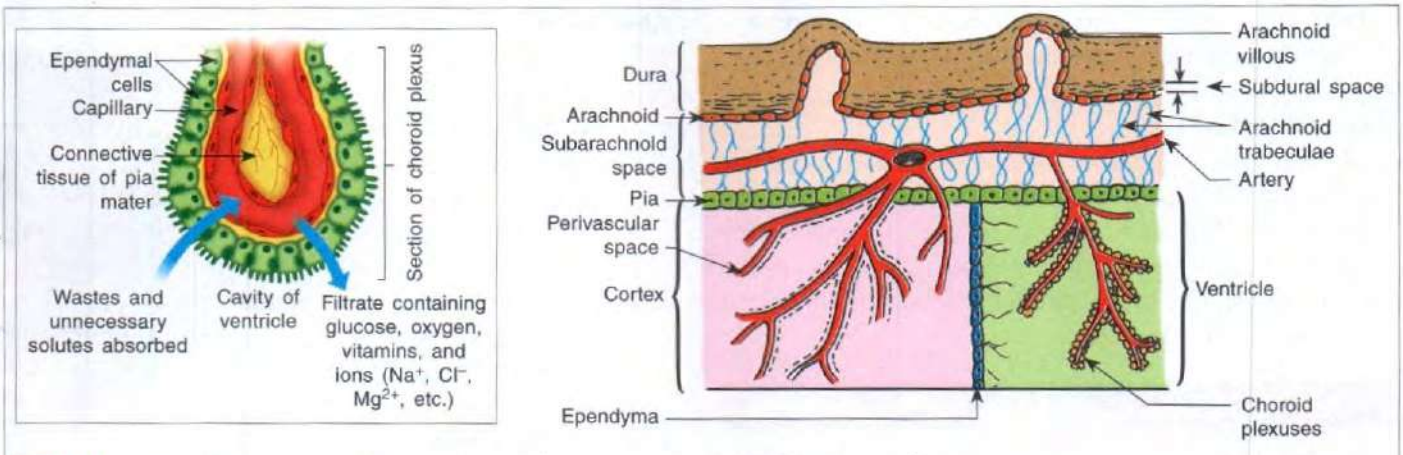


Fig. 43.5 Section of brain and investing membranes (**Inset:** Secretion from choroid plexus)

THE CEREBROSPINAL FLUID (CSF)

PHYSIOLOGICAL ANATOMY

1. 'CNS' is enveloped by the meninges; from outside to inside are termed: *dura, arachnoid* and *pia.* **(Fig. 43.5)** Dura ends at the lower border of 2nd sacral spine, while spinal cord ends at the lower border of 1st lumbar spine, therefore, spinal theca can be punctured in lower lumbar segments without fear of injury to the spinal cord.
2. The arachnoid is separated from the pia by ***subarachnoid space,*** which contains CSF. Arachnoid invests the spinal cord quite loosely. There are definite dilatations of subarachnoid space called ***cisternae;*** these are:
   (i) *Cisterna magna* is found in the interval between the medulla and under-surface of cerebellum.
   (ii) *Cisterna pontis* and *cisterna basalis.*
   Cisterna pontis lies on the ventral aspect of the pons and contains the basilar artery. The 'cisterna basalis' (interpeduncularis) is formed by the arachnoid bridging across the interval between the tips of the temporal lobes, and contains the circle of willis.
   Prolongation of the subarachnoid space extends along the sheaths of spinal and cranial nerves, particularly the optic nerve.
3. ***Pia*** invests the nervous substance very closely. As the arteries and veins enter and leave the brain substance they are surrounded by the ***Perivascular Spaces*** *i.e.* pia is loosely adherent to the blood vessels. These spaces extend down as far as arterioles and venules and above are continuous with subarachnoid spaces.
   Function: Perivascular spaces are modified lymphatic system for brain (since no true lymphatics are present in brain tissue). They help in transporting fluid proteins and waste products and extraneous particulate matter from the brain into the subarachnoid space.
4. ***Ventricles.*** They are series of interconnecting chambers within the brain. They are:
   (i) Lateral ventricle, chamber in central hemisphere.
   (ii) IIIrd ventricle, narrow chamber in mid brain, which communicates with lateral ventricle above by *Foramen of Monro* and below to IVth ventricle by *Aqueduct of Sylvius.*
   (iii) IVth ventricle, it lies between pons and medulla oblongata.
   Surface of ventricles are lined with a thin cuboidal epithelium called ***Ependyma.***
5. ***Choroid Plexuses.*** Some arteries (accompanied by covering of pia) pass through the brain substance to reach the lining, *Ependymal Layer* in ventricles (Lateral, IIIrd and IVth). They then break up into complex blood vessels and their epithelium becomes differentiated into cubical cells. This modified lining of blood vessels constitute *Choroid Plexuses* **(Fig. 43.5)**. It contributes to the formation of cerebrospinal fluid.
6. ***Arachnoid Villi.*** They are small finger like projections of arachnoidal trabeculae, lined by flat epithelium **(Fig. 43.6)**. These villi project into venous sinuses

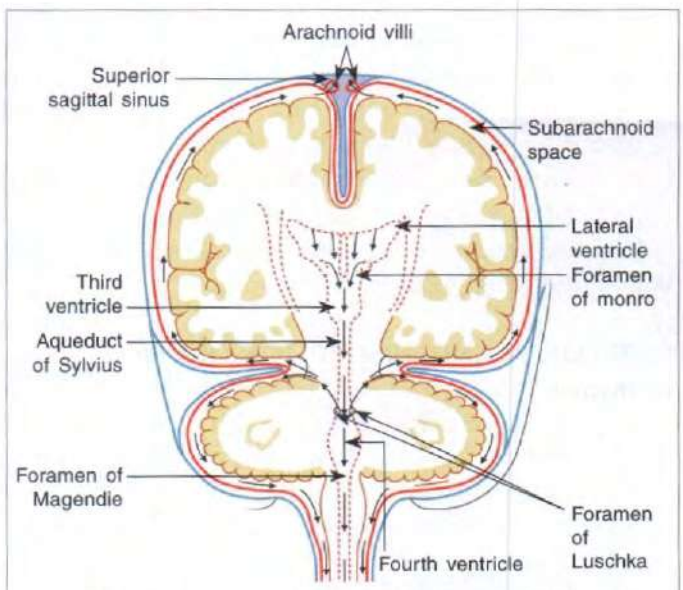


Fig. 43.6 Arrows showing the flow of cerebrospinal fluid within brain spaces.

and allow relatively free flow of 'CSF', proteins and particles less than 1 µm size into the blood.

CSF COMPOSITION

'CSF' is a clear colourless alkaline fluid with a specific gravity 1005-1008. It is almost *protein free* (20-30 mg/dL) and almost *cell free* (lymphocytes 0-5/µL). It contains less glucose (50 mg/dL) than plasma. Also contains some urea and creatinine. Hence it is similar to blood plasma but it does not clot. **(Table 43.8)**

Table 43.8: Composition of human CSF and plasma

Substance	CSF	Plasma	Ratio CSF to Plasma
Na^+ (mEq/L)	147	150	0.98
K^+ (mEq/L)	2.9	4.6	0.62
Ca^{2+} (mEq/L)	2.3	4.7	0.49
Cl^- (mEq/L)	113	99	1.14
HCO_3^- (mEq/L)	25.1	24.8	1.01
Protein (mg/dL)	25 (20-30)	6000	0.003
Glucose (mg/dL)	64 (50-85)	100	0.64
pH	7.33	7.40	0.99
Osmolality (mOsm/kg water)	289	289	1.0

Note

$[K^+]$, $[Ca^{2+}]$ and glucose is lower, $[H^+]$ is higher whereas $[Na^+]$ is similar to that in the plasma.

Volume: 130-150 mL of which 30 mL is in ventricular system and remainder in subarachnoid space.

Daily secretion: 500 to 550 mL.

Rate of formation: 0.2 - 0.3 mL/min.

Pressure: average: 130 mm H_2O (10 mmHg) in lateral lying position (range: 100-200 mm H_2O).

In sitting position CSF pressure is 200 mm H_2O higher than in the lying position.

FORMATION AND ABSORPTION OF CSF

Formation

1. 50% by choroid plexuses in the ventricles.
2. 40% by blood vessels of meningeal and ependymal lining of ventricles.
3. 10% by blood vessels of brain and spinal cord. The "CSF" is formed continuously by the secretory activity of the epithelial cells of the choroid plexuses of the intraventricular system (*evidence:* CSF composition differs from that of plasma).

Absorption

1. Mainly (80%) by arachnoid villi into venous (dural) sinuses and spinal veins; because:
   (i) hydrostatic pressure of "CSF" is more than venous pressure in sinuses, and
   (ii) it is also aided by osmotic pull of plasma proteins within plasma, in returning "CSF" to blood stream.
2. To minor degree *i.e.* remaining 20% may pass along the sheaths of cranial nerves into cervical lymphatics and also into perivascular spaces.

Route of Absorption

From choroid plexuses of ventricles to arachnoid villi. How? **(Fig. 43.6)**

The cerebrospinal fluid formed in the lateral *ventricles* via *foramen of monro* reaches IIIrd ventricle (combines with fluid secreted here), then via *aqueduct of sylvius* enters into *cisterna magna* (dilatation in subarachnoid space). The 'CSF' from other ventricles flows through the *foramen of magendie* and *Luschka* also to the *sub-arachnoid space*. From sub-arachnoid space it flows upwards towards cerebrum, where almost all arachnoid villi are located; and is absorbed through these villi into the cerebral venous sinuses.

Important Notes

1. **At a normal average CSF pressure of 130 mmH_2O, the CSF filtration and absorption are equal. (Fig. 43.7)**
2. **At CSF pressure less than 70 mmH_2O, CSF absorption stops and it starts accumulating in excessive amounts within brain spaces, a condition called *Hydrocephalus*.**

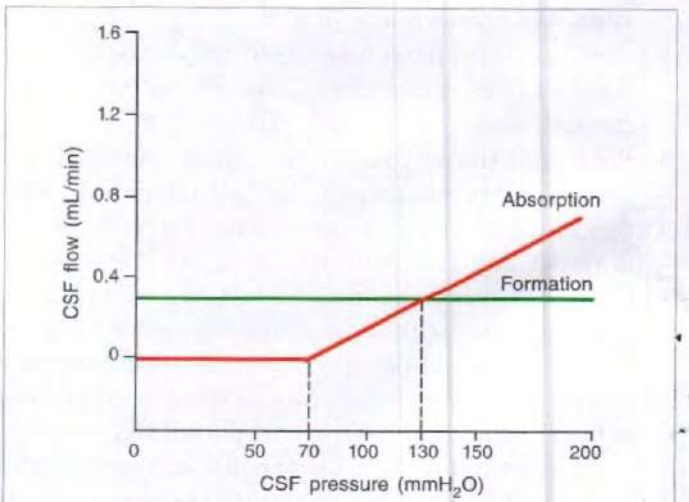


Fig. 43.7 CSF formation and absorption.
(a) At 130 mmH_2O pressure, CSF formation and absorption are equal;
(b) at 70 mmH_2O pressure, CSF absorption is zero.

FUNCTIONS OF CSF

1. It serves as a fluid *buffer* thereby provides optimum environment to neurons.
2. *Protective function:* supports the delicate brain in cranial vault. However, brain and CSF have approx. the same specific gravity, therefore, brain simply floats in the fluid.
3. It acts as a *reservoir* to regulate the contents of cranium *i.e.* it can compensate for fluctuation in amount of blood within skull, therefore, at any moment if blood volume of brain increases then 'CSF' drains away. Conversely, if brain's blood volume shrinks, more 'CSF' is retained. Thus, CSF keeps the total volume of cranial content constant.

Important Note

Because brain tissue and CSF are essentially incompressible, the volume of blood, CSF and brain in the cranium at any time must be relatively constant (*Monro-Kellie Doctrine*).

4. The highly selective permeability of brain capillaries prevents the brain from toxins and from fluctuations in neurotransmitters in the blood.
5. It helps transfer of waste products (*metabolic exchanges*) of brain into the blood.
6. It may serve as a medium for *nutrient exchanges* in the nervous system.

Important Note

Removal of CSF during lumbar *puncture* can cause a severe headache afterwards, because the brain hangs on vessels and nerve roots, and traction on these structures stimulates pain fibers.

MEASUREMENT OF CSF PRESSURE

1. Subject lies in lateral position (therefore, spinal fluid pressure equals pressure in cranial vault).
2. A spinal needle is inserted into spinal canal between L_4 and L_5 and is connected to glass tube.
3. Spinal fluid is allowed to rise in the tube as high as it will.
4. 'CSF' level height in the tube (in mm) above the level of the needle will give CSF pressure in mmH_2O.

CLINICAL ASPECTS

A. Causes of increase in CSF pressure

I. Physiological

1. Increase in venous pressure, for example, following coughing or crying or compression of internal jugular vein.
2. *Queckenstedt's Sign:* compression of internal jugular vein decreases absorption of CSF, as a result CSF pressure increases.

II. Pathological

1. Increase in rate of fluid formation *e.g.* inflammation of meninges.
2. Increased resistance to absorption through arachnoid villi; for example, brain tumours, haemorrhage or infection (cellular elements block the absorption).

B. Causes of decrease in CSF pressure

1. decrease in venous pressure;
2. decrease in rate of fluid formation.

Diagnosis of increase in CSF pressure by ophthalmoscopy: Look for ***Papilloedema*** *i.e.* swelling of optic disc. How?

Increased pressure of 'CSF' increases pressure in optic sheath, so blood flow in retinal veins decreases. This increases retinal capillary pressure throughout the eye producing retinal oedema. Tissue of optic disc being more distensible compared to rest of retina, therefore, becomes more oedematous and swells into the cavity of the eye.

C. The effect of I.V. injection of hypertonic solutions on the CSF pressure

1. I.V. injection of 50 mL of **10% NaCl solution** causes fall in CSF pressure for 2 hours due to absorption of fluid from CSF into the plasma. However, effect is temporary, because Na^+ and Cl^- eventually move into the CSF themselves and equilibrium is re-established

   Use: it is of value in conditions of raised intracranial pressure caused by cerebral tumours; therefore, helpful in:

   (i) relieving papilloedema;
   (ii) restoring consciousness; and
   (iii) intracranial operations made easier as bulging of the brain is prevented.
2. **Hypertonic glucose** I.V. injection likewise exerts similar effects but is of transient benefit as glucose gets metabolized.
3. **Hypertonic urea** exerts a more prolonged effect because of the low rate of penetration of urea into the CSF and the slowness of renal excretion of urea.

D. Hydrocephalus

This means pathological accumulation of CSF within brain spaces.

Types

1. *Communicating (or External) Hydrocephalus* *i.e.* excess of fluid accumulation in subarachnoid space.

Causes: when rate of CSF formation is more than rate of its absorption, *e.g.*

(i) Over development of choroid plexuses increases CSF formation; this fails to get absorbed by the total surface of arachnoid villi available for absorption;

(ii) Decrease absorptive capacity of arachnoid villi due to:

(a) thrombosis of venous sinuses, or

(b) inflammatory changes in meninges which causes occlusion of arachnoid villi.

2. *Non-communicating (or Internal) Hydrocephalus i.e.* excess of fluid accumulation in ventricular system proximal to block. Common sites of block are: foramen of monro; aqueduct of sylvius; foramen of luschka or magendie, and within ventricular system itself.

BLOOD BRAIN BARRIER (BBB)

The term applies to the barrier between blood and the brain tissue. In fact this barrier exists at two places within the brain. **(Fig. 43.8)**

(1) One located at the choroid plexus and CSF fluid interface. Some physiologists use separate term for this as *blood-CSF barrier* (choroid plexus is slowly permeable to small ions and lipid insoluble substances and is impermeable to large lipid-insoluble compounds); and

(2) Other is located between the CSF and brain capillaries elsewhere than the choroid plexuses ***(true blood brain barrier).***

However, both the barriers are similar and the *blood brain barrier (BBB)* is the most common term used to refer to the net exchange of substance between blood and the brain.

There is an H^+ gradient between brain ECF and blood; the pH of brain ECF is 7.33; whereas that of blood is 7.4.

In general rapidity with which substances penetrate brain tissue is inversely related to molecular size of substance and directly to lipid solubility of substance, thus water soluble polar compounds generally cross slowly.

Therefore, these barriers are:

(i) *Highly permeable to*: water, O_2, CO_2, sulpha drugs and erythromycin.

(ii) *Slightly permeable* to electrolytes : H^+, Na^+, K^+, Mg^{2+}, Cl^-, HCO_3^- and HPO_4^{2-} glucose and some drugs *e.g.* penicillin, chloromycetin, tetracyclin.

(iii) *Almost impermeable to*: arsenic, gold, sulphur; urea; catecholamines; proteins and bile salts.

Rate of transfer of substances

Certain compounds cross the BBB slowly, whereas closely related compounds enter rapidly *e.g.* amines like dopamine and serotonin penetrate to a very limited degree, but their corresponding acid precursors, L-dopa, and 5-hydroxytryptophan, enter with relative ease.

Variable permeability of brain capillaries is due to the fact that in these capillaries:

(i) the endothelial cells are surrounded by a continuous belt of tight junctions and do not allow the penetration of molecules with MW more than 2000;

(ii) the endothelial cells are covered by foot process of astrocytes. This covering is, however, incomplete and large enough to permit the substances with a MW 40,000.

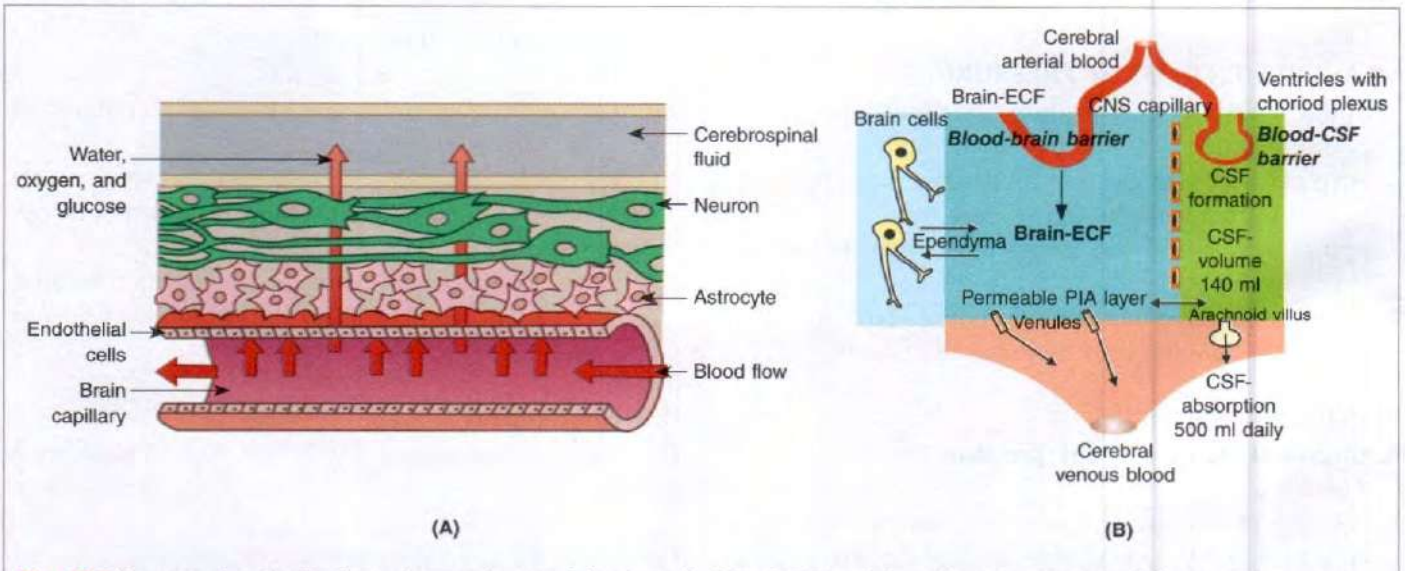


Fig. 43.8 Structures involved in the exchange of materials between the blood, CSF and the cells within the brain (A); Blood Brain Barrier and Blood-CSF barrier (B)

Important Note

Glucose is the major source of energy for neurons. It is transported across the walls of brain capillaries by glucose transporter (GLUT-1, page 260). Infants with congenital GLUT-1 deficiency develop decreased CSF glucose concentration in spite of normal blood glucose and they have delayed development and fits.

Development of BBB

BBB develops during early years of life; at this time cerebral capillaries are much more permeable than adulthood. Therefore, in severely jaundiced infants, bile pigments penetrate into nervous system and, in the presence of asphyxia, damage the basal ganglia ***(kernicterus)*** *(page 109)*. However, this is not true in jaundiced adults.

Functions of BBB

(1) BBB protects the sensitivity of the cortical neurons to ionic changes with fluid bathing them. This helps to maintain constancy of environment *i.e.* concentration of K^+, Ca^{2+}, Mg^{2+}, H^+ and other ions in the CNS;
(2) It protects the brain from endogenous and exogenous toxins in the blood; and
(3) It prevents the escape of neurotransmitters into the general circulation.

Clinical Importance of BBB

1. Selection of drugs during management of meningitis: Sulpha and erythromycin are most commonly given to treat meningitis which can easily cross the BBB.
2. BBB breaks down in the areas of irradiation, infection or tumours, therefore, localization of pathological area with accuracy is possible with dyes or radioactive iodine labelled with albumin.
3. BBB also breaks down by sudden marked increase in B.P. or by I.V. administration of hypertonic fluids.

Important Note

CSF in the ventricles and the subarachnoid space is separated from the brain by highly permeable structures, the ependyma and the pia respectively. Thus there is no *'CSF-brain barrier'* and a drug injected into the CSF by lumbar puncture reaches the brain and spinal cord easily.

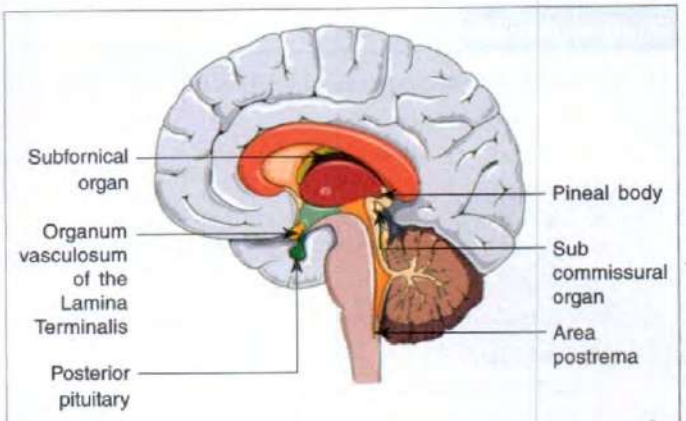


Fig. 43.9 Circumventricular organs

CIRCUMVENTRICULAR ORGANS

There are 4 small areas in or near the brainstem outside the BBB. These are the areas having fenestrated capillaries in which substances in the circulating blood can act to trigger changes in brain functions without penetrating the BBB (Fig. 43.9). These areas are:

1. *The Posterior Pituitary* (neurohypophysis) and the adjacent ventral part of the median eminence of the hypothalamus. This functions as ***neurohemal organs*** *i.e.* areas in which substances (polypeptides) secreted by neurons enter the circulation *e.g.* oxytocin and ADH enter the general circulation in the posterior pituitary (Also see to page 672).
2. *The Area Postrema*, a chemoreceptor zone that initiates vomiting in response to chemical changes in the plasma. *Chemoreceptor zone* means the area in which substance in the circulating blood can act to trigger changes in the brain function without penetrating the 'BBB'.
3. *The Organum Vasculosum of the Lamina Terminalis* (OVLT, supra optic crest). It is the site of the *specialized osmoreceptors* controlling thirst mechanism via ADH secretion (for detail refer pages 672 and 1008). Moreover, circulating IL-1 produces fever by acting on this organ.
4. *The Subfornical Organ* (intercolumnar tubercle). Circulating angiotensin II acts on area 3 and 4 to increase water intake.

Note

Subcommissural organ, does not have fenestrated capillaries and is not highly permeable. Thus it is not a part of circumventricular organs.

Study Questions

1. **Removal of CSF during lumbar puncture can cause a severe headache. Explain.**
2. **Write short notes on:**
   (i) Cerebral ventricles
   (ii) Composition and functions of CSF
   (iii) Formation and absorption of CSF
   (iv) Monro-Kellie doctrine
   (v) Causes of increase and decrease in CSF pressure
   (vi) Hydrocephalus
   (vii) Importance of circumventricular organs
   (viii) Choroid plexuses
   (ix) Blood-CSF barrier
3. **Why spinal theca is punctured in the lower lumbar region to collect the CSF?**
4. **What will happen and why?**
   (i) If CSF pressure is increased
   (ii) If blood brain barrier breaks
   (iii) If hypertonic saline is administered intravenously

MCQs

1. **CSF pressure in the lying down posture is ..... mmH_2O:**
   (a) 20-50
   (b) 50-100
   (c) 130-150
   (d) 150-200
2. **The amount of CSF which can easily be removed without producing any ill effects is ..... mL:**
   (a) 50
   (b) 75
   (c) 100
   (d) 150
3. **Blood brain-barrier is made up of:**
   (a) Astrocytes
   (b) Oligodendrocytes
   (c) Oligodendroglia
   (d) Microglia
4. **All *except* one area lie outside the blood brain barrier:**
   (a) Posterior pituitary
   (b) Area postrema
   (c) Thalamus
   (d) Organum vasculosum of lamina terminalis
5. **Blood brain barrier is *not* present in:**
   (a) Area postrema
   (b) Corpus striatum
   (c) Corpus callosum
   (d) Cerebral cortex
6. **Normal CSF volume is:**
   (a) 100-120 mL
   (b) 130-150 mL
   (c) 160-180 mL
   (d) 180-200 mL
7. **CSF differs from plasma in having higher concentration of:**
   (a) Na^+
   (b) K^+
   (c) Cl^-
   (d) Glucose
8. **pH of CSF is:**
   (a) 7.13
   (b) 7.23
   (c) 7.33
   (d) 7.43
9. **CSF is principally secreted by:**
   (a) Choroid plexus
   (b) Meningeal blood vessel
   (c) Ependymal lining of ventricles
   (d) Blood vessels of spinal cord

Answers

1. (c) 2. (a) 3. (a) 4. (c) 5. (a) 6. (b) 7. (c) 8. (c) 9. (a)

CUTANEOUS (SKIN) CIRCULATION

Characteristic features

1. *Skin weighs* approx. 2 kgm in an adult and skin blood flow depends on:
   (i) the requirements for the maintenance of body temperature (mainly), and
   (ii) metabolic activity of the skin itself.
2. Average *skin flow* of a naked man in resting thermal equilibrium *i.e. comfortable environmental temperature* of approx. 27°C is 10-15 mL/100 gm per min, therefore:
   (i) during exposure to cold, skin blood flow decreases to as low as 1 mL per 100 gm/ min; and
   (ii) during exposure to heat skin blood flow increases to as high as 150 mL /100 gm per min.
3. *Regional variations in skin blood flow*
   (i) Normally skin vessels are tonically under the influence of a 'sparse' sympathetic discharge. This is true for the skin as a whole and it is not correct for skin of the hands and feet.
   (ii) The vascular circuit of skin in hand, feet and ear lobes shows numerous A-V anastomoses (most numerous in the fingers, page 314) which are much less frequent in the skin of the rest of the body. These A-V anastomoses are richly innervated by sympathetic vasoconstrictor activity and are closed; that is why during heat stress the hands and feet skin flow increases markedly (70-80 mL/100 gm/min) whereas the skin which has few or no A-V anastomoses, blood flow increases to only 25-30 mL/100 gm/min under heat stress or after sympathetic blockade.
4. Ordinary pre-capillary resistance vessels and pre-capillary sphincters of the skin show a considerable *basal myogenic tone* (page 321) which can be modified considerably by the influence of post ganglionic sympathetic fibers. At 'comfortable environmental temperature', their discharge is only sufficiently effective to half the flow from that allowed by the basal myogenic tone itself.

Nervous Control of Skin Circulation

1. Normally the skin vessels are subjected to a low rate of sympathetic constrictor discharge which effectively limits the flow through them.
2. Skin vessels are not innervated by vasodilator nerves, however, *vasodilatation* in skin blood vessels is due to:
   (i) reduction of vasoconstrictor impulse activity;
   (ii) local production of bradykinin in sweat glands; and
   (iii) local vasodilator metabolites (page 321).

Control of vasoconstrictor activity to skin blood vessels is modified from:

(i) the hypothalamus in response to temperature changes which are recorded centrally; and
(ii) stimulation of lateral spinothalamic tract by the temperature receptors in the skin.

As heat load rises gradually

(a) 1st: A-V ananstomoses of hands, ear and feet dilate due to the reduction of their regional sympathetic discharge;
(b) 2nd: remainder of the skin vessels dilate due to progressive withdrawal of sympathetic vasoconstrictor activity;
(c) 3rd: sweat glands get activated due to bradykinin released from sympathetic discharge. This helps,
- to restore the thermal equilibrium; and
- also causes vasodilatation of skin vessels in the neighbourhood.

Important Note

During exercise when the body temperature rises, the cutaneous blood vessels dilate in spite of continuing nor-adrenergic discharge in other parts of the body since the rise in the hypothalamic temperature overrides the other reflex activity.

Cold Blue (or Grey) Skin: is one in which the arterioles are constricted and the capillaries dilated.

Warm Red Skin is one in which both arterioles and capillaries are dilated.

Factors Affecting Skin Blood Flow

1. *Exposure to cold stress,* via hypothalamic mechanism, stimulates sympathetic discharge. Therefore, skin blood vessels are strongly constricted and blood flow is directed to the deeper tissues. This decreases total skin blood flow less than 50 mL/min (*i.e.* 1 mL per 100 gm per min).

Clinical significance

(i) Cold produces vasoconstriction, therefore,
(a) decreases supply of nutrients to the skin; also
(b) decreases metabolic rate of the tissue and metabolite accumulation is very slow. As a result pre-capillary resistance vessels of the skin to maximal vasoconstrictor discharge are well sustained and do not "escape" to any great extent (compared to the skeletal muscle, pre capillary resistance vessels 'escape' from sympathetic vasoconstrictor discharge because of local accumulation of metabolites).

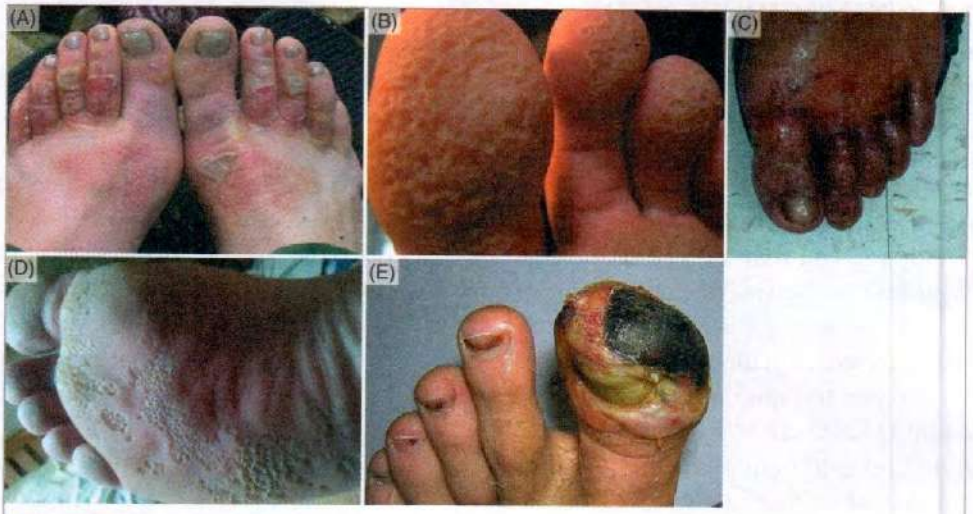


Fig. 43.10 Various stages of trench foot (A to E)

(ii) With **severe cold** *i.e.* when skin temperature falls less than 10°C, it causes:
   (a) tissue injury producing pain and release of histamine which excites the sensory terminals. Impulses travel via ***axon reflex*** (page 326) pathway to the A-V anastomoses to bring about vasodilatation, called ***cold vasodilatation***.
   (b) promotes the formation of plasma kinins which also produce vasodilatation, that is how, *prolonged exposure to cold*, specially in damp conditions produces skin lesions; for example, ***Trench Foot*** **(Fig. 43.10)**.

2. ***Exposure to heat stress*** via hypothalamic mechanism:
   (i) abolishes sympathetic discharge, therefore,
      (a) A-V anastomoses dilate widely and increases hand and feet skin blood flow markedly;
      (b) abolish basal myogenic tone, thus skin blood flow increases;
   (ii) activates sweating mechanism to cause sweating (maximum @ 2L/hour);
   (iii) increases skin blood flow due to release of a potent vasodilator, bradykinin.

   All these mechanisms increase total skin blood flow to as much as 3-4 L/min *i.e.* approx. 150 mL per 100 gm/min. This marked increase in skin blood flow will throw a big load on cardiovascular functions, *that is why men working under maximal heat loads may simply collapse with circulatory failure unless supervised adequately.*

3. ***Emotional factors*** on skin blood vessels are relayed from corticohypothalamic centers to the thoracolumbar sympathetic cell bodies and thence to the skin vessels. This produces ***blanching of the skin*** *(pale with fear)*. ***Phenomenon of blushing*** (emotional embarrassment). There are no specific vasodilator fibers to skin. However, blushing is due to bradykinin release (a most potent vasodilator) secondary to a brief cortico-hypothalamically controlled discharge of sympathetic cholinergic fibers to the sweat glands (page 332).

Triple Response *(Lewis T 1927)*

It is a three part response of the normal reaction to injury **(Fig. 43.11)**. For example, a firm strong stroke across the skin by a blunt pointed object (using a pencil point), evokes a series of response classified as:

1. *Red reaction i.e.* reddening at the site that appears in approx. 10 sec. This is followed in a few minutes by;
2. *Flare i.e.* redness spreading out from the injury, and
3. *Wheal i.e.* local diffuse swelling.

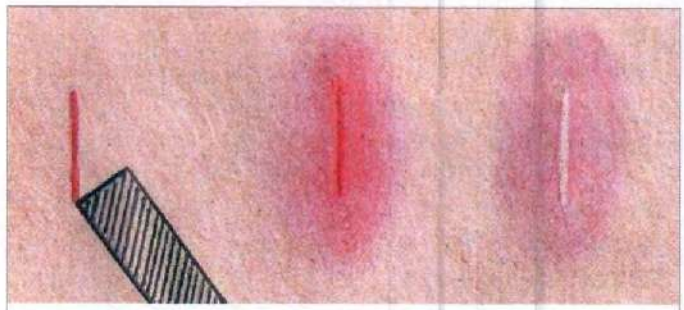


Fig. 43.11 Triple response

1. ***Red Reaction or Red Line***

   This is dilatation of pre-capillary sphincters directly and it characteristically outlines the stroke and is due to:
   (i) release of histamine; and/or
   (ii) polypeptides *e.g.* bradykinin

   (i) and (ii) released from the damaged skin.

   It is purely "passive" phenomenon and not mediated by nerves and is due to relaxation of the pre-capillary

sphincters. Local anaesthesia of skin does not prevent the red reaction.

2. ***Flare*** is due to dilatation of the (i) arterioles, (ii) terminal arterioles, and (iii) pre-capillary sphincters. They give rise to irregular erythematous area which surrounds the site of the red line.

Flare is characterised by:

(i) Skin temperature overlying the area is raised, because decrease in arteriolar resistance increases local blood flow.

(ii) Injection of local anaesthetic agents abolishes the reaction, therefore, it is mediated by nerves but it does not involve the CNS connections.

Mechanism: Response of arteriolar dilatation is mediated by a branching of the terminal axons of "C-fibers" of sensory nerve called ***Axon Reflex*** (page 326).

3. ***Wheal***

If stroke of stimulus has been strong enough, a blister like appearance develops spreading from the margins of the red line within the flare area. This is due to:

(i) increased capillary permeability produced by histamine and related substance released locally from mast cells and mediated via H_1 receptors, and

(ii) rise of capillary pressure due to dilatation of the pre-capillary resistance vessels.

(i) and (ii) cause transudation of fluid out of capillary which contains some amounts of protein.

Important Note

A painful injury leads to:

(i) diffuse nor-adrenergic discharge which produces generalised cutaneous vasoconstriction; and

(ii) local triple response.

Cutaneous hyperaemia: Refer to page 379.

Dermatographia

It is seen in individuals which are unusually prone to develop striking 'triple response' reactions **(Fig. 43.12)**. Exact cause is not known. It is possibly due to increased release of histamine from the damaged skin areas as intradermal injection of histamine in known amounts can stimulate the triple response.

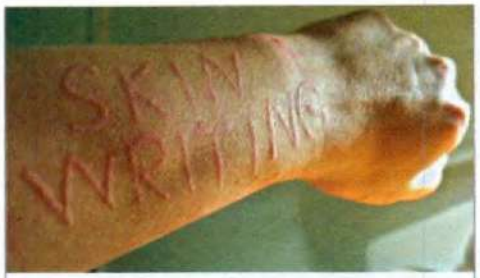


Fig. 43.12 Dermatographia

White Reaction

When a pointed object is drawn lightly over the skin, the stroke lines become pale (***white reaction***). This is due to contraction of pre-capillary sphincters, and blood drains out of the capillaries and small veins, the response appears in about 15 secs.

Study Questions

1. Give physiological basis of:
   (i) Blanching of skin
   (ii) Phenomenon of blushing
   (iii) Dermatographia
   (iv) Trench foot
   (v) Cold vasodilatation
   (vi) Flare and wheal
   (vii) Cold blue and warm red skin
   (viii) Red and white reaction
2. Write short notes on:
   (i) Effect of variation of environmental temperature on cutaneous circulation.
   (ii) How can vasodilatation in skin blood vessels be achieved?
   (iii) Role of skin to restore thermal equilibrium during heat stress.
   (iv) Factors affecting skin blood flow.
   (v) Triple response.

MCQs

1. Cold blue or grey skin is one in which:
   (a) Arterioles are constricted and capillaries dilated
   (b) Both arterioles and capillaries are dilated
   (c) Arterioles are dilated and capillaries constricted
   (d) Both arterioles and capillaries are constricted
2. Warm red skin is one in which:
   (a) Arterioles are constricted and capillaries dilated
   (b) Arterioles are dilated and capillaries constricted
   (c) Both arterioles and capillaries are dilated
   (d) Both arterioles and capillaries are constricted

3. **Phenomenon of blushing may occur due to:**
   (a) Activation of vasodilator nerves innervating the skin blood vessels
   (b) Bradykinin release
   (c) Cold vasodilatation
   (d) Activation of axon reflex
4. **The sequence of events *i.e.* red reaction (R), flare (F) and wheal (W) in triple response is:**
   (a) R, F, W (b) R, W, F (c) W, F, R (d) W, R, F
5. **Area of brain where blood brain barrier seems to be most permeable is probably located in the:**
   (a) Thalamus (b) Hypohythalamus (c) Brain stem (d) Basal ganglia

Answers

1. (a) 2. (c) 3. (b) 4. (a) 5. (b)

MUSCLE CIRCULATION

Characteristic Features

1. Skeletal muscles weigh approx. 30 kg in an adult *i.e.* 40% of body weight. With muscle **blood flow** is as under:
   (i) **At rest** – 800 mL per min *i.e.* 3-4 mL/min/100 gm.
   (ii) **In severe exercise**, increases to 20 L/min *i.e.* 100 mL/min/100 gm due to vasodilatation. This shows that *vascular resistance is high in resting muscle* and in fact muscle vascular circuit provides main contribution to the total peripheral resistance.
2. ***Vascular resistance*** offered by the muscle circuit is due to tonic sympathetic discharge on the *basal myogenic tone* of the pre-capillary resistance vessels (page 312). Sympathetic vasoconstrictor nerves richly innervate the pre-capillary resistance vessels (page 322), their discharge causes:
   (i) increase of the pre/post-capillary resistance ratio, which decreases the mean capillary pressure and thus helps to increase the uptake of tissue fluid;
   (ii) constriction of the post-capillary venules which helps to mobilize blood towards the heart.

   Normal sympathetic discharge occurs at the rate of 1 impulse/sec in recumbent position and increases to 2-3 impulses/sec in the upright position.
3. ***Factors affecting muscle vascular sympathetic vasoconstriction:***
   (i) *Abolished by* (a) Lumbar sympathectomy, (b) rise in body temperature which causes an inhibition of the normal bulbar vasomotor drive to the thoracolumbar sympathetic neurons. Both these factors increase the muscle blood flow.
   (ii) *Increased after* (a) haemorrhage; and (b) hypotension. Therefore, these conditions are associated with marked decrease in muscle blood flow.

   The maximal reduction of muscle blood flow with sympathetic activity can be to 15% of its normal value. Thus muscle blood flow decreases to 0.3-0.5 mL/min/100 gm.
4. ***Sympathetic vasodilator nerves*** (page 332), dilate the arterioles only (*not* the pre-capillary sphincters), therefore
   (i) vascular peripheral resistance of the muscle bed decreases; and
   (ii) bypassing the muscle capillaries they provide a thoroughfare channels across the venules.

Important Note

Sympathetic vasodilator nerves, prevent sudden rise in the systemic B.P. at the beginning of the exercise, otherwise increase in stroke volume and heart rate will produce a marked increase in B.P., unless the 'total peripheral resistance' is reduced.

The sympathetic vasodilator nerves are activated by cortico-hypothalamic-reticulo-spinal pathways (page 332) (which are quite separate from 'VMC'-thoracolumbar pathways). Therefore, these nerves are not influenced by medullary afferents (baroreceptor and chemoreceptor fibers). Thus, these afferents play no part in the changes of peripheral resistance. Sympathetic vasodilator excitation may increase the muscle blood flow from 3-4 mL/min/100 gm to 30 mL/min/100 gm.

Significance: mental stress; emotions; and stimulation of defence area in anterior hypothalamus are associated with muscle vasodilatation with somatomotor features of the alerting reaction; therefore, this system probably operates during emergencies.

5. ***Exercise hyperaemia***

   During exercise:
   (i) Muscle blood flow increases to 100 mL/min per 100 gm due to marked decrease of local vascular resistance following chemical effects. This produces dilatation of arterioles and pre-capillary sphincters;

increases surface area of capillary bed, which facilitates diffusion of O_2 and CO_2 from the active tissues.

(ii) Increase in systemic B.P. increases hydrostatic pressure in capillaries; which increases the fluid transfer from capillaries to the interstitium and finally plasma volume decreases (*haemoconcentration* of blood).

Exercise hyperaemia is due to:

(i) hyperosmolality of the interstitial fluid which:
  (a) inhibits myogenic pacemaker activity, and
  (b) relaxation of vascular smooth muscle cells;
(ii) rise of $[K^+]$ in the neighbourhood of the pre-capillary sphincters causes their relaxation;
(iii) O_2 lack (hypoxia);
(iv) increase in tissue pCO_2;
(v) increase temperature in active muscle.
(vi) fall in blood pH.

6. *Mechanical interference of muscle contractions*
   (i) With intense phasic muscle contraction, arterial blood flow decreases or may even stop.
   (ii) During relaxation period:
     (a) muscle blood flow increases, and
     (b) myoglobin acts as an O_2 acceptor and it yields its O_2 to the myofibrils during the subsequent muscle contraction period.
   (iii) If sustained contraction of muscle occurs for more than 10 sec, myoglobin supply of O_2 is exhausted and anaerobic metabolites accumulate causing fatigue and ischaemic pain.
7. *Reactive hyperaemia*
   Occlusion of arterial blood supply to a limb for 5 min.; then on releasing the blood supply, the limb shows *cutaneous hyperaemia* associated with *muscle hyperaemia* (*i.e.* muscle blood flow may increase to 35-40 mL/100 gm/min) due to:
   (i) local accumulation of metabolites, which causes dilatation of arterioles and pre-capillary sphincters;
   (ii) increased release of nitric oxide (page 322) secondary to local effect of hypoxia (*proof:* reactive hyperaemia is prevented if circulation of limb is occluded in an atmosphere of 100% O_2).

   Since these metabolites are quickly dissipated by the flushing effect of the increased blood flow, therefore, the reactive hyperaemia is of short duration.
8. *White muscles versus red muscles* and their blood supply (also see to page 171).

   A. White muscles

   They constitute 3/4th of the total muscle mass and show rapid phasic contractions. They have a resting blood flow of 3 mL/ min/100 gm, which may increase to 60-70 mL/min/100 gm in maximal exercise. These muscles are prone to ***O_2 debt*** (page 482).

   B. Red muscles

   They comprise 1/4th of the total muscle mass and are concerned with maintenance of posture. Their activity is of the steady prolonged type which requires a relatively low O_2 usage. Their resting blood flow is 20-30 mL/min/100 gm due to low basal myogenic tone. They have vascular bed 3 times the size of white muscles with high flow capacity, approx. 150 mL/min/100 gm. Because of greater surface area of the capillary bed and their lower O_2 requirement, these muscles are unlikely to be exposed to O_2 *debt*.

Study Questions

1. **Give normal value of skeletal muscle blood flow at rest and during severe exercise.**
2. **Write briefly about:**
   (i) Factors affecting skeletal muscle blood flow (ii) Exercise hyperaemia (iii) Reactive hyperaemia
3. **Give physiological significance of:**
   (i) White and red muscles
   (ii) Sympathetic constrictor and dilator innervation to skeletal muscle blood vessels.

MCQs

1. **White muscles are prone to oxygen debt because:**
   (a) They constitute the 3/4th of the total muscle mass (b) They are concerned with maintenance of body posture
   (c) Their resting blood flow is low being 3 mL/100 g/min (d) Their O_2 requirement is low

Answers

1. (c)

SPLANCHNIC CIRCULATION

Splanchnic circulation means drainage of blood from the liver, spleen, GIT and pancreas. The blood from spleen, GIT and pancreas drains via portal veins to the liver (***portal circulation***) and from the liver via hepatic veins to the inferior vena cava (IVC).

At rest splanchnic circulation is 1500 mL/min. It passes through liver, which receives 1200 mL/min (80%) of its blood from the portal veins; and remaining 300 mL/min (20%) from the hepatic artery.

INTESTINAL CIRCULATION

Characteristic features

1. The intestine receives blood via superior and inferior mesenteric arteries **(Fig. 43.13)**. The mucosal blood flow is much greater compared to the smooth muscle wall both at rest and after meals. (See to **table** below)
2. *Capillary filtration coefficient* (page 356) for mucosal capillaries is ten times than that for skeletal muscles, that is why the mucosal vessels have an enormous capillary surface available for absorption and pore bound secretion.
   During metabolic activity blood flow increases due to:
   (i) vagal activity
   (ii) humoral activity
   (iii) local release of bradykinin from mucosal glands; and
   (iv) local release of metabolites in GIT itself.
3. There is *counter current system* (page 543) of villous blood vessels in the small intestine, therefore,

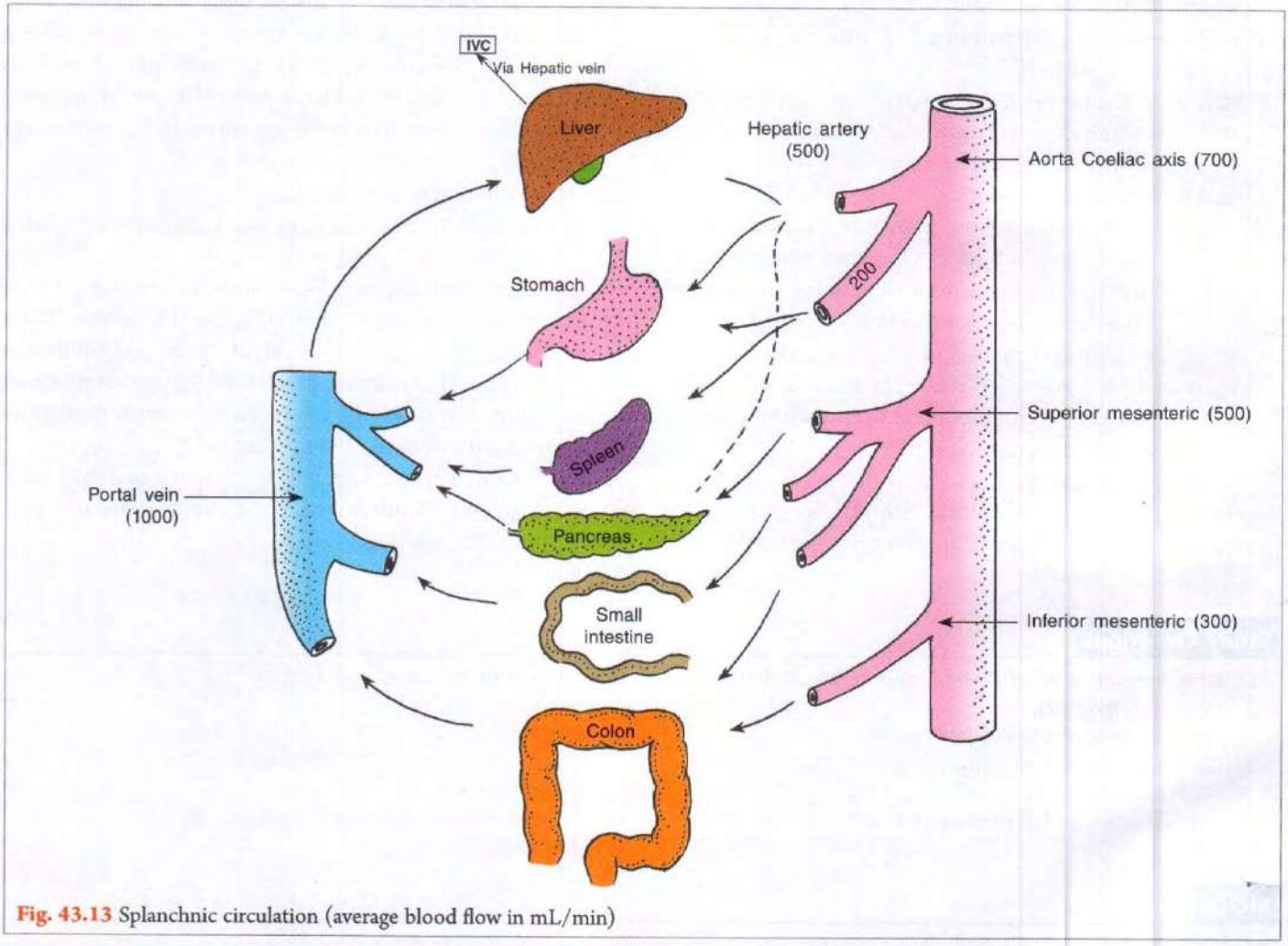


Fig. 43.13 Splanchnic circulation (average blood flow in mL/min)

	Blood flow at rest	Blood flow during maximum vasodilatation*
1. inner mucosa	50-60 mL/100 gm/min	300-400 mL/100 gm/min
2. outer smooth muscular wall	10 mL/100 gm/min	40 mL/100 gm/min

*(viz. due to increased metabolic activity seen after meals)

(i) Lipid soluble substances when carried in the *venous descending limb* of the vascular hairpin loop pass by diffusion across into the *ascending arterial limb* because of concentration gradient. As a result high concentration of absorbed substances are reached in the outer parts of the villi and then these substances leave relatively slowly via the venous drainage. That is how the counter current system of villous blood vessels slows down the entrance of rapidly absorbed solutes into the blood.

(ii) O_2 from the *ascending arterial limb* of the villi, diffuses across into the *venous descending limb.*

Thus, pO_2 at the tip of the villi is lower than that at their base.

4. GIT is *richly innervated* by sympathetic nerve fibers which are tonically active but provide only a moderate degree of splanchnic vascular resistance, therefore, sympathectomy causes only 25% increase in regional blood flow.

   Splanchnic nerve stimulation leads to ***double*** rise in systemic B.P.

   (i) 1st rise is immediate, due to vasoconstriction caused by stimulation of splanchnic nerves (***resistance effect***); and

   (ii) 2nd rise is due to liberation of epinephrine from adrenal medulla. It has to reach the heart through the blood flow before it can stimulate and cause rise in BP (***capacity effect***).

5. The intestinal circulation is capable of extensive ***autoregulation.***

HEPATIC CIRCULATION

Characteristic features

1. As stated above, liver receives 80% of its blood from the portal vein and remaining 20% from the hepatic artery.
2. Hepatic blood vessels are innervated by vasoconstrictor sympathetic nerves, therefore, fall in arterial B.P. stimulates sympathetic vasoconstrictor nerves to cause constriciton of hepatic as well as mesenteric arterioles and venules. As a result capacitance vessels constrict and mobilize the blood towards the heart. The contraction of capacitance vessels in splanchnic circulation can pump approx. 1L of blood into the arterial circulation in less than 1 minute, called *Reservoir Function of the Splanchnic Circulation*.

Important Note

In severe shock, hepatic blood flow gets reduced markedly and may produce patchy necrosis of the liver.

3. The mean pressure in the hepatic artery branches that converge on the hepatic sinusoids is 90 mmHg. Both arterial and hepatic blood flow join to produce a sinusoidal pressure called ***Hepatic Venous Pressure*** of 6-8 mmHg, showing there is a marked pressure drop along the hepatic arterioles. This pressure drop is adjusted so that *there is inverse relationship between hepatic arterial and portal venous blood flow* **(Table 43.9)**. This is brought about by different mechanisms:

   (i) by vasoconstrictor sympathetic nerve fiber innervation to sphincters in hepatic arterial system;

   (ii) by basal myogenic tone of vascular smooth muscles; and

   (iii) by production of vasodilator metabolites in the liver when its blood flow is decreased.

Table 43.9: Differences between hepatic artery and hepatic portal vein blood flow (Also refer to page 237)

Hepatic Artery Blood Flow	Hepatic Portal Vein Blood Flow
1. It contributes 20% of the total liver blood flow *i.e.* 300 mL/min.	1. It contributes 80% of the total liver blood flow, *i.e.* 1200 mL/min.
2. Blood flows with a pressure head of 100 mgHg, therefore, pre-capillary resistance in arterial system is high.	2. Blood flows with a pressure head of 7-12 mmHg (portal venous pressure), therefore, pre-capillary resistance in portal system is feeble.
3. Shows *autoregulation* of blood flow which is due to: (i) myogenic arterial tone, and (ii) balance between local vasodilator metabolites and myogenic arterial tone.	3. It does not show autoregulation of blood flow.
4. Maximum arterial dilatation increases the blood flow to more than 100 mL/100 gm/min.	4. It increases after meals due to functional hyperaemia in the GIT.
5. It usually increases when portal blood flow decreases, thus keeping the total hepatic blood flow constant.	5. No such phenomenon is seen

adjacent to, but not in the smallest pulmonary arterioles) produces an extraordinary tachypnoea (rapid, shallow respiration).
These nerve endings get stimulated by:
(a) multiple microemboli in the pulmonary small vessels; and
(b) intravenous injection of phenyl diguanide or 5 HT (serotonin).

12. ***Chemical effects on the lung vasculature***
(i) **Acute hypoxia**
(a) stimulates systemic chemoreceptors producing reflex sympathetic stimulation which leads to pulmonary vasoconstriction (reflex effect)
(b) direct action causes persistent pulmonary vasoconstriction.
(ii) **Chronic hypoxia** causes marked increase in pulmonary arterial pressure ***(pulmonary hypertension)***; later this results in right ventricular hypertrophy, right heart failure and pulmonary oedema.
That is why:
(a) thick pulmonary pre-capillary vessels develop in high altitude dwellers;
(b) children born and raised at high altitude show pulmonary hypertension.
(iii) **Acute hypercapnia:** Accumulation of CO_2 leads to a drop in pH, acidosis of any type produces pulmonary vasoconstriction.

13. ***Functions of pulmonary circulation*** – refer to page 404.

Study Questions

1. **Write briefly about:**
(i) Distensible low pressure system (ii) Pulmonary hypertension (iii) Characteristic features of pulmonary circulation
2. **Give physiological basis of pulmonary oedema.**
3. **How are lung alveoli kept dry?**
4. **Why does the right ventricle work less than the left ventricle?**

MCQs

1. **In pulmonary vascular bed:**
(a) Vascular resistance is approx. 3/4th of that in systemic circuit
(b) The flow per minute is much less than that in systemic circuit
(c) Approx. half of blood volume is accommodated
(d) Flow may increase several folds with little change in mean pulmonary artery pressure

2. **Difference of pulmonary microcirculation from systemic one is:**
(a) Resistance low, pulsatile flow
(b) Resistance low, capillary pressure low
(c) Capillary pressure high, pulsatile flow
(d) Resistance high, capillary pressure high

3. **Pulmonary wedge pressure corresponds to:**
(a) Right atrial pressure
(b) Right ventricular pressure
(c) Left atrial pressure
(d) Left ventricular pressure

4. **What keeps the alveoli dry?**
(a) Low pulmonary capillary pressure
(b) High pulmonary arteriolar pressure
(c) High plasma colloidal osmotic pressure
(d) All of the above

5. **Normal systolic pressure in pulmonary artery is:**
(a) 0-10 mmHg
(b) 10-20 mmHg
(c) 20-25 mmHg
(d) 90-120 mmHg

6. **Pulmonary arterioles are constricted by the following *except*:**
(a) Norepinephrine
(b) Epinephrine
(c) Angiotensin II
(d) Acetylcholine

Answers

1. (d) 2. (b) 3. (c) 4. (d) 5. (c) 6. (d)

Cardio-vascular Homeostasis i Health and Disease

Chapter 44

I. Regulation of blood volume
II. Compensations for gravitational effects
III. shock and syncope
IV. Heart failure
V. High blood pressure (hypertension)

REGULATION OF BLOOD VOLUME

Refer to Excretory System Unit (page 557).

COMPENSATIONS FOR GRAVITATIONAL EFFECTS

A. EFFECT OF RISING FROM THE SUPINE TO THE UPRIGHT POSITION

1. Changes which occur on assuming standing position from the supine are:

	Head	Feet
Mean arterial pressure	60-75 mmHg	180-200 mmHg
Venous pressure	zero	85-90 mmHg

(Also to see Table 43.7 page 366)

2. Venous pooling of blood in dependent parts (lower extremities) decreases stroke volume upto 40%; this decreases the cerebral blood flow, if decreases below 60% of flow in the lying position, signs and symptoms of cerebral ischaemia starts appearing. If compensatory mechanism fails consciousness may be lost.
3. *Compensatory changes*
   (i) The major compensatory changes are due to decrease in arterial B.P.
      (a) via baroreceptor mechanism produces tachycardia and vasoconstriction, this helps to maintain cardiac output;
      (b) prompt increase in circulating levels of renin and aldosterone; and
      (c) arteriolar constriction which helps to maintain normal B.P.
   (ii) Compensatory changes in cerebral circulation:
      (a) the arterial B.P. at head level decreases by 20-30 mmHg but jugular venous pressure decreases by 5-8 mmHg; therefore, drop in perfusion pressure (arterial minus venous pressure) gets reduced;
      (b) Intracranial pressure falls as venous pressure falls, therefore, cerebral vascular resistance is reduced; thus decreasing the pressure on cerebral blood vessels;
      (c) decrease in cerebral blood flow increases pCO_2 and decreases pO_2 and pH in brain tissue to produce cerebral vasodilatation; because of these autoregulatory mechanisms, cerebral blood flow decreases only 20% on standing; moreover,
      (d) amount of O_2 extraction from each unit of blood increases, therefore, cerebral O_2 consumption remains the same in the supine and the upright position.

B. EFFECTS DURING PROLONGED QUIET STANDING

This situation is met with military or police personnel standing at attention for long periods. This produces additional effect of increase in interstitial fluid volume in lower extremities which may cause fainting; falling to the horizontal position promptly restores venous return, cardiac output and cerebral blood flow to normal.

C. POSTURAL HYPOTENSION or ORTHOSTATIC HYPOTENSION

Definition

In some individuals, sudden standing causes decrease in systemic B.P., dizziness, dimness of vision and even fainting.

Causes

1. After surgical sympathectomy.
2. Patients receiving sympatholytic drugs.
3. In patients having diabetes mellitus or syphilis which damages sympathetic nervous system.
4. *Autonomic insufficiency* secondary to:
   (i) defect in the CNS with normal resting plasma nor-epinephrine levels which fail to rise with standing;
   (ii) defect in peripheral nerves and resting nor-epinephrine values are low with little or no response to baroreceptor stimulation.
5. Patients with primary hyperaldosteronism who show abnormal baroreceptor reflexes; but these patients generally do not have postural hypotension, because their blood volumes are expanded sufficiently to maintain cardiac output inspite of changes in position.

D. EFFECT OF GRAVITY (ACCELERATION AND DECELERATION) ON CVS

Forces acting on the body as a result of acceleration are expressed in *'g' units*, one 'g' being the force of gravity on the earth's surface.

'Positive' g is force due to acceleration acting in the long axis of the body, from head to foot. For example, acceleration in vehicles or elevators or rockets.

'Negative' g is the force due to acceleration acting in the opposite direction, either deceleration back into earth's atmosphere or deceleration in vehicles.

Effects of Positive 'g'

During exposure to *positive 'g'* for first few seconds blood is thrown into the lower part of the body, therefore, venous return to heart decreases resulting in fall in cardiac output and systolic B.P. Diastolic B.P. also decreases due to passive vasodilation. However, recovery occurs within another 10 to 15 seconds by activation of baroreceptor reflexes.

Notes

1. The cerebral circulation is protected by the fall in venous pressure and intracranial pressure; (page 366)
2. Cardiac output is maintained for some time, because,
   (i) blood is drawn from the pulmonary venous reservoirs; and
   (ii) the force of cardiac contraction is increased

Important Note

Acceleration producing more than 5 'g' produces *Black Out (vision failure)* in approx. 5 sec and finally leads to unconsciousness. Complete closure of peripheral retinal vessels occurs when perfusion pressure in these vessels falls below the intraocular pressure. This results in Black out.

Treatment

Use of ***antigravity 'g' suits*** *i.e.* double-walled pressure suits containing water or compressed air and regulated in such a way that they compress the abdomen and legs with a force proportionate to the positive 'g'. This decreases venous pooling and helps maintain venous return.

Effects of negative 'g'

1. Increase in cardiac output.
2. Increases cerebral arterial pressure, but cerebral vessels do not rupture because of corresponding increase in intracranial pressure. (page 366)
3. Intense congestion of the head and neck vessels and ecchymoses around the eyes because the eyes are not protected by the cranium. As a result the eyes become temporarily blind (***Red out***).
4. Severe throbbing head pain and finally produces mental confusion.

E. EFFECTS OF ZERO GRAVITY ON THE CVS: WEIGHTLESSNESS

This situation is met when an individual goes out of gravitational effect of the earth into space. For example, orbital flight to planets. 'Zero' gravity produces ***weightlessness***. Weightlessness upto one year produces only transient adverse effects on CVS, which is *characterized by*:

1. Transient postural hypotension.
2. Disuse muscular atrophy produces flaccidity and loss of muscle mass.
3. Some myocardial atrophy.
4. *Space motion sickness* secondary to unfamiliar pattern of motion signals.
5. Loss of plasma volume due to headward shift of body fluids with subsequent diuresis. (This is because of failure of gravity to cause hydrostatic pressure);
6. Steady loss of bone mineral with increased calcium excretion;
7. Loss of red cell mass and decrease in plasma lymphocytes.

All these defects disappears completely in 4-6 weeks time.

Note

Similar effects also occur in persons who lie in bed for prolonged period of time. (Also refer to page 704)

SHOCK AND SYNCOPE

Definition

'Shock' is a clinical syndrome characterized by impairment of adequate tissue perfusion primarily with ***low cardiac output*** (or acute circulatory failure).

Types of Shock

The various types of shock as classified on the basis of the causes are given the in **Table 44.1**.

The cardinal feature in all types of shock is low cardiac output.

HYPOVOLEMIC SHOCK

It is also called ***cold shock***.

Characteristic features

1. Hypotension,
2. Rapid and thready pulse,
3. Rapid, shallow breathing,
4. Cold, pale and moist (clammy) skin with greyish tinge (if associated with cyanosis),
5. Intense thirst,
6. Usually patients are 'restless' or alternately some are 'lethargic' (mentally dull with decreased sensibility) because of cerebral ischaemia and acidosis,
7. Prone to vomiting.

Hypovolemic shock is *subdivided into* different categories on the basis of causes like:

A. Haemorrhagic shock
B. Traumatic shock
C. Surgical shock
D. Dehydration shock.

A. Haemorrhagic Shock

It is a major form of hypovolemic shock. The effects of haemorrhage depend on the:

(i) amount and rapidity of blood loss, and
(ii) the efficiency of the compensatory power of the subject.

Therefore,

(1) If the haemorrhage is ***mild to moderate*** (5–15 mL/kg body weight, *i.e.* loss upto 10% to 30% of total blood volume) and subject is healthy, compensatory changes take place and normal condition is restored.
(2) If the haemorrhage is ***severe*** (30 mL/kg body weight, *i.e.* loss upto 40% of total blood volume), it may lead to circulatory collapse and death.

Mechanism of development of shock

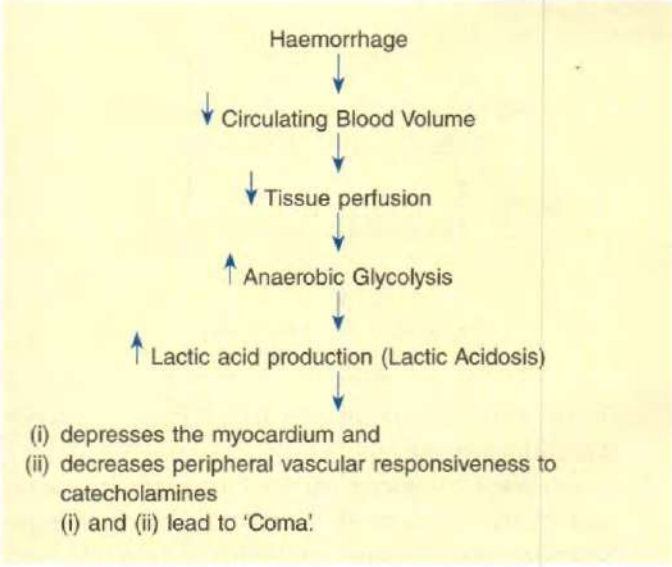


Table 44.1: Classification and major causes of various types of shock

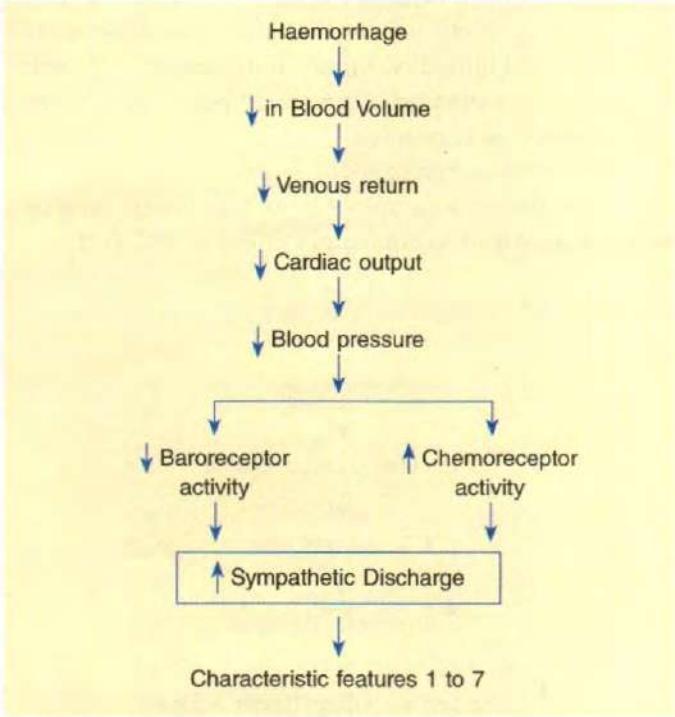
Classification	Definition (How low cadiac output: 'CO' develops?)	Causes/subdivisions
I. Hypovolemic Shock or Cold Shock	Amount of fluid in the vascular system is inadequate to fill it, resulting in decrease in circulating blood volume.	A. Haemorrhage → Haemorrhagic shock B. Trauma → traumatic shock C. Surgery → surgical shock D. Dehydration → dehydration shock
II. Distributive or Vasogenic or Low-Resistance Shock or Warm Shock	Size of capacitance vessels is increased by vasodilatation → ↓ 'CO' inspite of normal blood volume.	A. Fainting (syncope) → neurogenic shock B. Anaphylaxis → anaphylatic shock C. Sepsis → septic shock
III. Cardiogenic Shock	Inadequate pumping action of the heart as a result of myocardial abnormalities.	A. Myocardial infarction B. Congestive heart failure C. Arrhythmias
IV. Obstructive Shock	Obstruction to blood flow in the lungs or heart.	A. Tension pneumothorax B. Pulmonary embolism C. Cardiac tumour D. Cardiac tamponade

Pathophysiology of haemorrhagic shock

The sequence of events which follow haemorrhage are divided into two stages, viz.,

I. Rapid compensatory reactions, and
II. Long-term compensatory reactions.

I. RAPID COMPENSATORY REACTIONS: Sequence of Events



1. Tachycardia; this along with hypotension produces ***rapid thready pulse***.
2. Generalised vasoconstriction (except in brain and heart). It is most marked in kidneys, skin, subcutaneous tissue, pulmonary circuit and spleen.
3. Generalised venoconstriction.
4. Stimulates adrenal medulla to cause increase release of catecholamines.
5. Stimulates reticular activating system which produces restlessness, apprehension and irritability.
6. Stimulates respiratory centre → ***rapid shallow breathing***.
   2, 3, 4 and 5 all help to increase 'venous return' to the heart and eventually cardiac output increases.
7. Renal vasoconstriction results in:
   (i) Renal ischaemia with release of angiotensin II, which acts on subfornical organ to produce ***intense thirst*** (page 373).
   (ii) constriction of afferent arterioles → slight decrease in 'GFR'
   (iii) Constriction of efferent arterioles (to greater extent) produces fall in 'RPF'

   (ii) and (iii) → ↑ Filtration Fraction, which produces:
   (a) 'oliguria'
   (b) sodium retention
   (c) retention of nitrogenous substances in blood → Uremia. (page 538)
   (d) later on, severe renal tubular damage (***acute renal failure***) → loss of ability to concentrate the urine.

Important Note

A great majority of compensatory reactions are mainly due to increased Sympathetic Discharge in response to haemorrhage. Therefore,

1. Reflex warming of a shock patient either with water bottles or providing warm environment will abolish this sympathetic discharge and patient may die.
2. Sympathectomized animals cannot tolerate a blood loss of >30% and may die.

II. LONG-TERM COMPENSATORY REACTIONS. These include:

1. Restoration of Plasma Volume within 12-72 hours.

Mechanism

(i) By mobilization of tissue fluids. How? Haemorrhage produces:
   (a) ↓ blood volume → ↓ venous pressure.
   (b) ↑ sympathetic discharge → arteriolar constriction.

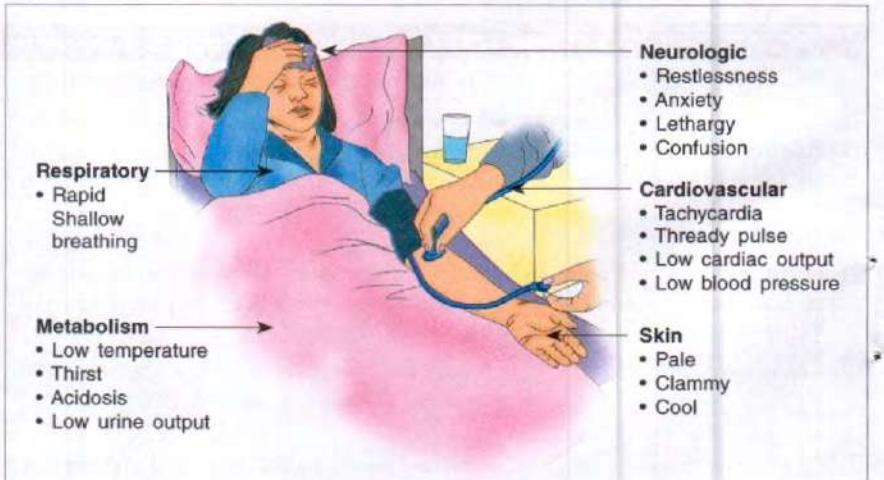


Fig. 44.1 Shock patient: characteristic features

(a) and (b) → ↓ capillary pressure → ↑ uptake of tissue fluids.

(ii) By retention of water and electrolytes by kidney. How? Refer to Fig. 44.2.

2. Restoration of Plasma Proteins over a period of 3-4 days.

Mechanism: Haemorrhage → Activate Liver to cause:

(i) ↑ protein synthesis

(ii) ↑ release of preformed proteins

3. Restoration of RBC mass in 4-8 weeks.

Mechanism: Haemorrhage → Hypoxia →

(i) ↑ erythropoietinogen synthesis by liver

(ii) ↑ renal erythropoietic factor (REF) release by kidneys

(i) and (ii) → ↑ erythropoietin production (peak on 10th day).

4. Restoration of B.P. over several months via kidneys' long-term regulatory mechanisms (page 352).

B. Traumatic Shock

It occurs due to injury causing 'severe' damage to muscle and bone.

Features

1. Frank bleeding into injured areas results in shock.

Note

The thigh muscles can accommodate 1 litre of extruded blood with an increase in only 1 cm of thigh diameter.

2. If there is extensive soft tissue and muscle crushing (called ***Crush Syndrome***), *myoglobin* leaks into circulation; it gets precipitated into renal tubules and clogs them resulting in renal damage.

C. Surgical Shock

This occurs as a result of external or internal blood loss caused by ruptured blood vessels during surgical procedures.

D. Dehydration Shock

It occurs as a result of fluid loss from:

(1) GIT due to prolonged vomiting or diarrhoea

(2) Kidneys due to:

(i) diabetes mellitus,

(ii) diabetes insipidus,

(iii) diuretic overdose,

(iv) adrenal insufficiency.

(3) Skin due to 'burns', heat stress (fever, exposure to heat) results in sweating.

Shock due to burns is characterised with ***haemoconcentration***. How? Burn causes:

1. Loss of protein rich fluid through the capillary wall.
2. Accumulation of cellular damage products in interstitial space.

1 and 2, increase osmotic pressure of the tissue fluid → rapid loss of fluid from circulation → *haemoconcentration*.

DISTRIBUTIVE SHOCK or VASOGENIC SHOCK or LOW RESISTANCE SHOCK

It is also called ***Warm Shock*** because skin is warm and *not* cold and moist as it is in hypovolemic shock.

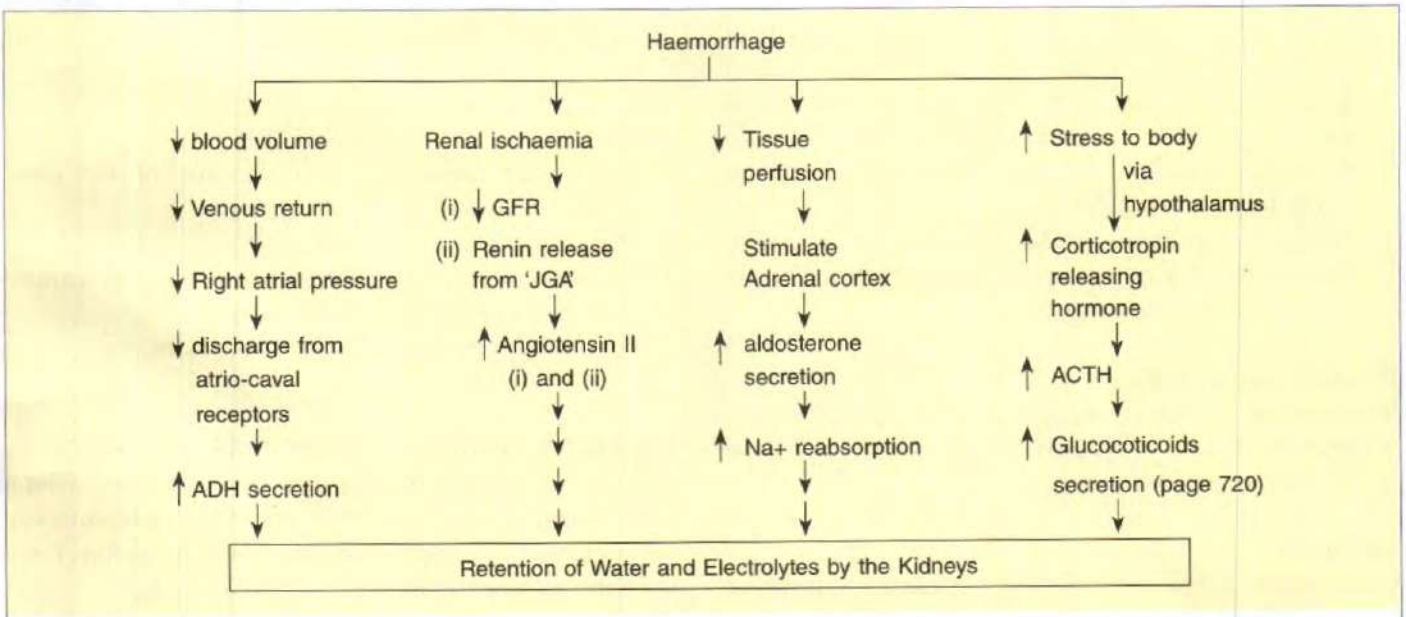


Fig. 44.2 Sequence of events involved to restore plasma volume following the haemorrhage
(↑ : increase; ↓ : decrease; JGA: Juxtaglomerular apparatus, page 504)

It includes:
A. Fainting or Syncope
B. Anaphylactic Shock
C. Septic Shock

A. Fainting or Syncope

It is sudden transient loss of consciousness due to decrease in cerebral blood flow. It is also called *Neurogenic Shock*. It is often benign and is most commonly associated with abrupt vasodilatation. This produces hypotension and generally associated with bradycardia. The attacks are short-lived and consciousness is restored in a few minutes.

Types

1. *Vaso-vagal syncope:* Here strong emotions such as overwhelming fear or grief causes activation of sympathetic vasodilator system producing fall in vascular resistance in skeletal muscles → marked decrease in peripheral resistance.
2. *Postural Syncope* is fainting due to pooling of blood in the dependent parts of the body on standing.
3. *Micturition Syncope* is fainting during urination, occurs in patients with *orthostatic hypotension* (page 396). It is due to combination of orthostatic and reflex bradycardia induced by emptying of urinary bladder in these patients.
4. *Carotid Sinus Syncope* is due to pressure on carotid sinus produced by tight collar →marked bradycardia and hypotension.
5. *Cough Syncope* occurs when the increase in intrathoracic pressure during straining or coughing is sufficient to block venous return.
6. *Effort Syncope* is fainting on exertion as a result of inability to increase cardiac output to meet the increased demands of the tissues. It is specifically common in patients with aortic or pulmonary stenosis.
7. *Deglutition Syncope* (rare) bradycardia and vasodilation occurs by swallowing.
8. *Neurocardiogenic Syncope i.e.* syncope of cardiac origin; for example, due to extreme bradycardia, heart block, massive heart attack etc.

B. Anaphylactic Shock

It is a rapidly developing severe allergic reaction. It occurs when an individual who has previously been sensitive to an antigen is re-exposed to it.

Mechanism

Generalized *antigen-antibody reaction* causes release of large quantities of histamine and other substances to produce:

(i) ↑ capillary permeability → ↓ blood volume; and
(ii) generalised arteriolar dilatation → ↓peripheral resistance.

(i) and (ii) lead to fall in BP (hypotension).

C. Septic Shock

Certain infections *e.g.* strangulated bowel, perforated duodenum → release of bacterial ***endotoxin*** by gram negative bacteria which get absorbed into systemic circulation. It results in

1. high fever,
2. peripheral arteriolar paralysis → marked vasodilatation,
3. depresses myocardium, and
4. increase capillary permeability → plasma leaks into the tissues → fall in B.P.

CARDIOGENIC SHOCK

It is caused when pumping action of the heart is inadequate, therefore, heart fails to pump out all venous return →

1. Marked decrease in cardiac output → shock,
2. Congestion of the lungs and viscera, that is why it is also called ***Congested Shock***.

Predisposing factors

1. Myocardial infarction → release of certain chemicals via Bezold-Jarisch reflex (page 331) → apnoea, marked bradycardia and hypotension. This makes the shock worse.
2. Congestive cardiac failure (CCF).
3. Arrhythmias.

OBSTRUCTIVE SHOCK

It occurs when cardiac output is decreased as a result of mechanical obstruction of left or right ventricular filling.

Causes

1. Tension pneumothorax with kinking of the great veins.
2. Massive pulmonary emboli.
3. *Cardiac tamponade i.e.* bleeding into the pericardium with external pressure on the heart.
4. Post end-expiratory pressure respiration.

REFRACTORY SHOCK or IRREVERSIBLE SHOCK

Definition: Persistent decrease in 'cardiac output' which fails to come back to normal either by compensatory mechanisms or by appropriate treatment. Therefore, there is no longer any response to:

(1) Vasopressor drugs,
(2) Fluid replacement, and
(3) Blood transfusion.

These measures can restore circulating blood volume to normal but cardiac output remains low. The condition is not only unique to haemorrhagic shock but occurs in other types of shock as well.

It was previously used to be called as ***irreversible shock***. However, with better understanding of pathophysiological mechanisms and improved treatment many patients can be saved. Therefore, ***refractory shock*** is more appropriate term.

Mechanism of Development

'Refractory shock' is the manifestation of late effects of sympathetic vasoconstriction ⟶ long sustained decrease of regional circulation ⟶ operation of *'positive' feed back mechanisms* (for details, refer Fig. 44.3).

Important Note

A late complication of shock is *acute respiratory distress* syndrome (ARDS) caused by damage to capillary endothelilal cells and alveolar epithelial cell, with release of cytokines; eventually resulting in acute respiratory failure with high mortality rate.

HEART FAILURE

Definition

Failure of heart to maintain an adequate cardiac output ('CO') for normal tissue perfusion throughout the body.

PATHOGENESIS AND PATHOPHYSIOLOGY

Primarily two mechanisms are involved in the production of heart failure:

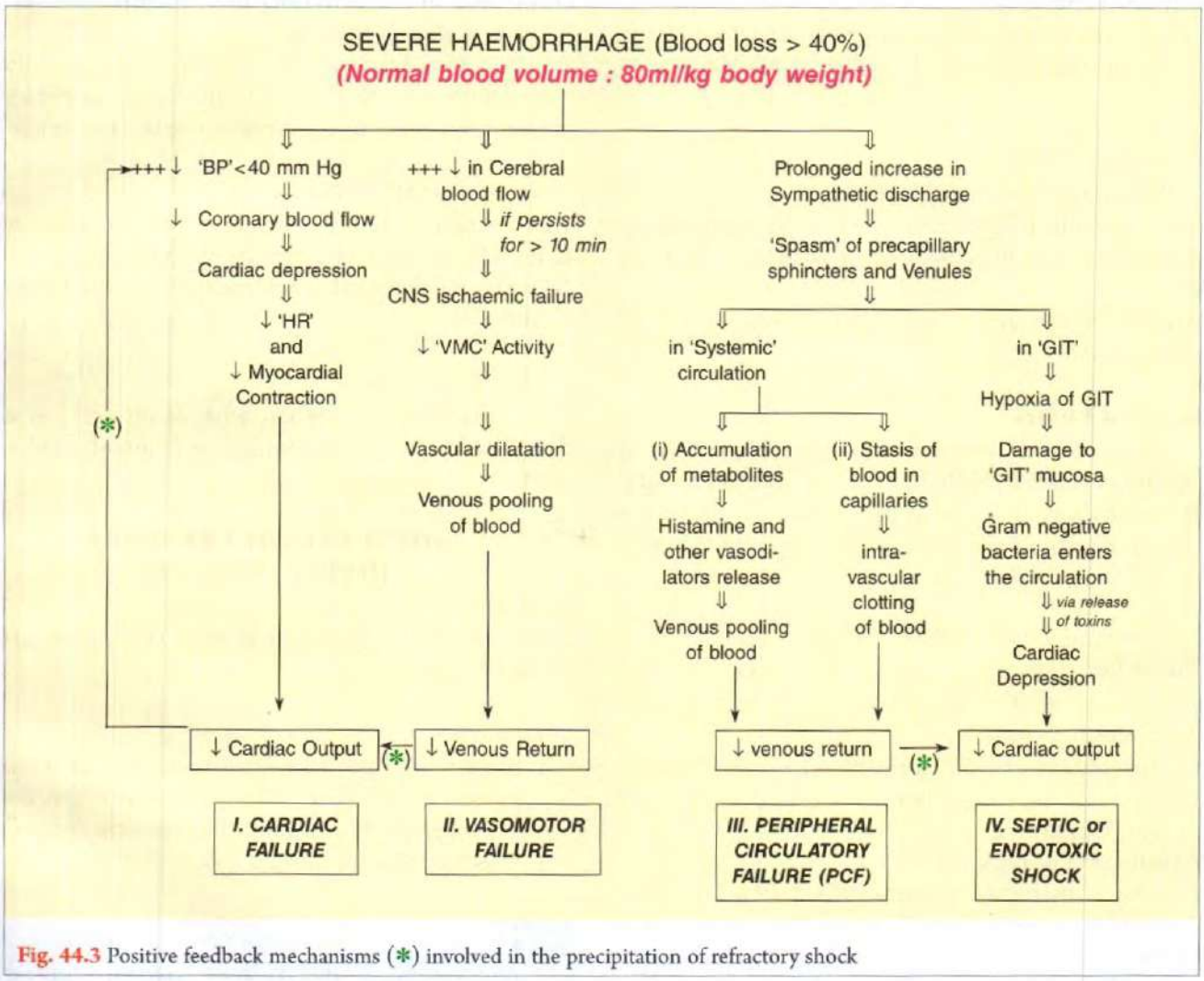


Fig. 44.3 Positive feedback mechanisms (*) involved in the precipitation of refractory shock

Important Note

I,II, III and IV are the main causes which precipitate 'refractory shock' and may lead to death.

(i) ***Systolic Heart Failure*** in which 'CO' is decreased due to poor ventricular contractility. Therefore, End-systolic ejection fraction *i.e.* percentage of blood ejected during systole decreases upto 20% (normal: 65%).

(ii) ***Diastolic Heart Failure*** in which 'CO' is decreased due to reduced ventricular filling.

Causes

Refer to **Table 44.1** (page 387).

Clinical Manifestations

Clinical manifestation of systolic and diastolic heart failure are popularly referred as *Forward Failure* and *Backward Failure* respectively.

A. Forward Failure

It is due to poor ventricular contractility, therefore, tissue perfusion throughout the body is grossly inadequate.

Signs and Symptoms

1. Generalised weakness.
2. Exertional or exercise intolerance.
3. Fall in systemic B.P. produces cerebral ischaemia which is associated with hypoxic symptoms on the CNS (page 459).
4. Increase in ventricular end diastolic volume (EDV) leads to *cardiomegaly*.

B. Backward Failure

It is due to reduced ventricular filling. This results in congestion in venous system with rise of venous pressure. Therefore, backward failure is also called as ***congestive cardiac (or heart) failure*** (CCF/CHF). It manifests in two forms:

1. Right ventricular failure (RVF)

Failure of 'RV' causes rise in systemic venous pressure. This produces signs and symptoms due to backward pressure which include:

(i) Transudation of fluid from blood vessels causing oedema of dependent parts (oedema over feet and sacral region).

(ii) Distension of neck veins.

(iii) Increase resistance to portal blood flow produces hepato-spleenomegaly (enlargement of liver and spleen).

2. Left ventricular failure (LVF)

Failure of 'LV' increases pulmonary venous pressure, which results in pulmonary venous distension and transudation of fluid into air spaces, called ***pulmonary oedema*** (also see to page 383). Pulmonary oedema is characterized by:

(i) *dyspnoea* (difficulty in breathing) on exertion,

(ii) *paroxysmal nocturnal dyspnoea (PND), i.e.* dyspnoeic attacks at night,

(iii) *orthopnoea i.e.* dyspnoea in lying down position (because when a person lies down the pulmonary blood volume increases by upto 400 mL - page 383),

(iv) *Frothy sputum* due to irritation of diaphragm.

COMPENSATORY MECHANISM

The various compensatory mechanisms which operate during heart failure are **(Fig. 44.4)**:

1. Frank Starling mechanism,
2. Baroreceptor reflex mechanism, and
3. Renal mechanism.

Limitations of compensatory mechanisms

1. Frank Starling Mechanism

Frank-Starling law will fail to operate in late stages of heart failure, due to 'cardiomegaly'; since benefit by this mechanism is offset by operation of Laplace Law (page 318).

2. Baroreceptor Mechanism

This mechanism fails to operate if MBP decreases below 40 mmHg due to decrease in coronary circulation, which decreases the myocardial contractility to cause further fall in MBP.

3. Renal Mechanism

This mechanism fails when renal failure sets in either due to severe renal ischaemia or accumulation of waste products in the kidneys.

HIGH BLOOD PRESSURE (HYPERTENSION)

Definition

Hypertension is a sustained increase of systemic arterial blood pressure.

Clinical types

I. *Primary or Essential hypertension:* It is of unknown causation *i.e.* cause of hypertension is not known. It is seen in 90% of total hypertensive individuals. It is treatable but not curable.

Note

In most persons, obesity and sedentary life style appear to play a major role in causing essential hypertension.

II. *Secondary hypertension:* It is due to some underlying cause.

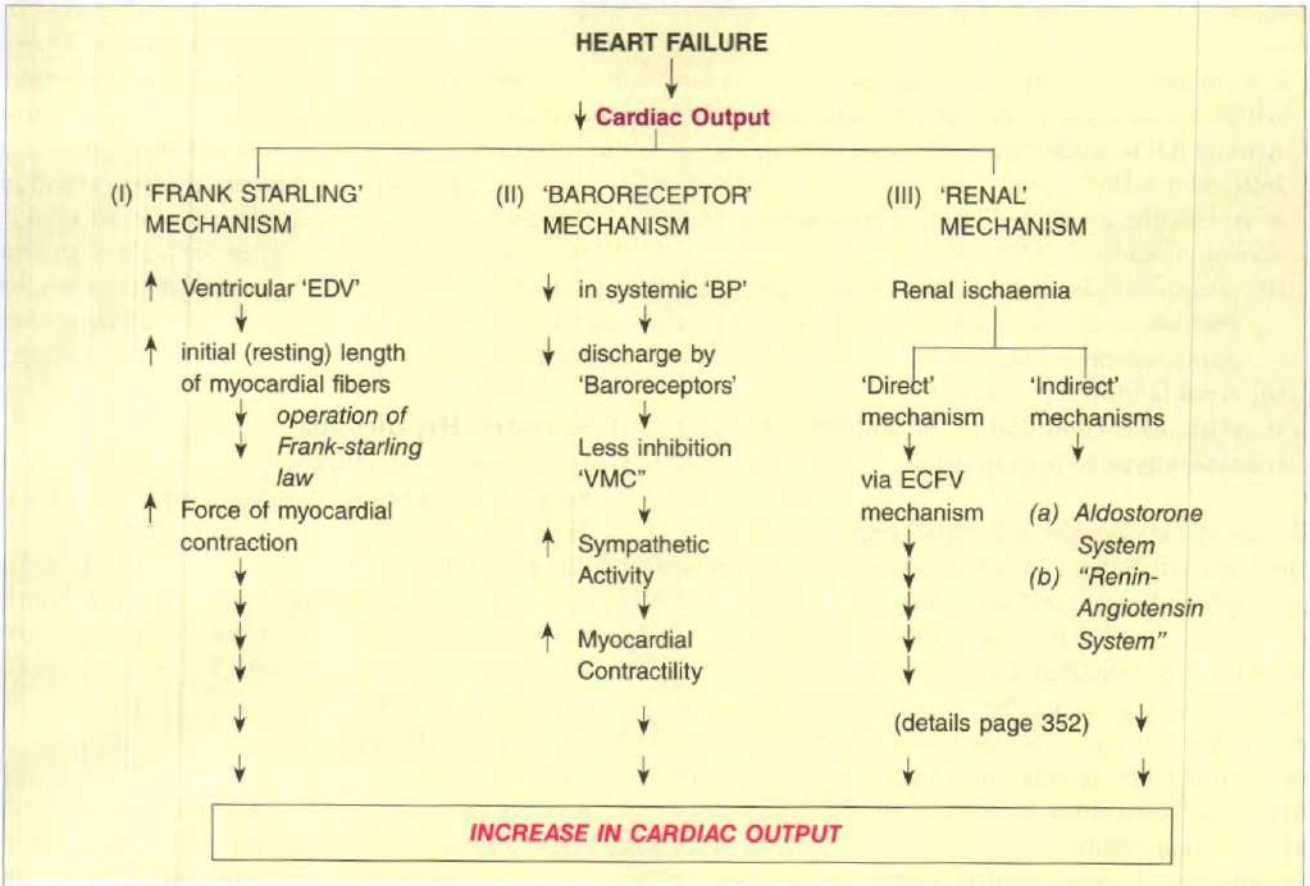


Fig. 44.4 Outline of compensatory mechanisms which operate during heart failure:

Note

In some of normal individuals, a transient rise in B.P. occurs in the doctor's chamber due to nervousness (*white coat hypertension*). However, such individuals have normal B.P. during their daytime activity.

I. Primary or Essential Hypertension

Definition

When arterial B.P. is persistently more than 150/90 mm Hg.

Forms

A. Benign type, and B. Malignant type

A. Benign Form of Essential Hypertension

It is seen in two stages: 'early' and 'late' stage.

1. In early stages

(i) Hypertension is moderate and increases upto 210/110 mmHg.

(ii) Specially 'SBP' fluctuates considerably; for example, it may be

(a) normal during sleep or emotional and physical rest, and

(b) increases markedly in stress states (cold, excitement, exercise, emotions, etc.).

Therefore, it is also referred as ***labile hypertension***. In this form, hypertension can continue indefinitely; but usually lasts for 10-15 years and then enters the late stage.

2. In late stages

(i) No fluctuation is seen with 'SBP' and hypertension becomes ***fixed*** in the abnormal higher range and cannot be reduced to normal by rest or sedatives.

(ii) It is associated with complications, for example:

(a) compensatory cardiac hypertrophy of either right or left ventricles.

(b) thickening of medial walls of small arteries and arterioles,

(c) myocardial infarction,

(d) renal changes *e.g.* increase in volume of night urine (nocturia); or albuminuria or mild haematuria.

(iii) If not treated, death occurs after few years due to:

(a) cardiac failure; or

(b) cerebro-vascular accidents (haemorrhage or thrombosis); or

(c) renal failure.

B. Malignant Form of Essential Hypertension

Features

1. It is called malignant form, because death occurs within 6 months to 2 years of its diagnosis.
2. Arterial B.P. is much higher, increases upto or above 260/150 mmHg.
3. It is usually associated with complications (see above); in addition, also shows:
   (i) peripheral vascular changes *e.g.* acute arteriolar necrosis specially in retinal vessels resulting in papilloedema; and
   (ii) renal failure (common).
4. It occurs as a complication of both essential and secondary type of hypertension.

Mechanism of Development of Essential Hypertension

Systemic arterial B.P. is a function of product of cardiac output (CO) and peripheral resistance (PR) *i.e.*

$$B.P. = CO \times PR$$

therefore, it is determined by 'CO' and 'PR'.

Since 'CO' is determined by heat rate (HR) and stroke volume (SV), whereas 'PR' by viscosity of blood and caliber of resistance vessels; therefore, hypertension can be produced due to either increase in any of these factors alone or in combination.

By definition, hypertension is sustained elevation of systemic arterial BP, but sustained increase in HR, SV and viscosity of blood usually *does not* occur in hypertensive individuals. The characteristic findings in these individuals being:

(1) cardiac output is normal;
(2) viscosity of blood is normal; and
(3) increased peripheral resistance (PR).

Increased 'PR' can be due to either sympathetic overactivity or influence of blood borne agents like angiotensin and catecholamines.

As persistent increase in their levels is not evident in hypertensive individuals, therefore, these may not be responsible for increased 'PR'.

Other causes of increased peripheral resistance

The pre-capillary resistance vessels specially arterioles offer a greater resistance to blood flow. It has been found that the increased arteriolar resistance in hypertensive individuals is mainly due to medial wall hypertrophy of the arterial system ***(arteriosclerosis)*** in response to long standing increase in pressure load. Various *predisposing factors* may be involved to cause arteriosclerosis:

1. Individuals are prone by reason of heredity.
2. Intermittent bouts of corticohypothalamic discharge; causes:
   (i) sympathetic vasoconstriction, and
   (ii) release of chemical agents like catecholamines, cortisol, ADH, etc.

As a result of these factors, arteriosclerosis in the resistance vessels increases arterial resistance. Therefore, these structural changes of arteries and arterioles will become widespread and decrease the distensibility of the vascular wall (generalised arteriosclerosis). That is why more pressure is required by the carotid sinus baroreceptors to discharge impulses so as to inhibit the 'VMC' (see Important Note, page 328). Thus, in chronic hypertensive individuals, *baroreceptor reflex mechanisms are* ***reset*** *at a higher pressure level to maintain an elevated rather than a normal B.P.*

II. Secondary Hypertension

Causes (in order of occurrence):

1. Renal diseases (most common cause).
2. Thyrotoxicosis.
3. Pill hypertension.
4. Adrenal medulla tumour – 'phaeochromocytoma'.
5. Adrenal cortex tumours *e.g.* Conn's syndrome; cushing syndrome.
6. Coarctation of aorta.
7. Severe polycythemia.

1. *Renal hypertension.* Kidney diseases like nephritis, cystic renal diseases; polynephritis, renal arterial stenosis etc. cause increased 'renin' release. This via renin-angiotensin system causes hypertension.

Important Note

Constriction of one renal artery causes a prompt increase in renin secretion and the development of sustained hypertension called *Goldblatt hypertension.*

2. *Thyrotoxicosis,* increases cardiac output to increase systolic and diastolic B.P.
3. *Pill hypertension.* Long term treatment with oral contraceptives containing oestrogen and progesterone produces hypertension by:
   (i) retention of fluid and electrolytes; and
   (ii) increased angiotensin II formation, specially by oestrogens.
4. *Tumour of adrenal medulla* – pheochromocytoma, increases nor-epinephrine release to produce hypertension.
5. *Adrenal cortex tumours*
   (i) Primary Aldosteronism (Conn's syndrome) is tumour of zona glomerulosa of adrenal cortex. Here hypertension is produced due to increased release of aldosterone which causes sodium and water retention.
   (ii) 'Cushing syndrome' following tumour of zona fasciculata and reticulosa of adrenal cortex causes increased glucocorticoid secretion. This produces hypertension due to salt retaining properties.

6. *Coarctation of Aorta.* A congenital narrowing of a segment of thoracic aorta, increases resistance to blood flow, thereby produces hypertension above the aortic constriction and hypotension below. Latter produces renal ischaemia which via renin-angiotensin system results in hypertension.
7. *Severe polycythemia* increases blood viscosity, which increases peripheral resistance to cause hypertension.

Study Questions

1. **Write short notes on:**
   (i) Orthostatic hypotension (ii) Weightlessness (iii) Salient features of shock
   (iv) Mechanism of development of shock (v) Pathophysiology of haemorrhagic shock (vi) Crush syndrome
2. **Give physiological basis of:**
   (i) Warm and cold shock (ii) Cardiogenic and obstructive shock (iii) Heart failure
   (iv) Pill hypertension (v) Congestive heart failure
   (vi) Orthopnoea and paroxysmal nocturnal dyspnoea
   (vii) Essential Hypertension (viii) Refractory shock (ix) Secondary hypertension
3. **Describe effect of positive and negative 'g' on CVS**
4. **Mention different types of syncope with one major feature of each type.**
5. **Classify severity of haemorrhage based on blood loss.**
6. **Why is reflex warming of a shock patient not advisable?**
7. **Define refractory shock. Briefly explain the positive feedback mechanisms involved in its precipitation.**
8. **Give various compensatory mechanisms which operate during heart failure. Give their limitations.**
9. **Mention mechanism of development of essential hypertension.**

MCQs

1. **Sudden standing can produce loss of circulating blood volume upto:**
   (a) 10% (b) 20% (c) 30% (d) 40%
2. **Sudden change of posture from supine to standing in a normal person, decreases cerebral blood flow by:**
   (a) 10% (b) 20% (c) 30% (d) No change
3. **'g' is the acceleration which a mass exhibits due to gravitational force. Numerically this value is:**
   (a) 760 cm/sec^2 (b) 760 mmHg (c) 981 cm/sec^2 (d) 380 cm/sec^2
4. **Weightlessness upto one year results in:**
   (a) Decrease in cardiac output (b) Hypotension
   (c) Decrease in gut motility (d) Osteoporosis
5. **Moderate haemorrhage is one in which blood loss is upto:**
   (a) 5-8 mL/kg body weight (b) 10-20 mL/kg body weight
   (c) 15-25 mL/kg body weight (d) Above 25 mL/kg body weight
6. **Loss of upto 10% of total blood volume over the period of 20 minutes causes:**
   (a) Decreased BP, normal venous pressure (VP) (b) Decreased BP, decreased VP
   (c) Normal BP, normal VP (d) Normal BP, decreased VP
7. **A great majority of compensatory reaction following haemorrhage are mainly due to:**
   (a) Retention of water and electrolytes by kidneys (b) Increased sympathetic discharge
   (c) Formation of angiotensin II (d) Release of vasopressin
8. **All are the causes of dehydration shock, *except*:**
   (a) Prolonged vomiting or diarrhoea (b) Diabetes mellitus
   (c) Burns (d) Perforated duodenal ulcer
9. **Warm shock is the term applied to the:**
   (a) Hypovolemic shock (b) Cardiogenic shock (c) Low resistance shock (d) Surgical shock
10. **Emotional fainting is associated with:**
    (a) Activation of cholinergic fibers originated in the spinal cord
    (b) Pooling of blood in the dependent parts of the body
    (c) Decreased myocardial contractility
    (d) Cardiac arrhythmias

11. **Which of the following statement is *false*?**
    (a) Obstructive shock may be caused by tension pneumothorax
    (b) Myocardial infarction predispose to congested shock
    (c) Reflex warming of a shock patient is helpful to maintain adequate tissue perfusion
    (d) Generalised antigen-antibody reaction produces anaphylactic shock
12. **All but one positive feedback mechanism involved in precipitation of refractory shock is:**
    (a) Cardiac failure (b) Vasomotor failure
    (c) Peripheral circulatory failure (d) Increased sympathetic discharge
13. **Which type of heart failure is most likely to be associated with pulmonary oedema?**
    (a) Heart failure resulting from an arteriovenous fistula (b) High cardiac output heart failure
    (c) Left heart failure without right heart failure (d) Right heart failure without left heart failure
14. **Limitations to the Frank-Starling Law to operate in late stages of heart failure is due to:**
    (a) Cardiomegaly (b) Fall in systemic mean blood pressure
    (c) Decreased myocardial contractility (d) Renal failure
15. **The commonest cause of sustained primary hypertension is:**
    (a) Unknown (b) Conn's syndrome
    (c) Renal disease (d) Phaeochromocytoma
16. **All the following predisposing factors may be responsible for the development of essential hypertension *except*:**
    (a) Individual are prone by reason of hereditary (b) Intermittent bouts of corticohypothalamic discharge
    (c) Generalised arteriosclerosis (d) High circulating levels of catecholamines
17. **The most common cause of secondary hypertension is:**
    (a) Renal diseases (b) Thyrotoxicosis (c) Oral contraceptive pills (d) Pheochromocytoma
18. **Orthostatic (postural) hypotension is common after:**
    (a) Sympathectomy (b) Autonomic insufficiency
    (c) Abnormal baroreceptor reflexes (d) Primary hyperaldosteronism
19. **One of the following is earliest indication of concealed acute bleeding is:**
    (a) Tachycardia (b) Postural hypotension (c) Oliguria (d) Cold clamy fingers
20. **Because of good reservoir system, a healthy man can tolerate safely acute blood loss of ..... % of body weight:**
    (a) 10-15 (b) 20-30 (c) 30-40 (d) 40-50
21. **The first reactive change to occur after haemorrhage is:**
    (a) Vasocontriction (b) Tachycardia
    (c) Raised cortisol levels (d) Raised catecholamine levels
22. **After haemorrhage, restoration of blood volume is due to:**
    (a) Arteriolar constriction (b) Shift of intracellular fluid to extra-cellular space
    (c) Venous return increases (d) Intravenous infusion
23. **Anaphylaxis is mediated by all *except*:**
    (a) Serotonin (b) Bradykinin (c) Histamine (d) Prostaglandin
24. **Which of the following statement is *false*?**
    (a) Obstructive shock may be caused by tension pneumothorax
    (b) Myocardial infarction predispose to congested shock
    (c) Reflex warming of a shock patient is helpful to maintain adequate tissue perfusion
    (d) Generalised antigen-antibody reaction produces anaphylactic shock
25. **Left ventricular failure tends to cause:**
    (a) No breathlessness in lying position (b) Decrease in ventricular end diastolic pressure
    (c) Paroxysmal nocturnal dyspnoea (d) Rise in lung compliance

Answers

1. (b) 2. (d) 3. (c) 4. (d) 5. (b) 6. (c) 7. (b) 8. (d) 9. (c) 10. (a) 11. (c) 12. (d) 13. (c) 14. (a) 15. (a)
16. (d) 17. (a) 18. (a) 19. (a) 20. (a) 21. (a) 22. (b) 23. (a) 24. (c) 25. (c)

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Unit VI

THE RESPIRATORY SYSTEM

Chapter 45: Physiological Anatomy of Respiratory System

Passage of air; Tracheo-bronchial tree; properties of gases; Non-respiratory functions of respiratory system.

Chapter 46: Mechanics of Respiration

Mechanism of breathing; Pressure changes during ventilation; Lung volumes and capacities; Alveolar surface tension (surfactant, hyaline membrane disease); Pressure-volume relationship (compliance); Work done during breathing: Airway resistance; Alveolar ventilation: dead space, V/P ratio, diffusion capacity of lungs.

Chapter 47: Transport of Gases

Oxygen transport: oxygen-haemoglobin dissociation curve; Carbondioxide transport.

Chapter 48: Regulation of Respiration

Nervous regulation of respiration: respiratory centres, genesis of respiration; Chemical regulation of respiration: chemoreceptors (peripheral and central); Physio-clinical aspects: dyspnoea, breath holding, asphyxia, drowning, periodic breathing.

Chapter 49: Hypoxia

Definition; types (hypoxic, anaemic, stagnant, histotoxic); effects; treatment: O_2 therapy; Cyanosis.

Chapter 50: Physiology of High Altitude

Effects during rapid and slow ascent: pulmonary oedema; motion-sickness; Acclimatization.

Chapter 51: Effects of High Atmospheric Pressure

Caisson's disease; Nitrogen narcosis; High pressure nervous syndrome; Air embolism.

Chapter 52: Pulmonary (Lung) Function Tests

Chapter 53: Physiology of Exercise

Grading; Cardio-respiratory adaptations to exercise; Physiological effects of physical training.

Chapter 54: Physiology of Yoga

General; History; Requirements for doing yoga exercises; Health Benefits; Yoga vs conventional exercises.

Chapter

45

Physiological Anatomy of Respiratory System

I. Introduction – passage of air

II. Tracheo-bronchial tree: Respiratory membrane; pleura
A. Weibel's lung model B. Histology C. Innervation

III. Properties of gases: partial pressures; composition of air

IV. Non-respiratory functions of respiratory system

leaf ≡ alveoli
branches ≡ other respir. parts

INTRODUCTION

Body tissues utilize oxygen and produce CO_2 as a result of metabolism. The main function of the respiratory system is to extract O_2 from the atmosphere, to deliver it to the tissues and to take out CO_2 from the tissues and discharge it into the atmosphere. (Fig. 45.1)

To achieve this, the following events occur:

1. The lungs expand *(inflate)*, as a result the atmospheric air, rich in O_2, enters the lungs.
2. The O_2 is extracted and transferred to the blood contained in the pulmonary capillaries.
3. The *oxygenated* blood then circulates to the tissues all over the body.
4. At the tissue level, the blood delivers O_2 to the tissues which utilize O_2 (for metabolic purposes) and produce CO_2. The CO_2 is delivered back into the blood.
5. The *deoxygenated* blood (CO_2 rich blood) brings the CO_2 to the lungs where CO_2 is diffused out of the lungs.
6. The lungs *deflate* and discharge impure air, rich in CO_2, to the atmosphere.

Thus, the entire respiration can be divided into *3 main divisions:*

1. **External Respiration** (*Ventilation* or *Breathing*) *i.e.* the absorption of O_2 and removal of CO_2 from the body as a whole.
2. **Transport of Gases** (O_2 and CO_2) in the Blood
3. ***Internal*** (or *cellular*) ***Respiration*** *i.e.* the utilization of O_2 and production of CO_2 by cells, and the gaseous exchange between the cells and their fluid medium.

Teacher's Emolument is Incremented.

PASSAGE OF AIR

The inspired air enters by *nose and mouth* and proceeds through *glottis* (the triangular space between vocal cords) to *trachea*. The trachea divides into 2 major branches, the 'bronchi' one going to each lung. Each bronchus divides

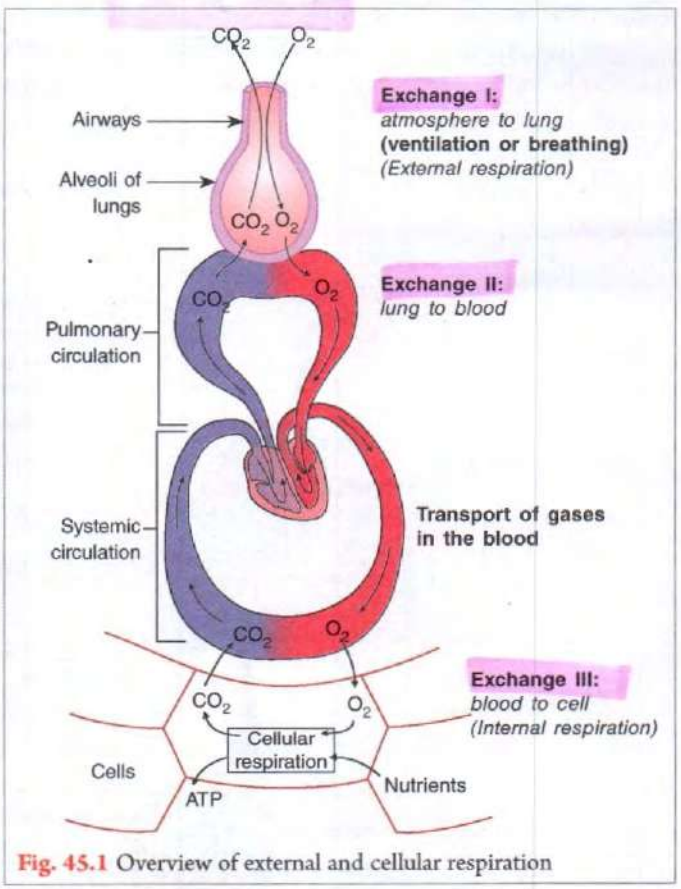


Fig. 45.1 Overview of external and cellular respiration

and re-divides to form many small branches, the *bronchioles*. Bronchioles finally give rise to *alveolar ducts* which lead via *atria* to tiny sac like structures called *pulmonary alveoli*. The two lungs contain approx. 300 million alveoli giving a surface area of more than 70 square meters. **(Fig. 45.2)**

The whole 'respiratory passage' *i.e.* branching network of trachea constitutes *tracheo-bronchial tree*.

TRACHEO-BRONCHIAL TREE

A. WEIBEL'S LUNG MODEL

Between the trachea and the alveolar sac, the air passage divides 23 times. *E.R. Weibel* (1963), a Swiss anatomist numbered each generation of tracheo-bronchial tree. Thus, the trachea is designated as generation 'zero' the two major divisions of the trachea, constitute the 'first' generation, and so on. The atria (alveolar sac) is the 23rd and the last generation.

On and from the 17th generation, few alveoli can be found on the bronchioles. Although the major portion of the O_2 and CO_2 exchange occurs in the alveoli (23rd generation), but some exchange begins to occur from the 17th generation, called the ***respiratory bronchioles***. The 16th generation bronchioles where no exchange of gases is possible are thus called ***terminal bronchioles***.

From functional point of view, therefore, the whole tracheo-bronchial tree can be divided into *two major* zones:

1. ***Conducting zone*** – This includes the portion of air passage upto the 16th generation where no exchange of gases is possible, called ***dead space***. This extends from the nose and mouth upto the *terminal bronchioles*. The total capacity of this zone is approximately 150 mL.
2. ***Respiratory zone*** – This includes the portion of air passage on and from the 17th generation where gaseous exchange takes place. This is made up of respiratory bronchioles, alveolar ducts and the alveoli. Its volume is approx. 4 litres.

B. HISTOLOGY (Table 45.1)

The wall of tracheo-bronchial tree is made up of fibers, cartilages, smooth muscles and epithelial lining containing glands and cilia.

'Cilia' beat towards the exterior *i.e.* towards the laryngeal side thereby preventing entry of foreign particles into the alveoli, called ESCALATOR action of cilia.

Alveolar lining epithelium

Alveolar lining epithelium is exceedingly thin, simple squamous type and consists of two types of cells:

(i) ***Type I cells*** are flat cells with large cytoplasmic extensions and are primary lining cells, covering about 95% of the alveolar epithelial surface area.

(ii) ***Type II cells (granular pneumocytes)*** - make up 5% of the alveolar epithelial surface area. These cells

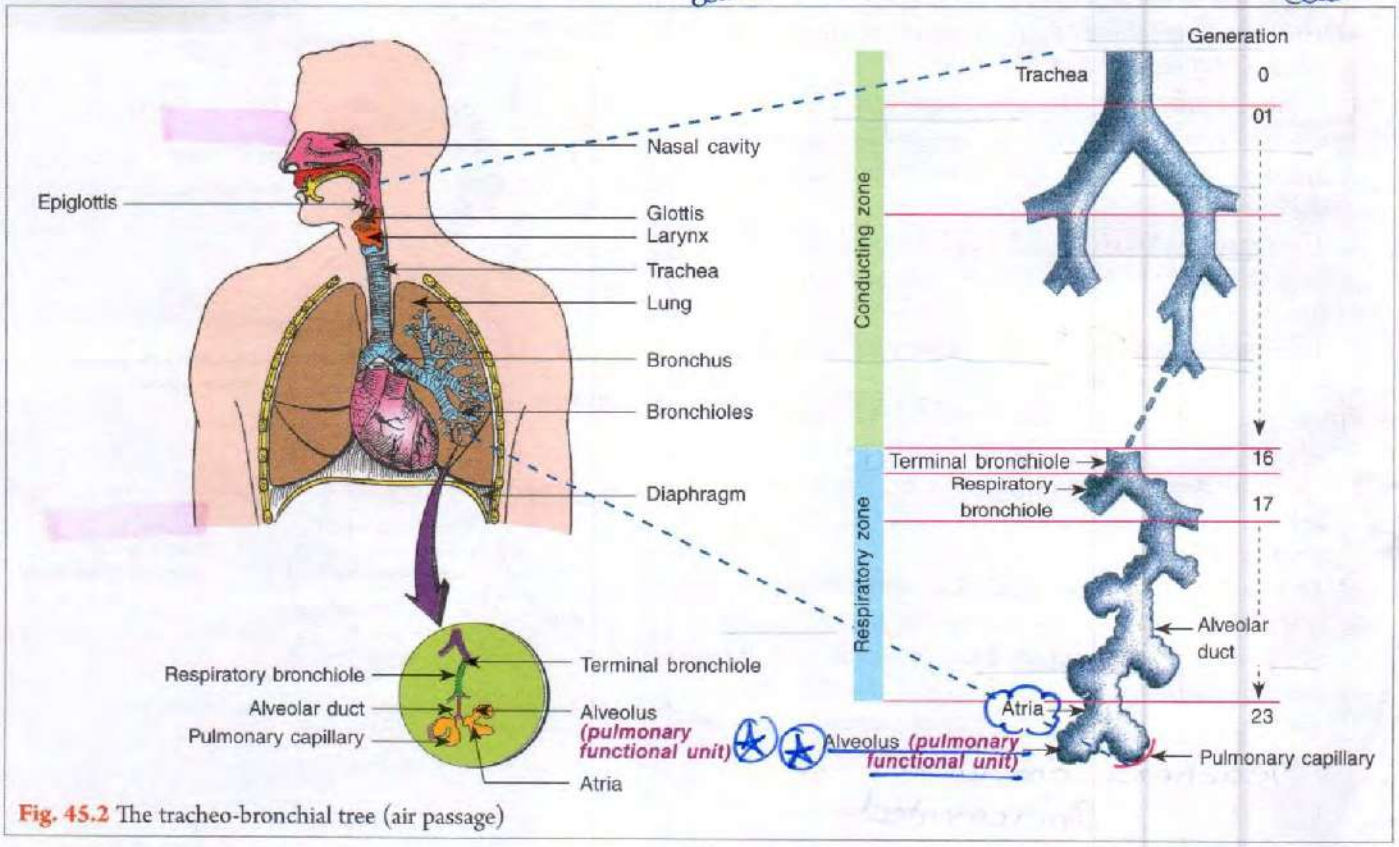


Fig. 45.2 The tracheo-bronchial tree (air passage)

are thicker and contain numerous lamellar inclusion bodies which secrete *surfactant*.

In addition, alveoli also contain:

(a) *pulmonary alveolar macrophages* (PAM), actively phagocytic cells

(b) lymphocytes

(c) *plasma cells:* very active cells; form and secrete immunoglobulins

(d) *amine precursor uptake and decarboxylation (APUD) cells:* These cells manufacture amines in addition to polypeptides. They store and secrete many biologically active peptides *e.g.* vasoactive intestinal peptide (VIP), substance P etc.

(e) mast cells. These cells contain heparin, histamine and 5 hydroxytryptamine (5 HT) that participate in allergic reaction.

Alveoli communicate with each other through small pores, called *pores of Kohn*.

C. INNERVATION

1. Two branches of autonomic nervous system (ANS):

(i) *Parasympathetic:* Its stimulation causes cholinergic discharge producing bronchoconstriction and increased bronchial secretion via muscuranic receptors. These nerves get stimulated by leukotrienes, irritants, chemicals *e.g.* SO_2, NO_2, lead, CO, hydrocarbons and by cool air during winter or exercise.

There is a *circadian rhythm* in *bronchial tone* with maximal constriction at 6 a.m. and maximal dilatation at 6 p.m.

(ii) *Sympathetic:* Its stimulation via adrenergic receptors (predominantly β_2) causes bronchodilation and increased bronchial secretions.

2. ***Non-cholinergic Non-adrenergic:*** Its stimulation produces bronchodilation due to release of mediator VIP (vasoactive intestinal peptide).

Note

VIP has been shown to be deficient or absent in a large number of patients with *bronchial asthma*.

THE RESPIRATORY MEMBRANE

The air in the alveoli is separated from the blood in the pulmonary capillaries by a 'wall' called *Respiratory Membrane* which consists of:

(i) Alveolar wall, and

(ii) Capillary wall.

Normally there is no fluid in this separating wall. The separating wall, called *alveolar-capillary membrane* or respiratory membrane has a thickness in the range of 0.3 to 1µm (average 0.5µm) **(Fig. 45.3)**. Due to its thinness, the gaseous exchange between the alveoli and blood capillaries is completed within fraction of a second. Thickness of either wall (alveolar or capillary wall) or accumulation of fluid within the wall, makes the gaseous exchange difficult.

PLEURA

The lungs are enveloped by 'pleura' which has two layers, parietal and visceral. **(Fig. 45.4)**

1. *Parietal Pleura.* It is adherent to the parieties *i.e.* inner side of the chest wall and the thoracic side of the diaphragm. Therefore, when these structures move, the parietal pleura has to move.
2. *Visceral Pleura.* It is adherent to the underlying viscus *i.e.* the lungs itself. Therefore, when the viscus (lungs) moves, it has to follow the viscus.

In between the two layers there is a potential space, called *Pleural Cavity*. This space is filled with a very small amount (approx. 2 mL) of serous lubricating fluid, called *Pleural Fluid*, which is dispersed throughout the pleural cavity. The fluid is adhesive and inexpansile and keeps the two pleurae together *i.e.* there is a large amount of traction between the two *(Hydraulic Traction)*. Therefore,

Table 45.1: Histological characteristics of tracheo-bronchial tree

	Trachea	Initial bronchi of few generations	Terminal bronchiole	Respiratory bronchiole	Alveoli
1. Cartilage	Present as rings, upto 20 in number and are deficient posteriorly	present	absent	absent	absent
2. Smooth muscle	little	little	largest in amount	more amount	absent
3. Lining epithelium	columnar	columnar	cuboidal	cuboidal	simple squamous
(i) Cilia	present	present	present	present	absent
(ii) Glands: mucous and serous	present	present	absent	absent	absent

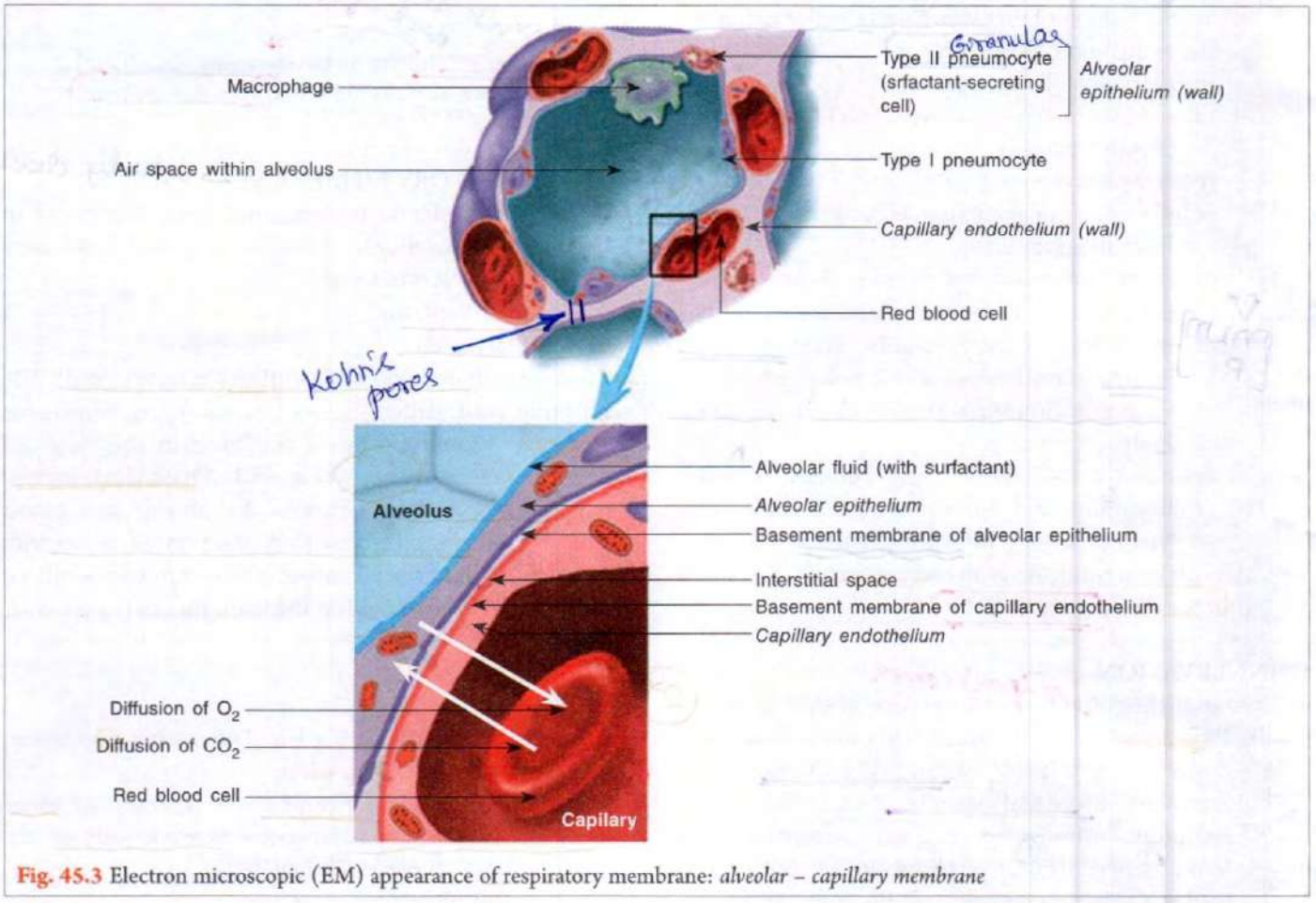


Fig. 45.3 Electron microscopic (EM) appearance of respiratory membrane: *alveolar – capillary membrane*

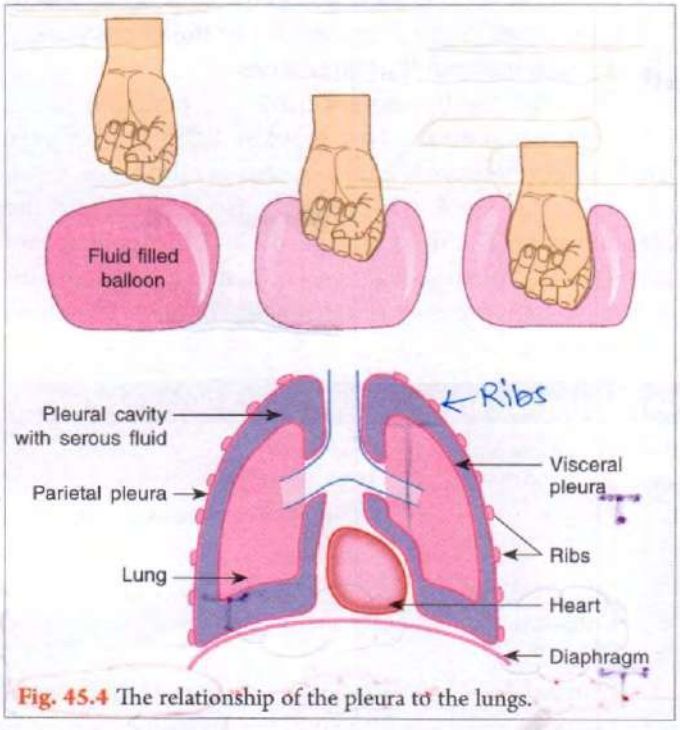


Fig. 45.4 The relationship of the pleura to the lungs.

when one moves, other follows. That is why the lungs slide easily on the chest wall but resist being pulled away from it in the same way that two moist pieces of glass slide on each other but resist separation.

PROPERTIES OF GASES

Partial Pressures

Unlike liquids, gases expand to fill the volume available to them, and the volume occupied by a given number of gas molecules at a given temperature and pressure is (ideally) the same regardless of the composition of the gas. Therefore,

$$P = \frac{nRT}{V}$$

where P = pressure
n = number of moles
R = gas constant
T = Absolute temperature
V = volume

Therefore, the pressure exerted by any one gas in a mixture of gases is called its *Partial Pressure*. It is equal to the total pressure times the fraction of the total amount of gas it represents.

Composition of dry air

O_2	:	20.98%
CO_2	:	0.03%
N_2	:	78.06%
Other inert constituents	:	0.93%

e.g. Argon and Helium etc.

The barometric pressure (P_B) at sea level is 760 mmHg (= 1 atmospheric pressure).

The partial pressure is indicated by symbol 'P'.

Calculation of partial pressure of a gas (pGas). It is done by measuring the percentage of gas and total atmospheric pressure, *i.e.*

$$P_{Gas} = \frac{\text{Percentage of gas}}{100} \times \text{total atmospheric pressure}$$

For example, percentage of O_2 in inspired air is 20.98%, then pO_2 (partial pressure of O_2) at sea level = 20.98/100 × 760 = 159.5 mmHg.

Dalton's Law – Total pressure exerted by a mixture of gases is equal to the sum of the partial pressures of all the gases present in it.

It is important to know the percentage of gases in inspired, expired and alveolar air and their corresponding partial pressures, because:

(1) Diffusion of gases depends on difference in partial pressure of gases. Gas moves from a region of high to low partial pressure in a liquid by diffusion.

(2) According to ***Henry's Law***, when temperature is kept constant, amount of gas dissolved in any solution is directly proportional to the partial pressure of the gas.

The water vapour in the air in most climates reduces these percentages and, therefore, the partial pressure of gases to a slight degree. The pH_2O at body temperature (37°C) is 47 mmHg. Therefore, partial pressure at sea level of other gases in the air reaching the lungs are shown in **Table 45.2.**

Since gas volumes vary with temperature and pressure and since the amount of water vapour in them varies, therefore, it is important to correct respiratory movements involving volume to a stated set of standard conditions, which are:

STPD – O°C, 760 mmHg, dry (standard temperature and pressure, dry).

BTPS – Body temperature and pressure, saturated with water vapour.

ATPS – Ambient temperature and pressure, saturated with water vapour.

NON-RESPIRATORY FUNCTIONS OF RESPIRATORY SYSTEM

Main function of the respiratory system in general and the lungs in particular is ***Gas Exchange.***

Non-respiratory functions of respiratory system include:

A. *Lung defence mechanisms i.e.* its own defence against inspired particulate matter.

B. *Functions of pulmonary circulation:* the storage and filtration of blood for systemic circulation.

C. *Metabolic and endocrine functions:* handling of vasoactive substances in the blood, formation and release of substances used in the alveoli or circulation.

A. LUNG DEFENCE MECHANISMS

Functions of respiratory passage

1. **Humidify and cool or warm the inspired air** so that even the very hot or very cold air approaches body temperature by the time it reaches the alveoli.

Table 45.2: Partial pressure of gases in various parts of cardio-respiratory system (in mmHg)

	Inspired air	Alveolar air	Arterial blood	Venous blood	Expired air
1. pO_2	158 (20.98%)	100-104 (14%)	98-100	40	116 (16%)
2. pCO_2	0.3 (0.03%)	40 (5.3%)	40	46	32 (4%)
3. pH_2O	Variable from (5.7 to 47)*	47 (6.2%)	47	47	47 (6.2%)
4. pN_2 {760-(1+2+3)}	Variable 596 (78.06%)	573 (74.5%)	578	627	565 (73.8%)
Total	760	760	760	760	760

* depending on the humidity of the atmosphere

Important Notes

1. Values in parenthesis indicate the percentage of total gas.
2. 'Composition' refers to the percentage of each gas in the air. *Composition of atmospheric air is essentially 'constant' everywhere and is independent of altitude.* At high altitude the air is thin (less dense).

2. Bronchial secretions contain secretory immunoglobulins (IgA) and other substances (nitric oxide etc.) that help resist local infection and maintain the integrity of the mucosa.
3. **Prevent foreign bodies from reaching the alveoli:**
   (i) particles >10 µm diameter – (a) strained out by hairs in the nostrils or (b) settle down on mucous membrane in the nose and pharynx;
   (ii) particles 2-10 µm diameter – fall on the walls of the bronchi as airflow slows in the smaller passages, there they initiate reflex bronchoconstriction and coughing; they are moved away from the lungs by the *ciliary escalator action*;
   (iii) particles <2 µm diameter – generally reach the alveoli, where they are ingested by the macrophages.

Important Note (APPLIED):

Ciliary immobility may be produced by various air pollutants or it may be congenital (*Kartagener's syndrome*), in which the axonemal dynein, the ATPase molecular motor, that produces ciliary beating is absent. This condition is also associated with infertility becasue of the lack of motile sperms.

(II) Pulmonary alveolar macrophages ('PAMS', dust cells) functions

1. These cells come originally from the bone marrow, they are actively phagocytic cells and ingest inhaled bacteria and small particles.
2. Help in processing inhaled antigens for immunologic attack.
3. Secrete substances:
   (i) that attract polymorphonuclear leucocytes to the lungs. The leucocytes, release proteases including elastase, which attacks the elastic tissues in the lungs resulting in *emphysema*. (Also see to pages 411, 417).
   (ii) that stimulate granulocyte and monocyte formation in the bone marrow.
4. They may release lysosomal products into extracellular space and produce inflammation. (This is particularly seen when macrophages ingest large amount of substances in cigarette smoke or silica or asbestos particles.)

B. FUNCTIONS OF PULMONARY CIRCULATION

1. *Reservoir for left ventricle* (LV) – If LV output becomes transiently greater than systemic venous return, LV output can be maintained for a few strokes by drawing out blood stored in the pulmonary circulation (Refer to page 382).
2. Pulmonary circulation *as a filter*. The particles filtered may include:
   (i) small fibrin or blood clots
   (ii) fat cells
   (iii) detached cancer cells
   (iv) gas bubbles
   (v) agglutinated RBC's, masses of platelets or WBC's
   (vi) debris from stored blood or intravenous solution; these are removed by
      (a) lytic enzyme in capillary endothelium
      (b) ingestion by macrophages, and
      (c) penetration into the lymphatic system.
3. *Fluid exchange and drug absorption.*
   (i) Low pulmonary hydrostatic pressure tends to pull fluid from alveoli into pulmonary capillaries and keeps the alveolar surface free from liquids (page 415). Water taken into the lungs is rapidly absorbed into the blood. This protects the gas exchange function of the lungs and opposes transudation of fluid from capillaries to the alveoli.
   (ii) Drugs that rapidly pass through the alveolar-capillary barrier by diffusion, rapidly enter the systemic circulation. Therefore, these are administered by inhalation *e.g.*
      (a) Anaesthetic gases, and
      (b) 'Aerosol' and other bronchodilators.

C. METABOLIC AND ENDOCRINE FUNCTIONS OF THE LUNGS

1. Substances *synthesized* and used in the lungs: *surfactant* (for details, refer page 414).
2. Substances synthesized or stored and released into the blood.
   (i) *Prostaglandins*, specially PGE_2 and $PGF_{2\alpha}$.

Note

The pulmonary epithelium contains *protease-activated receptors* (PARs) that when activated cause release of PGE_2, which in turn protects the *epithelial cells*.

   (ii) Histamine: It is released from the mast cells in the lungs in response to pulmonary embolism or anaphylaxis and produces bronchoconstriction.
   (iii) Kallikrein.
3. *Substances removed* from the blood: many vasoactive substances are inactivated, altered or removed from the blood as they pass through the lungs. *Site*: endothelial cells in the vessels of pulmonary circulation. For example,
   (i) Prostaglandins: PGE_1, PGE_2 and $PGF_2\alpha$ are completely removed in a single pass through the lungs.
   (ii) Bradykinin

(iii) Adenine derivatives
(iv) Serotonin*
(v) Nor-epinephrine*
(vi) Acetyl-choline

(*Lung decreases the amount of these vasoactive substances reaching the systemic circulation.)

4. Substances ***activated in*** the lungs (specially pulmonary circulation) *e.g.*

Angiotensin I		Angiotensin II
Decapeptide (inactive)	*Angiotensin Converting Enzyme (secreted by endothelial cells)* →	*Octapeptide* (active)

(For details refer to page 506)

5. Vasoactive hormones that pass through the lungs without being metabolized include: epinephrine, dopamine, oxytocin, vasopressin, and angiotensin II.
6. ***Storage of hormones*** and certain biologically active peptides in 'amine precursor uptake and decarboxylation' (APUD) cells and nerve fibers of lung *e.g.* vasoactive intestinal peptide (VIP), substance P, opioid peptides, cholecystokinin-pancreozymin (CCK-PZ) and somatostatin. These substances are later released into the systemic circulation.
7. Contain ***fibrinolytic system*** that lyses clot in the pulmonary vessels.

Study Questions

1. **Give physiological basis of:**
   (i) How the lungs slide easily on the chest wall
   (ii) Administration of certain drugs by inhalation
   (iii) Why no exchange of gases is possible at the level of conducting zone
   (iv) Asthma attacks are more common early in the morning
   (v) Composition of air at sea level and at high altitude
2. **Write short notes on:**
   (i) Terminal and respiratory bronchiole
   (ii) Alveolar lining epithelium
   (iii) Innervation of tracheo-bronchial tree
   (iv) Partial pressure of gases in inspired and expired air
   (v) Non-respiratory functions of lungs
   (vi) APUD and PAMS (Dust cells).
   (vii) Basic lung defence and metabolic function
   (viii) Pulmonary function unit
3. **What will happen and why, if**
   (i) excalator action of cilia is is deficient
   (ii) if foreign particles enters the pulmonary functional unit
4. **How lung protect itself from invading foreign substances.**
5. **Depict diagrammatically.**
   (i) Weibel lung model
   (ii) External and internal respiration
   (iii) Electron microscopic structure of respiratory membrane

MCQs

1. **Both the lungs contain about ..... million alveoli:**
   (a) 50 (b) 100 (c) 300 (d) 500
2. **Alveolar lining epithelium:**
   (a) Is exceedingly thin, simple squamous type
   (b) Contains type I granular pneumocytes
   (c) Contains cilia that beat towards the exterior
   (d) Contains numerous mucous and serous glands
3. **Sympathetic stimulation of the bronchus causes:**
   (a) Bronchial constriction
   (b) Increased secretion from glands
   (c) No effect
   (d) Bronchial dilatation

4. **Gaseous exchange across respiratory membrane is completed in:**
   (a) Less than 1 sec (0.5 sec) (b) 1 sec (c) 2 sec (d) 3 sec

5. **Which of the following is *true* about composition of venous blood?**

	pO_2 (mmHg)	pCO_2 (mmHg)
(a)	95	40
(b)	40	40
(c)	40	46
(d)	46	40

6. **Mark the *correct* response:**
   (a) Arterial pO_2 : 104 mmHg (b) Venous pCO_2 : 40 mmHg
   (c) Arterial pCO_2 : 40 mmHg (d) Venous pO_2 : 46 mmHg

7. **Particles falling on walls of the bronchi are prevented going to alveoli by:**
   (a) Bronchoconstriction (b) Coughing (c) Ciliary escalator action (d) All of the above

8. ***False* statement regarding dust cells in the lung:** (Tupper cap)
   (a) Also called PAM cells (b) Actively phagocytic cells
   (c) Process inhaled antigens for immunological attack (d) Originally come from the blood (Bone marrow

9. **Pulmonary functional unit is:**
   (a) Alveolus (b) Terminal bronchiole (c) Respiratory bronchiole (d) Alveolar duct

10. **Contraction of smooth muscle in respiratory tract occurs in response to the following, *except*:** (Broncho-constriction)
    (a) Irritation of bronchial mucosa (b) Stimulation of local beta adrenoceptors (Sympath.)
    (c) A decrease in the pCO_2 in the bronchial air (d) A cold stimulus to the bronchial mucosa

11. ***True* about non-cholinergic non-adrenergic nerves to lungs:**
    (a) Its stimulation produces bronchoconstriction
    (b) These nerves get stimulated by irritants and chemicals
    (c) Decreases bronchial secretions
    (d) Their activity is either deficient or absent in bronchial asthma patients

12. ***False* statement regarding the pleural fluid:**
    (a) A serous lubricating fluid (b) Normal amount 30-35 mL
    (c) Adhesive and non-expansible in nature (d) Keeps the two pleurae together

13. **The partial pressure of water vapour in the lungs:**
    (a) Increases with hyperventilation
    (b) Is relatively constant with changes in altitude
    (c) Is proportional to the CO_2 concentration
    (d) Becomes negative when barometric pressure falls below 47 mmHg

14. **The maximum particle size that reaches the alveoli is ..... diameter:**
    (a) 15 µm (b) 10 µm
    (c) 5 µm (d) 2 µm

15. **Substance not filtered in pulmonary circulation:**
    (a) Blood clots (b) Cancer cells
    (c) Plasma proteins (d) Gas bubbles

Answers

1. (c) 2. (a) 3. (d) 4. (a) 5. (c) 6. (c) 7. (d) 8. (d) 9. (a) 10. (b)
11. (d) 12. (b) 13. (b) 14. (d) 15. (c)

Chapter

46

Mechanics of Respiration

I. Mechanism of breathing
II. Pressure changes during ventilation
III. Lung volumes and capacities
IV. Alveolar surface tension: Surfactant; Hyaline membrane disease
V. Pressure volume relationship: compliance
VI. Work done during breathing: Airway resistance
VII. Alveolar ventilation: Dead space; V/P ratio; Diffusion capacity of lungs

1 MECHANISM OF BREATHING

Eupnoea means rhythmic breathing at rest. It consists of *Inspiration* and *Expiration*.

Inspiration is an *active* process. During inspiration the size of the thoracic cavity is increased by contraction of appropriate muscles. The parietal pleura follows the expanding chest wall. The visceral pleura also follows the parietal pleura and thus the *Elastic Lungs* also follow the thoracic expansion passively. The expansion of lungs is associated with a fall in the pressure in the lung parenchyma and atmospheric air is thereby drawn into the depths of the lungs.

Expiration is a *passive* process. At the end of inspiration, the muscle which contracts actively during inspiration relaxes and the *elastic recoil* of the thoracic wall and lungs cause passive expiration.

At rest an adult breathes at a respiratory rate of 12-14 breaths/min and the amount of air inspired or expired per breath (*i.e. Tidal Air*) is approx. 500 mL. Thus, 6-7 litres of air is breathed in or out of the lungs per minute called ***Pulmonary Ventilation***.

MECHANISM OF INSPIRATION

Inspiration is an *active process*. During inspiration the thorax is enlarged by:

A. movements of the ribs outwards and upwards (*Rib Movements*); and
B. descent of the diaphragm (*Diaphragmatic Movements*).
C. Accessory m.

A. Rib Movements

1. In quiet respiration, the first pair of ribs moves but little. In hypernoea their movements bring about increase in anteroposterior diameter of upper thorax.
2. The 2nd to 6th ribs slope obliquely downwards and forwards from their joints with the spinal column. On inspiration the ribs move upwards *(Pump Handle Movements)* to assume a more horizontal position due to contraction of *external intercostal muscles* (supplied by $T_{1,2}$) to cause increase in anteroposterior diameter of the chest **(Fig. 46.1)**. Moreover, by virtue of their curved (bowed) mid-part, cause an increase in the transverse diameter of the thorax.
3. The lower ribs (7th to 10th) also swing outwards and upwards *(Bucket Handle Movement)* in inspiration to cause increase in the transverse diameter of the thorax.

B. Diaphragmatic Movements

Diaphragm consists of muscle fibers which converge on the dome-shaped central tendinous portion. Muscle fibers arise from the,

(1) xiphisternum
(2) inner surface of lower six ribs, and
(3) lumbar vertebrae.

During inspiration, as a result of discharge in phrenic neurons ($C_{3,4,5}$), muscle fibers contract and draw the central tendon downwards by 1.5 cm in eupnoea, and by 7 cm in deep inspiration. This causes an increase in the vertical diameter of the thoracic cage **(Fig. 46.2)**. For each 1 cm descent 200-300 mL of air is sucked in, therefore, diaphragmatic movements account for as much as 75% of tidal volume in eupnoea.

Clinical significance

1. Either the diaphragm or the external intercostal muscles alone can maintain adequate ventilation at rest.

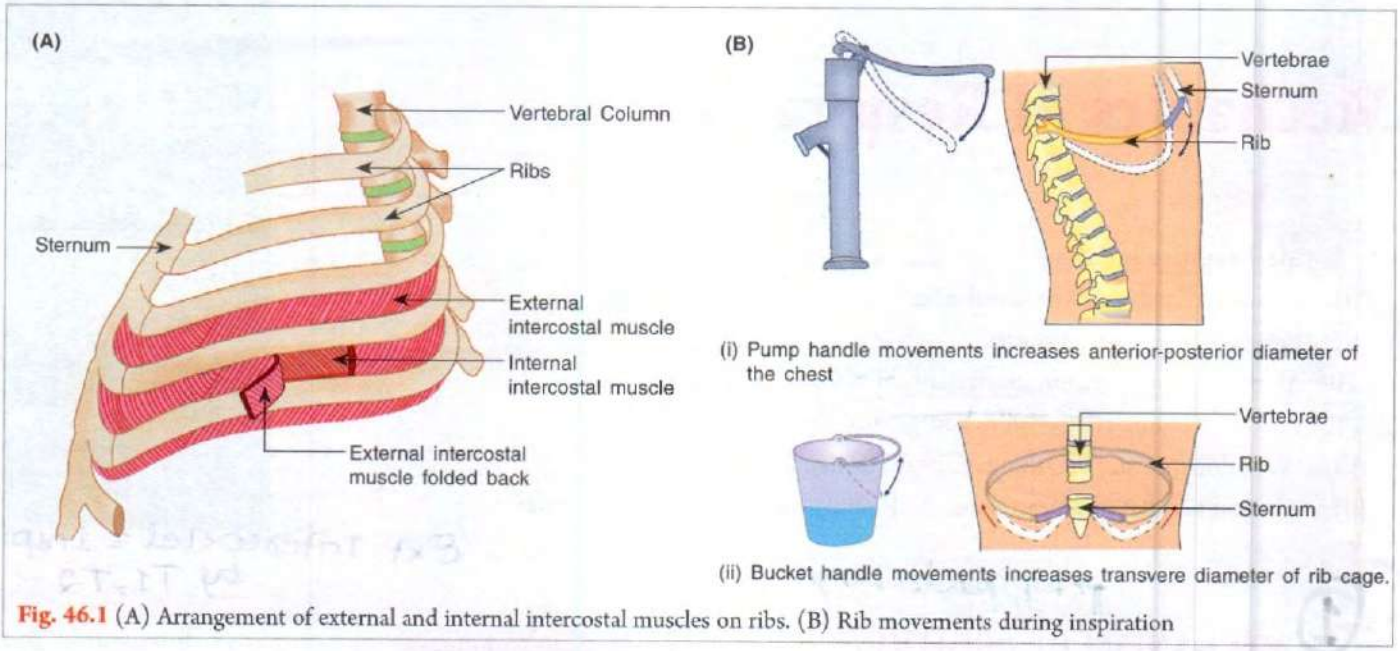


Fig. 46.1 (A) Arrangement of external and internal intercostal muscles on ribs. (B) Rib movements during inspiration

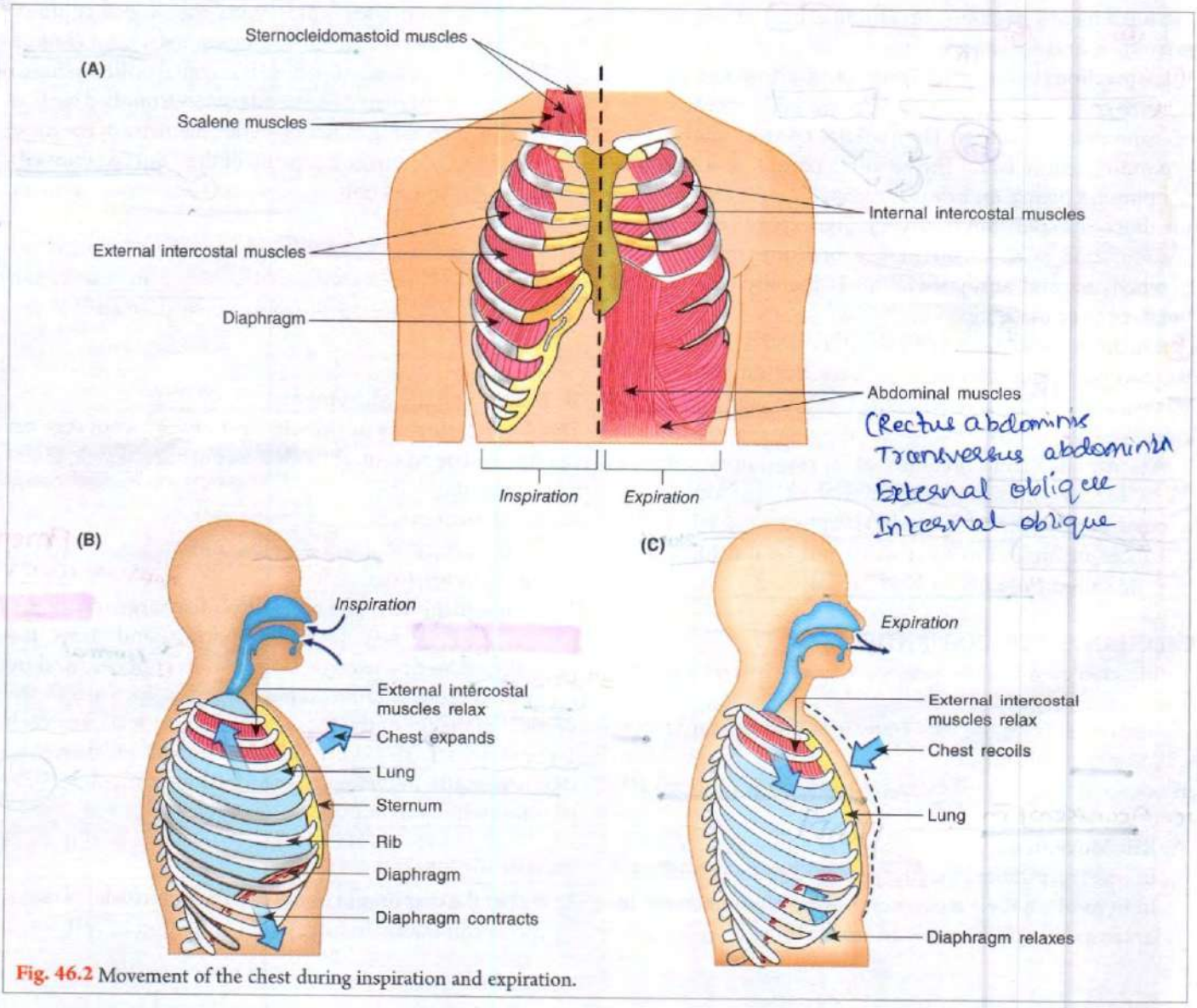


Fig. 46.2 Movement of the chest during inspiration and expiration.

2. In patients with bilateral phrenic nerve palsy but intact innervation of their intercostal muscles, respiration is somewhat laboured but adequate to maintain life.
3. Transection of spinal cord above the 3rd cervical segment is fatal without artificial respiration, but transection below the 5th cervical segment is not, because it leaves the phrenic nerve ($C_{3,4,5}$) that innervates the diaphragm intact.

Position of diaphragm: postural relationship

1. *Sitting:* most comfortable because diaphragm is down due to gravity and not pressing over abdominal contents. This also increases the reserve volume of lungs (page 411).
2. *Standing:* abdominal muscles contract to cause intra-abdominal pressure to increase, resulting in ascent of diaphragm.
3. *Lying:* most uncomfortable.

Accessory muscles of inspiration

1. **Scalene and sternocleidomastoid muscles** in the neck play little, if any, part in breathing at rest, but become active in voluntary static inspiratory efforts and help to elevate the thoracic cage during deep inspiration.
2. **Intrinsic muscles of the larynx:** Abductor muscles of vocal cords *i.e.* posterior cricoarytenoid, contracts early in inspiratory phase pulling the vocal cords apart and opening the glottis. It is supplied by recurrent laryngeal nerve, a branch of vagus nerve. Their paralysis leads to ***Inspiratory Stridor*** *i.e.* inspiration occurs with a loud sound.

MECHANISM OF EXPIRATION

In quiet breathing, expiration is a *Passive Process*, but during forced expiration, for example: voluntary expiratory efforts; during exercise; bronchial asthma etc.; the muscles of expiration contract, which include:

A. *Anterior abdominal wall muscles* (abdominal recti; transversus abdominis; internal and external oblique muscles); and

B. *Internal intercostal muscles*

1. Contraction of ***anterior abdominal wall muscles*** increases intra-abdominal pressure and draws the lower ribs down and medially, thereby pushing the diaphragm upwards, thus, aiding in expiration.
2. ***Internal intercostal muscles:*** They pass obliquely downwards and posteriorly from rib to rib. On contraction they pull the upper ribs down so that the ribs acquire the position as seen at the end of expiration. **(Fig. 46.1)**
3. ***Accessory muscles of expiration*** are adductor muscles of vocal cords. They begin to contract early in expiration, but their contraction is not complete. Therefore, it seems their main respiratory function is protective *i.e.* prevent entry of food and fluid into the trachea. When adductors are paralysed, fluid and food enter the trachea, causing aspiration pneumonia and oedema.

2 PRESSURE CHANGES DURING VENTILATION

With the initiation of breathing after birth the first inspiration causes enlargement of the chest. This produces expansion of lungs which are virtually dragged after the chest wall because of the adhesive and inexpansible properties of the pleural fluid.

The expansion of lungs is naturally attended by a fall in the pressure in the lungs parenchyma, called *Intra Pulmonary Pressure* or *Intra Alveolar Pressure*, so that air is pulled into the lungs via the tracheo-bronchial tree.

INTRA-PULMONARY PRESSURE (or INTRA-ALVEOLAR PRESSURE)

In quiet breathing, at end-expiration and at end-inspiration, as no air is going in and coming out of the lungs, therefore, intrapulmonary pressure is equal to the atmospheric pressure (normal: 760 mmHg). **(Fig. 46.3A)**

With the **beginning of inspiration**, as volume increases pressure decreases, therefore, the intra-pulmonary pressure decreases to approx. 1 mmHg below atmospheric pressure (*i.e.* 759 mmHg), but regains the full atmospheric pressure value at end-inspiration. When expiration follows passively, the elastic recoil of the lungs causes the intra-pulmonary pressure to swing slightly to the positive side (1 mmHg *i.e.* 761 mmHg) but regains the atmospheric pressure value at end-expiration.

Factors affecting intra-pulmonary pressure

1. ***Valsalva Manoeuvre*** *i.e.* forced expiration against a closed glottis, may produce a positive intra-pulmonary pressure of ≥ 100 mmHg above the atmospheric value. #: Normally, it must be +ve slightly only
2. ***Muller's Manoeuvre*** *i.e.* forced inspiration against a closed glottis, can reduce the intra-pulmonary pressure to ≤80 mmHg below the atmospheric value.

INTRA-PLEURAL PRESSURE (or INTRA-THORACIC PRESSURE)

The lungs and the chest wall are elastic structures. **At end-expiratory position**, the tendency of the lungs to recoil from the chest wall is just balanced by the tendency of the chest wall to recoil in the opposite direction (Refer

#: We usually talk pressures (assuming the reference std. atm. pr. = 0 mmHg

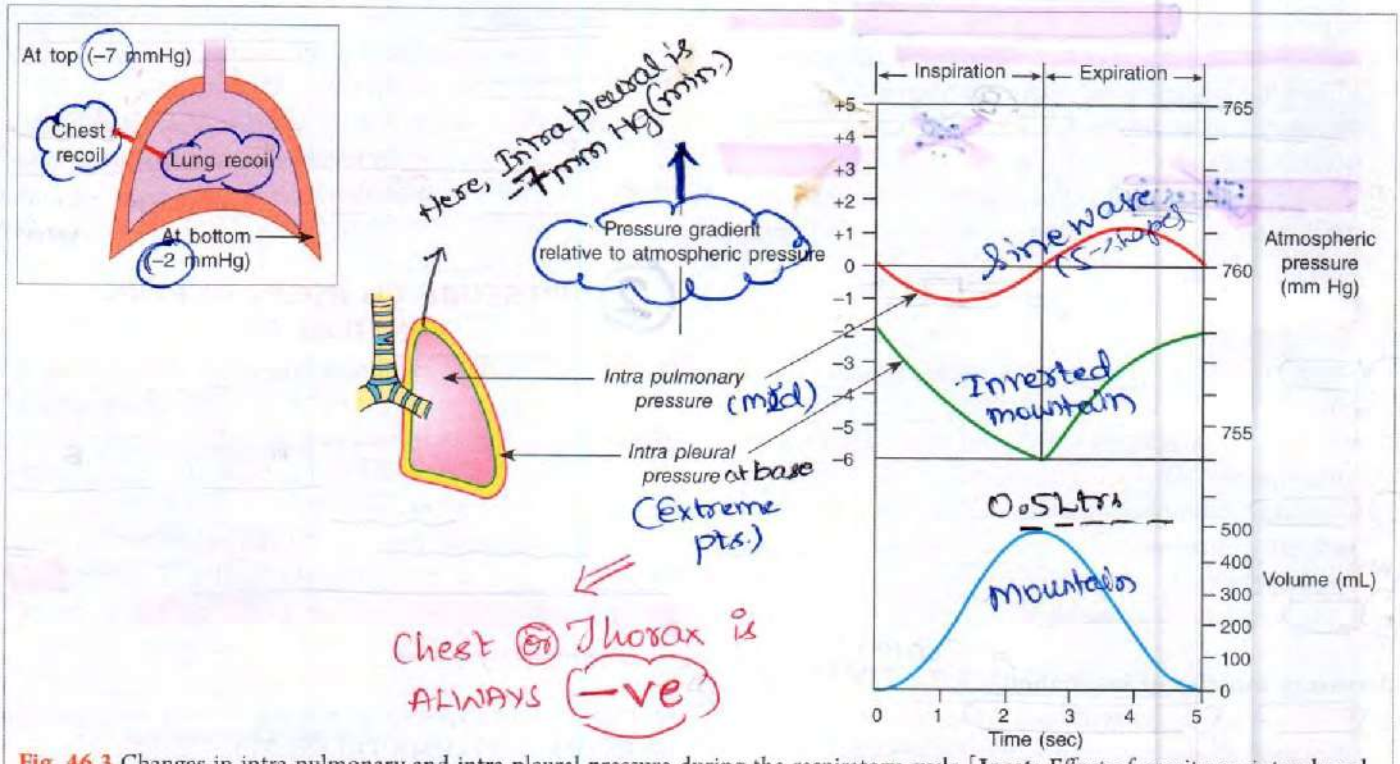


Fig. 46.3 Changes in intra-pulmonary and intra-pleural pressure during the respiratory cycle [**Inset:** Effect of gravity on intrapleural pressure]

(Fig. 46.3)). This causes a slight subatmospheric pressure to develop, say -2 mmHg between the two layers of pleura at the start of inspiration, called *intra-pleural pressure* or *intra-thoracic pressure*. (Fig. 46.3)

The negative intra-pleural pressure is directly proportional to the amount of thoracic expansion which occurs. Therefore, **during quiet inspiration** (*i.e.* at rest), the lungs are pulled into a more expanded position causing intra-pleural pressure to decrease to about –6 mmHg. At the end of inspiration, the lung recoil begins to pull the chest back to the expiratory position; and *at end-expiratory position* where the recoil pressure of the lungs and chest wall balance, intra-pleural pressure returns back to –2 mmHg.

Factors affecting intra-pleural pressure

A. Physiological factors

1. **Deep inspiration** decreases it as much as 30 mmHg subatmospheric *i.e.* 730 mmHg.
2. **Valsalva manoeuvre** (see above) or sudden forceful expiratory movements; for example, coughing, defecation etc., increase intra-pleural pressure by 60-70 mmHg *i.e.* it becomes 820-830 mmHg.
   Mechanism: Forceful expiration decreases thoracic volume causing lung deflation. This decreases the lung recoil forces and eventually causes intra-pleural pressure to rise.

Important Note

A large positive intra-pleural pressure produced by pressing over the great vessels within the thoracic cavity greatly reduces the venous return to the heart and cardiac output falls markedly. This produces cerebral ischaemia resulting in 'syncope' (transient loss of consciousness). Thus, severe bouts of cough or straining at stools may be dangerous in old persons or cardiac patients.

3. **Effect of gravity** – because of gravitational forces, intra-pleural pressure in standing position is more negative (-7 mmHg) at the apices of lungs compared to the bases (-2 mmHg) *i.e.* 5 mmHg greater at the bases of the lungs than at the apex. (Fig. 46.3-inset)

Clinical significance: Since the ***transmural pressure*** *i.e.* pressure difference between intra-pulmonary and intra-pleural pressure. It is a measure of the elastic forces in the lungs (called *recoil pressure*). It is less at the bases, therefore, the lungs are less expanded at the bases. This transmural pressure further decreases or may become negative at the end of forced expiration, thus, causing airways to close at the bases. This is why during first part of inspiration, more of the inspired gas goes to the apices than bases of the lungs.

B. Pathological Factors

1. **Emphysema** *i.e.* loss or decrease in lung elasticity, increases intra-pleural pressure, therefore, chest expands and becomes barrel-shaped.
2. **Injury to thoracic wall causes** air to enter between the two layers of pleura till intrapleural pressure equalises atmospheric pressure. This produces collapse of lungs as there are no opposing forces, a condition called as ***Pneumothorax.***

Measurement of intra-pleural pressure

Two methods :

1. By inserting a needle into intra-pleural space and by injecting a tiny bubble of air therein, whereupon suitable manometric recordings can be made by connecting the other end of the needle with a water manometer through a rubber tube.
2. By introducing air containing rubber balloon sealed over a catheter which is passed via the nostril into the lower part of thoracic oesophagus and recording *intra-oesophageal pressure*. This is equivalent to intra-pleural pressure, because at cricothyroid level there is closure of glottis and below it is closed by cardiac sphincter. Therefore, oesophagus (thoracic part) becomes a closed cavity. Oesophageal walls are quite lax and pressures are easily transmitted to the balloon.
   This method can be modified by connecting the outer end of the catheter to electrical transducers which provide more accurate record.

3 LUNG VOLUMES AND CAPACITIES

These can be divided into 2 major headings:

A. *Static lung volumes and capacities* (Time factor is not involved, therefore, expressed in mL or L).

B. *Dynamic lung volumes and capacities* (Time dependent, therefore, expressed in mL/min or L/min).

A STATIC LUNG VOLUMES AND CAPACITIES

Volumes (Fig. 46.4)

1. ***Tidal Volume*** *(TV).* It is the volume of air breathed in or out of lungs, during quiet respiration.
   Normal: 500 mL.
   (i) *Decreases* due to less contraction of respiratory muscles; *causes:* respiratory muscles weakness or depression of respiratory center.
   (ii) *Increases* in muscular exercise.
2. ***Inspiratory Reserve Volume*** *(IRV).* It is the maximal volume of air which can be inspired after completing a normal tidal inspiration *i.e.* inspired from the end-inspiratory position.
   Normal: 2000-3200 mL.
3. ***Expiratory Reserve Volume*** *(ERV).* It is the maximal volume of air which can be expired after a normal tidal expiration *i.e.* expired from the end-expiratory position.
   Normal: 750-1000 mL.
4. ***Residual Volume*** *(RV).* It is the volume of air which remains in lungs after a maximal expiration.
   Normal: 1200 mL.
5. ***Closing Volume*** *(CV).* It is the lung volume above residual volume at which airways in the lower, dependent parts of the lungs begin to close off because of the lesser transmural pressure in these areas (page 410).

Capacities (Fig. 46.4)

1. ***Inspiratory Capacity*** *(IC).* It is the maximal volume of air which can be inspired after completing tidal expiration *i.e.* from the end-expiratory position. It can be computed as: TV + IRV.
   Normal: 2500-3700 mL.
2. ***Expiratory capacity*** *(EC).* It is the maximal volume of air which can be expired after completing tidal

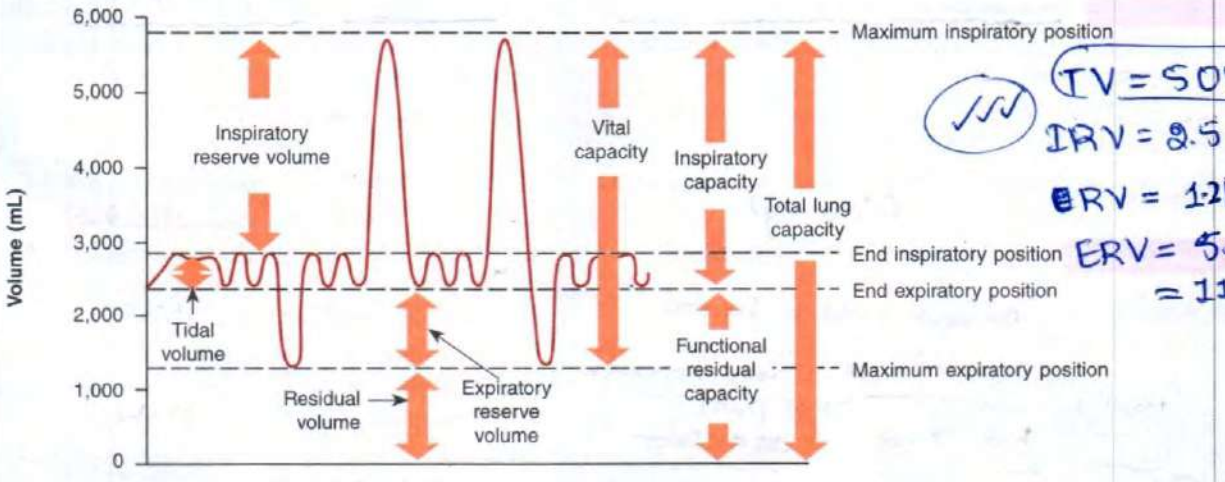


Fig. 46.4 Lung volumes and capacities (Abbreviations as given in the text)

inspiration *i.e.* from the end-inspiratory position. It can be computed as: TV + ERV.
Normal: 1250-1500 mL.

3. ***Vital Capacity*** *(VC).* It is the maximal volume of air which can be expelled from lungs by forceful effort following a maximal inspiration *(one stage VC).*
   Two stage VC. VC measured in two stages:
   Stage 1: Subject is asked to breath in maximally after a normal expiration; then he takes a few normal breaths.
   Stage 2: Subject is asked to breath out maximally after a normal expiration.
   Sum of stage 1 and 2 volumes gives the 'two stage VC'. It is slightly larger than 'one stage VC'.
   Normal: 4.8 litres in males and 3.2 litres in females.
   It can be computed as: TV + IRV + ERV.

Advantages of VC

1. It provides useful information about the strength of the respiratory muscles; therefore, maximum inspiratory and expiratory effort can be assessed.
2. It gives the useful information about other aspects of pulmonary function through FEV_1 (page 413).

Factors Affecting VC

A. Physiological

(i) Physical dimension *i.e.* size and development of the subject.
VC in males is 2.6 L/m² BSA; and in females, 2.1 L/m² BSA.
VC is more in males because of (a) large chest size, (b) more muscle power; and (c) more BSA (body surface area).

(ii) Age: VC decreases in old age due to loss of elasticity of lungs. (With aging, elastic fibers get replaced by fibrous tissue.)

(iii) Strength of respiratory muscles: VC increases in swimmers and divers.

(iv) Posture: in standing position VC is more as compared to sitting or lying positions, because in standing position,
(a) decrease in venous return, decreases the pulmonary blood flow and
(b) diaphragm descends down thus increasing inspiration.

(v) Pregnancy: VC decreases.

B. Pathological

(i) VC decreases in diseases of the respiratory apparatus. For example: poliomyelitis, pulmonary fibrosis, respiratory obstruction, emphysema, pleural effusion, pulmonary oedema, pneumothorax, etc.

(ii) Ascites, *i.e.* accumulation of fluid in the abdominal cavity causes VC to decrease.

4. ***Functional Residual Capacity*** *(FRC).* It is the volume of air which is contained in the lungs at end-expiratory position *i.e.* after completion of tidal expiration. It can be computed as: RV + ERV. *Normal:* 2.5 litres.
5. ***Total Lung Capacity*** *(TLC).* It is the volume of air contained in the lungs after a maximal inspiration. It can be computed as: VC + RV. *Normal:* 6 litres.

Note

With the exception of *FRC, RV* and *closing volume* all other lung volumes and capacities can be measured with the help of a simple spirometer.

Significance of FRC

FRC maintains the residual volume (RV) constant. Suppose there is no 'RV' in lungs, then, during inspiration gas for exchange is in excess and during expiration gas for exchange is 'nil'. As a result during inspiration partial pressure of gases in the alveolar air will be very high and very low during expiration, and death may occur. Thus, 'FRC' acts as a buffer and allows the continuous exchange of gases to occur even during expiration thereby prevents sudden changes in partial pressure of gases in the blood.

Factors affecting FRC

FRC is increased in conditions of hyperinflation of the lungs, which may result from:

1. Old age due to loss of elasticity
2. Emphysema,
3. Bronchial asthma, and
4. Atelectasis (areas of collapse of alveoli).

B DYNAMIC LUNG VOLUMES AND CAPACITIES

1. ***Timed Vital Capacity (TVC) or Forced Vital Capacity (FVC)***
   If VC is recorded on a kymograph (spirograph) at the known speed, volume of air expelled can be timed. Thus, FVC is the maximum volume of air which can be breathed out as 'forcefully' and 'rapidly' as possible following a maximum inspiration. Thus TVC is exactly similar to VC except that there is a special stress on "rapid, forcible and complete exhalation".
   Components of TVC (FVC) **(Fig. 46.5)**
   (i) FEV_1 (Forced Expiratory Volume in 1 sec) *i.e.* volume of FVC expired in 1st sec of exhalation. *Normal:* 80% of FVC.
   (ii) FEV_2 (Forced Expiratory Volume in 2 sec) *i.e.* volume of FVC expired in first two secs of exhalation. *Normal:* 95% of FVC.
   (iii) FEV_3 (Forced Expiratory Volume in 3 sec) *i.e.* volume of FVC expired in first three secs of exhalation. *Normal:* 98-100% of FVC.

Clinical Significance of TVC (FVC)

To distinguish between *'restrictive' and 'obstructive' lung disorders.* **(Fig. 46.6)**

In ***restrictive disorders*** (*e.g.* emphysema, kyphoscoliosis and ankylosing spondylitis *i.e.* arthritis of vertebral column) chest expansion is restricted. Therefore, VC decreases while FEV_1 is normal. TLC, flow rates and MVV (see below) also falls as VC decreases.

Note

Emphysema is a degenerative disorder and is characterised by a loss of lung elasticity and replacement of alveoli with large air sacs. Thus loss of lung elasticity prevents full expansion of lungs (i.e. *airway restriction*). Most common cause of emphysema is heavy cigarette smoking.

In ***obstructive disorders*** (*e.g.* bronchial asthma), inspiration is normal but expiration is obstructed, therefore, VC is normal while FEV_1 decreases.

Important Note

FEV_1 is a much more sensitive index (*i.e.* most reproducible) of the severity of obstructive lung disorders, but it does not allow for differentiation of the various causes of obstruction.

2. *Forced Expiratory Flow during 25-75% of Expiration ($FEF_{25-75\%}$)* (Fig. 46.7)

This is the mean expiratory flow rate during middle 50% of FVC. Some workers call it erroneously as ***Maximum Mid Expiratory Flow Rate*** (MMEFR). *Normal:* 300 L/min. It is a sensitive indicator of *small airway disease* where most of chronic obstructive pulmonary diseases (like bronchial asthma, emphysema) start.

The time taken for $FEF_{25-75\%}$ is called *Mid Expiratory Time (MET). Normal:* MET 0.5 sec; increases in obstructive lung disorders.

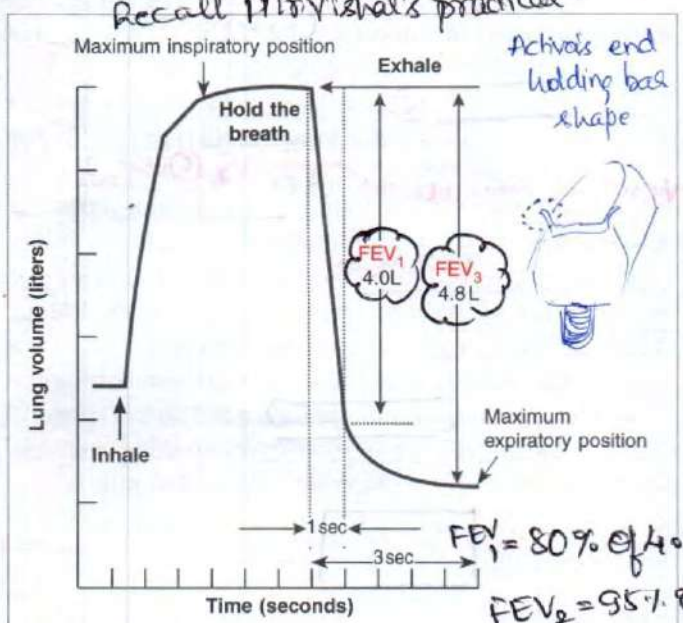


Fig. 46.5 Timed vital capacity record

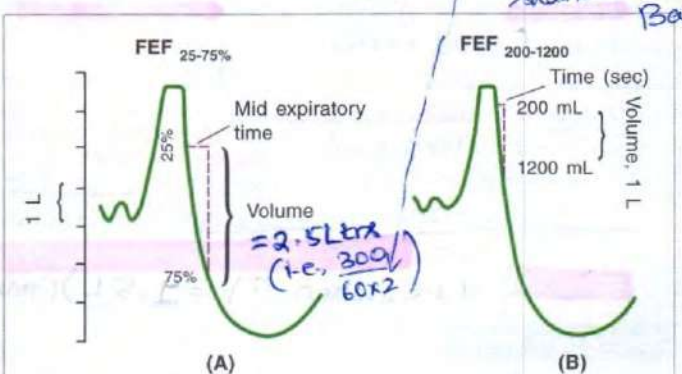


Fig. 46.7 Forced expiratory flow (FEF) during (A) 25–75%; and (B) 200–1200 mL of expiration

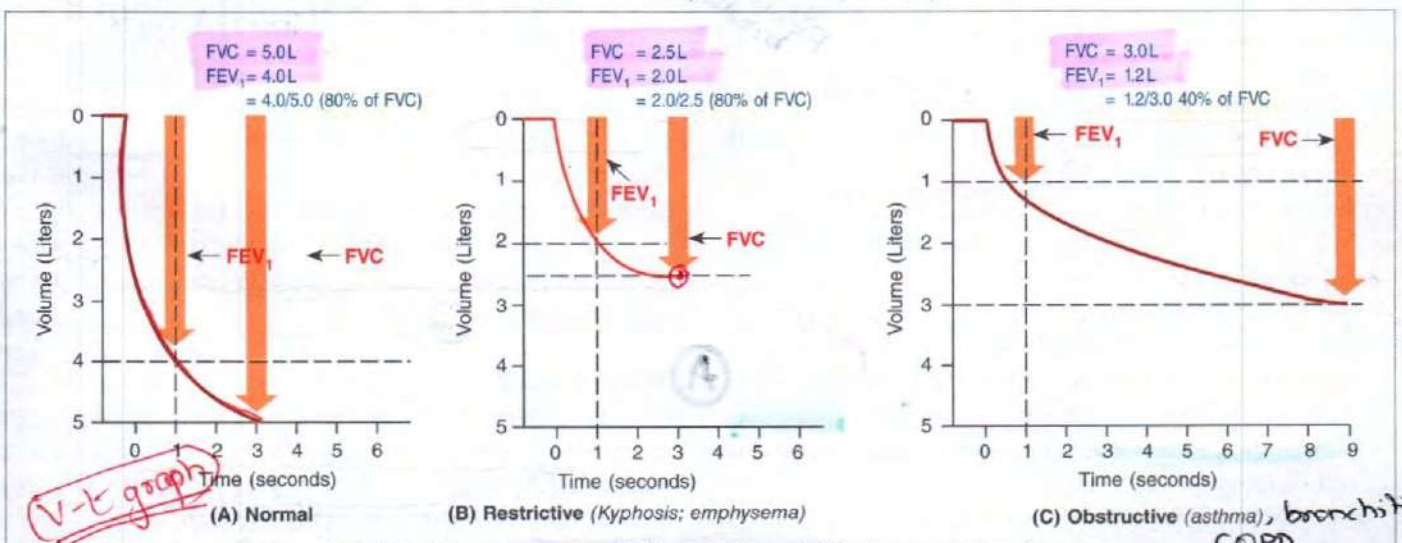


Fig. 46.6 Timed vital capacity (TVC) in (A) normal subject; (B) restrictive and (C) obstructive lung diseases

3. ***Forced Expiratory Flow During 200-1200 mL of Expiration ($FEF_{200-1200}$) (Fig. 46.7)*** → Tasneem
This is the mean expiratory flow rate between 200 to 1200 mL segment of FVC.
Normal: 350 L/min. *Disadvantage* over $FEF_{25-75\%}$: in initial phase of FVC, fall is very rapid, therefore, pressure difference is too much; thus, it may miss the small obstruction in the air passage if present.

4. ***Minute Ventilation (MV) or Pulmonary Ventilation (PV)*** → Saniya
This is the volume of air expired or inspired by the lungs in one minute. Therefore,
PV = TV × RR per min
= 500 × 12
= 6L/min, normally.

5. ***Peak Expiratory Flow Rate (PEFR)*** → Fouziya
This is the expiratory flow rate during the peak of FVC. *Normal*: 400–450 L/min.
It is a simple test of ventilatory function which is widely used in clinical practice and is recorded with the help of *Wright's peak flow metre*. Normal values are dependent on age, sex and body built. There may be a marked fall in its value in cases of obstructive airway disease.

6. ***Maximum Breathing Capacity (MBC) or Maximum Voluntary Ventilation or Maximum Ventilation Volume (MVV)*** → Lateef saab
It is the largest volume of air that can be moved into and out of the lungs in one minute by maximum voluntary effort. *Normal:* 90-170 L/min (average 100 L/min). (i.e., when TV = 2.5 L) (max)

Important Note

Hyperventilation if persists for 1–2 minutes, causes CO_2 washout and leads to respiratory depression. This may produce fainting; therefore, voluntarily it should be carried out for 15 sec only.

The ability to reach a high MVV *depends on*:
(i) muscular forces available,
(ii) the 'compliance' (distensibility) of the thoracic wall and lungs, and
(iii) the airway resistance set up.
MVV is *markedly decreased* in patients with:
(i) emphysema,
(ii) airway obstruction, and
(iii) poor respiratory muscle strength.

7. ***Pulmonary Reserve (PR) or Breathing Reserve (BR)***
That is, maximum amount of the air above the pulmonary ventilation, which can be breathed in and out of lungs in one minute.
It can be computed as: MVV – PV.
= 100L − 60L
= 40L.
→ DI = 40 × 100 = 40% ⇒ Dyspnea ✓

It is usually expressed as percentage of MVV *i.e.* (MVV–PV) × 100/MVV; and called as percentage pulmonary reserve or *Dyspnoeic Index (DI)*.
Normal % PR (DI) ≥ 60-70% (usually 90%).
If < 60%, *Dyspnoea* is usually present.

4 ALVEOLAR SURFACE TENSION

The lungs and the chest wall are elastic structures. The lungs are stretched when they are expanded at birth and at the end of resting expiration their tendency to recoil from the chest wall is just balanced by the tendency of the chest wall to recoil in the opposite direction. Therefore, if the chest wall is opened the lungs collapse and if the lungs lose their elasticity, the chest expands and becomes barrel shaped. [Emphysema] → No recoil capac. by spring

The tendency of the lungs to recoil back from the chest wall is governed by 2 forces: (Rubber ball)
(1) *Recoil of elastic tissue of the lungs*, and
(2) *Surface tension* within the alveoli, due to the fact that alveoli contain a very thin film of fluid lining their inner side.

Surface tension is due to the intermolecular attraction between the surface molecules and thus it tries to reduce surface area and collapses the lungs. Hence this surface tension must be reduced especially during expiration. Otherwise, the lungs will collapse. Moreover, this force opposes the expansion of the lungs during inspiration.

According to the *Law of Laplace* (page 318) in a spherical structure like alveoli, outer force or 'distending' pressure equals two times the tension divided by the radius,

$$i.e.\quad P = \frac{2T}{R}$$

where,
P = distending pressure (outer force)
T = tension on the walls due to surface tension
R = radius (inward force)

Surface tension (T) is the inward force. Therefore, with 'P' constant if 'T' is not reduced as 'R' is reduced during expiration, surface tension may overcome the distending pressure and then lungs will collapse. But in the lungs, with reduction of radius, there is reduction of surface tension by a surface tension lowering agent called *Surfactant*.

A SURFACTANT

It is a mixture of protein-lipid complexes made up of mainly *Dipalmitoyl Phosphatidyl Choline* (DPPC) lipid along with other lipids (phosphatidyl glycine; phospholipids; neutral lipids); proteins and carbohydrates. (PG, PL)

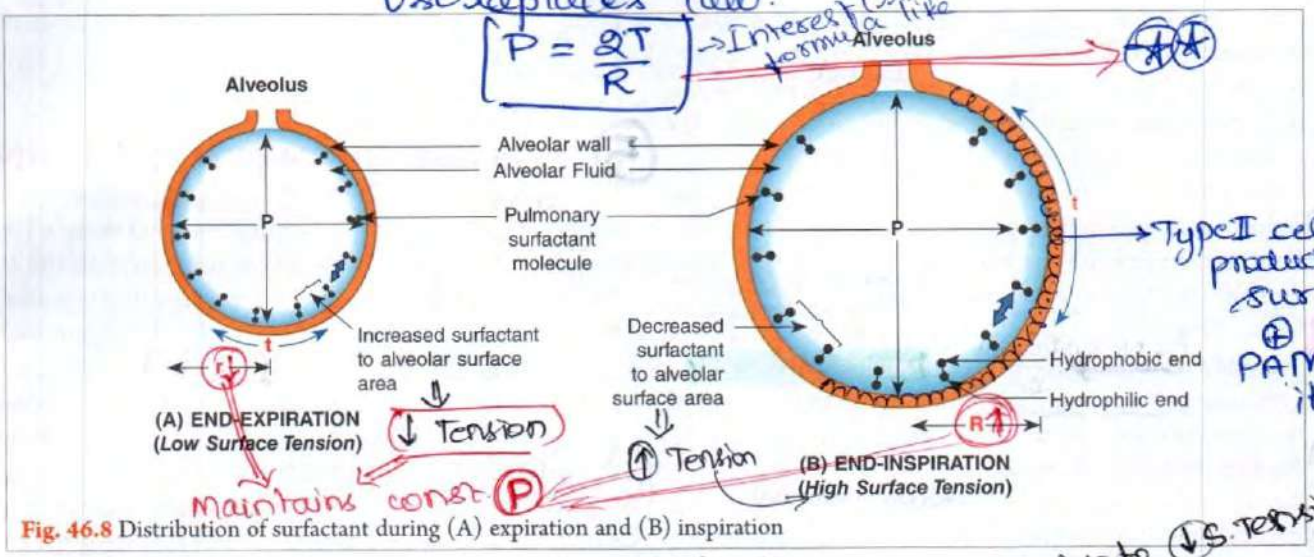


Fig. 46.8 Distribution of surfactant during (A) expiration and (B) inspiration

It is *produced by* granular pneumocytes *i.e.* alveolar lining epithelial type II cells and it comes to the surface by the process of exocytosis. It is removed by pulmonary alveolar macrophages (PAMS).

Actions of Surfactant and its Physiological Significance

1. There is a layer of fluid and air lining the alveoli which causes surface tension. Surfactant reduces the surface tension by forming a layer between the fluid lining the alveoli and alveolar air (Fig. 46.8). Thus, it prevents development of surface tension between the fluid and the air. Surface tension between the fluid and the air is 7-14 times more than that between the surfactant and the air.
2. Surface tension is inversely related to the concentration of surfactant per unit area. Surfactant molecules are spread apart as alveolar size increases during inspiration but come closer during expiration thereby *adjusting surface tension during breathing*. Its action is more effective during expiration than during inspiration. This can be shown by a simple *experiment in cats*. (Fig. 46.9)
   (i) The lungs are removed from the animal's body and are distended *with air* in increment volume to maximum while measuring the intra-pulmonary pressure each time.
   (ii) Repeat the experiment during successive deflation of the lungs.
   (iii) The graph between the intra-pulmonary pressure and the volume, called *pressure-volume (P-V) curve*. It measures both the components, the tissue elasticity and surface tension within the alveoli.
   (iv) The whole experiment is repeated by successively inflating and deflating the lungs *with saline*. As saline reduces the surface tension to nearly 'zero', therefore, pressure-volume curve obtained *with saline* measures the tissue elasticity only. Therefore,
      (a) the pressure-volume curve obtained during inflation and deflation of the lungs with saline are virtually identical, and
      (b) the whole curve shifts to the 'left' of the first curve, since the surface tension decreases to nearly 'zero' which opposes the expansion of the lungs during inspiration.
   (v) The difference between the two curves (curve A and B in Fig. 46.9) measures the elasticity due to surface tension. It is much smaller at smaller than at large lung volumes. Thus the surface tension is low when the alveoli are small.
3. *Surface tension keeps the alveoli dry* and thus helps in exchange of gases. How? Normal pulmonary capillary hydrostatic pressure is 8 to 10 mmHg and oncotic pressure is 25 mmHg.

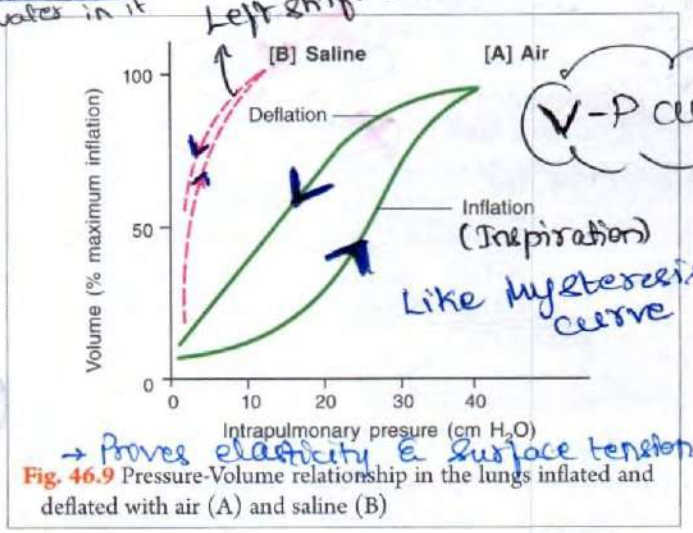


Fig. 46.9 Pressure-Volume relationship in the lungs inflated and deflated with air (A) and saline (B)

This produces an inwardly directed pressure gradient of about 15 to 17 mmHg. Therefore, low pulmonary hydrostatic pressure helps to pull fluid from alveoli into pulmonary capillaries and keeps the alveolar surface free of fluid (also see to page 383).

4. Surfactant *helps to prevent pulmonary oedema* by reducing surface tension. How?
If surfactant was not present, the un-opposed surface tension in the alveoli would produce a 20 mmHg force, which can draw out fluid from the blood capillaries into the alveoli (*i.e.* transudation of fluid) producing pulmonary oedema. Surfactant prevents this phenomenon by lowering the surface tension.

(B) *Factors Affecting Surfactant*

Surfactant *decreases* due to:
(1) long-term inhalation of 100% O_2, (as occurs during cardiac surgery)
(2) occlusion of main bronchus
(3) occlusion of one pulmonary artery,
(4) cigarette smoking, and
(5) cutting both the vagi.

Surfactant *increases* due to:
(1) thyroid hormones, increase the size and number of inclusion bodies in type II alveolar lining epithelial cells, and
(2) glucocorticoids, accelerate the maturation of surfactant.
(3) Absence of GM-CSF (page 66).

Applied

1. ***Hyaline Membrane Disease or Infant Respiratory Distress Syndrome (IRDS).*** It is a serious disease of newborn infants, due to deficiency of surfactant; or it develops in infants born before their surfactant system becomes functional. (*Surfactant is always present by 35 weeks of gestation*). Normally after birth, the infant makes several strong inspiratory movements and the lungs expand. Surfactant keeps them from collapsing again. But due to prolonged immaturity of epithelial Na^+ channels, Cl^- is secreted with the fluid within, the alveoli, resulting in deficiency of surfactant. Therefore, surface tension in the lungs of these infants is very high, and there are many areas in which alveoli are collapsed (*Atelectasis*). Also pulmonary oedema occurs and infants die of pulmonary insufficiency. Infants with "IRDS" show low thyroid hormone and cortisol levels in their plasma.
'IRDS' is also called *hyaline membrane disease* due to formation of *hyaline* (a translucent) membrane from albuminous intrapulmonary fluid in the walls of the alveoli and respiratory bronchioles..
2. ***Patchy Atelectasis*** is seen in patients who have undergone cardiac surgery during which a pump oxygenator is used and the pulmonary circulation is interrupted.

(5) PRESSURE VOLUME RELATIONSHIP: COMPLIANCE

A perfect elastic body like spring follows ***Hooke's Law*** *i.e.* length is directly proportional to force (within the elastic limit). Therefore, greater the force applied, greater will be the length, but once the elastic limits are crossed, the spring tears off. **(Fig. 46.10)**

This relationship indicates stiffness and expansion of the spring. If line on the graph shifts towards left, it shows spring is more expandable *i.e.* less stiff. Conversely, if line on the graph shifts to right, it shows spring is less expandable *i.e.* more stiff.

Same law can be applied to the lungs and thorax which are elastic structures. In lungs we measure 'volume' equivalent to the length and 'pressure' equivalent to the force. Therefore, by increasing the airway pressure, lung volumes can be increased. However, in lungs, the above relationship will not be perfectly linear, as lungs and thorax are not perfect elastic structures. Here, this relationship indicates the *distensibility* (or stretchability) of lungs and thorax. This distensibility is called ***compliance***. Therefore, compliance indicates the distensibility of the lungs and thorax.

(A) COMPLIANCE = $\Delta V / \Delta P$

Definition: The change in lung volume per unit change in airway pressure ($\Delta V / \Delta P$) is the 'distensibility' (compliance) of the lungs and the chest wall.

Where V = volume of the lung
P = airway pressure
Δ = the difference.

It is expressed as litre/cm H_2O.

Compliance is studied under two headings:
(A) Compliance of the lungs and the thoracic wall
(B) Compliance of the lungs only.

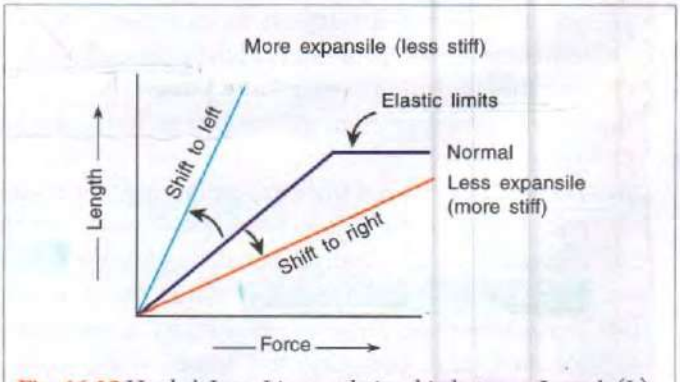


Fig. 46.10 Hooke's Law: Linear relationship between Length (L) and Force (F)

(A) *Compliance of the Lungs and the Thoracic Wall i.e.* lungs inside the thorax.

The lungs are visco-elastic structures. They expand to a certain volume by a certain increase in airway pressure. Similarly, the thorax is a visco-elastic structure so that greater the pressure in the lungs, greater is the expansion of the thoracic wall.

Normal values of compliance of lungs and thorax is 0.13 litre/cm H_2O *i.e.* when there is an increase of airway pressure by 1 cm H_2O then the volume of the lungs inside the thoracic wall increases by 0.13 litre.

(B) *Compliance of the Lungs only i.e.* lungs outside the chest wall.

Normal value: 0.22 litre/cm H_2O *i.e.* when airway pressure is increased by 1 cm H_2O, then the lungs expand by 0.22 litre.

Therefore, compliance of lungs alone = Approx. 2 times the compliance of lungs and thorax. This is so because, inside the thorax, some energy is required to expand the thorax also. Lungs can be distended to such a great degree due to:

(1) Presence of large number of elastic fibers throughout the lung tissue, and

(2) Peculiar arrangement of these elastic fibers; in the same way as an individual nylon fiber cannot be stretched to that extent but whole nylon sock can be stretched to a greater extent, because of the specific knitting arrangement of its fibers.

Measurement of Compliance

Compliance is measured always under static conditions *i.e.* when there is no airflow.

A. Measurement of Compliance of Lung and Thoracic Wall

The interaction between the recoil of the lungs and recoil of the chest wall can be demonstrated in the living subjects as under:

Procedure

1. The subject breaths through mouth with nose clipped through a spirometer from the end-expiratory position. Just beyond the mouthpiece there is a 'valve' and a pressure measuring device to measure the 'airway pressure'. **(Fig. 46.11 A)**
2. The subject inhales a known amount of air from the spirometer, the valve is shut, thus closing off the airways.
3. The subject then holds his breath and relaxes the respiratory muscles; any change in the 'airway pressure' is measured.
4. The whole procedure is repeated after actively inhaling or exhaling various amounts of air upto the maximum.
5. The curve of the *airway pressure* obtained in this way when plotted against volume is called, ***relaxation pressure curve of the total respiratory system*** **(Fig. 46.11 B)**.

Observations and Conclusion

1. It can be seen from the graph that, at end expiratory position when the lung volume is equal to the FRC, the corresponding 'airway pressure' is 'zero' mmHg *i.e.* equal to the atmospheric pressure.
2. The lung volume at which airway pressure is 'zero' mmHg is called *relaxation volume* which is equal to FRC. Therefore, relaxation volume is the point where the recoil of the chest and the recoil of the lungs balance.
3. Increase in lung volume above the relaxation volume, increases the 'airway pressure' because inspiratory muscles are relaxed. At the maximum inspiratory position, airway pressure increases to about 30 mmHg.
4. Conversely, decrease in lung volume, decreases the airway pressure, and at the maximum expiratory position it decreases to about 30 mmHg.
5. The ***pressure-volume relationship*** is almost linear within physiological limits above and below the relaxation point. **(Fig. 46.11 B)**

Factors affecting the combined compliance of lungs and thoracic wall

It is more informative to examine the whole pressure-volume curve. The curve if shifted:

(1) Downwards and to the right, indicates that the 'compliance' is decreased.

Causes

(i) Pulmonary congestion (lungs filled with blood),

(ii) Interstitial pulmonary fibrosis (stiffening and scarring of the lung).

(iii) Pulmonary oedema.

(2) Upwards and to the left, indicates that the 'compliance' is increased.

Causes

(i) Emphysema (page 411), and

(ii) Old age.

In these conditions, loss of elasticity occurs, therefore, more pressure is required to inflate the lungs. But, clinically, it is seen that compliance increases. Why? Not known; it may be due to some modification in the knitting arrangement of the elastic tissues. Therefore, effect of emphysema and old age on compliance is paradoxical.

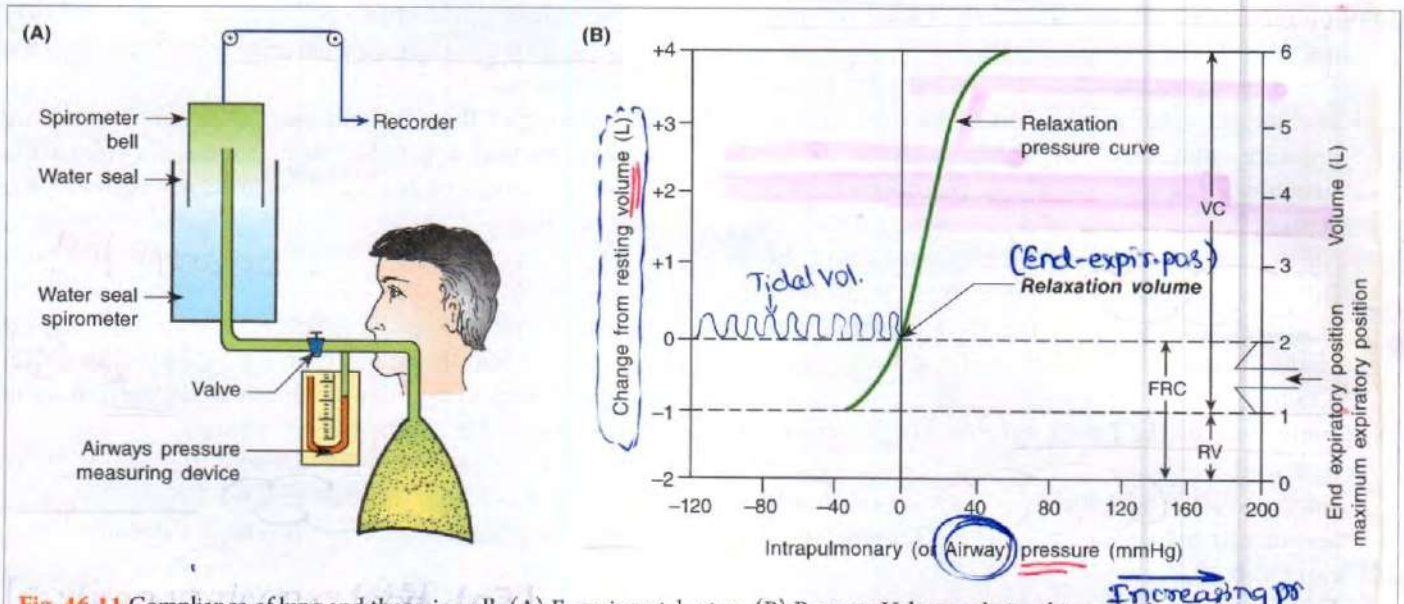


Fig. 46.11 Compliance of lung and thoracic wall: (A) Experimental set up (B) Pressure-Volume relationship and relaxation pressure curve of the total respiratory system.

→ Just see graph)

B. Measurement of Compliance of Lungs Alone

It can be measured by measuring intrapleural pressures at different lung volumes. Intrapleural pressure indicates 'distensibility' of lungs. With more inflation of lungs, intrapleural pressure is more negative since recoil forces are more. Therefore, intrapleural pressure gives an idea about the distensibility of the lungs. Intrapleural pressure is measured clinically by measuring *'intra-oesophageal' pressure.*

Procedure

1. Let the subject inhale certain volume of air from spirograph from end expiratory position (*i.e.* resting position) upto end inspiratory position*i.e.* 500 mL and then hold his breath. Therefore, any change in intrapleural pressure is not altered by the contraction of respiratory muscles.
2. Measure the intrapleural pressure for different air volumes inhaled and exhaled and plot a graph between the two. **(Fig. 46.12)**
3. *Observations and Conclusion*
   (i) AXB and BZA represent pressure volume relationship during inspiration and expiration respectively.
   (ii) *P-V relationship* is curved and different during inspiration and expiration *i.e.* at identical intrapleural pressure, the volume of lung is less in inspiratory phase than in the expiratory phase. This type of curve is called *Hysteresis Curve* by mathematician (Hysteros means to lag behind).
   (iii) The difference in the 'distensibility' (stretchability) of the lungs between inspiratory and expiratory phase accounts for the *Hysteresis Loop* of the compliance.

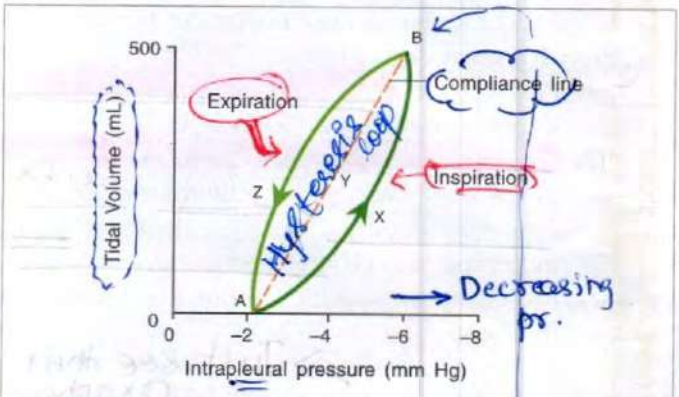


Fig. 46.12 Compliance of lungs alone: Pressure-Volume relationship

(iv) Had the lungs been a perfectly elastic structure then *P-V relationship* would have been linear; and straight line thus formed is called as ***Compliance Line*** (line AYB).
(v) But the line is curved both during inspiration and expiration because of two resistances (see below):
   (a) Viscous resistance, and
   (b) Airway resistance.

How to calculate the compliance of the lungs?

1. Take the point on the graph at the end of inspiration *i.e.* when there is no airflow, thus, there is no airway resistance and no viscous resistance.
2. Calculate ΔV and ΔP.
3. Calculate the compliance as $\Delta V/\Delta P$.
   Normal: 0.22 L/cm H_2O.

This compliance indicates the 'distensibility' of lungs alone due to elastic tissues only.

Factors affecting only the lung compliance

1. *Lung Volume.* An individual with only one lung has approx. 1/2 the change in lung volume (ΔV) for a given change in pressure (ΔP).
2. *Phase of respiratory cycle.* Compliance is slightly greater when measured during deflation than when measured during inflation. Compliance decreases with the inflation of the lungs as more pressure is required to distend the already distended lungs.
3. *Effect of Gravity.* In the standing position compliance is less at the apices of the lungs as there are more distended alveoli at the apex (Why?, page 410).
4. *Surface tension.* If surface tension is high as in deficiency of 'surfactant', more pressure is required to distend the lungs, therefore, compliance is decreased.

SPECIFIC COMPLIANCE

The compliance, when expressed as a function of 'FRC', is called *specific compliance*, i.e. compliance/FRC.

Advantage

In individuals with one lung only, lung compliance is approx. half of the normal compliance. This is inspite of the fact that the remaining lung may be healthy, with normal 'distensibility'.

Similarly in children because of smaller lung volume, compliance will be below normal inspite of normal distensibility. This fallacy is removed with 'specific' compliance since 'FRC' is proportionately reduced, and specific compliance remains essentially constant.

WORK DONE DURING BREATHING

To move the air into the lungs, the muscles of respiration have to do work to overcome all different forms of resistance.

Therefore, work is done by the respiratory muscles:

(A) in stretching the elastic tissue of the lungs and the chest wall, called *Elastic Resistance*. It is the force of elastic recoil exerted by the lung's elastic fibers and force of surface tension;

(B) in moving viscous material of lung tissues, called *Viscous Resistance*;

(C) when air flows through the airways, there is resistance to the airflow, therefore, work is done in moving air through the respiratory passage, is called *Airway Resistance*.

Distribution of various forms of resistances, when expressed as percentage of the total resistance, is:

1. *Elastic Resistance:* 65%,
2. *Non-Elastic Resistance:* 35%:
   (i) Viscous resistance: 7%,
   (ii) Airway resistance: 28%.

AIRWAY RESISTANCE

As we know flow in a tube, F = P/R.

Where,

P = pressure difference between two ends of the tube (P_1–P_2)

R = resistance to airflow

therefore,

R = P/F *i.e.*

$$\text{Airway resistance} = \frac{\text{pressure difference between atmospheric and alveolar pressure (cm } H_2O)}{\text{Airflow (L/sec)}}$$

Normal airway resistance is 1.5 to 2 cm H_2O/L/sec, *i.e.* when there is a fall of pressure by 2 cm H_2O, there is air flow of 1 L/sec (compared with smoker's pipe, for same flow rate, pressure difference of 500 cm H_2O is required).

Factors Affecting Airway Resistance

1. *Total cross-sectional area*

Resistance to the air flow is inversely related to the total cross-sectional area of the respiratory passage. This greatly increases from 2.5 cm^2 in the trachea to 11,800 cm^2 in the alveoli. Therefore, the resistance to the air flow is high in the 'conducting zone', whereas it is low in the 'respiratory zone'. *Why so?*

Where the diameter (cross-section) is bigger, the resistance against the air flow is smaller and vice versa. Although the diameter of the trachea is bigger than that of a single fine bronchus of any particular generation, yet the combined value of the diameter *i.e.* total cross-section, of the fine bronchi of a generation is always greater than that of the trachea. Thus the resistance to the air flow is greater when air is passing through the trachea and big divisions of the bronchi. Actually approx. 80% of the total airway resistance is offered by the trachea and the bigger divisions of the bronchi upto 7th generation.

2. According to *Poiseuille-Hagen formula*, the relation between the flow in a long narrow tube, the viscosity of fluid and the radius of the tube is expressed as follows:

$$F = (P_A - P_B) \times (\pi/8) \times (1/\eta) \times (r^4/L) \quad \ldots (i)$$

where

F = flow

$P_A - P_B$ = pressure difference between two ends of the tube

$\eta_{(eta)}$ = Viscosity

L = Length of the tube

r = radius of the tube

$$\text{Since Flow} = \frac{\text{Pressure difference between two ends of the tube } (P_A - P_B)}{\text{Resistance (R)}} \quad \text{.. (ii)}$$

Therefore, from (i) and (ii), it follows

$$R = \frac{8\eta L}{\pi r^4}$$

Thus, $R \propto$ 'η' and 'L'
$\propto 1/r^4$

Therefore, if the radius decreases by 'half', keeping the other factors constant, the resistance increases by '16 times'; but this does not occur in our body because as diameter of the airways is large, there is no difficulty in breathing.

Physiological significance

(i) *Duration of expiration is 1.2 times the duration of inspiration* because,
(a) expiration is a passive process, and
(b) airway resistance is high during expiration as diameter of airways decreases.

(ii) *In bronchial asthmatic patients* (or after histamine injection) bronchoconstriction develops and diameter of the airways decreases causing increased resistance to the air flow, eventually producing difficulty in breathing. However, inspiration is possible as expansion of bronchioles does take place but during expiration, the already constricted bronchioles are constricted further causing marked increase in airway resistance and there is extreme difficulty in expiration. Therefore, *bronchial asthma is regarded as a disease of expiratory obstruction.*

3. ***Type of flow***

Airway resistance is more in 'turbulent' flow (*e.g.* during rapid respiration) than in 'laminar' flow or streamline flow (*e.g.* in quiet breathing) (page 317).

The type of airflow depends on ***Reynold's Number (Re)***:

$$Re = \frac{V\, d\, \rho}{\eta}$$

where V = velocity of airflow
d = diameter
ρ = density of air
η = viscosity

Therefore, 'Re' increases when V,r,d increases or η decreases.

When 'Re' rises above 200 to 400, 'turbulent flow' will occur at some branches of tracheo-bronchial tree, but will die out along the smooth portion of the tree. However, when 'Re' exceeds 2000, there usually occurs turbulent flow even in a straight, smooth bronchi and its branches and the airway resistance increases.

Important Note

Increased atmospheric pressure, increases density of gas and can increase resistance to airflow. Therefore, when one goes for deep sea diving he must use low density gas like helium (page 473).

Calculation of Work Done

Since pressure times volume (gm/cm^2 × cm^3 = gm × cm) has the same dimensions as work (force × distance), the work of breathing can be calculated from the *relaxation pressure curve* (page 418) during inspiration and expiration.

Work Done during Inspiration

Area AXBCA represents total work done during inspiration **(Fig. 46.13)**. *Pressure-volume relationship* could be linear (straight line AYB) if lungs are perfectly elastic structures *i.e.* no *non-elastic* resistance is present. However, during inspiration the line is curved to overcome the non-elastic resistance.

Therefore, area AXBYA represents work done during inspiration to overcome *non-elastic resistance i.e.* airway resistance and lung viscosity.

Area AYBCA represents work done to overcome *elastic resistance.*

Energy is required to overcome these resistances which is provided by contraction of inspiratory muscles. Most of the energy is used in overcoming *elastic resistance.*

Work Done during Expiration

Less work is required during expiration because it is a passive process due to the passive recoil of the lungs. When the lungs are recoiling back some energy is required to overcome *non-elastic resistance i.e.* resistance due to airways and viscous material. Area AYBZA is the work done to overcome *non-elastic resistance* during expiration. Normally this falls within the area AYBCA.

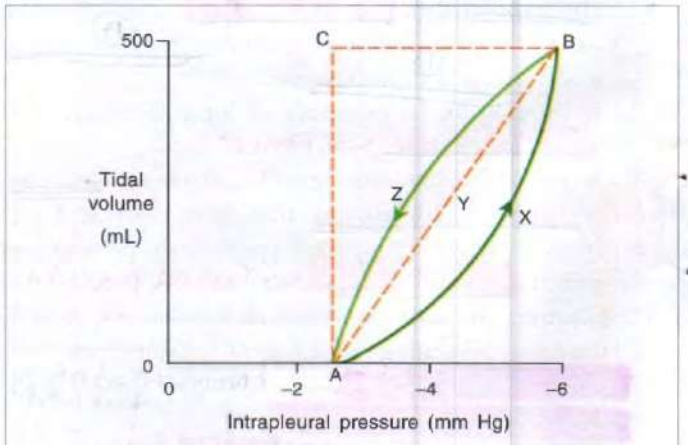


Fig. 46.13 Calculation of work done (Also see **Fig. 46.12**)

Since during expiration no contraction of muscles occurs then what is the source of energy?

Some amount of energy derived from contraction of inspiratory muscles is stored in the expanded elastic fibers and during expiration this energy is utilized to overcome the non-elastic resistance. The difference between the area AYBZA and AYBCA represents the work dissipated as heat.

The amount of elastic work required to inflate the whole respiratory system is less than the amount required to inflate the lungs alone, because part of the work comes from elastic energy stored in the thorax. The elastic energy lost from thorax is equal to that gained by the lungs.

Total amount of work done on lungs and thorax during normal respiration can be measured by placing the subject in a chamber called *RESPIRATOR* and by measuring continuously the pressure difference between lungs and outside body and measuring corresponding volume changes.

Therefore, total work done during normal respiration = Pressure × volume.

Normally total work done during quiet breathing ranges from 0.3 to 0.8 kg-mt/min. A healthy adult person at rest spends less than 5% of total oxygen consumption on breathing.

Factors affecting total work done

1. Increases markedly during exercise (physiological).
2. Causes of pathological increase:
   (i) Emphysema
   (ii) Bronchial asthma
   (iii) Congestive heart failure with dyspnoea
   (iv) Orthopnoea

Why does work done increases in these conditions?

The higher the breathing rate, the faster the flow rates and the larger the 'viscous work' area AXBYA. On the other hand, larger the tidal volume, the larger the 'elastic work' area AYBCA.

Important Note

This is why patients with severe airway obstruction often breath slowly; while patients with reduced compliance, tend to take small rapid breaths. In both cases, there is a trend to reduce the work done on the lungs.

7 ALVEOLAR VENTILATION

A ALVEOLAR VENTILATION (V_A)

Alveolar ventilation is the amount of air ventilating the alveoli per minute. Therefore,

Alveolar Ventilation
= (Tidal Volume – Dead Space) × Respiratory Rate
= (500 – 150) × 12
= 4.2 L/min.

Physiological significance of alveolar ventilation

Since respiration involves the gaseous exchange of O_2 and CO_2 by diffusion between the alveoli and pulmonary capillary blood, therefore, maintenance of volume of alveolar ventilation is of paramount importance. All the factors which affect the tidal volume (TV), dead space (DS) and respiratory rate (RR), affect the alveolar ventilation. How?

For example, it can be seen from Table 46.1 that in rapid shallow respiration (*Tachypnoea*), alveolar ventilation decreases though the pulmonary ventilation remains normal, as a result less air is available for exchange; while in slow and deep respiration, both alveolar and pulmonary ventilation are normal.

B DEAD SPACE

It is the amount of air in the 'respiratory passage' which does not take part in exchange of gases. It is of two types:
(1) *Anatomical dead space*
(2) *Physiological (total) dead space*

1. Anatomical Dead Space

It is the volume of air present in the 'conducting zone' of the respiratory passage *i.e.* from nose and mouth upto terminal bronchioles (page 400) where exchange of gases does not take place.

Normal value: 150 mL (approx. equal to the body weight in pounds).

2. Physiological Dead Space or Total Dead Space

It includes anatomical dead space plus volume of air in the alveoli which does not take part in exchange of gases (*i.e.* wasted alveolar ventilation).

Table 46.1: Effect of variation of tidal volume (TV), dead space (DS) and respiratory rate (RR) on alveolar ventilation

	Normal	Rapid shallow respiration i.e. Tachypnoea	Slow and deep respiration
TV	500 mL	200 mL	1000 mL
RR	12/min	30/min	6/min
DS	150 mL	150 mL	150 mL
Alveolar ventilation	4.2 L/min	1.5 L/min	5.1 L/min
Pulmonary ventilation	6 L/min	6 L/min	6 L/min

For example,

(i) Volume of inspired air which ventilates alveoli but receives no pulmonary capillary blood flow.

(ii) Some of the alveoli may be over ventilated *i.e.* volume of inspired air which ventilates alveoli in excess of that volume which is required to equilibrate with the blood.

Clinically anatomical and physiological dead spaces are same in healthy subjects. If ventilation and perfusion are not in equilibrium, then only they differ in volume.

Variations

1. **Physiological**
   (i) Sex: DS is more in males.
   (ii) Age: DS increases with age, because inflated lungs pull the airways thereby increasing the airway diameter.
   (iii) Body height: DS increases in proportion with increase in body height.
2. **Pathological**
   (i) Emphysema: loss of elasticity of lungs in emphysema decreases elastic recoil, this produces hyperinflation of lungs to cause increase in DS.
   (ii) Bronchiectasis: It is associated with dilated bronchi, thereby causes DS to increase.
   (iii) Pulmonary embolism which produces regional decrease in pulmonary vascular bed.

Measurement of Anatomical Dead Space

Two Methods: (1) Direct and (2) Indirect Method.

(1) *Direct Method:* It is based on ***Bohr's equation,*** which states that expired air volume equals the alveolar air volume and inspired air volume in dead space, *i.e.*

Expired air volume
= Alveolar air volume + DS, *or*
Expired air volume × CO_2 % in Expired air
= (Alveolar air volume × CO_2% in alveolar air) + (Dead space × CO_2 % in DS)
= (Expired air volume – DS) × CO_2 % in alveolar air
(Because alveolar air volume = Expired air volume minus DS, and CO_2% in inspired air is 0.03%, hence neglibile.)

$$\text{Thus DS volume} = \frac{\text{Expired air volume} \times (CO_2\ \%\ \text{in alveolar air} - CO_2\ \%\ \text{in expired air})}{CO_2\ \%\ \text{in alveolar air}}$$

Therefore, by measuring the expired air volume and CO_2 % in expired and alveolar air, DS volume can be determined.

(2) ***Indirect Method*** – *Single Breath Oxygen Technique*
Here, anatomical DS can be measured with the aid of 'nitrogen meter', which allows the rapid and continuous measurement of N_2 concentration of the inspired and expired air. When the subject is breathing atmospheric air, the N_2 concentration of expired air is approx. 80%. After quiet (resting) expiration the subject takes a 'deep' breath of pure (*i.e.* 100%) O_2 and then breathes out slowly and evenly. First portion of O_2 goes to the alveolar region and last O_2 portion will be lying in tracheobronchial tree.

Findings (refer **Fig 46.14A***)*

(i) during inspiration as pure O_2 is coming in, N_2 percentage will be 'zero' in N_2 meter;
(ii) during initial expiration, pure O_2 comes out from the dead space and N_2 percentage will be 'zero' (between I and II);
(iii) subsequently, the N_2 content of the remainder of expiration rises rapidly to reach a plateau at say 60% N_2.
(iv) If a square front were preserved between the dead space gas and the alveolar gas, the expired N_2

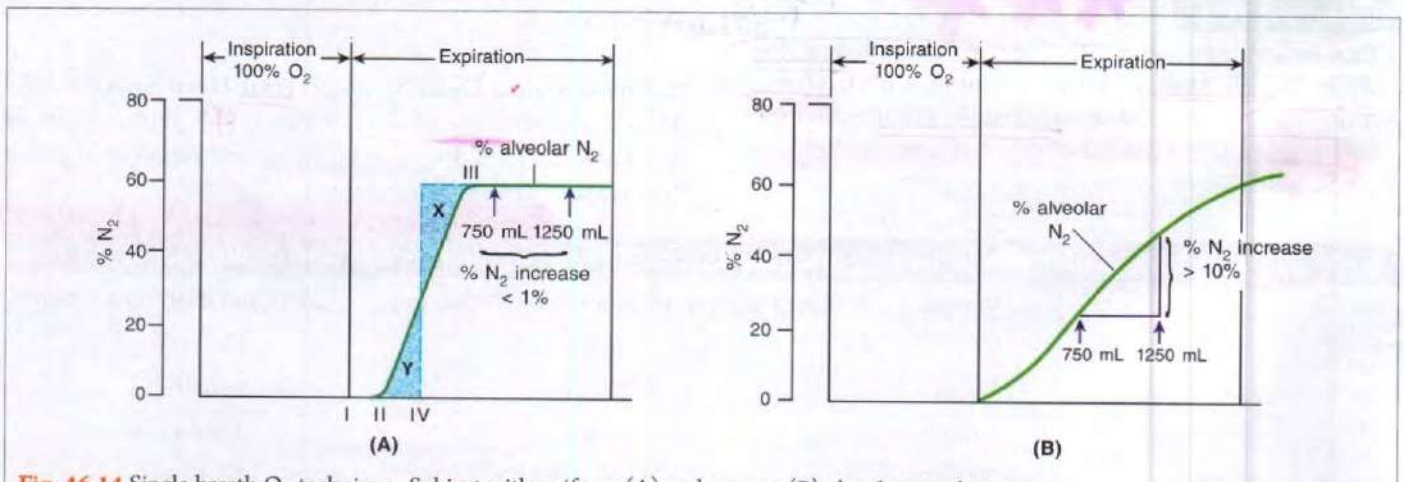


Fig. 46.14 Single breath O_2 technique: Subject with uniform (A) and uneven (B) alveolar ventilation

concentration would remain zero until a volume equal to that of the anatomical dead space was expired and would then rise sharply to the final plateau concentration. However, this is not the case, as there is some mixing between the DS and alveolar gases during the expiration (*i.e.* between II and III).

Therefore, by placing a vertical line on the record in such a position, that the shaded area 'X' is equal to the shaded area 'Y', the volume expired upto this point (IV) is the anatomical dead space.

Measurement of Physiological (Total) Dead Space

According to Bohr's equation, Expired air gas volume = Alveolar air volume + DS volume. Considering the partial pressure of gases instead of CO_2 %, because here exchange is taking place at alveolar level and gaseous exchange at respiratory membrane depends on difference in partial pressure of gases. Therefore,

Expired air volume × pCO_2 in Expired air
= Alveolar air volume × pCO_2 in Alveolar air + DS × pCO_2 in Inspired air (Negligible)
= (Expired air volume – DS volume) × Alveolar air pCO_2
= (Expired air volume – DS volume) × Arterial pCO_2
(because alveolar pCO_2 = Arterial pCO_2)

$$\text{Therefore, DS volume} = \frac{\text{Expired air volume} \times (pCO_2 \text{ in Alveolar air} - pCO_2 \text{ in Expired air})}{pCO_2 \text{ in Alveolar air}}$$

pCO_2 is chosen because CO_2 concentration of inspired air is negligible (0.03%), therefore, complete equilibrium is taking place at the alveolar level, while pO_2 varies from alveoli to pulmonary capillary blood.

Uniformity of Alveolar Ventilation

In healthy subject at rest, alveolar ventilation is uniform and averages 2 – 2.5 L/m^2/min. In diseased lungs there may be unevenness of ventilation. This can be detected by using 'single breath O_2 technique' (see above). In the healthy subject the N_2 concentration of the air expired rises very little (+1%) if measured between an expired volume of 750 mL and 1250 mL. In the diseased lung, for example, in asthma, emphysema, pneumothorax, fibrosis or congestive heart failure, the N_2 concentration rises appreciably as the expired air volume increases from 750 to 1250 mL **(Fig. 46.14B)**.

→ Govt made law. The law enforcement throughout LUNGS (country) is IMP.

VENTILATION PERFUSION RATIO: V/P RATIO

It is the ratio of *alveolar ventilation* to *pulmonary blood flow.*

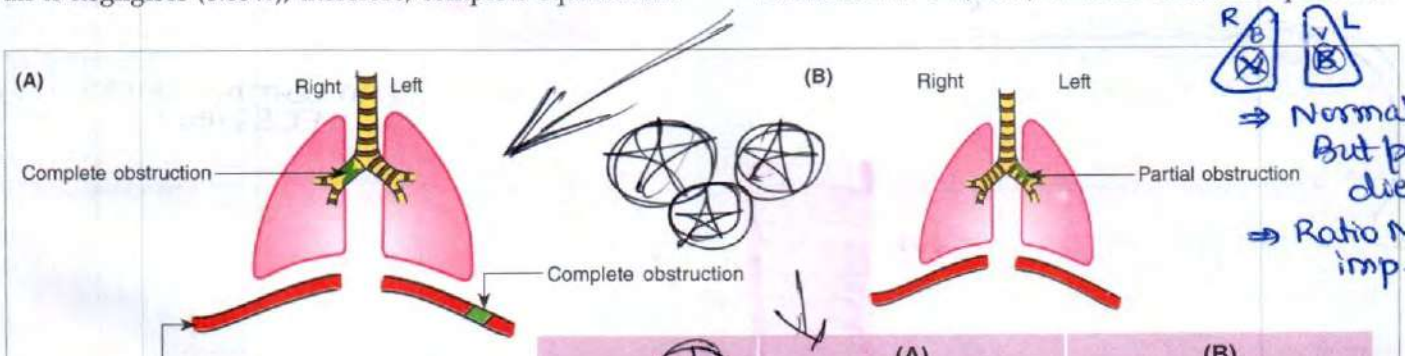
As alveolar ventilation is 4 L/min and pulmonary blood flow, 5 L/min, therefore, ventilation perfusion ratio = 4 L/min divided by 5 L/min = 0.8.

Each lung normally displays the same ratio, receiving 2 L of air and 2.5 L of blood respectively.

But total ratio is *not* important. More important is that whether this ratio is present uniformly throughout the lungs for proper oxygenation. If it is not present, the oxygenation will be defective.

Example 1 **(Fig. 46.15 A)**

Suppose in right lung, there is full obstruction so no air can enter into the lungs but pulmonary blood flow is 5 L/min. In case of left lung, there is complete obstruction to pulmonary blood flow and alveolar ventilation is 4 L/min, so total ventilation-perfusion



	(A)		(B)	
	Right Lung	Left Lung	Right Lung	Left Lung
Alv. vent (L/min)	—	4	3.5	0.5
Blood flow (L/min)	5	—	2.5	2.5
V/P ratio	zero	α	1.4 (> 0.8)	0.2 (< 0.8)
Total V/P ratio (both lungs)	4/5 = 0.8		4/5 = 0.8	

Fig. 46.15 Variations in V/P ratio

⇒ Normal ratio But person dies
⇒ Ratio NOT imp.

ratio is 4/5 = 0.8 *i.e.* normal, but patient will die of complete lack of O_2.

Example 2 **(Fig. 46.15B)** Suppose in right lung, alveolar ventilation is 3.5 L/min and in left lung due to partial obstruction it is 0.5 L/min, and pulmonary blood flow in both lungs is normal 2.5 L/min. Then, ventilation-perfusion ratio for right lung = 3.5/2.5 = 1.4 (more than 0.8), and for left lung = 0.5/2.5 = 0.2 (less than 0.8).

Though ventilation-perfusion ratio is more than normal in right lung, but it will go as ***wasted ventilation***, *i.e.* it will add to physiological DS and hence, oxygenation will be defective.

Factors affecting V/P Ratio

(1) Physiological Causes

Effect of gravity: In normal subjects during sitting or standing position there is a *steady* increase in alveolar ventilation as well as pulmonary blood flow from the apex to the base (bottom) of the lung; however, the alveolar ventilation does not increase *linearly* from apex to the base of the lung **(Fig. 46.16)**. Thus, the *V/P ratio declines in a linear fashion from the apices to the bases of the lung; How?*

(i) In apical region

Because of gravitational forces, intra-pleural pressure in standing position is more negative (–7 mmHg) at the apices of the lungs compared to the bases (– 2 mmHg). This causes the alveoli to expand, decreasing the alveolar ventilation at the apices of lungs. Perfusion is also reduced to a *greater extent* at the apex of the lungs relative to at the base because the upper portions of the lungs are well above the level of the heart as a result alveolar pessure exceeds the arterial pressure. Therefore, V/P ratio at apices is high (3.4) **(Fig. 46.16)**.

Important Note

High V/P ratio in apices predisposes to tuberculosis (T.B.), because high alveolar pO_2 provides favourable environment for the growth of tuberculosis bacteria.

(ii) In basal region

Increase in the alveolar ventilation is much less relative to perfusion causing V/P ratio to fall below normal to 0.63. Therefore, pO_2 in the alveoli falls because less O_2 is delivered to it and pCO_2 rises as less CO_2 is expired **(Fig. 46.16)**.

(2) Pathological Causes

All the factors which cause uneven alveolar ventilation or non-uniform blood flow to pulmonary circulation will alter the V/P ratio.

(i) Causes of uneven alveolar ventilation

(a) Bronchial asthma
(b) Pneumothorax
(c) Emphysema
(d) Pulmonary fibrosis
(e) Congestive heart failure.

(ii) Causes of non-uniform pulmonary blood flow

(a) Anatomical shunts *e.g.* Fallot's tetralogy (page 457)
(b) Regional decrease in pulmonary vascular bed *e.g.* emphysema
(c) Pulmonary embolism
(d) Increased pulmonary resistance due to fibrosis, pneumothorax, congestive heart failure etc.

Obviously, uneven alveolar ventilation may exist without any abnormality of pulmonary blood flow and vice-versa or both may be non-uniform *e.g.* as in emphysema.

Location	Alv. vent.	Blood flow	V/P ratio	pO_2	pCO_2	pN_2	pH_2O	Relative pressure
Upper zone (Apex)	1.92 ↓	0.56 ↓↓↓	3.4	132	28	553	47	Alv. P > Art. P > Venous P
Middle zone	4.0	5.0	0.8	100	40	573	47	Art. P > Alv. P > Venous P
Lower zone (Base)	6.5	10.3	0.63	89	42	582	47	Art. P > Venous P > Alv. P

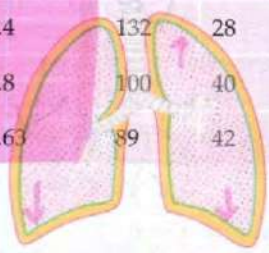


Fig. 46.16 Alveolar ventilation (L/min), blood flow (L/min), V/P ratio and gas tension (mmHg) at different zones of the lung in a normal upright subject (during sitting or standing) Alv. P and Art. P : Alveolar and Arterial pressure respectively.

Important Note

When a person is supine, blood flow is uniform throughout the lung. When a person is standing, blood flow is slowest at the apex and highest at the base of the lung due to the effect by gravity.

Assessment for uniformity of V/P ratio

(1) Measure physiological and anatomical DS. If physiological DS greatly exceeds anatomical DS, there must be alveoli which have a high V/P ratio.

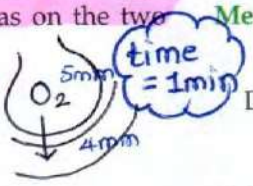
(2) Continuously measure the CO_2 content of expired air, normally it changes little in its CO_2 content during the completion of the breath. If the CO_2 content of the last part of the alveolar gas is much higher than the initial value, the V/P ratio is non-uniform, being high initially and low in the alveoli contributing to the last part of the tidal air.

i.e., Initial expired CO_2 content << Final expired CO_2 content

DIFFUSION CAPACITY OF LUNGS

Definition: It is the amount of a gas that crosses the *alveolar capillary membrane* (respiratory membrane) per minute per mmHg difference in partial pressure of gas on the two sides of the membrane.

Normal diffusion capacity for O_2 (D_{O_2}) = 20-30 mL/min/mmHg at rest. (NAIH)



Factors affecting diffusion capacity (D_C)

1. **Surface area and thickness of alveolar capillary membrane**

$$\text{Diffusion Capacity } (D_C) = \frac{\text{Surface area of alveolar capillary membrane (Normal: 70M}^2\text{)}}{\text{Thickness of alveolar capillary membrane (Normal: 0.5 µm)}}$$

In healthy subjects rapid diffusion of O_2 and CO_2 occurs within 0.3 secs across the alveolar capillary membrane. As D_C varies directly with surface area and inversely with thickness of the alveolar capillary membrane, therefore,

(i) D_C of lungs decreases when there is block in pulmonary capillary or block in the airway because of decrease in surface area of the membrane.

(ii) D_C of lungs increases markedly (>3 times) in exercise because of increase in surface area of the membrane due to (a) opening of closed capillaries, and (b) vasodilatation.

(iii) D_C of lungs decreases in pulmonary oedema due to collection of fluid in the alveolar capillary membrane which results in increased thickness of the membrane.

2. **Solubility** and square root of the **molecular weight (MW)** of the gas.

$$D_C = \frac{\text{Solubility of the gas}}{\sqrt{\text{MW of the gas}}}$$

Fick's law of diffusion

i.e. D_C is directly proportional to solubility of the gas.

As solubility of $CO_2 > O_2 > CO$ and MW: CO_2 (44); O_2 (32); CO (28)

Ultimately, it follows that $D_{O_2} = 1.23 \times D_{CO}$ and $D_{CO_2} = 24.6 \times D_{CO}$, and

$D_{CO_2} = 20.7 \times D_{O_2}$ *i.e.* diffusion capacity of CO_2 is approx. 20 times than that of O_2.

Clinical significance

In diseases due to defect in alveolar capillary membrane, diffusion capacity decreases and should produce retention of CO_2 with lack of O_2; but the patients suffer from only lack of O_2 with very little signs of retention of CO_2, because D_{CO_2} is 20 times than that of D_{O_2}.

$D_{CO_2} = 20 \times D_{O_2}$

Measurement of diffusion capacity

$$D_{O_2} = \frac{O_2 \text{ consumption/min}}{\text{partial pressure difference of } O_2 \text{ on two sides of the membrane (mmHg)}}$$

$$D_{O_2} = \frac{O_2 \text{ consumption/min}}{\text{Mean alveolar } pO_2 \text{ minus mean pulmonary capillary } pO_2}$$

O_2 consumption/min can be easily measured but it is difficult to measure the pulmonary capillary pO_2.

Therefore, D_{O_2} is measured indirectly by measuring D_C of carbon monoxide (D_{CO}).

$$D_{CO} = \frac{\text{CO uptake/min}}{\text{Mean alveolar pCO} - \text{Mean pulmonary capillary pCO}} \quad \text{.. (i)}$$

Important Note

CO is chosen because affinity of CO to combine with haemoglobin is 210 times more than that of O_2. But since CO is a poisonous gas, therefore, given in very little concentration.

pulmon. capil. $PCO_2 = 0$

Procedure

1. Subject takes very small amount of CO and then measure:
   (i) CO uptake/min
   (ii) mean alveolar pCO
   (iii) mean pulmonary capillary pCO is 'zero', because most of the CO combines with haemoglobin and amount of CO in plasma is zero.
   (As partial pressure exerted by a gas in blood is the property of dissolved gas.)
2. Calculate D_{CO} from (i), then $D_{O_2} = D_{CO} \times 1.23$.
   (The normal value of D_{CO} at rest is 25 mL/min/mmHg)

$\therefore D_{CO} < D_{O_2} < D_{CO_2}$ (1.23, 20)

Study Questions

1. **Draw well labelled diagram:**
   (i) Change in intra-pleural and intra-pulmonary pressure with respiratory cycle
   (ii) Timed vital capacity and its clinical significance
   (iii) Pressure-volume relationship of lungs alone
   (iv) Lung volume and capacities
   (v) Distribution of surfactant during inspiration and expiration
   (vi) Relaxation pressure curve of the total respiratory system
   (vii) Compliance of lungs alone
   (viii) Variation in V/P ratio in various zones of lungs
2. **Write short notes on:**
   (i) Major muscles involved in respiration and role of each.
   (ii) Factors affecting intra-pulmonary and intra-pleural pressure
   (iii) Surfactant and its actions. Explain the factors responsible for its maturation
   (iv) Hyaline membrane disease
   (v) Factors affecting vital capacity
   (vi) Advantage of residual volume to the lungs
   (vii) Factors affecting compliance of lungs
   (viii) Specific compliance and its significance
   (ix) Factors affecting total dead space
   (x) V/P ratio and its physio-clinical significance
   (xi) Factors affecting V/P ratio
   (xii Factors affecting diffusion capacity of lungs
   (xiii) Factors determining alveolar ventolation
   (xiv) Airway resistance and factors affecting it
3. **Give physiological basis/Explain:**
   (i) Most comfortable position of the diaphragm is in sitting position
   (ii) Valsalva and Muller's manoeuvre
   (iii) Straining at stool is not advisable in old patients
   (iv) Compliance of lungs alone is greater than the combined compliance
   (v) Lungs compliance increases in old age
   (vi) Airway resistance is high in conducting zone as compared to the respiratory zone
   (vii) Expiratory phase is longer than the inspiratory phase of respiratory cycle
   (viii) Bronchial asthma is a disease of expiratory obstruction
   (ix) During deep sea diving, one must use low density gas
   (x) Why patients with severe airway obstruction often breathe slowly
   (xi) High V/P ratio at the apices of the lung
   (xii) Patients with defects in respiratory membrane suffer initially only from hypoxia symptoms and not of hypercapnia.
   (xiii) Pressure changes during ventilation
   (xiv) What keep the alveoli dry?
4. **Justify/Explain:**
   (i) Lungs first expand and facilitate air entry or entry of air into the lungs makes them expand.
   (ii) Which is more dangerous situation, rapid shallow respiration or slow deep respiration.
5. **What will happen:**
   (i) If bilateral paralysis of diaphragm muscle occurs
   (ii) if surfactant is deficient at birth
   (iii) if intrapulmonary pressure increases
   (iv) if pleura gets damaged
   (v) if intrapleural pressure is increased
   (vi) if FEV1 decreases but FVC is normal
   (vii) if lungs lose their elasticity
   (viii) if chest wall is opened during operative procedure
   (ix) if airway resistance is increased?
   (x) to respiration if spinal cord gets transected above 3rd cervical segment?
   (xi) to respiration if atmospheric pressure increases
6. **Give physio-clinical significance of:**
   (i) Vital capacity
   (ii) Timed vital capacity
   (iii) Functional residual capacity
   (iv) $FEF_{25-75\%}$
   (v) $FEF_{200-1200\ ml}$
   (vi) Dyspnoeic index
   (vii) Alveolar ventilation
   (viii) Surfactant
   (ix) Dead space
   (x) Reynold's number to type of airflow
   (xi) Diffusion capacity of carbon monoxide

MCQs

1. **Eupnoea means:**
   (a) Rhythmic normal breathing at rest
   (b) Difficulty in breathing
   (c) Cessation of breathing
   (d) Consciousness of breathing at rest

2. **For each 1 cm descent of diaphragm, the amount of air sucked into the lungs is:**
   (a) 100-200 mL (b) 200-300 mL (c) 300-400 mL (d) 400-500 mL

3. **Tendency of the lungs to recoil from the chest wall is balanced by the tendency of chest wall to recoil in the opposite direction at:**
   (a) End expiratory position (Relaxing volume)
   (b) Maximum expiratory position
   (c) End inspiratory position
   (d) Maximum inspiratory position

4. **Which of the following volume is expected to be equal in both men and women?**
   (a) Inspiratory reserve volume
   (b) Tidal volume
   (c) Residual volume
   (d) Vital capacity

5. **Vital capacity provides useful information about:**
   (a) Size and development of the chest wall
   (b) Strength of respiratory muscles
   (c) Patency of respiratory passage
   (d) Pulmonary ventilation

6. **The volume of gas contained in the lung at the end of maximum inspiration is:**
   (a) Functional residual capacity
   (b) Inspiratory capacity
   (c) Inspiratory reserve volume
   (d) Total lung capacity

7. **Spirometry measures all *except*:**
   (a) Vital capacity
   (b) Inspiratory reserve volume
   (c) Functional residual capacity
   (d) Functional residual volume

8. **Timed vital capacity differs from vital capacity:**
   (a) Higher volume of air breathed out
   (b) Special stress on rapid, forcible and complete exhalation
   (c) Cannot be measured by simple spirometer
   (d) Provides useful information about strength of respiratory muscles

9. **Most sensitive index to assess severity of obstructive lung disorders is:**
   (a) Forced vital capacity
   (b) FEV_1
   (c) Maximum mid expiratory flow rate
   (d) Functional residual capacity

10. **The most important factor that tends to collapse the lungs is the:**
    (a) Intrapleural fluid pressure
    (b) Total intrapleural pressure
    (c) Surface tension of the alveolar fluid
    (d) Tension in the intercostal muscles

11. **Surfactant causes:**
    (a) Increased compliance of lungs
    (b) Decreased compliance of lungs
    (c) Secreted by bronchus
    (d) Absent at birth

12. **The size and number of inclusions in the type II alveolar epithelial cells producing surfactant are increased by:**
    (a) Growth hormone (b) Androgens (c) Thyroxine (d) Insulin
    + Cortisol

13. ***False* statement for relaxation volume of lungs is:**
    (a) Lung volume at which airway pressure is 'zero' mmHg
    (b) Equal to FRC
    (c) Point where the recoil of the chest and recoil of the lungs balance
    (d) 1250 mL

14. **Maximum effort during normal respiration is done for which purpose?**
    (a) Lung elasticity
    (b) Respiratory air passages
    (c) Alveolar air spaces
    (d) Creating negative pleural pressure

15. **Major percentage of airway resistance is offered by the:**
    (a) Trachea and bigger bronchi ($\because R \propto \frac{1}{SA}$)
    (b) Terminal bronchioles
    (c) Respiratory bronchioles
    (d) Alveolar ducts

16. **A swimmer breathing through a pipe has a respiration rate of 10/min, a tidal volume of 550 mL and an effective anatomic dead space of 250 mL. What is his alveolar ventilation?**
    (a) 2500 mL/min (b) 3000 mL/min (c) 3500 mL/min (d) 4000 mL/min

17. **Ventilation/perfusion ratio is ratio of:**
    (a) Pulmonary ventilation to pulmonary blood flow
    (b) Minute ventilation to pulmonary capillary pressure
    (c) Alveolar ventilation to pulmonary blood flow
    (d) Expired air volume to intra-pulmonary pressure

18. **In health, physiological dead space is:**
(a) Double than that of anatomical dead space
(b) Less than that of anatomical dead space
(c) Triple than that of anatomical dead space
(d) Equal to anatomical dead space

19. **Diffusion capacity is the volume of gas diffusing:**
(a) Each second for pressure difference of 100 mmHg
(b) Each minute for pressure difference of 1 mmHg
(c) Each minute for pressure difference of 100 mmHg
(d) Each second for pressure difference of 1 mmHg

20. **Diffusion capacity of CO_2 is approx. 20 times than that of O_2 because of:**
(a) Higher solubility coefficient
(b) Low solubility coefficient
(c) Smaller particle size
(d) Larger surface area of respiratory membrane available for CO_2

21. **Bilateral phrenic nerve paralysis results in:**
(a) Death
(b) Adequate ventilation to maintain life
(c) Is fatal without artificial respiration
(d) Normal respiration

22. **Respiratory movements are associated with fluctuations in blood pressure. The highest fall in cardiac output occurs at the time of the:**
(a) Late inspiration-early expiration
(b) Forceful expiration
(c) Beginning of inspiration
(d) Late expiration-early inspiration

23. **Which of the following pulmonary volume for average young man is true?**
(a) Tidal volume: 1200 mL
(b) Inspiratory reserve volume: 500 mL
(c) Residual volume: 3000 mL
(d) Expiratory reserve volume: 1000 mL

24. **Closing volume of lung determines:**
(a) Transmural pressure (b) Residual volume (c) Small air way resistance (d) Dead space

25. **All are the causes of decrease in vital capacity *except*:**
(a) Old age (b) Pregnancy (c) Lying position (d) Divers

26. **Vital capacity is decreased but timed vital capacity is normal in:**
(a) Bronchial asthma (b) Scoliosis (c) Chronic bronchitis (d) Respiratory muscles weakness

27. **Maximum mid expiratory flow rate (MMEFR) indicates flow obstruction in:**
(a) Large airways (b) Small airways (c) Trachea (d) Trachea and bronchi

28. **In the event of lungs losing their elasticity:**
(a) Chest expands and becomes barrel shaped
(b) Lungs collapse
(c) FRC decreases
(d) Vital capacity increases

29. **All statements are *true* in an infant with hyaline membrane disease *except*:**
(a) Associated with low thyroid hormone
(b) Surface tension of the alveoli is low
(c) It is a serious disease
(d) There is patchy atelactasis

30. **Lungs which easily expand:**
(a) Have low compliance
(b) Have high compliance
(c) Owe their distensibility to a low level of surfactant
(d) Have high collagen/elastic fibre ratio

31. **Elastic resistance during breathing is exerted by all of the following *except*:**
(a) Lung's elastic fibers
(b) Force of surface tension
(c) Force of elastic recoil of lungs and chest wall
(d) Viscous material of lung tissues

32. ***False* about bronchial asthma is:**
(a) Diameter of small airways decreases
(b) Airway resistance is high during inspiration
(c) Normal expansion of bronchioles does take place during inspiration
(d) Regarded as a disease of expiratory obstruction

33. **A person with rapid shallow breathing has his alveolar ventilation:**
(a) Normal (b) Increased (c) Decreased (d) Equal to pulmonary ventilation

Answers

1. (a)	2. (b)	3. (a)	4. (b)	5. (b)	6. (d)	7. (c)	8. (b)	9. (b)	10. (c)	11. (a)	12. (c)	13. (d)	14. (a)	15. (a)
16. (b)	17. (c)	18. (d)	19. (b)	20. (a)	21. (b)	22. (b)	23. (d)	24. (a)	25. (d)	26. (b)	27. (b)	28. (a)	29. (b)	30. (b)
31. (d)	32. (b)	33. (c)												

Chapter

47

Transport of Gases

I. Oxygen Transport: Oxygen-haemoglobin dissociation curve

II. Carbondioxide Transport

OXYGEN TRANSPORT

INTRODUCTION

1. **Distribution of oxygen in the body**

	pO_2 (mmHg)	O_2 content
(i) Inspired air	158	21 mL/dL
(ii) Expired air	116	16 mL/dL
(iii) Alveolar air	100-104	13-14 mL/dL
(iv) Arterial blood	98-100	19 mL/dL
(v) Venous blood	40	14 mL/dL

Therefore,

(i) for each 100 mL of inspired air, 5 mL of O_2 is extracted by the blood; and

(ii) for each 100 mL of arterial blood, 5 mL of O_2 is extracted by the tissues.

2. **Significance of alveolar pO_2**

(i) pO_2 difference across the alveolar capillary membrane determines the diffusion of O_2.

(ii) It is practically kept constant, because air is continuously going to the blood and coming from inspired air.

(iii) It determines the pO_2 and O_2 content of arterial blood.

3. **Arterial pO_2** is 98-100 mmHg. It should be equal to alveolar pO_2 but attains low value due to the fact that the blood leaving the pulmonary capillaries with oxygen tension equal to the alveolar pO_2 is joined by:

(i) venous blood from the bronchial circulation (normally there is extensive anastomosis between the bronchial and pulmonary capillaries), and

(ii) venous blood from anterior cardiac and thebesian veins, which directly empty into the cavities of the left heart.

Arterial pO_2 decreases when inspired air pO_2 decreases at high altitude.

Arterial pO_2 remains unaltered even during exercise, as velocity of blood flow increases during exercise.

4. **Venous blood pO_2** at rest is 40 mmHg. It varies according to degree of body activity, since active tissue will utilize more O_2 and pO_2 in venous blood decreases *e.g.*

(i) *in moderate activity* venous pO_2 = 25 mmHg

(ii) *in violent exercise* venous pO_2 = 15 mmHg

CARRIAGE OF OXYGEN IN THE BLOOD

O_2 is carried in the blood in 2 forms:

(A) in dissolved form; and

(B) in combination with haemoglobin.

A. Dissolved Form

In dissolved form, amount of O_2 is 0.3 mL per 100 mL of blood per 100 mmHg pO_2. Amount of dissolved O_2 increases in linearity with arterial pO_2 *i.e.* greater the arterial pO_2, more the amount in dissolved form. The tension (partial pressure) exerted by a gas in blood is the property of dissolved gas and not the combined form (*Henry Law*, page 403).

B. Combined with Haemoglobin

Each haemoglobin molecule has 4 heme groups which have an iron in ferrous form. Sixth valency bond of each Fe^{2+} combines with 1 mole (2 atoms) of O_2. Therefore, 4 moles (8 atoms) of O_2 combine with one mole of haemoglobin. The reaction is rapid requiring <0.01 sec. The deoxygenation (reduction) of Hb_4O_8 is also very rapid. The O_2 carrying power of haemoglobin is given by ***Oxygen Haemoglobin Dissociation Curve*** *i.e.* the curve relating percentage O_2 saturation of the haemoglobin to the pO_2. It has a characteristic *sigmoid* shape. **(Fig. 47.1)**

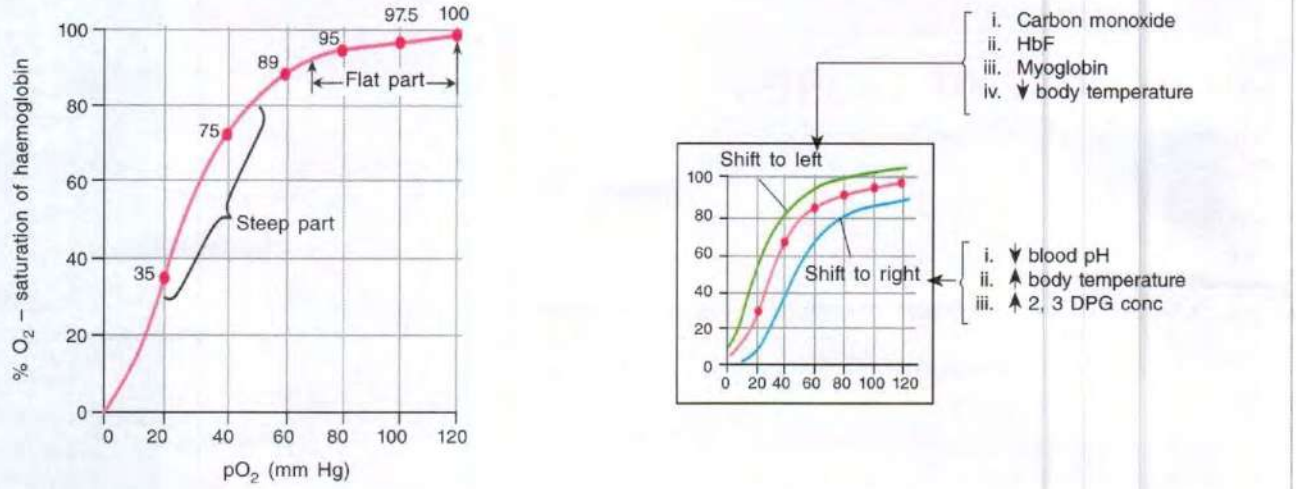


Fig. 47.1 Oxygen-haemoglobin dissociation curve at pCO_2 40 mmHg, pH 7.4 and temperature 37°C; and factors affecting it (inset)

Why curve is sigmoid shape?

(1) **Due to shifting affinity of haemoglobin for O_2.**

In deoxyhaemoglobin, the globin portion is tightly bound in a *Tense (T) Configuration* thereby reduces the affinity of the molecule for O_2. Combination of molecule with O_2 releases the bonds holding the globin units, producing a *Relaxed (R) configuration*. This results in exposure of more O_2 binding sites and affinity of haemoglobin molecule with O_2 increases. However, all 4 atoms of Fe^{2+} do not combine with O_2 immediately and simultaneously. If they do so then line should be vertical. The combination is a step-wise process and affinity for O_2 is different at different steps *e.g.* combination of 1st heme in the haemoglobin molecule with O_2 increases the affinity of the 2nd heme for O_2; and oxygenation of the 2nd increases the affinity of the 3rd and so on. Therefore, the affinity of haemoglobin for the 4th O_2 molecule is many times that for the 1st. This shifting affinity of haemoglobin for O_2 due to *T-R interconversion* produces characteristic sigmoid shape:

$$Hb_4 + O_2 \rightleftharpoons Hb_4O_2$$
$$Hb_4O_2 + O_2 \rightleftharpoons Hb_4O_4$$
$$Hb_4O_4 + O_2 \rightleftharpoons Hb_4O_6$$
$$Hb_4O_6 + O_2 \rightleftharpoons Hb_4O_8$$

(2) If we increase the **concentration of salts and electrolytes**, the curve becomes more sigmoid shape, therefore, it may be due to presence of salts.

At maximum pO_2 of approx. 120 mmHg, haemoglobin gets saturated to full capacity and at this stage 1 gm of haemoglobin can combine with 1.34 mL of O_2.

As normal average haemoglobin concentration is 15 gm/dL, therefore, 100 mL of blood can carry $1.34 \times 15 = 20$ mL of O_2 at full saturation, this determines the *O_2 carrying capacity of blood.*

Important Note

99% of the O_2 that dissolve in the blood combines with the haemoglobin; and presence of haemoglobin within the RBCs increases the O_2 carrying capacity of the blood 70-folds.

Factors affecting O_2-haemoglobin dissociation curve

(A) ***Shift to Right*** *i.e.* at any pO_2, the O_2 content that can be held by blood decreases, causing "unloading" of O_2.

Causes

1. *Fall in blood pH* due to (i) increased CO_2 or (ii) presence of any acid in blood
2. *Increase in body temperature*
3. *Increase in concentration of 2, 3, diphosphoglyceric acid (2, 3 DPG).*

2, 3 DPG, a product of glycolysis, is very plentiful in RBCs. It is a highly charged anion that binds to the β-chains of deoxyhaemoglobin. It competes with O_2 for the binding sites on the haemoglobin molecule and, therefore, at a given pO_2 the percentage saturation of haemoglobin with O_2 will be reduced in the presence of 2, 3 DPG. **(Table 47.1)**

All the factors which shift the O_2-haemoglobin dissociation curve to right, decrease the affinity of haemoglobin for O_2, therefore, a higher pO_2 is required for haemoglobin to bind a given amount of O_2. Therefore, CO_2 enters the blood from tissues and helps unloading of O_2. This phenomenon is called ***Bohr Effect*** (*Bohr* 1904), *i.e.* the decrease in O_2 affinity of haemoglobin when pH of blood falls (or in simple words, Bohr's effect is *loading of CO_2 to blood causes unloading of O_2*, a phenomenon seen at tissue level).

Table 47.1 : Factors affecting concentration of 2, 3 DPG in the RBCs

Decrease	Increase
(i) Acidosis (low blood pH) – inhibits glycolysis in RBCs	(i) Hormones: thyroid, androgen; GH
(ii) Blood stored in acid citrate buffer in blood bank. (This is not seen if the blood is stored in citrate phosphate-dextrose solution. Also see to page 111)	(ii) Anaemia
	(iii) Exercise
	(iv) High altitude
	(v) Increased body temperature
	(vi) Chronic hypoxia

Important Notes

(i) Reduced haemoglobin (deoxyhaemoglobin) binds H^+ more actively than does oxyhaemoglobin.

(ii) The exercising muscles which are acidic, hot and have high pCO_2 benefit from increased unloading of O_2 in their capillaries.

(B) ***Shift to Left*** *i.e.* affinity of haemoglobin to combine with O_2 increases, causing less release of O_2 to the tissues.

Causes

1. Carbon monoxide
2. Foetal haemoglobin (HbF)
3. Myoglobin
4. Decrease in body temperature.

(1) Effect of carbon-monoxide (CO)

CO shifts the curve to 'left' due to inhibition of synthesis of 2, 3 DPG. Affinity of 'CO' to combine with haemoglobin is 210 times more than that of O_2. Higher affinity of CO for haemoglobin produces large proportions of haemoglobin as carboxy-haemoglobin (COHb) and, therefore, haemoglobin is unavailable for O_2 carriage.

(2) Foetal haemoglobin (HbF)

Affinity of HbF for 2, 3 DPG is considerably less than that of HbA (because of poor binding of 2, 3 DPG by the γ-polypeptide chain). Therefore, HbF shifts the curve to left *i.e.* a lower pO_2 is required to bind a given amount of O_2. Thus, affinity of HbF to combine with O_2 is more than that of adult haemoglobin (HbA). At pO_2 20 mmHg where HbA is only 35% saturated with O_2, HbF is more than 70% saturated, that is why HbF can store more O_2. However, foetus never suffers from hypoxia, as it requires less O_2 for having low metabolic activities.

(3) Role of Myoglobin

(i) It is an iron-containing pigment found in greater quantities in muscles specialized for sustained contraction *e.g.* muscles of leg and heart muscles.

(ii) It contains only one heme group with one polypeptide chain *i.e.* one atom of iron per molecule, therefore, its MW is 1/4 MW of haemoglobin.

(iii) It takes up O_2 at low pressure much more readily than does blood *i.e.* rate of association of myoglobin with O_2 is very fast. Thus, its dissociation curve is a *rectangular hyperbola* rather than a sigmoid curve. (Fig. 47.2)

(iv) It does not show Bohr effect, as a result even at pO_2 of 40 mmHg it is 95% saturated with O_2; and when pO_2 falls below 5 mmHg, it becomes < 60% saturated. Therefore, myoglobin acts as a temporary O_2 storehouse in the muscles.

Physiological significance of O_2-haemoglobin dissociation curve

It consists of 2 parts:

1. *Flat (horizontal) top part* in 70-100 mmHg range, and
2. *Steep part,* below 40 mmHg.

1. *Significance of flat top part.* Suppose pO_2 of inspired air falls from 100 to 70 mmHg, then

(i) Percentage saturation of haemoglobin falls from 97.5 to 92.5% *i.e.* amount of oxygen carried by the blood does not change much even if pO_2 drops to 70 mmHg; and

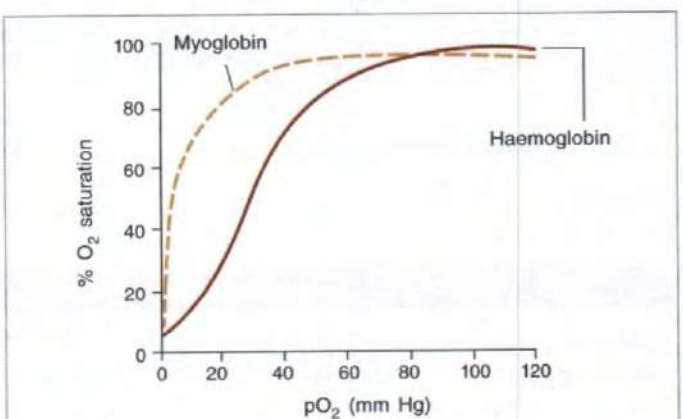


Fig. 47.2 Dissociation curve for haemoglobin and myoglobin at 37°C; pCO_2 40 mmHg and pH 7.4.

(ii) O_2 content of arterial blood falls from 20 mL/dL to 18 mL/dL only. Thus at moderate altitude (upto 12000 feet) subjects suffer little impairment in their uptake of O_2 by the body, *i.e.* can tolerate changes in atmospheric pressure without compromising the O_2 carrying capacity of haemoglobin.

2. *Significance of steep part.* When pO_2 falls below venous pO_2 40 mmHg, curve becomes steeper causing more release of O_2 from haemoglobin to prevent tissues suffering from hypoxia.

Fall in pH, rise in CO_2 and rise in body temperature, *shift the curve to 'right'* but flat part does not undergo any significant change; only steep part becomes more steeper and is affected the maximum, thus causing more release of O_2.

Significance of P_{50}: P_{50} means the pO_2 at which the haemoglobin is half (50%) saturated with O_2. Its normal value is 26 mmHg, at pCO_2 40 mmHg, pH 7.4 and temperature 37°C. It is a convenient index to tell the haemoglobin affinity for O_2. Haemoglobin affinity for O_2 is an *inverse function* of P_{50} value *i.e.* higher the P_{50}, the lower the affinity of haemoglobin for O_2.

VEHICLES FOR OXYGEN TRANSPORT

Vehicles for the transport of O_2 are:

1. *Plasma*
2. *Haemoglobin solutions*, and
3. *Blood*

Which vehicle is ideal for transport of O_2, can be determined from the *oxygen-dissociation curve, i.e.* determining O_2 content at different pO_2 for each vehicle. The vehicle which can give off more O_2 to tissues at lower pO_2 is the ideal vehicle for transport of O_2. **(Table 47.2)**

Therefore, blood is an ideal vehicle for transport of O_2, to load itself in lungs with O_2 and to unload O_2 in tissues.

CARRIAGE OF OXYGEN IN THE BODY

A. In the Tissues

1. ***At Rest* (Table 47.3)**

(i) Because of partial pressure gradient, at rest, tissues remove 5 mL of O_2 for each 100 mL of blood passing through them.

As cardiac output is 5 L/min, therefore, approx. $5/100 \times 5000 = 250$ mL of O_2/ min is transported from blood to the body tissues, called ***O_2 consumption*** of the whole body at rest.

(ii) *Coefficient of O_2 utilization* *i.e.* percentage of O_2 utilized out of the amount which is made available to the tissues, *i.e.*

O_2 taken by tissues/O_2 content of arterial blood: $5/19 = 0.26$ or 26%.

2. ***During Activity***

Changes which take place in the body are:

(i) Increase in CO_2 production

(ii) Rise in body temperature

(iii) Increase in H^+ concentration

Factors (i) to (iii) shift ***Oxygen-haemoglobin dissociation*** curve to right, therefore, more O_2 is given off to tissues at any pO_2. This can increase O_2 uptake by tissues to 750 mL/min *i.e.* 3 times the resting level.

(iv) Increase capillary density (increase in number of open capillaries).

(v) Local arteriolar dilatation.

Factors (iv) and (v) increase blood flow to the tissues.

Table 47.2: Amount of oxygen held by different vehicles

Vehicle	Amount of Oxygen: In arterial blood at pO_2 100 mmHg	Amount of Oxygen: In venous blood at pO_2 40 mmHg	Amount of Oxygen: Given to tissues	Remarks
1. In plasma	0.3 mL/dL	0.12 mL/dL	0.18 mL/dL	(1) and (2) are insufficient to meet the tissue demand, therefore, they are not good vehicles for O_2 transport.
2. In haemoglobin solution	19-19.5 mL/dL	18 mL/dL	1-1.5 mL/dL	
3. In Blood	19 mL/dL	14 mL/dL	5 mL/dL	Ideal vehicle for O_2 transport.

Table 47.3: Carriage of oxygen in the blood

	pO_2	O_2 content	Form in which O_2 is carried
Arterial blood	100 mmHg	19 mL/dL	(i) 0.3 mL/dL in dissolved form (ii) 18.7 mL/dL bound to haemoglobin
Venous blood	40 mmHg	14 mL/dL	(i) 0.12 mL/dL in dissolved form (ii) 13.88 mL/dL bound to haemoglobin

(vi) Depending on degree of activity, O_2 tension within tissues may fall to 'zero'. This results in very steep O_2 pressure gradient between blood and tissues, thereby causing rapid diffusion of O_2. Thus, coefficient of O_2 utilization varies from 65-80%.

(vii) Increase in RBC count due to splenic contraction.

Factors (iv) to (vii) increase O_2 delivery to tissues by another 5 times. This along with 3 times more O_2 extraction by the tissues eventually increases the O_2 transport to tissues by 15 times.

B. In the Lungs

Venous blood pO_2 is 40 mmHg and alveolar air pO_2 is 100 mmHg.

Thus, because of pressure gradient O_2 rapidly diffuses from alveoli through the thin pulmonary and capillary endothelium into the plasma, therefore, arterial blood finally leaves the lungs almost fully saturated with O_2 (97% saturated), at pO_2 100 mmHg with O_2 content of 19 mL/dL; 0.3 mL/dL in dissolved form and 18.7 mL/dL bound to haemoglobin.

CARBON DIOXIDE TRANSPORT

INTRODUCTION

Tissue activity produces CO_2 which enters the blood due to:

(1) Difference in pCO_2 between arterial blood and tissues. Arterial blood pCO_2 is 40 mmHg and tissues pCO_2 is 46 mmHg.

(2) CO_2 has high diffusion coefficient, 20 times more than that of O_2; therefore, even this small pressure gradient of 6 mmHg is sufficient for CO_2 transport.

(3) Decrease in O_2 content, shifts *"CO_2 dissociation"* curve to 'left', causing further loading of CO_2 from the tissues to the blood.

CARRIAGE OF CO_2 IN THE BLOOD

(1) CO_2 content of arterial blood is 48 mL/dL and that of venous blood is 52 mL/dL. Therefore, each 100 mL of arterial blood which passes through tissues picks up 4 mL of CO_2.

(2) As a rule, CO_2 first gets accommodated in plasma; when plasma becomes fully saturated, then it is accommodated in the RBCs. Thus, of the total 4 mL/dL of CO_2 transported in the blood, 60% (2.4 mL/dL) is transported in plasma and remaining 40% (1.6 mL/dL) within the RBCs.

(3) CO_2 is carried in the plasma and RBCs in 3 forms:
(i) In dissolved form (0.3 mL/dL)
(ii) As carbamino compounds (0.7mL/dL), and
(iii) As bicarbonate (3 mL/dL).

(i) ***In dissolved form*** *(0.3 mL/dL)*

(a) *In plasma*

CO_2 as it enters the plasma, (1) a part goes to solution as CO_2, and (2) remaining in small amounts with water forms carbonic acid (H_2CO_3).

$$CO_2 + H_2O \xrightarrow{\text{15-20 min}} H_2CO_3$$

This is a slow reaction because of absence of enzyme carbonic anhydrase (CA) in plasma and only 0.2 mL/dL of CO_2 is transported in plasma in dissolved form.

(b) *In RBCs*

Here, CO_2 gets rapidly hydrated to form H_2CO_3 due to presence of enzyme 'CA' in the RBCs. However, only 0.1 mL/dL of CO_2 is transported in the RBCs in dissolved form.

$$CO_2 + H_2O \xrightarrow{\text{'CA'}} H_2CO_3 \quad \text{(rapid reaction)}$$

(ii) ***As carbamino compounds*** *(0.7 mL/ dL)*

(a) *In plasma*

CO_2 combines directly with plasma proteins to form 'cabamino protein'. This is very slow reaction, therefore, only 0.1 mL/dL of CO_2 is transported this way.

$$CO_2 + PrNH_2 \longrightarrow PrNHCOOH$$

(b) *In RBCs*

CO_2 with amino group of haemoglobin forms 'carbamino haemoglobin'; comparatively, a fast reaction and 0.6 mL/dL of CO_2 is transported in this way.

$$CO_2 + HbNH_2 \longrightarrow HbNHCOOH$$

(iii) ***As bicarbonates*** *(3 mL/dL)*

(a) *In plasma*

CO_2 is carried in plasma as sodium bicarbonate ($NaHCO_3$). How?

– *by phosphate buffer*

$$CO_2 + H_2O \downarrow$$

$$H_2CO_3 + Na_2HPO_4$$
(disodium hydrogen phosphate – basic)

$$NaHCO_3 + NaH_2PO_4$$
(mono sodium dihydrogen phosphate – acidic)

– *by protein reduction*

HCO_3^- and proteins both act as acid and compete for the base. During this process, proteins get

reduced and some Na^+ from proteins shifts to HCO_3^- forming $NaHCO_3$. 2.1 mL/dL of CO_2 is transported in plasma by these mechanisms.

(b) *In RBCs*

CO_2 is carried within the RBCs as potassium bicarbonate ($KHCO_3$). How?

Within RBCs, (1) haemoglobin is a strong H^+ acceptor and (2) RBCs are rich in enzyme carbonic anhydrase (CA), therefore, irreversible reaction can take place within 1-2 sec.

$$CO_2 + H_2O \xrightarrow{\text{'CA'}} H_2CO_3 \longrightarrow H^+ + HCO_3^-$$

H^+ are buffered by haemoglobin rapidly and prevent the reaction in the opposite direction, as a result, haemoglobin is reduced but HCO_3^- formation is rapid and in large amounts due to presence of 'CA'. HCO_3^- combines with intracellular K^+ forming $KHCO_3$. By this process 0.9 mL/dL of CO_2 is transported in the RBCs.

VEHICLES FOR CO_2 TRANSPORT

Vehicles for transport of CO_2 are: (a) *plasma*, (b) *bicarbonate solution* and (c) *blood*. Which vehicle is ideal for transport of CO_2 can be determined by studying the *'CO_2-dissociation' curve i.e.* curve relating CO_2 concentration to pCO_2. (**Table 47.4, Fig. 47.3**)

In tissue capillaries, blood O_2 concentration decreases producing deoxygenated (reduced) blood with pO_2 40 mmHg. In lung capillaries, blood O_2 concentration increases and blood becomes oxygenated with pO_2 100 mmHg. So, two separate "CO_2 dissociation" curves are there; one for deoxygenated blood with pO_2, 40 mmHg and second for oxygenated blood with pO_2 100 mmHg.

1. In deoxygenated blood with maximum pCO_2 60-67 mmHg, CO_2 content is 65 mL/ dL, called the ***maximum venous point***.
2. In oxygenated blood at pCO_2 40 mmHg, CO_2 content is 48 mL/dL, called the ***arterial point***.

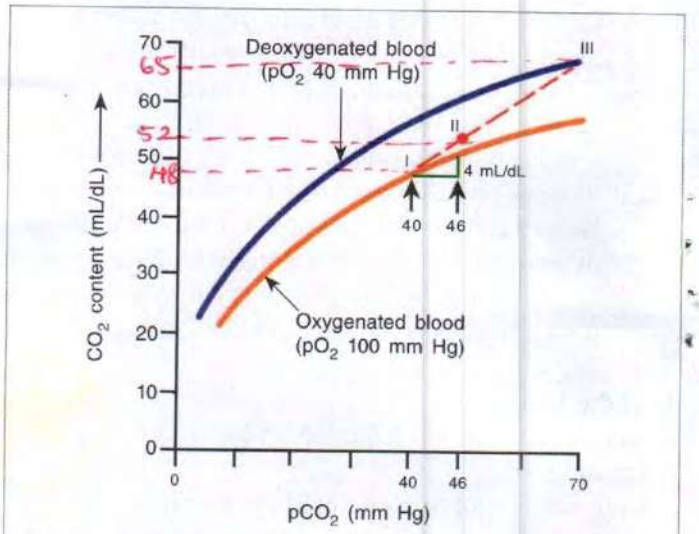


Fig. 47.3 'CO_2 dissociation' curve in whole blood (i) Arterial point, (ii) venous point; (iii) Maximum venous point; Dotted line (i) to (iii) is Physiological CO_2 dissociation curve.

If we join the *maximum venous point* and *arterial point*, which corresponds to extreme CO_2 levels in the body respectively, it will roughly reflect changes between pCO_2 and CO_2 content in the body and is called the ***Physiological CO_2 Dissociation Curve***. At rest with venous pCO_2, 46 mmHg, where it cuts the "physiological dissociation curve", CO_2 content is 52 mL/dL, called the ***venous point***. It can be seen that, when the haemoglobin is oxygenated, the CO_2 dissociation curve shifts to 'right' *i.e.* the blood begins to lose some CO_2 as it becomes oxygenated. This is called ***Haldane Effect***. In simple words *loading of oxygen to the blood causes unloading of CO_2*, a phenomenon seen at the lung level.

Factors affecting CO_2 dissociation curve

1. **Increase in body temperature** causes release of O_2 from blood, this shifts the curve to 'left' *i.e.* larger amount of CO_2 can be taken at a given pCO_2.

Table 47.4: Amount of CO_2 held by different vehicles

	Amount of CO_2			
Vehicle	**In venous blood at pCO_2 46 mmHg**	**In arterial blood at pCO_2 40 mmHg**	**Taken by the blood**	**Remarks**
(a) In plasma	1.8 mL/dL	1.6 mL/dL	0.2 mL/dL	CO_2 transport is very less, therefore not a good transport medium for CO_2.
(b) In bicarbonate solution	48 mL/dL	48 mL/dL	Nil	When pCO_2 increases 40 mmHg there is no further transport of CO_2, therefore it is also not a good transport medium.
(c) In blood	52 mL/dL	48 mL/dL	4 mL/dL	Ideal transport medium for CO_2

2. Decrease in pO_2 shifts the curve to 'left', thereby helps in loading of CO_2.
   This shows deoxygenation of haemoglobin *i.e.* reduced haemoglobin carries more CO_2 at any level of pCO_2.

Chloride Shift or Hamburger Phenomenon
(Hamburger 1918) **(Fig. 47.4)**

1. As the blood passes through the capillaries, the rise in the HCO_3^- content of RBC is much greater than that in the plasma. Approx. 70% of HCO_3^- formed in RBCs, enters the plasma along its concentration gradient. Therefore, electrical equilibrium is disturbed within RBCs.
2. Normally protein anions cannot cross the cell membrane; and Na^+ and K^+ do not diffuse freely due to the operation of Na^+–K^+ pump. Therefore, electrochemical neutrality is maintained quickly within 1 sec by diffusion of Cl^- from plasma into RBC *(Chloride Shift)*. This process is mediated by *anion exchanger – 1 (AE –1)*, which continues till electrical equilibrium is attained within RBCs. The Cl^- content of RBC in venous blood is, therefore, significantly greater than the arterial blood.
3. For each CO_2 molecule added to the RBC, there is an increase of one osmotically active particle, either an HCO_3^- or Cl^- in the RBC. Therefore, RBC takes up water and increases in size. Thus there are swollen RBCs in venous blood as compared to arterial blood, that is why *haematocrit of venous blood is normally 3% greater than that of the arterial blood*. In the lungs, the Cl^- moves out of the RBCs and they shrink.

CARRIAGE OF CO_2 IN THE LUNGS

1. Venous blood pCO_2 is 46 mmHg with CO_2 content 52 mL/dL; whereas alveolar air pCO_2 is 40 mmHg with CO_2 content 48 mL/dL. Therefore, for each 100 mL of venous blood, while passing through lungs releases 4 mL CO_2.
2. Since cardiac output is 5 L/min, "CO_2 output" from the body is $4 \times 5000/100 = 200$ mL/min.
3. The sequence of reactions gets reversed when pulmonary blood reaches the alveoli *i.e.* of 4 mL, (i) 0.3 mL (7%) comes from breakdown of CO_2 in dissolved solution; (ii) 0.7 mL (18%) from carbamino compounds and (iii) 3 mL (75%) from bicarbonates.
4. Sequence of events in the lungs is as follows:
   (i) A part of CO_2 from dissolved solution and carbamino compound breaks up to liberate CO_2.
   (ii) Haemoglobin becomes oxygenated forming oxyhaemoglobin (acidic) which increases the acidity of the cell to mobilize Cl^- shift in the reverse order. Cl^- of KCl comes out of the cell, reacts with $NaHCO_3$ in plasma, forming NaCl and liberating HCO_3^-.
   (iii) HCO_3^- from plasma enters the cells, combines with free K^+ forming $KHCO_3$.
   (iv) Within the RBCs, oxyhaemoglobin (HbO_2) being stronger acid than carbonic acid (H_2CO_3), releases H^+ from haemoglobin. The released H^+ joins with HCO_3^- released from $KHCO_3$ forming H_2CO_3 and free K^+ with HbO_2 forms $KHbO_2$.
   (v) H_2CO_3 is broken up by the carbonic anhydrase in RBC into water and CO_2. CO_2 diffuses out into the plasma and since there is nothing to fix it, CO_2 there is liberated through the lungs along the pressure gradient.

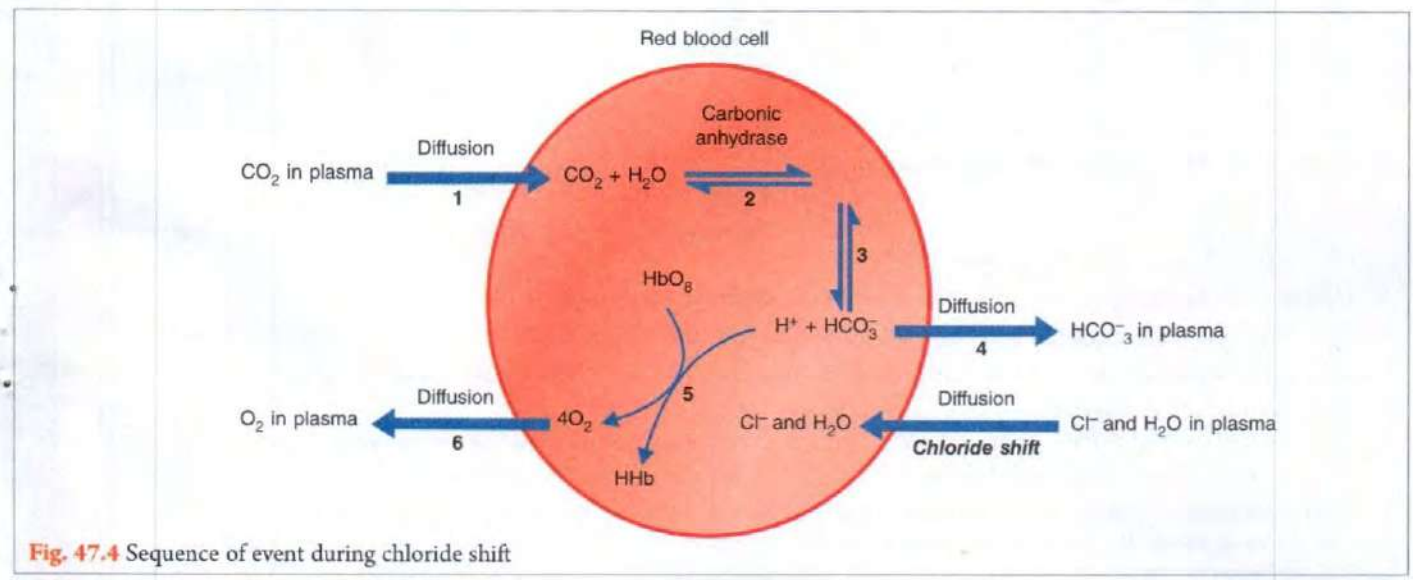
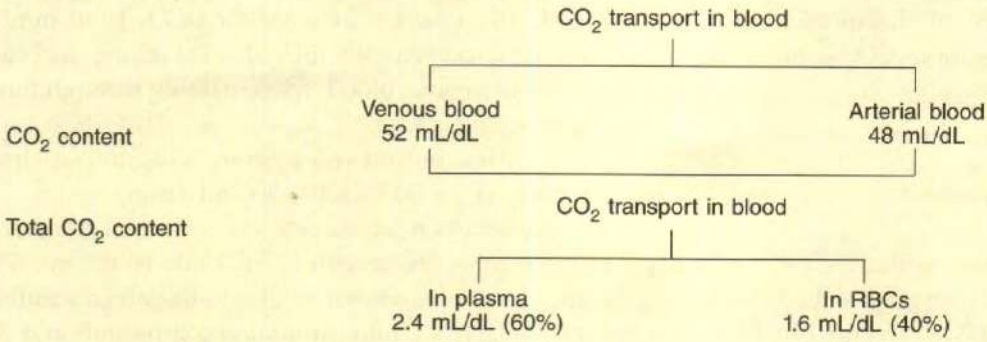


Fig. 47.4 Sequence of event during chloride shift

Summary of CO_2 transport



Forms of CO_2 carriage	In plasma	In RBCs	Total
A. In dissolved form	(i) as dissolved solution (ii) as carbonic acid (H_2CO_3) 0.2 mL/dL (1.8-1.6)	as carbonic acid 0.1 mL/dL (0.9-0.8)	0.3 mL/dL (2.7-2.4) *i.e.* 7%
B. As carbamino compound	As carbaminoprotein 0.1 mL/dL (1.1-1.0)	As carbamino-haemoglobint 0.6 mL/dL (2.6-2.0)	0.7 mL/dL (3.7-3.0) *i.e.* 18%
C. As bicarbonates	As $NaHCO_3$ (i) by phosphate buffer (ii) by protein reduction 2.1 mL/dL (35.2-33.1)	As $KHCO_3$ 0.9 mL/dL (10.4-9.5)	3 mL/dL (45.6-42.6) *i.e.* 75%

{Values in parenthesis indicate venous minus arterial blood levels in mL/dL.}

Study Questions

1. **Give physiological basis of:**
   (i) Arterial pO_2 is less than alveolar pO_2
   (ii) Sigmoid shape of oxygen-haemoglobin dissociation curve
   (iii) Oxyhaemoglobin binds less H^+ than reduced haemoglobin.
   (iv) Myoglobin acts as temporary O_2 storehouse
   (v) Haemoglobin in the blood is an ideal vehicle of O_2 transport
   (vi) PCV of venous blood is greater than that of arterial blood.
   (vii) Alveolar pO_2
   (viii) CO_2 dissociation curve
2. **Write short notes giving physiological significance:**
   (i) Bohr's effect (ii) Haldane effect (iii) P_{50}
   (iv) CO_2 transport in blood (v) Role of carbonic anhydrase in RBCs (vi) Hamburger phenomenon
   (vii) Oxygen-haemoglobin dissociation curve.
3. **Describe the factors that determine the amount of oxygen in the blood.**
4. **How does haemoglobin facilitates the transport of oxygen and carbondioxide?**
5. **Give distribution of oxygen in the body. Describe briefly how is it carried in the blood.**
6. **Draw well labelled diagram:**
   (i) Oxygen haemoglobin dissociation curve (ii) Dissociation curve for haemoglobin and myoglobin
   (iii) CO_2 dissociation curve in whole blood (iv) Sequence of events during chloride shift
7. **What will happen and why to oxygen haemoglobin dissociation curve:**
   (i) If concentration of salts and electrolytes is increased in blood (ii) During exercise
   (iii) At high altitude (iv) Presence of CO is blood (v) In HbF

MCQs

1. **The partial pressure of oxygen in the arterial blood is normally lower than that of the alveolar gas primarily because:**
   (a) The lungs use oxygen
   (b) Venous blood moves through the myocardium directly into the cavities of left heart
   (c) Some portions of the lungs are ventilated but not perfused
   (d) Some portions of the lungs are perfused but not ventilated

2. **The amount of oxygen carried in blood in the dissolved form is ..... mL/100 mL of blood per 100 mmHg:**
   (a) 3 (b) 0.3 (c) 0.03 (d) Less than 0.03

3. **Saturation of haemoglobin with oxygen in arterial and venous blood respectively is:**
   (a) 100%; 50% (b) 97%; 75% (c) 75%; 60% (d) 50%; 50%

4. **A shift of O_2 haemoglobin dissociation curve of blood to the right is a feature *not* found:**
   (a) With rise in temperature (b) When foetal blood is replaced by adult blood
   (c) In pulmonary capillaries (d) In hypercapnia

5. **Which causes a left shift of the oxygen haemoglobin dissociation curve?**
   (a) Decreased diphosphoglycerate (b) Increased 2,3 DPG
   (c) Carbon monoxide (d) Increased lactate

6. ***Not true* of myoglobin:**
   (a) Found in greater quantities in muscles specialized for sustained contraction
   (b) It takes up O_2 at low pressure
   (c) Its O_2-dissociation curve is a rectangular hyperbola
   (d) It shows Bohr's effect

7. **Physiological significance of flat top part of O_2-haemoglobin dissociation curve is:**
   (a) Causes more release of O_2 from haemoglobin when pO_2 falls
   (b) Shifting of curve to right makes this portion steeper
   (c) Amount of O_2 carried by blood does not change with fall in pO_2
   (d) Uptake of O_2 by body decreases at high altitude

8. **Coefficient of oxygen utilization at rest is:**
   (a) 1.0 (b) 0.75 (c) 0.52 (d) 0.26

9. **Percentage contribution of each form in which CO_2 is carried in the blood is:**

	In Dissolved form	As Carbamino compound	As bicarbonates
(a)	7	18	75
(b)	18	7	75
(c)	20	20	60
(d)	75	7	18

10. **What is Haldane effect?**
    (a) Loading of CO_2 to the blood causes unloading of O_2
    (b) Loading of O_2 to blood causes unloading of CO_2 (AT LUNGS)
    (c) Binding of CO to haemoglobin displaces O_2
    (d) Decrease in O_2 affinity of haemoglobin when pH of blood falls

11. **Which is *untrue* about chloride shift?**
    (a) Essentially complete in 1 sec
    (b) Chloride content of arterial blood is more than venous blood
    (c) Chloride content of venous blood is more than arterial blood
    (d) Associated with diffusion of Cl^- from plasma into the red cells

12. **Hematocrit of venous blood is:**
    (a) 3% greater than arterial blood (b) 3 times greater than arterial blood
    (c) 3% less than arterial blood (d) 3 times less than arterial blood

13. **What happens when blood passes through the systemic capillaries?**
    (a) pH rises (b) O_2-haemoglobin dissociation curve shifts to left
    (c) HCO_3^- pass from RBCs to plasma (d) Cl^- concentration in RBCs falls

14. **Shifting of O_2-haemoglobin curve to right means:**
    (a) Decrased O_2 delivery to tissues (b) Increased O_2 delivery to tissues
    (c) Loading of CO_2 to blood (d) Loading of O_2 to blood

15. **Myoglobin binds with:**
(a) 1 mol of oxygen per mol
(b) 2 mol of oxygen per mol
(c) 3 mol of oxygen per mol
(d) 4 mol of oxygen per mol

16. **P_{50} means:**
(a) pO_2 at which the haemoglobin is half saturated with O_2
(b) At pO_2 of 50 mmHg, haemoglobin is half saturated with O_2
(c) Amount of O_2 carried by blood does not change much even if pO_2 falls to 50 mmHg
(d) All of the above

17. **Net diffusion of CO_2 from venous blood to alveolar spaces occurs because:**
(a) Diffusibility of CO_2 in plasma is less than in the gaseous phase of alveoli
(b) Of its large kinetic activity
(c) The alveolar pCO_2 is lower than venous blood
(d) Of its very light molecular weight

18. **The most important factor in transport of CO_2 as bicarbonate is:**
(a) Affinity to haemoglobin
(b) Basic nature of HCO_3^-
(c) Increased solubility of CO_2
(d) Carbonic anhydrase in RBC (5000 times faster)

Answers

1. (b)	2. (b)	3. (b)	4. (c)	5. (c)	6. (d)	7. (c)	8. (d)	9. (a)	10. (b)
11. (c)	12. (a)	13. (c)	14. (b)	15. (a)	16. (a)	17. (c)	18. (d)		

Chapter

48

Regulation of Respiration

I. i) Nervous regulation of respiration
   ii) Respiratory centres and factors affecting it
   iii) Genesis of respiration
II. Chemical regulation of respiration
   Respiratory chemoreceptors: peripheral, central
   Chemical factors affecting respiration: O_2, CO_2; H^+
III. Physio-clinical aspects
   Dyspnoea; Apnoea; Breath holding; Asphyxia; Drowning; Periodic breathing

The normal rate of respiration in adults is 12-16/min, with a tidal volume of approx. 500 mL. The rate and depth of respiration *i.e.* total ***pulmonary ventilation*** can be adjusted to the requirements of the body. Spontaneous respiration is produced by rhythmic discharge of motor neurons that innervate the respiratory muscles. This discharge is totally dependent on nerve impulse from the brain. The rhythmic discharge from the brain that produces spontaneous respiration is regulated by two mechanisms:

I. *Nervous regulatory mechanism*, and
II. *Chemical regulatory mechanism*.

NERVOUS REGULATION OF RESPIRATION

This is brought about by two systems:

A. System responsible for ***automatic*** control of respiration is initiated by rhythmic discharge from 'pacemaker cells' in the ***pre-Botzinger complex***. It is located in the medulla near the nucleus ambiguus.
B. System responsible for ***voluntary*** control of respiration is located in the cerebral cortex.

A. AUTOMATIC CONTROL OF RESPIRATION

The collection of certain groups of neurons in (i) medulla, and (ii) pons constitutes the *'medullary'* and *'pontine'* respiratory centres respectively.

Medullary respiratory centre

It is located in the ventrolateral medulla overlying the olivary nucleus. The respiratory neurons show rhythmic discharge with varying frequencies and are of two types:

(1) those that discharge during inspiration only, *I-neurons*, and
(2) those that discharge during expiration only, *E-neurons*.

These neurons are located in two groups in the medulla. **(Fig. 48.1)**

1. The *dorsal group* of neurons are located in and near the nucleus of tractus solitarius. They are made up of primarily *I-neurons* and provide rhythmic drive to the diaphgram via the phrenic motor neurons. They receive afferents from the airways and chemoreceptors (aortic and carotid bodies via the Xth and IXth cranial nerve respectively (page 444).
2. The *ventral group* of neurons extends through the nucleus ambiguus and nucleus retroambiguus. It has two divisions:
   (i) *Cranial* division innervates the accessory muscles of respiration via vagus (X) nerve.
   (ii) *Caudal* division provides the inspiratory and expiratory drive to the motor neurons supplying the intercostal muscles.

Note

In addition, dorsal and ventral group of respiratory neurons project to the pre-Botzinger pacemaker neurons.

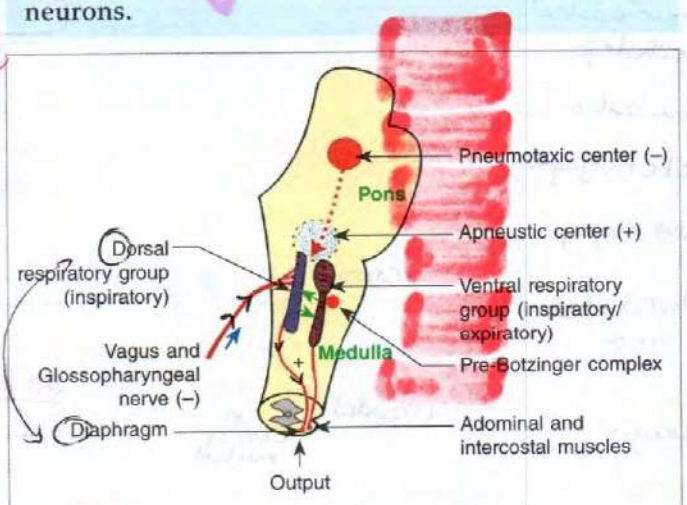
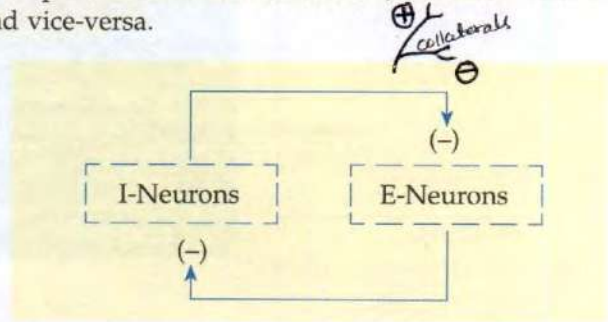


Fig. 48.1 Pontine and medullary respiratory centres

The 'I' and 'E' neurons have inhibitory connections to each other *i.e.* there exists *reciprocal innervation* between the two. Therefore, the motor neurons to the expiratory muscles are inhibited when those supplying the inspiratory muscles are active and vice-versa; this 'reciprocal' innervation is mediated via collaterals from excitatory pathway that synapse on inhibitory interneurons. Thus, an impulse that stimulates the one, will inhibit the other and vice-versa.



The area in the medulla that is concerned with respiration has classically been called the *respiratory centre.*

Pontine respiratory centre

1. An area in the lower pons contains neurons which are tonically active and activate the 'I-neurons' in medulla. This area was previously referred to as *apneustic centre.* The activity in these neurons is inhibited by afferents in the vagus (X) nerve from the airways and lungs. Therefore,
   (i) if vagi are intact, regular rhythmic respiration continues; and
   (ii) if vagi are cut, arrest of respiration occurs in inspiration, called *apneusis.*
2. An area in the upper pons, in the medial parabrachial nucleus, contains both I and E neurons, called *pneumotaxic centre.* These neurons are active in both phases of respiration. It inhibits the neurons in the lower pons, thus prevents *apneusis.*

Important Note

All these respiratory centres are bilaterally represented in the brain stem with same sided control and freely communicate with each other.

The rhythmic discharge of the neurons in the medullary respiratory centre is spontaneous, but it is modified by:

1. neurons in the pons; and
2. by afferents in the vagus nerves from receptors in the airways and lungs.

These influences can be shown by evaluating the results from the following set of animal experiments (Fig. 48.2):

Experiment 1st Set: Complete transection of brain stem:

1. below the medulla (*section 'D'*), stops all respiration (*apnoea*)
2. above pons - between 2 colliculi *i.e.* ***Decerebration*** (Section 'A') when both vagi are also cut; regular breathing continues (***eupnoea***).

Conclusion: Respiratory centres are situated in between these two sections *i.e.* in upper 2/3rd of medulla and pons.

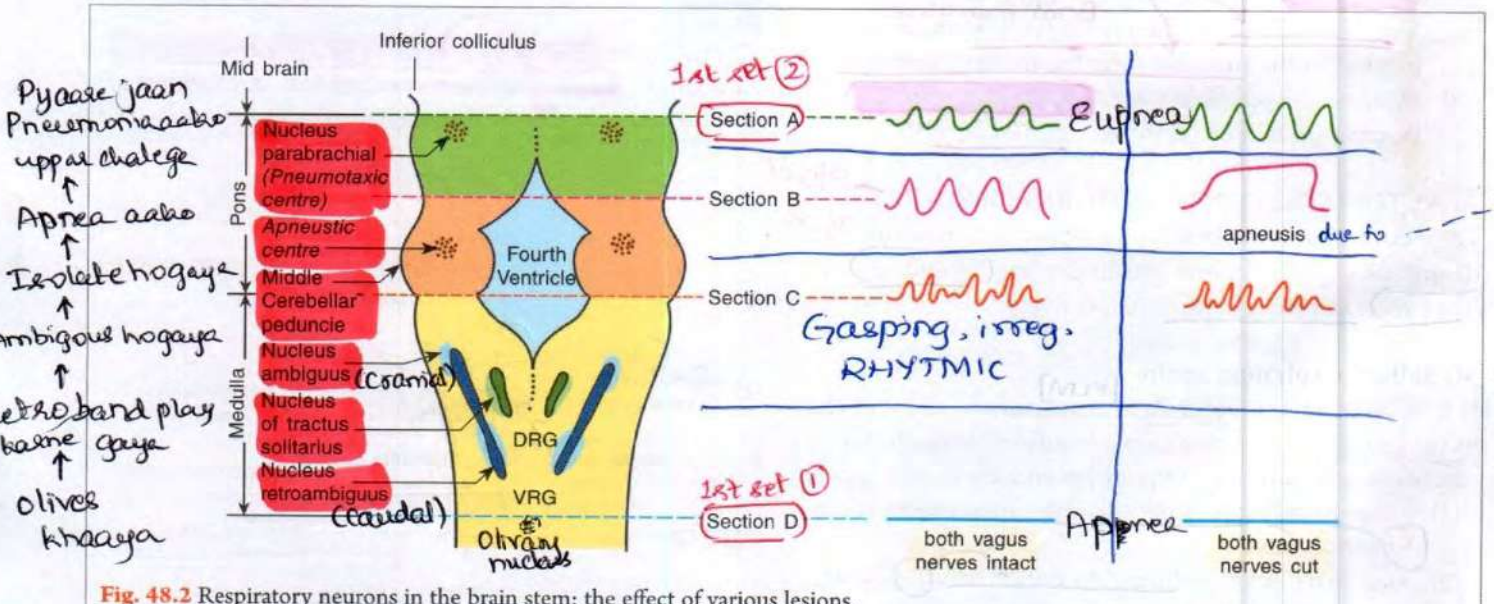


Fig. 48.2 Respiratory neurons in the brain stem: the effect of various lesions.
DRG and VRG – dorsal and ventral group of respiratory neurons

Experiment 2nd Set: Electrical stimulation of different areas in the medullary region helps in location of inspiratory and expiratory centres by producing prolonged inspiration and expiration respectively.

Experiment 3rd Set: Transection in the inferior portion of the pons *i.e.* when all pontine tissue is separated from the medulla (*section C*); respiration continues whether or not the vagi are intact. This respiration is somewhat irregular and gasping, but it is rhythmic *i.e.* inspiration is regularly followed by expiration.

Conclusions

1. *Respiratory centre* neurons are capable of spontaneous rhythmic discharge though it is irregular.
2. *Self limitation* is shown by the respiratory centre neurons *i.e.* when inspiration is complete then expiration starts (evidence for reciprocal innervation).
3. That the influence of other parts is apparently there which makes the rhythmic discharge of the medullary neurons smooth and regular.

Experiment 4th Set: Transection in the midpons (*Section 'B'*):

1. With vagi cut, there occurs arrest of respiration in inspiration (*apneusis*).
   Conclusion: In lower pons there lies a centre which is tonically active and activates inspiratory centre, called *apneustic center.*
2. If vagi are intact, regular rhythmic respiration continues however it becomes slower and deeper.
   Conclusion: 'Apneustic' centre is inhibited by vagus.

Experiment 5th Set: Decerebration (*section 'A'*), exhibits normal rhythmic breathing of a reasonable pattern, however, the depth of respiration is increased and rate is decreased after vagotomy.

Conclusion: There is certain area in the upper pons which inhibits the neurons in lower pons and thus prevents apneusis. This area is called *Pneumotaxic Center* and is located in the nucleus parabrachialis.

B. VOLUNTARY CONTROL OF RESPIRATION

Respiration can be modified both in rate and/or depth at will for a specific period only; for example, voluntary hyperventilation, breath holding, forceful inspiratory or expiratory efforts etc. The pathway for such a control is via *corticospinal tract* (page 899) which originates from the cerebral cortex to end on spinal motor neurons innervating the respiratory group of muscles. Thus, this pathway bypasses the medullary respiratory neurons.

Genesis of Respiration

A. Genesis of inspiration

The rhythmic discharge concerned with respiration is spontaneous (page 439). In addition, inspiratory centre although has its own rhythmicity, is activated by 'apneustic' centre *i.e.* neurons in lower pons which are tonically active. It stimulates the inspiratory centre. Inspiratory centre discharges over pathways in the spinal cord to $C_{3,4,5}$ and $T_{1,2}$ (anterior horn cells), therefore, inspiration starts. **(Fig. 48.3)**

B. Genesis of expiration The inspiration must be inhibited for expiration to proceed. How?

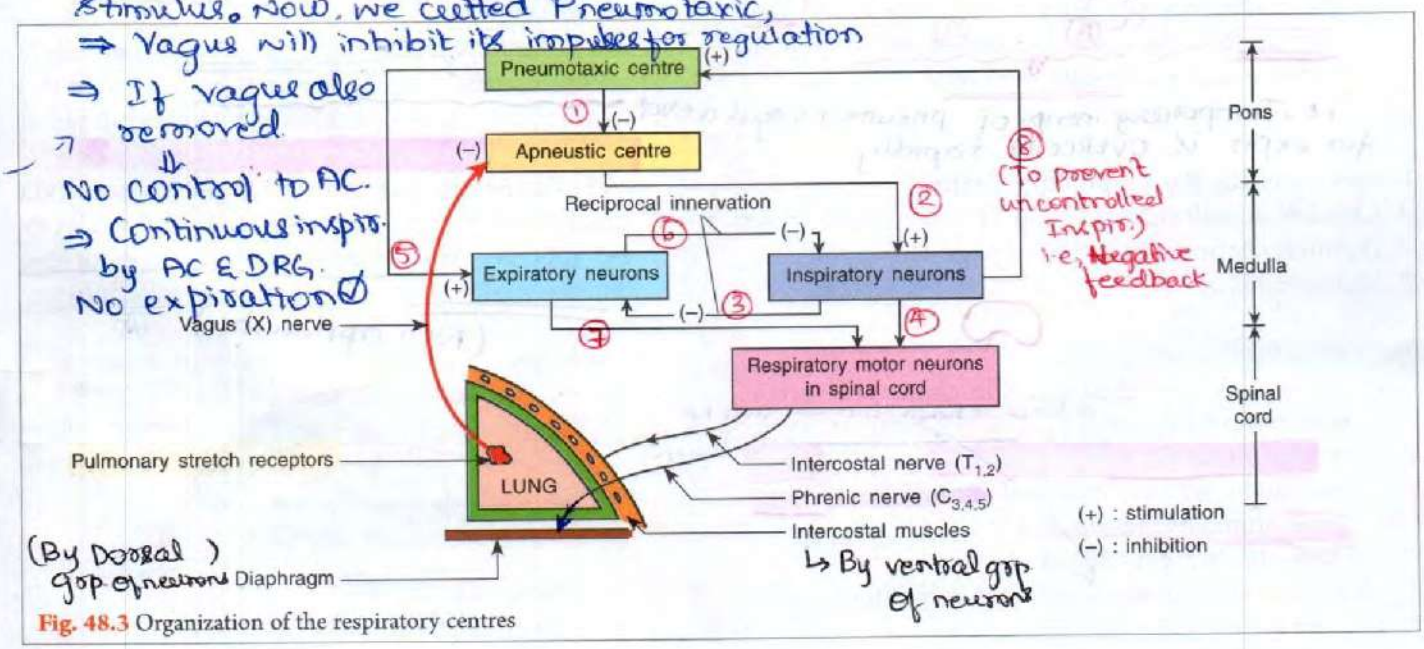


Fig. 48.3 Organization of the respiratory centres

1. I-neurons in medulla send excitatory impulses to 'pneumotaxic' centre which, in turn, discharges inhibitory impulses to 'apneustic' centre.
2. The ***pulmonary stretch receptors*** in the lungs which get stimulated during inspiration send inhibitory impulses via vagus to 'apneustic' centre.
3. The 'pneumotaxic' centre stimulates expiratory centre which reciprocally inhibits inspiratory centre. 'Apneustic' centre gets inhibited and ceases to activate the inspiratory centre. As a result, inspiratory centre stops discharging and expiration follows passively.

'Expiration' is passive in eupnoea, however, when expiration is active (*e.g.* voluntary hypernoea or during exercise) probably the 'pneumotaxic' centre relays excitatory effects originating from higher centres to expiratory centre.

Regulation of rate and depth of respiration

The exact mechanism responsible for the spontaneous discharge of the medullary neurons is unknown.

The inspiratory neurons are characterized by bursts of activity interspersed with periods of rest 12-15 times/min. Unlike the inspiratory neurons, the expiratory neurons do not discharge spontaneously, but they can be excited via afferents that converge on them and the inspiratory neurons from many sources. When the activity of the inspiratory neurons is increased, the rate and the depth of respiration are increased.

(1) The depth of respiration is increased because the lungs are stretched to a greater degree before the amount of vagal and 'pneumotaxic' centre inhibitory activity is sufficient to overcome the more intense inspiratory neuron discharge.

(2) The respiratory rate is increased because the 'after discharge' in the vagal and pneumotaxic afferents to the medulla is rapidly overcome.

Factors Affecting the Respiratory Centre

A. Chemical Stimuli (CO_2, O_2 and H^+) (for details, refer chemical control of respiration, page 444)

B. Non-chemical stimuli.

1. Afferents from higher centres

(i) **Cerebral cortex:** Electrical stimulation of certain areas of cerebral cortex *e.g.* anterior cingulate gyrus or ventral surface of frontal cortex will inhibit respiration; whereas electrical stimulation of motor cortex stimulates respiration.

These effects are mediated via:

(a) Corticobulbar tracts acting on respiratory centres in the medulla; and

(b) Corticospinal tracts which modify the activity of spinal motor neurons innervating the respiratory muscles (pathway for 'voluntary control' of respiration).

(ii) **Hypothalamus and limbic system**

(a) Pain and emotional disturbances (fear, anxiety, anger etc.) act through hypothalamus and limbic system to stimulate the respiration.

(b) Fever acts via anterior hypothalamus, which acts on respiratory centre and produces **tachypnoea** (*rapid, shallow respiration*).

2. Vagal afferents from inflation and deflation receptors in the lungs

These are stretch receptors located in the smooth muscles of the airways. They relay in the medulla via myelinated afferents in the vagi.

(i) 'Steady' inflation of the lungs stimulates 'stretch' receptors, impulses travel via vagi to inhibit the 'apneustic' centre, thus, inhibiting inspiration producing prolonged expiration, called ***Hering-Breuer Inflation Reflex*** (*Hering, E and Breuer, E* 1868).

Physiological significance of Hering-Breuer inflation reflex

This reflex is absent in healthy eupnoeic man with tidal volume (TV) of 500 mL. The threshold level of the reflex is at TV approx. 1-1.5 litres. Therefore, this reflex may exert a considerable influence in determining the pattern of breathing when respiration is increased such as during exercise. Thus prevents excessive lung inflation.

(ii) ***Hering-Breuer deflation reflex*** is a decrease in the duration of expiration produced by marked deflation of the lungs.

(iii) Marked inflation (*hyperinflation*) of lungs, stimulates unmyelinated vagal afferent nerve endings (pulmonary C fibers), located in alveolar wall called ***juxta-pulmonary capillary receptors*** or ***J-receptors*** (*A.S. Paintal* 1955) **(Fig. 48.4)**. Impulses travel via vagal fibers of small diameter (conduction velocity 2-3 mts per sec), reinforcing the action of the 'pneumotaxic' centre in producing intermittency

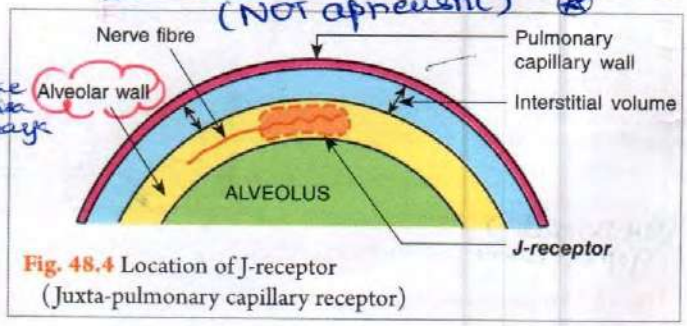


Fig. 48.4 Location of J-receptor (Juxta-pulmonary capillary receptor)

of inspiratory neuronal discharge, thus producing reflex apnoea, followed by tachypnoea (shallow, rapid breathing), hypotension and bradycardia.

J-receptors are primarily sensitive to the content of interstitial fluid between the capillary endothelium and alveolar epithelium, therefore, they respond better to:

(i) pulmonary congestion,
(ii) pulmonary embolization,
(iii) pulmonary oedema, and
(iv) inhalation of strong irritants or chemicals.

J-receptors may have a physiological role in severe exercise, which cause an increase in interstitial fluid in the lungs. J-*receptors* get stimulated, inhibiting spinal stretch reflex and thus limit the power of contraction of skeletal muscle, called *J-reflex*.

Role of vagi in regulation of respiration

(i) Stretching of the lungs during inspiration initiates impulses in vagal afferent fibers. These impulses in turn inhibit inspiratory discharge and expiration begins.

(ii) Hering-Breuer inflation as well as deflation reflexes are mediated by vagus as both disappear after cutting vagi.

(iii) *After cutting the vagi*, respiration becomes deep and slow. 'Deep' because:
(a) vagal feedback activity to inspiratory discharge gets abolished;
(b) more time is required by 'pneumotaxic' centre to inhibit 'apneustic' centre.

'Slow' because of fall in alveolar pCO_2.

3. Afferents from proprioceptors

Active or passive movement of joints, stimulates 'proprioceptors' in muscles, tendons and joints; afferent impulses stimulate the inspiratory neurons to increase the rate and depth of respiration. This effect helps increase ventilation during exercise.

4. Afferents from pharynx, trachea and bronchi

Throughout the airways, from trachea to respiratory bronchioles, there are endings of myelinated vagal afferents that function as ***Irritant Receptors***. Stimulation of these receptors(e.g. by dust, pollen) in the conducting zone of respiratory passage produces coughing or sneezing, while in the respiratory zone it produces *tachypnoea* (rapid, shallow breathing) and bronchoconstriction.

(i) *Cough Reflex:* It begins with a deep inspiration followed by forced expiration against a closed glottis. This increased intrapleural pressure ≥ 100 mmHg. The glottis is then suddenly opened producing an explosive outflow of air.

(ii) *Sneezing Reflex:* It is a similar expiratory effort with a continuously open glottis.

(iii) *Mechanism of tachypnoea and bronchoconstriction:* Stimulation of irritant receptors in the respiratory zone of respiratory passage stimulates inspiratory motor neurons producing rapid, shallow breathing. Broncho-constriction is produced by release of histamine and other chemical substances in the lungs thereby limiting the irritants to reach the gas-exchanging surface ('protective' mechanism).

(iv) *Swallowing (Deglutition) Reflex:* During a swallowing movement, respiration is inhibited in whatever stage of the cycle the swallowing was initiated (***Deglutition 'apnoea'***). Afferent impulses travel in glosso-pharyngeal (IX) nerve which inhibit the respiratory centres. The reflex is protective in nature and prevents aspiration of food particles into the respiratory passage.

(v) *Hiccup:* It is a spasmodic contraction of the diaphragm that produces an inspiration during which the glottis suddenly closes with production of sound. They often respond to breath holding that increases arterial pCO_2.

(vi) *Yawning:* Physiological basis of yawning is not known. Here underventilated alveoli have a tendency to collapse which get reflexely stretched open by deep inspiration and thus prevent the development of atelectasis (collapse of alveoli). It also increases the venous return to the heart.

5. Afferents from baroreceptors and chemoreceptors

(i) *Baroreceptors* are stretch receptors situated in the aortic arch and carotid sinus. These get stimulated by high blood pressure (impulses travel via vagus from aortic arch and in IX nerve from carotid sinus) and cause inhibition of respiration by inhibiting respiratory center.

Important Note

Injection of adrenaline (epinephrine) in high doses raises the systemic arterial blood pressure, which in turn inhibits the respiration producing apnoea, called *Adrenaline Apnoea*. However, in small doses, adrenaline stimulates respiration by stimulation of peripheral chemoreceptors.

(ii) *Chemoreceptors* (carotid and aortic bodies) get stimulated by chemicals (*e.g.* lack of O_2 or excess of CO_2), send impulses to respiratory centre to cause increase in rate and depth of respiration. Impulses travel in X (vagus) nerve from aortic bodies and in IX nerve from carotid bodies.

CHEMICAL REGULATION OF RESPIRATION

The chemical regulatory mechanism adjusts ventilation in such a way that the alveolar pCO_2 is kept constant at normal value of 40 mmHg. It also maintains the tension of O_2, CO_2 and H^+ of blood. For example: Fall in arterial pO_2 or pH, or rise in arterial pCO_2, stimulates the respiratory neurons in the medulla to increase *rate and depth* of (Tidal volume) respiration. Body O_2 supply increases and CO_2 is washed out, restoring the normal arterial pO_2, pH and pCO_2.

These changes are mediated via *respiratory chemoreceptors i.e.* receptor cells in the carotid and aortic bodies, and in medulla that are sensitive to changes in the blood chemistry and initiate impulses that stimulate respiratory centre.

RESPIRATORY CHEMORECEPTORS

These are of 3 types:

A. *Peripheral Chemoreceptors,*
B. *Medullary (or Central) Chemoreceptors,* and
C. *Pulmonary and Myocardial Chemoreceptors.*

A. Peripheral Chemoreceptors: Carotid and Aortic Bodies

(Discovered by *Heymans, C and Neil, E* in 1930 for which they got the Nobel Prize.)

There is a **carotid body** near the common carotid artery bifurcation on each side and there are usually two or more aortic bodies near the arch of aorta **(Fig. 48.5)**. Each carotid and aortic body (*glomus*) contains two types of cells, Type I and II cells. These cells are surrounded by fenestrated sinusoidal capillaries. Type II cells are glial cells (supporting cells) that support the type I cells **(Fig. 48.6)**.

In the *carotid body* unmyelinated endings of carotid sinus nerve, branch of glossopharyngeal (IX) nerve, are found at intervals between type I and II cells. Type I cells contain catecholamines (probably dopamine). When exposed to hypoxia, they release catecholamines that stimulate the carotid sinus nerve via D_2 receptors.

Mechanism of release of neurotansmitter by Type I cells

Hypoxia → decrease activity of O_2-sensitive K^+ channels → decrease K^+ efflux → increase Ca^{2+} influx (via L - type Ca^{2+} channels) → depolarization of cell membrane → release of neurotransmitter → stimulate afferent nerve endings.

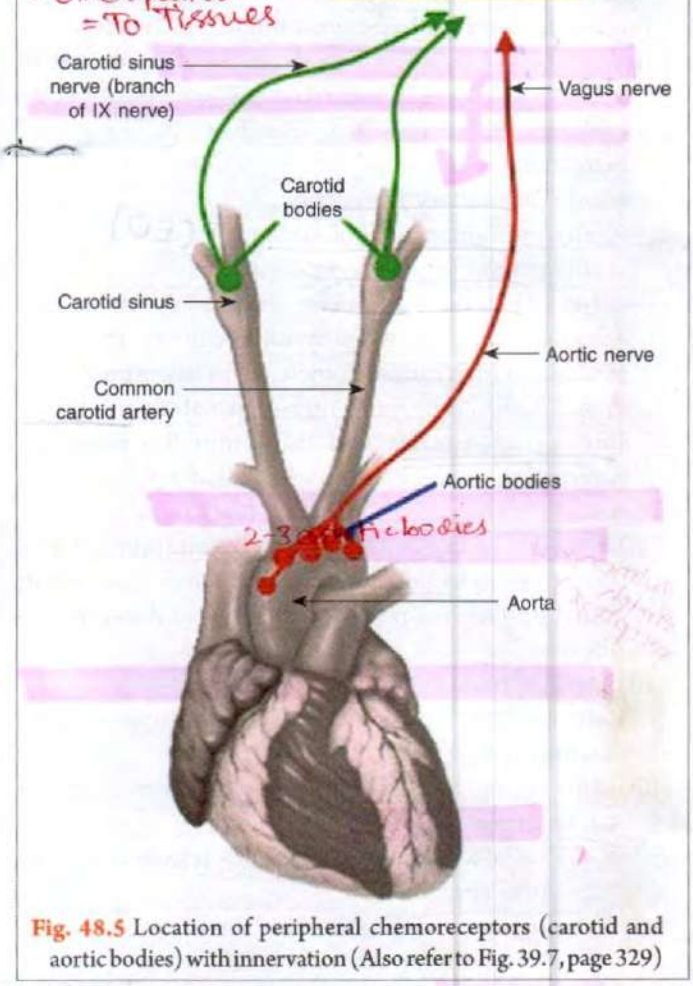


Fig. 48.5 Location of peripheral chemoreceptors (carotid and aortic bodies) with innervation (Also refer to Fig. 39.7, page 329)

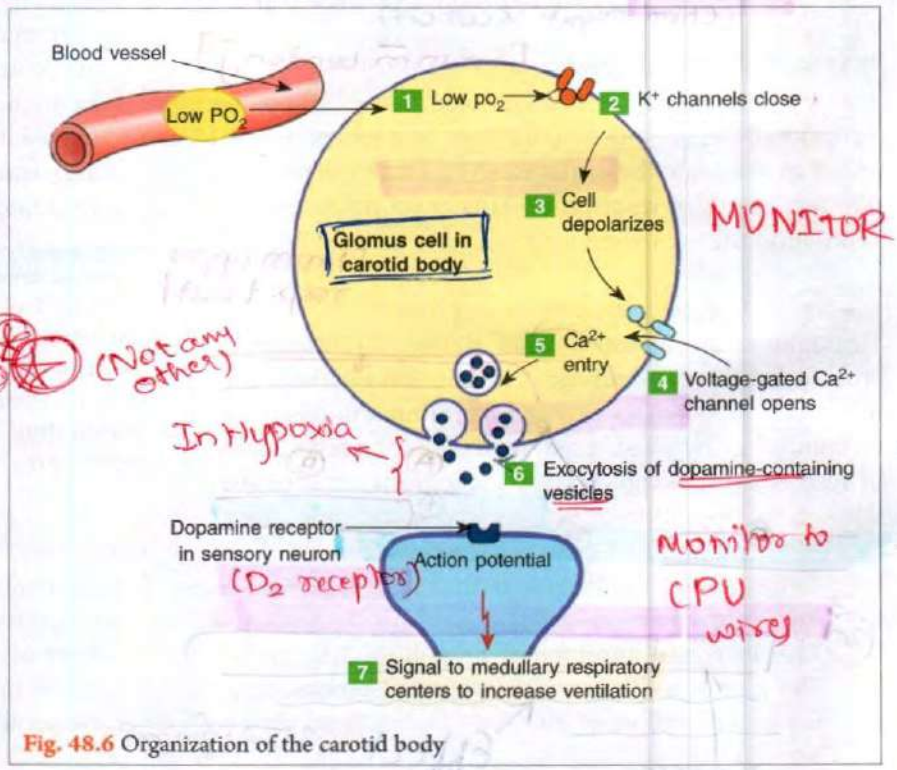


Fig. 48.6 Organization of the carotid body

Blood flow in each carotid body (weighing 2 mg) is approximately 0.04 mL/min (or 2000 mL/ 100 gm/ min). This blood flow is highest in the body, compared with brain 54 mL/ 100 gm/ min, kidney 420 mL/100 gm/min and heart 84 mL/ 100 gm/min. Therefore, the O_2 needs of the cells can be met largely by *dissolved* O_2 alone.

The receptors are ***not stimulated*** in anaemia or carbon-monoxide (CO) poisoning because:

(1) Although the combined oxygen in the blood is markedly reduced yet the amount of dissolved O_2 reaching the receptors is normal. (The partial pressure exerted by a gas in blood is the property of dissolved gas and not the combined form, *Henry Law*, page 403).

(2) Due to further increase in blood flow in anaemia.

Peripheral chemoreceptors get ***stimulated by:***

(1) *Hypoxia:* when arterial pO_2 decreases, the amount of dissolved O_2 decreases and A-V O_2 difference in carotid bodies falls below 0.2 mL/dL.

(2) *Vascular stasis:* The amount of O_2 delivered to receptors per unit of time is decreased.

(3) *Asphyxia:* Combination of O_2 lack plus CO_2 excess in the blood.

(4) *Drugs* (cyanide, nicotine, lobeline etc.) prevent O_2 utilization at the tissue level.

(5) *Increase in plasma* K^+ levels, such as during exercise, contributes to exercise-induced hyperpnoea.

Important Note

Increase in rate or depth of respiration regardless of patient's subjective sensation is called *Hyperpnoea;* whereas increase in rate and depth of respiration is called *Hyperventilation.*

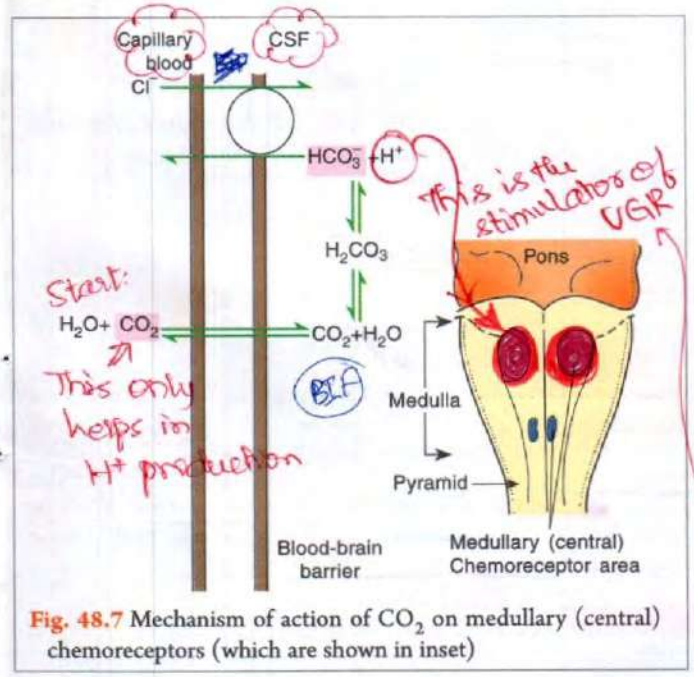


Fig. 48.7 Mechanism of action of CO_2 on medullary (central) chemoreceptors (which are shown in inset)

Applied Aspect: *Effect of denervation* (or removal) of peripheral chemoreceptors *i.e.* carotid and aortic bodies. There is a little change in ventilation at rest, but the ventilatory response to change in blood chemistry *i.e.* hypoxia, hypercapnia and acidemia, is lost by approx. 30%; rather they may cause direct depression of the respiratory centre.

(Refer **Table 48.1** *for major differences between carotid and aortic bodies.*)

Table 48.1: Differences between carotid and aortic bodies

	Carotid bodies	Aortic bodies
1. Location	These are located near common carotid artery bifurcation on each side.	These are located near the arch of aorta.
2. Innervation	Carotid sinus nerve branch of glosso-pharyngeal (IX) nerve.	Aortic nerve branch of vagus (X) nerve.
3. Effect of stimulation	Seven times more effective in stimulating respiration than the aortic bodies. They increase both rate and depth (*i.e.* tidal volulme) of respiration.	They increase only the frequency of respiration with small increase in ventilation.

B. Medullary (Central) Chemoreceptors

These chemoreceptors are located on the ventral surface of the medulla near the respiratory centre but separate from it (Fig. 48.7). They get stimulated by the H^+ concentration of cerebrospinal fluid (CSF) and brain interstitial fluid. The magnitude of stimulation is directly proportional to the local increase in H^+ concentration, which in turn increases in linearity with arterial pCO_2. These chemoreceptors get *inhibited by* anaesthesia, cyanide and during sleep.

Mechanism of H^+ formation

CO_2 readily penetrates membranes, including blood-brain and blood-CSF barriers, whereas H^+ and HCO_3^- penetrate slowly. The CO_2 that enters the brain and CSF is promptly hydrated to form carbonic acid (H_2CO_3) which dissociates into H^+ and HCO_3^-. Thus, local H^+ concentration increases.

$$CO_2 + H_2O \longrightarrow H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

(carbonic acid)

Evidence: Experimentally produced changes in pCO_2 of CSF have minor variable effects on respiration as long as H^+ is held constant, but any increase in CSF H^+ concentration stimulates respiration. Therefore, the effect of CO_2 on respiration are mainly due to its movement into the CSF and brain interstitial fluid, where

it increases H^+ concentration and stimulates medullary chemoreceptors.

C. Pulmonary and Myocardial Chemoreceptors

Injection of veratridine or nicotine into pulmonary circulation stimulates chemoreceptors of some type in pulmonary vessels producing bradycardia, hypotension and apnoea followed by tachypnoea (rapid, shallow breathing), called ***pulmonary chemoreflex***. A similar reflex response is produced when these agents are introduced into coronaries supplying the left ventricle, called *coronary chemoreflex* or *Bezold-Jarisch reflex* (page 331).

This reflex plays no physiological role and occurs in pathological states like pulmonary congestion or embolism, and after myocardial infarction.

Summary:

Respiratory chemoreceptors - Refer Table 48.2.

CHEMICAL FACTORS AFFECTING RESPIRATION

These are:

A. *Effect of Hypoxia,*

B. *Effect of CO_2,*

C. *Effect of H+ concentration.*

A. Effect of Hypoxia on Respiration

Decrease in pO_2 of inspired air (IpO_2) decreases arterial pO_2, this via peripheral chemoreceptors stimulates the respiratory centre resulting in rise in pulmonary ventilation. If decrease in IpO_2 is above 60 mmHg, only slight increase in ventilation occurs; however, rise is marked when IpO_2 falls below 60 mmHg (Fig. 48.8). This is because:

(1) Hypoxia increases the amount of reduced haemoglobin in blood, which is a weaker acid than oxyhaemoglobin; it binds H^+ readily. The fall in H^+ concentration tends to inhibit respiration.

(2) Any increase in ventilation due to hypoxic stimulation on respiratory centre lowers the alveolar pCO_2, which also tends to inhibit respiration.

However, when arterial pO_2 falls below 60 mmHg, hypoxic stimulation of peripheral chemoreceptors is so strong that it overrides the above inhibitory effects to produce marked increase in pulmonary ventilation.

Mechanism of action: Hypoxia stimulates respiration via stimulation of peripheral chemoreceptors. This can be demonstrated by the following experiment.

During recording of pulmonary ventilation in a subject at rest, ask him to inhale 100% N_2, this produces hypoxia with marked increase in respiration. If we denervate the

SUMMARY

Table 48.2: Respiratory Chemoreceptors – Salient features

	Peripheral chemoreceptors i.e. Aortic and Carotid bodies	Medullary (central) chemoreceptors	Pulmonary and Myocardial chemoreceptors
1. Location	There are two or more aortic bodies near arch of aorta; and two carotid bodies, one on each side near bifurcation of common carotid artery.	These are located on the ventral surface of medulla near respiratory centre but separate from it.	These are located in pulmonary and coronary blood vessels.
2. Innervation	Aortic bodies by aortic nerve (branch of X nerve); carotid bodies by carotid sinus nerve (branch of IX nerve).	Nerve fibers from here directly project over to the respiratory centre.	Vagus (X) nerve.
3. Stimulated by	(i) increase arterial pCO_2 (ii) decrease arterial pO_2 (iii) cyanide (iv) nicotine, and (v) lobeline	Increase in H^+ concentration in CSF and brain interstitial fluid, which parallels arterial pCO_2; magnitude of stimulation is proportional to increase in H^+ locally. $CO_2 + H_2O \longrightarrow H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$ *Inhibited by:* anaesthesia, cyanide and during sleep.	Injecting veratridine or nicotine in these vessels.
4. Effect of stimulation	(i) increase in rate (mainly) and depth of respiration; hypertension; tachycardia (ii) regulates the respiration from *breath to breath* (iii) provides initial drive to increase respiration in response to increase in arterial pCO_2, and contributes to 20% increase in pulmonary ventilation.	(i) increase in rate and depth of respiration only (ii) regulates the respiration from *minute to minute*. (iii) provides late drive to increase respiration in response to increase in arterial pCO_2; and contributes to remaining 80% increase in pulmonary ventilation.	Bradycardia; hypotension; apnoea followed by tachypnoea, called "pulmonary chemoreflex" or coronary chemoreflex *(Bezold Jarisch reflex)*. Plays no physiological role, pathological reflex only.

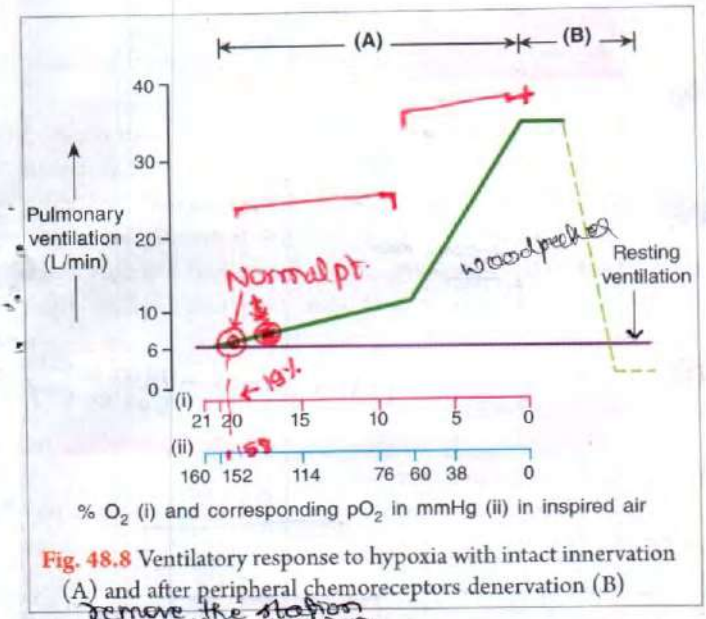


Fig. 48.8 Ventilatory response to hypoxia with intact innervation (A) and after peripheral chemoreceptors denervation (B)

peripheral chemoreceptors, then hypoxia cannot increase pulmonary ventilation, rather it falls below normal. This is because severe hypoxia causes direct inhibition of respiratory centre **(Fig. 48.8)**.

B. Effect of CO_2 on Respiration

Arterial pCO_2 is kept constant at normal value of 40 mmHg. How it is maintained?

Rise in arterial pCO_2 (due to any cause e.g. increased tissue metabolism or rise in $IpCO_2$)
↓
Stimulates chemoreceptors [central]
↓
Stimulates respiratory centre (pulmonary ventilation increases)
↓
Increase CO_2 washout until arterial pCO_2 becomes normal

Therefore,

(1) Inhalation of CO_2 upto 4%, increases pCO_2 of inspired air to 15 mmHg (normal: 0.3 mmHg) and pulmonary ventilation increases by 2 times *i.e.* to 12 L/min. However, alveolar pCO_2 increases slightly by 3 mmHg. The hyperventilation persists as long as CO_2 is inhaled **(Fig. 48.9)**.

(2) Inhalation of CO_2 between 4-7% increases the pulmonary ventilation markedly in linearity with increase in concentration of CO_2 in inspired air.

Limitation to this linear relationship

When the CO_2 concentration of the inspired gas is more than 7%, inspired air pCO_2 approaches close to the alveolar pCO_2, as a result elimination of CO_2 becomes difficult which causes alveolar and arterial pCO_2 to rise abruptly inspite of hyperventilation. The accumulation of CO_2 in the body (*Hypercapnia*) depresses the CNS, including respiratory centres producing headache, confusion, dizziness, apnoea and eventually coma, called

CO_2 narcosis

Clinical significance: If any comatose patient is brought to the hospital with respiratory depression, CO_2 inhalation can be given to stimulate the respiration but only after assessing his CO_2 content of the blood, otherwise patient will die of CO_2 *narcosis*.

Important Note

(A) In febrile patients there is 13% increase in CO_2 production for each 1°C rise in temperature, and a

(B) high carbohydrate intake increaes CO_2 production because of the increase in RQ. (Respiratory Quotient)

Mechanism of action: CO_2 increases pulmonary ventilation by stimulating the central chemo receptors primarily, though it is capable of increasing the pulmonary ventilation by stimulating the peripheral chemoreceptors as well.

Proof: There is **no** change in pulmonary ventilation responses to inhaling CO_2 in increment concentrations before and after removal of peripheral chemoreceptors. It means there are some other chemoreceptors in the body other than peripheral chemoreceptors that can sense the increase in arterial pCO_2. When central chemoreceptors are

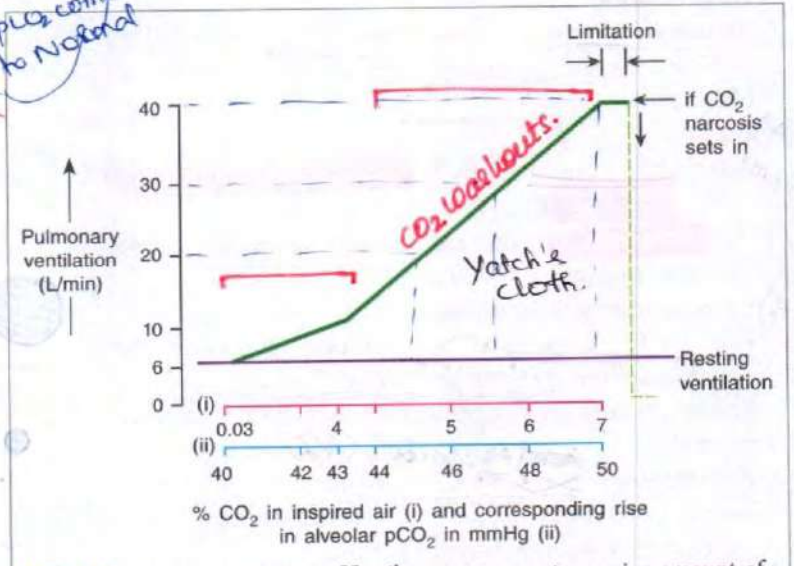


Fig. 48.9 CO_2 response curve: Ventilatory response to varying amount of CO_2 inhalations

depressed by anaesthesia, then CO_2 increases respiration through stimulation of peripheral chemoreceptors.

C. Effect of H^+ Concentration on Respiration

H^+ or HCO_3^- **cannot** normally act through modification of central chemoreceptors, because H^+ enters CSF very slowly.

In general, *Acidosis* (increased H^+ concentration in blood) produces pronounced respiratory stimulation resulting in hyperventilation; and conversely, *Alkalosis* (decreased H^+ concentration in blood) depresses respiratory centre producing hypoventilation. These effects are mediated via peripheral chemoreceptors.

Examples:

1. ***Metabolic Acidosis*** *i.e.* decrease in HCO_3^- concentration in blood secondary to increase H^+ concentration in blood.

A) *Causes:*

(i) Diabetes mellitus due to accumulation of acidic ketone bodies in the circulation.

(ii) Renal failure, kidneys fail to excrete its normal quota of H^+.

(iii) Severe muscular exercise leads to accumulation of lactic acid.

(iv) Starvation producing keto-acids.

(v) Infantile diarrhoeas associated with loss of $NaHCO_3$.

B) *Compensatory mechanism:*

Rise in H^+ concentration in blood, via peripheral chemoreceptors causes prolonged respiratory centre stimulation; therefore, CO_2 is washed out and alveolar pCO_2 decreases with subsequent decrease in arterial pCO_2 producing compensatory fall in blood H^+ concentration.

2. ***Metabolic Alkalosis*** *i.e.* increase in HCO_3^- concentration in blood secondary to decreased H^+ concentration in blood.

A) *Causes:*

(i) Severe vomiting due to pyloric or high intestinal obstruction.

(ii) Loss of HCl from the body (due to any cause) resulting in loss of H^+.

B) *Compensatory mechanism:*

Fall in H^+ concentration in blood depresses the respiration, so alveolar and arterial pCO_2 increases, finally restoring H^+ concentration of blood towards normal.

3. ***Respiratory Alkalosis*** *i.e.* increased pulmonary ventilation which occurs due to causes other than increase in arterial H^+ concentration.

A) *Causes:*

(i) Voluntary hyperventilation.

(ii) Chronic O_2 lack as seen at high altitude.

(iii) Neurotic patients who chronically hyperventilates.

B) *Compensatory mechanism:*

With increased pulmonary ventilation, CO_2 is washed out, so alveolar and arterial pCO_2 fall, as a result respiration slows down, CO_2 gets retained restoring H^+ concentration of blood towards normal.

4. ***Respiratory Acidosis*** *i.e.* decreased pulmonary ventilation which occurs due to causes other than decrease in arterial H^+ concentration.

A) *Causes:*

(i) Emphysema

(ii) Depression of respiratory centre by morphine etc.

B) *Compensatory mechanism:*

Hypoventilation causes CO_2 accumulation in the body, alveolar and arterial pCO_2 increases, as a result respiration increases, CO_2 gets washed out restoring H^+ concentration of blood towards normal. (Also refer to pages 562 to 564 for renal compensatory mechanisms).

Interaction of Chemical Factors in Regulation of Respiration

A. Interaction of CO_2 and O_2

1. The effects on ventilation of decreasing the alveolar pO_2, while holding the alveolar pCO_2 constant **(Fig. 48.10)**.

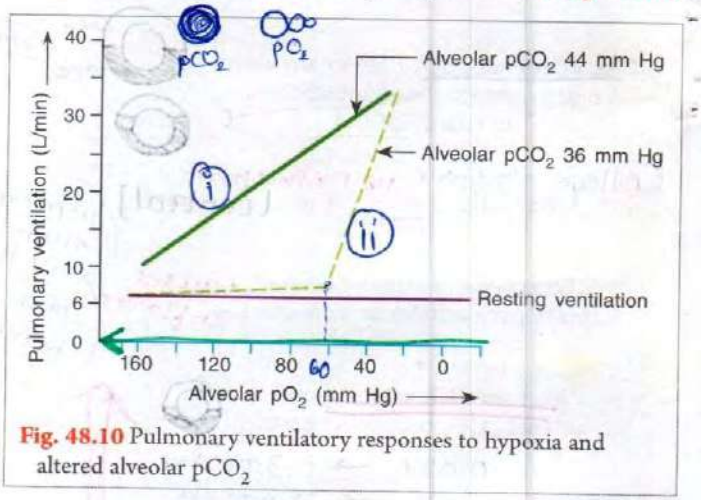


Fig. 48.10 Pulmonary ventilatory responses to hypoxia and altered alveolar pCO_2

(i) When the alveolar pCO_2 is held constant at a level 2-3 mmHg above normal, there is an inverse relationship between ventilation and the alveolar pO_2 even in the 90-110 mmHg range.

(ii) When the alveolar pCO_2 is held constant at a level 2-3 mmHg below normal, there is no stimulation of ventilation by hypoxia until the alveolar pO_2 falls below 60 mmHg.

2. The effects on ventilation to varying amount of inspired CO_2 while the alveolar pO_2 is held constant below the normal level **(Fig. 48.11)** shows that the slope of CO_2 *response curve* increases *i.e.* becomes more steeper when

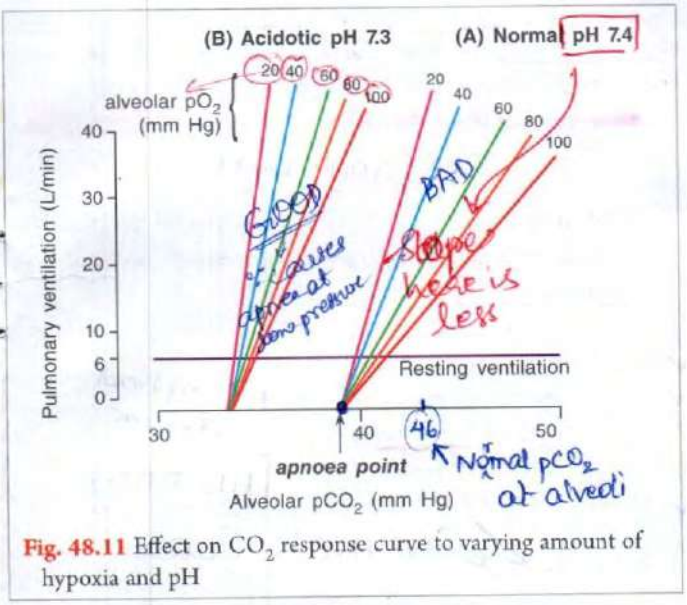


Fig. 48.11 Effect on CO_2 response curve to varying amount of hypoxia and pH

the alveolar pO_2 is decreased (compare with **Fig. 48.9**). Therefore, hypoxia makes the individual more sensitive to increase in arterial pCO_2.

Important Note

All the ventilation volume/pCO_2 lines though differing in slope according to the pO_2, when extrapolated to a zero ventilation meet at a common point. This point corresponds to an alveolar pCO_2 value on X-axis, which may be regarded as that at which CO_2 causes no respiratory stimulation of itself. This point of intersection is called the *Apnoea Point*. It can be seen, that in normal individuals, this threshold value is just below the normal alveolar pCO_2, indicating that normally there is a very slight but definite "CO_2 drive" of the respiratory center (Also see to page 448).

B. Interaction of CO_2 and H^+

The stimulatory effect of H^+ and CO_2 on respiration shows *additive effect*. When the subject becomes acidotic, CO_2 *response curve* shifts to the 'left' without a change in slope, *i.e.* the same amount of respiratory stimulation is produced by lower arterial pCO_2 levels **(Fig. 48.11)**.

C. Interaction of CO_2 and Body Temperature

The effect of pCO_2 on pulmonary ventilation, when measured with increase in body temperature, the "CO_2 response curves" shifts to the 'left' *i.e.* the lines become more steeper, indicating that the sensitivity of action of CO_2 on respiration becomes more with increase in body temperature **(Fig. 48.12)**.

Summary

Sensitivity of action of CO_2 on respiration:

(1) **increased by**: hypoxia, hyperthermia; and

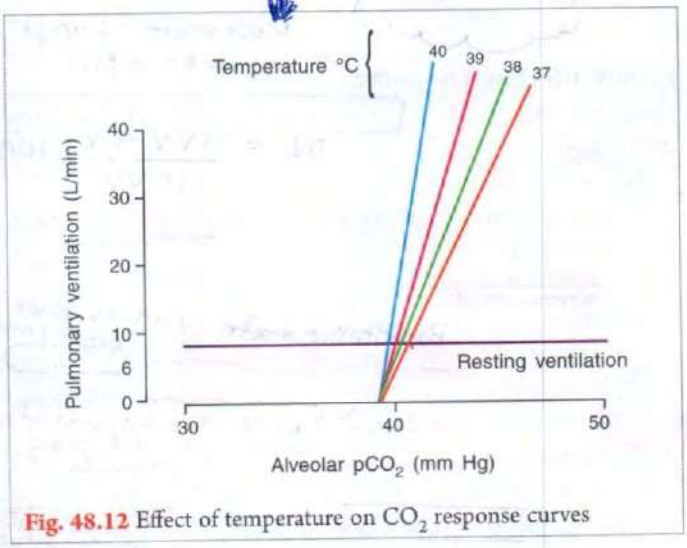


Fig. 48.12 Effect of temperature on CO_2 response curves

Summary: Regulation of Respiration

- 'Nervous' Regulatory Mechanisms
  - 'Voluntary' control system (in cerebral cortex)
  - 'Automatic control system (in brain stem)
    - 'Medullary' Respiratory centre
      - I-Neurons (Reciprocal innervation)
      - E-Neurons
    - 'Pontine' respiratory centre
      - 'Apneustic' centre (in lower pons)
      - 'Pneumotaxic' centre (in upper pons)
- 'Chemical' Regulatory Mechanisms
  - mediated via
    - 'Peripheral' chemoreceptors
    - Medullary (central) chemoreceptors
    - Pulmonary and myocardial chemoreceptors

(2) **decreased by:** sleep, hypothermia, anaesthesia. Sensitivity decreases as central chemoreceptors get inhibited by these agents.

PHYSIO-CLINICAL ASPECTS

A. DYSPNOEA

Definition. Dyspnoea literally means difficulty in breathing. It is defined as the breathing in which subject is conscious of shortness of breath *i.e.* when breathing enters consciousness, it becomes unpleasant and produces discomfort.

However, this definition is not correct, because there can be difficulty in breathing even in unconscious states *e.g.* in diabetic coma.

One is not aware of his respiration till resting pulmonary ventilation becomes more than double. Real dyspnoea (*i.e.* uncomfortable breathing) occurs when pulmonary ventilation is increased to 3-4 times. This level of pulmonary ventilation when person becomes dyspnoeic is called *Dyspnoeic Point*.

Factors affecting Dyspnoea

Dyspnoea occurs when Dyspnoeic Index falls below 60% (page 414).

$$DI = \frac{MVV - PV}{MVV} \times 100\%$$

Therefore, dyspnoea occurs when:

1. maximum Voluntary Ventilation (MVV) is less than normal,
2. vital capacity decreases causing MVV to decrease, and
3. Increase in pulmonary ventilation (PV) by 4-5 times.

(1) *Physiological Causes:* Severe exercise increases pulmonary ventilation.

(2) *Pathological Causes:*

(i) Bronchial asthma due to constriction of bronchioles, decreases VC.

(ii) Emphysema (loss of elasticity of lungs), decreases VC.

(iii) Metabolic acidosis increases pulmonary ventilation. (Daadi)

(iv) ***Cardiac Dyspnoea***. It is due to heart failure, this produces:

(a) Stagnant hypoxia (Ischaemic)

(b) Cardiac failure
↓
Pulmonary congestion
↓
Stimulate 'J-receptors'
↓
Tachypnoea (Rapid, shallow respiration) → ↑s pulmonary ventilation
↓
dyspnoea

Therefore, cardiac failure patients like to sit rather than lie down, because lying down position increases pulmonary congestion and aggravates dyspnoea.

Dyspnoea in lying down position is called *Orthopnoea* (page 392).

B. APNOEA

Definition: Apnoea is the term used to describe inhibition or stoppage of respiration.

Causes:

1. Deglutition (swallowing)
2. After hyperventilation
3. Hering-Breuer deflation reflex
4. Bezold-Jarisch reflex
5. During sleep (sleep apnoea)

Sleep Apnoea: In few individuals, respiration gets depressed with periods of apnoea during sleep, called sleep apnoea. It may be due to:

(i) Respiratory failure secondary to depression of central chemoreceptors.

(ii) In addition, during sleep there may be failure of genioglossus muscle to contract. Normally this muscle keeps the tongue forward, when it fails to contract, the tongue falls backwards and obstructs the airways. (obstructive sleep apnoea-most common during REM sleep; page 986)

This ***sleep apnoea syndrome*** can occur at any age. The symptoms include loud snoring morning headache and fatigue. When severe and prolonged, the patient may be hypertensive, polycythemic, hypoxemic and hypercapnoeic. The apnoeic episodes are most common during REM sleep (page 986). A form of sleep apnoea, more commonly seen in premature infants, is called ***sudden infant death syndrome (SIDS)***. High incidences are seen in infants of mothers who smoke or by an infant who sleep in prone position.

C. BREATH HOLDING

Respiration can be voluntarily inhibited for a period of 45-55 secs in normal healthy subjects.

The point at which breathing can no longer be voluntarily inhibited is called the ***Breaking Point***. It is due to:

1. increased arterial pCO_2 and
2. decreased arterial pO_2.

Thus, breath holding can be ***increased***:

1. If prior to breath holding, 100% O_2 is inhaled, this increases alveolar pO_2; the breath holding can be increased by 15-20 secs. ⇒ 1 min
2. If prior to breath holding, hyperventi-late room air for 1 min; therefore, CO_2 is washed off, arterial

pCO_2 decreases and breath holding can be increased by 1 min.

3. After removal of carotid bodies.
4. Psychological factors: subjects can hold their breath longer when they are told their performance is very good although it may not be so.

D. ASPHYXIA

It is produced by occlusion of airways. This results in hypoxia (lack of O_2) and hypercapnia (excess of CO_2).

Experimentally, asphyxia can be produced in an animal by connecting him to a closed bag for respiration, after some time O_2 gets utilized and CO_2 increases producing:

(1) initially there will be marked stimulation of respiration with violent respiratory efforts;
(2) blood pressure and heart rate increases;
(3) blood pH falls;
(4) increased catecholamine secretions (epinephrine and nor-epinephrine);
(5) later on, when CO_2 increases above a certain level, it
   (i) depresses respiratory center and VMC resulting in death, and
   (ii) moreover, severe hypoxia also produces cardiac arrest in 4-5 minutes.

Treatment: by 'artificial respiration' subject may be able to survive, but care has to be taken for:

(1) hypoxic damage of myocardium; and
(2) increased epinephrine and nor-epinephrine secretion may cause ventricular fibrillation due to multiple ectopic foci.

E. DROWNING

Effects of drowning:

1. In approx. 10% of drowning cases, the first gasp of water after losing struggle not to breath, triggers severe laryngospasm and further entry of water into the lungs is prevented, which produces asphyxia and finally death, without any water in the lungs.
2. In remaining 90% of drowning cases, muscles of glottis relaxes and water enters the lungs:
   (i) if *fresh water* enters; being 'hypotonic' gets rapidly absorbed and enters the circulation causing *Intravascular Hemolysis;*
   (ii) if *sea water* enters; being 'hypertonic' drains the water from the circulation into the lungs, thus blood volume decreases.

Treatment: Artificial respiration.

F. PERIODIC BREATHING

Definition: It is the repeated sequence of apnoea followed by respiration.

Classification: Two types:

1. Cheyne-Stokes Respiration (*gradual onset*)
2. Biot's Breathing (abrupt onset)

1. Cheyne-Stokes Respiration

Definition: The repeated sequence of gradual onset of apnoea followed by gradual restoration of respiration is called cheyne-stokes respiration.

Causes:

A. *Physiological*
   (a) Voluntary hyperventilation
   (b) High altitude
   (c) During sleep in some normal individuals

B. *Pathological*
   (a) Heart failure (left ventricular failure)
   (b) Brain damage
   (c) Uremia

Important Note

Some of the patients with Cheyne-stokes respiration have increased sensitivity to CO_2 due to distrutpion of nerve pathways that normally inhibit respiration.

a. Voluntary Hyperventilation

Voluntary hyperventilation for 2 minutes produces apnoea followed by respiration. This cycle keeps on repeating but with a decreasing duration of apnoea until the respiration comes back to normal. **(Fig. 48.13)**

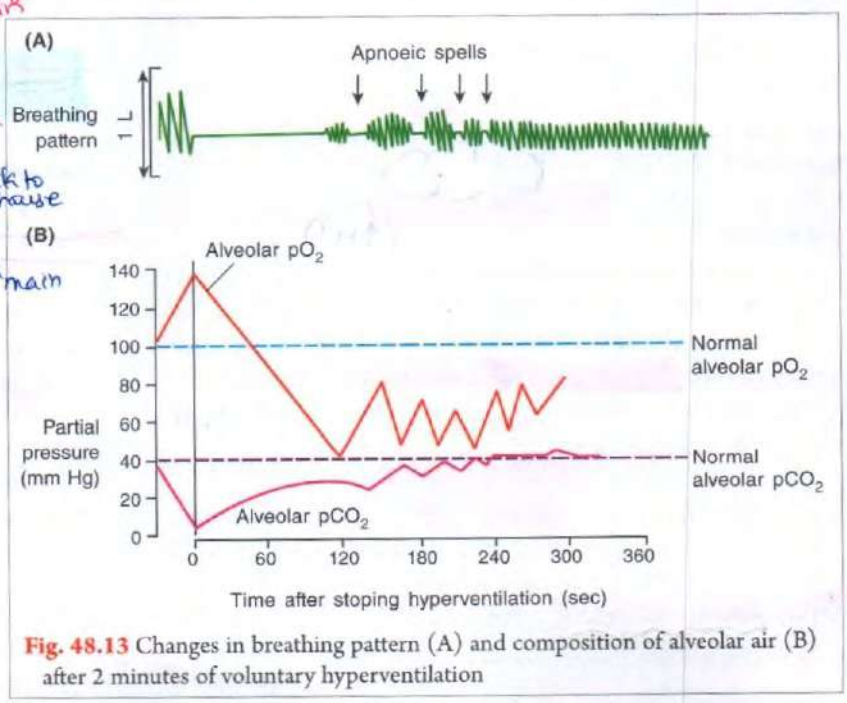


Fig. 48.13 Changes in breathing pattern (A) and composition of alveolar air (B) after 2 minutes of voluntary hyperventilation

Mechanism of development of apnoea: At the end of hyperventilation,

(i) CO_2 wash out decreases alveolar pCO_2 to approx. 15 mmHg; and

(ii) alveolar pO_2 can increase to a maximum of 140 mmHg only, because normal atmospheric pressure is 760 mmHg due to $pN_2 + pH_2O + pO_2 + pCO_2$.

Out of this, 573 + 47 = 620 mmHg is contributed by pN_2 and pH_2O remains constant and cannot be changed, and the remaining 140 mmHg left is contributed by pO_2 and pCO_2. Now by hyperventilation, even if whole CO_2 gets removed, O_2 will contribute only 140 mmHg pressure.

Therefore, apnoea can be due to either increased alveolar pO_2 or decreased pCO_2; former cannot produce apnoea because inhalation of 100% pure O_2 causes no apnoea. Thus, apnoea is due to decreased alveolar pCO_2 (*proof:* if hyperventilation is performed after inhalation of 5% CO_2, there is no apnoea).

Sequence of events following hyperventilation apnoea results in:

(i) CO_2 accumulation in the body with gradual increase in alveolar pCO_2. As long as increase in alveolar pCO_2 remains below the normal level of 40 mmHg *i.e.* below the 'threshold' level of stimulation of respiratory centre, apnoeic spells are there.

(ii) Fall in alveolar pO_2 to 60 mmHg (hypoxia) via peripheral chemo-receptors stimulates the respiratory centre producing hyperventilation and alveolar pO_2 increases but alveolar pCO_2 falls resulting in apnoea again.

This sequence of apnoea followed by respiration is repeated until and unless alveolar pCO_2 comes back to normal value of 40 mmHg.

Physio-clinical Significance

Hyperventilation due to any cause results in ***hypocapnia*** (page 563). The effects of hypocapnia are:

1. ***On Respiration:*** Respiratory alkalosis (page 448)
2. ***On CNS***
   (i) decrease in cerebral blood flow by 30% or more because of direct constrictor effect of hypocapnia on the cerebral vessel. The symptoms produced are due to cerebral ischaemia/hypoxia (page 458)
   (ii) ***On CVS:*** Constrictor effect on peripheral blood vessels produces:
      (a) increase in cardiac output
      (b) increase in systemic BP (to slight degree due to simultaneous depression of VMC which tends to decrease the BP).
   (iii) Alkalaemic tetany - Refer page 710.

b. Heart Failure

It produces *cheyne-stokes respiration* specially left ventricular failure (LVF). How?

LVF is associated with pulmonary congestion producing hypoxia; respiratory centre gets stimulated and ventilation increases, alveolar pO_2 increases and pCO_2 falls, therefore arterial pCO_2 falls.

As in heart failure, the circulation is slowed down from lungs to brain, therefore, when such individuals hyperventilate, it takes longer than normal time for the blood with a low pCO_2 to reach the brain. When this blood reaches the brain, the low pCO_2 inhibits the respiratory centre producing 'apnoea'.

However, hypoxia is maintained because of pulmonary congestion, therefore, sequence of apnoea followed by respiration keeps repeating till alveolar pCO_2 comes back to normal or as long as the heart failure is not corrected.

c. Brain Damage

If there is damage of supramedullary inhibitory pathways, the medullary chemoreceptors become more sensitive to the action of CO_2; pulmonary ventilation increases, CO_2 is washed out and alveolar pCO_2 falls producing apnoea. As a result, CO_2 accumulates, alveolar pCO_2 rises and respiration gets stimulated. Thus, the sequence of apnoea followed by respiration keeps on repeating.

Effects of Periodic Breathing

1. Fall in arterial pCO_2 causes severe vasoconstriction of cerebral blood vessels, as a result cerebral blood flow decreases producing dizziness.
2. Hyperventilation leads to respiratory alkalosis, therefore, more ionisation of proteins occurs; protein anions bind more Ca^{2+} and ionised Ca^{2+} decreases in the body resulting in *Tetany* (neuro-muscular hyperexcitability, muscle spasm, etc., page 710).

2. Biot's Breathing

This is a type of periodic breathing in which there are 3-4 cycles of normal respiration followed by abrupt onset of apnoea and again abrupt onset of normal respiration. This cycle is repetitive **(Fig. 48.14)**.

Seen in:

(i) meningitis, and

(ii) diseases affecting the medulla.

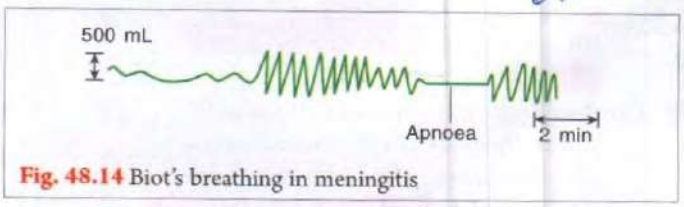


Fig. 48.14 Biot's breathing in meningitis

Study Questions

1. **Draw well labelled diagrams:**
   (i) Physiological organization and relationship between respiratory centres
   (ii) Ventilatory response to hypoxia with intact innervation and after peripheral chemoreceptors denervation
   (iii) CO_2 response curve
   (iv) Effect on CO_2 response curve to varying amount of hypoxia.
   (v) Organization of the carotid body
2. **Write briefly about:**
   (i) Automatic and voluntary control of respiration
   (ii) Genesis of respiration
   (iii) Pulmonary stretch receptors
   (iv) Non-chemical stimuli affecting respiratory centre
   (v) Hering-Breuer reflex
   (vi) J-Reflex
   (vii) Role of vagi in regulation of respiration
   (viii) Deglutition reflex
   (ix) Respiratory chemoreceptors
   (x) Pulmonary chemoreflex
   (xi) CO_2 narcosis
   (xii) Apnoea point
   (xiii) Sleep apnoea syndrome
   (xiv) Breath holding
   (xv) Cheyne-Stokes respiration.
   (xvi) Irritant receptor
   (xvii) Periodic breathing
   (xviii) Factors affecting dyspnoea
3. **What will happen:**
   (i) To respiration after cutting vagi
   (ii) To respiration if adrenaline is administered in low and high dosage respectively.
   (iii) If peripheral chemoreceptors are removed
   (iv) To pulmonary ventilation by inhalation of CO_2 in varying concentrations
   (v) If alveolar pCO_2 falls below normal levels.
   (vi) To respiration in a febrile person
   (vii) To respiration during disturbances in the body metabolism
4. **Give physiological basis of:**
   (i) Cough and sneezing reflex
   (ii) Tachypnoea and bronchoconstriction
   (iii) Yawning and hiccup
   (iv) Adrenaline apnoea
   (v) Respiratory chemoreceptors are not stimulated in anaemia or carbon-monoxide poisoning
   (vi) Mild to moderate hypoxia stimulates the respiration but severe hypoxia depresses it
   (vii) Pulmonary ventilation is not much affected until IpO_2 falls below 60 mmHg
   (viii) CO_2 increases pulmonary ventilation primarily by stimulating central chemoreceptors
   (ix) Sudden infant death syndrome
   (x) Mechanism of development of apnoea at the end of hyperventilation
   (xi) Alveolar pO_2 can be increased to a maximum of 140 mmHg only.
   (xii) Hering-Breuer reflexes
   (xiii) J-reflex
   (xiv) Breaking point during breath holding
5. **Give an account of effect on CO_2 response curve to varying amount of hypoxia.**
6. **Define apnoea. Give its physiological causes.**
7. **Give experimental evidence of:**
   (i) Reciprocal innervation,
   (ii) Respiratory centre neurons are capable of spontaneous discharge,
   (iii) Medullary respiratory centre discharge get influenced by other inputs.
   (iv) Central chemoreceptors get activated by local increase in H^+ concentration,
   (v) Hypoxia stimulates respiration via stimulation of peripheral chemoreceptors,
   (vi) There is very slight but definite hypoxic and CO_2 drive of respiratory centre in normal individuals.
8. **How is voluntary control of respiration brought about? What are its limitations?**
9. **Give an account of chemical regulation of respiration. What is its main aim?**

MCQs

1. **The inherent rhythmicity of respiration comes from:**
   (a) Vagus nerve (b) Pons (c) Cerebral cortex (d) Medulla
2. ***Not true* of medullary respiratory centre:**
   (a) Inspiratory neurons show rhythmic discharge with varying frequencies
   (b) I and E neurons have inhibitory connections to each other
   (c) Also called classical respiratory centre
   (d) Expiratory neurons discharge spontaneously
3. **Pulmonary stretch receptors:**
   (a) Get stimulated during expiration
   (b) Get stimulated both during inspiration and expiration
   (c) Send inhibitory impulses via vagus to apneustic centre
   (d) Stimulate the inspiration
4. ***Not true* of Hering-Breuer inflation reflex:**
   (a) Absent in healthy eupnoeic individual
   (b) Threshold level is at tidal volume 500 mL
   (c) Determine pattern of breathing during exercise
   (d) Inhibits inspiration
5. **J-receptors stimulation include all *except*:**
   (a) Inhibits spinal stretch reflex
   (b) Limits the power of contraction of skeletal muscles
   (c) Play important role in severe exercise
   (d) Initiated by hypoxia
6. **Stimulation of irritant receptors in the respiratory zone of air passage produces:**
   (a) Sneezing
   (b) Coughing
   (c) Tachypnoea
   (d) Apnoea
7. **Blood flow in each 2 mg carotid body is ..... mL/min:**
   (a) 0.01
   (b) 0.04
   (c) 0.08
   (d) 0.16
8. **The respiratory chemoreceptors have all the features, *except*:**
   (a) Have blood flow rate similar to that of brain
   (b) More influenced by arterial pO_2 than by arterial oxygen content
   (c) Stimulated by a rise in blood hydrogen ion concentration
   (d) Wholly responsible for stimulation of ventilation in response to hypoxia
9. **Carotid bodies differ from aortic bodies in all of the following *except*:**
   (a) More effective in stimualting respiration
   (b) Increases both rate and depth of respiration
   (c) Get stimulated by hyperkalemia
   (d) Innervated by IX cranial nerve
10. **There is *no* stimulation of ventilation by hypoxia until the alveolar pO_2 falls below:**
    (a) 60 mmHg
    (b) 58 mmHg
    (c) 50 mmHg
    (d) 45 mmHg
11. **Raised alveolar pCO_2 though a respiratory stimulant, can cause respiratory depression when the level exceeds:**
    (a) 10 mmHg
    (b) 20 mmHg
    (c) 40 mmHg
    (d) 60 mmHg
12. **Which is *not* a cause of physiological apnoea?**
    (a) Sleep
    (b) Deglutition
    (c) After hyperventilation
    (d) Bezold-Jarisch reflex
13. **Breath holding time can be increased by all *except*:**
    (a) After removal of carotid bodies
    (b) Psychological factors
    (c) Inhalation of 100% O_2 at 3 atmospheric pressure
    (d) Hyperventilating room air
14. **Maximum voluntary ventilation effort, alveolar pO_2 can be increased to a maximum value of:**
    (a) 100 mmHg
    (b) 120 mmHg
    (c) 140 mmHg
    (d) 160 mmHg
15. **Cheyne-Stokes respiration means:**
    (a) Apnoea followed by normal respiration
    (b) Repeated seqence of apnoea followed by respiration
    (c) Repeated sequence of gradual onset of apnoea followed by gradual restoration of respiration
    (d) 3-4 cycles of normal respiration followed by abrupt onset of apnoea
16. **Destruction of pneumotaxic centre in pons causes:**
    (a) Apnoea
    (b) Forceful respiration
    (c) Apneustic respiration
    (d) Accelerated respiration

17. **Tachypnoea means:**
    (a) Normal breathing at rest
    (b) Stoppage of breathing
    (c) Difficulty in breathing
    (d) Rapid, shallow breathing

18. **One of the following *does not* stimulate peripheral chemoreceptors:**
    (a) Hypoxia
    (b) Hypocapnia
    (c) Acidosis
    (d) Low perfusion pressure

19. **Central chemoreceptors:**
    (a) Innervated by vagus (X) nerve
    (b) Get stimulated by decreased blood pH
    (c) Inhibited during sleep
    (d) Stimulation produces bradycardia, hypotension and apnoea

20. **Most potent respiratory stimulant is:**
    (a) Oxygen
    (b) Carbon dioxide
    (c) H^+
    (d) K^+

21. **Maximum concentration of CO_2 in inspired air that results in hyperventilation and beyond which it produces depression of CNS is:**
    (a) 1%
    (b) 4%
    (c) 7%
    (d) 10%

22. **Raised alveolar pCO_2 though a respiratory stimulant, can cause respiratory depression when the level exceeds:**
    (a) 10 mmHg
    (b) 20 mmHg
    (c) 40 mmHg
    (d) 60 mmHg

23. **Uncomfortable breathing occurs when pulmonary ventilation is increased:**
    (a) 2 times
    (b) 3 times
    (c) 4-5 times
    (d) None of the above

24. **Respiration can be voluntary inhibited for a period of:**
    (a) 45-55 secs
    (b) 60-75 secs
    (c) 75-90 secs
    (d) More than 90 secs

25. **Cheyne-Stokes respiration is characterized by:**
    (a) Continuous hyperpnoea
    (b) Increased sensitivity of respiratory centre
    (c) Fluctuating pO_2 and stable pCO_2
    (d) Decreased alveolar pCO_2 below threshold level of stimulation of respiratory centre

Answers

1. (b)	2. (d)	3. (c)	4. (b)	5. (d)	6. (c)	7. (b)	8. (a)	9. (c)	10. (a)
11. (d)	12. (d)	13. (c)	14. (c)	15. (c)	16. (c)	17. (d)	18. (b)	19. (c)	20. (b)
21. (c)	22. (d)	23. (c)	24. (a)	25. (d)					

Chapter 49

Hypoxia

I. Definition
II. Types: Hypoxic, Anaemic, Stagnant, Histotoxic
III. Effects of hypoxia
IV. Treatment of hypoxia
- Inhalation of 100% pure O_2
- Hyperbaric O_2 therapy

V. Applied Aspects: Cyanosis

RBC standing still without oxygen, Hb due to attack of bact.

See table in pg. 460 in case you don't have time

RBC ≡ Tyre ↳ O_2 ≡ Air, Hb ≡ Black colour

DEFINITION

Deflated Tyre

Hypoxia means the lack of oxygen at tissue level.
Anoxia means complete absence of oxygen in the tissues.

TYPES

No air

A. *Hypoxic hypoxia*
B. *Anaemic hypoxia*
C. *Stagnant (ischaemic) hypoxia*
D. *Histotoxic hypoxia*

A. HYPOXIC HYPOXIA → Type of Snow city

I Definition

It is characterized by a low arterial pO_2 when O_2 carrying capacity of blood and rate of blood flow to tissues are normal or elevated.

RC

"An inflated tyre on ICE moves faster"

II Characteristic feature (Refer to Table below) (Fig. 49.1:B)

Thus, ***characteristic features*** of hypoxic hypoxia are:
(1) low arterial pO_2,
(2) low arterial O_2 content,
(3) low arterial % O_2-saturation of haemoglobin, and
(4) low A-V pO_2 difference.

PO_2 ↓, O_2 content, A-V

III Causes

Ooty - Polluted place - small cottage

1. **Low pO_2 in inspired air**, for example:
   (i) High altitude (more than 10000 feet or 3000 mts above sea level).
   (ii) Breathing in a closed space.
   (iii) Breathing an artificial gas mixture containing low pO_2.
2. **Decreased pulmonary ventilation** as seen in:
   (i) Airway obstruction. ↳ Udta Punjab
   (ii) Weakness/paralysis of respiratory muscles.
   (iii) Depression of respiratory centres by drugs specially morphine. (R. acidosis)
3. **Defect in exchange of gases** through alveolar-capillary membrane. This includes all the factors affecting V/P ratio (page 424).

Characteristic features: Hypoxic Hypoxia (Fig. 49.1 - A and B)

Feature	Normal		Hypoxic Hypoxia	
	Capillary		Capillary	
	Arterial End	Venous End	Arterial End	Venous End
1. pO_2 (mmHg)	100	40	55 (Half + 5)	25
2. % O_2-saturation of haemoglobin	97.5% (≈98%)	75% (73%)	85% (↓ 15%)	45% (↓ 30%)
3. O_2 content (mL/dL)	19	14	14	9
Therefore, at tissue level (i) O_2 utilization	19-14 = 5 mL/dL		14-9 = 5 mL/dL	
(ii) A-V pO_2 difference	100–40 = 60 mmHg		55–25 = 30 mmHg	

Recall 9th std text

1, 2, 3, 4ii

only thing that remained same

Half

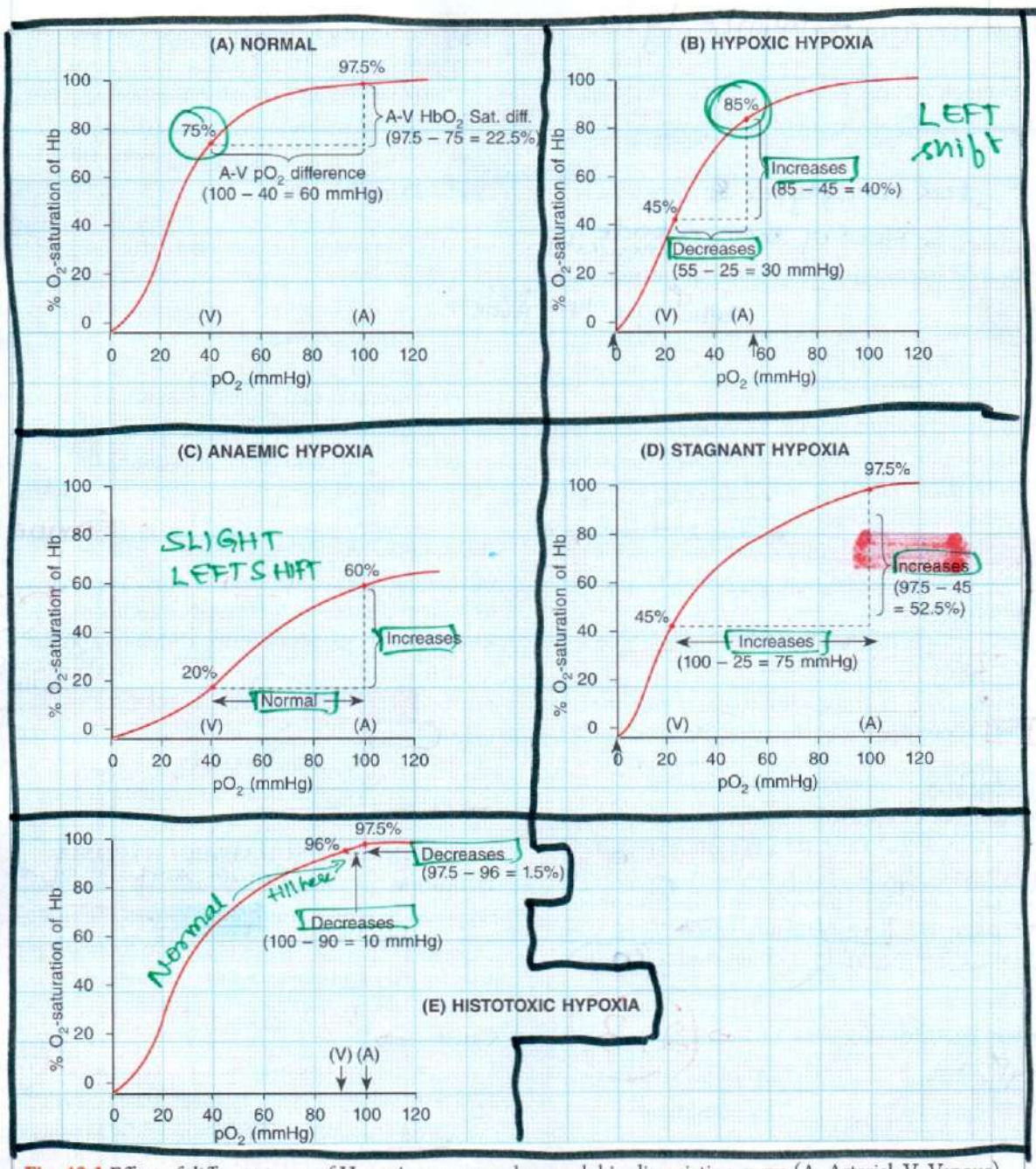


Fig. 49.1 Effect of different types of Hypoxia on oxygen-haemoglobin dissociation curve (A: Arterial, V: Venous)

4. **Venous-arterial shunts** *i.e.* venous blood enters arterial blood without going into the lungs, therefore, arterial pO_2 decreases. For example: cyanotic congenital heart disease (***Fallot's Tetrology***).

Persistent [In Patent Ductus Arteriosus]

Pathophysiology

'Hypoxic hypoxia'

↓ *Via peripheral chemoreceptors stimulates*

Respiratory centre

Pulmonary ventilation increases

↓

CO_2 wash out from the body

↓

Arterial pCO_2 decreases

↓

'Oxygen-haemoglobin curve' shifts to left (Bohr's effect)

↓

Less release of O_2 from haemoglobin (O_2 affinity to Hb ↑)

↓

Tissue hypoxia

Dr. Padma priya mam

B. ANAEMIC HYPOXIA

Definition

Hypoxia in which arterial pO_2 is normal but the amount of haemoglobin available to carry O_2 is reduced.

Causes

1. **Anaemia**
2. **Haemorrhage**
3. Conversion of **haemoglobin** to some **abnormal** form. For example:
   (i) **Methaemoglobin** *i.e.* iron in the haemoglobin is present in ferric (Fe^{3+}) form instead of ferrous (Fe^{2+}) form.
   (ii) **Carboxy (Carbonmonoxy) Haemoglobin**: COHb *i.e.* haemoglobin combines with 'CO'. The affinity of haemoglobin for 'CO' is 210 times its affinity for O_2 and COHb so formed liberates O_2 very slowly. COHb is *cherry red* in colour and visible in the skin, nail beds and mucous membranes. 'CO' produces severe hypoxia by:
   (a) preventing the haemoglobin to combine with O_2, and
   (b) shifting O_2-haemoglobin dissociation curve to left, therefore, less release of O_2.

Important Note

The symptoms of CO poisoning are those of any type of hypoxia (page 459). In addition, chronic exposure to sublethal concentrations of CO produces: progressive brain damage, mental changes and Parkinsonism like state (page 997). Death results when 70–80 % of the circulating haemoglobin is converted to COHb.

Characteristic Features (Fig. 49.1 - C)

Feature	Anaemic Hypoxia	
	Capillary	
	Arterial End	Venous End
1. pO_2 (mmHg)	100	40
2. % O_2-saturation of haemoglobin	60% (decreases)	20% (decreases)
3. O_2 content (mL/dL)	10	5
4. A-V O_2 diff.	= 5 mL	

1. As haemoglobin content is less than normal or it is converted to some abnormal form, thus, normal haemoglobin content will reduce to less than 50% of normal.
2. Arterial pO_2 is normal (100 mmHg), as there is no defect in alveolar ventilation or pulmonary blood flow.
3. Arterial percentage saturation of haemoglobin with oxygen decreases as its concentration in blood is low (about 50% of normal) due to anaemia.
4. O_2 content of arterial blood is less than normal, approximately 10 mL/dL at arterial pO_2 100 mmHg. Therefore, when this arterial blood reaches the tissues, the tissues will take up 5 mL/dL of O_2 (O_2 utilization of resting tissues), hence in venous blood:
   (i) pO_2 : 40 mmHg
   (ii) % O_2-saturation of haemoglobin: decreases, and
   (iii) O_2 content: 10–5 = 5 mL/dL.

Thus **characteristic features** of anaemic hypoxia are:
(1) normal arterial pO_2
(2) arterial O_2 content, moderately reduced
(3) arterial % O_2-saturation of haemoglobin: decreases, and
(4) A-V pO_2 difference is normal, 100 – 40 = 60 mmHg.

Pathophysiology

In anaemic hypoxia at rest, hypoxia is not severe, because in anaemia there is increased amount of 2,3 DPG in the RBCs which combines with oxyhaemoglobin and results in increased liberation of O_2.

$$HbO_2 + 2,3\ DPG \rightarrow Hb\ 2,3\ DPG + O_2$$

But during exercise when there is increased O_2 demand by tissues due to increased O_2 consumption, the tissue demand is not met fully and severe hypoxia develops.

C. STAGNANT (ISCHAEMIC) HYPOXIA

Definition

Hypoxia in which the blood flow to the tissues is so low that adequate O_2 is not delivered to them despite a normal arterial pO_2 and haemoglobin concentration.

Causes

1. **Circulatory failure**
2. **Haemorrhage**, via baroreceptors produces reflex vasoconstriction and thus blood flow to tissues decreases,
3. **Congenital heart failure**. It is associated with pulmonary congestion which produces defect in oxygenation, therefore, patient also suffers from hypoxic hypoxia in addition to stagnant hypoxia.

Characteristic Features (Fig. 49.1 - D)

Feature	Stagnant (Ischaemic) Hypoxia	
	Capillary	
	Arterial End	Venous End
1. pO_2 (mmHg)	100	25
2. % O_2-saturation of haemoglobin	97.5%	45%
3. O_2 content (mL/dL)	19	9

1. Since there is no defect in oxygenation of blood, % O_2-saturation of haemoglobin; pO_2 and O_2 content of arterial blood are normal.
2. As blood stays for a longer time at tissue level, therefore,
   (i) resting O_2 uptake by tissue increases from 5 mL/dL to 10 mL/dL;
   (ii) accumulation of CO_2 in tissues shifts 'oxygen-haemoglobin dissociation' curve to 'Right', more O_2 is released from haemoglobin, therefore, venous blood is further reduced.

Hence in venous blood:
(i) pO_2 : 25 mmHg,
(ii) % O_2-saturation of haemoglobin: 45%, and
(iii) O_2 content: 19–10 = 9 mL/dL.

Thus, **characteristic features** of stagnant hypoxia are:
1. Normal arterial pO_2,
2. Normal arterial O_2 content,
3. Normal arterial % O_2-saturation of haemoglobin, and
4. A-V pO_2 difference: 100–25 = 75 mmHg, more than normal.

D. HISTOTOXIC HYPOXIA

Definition

Hypoxia in which the amount of oxygen delivered to the tissues is adequate, but because of the action of a toxic agent the tissue cells cannot make use of the O_2 supplied to them.

Causes

Cyanide poisoning: It produces hypoxia at tissue level causing poisoning of cellular enzymes specially *cytochrome oxidase*, and also produces tissue oedema.

Characteristic Features (Fig. 49.1 - E)

Feature	Histotoxic Hypoxia	
	Capillary	
	Arterial End	Venous End
1. pO_2 (mmHg)	100	90
2. % O_2-saturation of haemoglobin	97.5%	96%
3. O_2 content (mL/dL)	19	18.5

Since tissues cannot utilize O_2 due to cyanide poisoning, so practically there is no difference of A-V pO_2. If tissues take 0.5 mL/dL of O_2, then % O_2-saturation of haemoglobin is 96% with O_2 content 18.5 mL/dL and pO_2 90 mmHg.

Thus, **characteristic features** of histotoxic hypoxia are:
1. Normal arterial pO_2,
2. No difference of O_2 content of arterial and venous blood,
3. Normal arterial % O_2 saturation of haemoglobin
4. A-V pO_2 difference: 100 – 90 = 10 mmHg *i.e.* less than normal.

Summary

Table 49.1 gives summary of different types of hypoxia.

2. EFFECTS OF HYPOXIA

1. On Respiration

All types of hypoxia except anaemic hypoxia stimulate peripheral chemoreceptors to increase respiration. The rate of increase in ventilation is in proportion to the severity of the hypoxia of the peripheral chemoreceptors.

Ventilation rate ∝ Hypoxial severity

2. On CNS

In all types of hypoxia, brain is affected 'first' and the symptoms produced are more or less like those of *overdose of alcohol*, viz.,
(i) drowsiness, impaired judgement, dull pain sensibility,
(ii) depression or excitement,
(iii) headache, loss of self control, disorientation, loss of time sense, and
(iv) talkativeness, emotional outburst of laughing, shouting or crying and fixed ideas.

A person thinks that his mind and judgement is clear inspite of his dangerous behaviour.

At approx. 18,000 feet (5,400 mts) when arterial pO_2 falls to 40 mmHg and percentage O_2-saturation of haemoglobin is 70%, *hypoxic hypoxia* occurs, producing signs and symptoms of CNS involvement which includes:
(i) Tremors,
(ii) *Cheyne-Stoke respiration* (page 451) specially during sleep, and
(iii) Errors in simple manipulation.

When % O_2-saturation of haemoglobin falls below 60%, there is unconsciousness within 20 seconds, causing death in 4 to 5 minutes.

3. On CVS

Hypoxia (*except anaemic*) ⟶ stimulates peripheral chemoreceptors ⟶ stimulate,
(i) vasomotor centre (VMC) ⟶ increases heart rate and blood pressure
(ii) respiratory centre ⟶ increases rate and depth of respiration.

Therefore, severe hypoxia causes increase in heart rate, systemic arterial blood pressure and respiration.

4. Other associated symptoms due to hypoxia are: nausea, vomiting and anorexia.

Table 49.1: Summary: Characteristic features of different types of hypoxia

	Hypoxic hypoxia	Anaemic hypoxia	Stagnant (ischaemic) hypoxia	Histotoxic hypoxia
1. *Definition* based on: (i) arterial pO_2 (ii) haemoglobin content (iii) Rate of blood flow to tissues	(i) ↓s; (ii) and (iii) are normal; *i.e.* low arterial pO_2 when O_2 carrying capacity of blood and rate of blood flow to tissues are normal.	(ii) ↓s; (i) and (iii) are normal; *i.e.* ↓ amount of haemoglobin available to carry O_2 in spite of normal arterial pO_2 and normal rate of blood flow to tissues.	(iii) ↓s; (i) and (ii) are normal; *i.e.* rate of blood flow to tissues ↓s so that adequate O_2 is not delivered to them in spite of normal arterial pO_2 and haemoglobin content.	(i), (ii) and (iii) are normal; *i.e.* normal O_2 delivery to tissues but they cannot make use of O_2 supplied to them due to action of toxic agent.
2. *Causes*	(i) ↓IpO_2 (ii) ↓pulmonary ventilation (iii) defective V/P ratio	Total haemoglobin content ↓s due to: (i) anaemia (ii) haemorrhage (iii) presence of abnormal haemoglobin: COHb or methaemoglobin.	(i) circulatory failure (ii) haemorrhage (iii) heart failure	Cyanide poisoning.
3. *Arterial pO_2*	↓s (as IpO_2 ↓s)	Normal	Normal	Normal
4. *Arterial O_2 content*	↓s	Markedly reduced	Normal	Normal
5. *Arterial %-O_2 saturation of haemoglobin*	↓s	↓s	Normal	Normal
6. *A-V pO_2 difference*	↓s	Normal	More than normal	Less than normal
7. *Cyanosis* *(page 461)*	Present	Absent	Present	Absent (as reduced haemoglobin is produced in small amounts).
8. *Stimulation of peripheral chemoreceptors*	Present (as dissolved O_2 in plasma ↓s)	Absent (dissolved O_2 in plasma is sufficient to meet receptor demand).	Present (because arterial pCO_2 increases and pO_2 ↓s).	Present (cyanide ↓s O_2 utilization at tissue level.

(↓ : decrease; ↑ : increase)

(3) TREATMENT OF HYPOXIA

Basic treatment of hypoxia includes:

1. Treatment of the underlying cause. It depends on the type of hypoxia.
2. Oxygen therapy

OXYGEN THERAPY

Simple *O_2 therapy* will not help in the treatment of hypoxia because diffusion across 'respiratory membrane' depends upon partial pressure of a gas, therefore, alveolar pO_2 can be increased either:

A. by inhalations of 100% pure O_2, or

B. by inhalation of 100% O_2 at high barometeric pressure, called ***hyperbaric oxygen therapy.***

A. Inhalation of 100% pure O_2 at 1 atmospheric pressure *i.e.* at 760 mmHg

This is only useful in *hypoxic hypoxia*, specially when it is due to decreased IpO_2. However, it has limited value in anaemic, stagnant and histotoxic hypoxia.

Dangers of inhalation of 100% pure O_2: Oxygen toxicity

Inhalation of 100% pure O_2 in normal individuals may cause decrease in respiration, suggesting normally there is very slight but definite some hypoxic chemoreceptor drive of the respiratory centre (Also see to page 469). 100% O_2 also produces toxic effects on bacteria, fungi and cultured cells due to production of superoxide anion (O_2^-) and H_2O_2. Therefore, administration of 80-100% O_2 in adult humans:

1. For more than 8 hours will stimulate the 'irritant' receptors in the respiratory passage (page 443). Depending on the site of stimulation it can produce nasal congestion, sore throat, substernal discomfort, sneezing, coughing, tachypnoea with bronchoconstriction.
2. For more than 24 hours causes *broncho-pneumonia* by:
   (i) inhibiting ability of lung macrophages to kill bacteria, and
   (ii) decreasing production of 'surfactant'.

   Newborn infants are very sensitive to O_2 *toxicity*, and should never be given more than 40% O_2 inhalations as this may lead to proliferation of retinal vessels with the formation of fibrous tissues *(retrolental fibroplasia)* producing permanent blindness. It may also leads to formation of lung cysts and densities *(broncho pulmonary dysplasia)*

Important Note

If hypoxia is associated with 'hypercapnia' (increased CO_2 in the body), as seen in 'severe pulmonary failure', O_2 therapy may cause death. How? In this situation, whatever respiration is there, it will be due to hypoxic stimulation of peripheral chemoreceptors. If 'O_2 therapy' is given at this stage respiration will be inhibited as hypoxic drive will be overcome soon causing death due to direct depression of respiratory centre by severe hypoxia and hypercapnia.

B. Inhalation of 100% pure O_2 at high barometric pressure: *Hyperbaric O_2 therapy*

Its main aim is to increase the amount of dissolved O_2 in plasma. Therefore, it is useful in:

1. Anaemic hypoxia (specially due to 'CO' poisoning or severe blood loss),
2. Stagnant hypoxia, and
3. Histotoxic hypoxia (Radiation induced tissue injury and gas gangrene)

Mechanism of action

At sea level, barometeric pressure is 760 mmHg or 1 atmospheric pressure with arterial pCO_2 40 mmHg and pH_2O 47 mmHg. Therefore, inhalation of 100% O_2 at 1 atmospheric pressure can increase arterial pO_2 to a maximum of 673 mmHg {760 – (40 + 47)}.

As normal dissolved O_2 in plasma is 0.3 mL per 100 mL per 100 mmHg pO_2 or 0.003 per 100 mL per mmHg pO_2. Thus,

1. At 'one' atmospheric pressure by inhalation of 100% O_2, the level of dissolved O_2 can be increased only to approx. 2 mL/dL of blood (673 × 0.003); but since this may not be sufficient to meet the tissue's resting O_2 demand of 5 mL/dL of blood, therefore, patient is asked to inhale 100% O_2 in a chamber at high pressure.
2. At 'two' atmospheric pressure, by inhalation of 100% O_2, arterial pO_2 can be increased to: 2 × 673 = 1346 mmHg and dissolved O_2 level increases to, 1346 × 0.003 = 4.038 mL/ dL of blood. This is still inadequate for normal tissue resting O_2 demand.
3. At 'three' atmospheric pressure, inhalation of 100% O_2, causes arterial pO_2 to increase to: 3 × 673 = 2019 mmHg and dissolved O_2 increases to 2019 × 0.003 = 6.057 mL/dL of blood.

However *oxygen toxicity* may occur early (from few to 30 minutes) at high barometeric pressure (above 3 atomospheric), therefore, one has to be very careful in increasing the pressure specially beyond 2 atmospheric.

Whenever, $pO_2 \geq 1500$ mmHg, in addition to 'O_2 toxicity' as described above, it can also produce derangement of cerebral activity which includes:

1. *muscular twitching*, ringing in ears, dizziness,
2. *jerking* (cog-wheel) type of respiration,
3. *convulsions and unconsciousness*.

It may be due to:

(i) decrease in brain GABA and ATP content
(ii) inhibition of tissue enzymes activity in the body, and
(iii) cerebral vasoconstriciton which causes:
   (a) decrease in cerebral blood flow,
   (b) accumulation of acid products near respiratory centre increases pulmonary ventilation, therefore, alveolar pCO_2 falls, further constricting the cerebral vessels.

Important Note

Since the harmful effects of breathing O_2 are proportionate to the arterial pO_2, they can be prevented by decreasing the concentration of O_2 in the gas mixture to 20% or less.

APPLIED ASPECT: CYANOSIS

Definition

Cyanosis is the bluish colouration of skin and/or mucus membrane due to the presence of at least 5 gm of *reduced haemoglobin* per 100 mL of blood in the capillaries.

Reduced haemoglobin is 'blue' (or dark) in colour. The skin and mucus membrane take blue colour when reduced haemoglobin is present in the *capillaries*.

Sites

Cyanosis is commonly seen at sites where the skin is thin, for example **(Fig. 49.2)**:

1. Mucus membrane of under surface of tongue
2. Lips
3. Ear lobes
4. Nail beds
5. Tip of Nose.

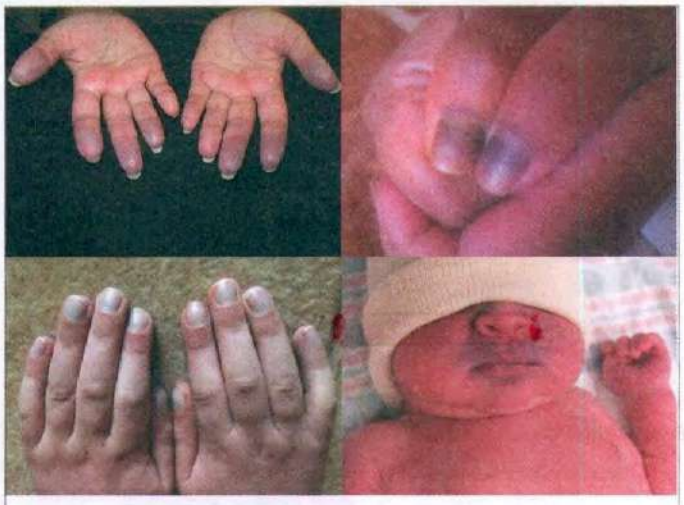


Fig. 49.2 Cyanosis sites

Pathophysiology: Criteria for cyanosis occurrence

Occurrence of cyanosis depends on:

1. Total amount of haemoglobin in the blood,
2. The degree of haemoglobin unsaturation, and
3. The state of capillary circulation.

Criteria 1

5 gm/dL of haemoglobin when fully saturated with O_2 will carry: 5 × 1.34 = 6.7 mL of O_2. This implies, O_2 "Unsaturation" of 6.7 mL per 100 mL of blood will produce cyanosis.

Criteria 2

Haemoglobin when fully saturated with O_2 is called 100% saturation of haemoglobin. As in normal adults average haemoglobin content is 15 gm/dL and for cyanosis to occur, at least 5 gm/dL of reduced haemoglobin is required in the capillaries. Therefore, in the remaining haemoglobin content of 10 gm/dL, percentage saturation of haemoglobin will be approx. 66% (100 × 10/15).

Thus, less than 66% saturation of haemoglobin with O_2 will produce cyanosis.

Causes of Cyanosis

1. *Hypoxic Hypoxia:* Since arterial pO_2 is less than normal due to the defective oxygenation of blood, hence more of reduced haemoglobin will be there; when it exceeds 5 gm/dL, cyanosis develops.
2. *Stagnant Hypoxia:* When blood flow through the capillaries becomes very low or nearly becomes stagnant, more oxygen is extracted from the blood causing increase in reduced haemoglobin producing cyanosis.
3. *Polycythemia:* There is increase in total haemoglobin content, therefore, polycythemia aggravates the cyanosis.

Important Note

Hypoxic hypoxia produces cyanosis when reduced haemoglobin exceeds 5 gm/dL. It also produces 'polycythemia' due to liberation of erythropoietin which in turn aggravates the cyanosis. Therefore, cyanosis is a common occurrence in hypoxic hypoxia.

4. *Local Factors*

(i) **Exposure to mild cold** (*i.e.* at approx. 20°C) produces cyanosis. How?

Cold by producing cutaneous vaso-constriction (arteriolar as well as venous) decreases tissue blood flow (stagnant hypoxia). Therefore, more and more O_2 is extracted by the tissues; amount of reduced haemoglobin increases and cyanosis develops.

(ii) **Exposure to severe cold** (at approx. 10°C or below) produces ***no*** cyanosis. Why?

(a) Oxygen-haemoglobin dissociation curve shifts to left, thus, prevents release of O_2 from the haemoglobin.

(b) O_2 consumption of tissues decreases markedly.

Both the above factors decrease the amount of reduced haemoglobin, hence no cyanosis develops.

Important Notes

1. *Anaemic Hypoxia:* Cannot cause cyanosis, as total haemoglobin content is low, enough reduced haemoglobin is not formed to produce blue colour.
2. In 'CO' poisoning the colour of reduced haemoglobin is obscured by the "cherry red" colour of carbonmonoxy haemoglobin.
3. *Histotoxic Hypoxia:* Cannot cause cyanosis, as O_2 is not taken up by tissues. Therefore, reduced haemoglobin is either not produced or produced in very small amounts (< 5 gm/dL).
4. In the absence of local factors, presence of cyanosis indicates hypoxia (hypoxic or stagnant), whereas absence of cyanosis does not indicate absence of hypoxia.
5. High circulating levels of methaemoglobin produces discolouration of skin and mucous membrane similar to cyanosis (page 59).

Study Questions

1. **Give physiological significance of:**
   (i) Simple and Hyperbaric oxygen therapy
   (ii) Retrolental fibrosis.
2. **Write short notes on:**
   (i) Pathophysiology of hypoxic hypoxia
   (ii) Effects of hypoxia
   (iii) Oxygen toxicity
   (iv) Criteria for occurrence of cyanosis.
3. **Give physiological basis of:**
   (i) Cyanosis is not seen in anaemic and histotoxic hypoxia
   (ii) Peripheral chemoreceptors are not stimulated in anaemic hypoxia
   (iii) Neonates should never be given 100% O_2 inhalations
   (iv) O_2 therapy may cause death in an individual with severe respiratory failure
   (v) Death may occur with hyperbaric O_2 therapy
   (vi) Exposure to mild cold produces cyanosis while it is not seen with severe cold
   (vii) Cyanosis is a common occurrence in hypoxic hypoxia
   (viii) Inhalation of 100% pure O_2 in normal individuals may cause decrease in respiration.
   (ix) Cyanosis
4. **Define hypoxia. Give its types. Describe any one of them in detail.**
5. **Draw labelled diagrams to show effect of different types of hypoxia on Oxygen. Haemoglobin dissociation curvve.**
6. **Define cyanosis. Mention its sites. List major causes of its occurrences.**

H (1) → Snow city
A (2) → White tigers
S (3) → Rough sliding roof
H (4) → Rejected types by people because of oldness.

MCQs

1. **Hypoxia is:**
   (a) Complete absence of O_2 at tissue level
   (b) Lack of O_2 at tissue level
   (c) Lack of O_2 and accumulation of CO_2 in the tissues
   (d) Low pO_2 in inspired air
2. **Decreased arterial pO_2 with normal O_2 carrying capacity of blood and rate of blood flow to tissues is:**
   (a) Hypoxic hypoxia (b) Anaemic hypoxia (c) Stagnant hypoxia (d) Histotoxic hypoxia
3. **All features can be found in hypoxic hypoxia *except*:**
   (a) Low arterial pO_2
   (b) Low arterial O_2 content
   (c) Normal haemoglobin content
   (d) Low A-V pO_2 difference
4. **Inhalation of 100% O_2 at 1 atmospheric pressure increases the alveolar pO_2 to about ..... mmHg:**
   (a) 473 (b) 560 (c) 673 (d) 760
5. **What is *not true* of carbon monoxide poisoning?**
   (a) Affinity of haemoglobin is more for CO than O_2
   (b) Its poisoning produces cherry red colour to skin
   (c) Combines with haemoglobin to form carbamino haemoglobin
   (d) Cigarette smoke emits CO
6. **Cyanide poisoning causes death due to:**
   (a) Respiratory depression
   (b) Brainstem depression
   (c) Destruction of cytochrome oxidase
   (d) Circulatory collapse
7. **Peripheral chemoreceptors are *not* stimulated in which type of hypoxia?**
   (a) Hypoxic (b) Anaemic (c) Stagnent (d) Histotoxic
8. **Earliest effect of hypoxia:**
   (a) Increase in heart rate and B.P.
   (b) Increase in pulmonary ventilation
   (c) Decrease in brain higher function
   (d) Cheyne-Stoke respiration
9. **Oxygen therapy has significant value in all the following types of hypoxia *except*:**
   (a) Atmospheric hypoxia
   (b) Hypoxia due to pulmonary oedema
   (c) Hypoxia due to decreased haemoglobin in the blood
   (d) Histotoxic hypoxia due to cyanide poisoning

10. **Cyanosis is:**
    (a) Substernal discomfort
    (b) Bluish discolouration of skin and/or mucus membrane
    (c) Presence of ≥5 gm/dL of reduced haemoglobin in blood
    (d) Ringing in ears

11. **Exposure to severe cold produces *no* cyanosis because:**
    (a) Tissue blood flow decreases markedly
    (b) Less O_2 is extracted by the tissues
    (c) Less O_2 is delivered to the tissues
    (d) (a) and (c) are correct

12. ***Not a true* statement regading absence of cyanosis in an individual:**
    (a) Indicate absence of hypoxia
    (b) Reduced haemoglobin in the capillaries is less than 5 gm/dL
    (c) O_2 unsaturation of blood must be below 6 mL/dL
    (d) Percentage saturation of haemoglobin is above 66%

13. **Causes of hypoxic hypoxia include all except:**
    (a) High altitude
    (b) Emphysema
    (c) Pulmonary fibrosis
    (d) Carbon monoxide poisoning

14. ***Not* a characteristic feature of anaemic hypoxia:**
    (a) Produces severe hypoxia during exercise
    (b) Associated with increase amount of 2,3 DPG in the RBC
    (c) Normal arterial O_2 content
    (d) Normal arterial pO_2

15. **All can lead to cyanosis *except*:**
    (a) Cyanide poisoning
    (b) Polycythemia
    (c) Defective V/P ratio
    (d) Heart failure

16. **Severe hypoxia may produce:**
    (a) Hypertension
    (b) Hypotension
    (c) BP fall followed by BP rise
    (d) No change

17. **Inhalation of 100% O_2 at sea level for 24 hours may lead to *all except*:**
    (a) Nasal congestion and respiratory tract irritation
    (b) Coughing
    (c) Decreased surfactant production
    (d) Convulsions, acidosis and coma

18. **Hyperbaric oxygenation is useful in *all except*:**
    (a) Congenital heart disease
    (b) Gas gangrene
    (c) Carbon monoxide poisoning
    (d) Nitrogen toxicity

19. **In which type of hypoxia, cyanosis is *not* seen?**
    (a) Histotoxic
    (b) Anaemic
    (c) Stagnant
    (d) Hypoxic

20. **A premature infant should *not*:**
    (a) Be exposed to 100% O_2 for a prolonged period
    (b) Have a carbohydrate diet
    (c) Have a diet poor in fats
    (d) Maintained in a closed room at ambient temperature of 20°C

Answers

1. (b)	2. (a)	3. (c)	4. (c)	5. (c)	6. (c)	7. (b)	8. (c)	9. (d)	10. (b)
11. (d)	12. (a)	13. (d)	14. (c)	15. (a)	16. (a)	17. (d)	18. (d)	19. (b)	20. (a)

Chapter

50

Physiology of High Altitude

I. Introduction

II. Effects during rapid ascent: pulmonary oedema

III. Effects during slow ascent: motion sickness

IV. Acclimatization

INTRODUCTION

1. **Barometric pressure** falls with increasing altitude but composition of air remains the same as that present at the sea level. Therefore, total pressure of the air (P) will be equal to the sum of partial pressures of the gases it contains (*Dalton's Law*, page 411) *i.e.*

$$P = pO_2 + pCO_2 + pN_2 + pH_2O$$

2. **pH_2O** does not depend on high altitude, because it is entirely determined by the body temperature. As body can regulate its temperature at 37°C, irrespective of altitude, therefore, pH_2O remains constant at 47 mmHg.
3. **Alveolar pCO_2** depends upon the balance between metabolism of the body and respiration. Therefore, when body metabolism increases, alveolar pCO_2 increases causing pulmonary ventilation to increase; CO_2 is washed out, restoring alveolar pCO_2. Opposite is seen when body metabolism decreases.

   As the metabolic production of CO_2 does not alter with increasing altitude, alveolar pCO_2 will not change until the breathing is affected by some other causes *e.g.* hypoxia. It has been known that hypernoea (increase in breathing) occurs at high altitude when arterial pO_2 falls below 60 mmHg, resulting in fall in alveolar pCO_2. (page 446)
4. From the above, it is clear that with increasing altitude, (i) barometric pressure decreases, so the total pressure of the air decreases and (ii) pH_2O and pCO_2 remain constant. Therefore, there occurs progressive decrease in pO_2 and pN_2 with increase of height. *A height in excess of 10,000 feet (3,000 mts) above the sea level is defined as* ***high altitude*** (Fig. 50.1).

   As decrease in pN_2 produces no ill effects on the body, therefore, effects of high altitude are entirely due to decrease in pO_2 in the inspired air *i.e.* effects are same as of ***Hypoxic Hypoxia*** (page 479).
5. The effects of *hypoxic hypoxia* at high altitude will depend upon (Table 50.1):
   (i) the rate at which the hypoxia develops *i.e.* whether the onset is sudden (*Rapid Ascent*) or gradual (*Slow Ascent*); and
   (ii) duration of exposure: long-term stay *e.g.* permanent residents at high altitude (*High Altitude Native*).

Hazards of Rapid Ascent

Rapid ascent more than 10,000 feet (3,000 mts) can produce ***Pulmonary Oedema***. It is seen in individuals,
(1) who engage in heavy physical work during 1st 3 days after arrival, seen in 75-80% subjects;
(2) who are 'acclimatized' to high altitudes; spend 2 weeks or more at sea level, and then reascend.

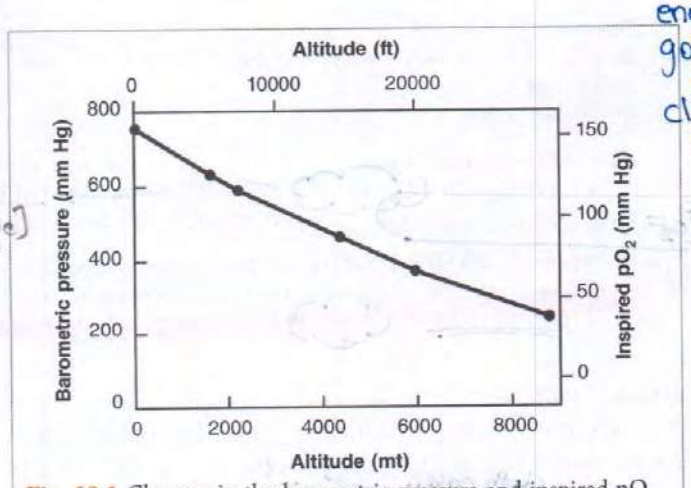


Fig. 50.1 Changes in the barometric pressure and inspired pO_2 with the increasing altitude

Table 50.1: Salient features of hypoxic hypoxia at high altitude

Type of ascent	Degree of hypoxia	Body's Compensatory mechanisms	Effects of hypoxia
I. 'Rapid' Ascent	Severe	Nil	Severe
II. 'Slow' Ascent	Less	Present to some extent	Moderate
III. Permanent residents at high altitude (High altitude natives)	Least	Well developed	Nil due to *Acclimatization (page 488)*

EFFECTS OF HIGH ALTITUDE DURING RAPID ASCENT

Table 50.2: Effects of high altitude

Level of altitude	Barometric pressure (mmHg)	Inspired air pO_2 (mmHg)	Alveolar pO_2 (mmHg)	Alveolar pCO_2 (mmHg)	% – O_2 saturation of haemoglobin	Effects
Sea Level	760	160	100-104	40	100%	Nil
1. Upto 5000 feet (1500 mts)	630	128	80	40	95%	No effects are produced on the body as long as alveolar pO_2 is above 60 mmHg with normal alveolar pCO_2 of 40 mmHg.
2. Upto 10,000 feet (3000 mts)	520	104	60	40	89%	Usually no effects are seen, except only at night there may be some reduction in visual capacity. Therefore, rapid ascend upto 10,000 feet is a *SAFE ZONE OF ASCENT.*
3. Upto 12,000 feet (3600 mts)	490	98	55	slightly ↓s (below 40)	85%	Effects of hypoxia develop which manifest in the form of tachycardia, hypertension, increase in pulmonary ventilation (for details, refer to page 459).
4. Upto 18,000 feet (5400 mts).	400	80	40	30	70%	Above changes plus hypoxic symptoms due to involvement of CNS (page 459).
5. Upto 20,000 feet (6000 mts)	350	70	< 40	< 30	< 70%	Hypoxic symptoms due to involvement of CNS aggravate; *if %-O_2 saturation of haemoglobin falls below 60%, it produces unconsciousness.*
6. Between 25,000-30,000 feet (7500-9000 mts)	← further falls →				falls below 60%	Beyond this, O_2 therapy has to be started. Therefore, called *Critical Survival Altitude.*

7. With 100% O_2-inhalation, one can go till 45,000 feet (13,500 mts) only, because at this height barometric pressure falls to 110 mmHg. This 110 mmHg has to accommodate pH_2O, 47 mmHg; and pCO_2, 30 mmHg. Therefore, pO_2 is left with 110 – (47 + 30) = 33 mmHg only. When pO_2 falls below 40 mmHg, impairment of mental function occurs.
8. At approx. 63,000 feet (18,900 mts), barometric pressure falls to 47 mmHg and water will occupy all the pressure; as a result body fluids will boil at body temperature (theoretical). Therefore, above 40,000-45,000 feet, creation of an artificial atmosphere in a pressurised cabin (*spaceship*) is necessary and in such a cabin one can go upto any altitude.

Cause of pulmonary oedema

It is not due to cardiovascular or lung disease because it responds to rest and O_2. It does not develop in individuals who ascend to high altitude gradually and avoid physical exertion for the first few days of high altitude exposure.

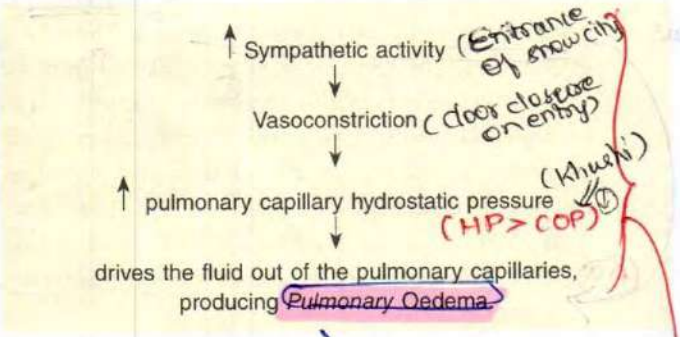
Therefore, probable causes of pulmonary oedema are:

1. **Hypoxia** gets aggravated by work and can increase membrane permeability of pulmonary capillaries producing *Pulmonary Oedema*. Normally, pulmonary capillary hydrostatic pressure of less than 10 mmHg

and osmotic pressure of 25 mmHg, keeps the alveoli dry.

2. At high altitude, sympathetic activity increases because of:
   (i) hypoxic stimulation of the VMC
   (ii) cold' (the temperature falls by 2°C for every 1,000 feet *i.e.* 300 mts. increase in altitude), and
   (iii) increase physical work.

Therefore,



Important Note

If hypoxia is very severe, it will produce generalised oedema.

Characteristics of pulmonary oedema

1. It is associated with *pulmonary artery hypertension*. Ca^{2+} channel blocking drug (*e.g.* Nifedipine), decreases the pulmonary oedema by reducing pulmonary artery pressure.
2. It has *high protein content*.

EFFECTS OF HIGH ALTITUDE DURING SLOW ASCENT

Effects of hypoxia are not very severe here as body gets some time to adjust to hypoxia. For example, in 1953, Edmond Hillary, at the summit of Mount Everest, approx. 29,028 feet (8848 mts), could walk nicely for 10 minutes. After that he had to take O_2.

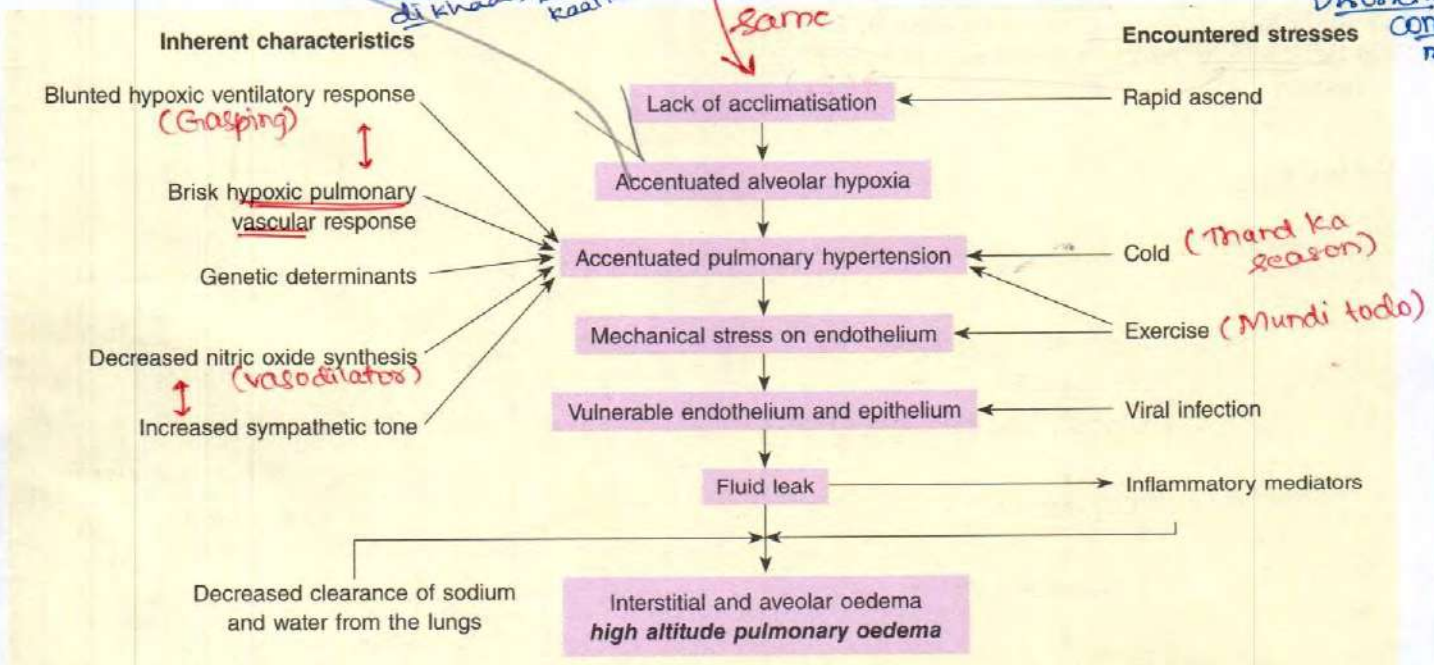
At high altitude, a person suffers from *Mountain Sickness*. It starts approx. 8-12 hours after arrival at high altitude and lasts for about 4-8 days.

It is *characterised by*: Nausea, vomiting, headache, insomnia, dyspnoea and irritability.

Exact cause of mountain sickness is not known, but it appears to be associated with cerebral oedema or alkalosis.

Mechanism of development of cerebral oedema

Decrease in arterial pO_2 with increasing altitude leads to arteriolar dilation; once autoregulatory mechanism is reached, capillary pressure increases causing transudation of fluid in brain tissue. If not treated, it may cause ataxia, disorientation, coma and finally death due to herniation of brain tissue through the tentorium.



Summary: Pathophysiology of pulmonary oedema.

Signs and symptoms of mountain sickness can be prevented if:

(1) cerebral oedema is reduced by administration of large doses of glucocorticoids; or nifedipine (a Ca^{2+} channel blocker).

(2) respiratory alkalosis, that results from hyperventilation (page 448), is prevented by treatment with acetazolamide, this by inhibiting carbonic anhydrase, decreases H^+, and increase HCO_3^- excretion through kidneys. Thus stimulating respiration and decreases the formation of CSF.

ACCLIMATIZATION

Definition

Various physiological readjustments and compensatory mechanisms in the body that reduce the effects of hypoxia in permanent residents at high altitude is called '*acclimatization*' at high altitudes.

These processes permit a normal and prolonged life in people living at high altitudes. There are permanent residents in Himalayas approx. 18,000 feet with pO_2 below 40 mmHg leading normal life.

Acclimatization is possible by following processes:

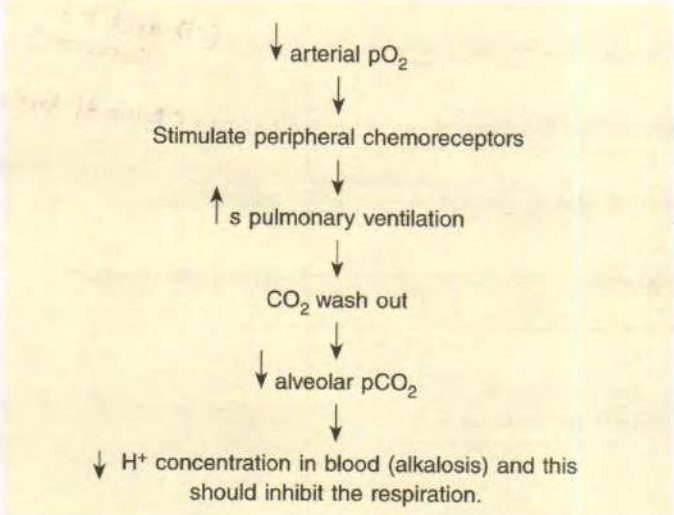
1. Increase in pulmonary ventilation

There is more increase in pulmonary ventilation in acclimatized subjects at high altitude than in an unacclimatized subject.

Note

Resting pulmonary ventilation may increase by 5 folds to 60 litres/min. primarily due to increase in tidal volume which may increase to 50% of vital capacity.

Mechanism



But it has been seen that in acclimatized subjects there is sustained increase in pulmonary ventilation inspite of decrease in alveolar pCO_2 or alkalosis. Why?

At sea level, normal arterial pO_2 and pCO_2 are 100 and 40 mmHg respectively. **(Fig. 50.2)**

(i) Rapid ascend in *unacclimatized subjects*, upto 10,000 feet decreases arterial pO_2 to 60 mmHg while pCO_2 is kept normal. Therefore, no increase in pulmonary ventilation occurs, but after 10,000 feet art. pCO_2 falls rapidly due to increase in pulmonary ventilation.

(ii) In *acclimatized subjects*, the sensitivity of respiratory centre to 'hypoxia' increases. Therefore, even with slight decrease of arterial pO_2, pulmonary ventilation increases and alveolar pCO_2 falls. This increase in pulmonary ventilation is maintained by active regulation of pH of CSF and blood to normal levels. How?

(a) *Regulation of pH of CSF*: It is maintained at normal level by either of the following mechanisms:

- active transport of HCO_3^- out of CSF; or
- active transport of H^+ into CSF; or
- 'hypoxia' causes lactic acid formation; thus, increases H^+ concentration in surrounding central chemoreceptors area will try to maintain increased pulmonary ventilation (interaction of H^+ and CO_2 is additive);
- although there is lowering of alveolar pCO_2 yet 'hypoxia' increases the sensitivity of pCO_2 action on central chemoreceptors.

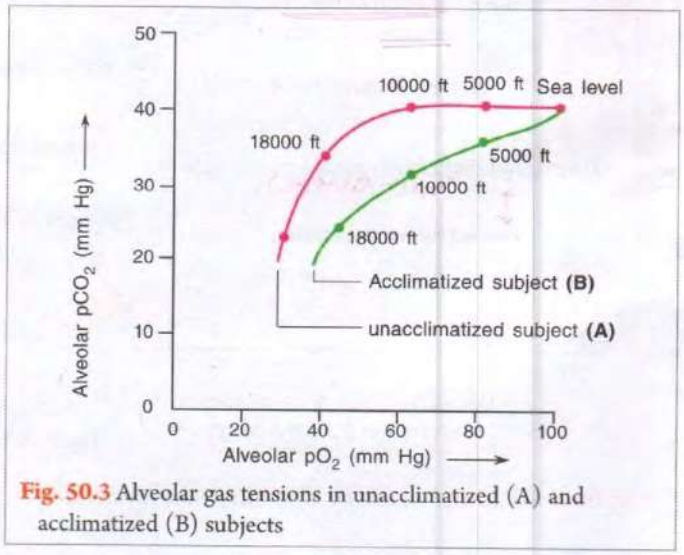


Fig. 50.3 Alveolar gas tensions in unacclimatized (A) and acclimatized (B) subjects

Important Note

People acclimatized at high altitude when come to sea level, increased pulmonary ventilation is maintained for some time, inspite of normal inspired air pO_2, because any reduction in breathing which may result from this oxygenation, will increase arterial pCO_2. This, in turn, increases H^+ concentration in CSF, stimulating central chemoreceptors and pulmonary ventilation increases.

(b) *Regulation of blood pH:* Though with fall of arterial pCO_2, there should be alkalosis but normal blood pH is maintained as kidneys actively excrete more HCO_3^- in urine producing *Alkaline Urine*.

Important Note

Acclimatized subjects can also suffer from 'mountain sickness'; causes are:

(i) if renal failure occurs, then normal blood pH cannot be regulated;

(ii) decrease in sensitivity of peripheral chemoreceptors to hypoxia; then sustained increase in pulmonary ventilation cannot be maintained;

(iii) failure of cardiovascular regulatory mechanisms; so increased tissue vascularity cannot be maintained.

2. Decrease affinity of the haemoglobin for O_2 under hypoxic conditions

Within few hours after one is exposed to hypoxia at high altitude, there is increased amount of 2,3 DPG in RBCs secondary to rise in blood pH. This shifts O_2-haemoglobin dissociation curve to right, releasing more O_2 from haemoglobin.

$$(HbO_2 + 2,3\ DPG \longrightarrow Hb\ 2,3\ DPG + O_2)$$

3. Rise in haemoglobin concentration

'Hypoxia' is a powerful stimulus for erythropoietin secretion thus activates the erythropoiesis; and O_2 carrying capacity of haemoglobin increases.

This process is slow to begin with and starts only after 2-3 weeks; it reaches to peak after several months. Therefore, in high landers haematocrit (PCV) increases to 60%, haemoglobin to 20 gm/dl and RBC count to 7.5–8 million/μL.

4. Increased vascularity of hypoxic tissues

'Hypoxia' increases tissue capillary density *i.e.* more capillaries open up (normally, at rest only 25% of capillaries are open). Moreover, hypoxia also causes vasodilatation. Therefore, more O_2 can be supplied to the tissues.

5. Increased diffusion capacity of lungs for O_2

Due to:

(i) increase in number of pulmonary capillaries secondary to increase in pulmonary artery pressure;

(ii) pulmonary vasodilatation;

(iii) pulmonary blood flow increases (as erythropoiesis expands the capillaries).

6. Changes at tissue level to reduce the effect of hypoxia are:

(i) increase in number of mitochondria, which are the sites of oxidative reactions; (ii) increase in cytochrome oxidase; (iii) increase in synthesis of myoglobin (O_2 storing pigment).

Summary

Table 50.3: Differences between 'High Altitude Native' and 'Newcomer to High Altitude'

	High altitude native	Newcomer to high altitude
1. Increase in pulmonary ventilation	More, therefore, (a) chest becomes enlarged (*barrel shape*) (b) alveolar ventilation is more (c) FRC is more.	Less, therefore, (a) chest is smaller in size (b) alveolar ventilation is less (c) FRC is less.
2. Response to hypoxic stimulation	More, therefore, hypocapenic alkalosis is less.	Less.
3. Urine reaction	Alkaline.	Acidic.
4. Affinity of haemoglobin for O_2	Less, therefore, more O_2 is released from haemoglobin.	More, therefore, body tissue suffers from 'hypoxia'.
5. RBC count	Higher, therefore, PCV increases. Because of polycythemia these individuals have red cheeks.	Low.
6. Organ vascularity	More.	Less.
7. Mitochondria and myoglobin content of muscles	High.	Low.
8. Diffusion capacity of lungs for O_2	More.	Less.

Study Questions

1. Write short notes on:
   (i) Hazards of rapid ascent (ii) Mountain sickness (iii) Mechanism of acclimatization.
2. Depict, diagrammatically, alveolar gas tension in unacclimatized and acclimatized subject at different altitudes.
3. Give main differences between high altitude native and a newcomer to high altitude.
4. Give physiological basis of:
   (i) Safe zone of ascent (ii) Critical survival altitude (iii) High altitude
   (iv) Pulmonary oedema during rapid ascent (v) Cerebral oedema during slow ascent
   (vi) Increased pulmonary ventilation in an acclimatized person, when he comes to sea level.
   (vii) Alkaline urine in an acclimatized person.
5. How high one can ascent with 100% oxygen inhalation? Explain.
6. Justify, with increasing altitude, partial pressure of all gases in the air decreases, then why are symptoms produced due to hypoxic hypoxia.
7. Describe briefly, pathophysiology of pulmonary oedema at high altitude.

MCQs

1. Composition of atmospheric air at 5000 mts above sea level is:
   (a) O_2 : 16%; CO_2 : Nil; N_2 : 84%
   (b) O_2 : 8%; CO_2 : Nil; N_2 : 92%
   (c) O_2 : 4%; CO_2 : 0.03%; Remaining: N_2
   (d) Same, as that present at sea level
2. A height in excess of ..... mts above sea level is defined as high altitude:
   (a) 500 (b) 1000 (c) 2000 (d) 3000
3. Safe zone of ascent is upto ..... mts:
   (a) 500 (b) 1000 (c) 2000 (d) 3000
4. Sudden exposure of an unacclimatized subject to 6000 mts (20,000 feet) would produce after 10 minutes:
   (a) Improved night vision
   (b) Acidosis
   (c) Nitrogen bubbles in the blood
   (d) Unconsciousness
5. Critical survival altitude is between
   (a) 1500-3000 mts (b) 3000-5400 mts (c) 6000-7500 mts (d) 7500-9000 mts
6. Pulmonary oedema seen in individuals during rapid ascent is due to:
   (a) Cardiovascular insufficiency
   (b) Pulmonary insufficiency
   (c) Increased sympathetic activity
   (d) Hypercapnia
7. *Not* a feature of pulmonary oedema at high altitude:
   (a) Associated with pulmonary artery hypertension
   (b) Seen in individuals who engage in heavy physical work after arrival to high altitude
   (c) Due to cardio-respiratory insufficiency
   (d) It responds to rest and O_2 therapy
8. What is *not* seen in acclimatization to a high altitude:
   (a) Hyperventillation
   (b) Polycythemia
   (c) O_2 dissociation curve shifts to right
   (d) Decreased density of systemic capillaries
9. In high altitude acclimatized individual increase in diffusion capacity is due to following *except*:
   (a) Increased permeability of alveolar capillary membrane
   (b) Increased alveolar capillary pressure gradient
   (c) Greater number of open capillaries
   (d) Capillary dilatation
10. High altitude acclimatization may be facilitated by all, *except*:
    (a) Increased production of red blood cells
    (b) Increased alveolar ventilation
    (c) Growth of new blood vessels
    (d) Growth of new skeletal muscle fibers

11. **Which of the following in the body *does not* vary with increase in altitude?**
    (a) pO_2 (b) pCO_2 (c) pN_2 (d) pH_2O

12. **As one ascends higher than 3000 metres (10,000 feet) above the sea level, alveolar gases change as follows:**

	pO_2	pCO_2
(a)	Decrease	Increase
(b)	Decrease	Slight decrease
(c)	Increase	Increase
(d)	Increase	Decrease

13. **A person rapidly ascends to 3600 mts (12,000 feet) and develops acute breath-lessness; this is due to:**
    (a) Decreased pulmonary blood flow
    (b) Stimulation of peripheral chemo-receptor
    (c) Decreased hypoxic stimulation of respiration
    (d) Mechanical interference of thorax

Answers

1. (d) 2. (d) 3. (d) 4. (d) 5. (d) 6. (c) 7. (c) 8. (d) 9. (b) 10. (d)
11. (d) 12. (b) 13. (b)

Chapter 51

Effects of High Atmospheric Pressure

I Caisson's disease

II Nitrogen narcosis

III High pressure nervous syndrome

IV Air embolism

High atmospheric pressure is met in:

1. ***Deep sea diving:*** For every 33 feet (10 mts) deep down in the sea there will occur an increase in pressure by 1 atmospheric pressure. In fresh water it is for every 10.4 mts.
2. ***Caisson's workers:*** Men who dig under water tunnel are in a chamber called *Caisson*. In this chamber atmospheric pressure is high to prevent entry of water.
   When one is exposed to high atmospheric pressure, one may develop symptoms either during exposure or when one comes to surface suddenly. For example, during sudden ascent *i.e.* during sudden reduction of pressure.

EFFECTS OF HIGH ATMOSPHERIC PRESSURE

1. Volume of gases show inverse relationship to atmospheric pressure (*Boyle's law*). Therefore, at two atmospheric pressures, the volume becomes 'half' causing lungs to become small and can produce serious damage.
2. Increase in atmospheric pressure increases the partial pressure of nitrogen, oxygen and CO_2 to equalize the increased pressure on chest wall and abdomen. Main danger is due to high tension of O_2 and N_2, and not due to CO_2 because body does not allow it to accumulate by causing hyperventilation.
3. Increased O_2 tension in blood produces *Oxygen Toxicity* (page 461); whereas increase in N_2 (pN_2) produces ***Caisson's Disease*** **(Fig. 51.1).**

CAISSON'S DISEASE or DECOMPRESSION SICKNESS or DYSBARISM or THE BENDS or DIVER'S PALSY

Dysbarism means the effects of a pressure difference between the ambient pressure and the pressure of the dissolved and free gases in the body. It is caused by increased pN_2 in blood. N_2 has two main characteristic features:

(1) it passes through cell membranes very slowly; and

(2) it is about 5 times more soluble in fat than water.

Therefore, when a person breathes air under high pressure for a long time, the amount of N_2 dissolved in the body fluids increases. Moreover, N_2 gets dissolved in fat because fat has more blood supply.

Characteristic Features: N_2 NARCOSIS

1. During exposure, when the body is exposed to high atmospheric pressure, say 3-4 atmospheric pressure, (*i.e.* at a depth of 25-30 mts in the sea) N_2 produces ***Nitrogen Narcosis*** due to high pN_2. It is characterized by:
   (i) Euphoria *i.e.* sense of well being.,
   (ii) Impairment of mental functions: intelligence, and
   (iii) Symptoms resembling of alcohol intoxication (page 459).

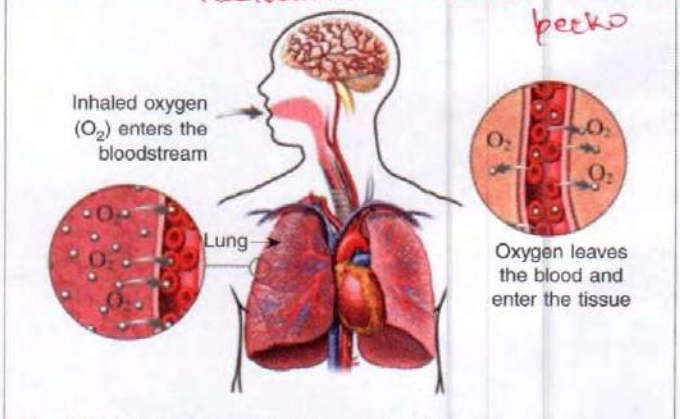


Fig. 51.1 Mechanism of development of Caisson's disease

comes out of shed quickly

2. When diver suddenly comes to the surface, dissolved N_2 comes out in fluid and forms bubbles in the tissues and blood due to reduction of pressure suddenly. The bubbles are primarily of N_2; because, body can get rid of other gases very easily.
3. **Bubbles of N_2** when present in: ⇒ reaction to it
   (i) Fat depot, press the nerves producing *sensory and motor disturbances.*
   (ii) Myelin sheath of
      (a) sensory nerves produce disturbance or loss of sensations, paraesthesia, itching etc.
      (b) motor nerves produce motor paralysis (temporarily), called *Diver's Palsy*.
   (iii) Blood capillaries block them; this blockage:
      (a) in brain produces sensory and motor disturbances (see above);
      (b) in lungs produces dyspnoea, called *Chokes*;
      (c) in heart leads to myocardial infarction; and
      (d) in joints and muscles of limbs causes severe pain, called *Bends* **(Fig. 51.2)**.

Treatment and Prevention

1. Subject should come to the surface slowly. When the subject complaints of pain in muscles and joints, put him into a *Compression Chamber.* 'Recompression' is done first to dissolve the N_2 bubbles, then slow 'decompression' is done, so the body can get rid of N_2 slowly.
2. *Nitrogen narcosis* can be avoided by breathing *O_2-Helium mixture*, instead of N_2 because helium being low density gas is less soluble in fat than N_2. However, at high atmospheric pressure such mixture can produce ***High Pressure Nervous Syndrome (HPNS)*** which is characterized by tremors, drowsiness or loss of sleep and depression of 'α' activity in EEG. Why?

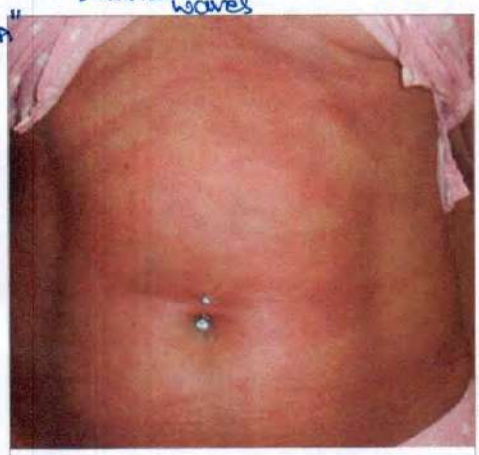


Fig. 51.2 Bends in Caisson's disease

Not known. However, gases like N_2, xenon, krypton, argon, neon and helium that are physiologically inert at normal atmospheric pressure act like anaesthetic agents at high pressure. Thus the anaesthesia may be due to their action on nerve cell membranes.

Clinical significance – Air Embolism, *i.e.* air entry into the blood circulation.

Causes

1. Deep sea divers or ***caisson's workers,*** when under the water, breathe *O_2-He mixture* at high pressure to equalise the high surrounding pressure on chest wall and abdomen. If they come to the surface suddenly holding the breath, there is sudden expansion of gases in the lungs causing rupture of pulmonary capillaries (arteries and veins). Air enters the blood circulation producing air embolism. This air embolism, which is due to the sudden fall in pressure, is called ***Explosive Decompression.***
2. In rockets etc., high pressure is maintained, according to the height of ascent. In the pressurised cabin if there is any leakage, sudden reduction of pressure causes expansion of gases in the lungs and 'air embolism' develops.

SCUBA Diving

SCUBA **(Fig. 51.3)** *i.e. self-contained underwater breathing apparatus*, consists of a cylinder and valve system for breathing that is carried by the divers under the water. It is a compact arrangement to carry compressed air so that more air can be carried in less volume. The cylinder of compressed air is connected via a mask and tube for breathing through a valve. The valve permits an appropriate amount of compressed air to be delivered to the diver and the expired air is released into the surrounding water.

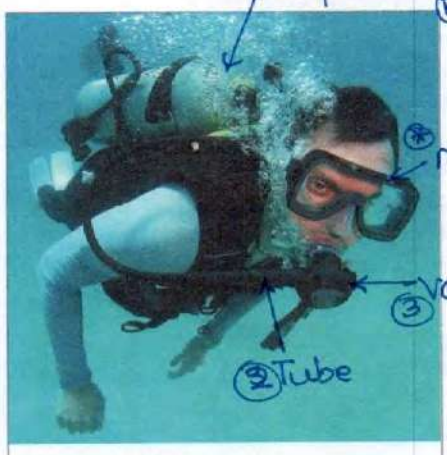


Fig. 51.3 SCUBA apparatus

Sudden reduction of HIGH Atm pressure causes AIR EMBOLISM

Study Questions

1. **Give physiological basis of:**
   (i) Caisson's worker (ii) Diver's palsy (iii) Chokes (iv) Bends
   (v) Air embolism (vi) Dysbarism (vii) Explosive decompression.
2. **Write short notes on:**
   (i) Nitrogen narcosis (ii) High pressure nervous syndrome.
3. **Explain: dangers of high atmospheric pressure are never due to high arterial pCO_2.**

MCQs

1. **A person working in 20 mts deep in sea water is exposed to pressure of:**
   (a) 1 atmosphere (b) 2 atmosphere (c) 3 atmosphere (d) 4 atmosphere
2. **Caisson's workers are:**
   (a) Men who dig under water tunnel
   (b) Workers working in a chamber in which atmospheric pressure is high
   (c) Deep sea divers
   (d) SCUBA divers
3. **In deep sea diving, the main danger is from:**
   (a) O_2 and N_2 (b) CO_2 and O_2 (c) N_2 and CO_2 (d) O_2, CO_2 and N_2
4. **Air embolism due to sudden fall in pressure is called:**
   (a) Decompression sickness (b) Explosive decompression
   (c) Dysbarism (d) The bends
5. **SCUBA:**
   (a) It is meant to carry compressed air by divers
   (b) Here diver is asked to breathe O_2-helium mixture at high atmospheric pressure
   (c) It refers to inhaling 100% O_2 at sea level
   (d) None of the above
6. **Dysbarism is due to:**
   (a) Sudden fall in pressure of ambient gases in body (b) Gradual fall in pressure of ambient gases in body
   (c) Gradual rise in pressure of ambient gases in body (d) Sudden rise in pressure of ambient gases in body
7. **The ambient pressure increased by ..... atmosphere for every 10m of depth in sea water and every 10.4 m of depth in fresh water:**
   (a) 0.3 (b) 0.8 (c) 1.0 (d) 1.8
8. **If atmospheric pressure is doubled:**
   (a) Lung becomes large (b) Lung volume is increased to 4 times
   (c) Lung volume becomes half (d) No change occurs in vital capacity
9. **Decompression sickness:**
   (a) Results from CO_2 bubbles in the body fluids (b) Can be prevented by rapid decompression
   (c) Is characterized by pains and some-times paralysis (d) Can occur if one descends a mountain too rapidly
10. ***False* about high pressure nervous syndrome:**
    (a) Due to increased pN_2 in blood
    (b) Caused by breathing O_2-helium mixture at high atmospheric pressure
    (c) Characterized by tremors and drowsiness
    (d) Associated with depressed α-activity in EEG

Answers

1. (c)* 2. (b) 3. (a) 4. (b) 5. (a) 6. (a) 7. (c) 8. (c) 9. (c) 10. (a)

* Add 1 atmospheric pressure of sea level to the pressure increased in 20 mts. deep in sea water.

Chapter

52

Pulmonary (Lung) Function Tests

I. Tests to assess ventilatory functions of lungs

II. Tests to assess gaseous exchange across the lungs

III. Tests to assess transport of gases in the body

Pulmonary function tests (PFT) provide a quantitative and objective assessment of the physiological derangement associated with pulmonary diseases. While these tests do not give a specific etiological or pathological diagnosis, the **reasons for pulmonary function testings** are:

1. Identification of cause of respiratory symptoms.
2. Diagnosis of functional abnormalities such as
   (i) obstructive, restrictive or reactive diseases;
   (ii) ventilation-perfusion mismatching;
   (iii) abnormalities in the control of ventilation; and
   (iv) occupational-related pulmonary disabilities.
3. Diagnosis of severity of dysfunction, including subclinical abnormalites.
4. Identification of and screening of unsuspected diseases.
5. Assessment of reversibility of airway obstruction.
6. Assessment of airway sensitivity.
7. Evaluation of effectiveness of short-term and long-term therapy.
8. Long-term follow-up.

On the basis of these requirements the various PFT can be broadly **classified into** three groups:

I. Tests to assess ventilatory functions of lungs;
II. Tests to assess gaseous exchange across the lungs, and
III. Tests to assess transport of gases in the body.

I. TESTS TO ASSESS VENTILATORY FUNCTIONS OF LUNGS

A. Assessment of the expansion of lungs and chest wall

1. Measurement of pressure changes during ventilation (page 409). For example:
   (i) intra-pulmonary (intra-alveolar) pressure; and
   (ii) intra-pleural (intra-thoracic) pressure.
2. Measurment of compliance (page 416)
   (i) compliance of lungs and chest wall; and
   (ii) compliance of lungs alone.

B. Assessment of restrictive and obstructive ventilatory defects

1. Measurement of static and dynamic lung volumes and capacities by 'spirometry' (page 411)
2. Measurement of airways resistance (page 419)

These provide a fairly good idea of the physical fitness in normal and the type and extent of derangement of lung functions in patients.

II. TESTS TO ASSESS GASEOUS EXCHANGE ACROSS THE LUNGS

1. Measurement of 'functional residual capacity' – FRC
2. Measurement of 'dead space' (DS) and uniformity of 'alveolar ventilation' (page 421 and 422 respectively).
3. Measurement of diffusion capacity of lungs (page 425).

III. TESTS TO ASSESS TRANSPORT OF GASES IN THE BODY

1. Measurement of gas tension, for example, pO_2 and pCO_2 in inspired, expired and alveolar air.
2. Measurement of gas tension and acid-base status of the blood.

Important Note

The importance of a careful history, physical examination and X-ray chest should not be underestimated.

HR acts as limiting factor of ↑ in CO & SV

Increased HR during exercise is due to

1. **Neurogenic Control**
   (i) *Central reflexes:*
   (a) increased activity of limbic system and motor cortex due to *Psychic stimuli*; this acts directly on medulla;
   (b) decrease in vagal tone (↓)
   (ii) *Peripheral reflexes* originating from:
   (a) muscle spindles
   (b) muscle-tendon receptors
   (c) organ of corti.
2. ***Circulating Hormones.*** Liberation of (i) catecholamines from adrenal medulla and (ii) T_4 from thyroid gland in response to stress.
3. ***Increase in body temperature.***
4. ***Chemical changes*** occurring in the blood (↓ arterial pO_2; ↓ pH and ↑ arterial pCO_2).
   Hypoxia Acidosis Hypercapnea

B. STROKE VOLUME

160-180mL

Normal: 80-90 mL (at rest). Increases to twice the normal value during exercise due to:

1. **Increase in venous return** (VR), which is maintained by:
   (i) Muscle pump
   (ii) Negative intra-thoracic pressure
   (iii) Modification of tone in capacitance (veins) vessels. Thus, helps in redistribution of blood to active tissues from inactive tissues.
2. **Increase myocardial contractility** by epinephrine and nor-epinephrine from sympathetic nervous system and adrenal medulla.
   Once HR increases to 120/min, there is little or no further increase in stroke volume (limited by Starling Law of the Heart) (page 182).

Important Note

As stroke volume depends on the heart size, *i.e.* why it is larger in
(i) 'males' compared to female; and
(ii) 'Athletes', due to low resting HR that increases end diastolic volume producing enlargement of the heart.

C. CARDIAC OUTPUT

25-30L

Normal: 5-6 litres/min (resting); it increases to 5-6 times during maximum exercise due to increase in heart rate and stroke volume.

That is why higher values of maximum cardiac output can be achieved in youngs compared to old subjects due to higher maximum HR in youngs.

D. BLOOD PRESSURE (BP)

1. In Systemic Circulation → Normal: 100-140 mm Hg

(i) **Systolic BP (SBP)** increases in linearity with severity of exercise at all ages and may increase to 200 mmHg (Fig. 53.1). Increase is more in older subjects, because resting SBP is more due to atherosclerosis.

[#: opp. effect of HR]

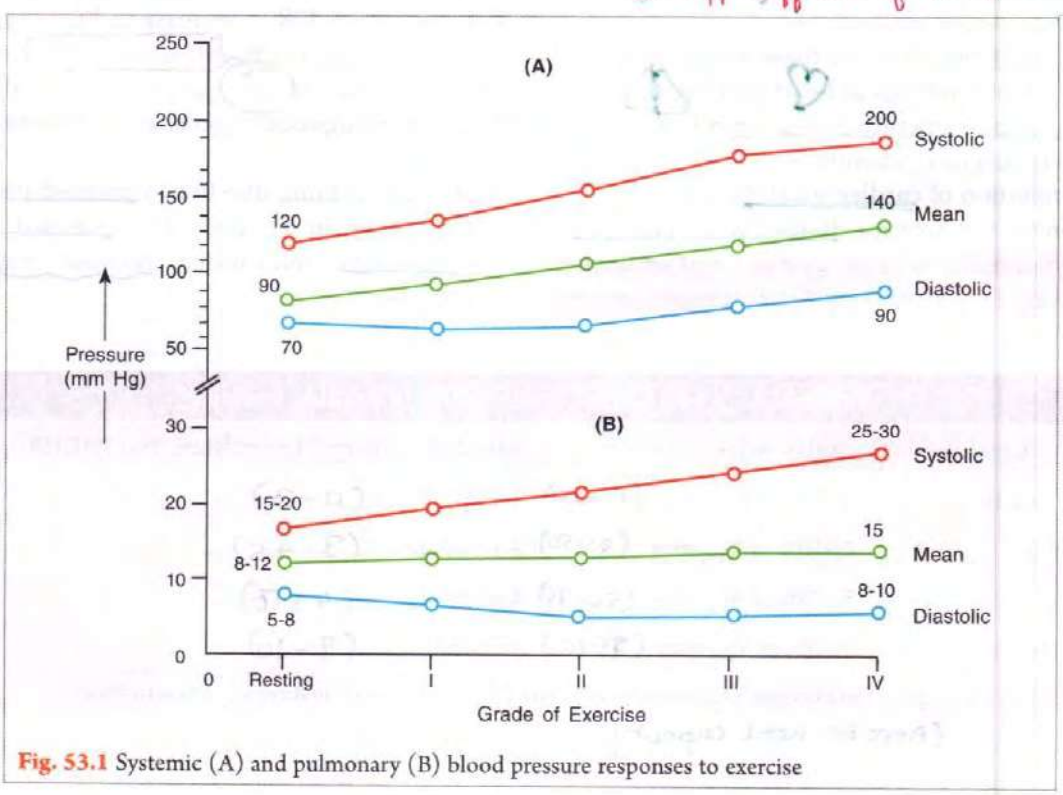


Fig. 53.1 Systemic (A) and pulmonary (B) blood pressure responses to exercise

Increase is due to:
(a) increase in cardiac output, and
(b) vasoconstriction in non-working muscles.

(ii) **Diastolic BP (DBP)**
(a) No change in mild and moderate exercise because total resistance falls considerably due to drastic vasodilatation in working muscles.
(b) Slight increase, in severe exercise due to vasoconstriction in non-working muscles and skin.

(iii) **Mean BP (MBP)** *i.e.* DBP + 1/3 pulse pressure; it increases from 90 mmHg (resting) to 140 mmHg during heavy exercise (also see to page 347).

2. In Pulmonary Circulation

Resting pulmonary artery

SBP	:	15-20 mmHg
DBP	:	5-8 mmHg
MBP	:	8-12 mmHg

During mild and moderate exercise, 'MBP' only increases to 15 mmHg due to intra-thoracic location of blood vessels which provides low resistance to blood flow in pulmonary circuit. Therefore, it can accept a several-fold increase in cardiac output with only mild increase in pulmonary artery pressure. However, during *heavy exercise* limits to vascular compliance are approached and pulmonary artery pressure increases markedly.

E. BLOOD FLOW

1. Muscle Blood Flow

Resting: 3-4 mL/100 gm/min.

During heavy exercise, increases by *25-30 times* to approx. 100 mL/100 gm/min.

Increase is due to *increase in capillary density i.e.* opening of more capillaries. Why? (refer to page 379)

Muscle blood flow increases before the start of exercise and continues to increase until a plateau is reached *i.e.* a *steady state* between inflow and outflow is reached. This period usually takes 1-2 minutes in mild and moderate exercise and longer in severe exercise.

A-V Oxygen Difference

At rest: 19 – 14 = 5 mL/dL with 26% co-efficient of O_2 utilization (100 × 5/19) (page 432).

During maximum exercise it increases by 3 times to 13-15 mL/dL with 80% coefficient of O_2 utilization; because of O_2-haemoglobin dissociation curve shifts to right due to:
(i) rise in temperature
(ii) ↑ arterial pCO_2
(ii) ↓ arterial pO_2
(iv) ↓ arterial pH

Note

The average distance between the blood capillary and the active muscle cells also decrease in exercising muscles.

Therefore, more O_2 is given to the tissues at any pO_2. 25-30 times increase in muscle blood flow and 3 times increase in A-V O_2 difference increases the ***muscle metabolism*** by approx. 100 times during severe exercise.

2. Coronary Blood Flow

At rest: 250 mL/min *i.e.* 60-70 mL/100 gm per min with 70-80% coefficient of O_2 utilization.

During maximum exercise, it increases by 5 times with 100% coefficient of O_2 utilization due to:
(i) increased coronary blood flow, and
(ii) coronary vasodilatation by:
(a) catecholamines
(b) hypoxia
(c) fall in blood pH
(d) ATP and ADP.

3. Pulmonary Blood Flow

At rest: 350-800 mL/min; increases in linearity with increase in cardiac output to 1400 mL/min during severe exercise.

4. Skin Blood Flow

Resting: 500 mL/min; depends on cooling power of surrounding air and overall metabolic rate of the body.
(i) Slight decrease, at the beginning of exercise due to reflex vasoconstriction.
(ii) Later increases upto 7 times due to stimulation of hypothalamus secondary to increase in body temperature (page 375). This produces vasodilatation and helps heat loss and transport of metabolites.
(iii) During severe exercise, decreases due to vasoconstriction of skin blood vessels.

5. Adipose Tissue Blood Flow

Increases by 4 times during exercise.

Advantage: Helps to deliver fatty acids mobilized from triglyceride stores to the working muscles.

6. Brain Blood Flow

Resting: 750 mL/min; no change during any grade of exercise.

F. BLOOD VOLUME

1. Decreases by 15% causing *haemoconcentration* of blood due to:
(i) increased hydrostatic pressure in capillaries causing loss of plasma water;

(ii) accumulation of osmotically active metabolites in tissue spaces (*e.g.* K^+, PO_4^{3-}, Lactic Acid), causing drainage of plasma water from capillaries.

Advantages

(i) O_2 carrying capacity of blood increases because of increased concentration of RBCs.

(ii) Increase in acid buffering capacity due to increased plasma proteins.

Disadvantages

Increases viscosity of blood, therefore, blood flow decreases.

2. WBC count increases, due to washing out of WBCs, from storage places and bone marrow.

Important Note

During isometric muscle contraction, the HR rises (due to psychic stimuli); SBP and DBP rises shortly; SV changes relatively little, and blood flow to the steady contracting muscle is reduced. (When a contracting muscle develop more than 70% of its maximal tension, blood flow is completely stopped as a result of compression of its blood vessels.)

7. Visceral Blood Flow

	Exercise Level		
	Resting	Mild and Moderate	Severe
(i) Kidneys	1100 mL/min	No change	decreases by 50-80%
(ii) Portal venous blood (spleen and GIT)	1200 mL/min	No change	decreases by 80% due to stimulation of sympathetic nervous system
(iii) Splanchnic (Portal + Liver)	1500 mL/min	No change	-do-

Important Note

Athletic Pseudonephritis: Prolonged, heavy exercise increases proteins, cells and other abnormal substances in urine due to:

(i) decrease in renal blood flow (RBF) produces glomerular capillary hypoxia, and increases permeability to large molecules;

(ii) increase in plasma proteins during exercise;

(iii) decreased rate of RBF, thus larger time is required to filter these molecules.

3 RESPIRATORY ADAPTATIONS TO EXERCISE

A. PULMONARY VENTILATION (PV)

At rest: Pulmonary ventilation is 6 litres per min with frequency of breathing as 12 per min and tidal volume as 500 mL.

During maximum exercise it *increases by 20-25 times* to approx. 100 litres/min due to **(Fig. 53.2)**:

(1) increase in frequency of breathing to 40-45 breaths/min; and

(2) tidal volume increases from 10-15% to approx. 50% of vital capacity.

Pulmonary ventilation increases in parallel with the increase in O_2 consumption (V_{O_2}) during exercise. It also increases in parallel with increase in CO_2 output (V_{CO_2}) except during heavy exercise when pulmonary ventilation increases disproportionately due to anaerobiosis of the working muscles, which contributes an extra drive to respiratory centre. The cause of such a drive is:

(1) H^+ concentration in the blood and/or

(2) CO_2 release as a consequence of high blood lactate level.

However, increase in pulmonary ventilation during maximum exercise is always of a lesser extent than maximum voluntary ventilation (MVV). It shows that respiration is not the main limiting factor in muscular exercise.

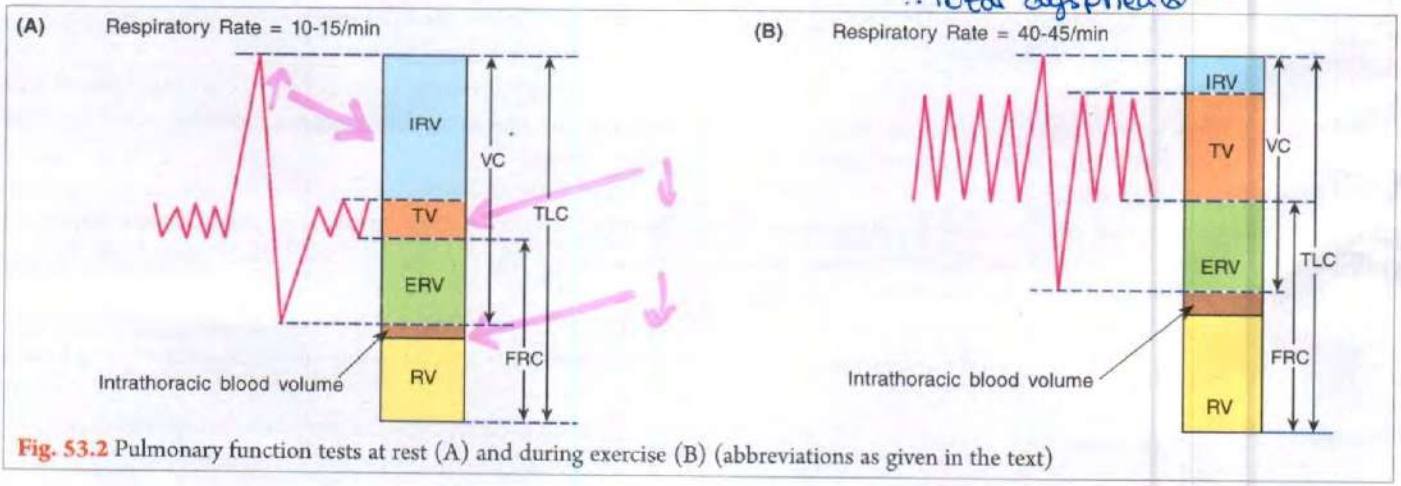


Fig. 53.2 Pulmonary function tests at rest (A) and during exercise (B) (abbreviations as given in the text)

Control of pulmonary ventilation during exercise

1. **Psychic stimuli:** stimulation of respiratory centre by increased activity in the motor cortex.
2. **Afferent stimuli from proprioceptors** in moving muscles, tendon and joints to the brain.
3. **Stimulation of carotid bodies,** secondary to changes in blood chemistry (↓ arterial pO_2; ↓ pH; ↑ arterial pCO_2; ↑ serum K^+). These changes also increase the sensitivity of respiratory centre to CO_2.
4. **Accumulation of lactic acid** in blood.
5. **Increased body temperature** - stimulates
   (i) respiratory center directly
   (ii) sensitizes the responses of respiratory mechanisms to arterial pCO_2.
6. **Increase in plasma potassium** level.

Important Note

Peripheral reflexes control the respiration at the beginning of exercise while chemical and thermal reflexes control the respiration during the exercise.

B. PULMONARY DIFFUSION CAPACITY FOR OXYGEN (D_{O_2}).

At rest: D_{O_2} = 20-30 mL/min/mmHg.

During maximum exercise, D_{O_2} increases above 3 times due to:

(1) increased blood perfusion around the air sacs in the lungs; and

(2) opening of more capillaries.

(1) and (2) increase surface area of contact between alveoli and pulmonary capillaries.

C. OXYGEN CONSUMPTION (V_{O_2})

Resting V_{O_2} = 250 mL/min (page 432).

During heavy exercise may increase to 15-20 times due to:

(1) **3 times** increase in A-V O_2 difference.

(2) **5 times** increase in O_2 delivery to the tissues due to:

(i) increase in cardiac output

(ii) marked increase in alveolar ventilation

(iii) increase in capillary density

(iv) increase RBC count due to splenic contraction.

Therefore, maximum O_2 consumption (V_{O_2} max.) depends on muscle mass and functional dimensions of O_2 transporting system.

V_{O_2} Max - maximum O_2 consumption

When steady state V_{O_2} is plotted against the rate of work performed, the O_2 cost of exercise is linearly related to the rate of work upto a limited value, above which a further increase of workload does not bring about further increase in V_{O_2}. This point (level) of V_{O_2} is defined as V_{O_2} max. for a particular type of exercise. It is the product of maximal cardiac output and maximal oxygen extraction by the tissues.

The V_{O_2} max. of an individual determines the maximum ***aerobic work capacity*** *i.e.* it is the best physiological indicator of aerobic work capacity in man. It is defined as the highest attainable rate of aerobic metabolism during performance of rhythmic muscular work that exhausts the subject within 5-10 minutes.

V_{O_2} max. increases during childhood and reaches its peak value during early adulthood, after that a gradual and steady decline takes place with the increasing age.

Whether V_{O_2} max. *is limited by* heart or by the lungs is still an open question:

1. Pulmonary factors impose no limitation to O_2 transport, therefore, capacity of the heart to increase the cardiac output may be the factor most frequently considered as the main limiting factor.
2. The ability of the active tissues to extract O_2 delivered by CVS or peripheral factors (muscle mass) are other possible limiting factors.

Criteria for establishing that V_{O_2} max. has been achieved

1. O_2 consumption (V_{O_2}) reaches a plateau
2. Achievement of maximum heart rate: 220 – Age (years)
3. *Respiratory quotient* (RQ) increases more than 1.15.
4. Blood lactic acid increases more than 70-80 mg/dL (normal 20-40 mg/dL).

Note

All cardio-respiratory changes in response to exercise returns to pre-exercise level within 4-5 minutes after stoppage of the exercise.

④ PHYSIOLOGICAL EFFECTS OF PHYSICAL TRAINING

Physical performance or *fitness* is inversely related to *O_2-deficit*.

What is 'O_2 deficit'?

In rhythmic dynamic muscular work, regardless of the level of exercise, O_2 consumption (V_{O_2}) increases during first 2 to 4 minutes of exercise, then reaches a plateau *i.e.* *steady state* level. Therefore, an *O_2-deficit* is established at the beginning of exercise, called *Adaptation Phase*, which remains throughout the whole exercise period (Fig. 53.3).

Causes of 'O_2-deficit'

It takes a few seconds for the circulation to deliver the extra O_2 required by the working muscles. Therefore,

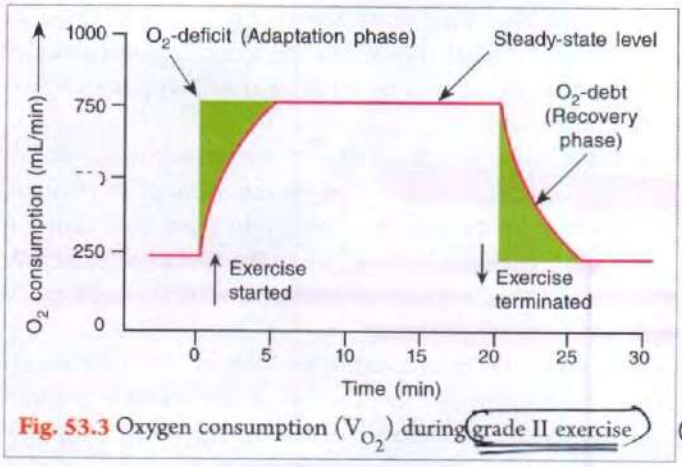


Fig. 53.3 Oxygen consumption (V_{O_2}) during grade II exercise

during this period ATP is primarily produced by anaerobic mechanisms *i.e.* breakdown of creatine phosphate and muscle glycogen.

This *O_2-deficit* is repaid after the stoppage of exercise, in the form of ***O_2-debt***. Therefore, the extra amount of V_{O_2} during recovery above the resting level, is called *Recovery Phase*. This extra O_2 is used:

(1) to regenerate depleted stores of ATP and creatine phosphate;
(2) to resupply O_2 to myoglobin in muscles; and
(3) to resupply dissolved O_2 in tissue fluids and blood.

Physical training decreases 'O_2-deficit' *i.e.* anaerobic mechanisms at the onset of exercise. This can be achieved by *warm up* and training. How?

A. WARM UP EFFECTS

1. Increases the blood flow and nutrients to working muscles.
2. Increases level of mitochondrial enzymes and energy stores causing lesser use of anaerobic work.
3. Prevents heart damage during 1st few seconds of heavy exercise; otherwise, there will be inadequate blood flow to the heart.
4. Prevents muscular or connective tissue injuries.

B. PHYSICAL TRAINING EFFECTS

1. Psychology of the individual improves with training causing **decrease in 'psychic stimuli'** to VMC and respiratory centres. Therefore, during exercise there is
   (i) less increase in sympathetic activity; and
   (ii) less decrease in parasympathetic activity.
2. **Optimal blood flow distribution** occurs *i.e.* cardio-respiratory response reaches a steady-state early.
3. **Greater fats are used for energy**, sparing glycogen. How? *Respiratory Quotient* (RQ), i.e. ratio of CO_2 production to O_2 consumption at rest. (*Normal* 0.8 on a mixed diet). 'RQ' for 100% protein utilization is 0.83; for carbohydrates 1.0 and for fats 0.7. Because of aerobic training, RQ decreases causing oxidation of more fats. Therefore, increased fatty acids are mobilized from tissues stores into the blood, sparing glycogen. As physical performance is a direct function of glycogen stores, therefore, endurance of the individual increases.
4. **Higher V_{O_2} max can be achieved** due to:
   (i) Increase in maximum cardiac output. This is seen secondary to increase in stroke volume. Stroke volume increases as end-diastolic volume increases by better venous return to the heart.
   (ii) Increase in A-V O_2 difference, because
      (a) capillary density increases
      (b) increase in number and size of mitochondria, and
      (c) myoglobin stores increase.
   (iii) Less increase in both systolic and diastolic blood pressure because arterioles are opened more completely in non-working organs, thus, peripheral resistance decreases.
   (iv) Less increase in pulmonary ventilation because of less accumulation of metabolites and lactic acid. Therefore, less stimulation of respiratory centre.
   (v) More increase in diffusion capacity of lungs for oxygen because pulmonary capillary density increases.

Important Note

Regular exercise improves coronary perfusion secondary to prodouction of prostacyclin and nitirc oxide by the endothelium of coronary vessels in response to shear stress.

Summary

In trained individuals with onset of exercise:
1. Psychology improves and psychic stimuli are less;
2. Cardio-respiratory responses reach a steady state early;
3. Optimal blood flow distribution, and
4. Mobilization of 'FFA' occurs rapidly.

Therefore, these factors help in decreasing the dependence on anaerobic mechanism.

Important Note

The athletes have lower HR, greater end-systolic ventricular volume and greater stroke volume at rest. Therefore, they can achieve a given increase in cardiac output by further increase in stroke volume without increasing their HR to that extent as an untrained individual.

Study Questions

1. Give WHO classification of grading of exercise. Why is it required?
2. What will happen to the following parameters during Grade IV (very severe exercise); give physiological basis of each.
   (i) Heart rate, stroke volume and cardiac output
   (ii) Visceral and skin blood flow
   (iii) BP in systemic and pulmonary circulation
   (iv) Muscle metabolism
   (v) Oxygen consumption
   (vi) Pulmonary ventilation.
   (vii) A-V O_2 difference in coronaries
3. Write short notes on:
   (i) Importance of exercise
   (ii) Cardio-respiratory changes during moderate exercise
   (iii) V_{O_2} max
   (iv) Effects of physical training
   (v) O_2 deficit and O_2 debt
   (vi) Athletic pseudonephritis.
4. Give physiological significance of:
   (i) Warm up during exercise
   (ii) Steady state exercise
5. Mention merits and demerits of haemoconcentration of blood which occurs during severe exercise.
6. Describe briefly the causes of increase in heart rate and pulmonary ventilation during exercise.
7. Why can a young person achieve higher cardiac output during exercise as compared to old subject?
8. Briefly explain the main limiting factors during muscular exercise.
9. Differentiate between:
   (i) Cardio-respiratory adaptation to exercise in untrained and trained individuals
   (ii) Oxygen deficit and oxygen debt.
   (iii) Isotonic versus isometric exercise.
10. Justify, how long beneficial effects of regular exercise on the body will last after its stoppage.

MCQs

1. Metabolic requirements of the body during exercise are met by:
   (a) O_2 delivery system to the tissues
   (b) Increased tissue perfusion
   (c) Cardio-respiratory system functioning
   (d) Capacity to extract O_2 from the atmosphere
2. Expenditure of energy during moderate exercise is:
   (a) Less than 3 METS
   (b) 3–4.5 METS
   (c) 4.6–7 METS
   (d) More than 7 METS
3. All factors contribute to increase in heart rate during exercise *except*:
   (a) Psychic stimuli
   (b) Stimulation of cardiac vagal centre
   (c) Catecholamine
   (d) Change in blood chemistry
4. Cardiac output increases to ..... times during maximum exercise:
   (a) 2-3 (b) 3-4 (c) 4-5 (d) 5-6
5. The prime regulator of blood flow through exercising muscles is:
   (a) Venous tone
   (b) Sympathetic control
   (c) Vasodilator metabolites
   (d) Parasympathetic control
6. During maximum exercise, coefficient of O_2 utilization of myocardium is:
   (a) 26%
   (b) 52%
   (c) 78%
   (d) 100%
7. Decrease in blood volume during exercise occurs due to:
   (a) Perspiration
   (b) Polyuria
   (c) Increased capillary hydrostatic pressure
   (d) Hyperkalemia
8. Exercising minute ventilation may increase upto ..... times of resting ventilation
   (a) 5-6 (b) 6-10 (c) 10-20 (d) 20-25
9. During exercise, O_2 consumption of the body may increase from 250 mL/min at rest to as high as:
   (a) 4 L/min (b) 10L/min (c) 20 L/min (d) 30 L/min

10. **Steady state exercise is:**
    (a) Very severe exercise
    (b) Moderate to severe exercise
    (c) O_2 consumption reaches a maximum level
    (d) O_2 consumption reaches a plateau for a particular level of exercise

11. **An athlete is considered to have performed better than others for the same turn out of effort, if:**
    (a) Increase in heart rate is less (Psychic stimuli)
    (b) Increase in heart rate is more
    (c) Pulmonary ventilation achieved is close to the predicted maximum breathing capacity
    (d) Oxygen consumption and CO_2 output is less

12. **Trained athletes compared to untrained individuals have:**
    (a) Larger stroke volume and lower heart rate
    (b) Smaller stroke volume and higher heart rate
    (c) Smaller size of heart
    (d) Decreased incidence of severity of myocardial infarction

13. **Maximum heart rate that can be achieved during very heavy exercise is:**
    (a) 100 beats/min (b) 125 beats/min (c) 150 beats/min (d) 220–Age (years)

14. **In athletes bradycardia is because of:**
    (a) Increased sympathetic tone
    (b) Increased vagal tone
    (c) Decreased cardiac output
    (d) Low venous return

15. **Tachycardia at the onset of exercise is due to stimulation of:**
    (a) Chemoreceptors (b) Baroreceptors (c) Stretch receptors (d) Joint proprioceptors

16. **Maximum stroke volume which can be achieved during exercise is:**
    (a) 100 mL (b) 130 mL (c) Twice the normal (d) 200 mL

17. **What limits the increase in stroke volume during exercise?**
    (a) Decrease in myocardial contractility
    (b) Frank-Starling Law of the heart
    (c) Tone of capacitance vessels
    (d) Muscle pump

18. **What happens to diastolic blood pressure during mild to moderate exercise?**
    (a) Increase slightly (b) Moderate increase (c) Decreases (d) No change

19. **During exercise, A-V O_2 difference:**
    (a) Increases
    (b) Decreases
    (c) Remains same
    (d) First decreases then increases

20. **During exercise, blood flow *does not* decrease in:**
    (a) Cutaneous circulation
    (b) Hepatosplanchinic circulation
    (c) Coronary circulation
    (d) Renal circulation

21. **Oxygen debt:**
    (a) Is impossible to incur when breathing pure oxygen
    (b) Can never occur in a healthy individual
    (c) Is often evidenced by an increase in lactic concentration in the blood
    (d) Is caused by lack of anaerobic metabolism

22. **Time required to repay an oxygen debt following moderate physical exercise is:**
    (a) 10-15 minutes
    (b) 20-25 minutes
    (c) 30-35 minutes
    (d) That required to resynthesize ATP and creatine phosphate

23. **Physical training is greatly influenced by:**
    (a) High carbohydrate diet (b) High protein diet (c) High fat diet (d) Mixed diet

Answers

1. (c)	2. (b)	3. (b)	4. (d)	5. (c)	6. (d)	7. (c)	8. (d)	9. (a)	10. (d)
11. (a)	12. (a)	13. (d)	14. (b)	15. (d)	16. (c)	17. (b)	18. (d)	19. (a)	20. (c)
21. (c)	22. (d)	23. (a)							

Chapter 54

Physiology of Yoga

I. General
II. Yoga History
III. Requirements for doing Yogic Exercises
IV. Type of Yogic Exercises
A. Asanas or Body Postures
B. Pranayama or Breathing Exercises
C. Purification (Cleansing) Practices or Kriyas
D. Music and Sound Therapy
E. Deep Relaxation
V. The Health Benefits of Yoga Practice
VI. Yoga in Health and Disease
VII. Yoga Versus Conventional Exercise

GENERAL

WHAT IS YOGA ?

Yoga is a Sanskrit word meaning *union*. It is *Hindu spiritual* (religious belief) and self discipline method of integrating the body, breath and mind. Yoga essentially **involves**:

1. Adopting certain simple to complex body postures (***Asanas***) and maintaining the same for set periods of time.
2. Controlled breathing;
3. Voluntary concentration of thoughts (***meditation***) or ***Raja Yoga*** ; and / or
4. Repeated recital of phrases called ***mantras***.

Yoga is thus described as comprising a rich treasure of physical and mental techniques that can be effectively used to create physical and mental well being. Since its introduction into modern culture, yoga has enjoyed a tremendous growth in popularity as an adjunct to healthy living.

Note

The branch of yoga that work with both adults and children is called *Hatha yoga*.

THE PURPOSE OF YOGA

1. To achieve highest potential
2. To experience enduring (*i.e.* permanent/ lasting) health and happiness.
3. To improve the quality of life.
4. To extend healthy, productive years far beyond the accepted norms.

Important Note

Because yoga works on so many different levels it has great potential as an effective therapy for chronic diseases and conditions that do not respond well to conventional treatment methods.

General characteristics of yogic practices

1. The yogic system of health involves the exercise of all types of muscles of the body (skeletal, cardiac and smooth)
2. The associated internal pressure such as intrathoracic and intra-abdominal pressure changes form the basis of yoga system of health.
3. Very little expenditure of energy is involved.
4. All walk of life of people and of all ages can practice yogic exercises easily and effectively.
5. Its ***main aim*** is to achieve the highest level of integration through the control of the modification of mind.
6. All yogic practices are complementary to each other.
7. The nature of yogic practices is ***psycho-neurophysical***.

Experience after yoga practice

Yoga is not just a slow-motion *calisthenics* (*i.e.* freehand exercises performed in a rhythmic sequence). Anyone who practices yoga correctly soon begins to appreciate the depth and breath of the following **benefits**:

1. The relaxation and softening of deep inner tension and blockages;
2. Sense of body – mind equilibrium ; and
3. Feeling of energetic light – heartedness.

Studies conducted revealed that 1 hr/day of yogic practice for a period of 6 months leads to *increase in parasympathetic activities*, provides *stability of autonomic balance* during stress, improves thermoregulation efficiency and *cognitive functions* such as : concentration, memory, learning and vigilance. Clinical studies have also demonstrated the *therapeutic potentials* of yogic practices in the treatment of:

1. Chronic obstructive pulmonary disease such as Bronchitis and Asthma.
2. Diabetes melllitus.
3. Low backache.
4. Stress related psychosomatic disorders.
5. Coronary artery disease.
6. GIT disturbances
7. Postural defects and joint pain etc.

Risks of Yoga Practice

1. Potential to cause musculo-skeletel injury
2. Potential to induce arrhythmias
3. Dehydration
4. Fluctuations of the haemodynamic parameters
5. Thrombo-embolic phenomenon due to intimal tear resulting in *Stroke*.

Such risks may occur particularly in those individuals who are physically unfit or weak or yogic practices if not performed within strict limits of safety.

YOGA HISTORY

Yoga is a tradition of health and spirituality that evolved in the Indian peninsula over a period of some 5000 years. It has emerged from the earliest known human civilizations of the Indus valley region. History of yoga traditions starts with the ***yoga sutra*** (i.e text on the philosophy of classical yoga between 200 BC and 300 AD), written by *Patanjali*, a renowned yoga teacher and Hindu philosopher. Yoga entered the western mainstream through the work of *Swami Vivekananda* who popularized Eastern Hindu philosophy in the late 19th and early 20th centuries.

REQUIREMENTS FOR DOING YOGIC EXERCISES

1. Always use an exercise mat
2. Room should be well ventilated and clean
3. Yoga should be done on an empty stomach in peaceful surroundings, wear clean, simple and least cumbersome dress.
4. One should not speak while doing yoga.
5. All exercises should be performed slowly, gently, smoothly and not to be taken to the point of exhaustion.
6. All exercises should be performed with the right attitude i.e with conscious awareness of spiritual aim.
7. Inconvenient postures should not be attempted.
8. It is preferably be performed in standing posture at the same time and at a fixed place *every day for about an hour*. The best timing is day break time (***Dawn***).
9. Learn the yogic practices first; persons who are weak or those who have recovered from illness should take help of yoga therapist.

TYPES OF YOGIC EXERCISES

The basic yoga methods can be divided into *five* basic areas of practice:

A. Asanas or Body postures
B. Pranayama or Breathing exercises
C. Purification (Cleansing) practices *or* kiryas
D. Music and sound therapy; and
E. Deep relaxation

Important Notes

Taking into account *Ashtanga yoga*, yogic practices should also include:

1. Practice of *Yama* to increase the power of concentration, mental purity and steadiness. It comprises :
   (i) *Ahimsa* (not to harm others)
   (ii) *Satya* (to be truthful)
   (iii) *Asteya* (not to steal)
   (iv) *Brahmacharya* (celibacy); and
   (v) *Aparigarha* (not to possess beyond actual needs).
2. *Niyamas* –these are
   (i) *Shauch* (external and internal purification)
   (ii) *Santosh* (contentment)
   (iii) *Tapa* (to make right efforts to achieve goals)
   (iv) *Swadhyaya* (to study authentic texts and religions scriptures so as to acquire correct knowledge of self and the supreme divinity)
   (v) *Ishwar Pranidhan* (complete surrender to the divine will).

A. ASANAS or BODY POSTURES

Asana literally means 'posture' or 'pose'. The principle of yoga practice involves the adoption and maintenance of a particular posture of the body, which is both steady and comfortable (called *psycho-physical posture*). Along with controlled breathing techniques it forms the basis of Yoga's ***mind-body integration*** work.

More than a hundred classical postures, with as many variations have been described. Of these, only few are recommended and are being useful for regular practice. The classic texts advice each ***asana*** to be maintained for a period of 5-20 breaths.

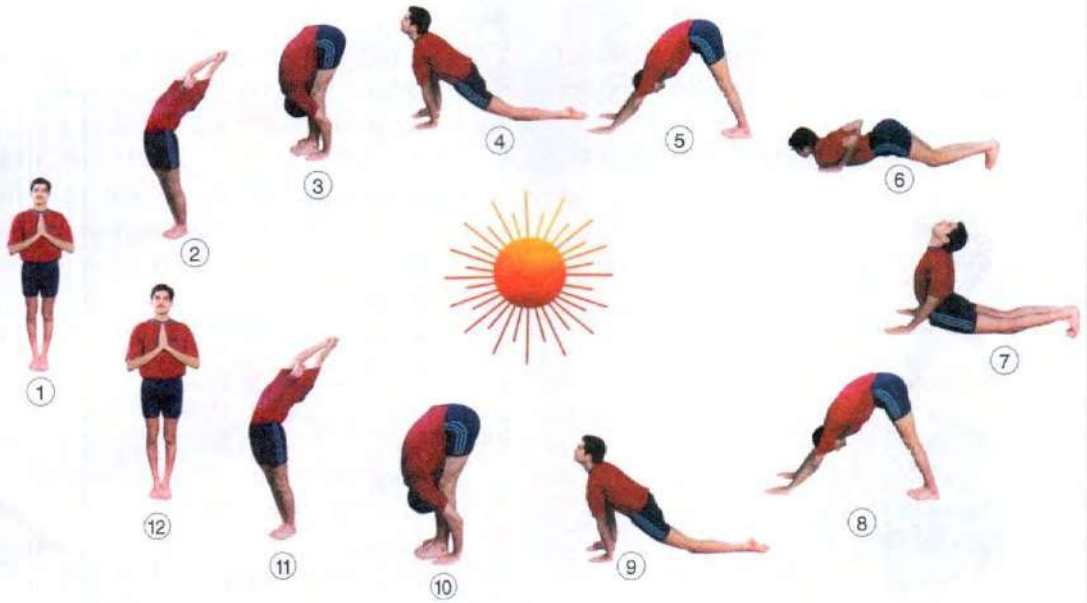


Fig. 54.1 Surya Namaskar (Salutation to Sun)

TYPES OF ASANAS

I. SURYA NAMASKAR *(salutation to sun)*

It means to greet. It is done facing the sun **(Fg. 54.1)**. It energises the entire *neuro-glandular* and *neuro-musular* system of the body. Its regular practice ensures a balanced supply of oxygenated blood and perfect harmony to all the systems of the body, thus strengthening the entire psychosomatic system of human constitution.

II. PADAMASANA (Fig. 54.2)

Steps

1. Sit in the position as shown in figure.
2. Keep head, neck and back absolutely straight.
3. Place the hands on the knees; breath normally.
4. Maintain this position for 4 to 5 minutes and meditate with eyes closed.
5. ***Benefit:*** This asana is primarily meant for concentration and helpful in relieving the physical, nervous and emotional problems.

Fig. 54.2 Padamasana

III. DHANURASANA (Fig. 54.3)

Steps

1. Lie prone with face down on the floor. Arch back with hands holding the ankle.
2. Inhale as you arch and exhale when the final position is reached.
3. Maintain this position for 1 to 2 minutes.

Benefits

(i) It provides the blood circulation and strengthens the abdomen muscles.
(ii) It helps to remove constipation.
(iii) It helps to maintain the spine erect and flexible.
(iv) It is particularly helpful to control blood sugar levels in diabetic patients.

Fig. 54.3 Dhanurasana

IV. VAJRASANA (Fig. 54.4)

Steps

1. Kneel down with the right leg folded on itself; turn it back and place the sole under the buttocks with toes pointing inwards.
2. Similarly, arrange the left leg under the left buttock.

Fig. 54.4 Vajrasana

3. Allow to rest your body weight between these two heels; keep the upper part of the body absolutely erect.
4. Place the hands on the thighs with palms down; relax with eyes closed.

Benefits

(i) It strengthens the thigh muscles and produces alertness.
(ii) It keeps all the body flexible, thereby prevents stiffening of joints.
(iii) It improves digestion and blood circulation.

V. BHUJANGSANA (Fig. 54.5)

Steps

1. Lie down on the abdomen with feet together; arms should be by the side of the body and forehead resting on the ground.
2. Raise the head and bend the neck backwards; simultaneously raise the chest with the help of the arms and lift the back further without raising the navel.
3. Maintain this posture 1-2 minutes and then reverse to bring the body slowly back to the ground.
4. Relax the arms and place them by the sides of the thighs.

Benefits

(i) It keeps the spine flexible and strengthens the back and abdomen muscles.
(ii) It helps to tone up the organs of the abdomen, increases appetite and prevents constipation.

VI. HALASANA (Fig. 54.6)

Steps

1. Lie flat on the back with arms straight by the side of your body and palms touching the ground.
2. Slowly raise the legs together without bending knees and bring them in a vertical position.
3. Now press the hands and without bending the legs take them backwards as far as possible towards the head till the toes touch the ground.
4. Bring the arms from the side of the body beyond the head. Make the finger lock and taking its support at the head bend the back further.
5. Slowly and gradually follow the reverse steps and return to the starting position.

Fig. 54.5 Bhujangsana

Fig. 54.6 Halasana

Benefits

(i) It helps to maintain flexibility of the spine.
(ii) It helps to strengthen the abdominal muscles and prevents constipation.
(iii) It helps to remove all gastric discomfort.
(iv) It is useful in controlling the high blood sugar levels.
(v) It helps to prevent protrusion of the belly.

VII. PAVANMUKTASANA (Fig. 54.7)

Steps

1. Lie flat on the back with head resting on the ground.
2. Raise both the legs and bend them at the knee; hold them between the arms and press them firmly on the abdomen with both hands.
3. Touch the chin with your knees and maintain this position for 1-2 minutes.
4. Breath in and out slowly and deeply.

Benefits

(i) It helps in expulsion of gas from the abdomen. (*Pavan* means *wind* and *mukta* means *release*)
(ii) It helps in strengthening the muscles of back and abdomen.
(iii) It also helps to remove constipation and abdominal fat.

Fig. 54.7 Pavanmuktasana

VIII. SHAVASANA (Fig. 54.8)

Steps

1. Lie flat on the back with all 4 limbs in a relaxed position.
2. Close the eyes and breath slowly and deeply.
3. Relax all body parts by loosening them.
4. Meditate in this position. This will help you further in relaxation of muscles of your body.

Benefits

(i) *Shava* means a dead body, thus this asana helps to have complete relaxation of mind and the body.
(ii) It also helps a person to free himself from the mental and physical stresses, therefore, more advantageous in reducing the raised blood pressure and relief from insomnia (lack of sleep).

Fig. 54.8 Shavasana

IX. SHALABHASANA (Fig. 54.9)

It is so called, since the body takes the shape of a *Locust* in the final position of the asana. (*Shalabha* means a locust *i.e.*, a tropical grasshopper)

Steps

1. Lie flat on abdomen with the chin resting on the ground and the arms extended on the sides; keep the feet together with the toes pointing backward.
2. Keeping the knees straight, raise both the legs with maximum effort.
3. Return to step 1 slowly by making reverse movements.

Benefits

(i) It improves the functioning of abdominal organs like liver, pancreas, and the intestine.
(ii) It also increases the flexibility of the spine.

Fig. 54.9 Shalabhasana

X. PADAHASTASANA (Fig. 54.10)

Pada means feet and *Hasta*, the hands. Thus one has to stand erect touching the feet with his hands.

Steps

1. Stand erect with feet close together and hands by the side of the legs; look straight.
2. Slowly bend forward without bending the knee; try to touch the feet with fingers.
3. Bring the forehead between the arms and keep it close to knees.
4. Maintain this position for some time and then return gradually to original position.

Benefits

(i) It increases the flexibility of the spine and strengthens the muscles of the back.

(ii) It also improves digestion and removes the extra fat from the abdomen.

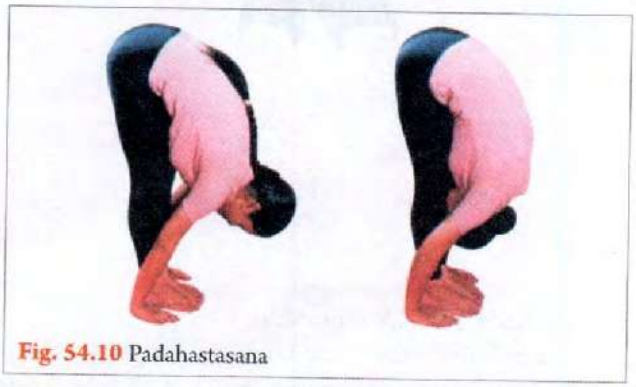


Fig. 54.10 Padahastasana

XI. TRIKONASANA *i.e., A Triangular form* (Fig. 54.11)

Steps

1. Stand erect with feet close together and arms by the sides of the legs.
2. Move the legs outward and raise the arms sideways.
3. Slowly bend the body forward and touch the right toes with the fingers of the left hand.
4. Raise the right hand up and keep looking at it.
5. Maintain this position for some time then return to the original position.

Benefits

It makes the spine flexible and reduces the waist line.

XII. VRISHASANA *i.e.* a tree (Fig. 54.12)

Steps

1. Adopt the position a step '1' above.
2. Raise the right leg and place the foot on the inner aspect of the left thigh.
3. Perform *'namaskar mudra'* in front of the chest.
4. Maintain this position for 30-60 seconds.
5. Return to original position; repeat the steps 2 to 4 with the left leg.

Benefits

(i) It strengthens the posture maintaining muscles *i.e.* muscles of the back and the legs.

(ii) It improves coordination between the body and mind.

XIII. PASCHIMOTTANASANA (Fig. 54.13)

Steps

1. Sit in fully relaxed position with legs stretched forward and hand by the sides with palm placed on the ground.
2. Slowly bend the body forward as far as possible.
3. Place the forehead on the knees and try to hold the great toe with fingers hooked together.
4. Maintain this position for 30-60 seconds, then return to the original position.

Fig. 54.13 Paschimottanasana

Benefits

(i) Since *Paschimottanasana* means stretching of back, this exercise improves the flexibility of spine and strengthen the back muscles, and prevents sciatica.

(ii) It removes constipation and gastic problems such as acidity, indigestion and flatulence.

(iii) It also strengthens the abdominal muscles.

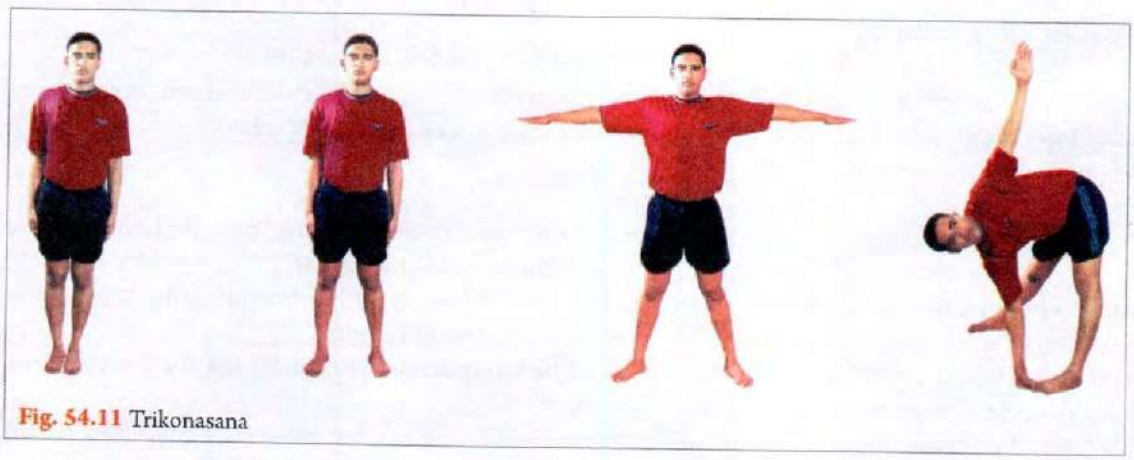


Fig. 54.11 Trikonasana

Fig. 54.12 Vrishasana

Fig. 54.14 Mayurasana

XIV. MAYURASANA (Fig. 54.14)

Steps

1. Kneel on the floor, bend forward and place the palms on the floor with the fingers pointing inwards.
2. Bend the elbows and rest the belly on the elbows. Give support to the chest with upper arms.
3. Stretch the legs straight.
4. Exhale, raise the legs from the floor with feet together. Bring the body in a position parallel to the floor and keep the balance.
5. Maintain this position for 30-60 seconds. Keep breathing normally.
6. Return to original position.

Benefits

(i) It strengthens the abdominal organs.
(ii) It improves digestion and also cures gastric discomfort and helps to control blood sugar.

XV. SHIRSHASANA (Fig. 54.15)

Steps

1. Kneel on the floor; interlock the fingers and place the forearms on the mat by keeping the distance between the elbows equal to the width of the shoulders.
2. Place the top of the forehead on the mat in such a way that the palms touch the top of the head.
3. Exhale, bend the knees and raise the legs together from the floor with a gentle swing. First raise the legs close to the hips and keep the balance.
4. Now unfold the bent legs; stretch the legs and point the toes upwards.
5. Keep the body perpendicular to the floor. Breathe normally and stay in this position for 30-60 seconds.
6. Bend the knees, lower the hips and come back to the original position slowly.
7. *Caution:* A person with high or low blood pressure should not do this asana.

Benefits

(i) It helps to improve memory, sleep and vitality.
(ii) It also give relief to cough, cold and sore throat.
(iii) It is particularly beneficial in preventing and treating hypertension.

Asanas can be subdivided into two categories: *Active* and *Passive*

A. ***Active postures*** tone specific muscles and nerve groups, benefit organs and endocrine glands, and activate brain cells.

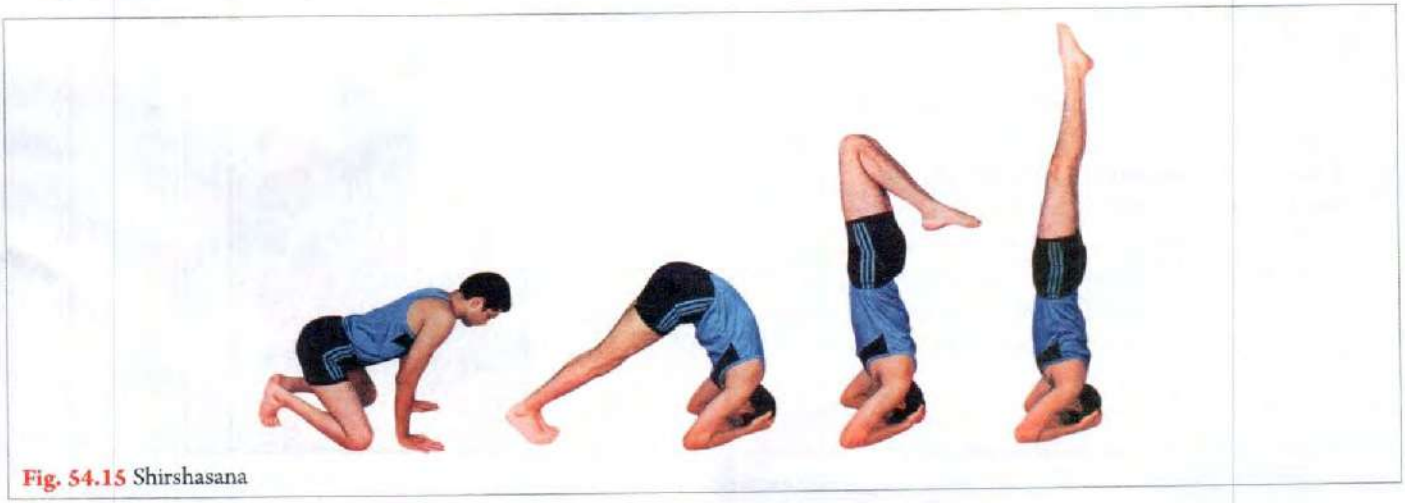


Fig. 54.15 Shirshasana

Fig. 54.16 Pranayama

B. Passive postures are employed primarily in meditation, relaxation, and pranayama practices.

Note

One may *not* be able to safely or comfortably do the posture the way the instructor is doing it. When you are practicing a posture, do what you can without creating more pain; modify or change the position so it feels good for you.

Important Note

The complete set of yoga asanas covers the entire human anatomy (from the top of the head to the tips of the toes). Regular practice helps to correct postural and systemic irregularities, and to maintain the entire physiology in peak condition. The *greatest benefit* from practicing asanas comes when we *learn how to relax* in a given posture rather concentrating the mind totally focused on a single object.

B. PRANAYAMA or *BREATHING EXERCISES*

Pranayama is the science of proper breathing. Breath is the main source of nourishment for all the cells of the body and we cannot live without oxygen for more than a few minutes Therefore,

1. By learning how to increase total lung capacity plus specific pranayama practices, we can increase the flow of vital energy to various organs in our bodies, build our immunity to disease, and overcome many physical ailments.
2. By regulating the breath and increasing oxygenation to brain cell, we help to strengthen and revitalize both the voluntary and antonomic nervous system. When practiced consistently, pranayama also has a powerful stabilizing effect on the mind and emotions thus promoting calm and relaxation.

Technique (Fig. 54.16)

The yogic breathing technique of pranayama involves a *slow deep breath* (about 6 min) inspired with the predominant use of the abdominal musculature and the diaphragm. The breath is held momentarily in full inspiration within the limits of comfort and allow slow and spontaneous exhalation. Again respiration is paused within the limits of comfort in full exhalation. *Observe the breath*, feel for the cool air passing through the nostrils during inspiration, and warm air leaving it during expiration.

Note

Pranayama and Asanas work hand-in-hand to balance and integrate different physiological functions and to help dissolve emotional blocks and negative habit patterns that can obstruct the flow of vital energy within the body.

C. PURIFICATION (CLEANSING) PRACTICES or *KRIYAS*

Purification practices are advanced form of yogic exercises. They clean the inside of the body and include:

1. A ***pranayama*** practice for eliminating excess of secretions (phlegm) and mucus from the respiratory system. It cleans the nasal passage and helps in rhinitis, sinusitis, headache, insomnia etc.

   Method: **Nasal cleaning** or **Jal/Water neti**-here water is poured into one nostril and allowed to flow out of the other. **(Fig. 54.17)**

Fig. 54.17 Jal (water) neti

2. An *eye cleaning exercise* (***Tratake***) It consists of gazing steadily without blinking at an object placed at eye level at a distance of approx 1mt. for a minute. Then the eyes are closed and the object is visualized mentally.
3. Technique for *isolating and rolling the abdominal muscles,* This gives a powerful self-massage to the organs of the abdomen, resulting in improved digestion and relief from constipation.

Methods

(i) *Stomach cleaning or Dhauti* – Here *dhauti* (or a piece of soft cloth) soaked in salty water is swallowed and then pulled out. **(Fig. 54.18)** It cleans the stomach and helps in flatulence and acidity complains.

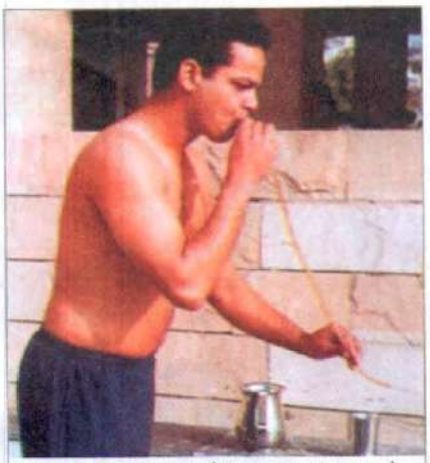


Fig. 54.18 Dhauti (Stomach cleaning)

(ii) *Colonic cleaning or Basti* – A person sits in a tub filled with water and create negative pressure in the abdomen, as a result, water is sucked into the rectum and colon and then eliminated.

(iii) *Abdominal churning or Nauli* – It is performed standing with knees flexed and hands resting on the thighs. After a maximal expiration, the abdominal muscles are forcefully contracted so as to form a central ridge and are then pushed in and out. **(Fig. 54.19)**

4. *Cleansing of the Head or Kapalbharti* – It is performed sitting with one nostril plugged at a time while breathing in and out with the other nostril forcefully and maximally. It is essentially a hyperventilation technique. It washes of carbon-dioxide and leads to a feeling of emptiness in the head.

Note

Purification/cleansing practices being advanced yogic techniques are by no means recommended for a beginner.

D. MUSIC AND SOUND THERAPY

It include rhythm and melody, combined with hand movements and sound combination. It help to develop concentration, breath coordination, communication and motor skill, as well as appreciation for the essentials of tone and harmony. It can also produce a calming and healing effect on the various system and psyche.

Note

By combining sound therapy techniques with traditional yoga practices, such as chanting mantras and sounding musical notes, it is possible to create an ideal learning environment for all levels of yoga practices.

E. DEEP RELAXATION

It is traditionally the conclusion and peak of every yoga session. During 10-20 minutes of complete silence and immobility, deep relaxation allows the body to absorb all the benefits of previous asanas, pranayama and purification (cleansing) practices.

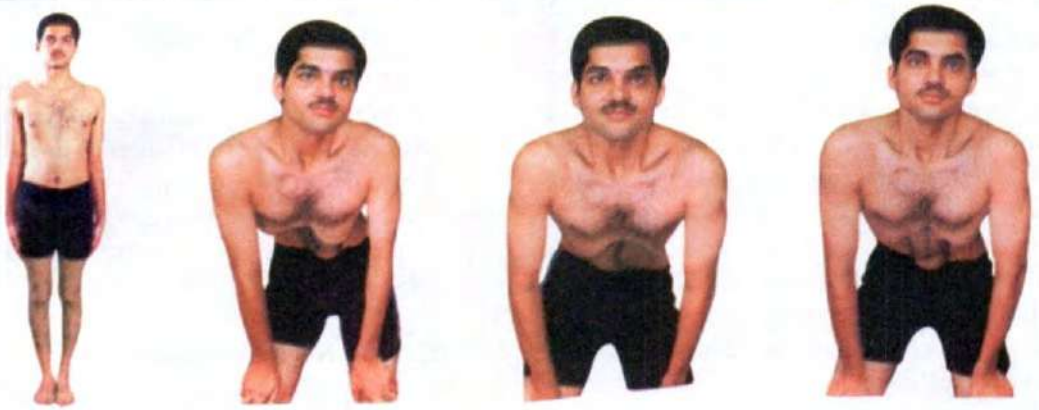


Fig. 54.19 Nauli (Abdominal cleaning)

It is necessary to learn how to relax after a period of activity to recoup the energy and vitality expended during the day.

1. For *infants and toddlers*, soft music is combined with massage of the feet and nape of the neck to help induce relaxation.
2. For *children and adults*, deep relaxation begins as they lie down on their backs with palms up and legs spread one to two feet apart. Using soft background music and muted lighting, encourage the release of physical tension and mental stress.

Note

Relaxation practices in yoga are different than sleeping when properly done. Deep relaxation can become a powerful meditation practice. This help to develop confidence and stabilize the minds awareness in pool of deep tranquility (calmness) and peace.

THE HEALTH BENEFITS OF YOGA PRACTICE

The yoga begins by working with the body on a structural level. The yogic practices stimulate and balance all the systems of the body. The end result is increased mental clarity, emotional stability and a greater sense of overall well being. A few month practice of yoga (asanas or pranayama or meditation or various combinations of these) triggers ***neurohormonal mechanisms*** (see below) that bring about health benefits.

I. PHYSIOLOGICAL BENEFITS

1. Effects on the Nervous system

(i) In general, yogic practices bring about *stable autonomic nervous system equilibrium* with a tendency toward *parasympathetic nervous system dominance*. This result in: (also refer to page 927)
  (a) Decrease in heart rate and blood pressure (of special significance in hypertensive)
  (b) Decrease in respiratory rate
  (c) Improvement in GIT and excretory functions
  (d) Improvement in sexual activity
  (e) Favours storage of absorbed nutrients
  (f) Decrease in oxygen consumption (fall in metabolic rate)
  (g) Galvanic Skin Response (GSR) increases indicating lower reactivity to stressful stimuli and greater stability of the sympathetic nervous system.

(ii) *E.E.G.* Predominance of alpha waves; theta, delta and beta waves also increases (during various stages of meditation). This indicates a more relaxed state of mind and result in:
  (a) *Sensory attenuation* i.e minimal sensory input (less distraction, better concentration and pain decreases)
  (b) *Motor attenuation* i.e minimal motor input (comfortable posture)

(iii) *Higher function of the nervous system* (also refer to page *1031*)
  (a) Improvement of higher intellectual activities such as: emotional affects, motivation, behavior, learning efficiency, personality, memory, moral and social sense etc.
  (b) Improvement in cerebral blood flow with synchronized neuronal activity.
  (c) Depth perception (page 1101) and critical fusion frequency (page 1094) improves, indicating reduced fatigue and stress level.

2. Effects on the CVS

(i) Heart rate and systemic BP decreases
(ii) Cardiovascular efficiency increases i.e a given level of exercise is associated with a smaller increase in above parameters. This may be attributed to *increase baroreflex sensitivity*.

3. Effects on Respiratory System

(i) Respiratory rate decreases
(ii) Tidal volume increases
(iii) Vital capacity increases
(iv) Breath holding time increases
(v) Maximum breathing capacity (MBC) increases
(vi) Respiratory efficiency increases and respiration become more smooth.

Mechanism

Parasympathetic nervous system dominance with decrease in sympathetic activity.

4. Effects on Metabolic Rate

(i) Metabolism is a good indicator of the rate at which we live. Yogic exercises slow down metabolism by decreasing the oxygen consumption. The fall in the metabolic rate is much greater and more steep than during sleep. Further, there is no fall in the internal body (core) temperature.
(ii) Higher maximum oxygen consumption (page *481*) can be achieved due to increase in cardio-respiratory efficiency.

5. Effects on Neurohormonal Activity

Regular yogic practice result in reduction in intrinsic neurohormonal activity such as:
(i) Decrease in urinary exertion of catecholamines

(epinephrine, nor-epinephrine and dopamine); aldosterone.
(ii) Serum catecholamines, testosterone and luteinizing hormone levels decreases
(iii) Increase in the urinary excretion of cortisol.
(iv) Thyroxine increases
(v) Oxytocin increases
(vi) Prolactin increases
(vii) Decrease in resting glucocorticoid levels. But its secretion increases in response to acute challenge indicating the improved ability to cope up with stress.
(viii) Decrease in fasting blood glucose level

6. Effects on skeleto-muscular system

(i) E.M.G. activity decreases
(ii) Musculo – skeletal flexibility and joint range of movement increases
(iii) Strength and resiliency increases
(iv) Endurance increases
(v) Energy level increases

7. Effects on Digestive System

Proper digestion requires a proper diet as well as proper exercise and posture. With a straight spine, abdomen has the space it needs to carry out the digestive processes without the ribs and upper internal organs interfering. Yoga helps digestion by:

(i) Increase blood flow to GIT
(ii) Stimulate peristalsis
(iii) Relax digestive system and leads to more effective elimination.

Note

To develop a strong digestive tract, exercises such as yoga, sit-ups, push-ups, aerobics and swimming are the most beneficial. *Caution:* Avoid yoga asanas if you have or had hiatus hernia, abdominal surgery, abdominal inflammation, hyperthyroid, severe back problems or pregnancy.

II. PSYCHOLOGICAL BENEFITS

1. Somatic and kinesthetic awareness increases
2. Mood improves and subjective well being increases
3. Social adjustment increases
4. Anxiety and depression decreases
5. Hostility (unfriendliness) decreases
6. Psychomotor functions improves such as:
   (i) Grip strength increases
   (ii) Fine skill movements improves
   (iii) Endurance increases
   (iv) Integrated functions of body parts improves

III. BIOCHEMICAL BENEFITS

The biochemical profile improves indicating an *antistress* and *antioxidant effect* important in the prevention of degenerative diseases.

1. Haematocrit, haemoglobin and lymphocyte count increases
2. TLC decreases
3. Total serum protein and vitamin C increases
4. Glucose and sodium levels decreases
5. Lipid profile:
   (i) Total cholesterol decreases
   (ii) Triglycerides decreases
   (iii) LDL and VLDL decreases
   (iv) HDL increases.

YOGA IN HEALTH AND DISEASE

Psychological stress and faulty life style are the major contributors to many diseases of modern civilization such as: ***obesity, hypertension, coronary artery disease*** and ***diabetes mellitus***, *Mindfullness-bases* (*i.e.* cautiously, or careful based) *stress reduction* such as yoga has been shown to play a major role in recovery and contribute to the general health. It has gained immense popularity as a form of recreational activity all over the world. Its possible contributions to healthy living have been studied and many interesting scientifically based revelations have been made.

1. Obesity and Body Weight

A reliable indicator for body fat is *Body Mass Index (BMI)* (page *781 and 1042*). In many individuals attempts at weight reduction have proved to be very challenging and often unfruitful. Yoga has been found to be particularly helpful in the management of obesity. By practicing yoga for *a year* help significant improvement in the ideal body weight and body density.

Mechanisms

(i) By simple excess calorie expenditure
(ii) Life style modification
(iii) Improvement in dietary habits
(iv) Positive body-mind equilibrium

2. Hypertension

Impaired baroreflex sensitivity has been increasingly postulated to be one of the major causative factors of essential hypertension. A short period (*3 months*) of regular yogic practice for 1 hr/day is effective in controlling blood pressure in such individuals.

Mechanisms

(i) Restoration of baroreflex sensitivity with autonomic readjustments.

(ii) Progressive reduction of sympatho-adrenal and rennin-angioten sin activity.

Important Note

Cardio-vascular response to *shirshasana* (head-down –body-up-postural exercise) has been shown to be particularly beneficial in preventing and treating hypertension associated left ventricular hypertrophy and diastolic dysfunction.

3. Coronary Artery Disease (CAD) / Ischaemic Heart Disease (IHD)

Reduced heart rate variability and baroreflex sensitivity are powerful predictors of CAD/IHD. Further *increased* intrinsic neurohormonal activity due to general stress in life also contributes to increased risk of myocardial infarction.

Mechanisms

(i) ***Slow breathing yogic exercises*** along with recitation of *mantras* increases heart rate variability and baroreflex sensitivity by re-synchroning inherent cardiovascular rhythms. This also decreases RR interval in ECG, and reduces both systolic and diastolic blood pressures.

(ii) Regular practice of yoga has shown to **improve serum lipid profile** in the patients with known IHD as well as in healthy individuals.

(iii) Yoga based guided relaxation help in the **reduction of sympathetic activity** with reduction in heart rate, GSR, oxygen consumption and increase in tidal volume. The clinical neurohornomal activity, thus facilitating protection against IHD and myocardial infarction.

(iv) Yoga exercise **increases regression and retards progression of atherosclerosis** in patents with CAD. Lipid-lowering and plaque –stabilizing effects of yoga exercise seems to be simlar to that of *statin drugs* (HMG CoA reducatase inhibitors). The changes brought about by the practice of yoga in the *millieu interieur* by a neurohormonal mechanism seem to be responsible for statin like actions.

(v) Yoga is also shown to have the ability to **control the sympathetic overdrive** thus mimicking beta blockade.

4. Diabetes Mellitus

Maturity onset diabetes can usually be controlled through dietary life style changes. While yoga cannot 'cure' diabetes, it can bring lifestyle changes necessary to keep diabetes symptoms in check. Yoga exercises tone and shape the body, improve posture and circulation (specially to the extremities) and contribute to feeling of well-being.

Yoga is considered as a beneficial adjuvant for NIDDM patients (page 747). It helps to reduce the frequency of hyperglycemia and need for oral hypoglycemics to maintain adequate blood sugar levels.

Mechanisms

Neurohormonal modulation involving insulin and glucagon activity.

Important Note

Most effective asana for controlling blood sugar levels in diabetics is *Dhanurasana*. This is true specially if fasting blood glucose level is less than 250mg/dL.

5. Skeleto- muscular disorders

Yoga has been beneficial for skeleto – muscular disorders such as *backache, spondolytis (cervical/lumbar), arthritis etc.* Asanas strengthen back muscles and relieve backache. They also help reliving muscular spasm by increasing flexibility, decrease in body weight and stress, and producing relaxation of the body as a whole.

6. Cancer Recovery

Individuals diagnosed with cancer, receiving chemotherapy or radiation treatment, recovering from surgical tumor removal or in remission may be dealing with symptoms or side effects, anxiety or emotional issues. Depending on what parts of your body are affected, what type of cancer you have (or had), and your physical abilities; yogic practice will be specific for you. Find out what works for you and what helps you to move in a positive direction. Yoga helps an adjunct to medical treatment by :

(i) ease the symptoms
(ii) give more energy
(iii) calm the mind; and
(iv) give tools for accepting, loving and motivation.

7. Psychiatric Disturbances

Yoga practice has been long used for stress reduction as well as for prevention and treatment of psychological disturbances. Yogic exercises and relaxation improve insomnia, tension headache and irritable bowel or bladder.

YOGA VERSES CONVENTIONAL EXERCISE

YOGA	CONVENTIONAL EXERCISE
1. Performed with the right/positive attitude preceeded by purification of behaviors	1. Performed without any such attitude. *(Note: Any conventional exercise performed with spiritual aim may be considered yogic)*
2. Parasympathetic nervous system dominates	2. Sympathetic nervous system dominates
3. Subcortical regions of brain dominates	3. Cortical regions of brain dominates
4. Provides normalization of muscle tone; low risk of injuring muscles and ligaments	4. Associated with increased muscle tension with higher risk of injury
5. Low caloric consumption	5. Moderate to high caloric consumption
6. Effort is minimized, relaxed	6. Effort is maximized
7. Energizing (breathing is natural or controlled)	7. Fatiguing
8. Balanced activity of opposing muscle groups.	8. Imbalanced activity of opposing muscle group
9. Non-competitive ; process – oriented	9. Competitive ; goal-oriented
10. Awareness is internal eg focus is on breath and infinite	10. Awareness is external eg focus is on reaching the finish line etc.
11. Limitless possibilities for growth in self awareness	11. Boredom factor.

Study Questions

1. Explain:
   (i) Yoga, Yama, Niyama, Pranayama and Kriya
2. Give physiological basis of :
   (i) Improvement in higher intellectual activities with yoga.
   (ii) How does yoga help in weight reduction.
3. Write briefly about :
   (i) General characteristic and requirements for doing yogic practices.
   (ii) Therapeutic potentials of yogic practices. (iii) History of yoga (iv) Techniques of doing pranayama
   (v) Cleansing practices (vi) Benefits of yoga practices.
4. Name the asanas that help in improving spine flexibility and produces strength to the muscles of the back.
5. How does yoga help to develop a strong digestive system?
6. List the major disorders caused by psychological stress and faulty life styles. Describe briefly the role of yogic exercise in overcoming them.
7. Describe briefly the part played by yoga in cancer recovery.

MCQs

1. The main aim of yoga practices is :
   (a) to achieve highest level of integration of body and mind.
   (b) to control breathing.
   (c) to adopt and maintain certain simple to complex body postures.
   (d) to improve quality of life.
2. Asana that energises the entire neuro-gladular and neuro-muscular system of the body is :
   (a) Surya namaskar (b) Dhanurasana (c) Halasana (d) Shavasana
3. All are the basic areas of practice of yoga *exception*:
   (a) Asanas (b) Pranayama (c) Yama (d) Music and sound therapy
4. All asana improve the functioning of the abdominal organs *except*:
   (a) Dhanurasana (b) Halasana (c) Shalabhasana (d) Trikonasana

5. **Pranayama:**
   (a) involves rapid and deep breathing for about 6 minuts. (b) is science of proper breathing.
   (c) is advanced form for yogic exercise. (d) is necessary to learn how to relax after a period of acitivity.
6. ***Not* a part of kriya:**
   (a) pranayama (b) jal neti (c) kapalbharti (d) deep relaxation practice
7. **Deep relaxation practice is:**
   (a) traditionally the conclusion and peak of every yoga session.
   (b) to recover the energy expended doing the day.
   (c) to regain vitality.
   (d) all of the above are true.
8. **Minimum duration for which yogic exercise need to be performed to trigger neuro-hormonal mechanisms that bring about health benefits is:**
   (a) 2 months (b) 4 months (c) 6 months (d) 8 months or above
9. **All are disease of modern civilization *except*:**
   (a) hypertension (b) skeleto-muscular disorders (c) diabetes mellitus (d) coronary artery disease
10. **Yoga differs from conventional exercise that in yoga:**
    (a) sympathetic nervous system dominates (b) is goal oriented
    (c) Provides normalization of muscle tone (d) awareness is external

Answers

1. (a) 2. (a) 3. (c) 4. (d) 5. (b) 6. (d) 7. (d) 8. (c) 9. (b) 10. (c)

1

Unit VII

THE EXCRETORY SYSTEM

↳ Go as Per the teaching schedule.

Chapter 55: Physiological Anatomy of the Kidney

Kidney structure: Nephron; organization and function of glomerulus; types of nephrons; Juxtaglomerular apparatus

Kidney functions

Blood supply of kidney: Renal blood vessels; peculiarities of renal circulation

Chapter 56: Mechanism of Formation of Urine

Glomerular filtration: GFR; Glomerular filtration versus systemic filtration; filtration fraction

Reabsorption and secretion in renal tubules of glucose; Na^+; K^+; HCO_3^-; H^+; Cl^- and water

Chapter 57: Renal Clearance

Significance

Applications: as a measure of : GFR, tubular secretory capacity, RPF, RBF, osmotic and free-water clearances, excertion of waste products; Uremia; Dialysis therapy

Chapter 58: Mechanism of Concentration and Dilution of Urine – The Counter Current System

Counter current system: counter current multipliers and exchangers; Role of urea

Diuresis: Water versus Osmotic; Diuretics

Chapter 59: Acidification of Urine

Renal regulation of acid-base balance: buffer system in the kidneys; titratable acidity; excretion of H^+

Chapter 60: Regulation of Volume and Concentration of Body Fluids

Regulatory mechanisms: Defence of tonicity, volume and H^+ concentration

Applied: dehydration; overhydration; acidosis; alkalosis; Anion Gap

Chapter 61: Kidney (Renal) Function Tests

Urine and blood examination

Renal clearance tests: for glomerular and tubular functions

Miscellaneous tests

Chapter 62: Physiology of Micturition

Definition; Physiological anatomy, nerve supply and postural activity of urinary bladder

Micturition reflex

Mechanism of voluntary micturition and its reflex control

Applied: Deafferentation; denervation

Chapter 63: Regulation of Body Temperature in Humans

Normal value; factors affecting

Body heat production and heat loss

Temperature regulating mechanisms

Applied Aspect: fever; hypothermia

✓ Physiol. anat. of Kidneys

17/03 → ✓ GFR

18/03 → ✓ Practical instruct - Cranial n - 2

21/03 → ✓ Formation of urine - Tubular absorp

22/03 → ✓ Formation of urine - concentration

23/03 → Micturition, Disorders, Cystometrogram

24/03 → Fluid balance + Electrolyte balance

25/03 → Acid-base balance

27/03 → Renal function tests & Renal clearance

28/03 → Temperature regulation

See the videos in youtube saved in favourites BEFORE STARTING

- Filtration fraction = 0.16 - 0.20 (or) 16% - 20%
- Renal plasma flow : 600-700mL
- Renal blood flow : 1250mL
- Glomerular filtration rate : 125ml/min (or) 180L/min
- Urine output : 1.5 - 2.5 L / day (or) 1mL / min.

- $C_x = \frac{U_x \times V}{P}$; $BV = \frac{PV}{1 - Hct.}$ (or) $RBF = \frac{RPF}{1 - Hct}$; $\text{Filtration fraction} = \frac{GFR}{RPF}$

- $ERPF = \frac{RPF}{E.R}$

- Filtered Load = GFR x Plasma conc. of solute (P_x)
- Excretion rate = $U_x \times V$

Chapter 55

Physiological Anatomy of the Kidney

I. Kidney Structure
Nephron
Types of Nephrons
Organization and Function of Glomerulus
Juxtaglomerular Apparatus

II. Kidney functions

III. Blood Supply of the Kidney
Peculiarities of Renal Circulation
Renal Blood Vessels

(A) (1) KIDNEY STRUCTURE

GROSS STRUCTURE

1. The two kidneys, each weighing 150 gms in adults are located retroperitoneally in the upper dorsal region of the abdominal cavity, on either side of the vertebral column. The kidneys are bean-shaped organs, approx. 10 cm long, 5 cm wide and 2.5 cm thick. The right kidney is usually slightly lower than the left because of the considerable space occupied by the liver. *Vertical section* of the kidneys shows: (Fig. 55.1)
   (i) *Outer cortex* – reddish in colour; and
   (ii) *Inner medulla* – pale in colour. It contains 10-15 *Pyramids* which terminate medially in the renal *papillae*. Papillae projects into 'calyces'; such 10-15 minor calyces join to form two *major calyces* which come out through the *pelvis* of kidney to the widened end of the *ureter*.
2. The ureters exit from the *hilus* of the kidney and pass to the bladder. The blood vessels, lymphatics and nerves enter into or exit from the kidney via the hilus.

(B) MICROSCOPIC STRUCTURE

The basic *functional unit* of the kidney is the *nephron*. There are approximately 1 to 1.3 million nephrons in each kidney which drain into the renal pelvis. Total length of a nephron ranges from 45 to 65 mm.

The different parts of the nephron are: *Bowman's capsule; Glomerulus; The proximal convoluted tubule* (PCT): length

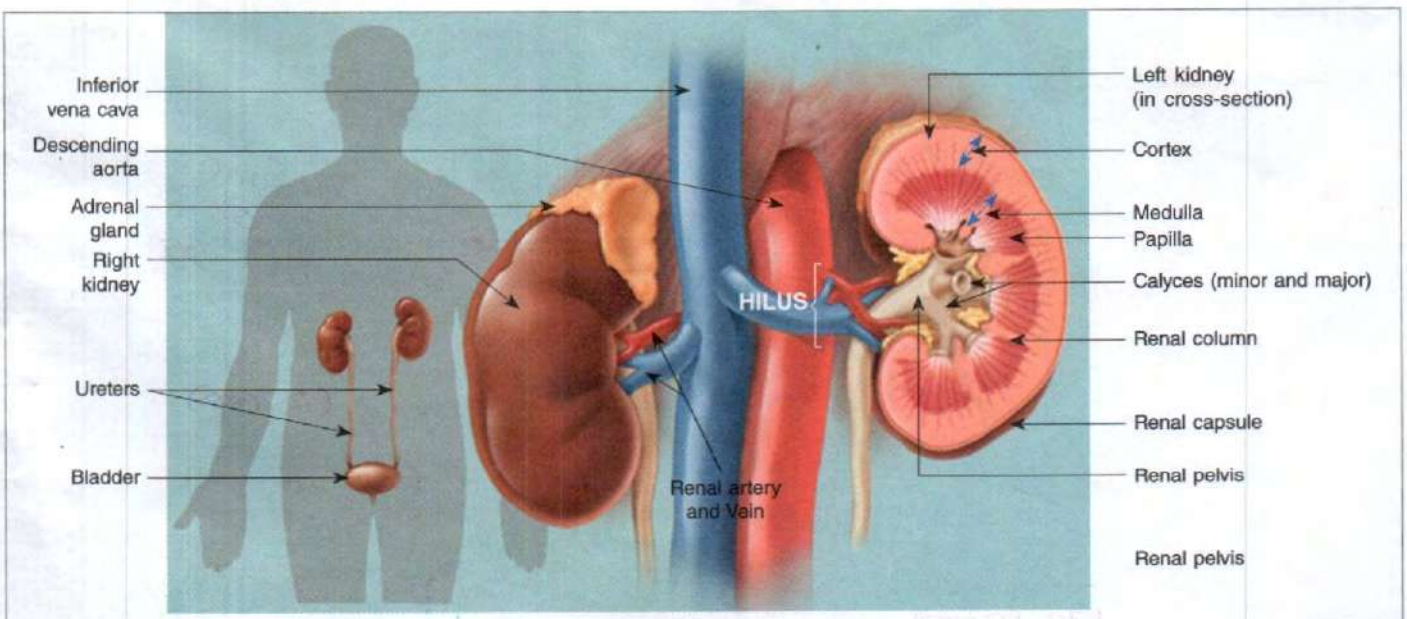


Fig. 55.1 Structure of the kidney

(15 + 20 + 5 + 20)

15 mm; *The loop of Henle:* length 14-26 mm; *The distal convoluted tubule* (DCT): length 5 mm, and *The collecting tubules:* length 20 mm. (Fig. 55.3)

A. Bowman's Capsule *i.e.* the initial dilated part of the nephron. Its epithelial cell lining is about 5 µm thick.

B. Glomerulus: It is about 200 µm in diameter and formed by the invagination of a tuft of capillaries into the Bowman's capsule. The capillaries are supplied by ***afferent arteriole*** and the blood leaves from the tuft by ***efferent arteriole***.

Bowman's capsule and the glomerulus together constitute the ***Malpighian Corpuscle*** (after *Marcello Malpighi* 1666).

1 Organization and function of the Glomerulus

The Bowman's capsule has two layers: (1) Visceral and (2) Parietal.

1. *Visceral cell layer* is very closely applied to the loops of the capillaries so as to surround each loop on all sides. It is continuous at the site of entrance of the afferent and efferent arterioles with the parietal layer. (Fig. 55.2)
2. *Parietal cell layer* is applied to the Bowman's capsule proper and forms the outer lining of the glomerulus. It is continuous with the proximal convoluted tubule.

A space is present between the visceral and parietal layers of the Bowman's capsule, called ***Bowman's Space***. The structures intervening the blood within the capillary loop and Bowman's space is called ***Glomerular membrane*** or ***Glomerular capillary wall***.

2 Structure of Glomerular Membrane

1. It is an extremely thin membrane and is made up of *five* layers (Fig. 55.2):

Layer 1: *Foot processes of podocytes.* Visceral epithelial cell layer covering the capillaries is not continuous. It gives out series of processes called *pedicles* (feet), interdigitating upon capillary surface to form *filtration slits* (25 nm wide) along the capillary wall.

Layer 2: *Lamina rara externa or outer cement layer.* Overlying this are the foot processes of podocytes.

Layer 3: *Lamina densa* – dense structural portion of the basement membrane.

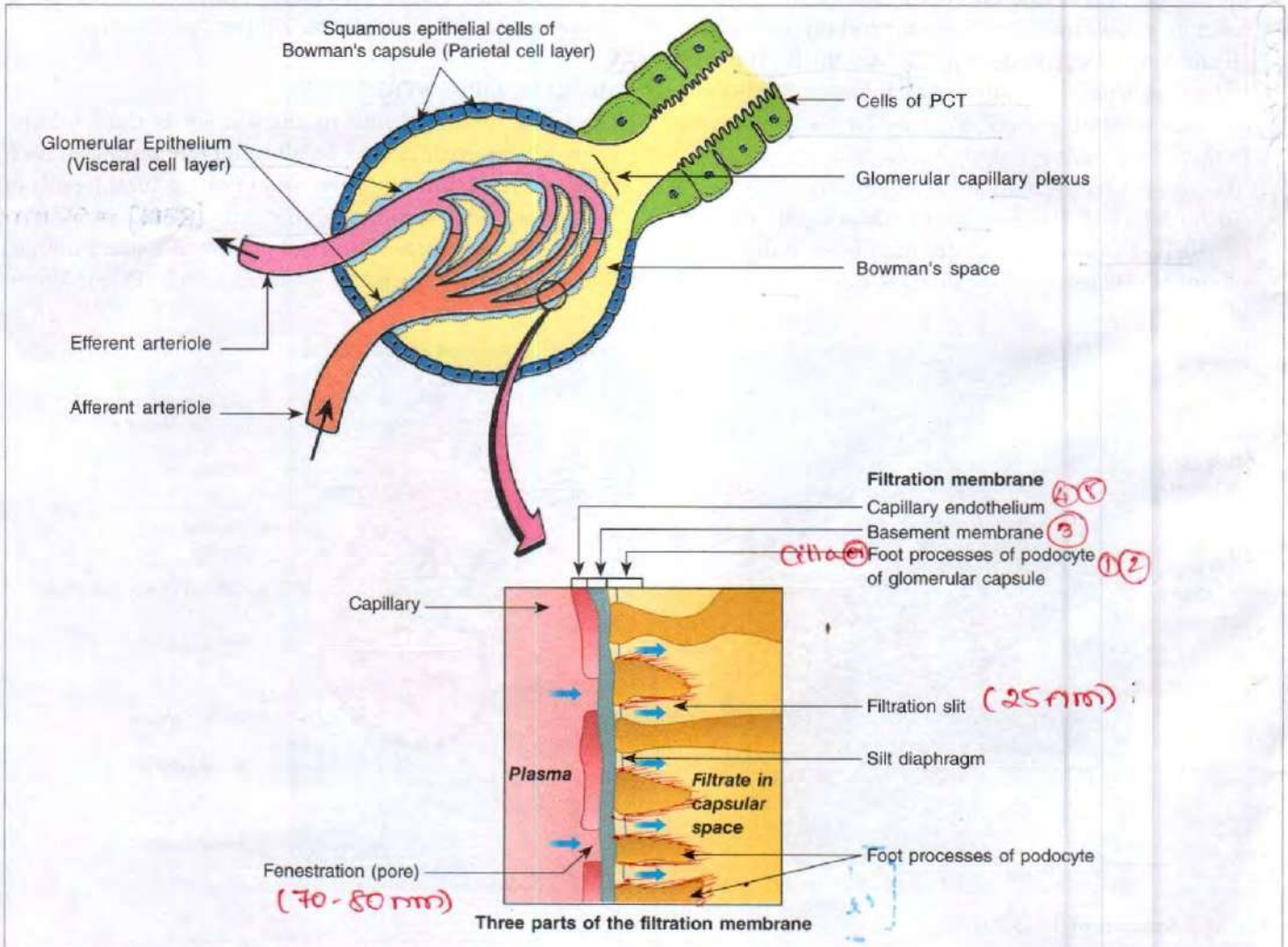


Fig. 55.2 Organization of the glomerulus

Layer 4: ***Lamina rara interna or inner cement layer.*** It provides a bed for the capillary endothelium.

Layer 5: ***Endothelial cell layer.*** The endothelium of the glomerular capillaries is fenestrated with pores of 100 nm in diameter. These features allow plasma filtration with retention of plasma proteins and blood cells.

2. The **glomerular capillaries** form a freely branching anastomotic network. Each glomerulus contains six lobules and each of these consists of 3-6 capillary loops *i.e.* 20-40 loops in all. Many anastomoses occur between the capillaries within any one lobule. Arrangement of afferent and efferent arterioles within the glomerulus allows the maintenance of a much ***higher pressure (about 45 mmHg)*** in the glomerular capillaries than in capillaries elsewhere (pressure in systemic capillaries is 25 mmHg). This high capillary pressure is well adapted for filtration function which the glomeruli subserve.
3. The *major function* of glomerular membrane is to produce an ***ultrafiltrate*** *i.e.* the glomerular filtrate will contain all the constituents of plasma except proteins. Functionally, the glomerular membrane permits the free passage of neutral substances upto 4 nm in diameter and almost totally excludes those with diameter greater than 8 nm.

Note

The total area of glomerular capilllary membrane across which filtration occurs is about 0.8 square meters.

Important Note

Filtration under pressure is called *ultrafiltration.* As a result water and small molecules filter through the glomerular membrane rapidly whereas the proteins and blood do not.

C. **Proximal Convoluted Tubule (PCT).** ***Features:*** **(Fig. 55.3)**

1. PCT lumen is continuous with that of Bowman's capsule.

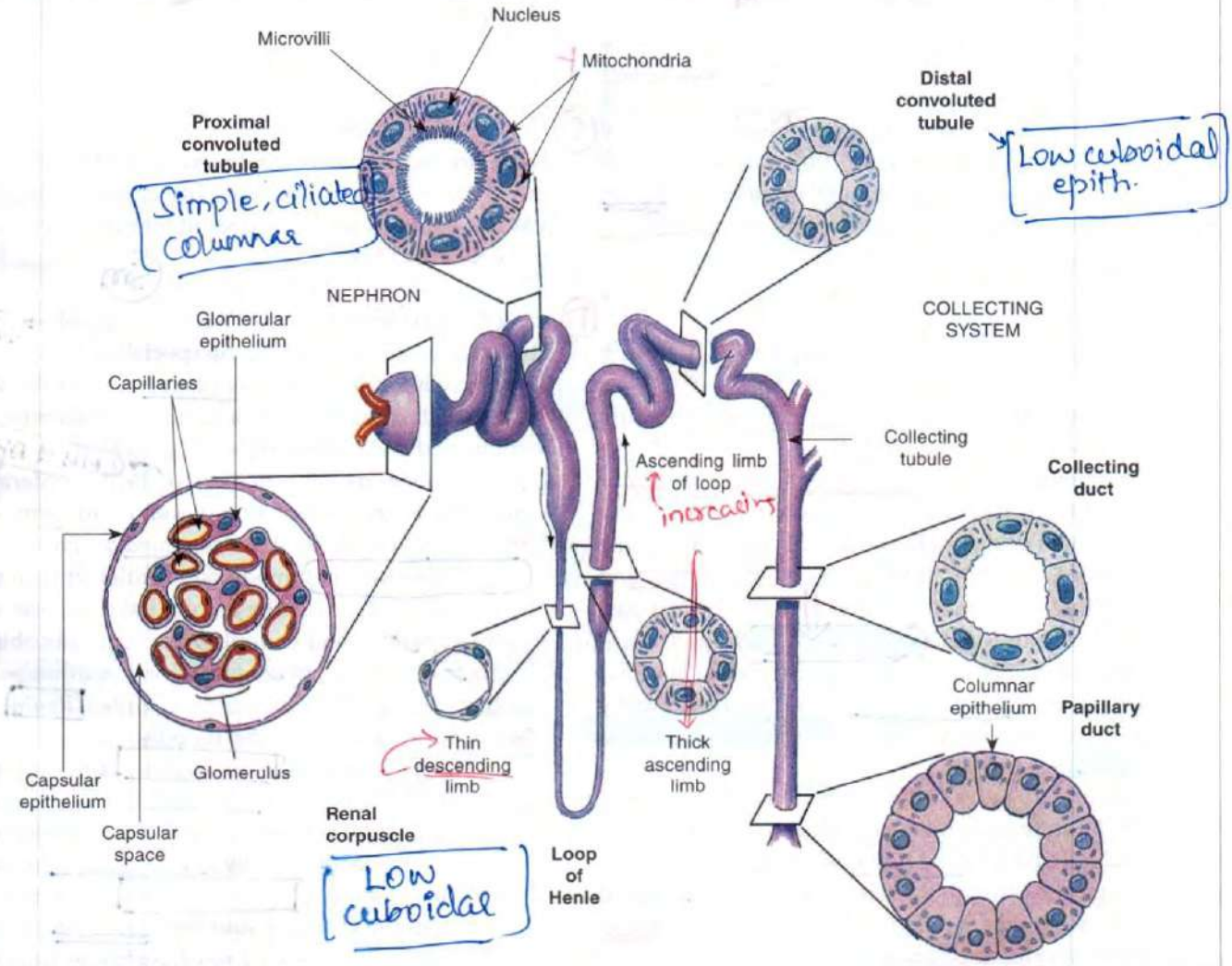


Fig. 55.3 Histology of a typical Nephron

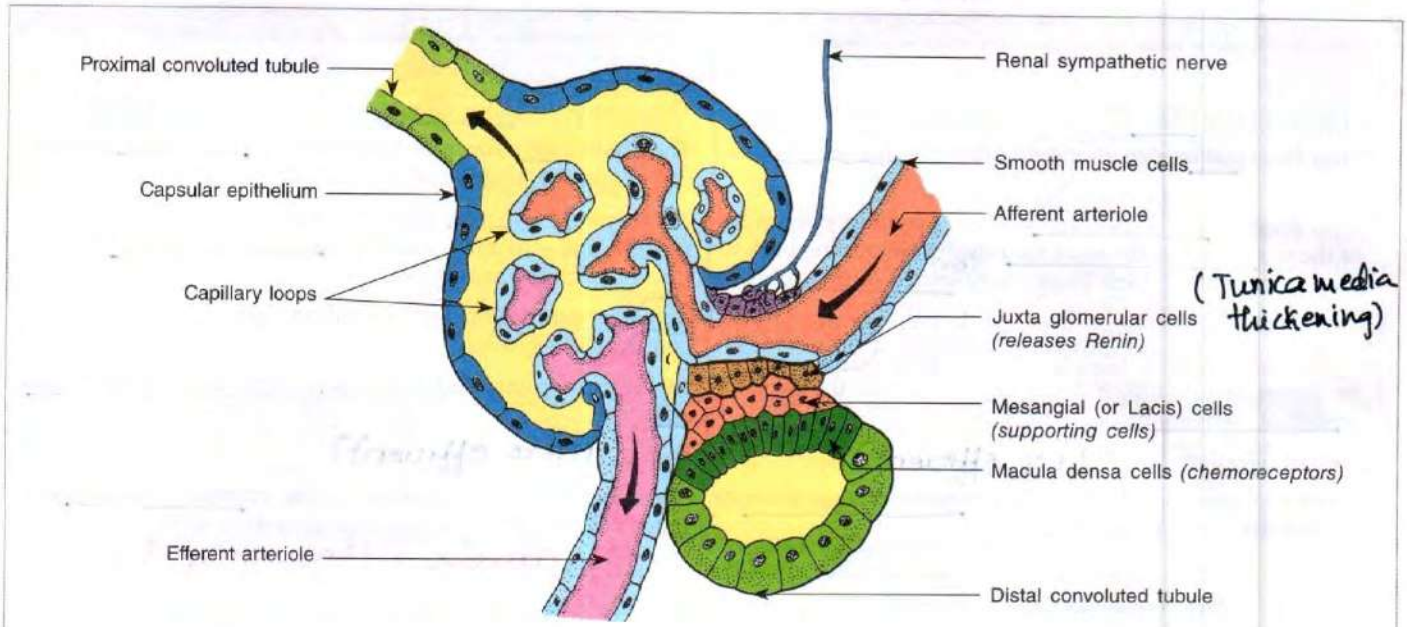


Fig. 55.5 Structure of Juxta glomerular apparatus (JGA)

(iii) They also secrete various substances and take up immune complexes.

The JGA regulates the renin secretion into the blood stream.

Normal plasma renin level: 200 ng/dL.

Regulation of Renin Secretion

Inhibition

1. Inhibition of JG cells by:
   (i) Stretch due to increase in afferent arteriolar pressure
   (ii) Angiotensin II (via negative feedback)
   (iii) Vasopressin (ADH).
2. Increased rate of Na^+ and Cl^- reabsorption across macula densa.
3. Atrial natriuretic peptide: ANP (page 559)

Stimulation

1. Stimulation of JG cells by:
   (i) increase in sympathetic activity via renal nerves (hypovolemia, hypotension)
   (ii) increase in circulating catecholamines (act via β_1-adrenergic receptor on JG cells)
   (iii) prostaglandins (specially prostacyclin)
2. Sodium depletion; Diuretics; Congestive cardiac failure. (CCF)

Note

The rate of renin secretion at any given time is determined by the summated activity of all the factors mentioned above. Also refer to page 726.

Renin-Angiotensin System

Angiotensinogen (α_2–globulin substrate in the circulating plasma)

↓ Renin an enzyme from JGA

Angiotensin I (inactive decapeptide)

↓ angiotensin converting enzyme (ACE) in the lungs, kidney and plasma

Angiotensin II (octapeptide; 1/2 life 1-2 min)

↓ angiotensin converting enzyme in lungs

Angiotensin III

Angiotensin II and III get rapidly destroyed by circulating angiotensinase to inactive metabolites.

Actions of Angiotensin II (*Mode of action: page 23*)

1. Most potent pressor substance (acts via AT_1 receptors, page 726) and by causing generalised arteriolar constriction increases both systolic and diastolic blood pressure; *4 to 8 times more potent than nor-epinephrine (NE).*

Note

Angiotension II pressor activity is decreased in sodium depleted individuals and in patients with cirrhosis of liver.

2. Stimulates zona glomerulosa of adrenal cortex to increase aldosterone secretion (page 726), which then leads to increased sodium reabsorption by the kidneys.

This action of aldosterone is slow because it requires new protein synthesis.

3. Facilitates the release of NE by a direct action on post-ganglionic symphathetic neurons.
4. Contraction of mesangial cells with a resultant decrease in GFR.
5. Increases $Na^+ - H^+$ exchange in the PCT, thus directly increases Na^+ reabsorption by the kidneys.
6. Acts on circumventricular organs (page 373) in the brain to cause increased water intake; increases BP; increases ADH and ACTH secretion.

Important Note

Angiotensin III has about 40% of the pressor activity of angiotensin II but 100% of the natural aldosterone stimulating activity.

(2) KIDNEY FUNCTIONS [GHEE]

A. Excretion of Metabolic Waste products. The *main* function of kidney is to filter the blood and reject its waste products in dissolved form in the urine. How?

1. Renal blood flow (RBF) is about 1.2 L per min *i.e.* approx. 1700 L of blood is filtered by the kidneys per day. Since glomerular filtration rate (GFR) is 125 mL/min *i.e.* about 180 L/day of cells and protein free filtrate are formed; whereas normal urine volume is 1 to 1.5 L/day. Thus, more than *99% of the filtrate is normally reabsorbed.* HOW?
2. On the part of the renal tubular cells, the essential constituents of this filtrate are restored to the circulation together with water; and ***waste products*** *e.g. urea* (non-toxic product of protein metabolism), *uric acid* (end product of purine metabolism), *creatinine* (an endogenous anhydride of muscle creatine), K^+ and H^+ are only partly absorbed. In fact, the tubular cells actively *secrete* K^+ *and* H^+ into the tubular fluid.
3. These rejected waste substances osmotically attract some water and in 24 hours approximately 1–1.5 L of hypertonic urine is finally passed by the kidney into the ureter which is to be voided periodically by the urinary bladder.
4. Minimal or '*obligatory*' volume of the fluid to excrete these waste substances is 500–600 mL/day (page 529).
5. *Kidneys cannot concentrate urine more than 5 times the osmolar concentration of the plasma* (normal osmolar concentration of plasma is approx. 300 mosm/kg water).

Important Note

Bilateral nephrectomy or renal failure leads to progressive rise in blood urea level, K^+ and H^+, which within a few days produces convulsions, coma and death.

B. Homeostatic function i.e. to maintain the constancy of body internal environment (*Millieu interior*, page 50) by the following mechanisms.

1. *Regulation of volume and concentration of body fluid* (Refer to page 557); and
2. *Regulation of Acid-Base Balance* (Refer to page 551)

C. Endocrine function. Kidneys are endocrine organs secreting:

1. *Renin* – it is a major component of the Renin-angiotensin-aldosterone mechanism and helps to *regulate B.P.*
2. *Renal erythropoietic factor* (REF, *erythropoietin*, page 68). In foetuses and neonates REF is primarily secreted by the liver. REF increases the number of circulating erythrocytes.
3. *1,25 DHCC (Dihydroxycholecalciferol)*

   Dietary Vitamin D_3 (cholecalciferol; cannot be used by body as such)

   ↓ *25 hydroxylase in liver, first hydroxylation*

   25 HOCC (hydroxycholecalciferol, 2-5 times more effective than vitamin D_3 in preventing rickets)

   ↓ *1-α hydroxylase in kidney PCT, second hydroxylation*

   1,25 DHCC (biologically most active form of vitamin D; 100 times more potent than 25 HOCC).

D. Gluconeogenesis function. The kidney acquires the important ability to synthesize and secrete glucose produced from non-carbohydrate sources (*e.g.* glutamine) only in unusual circumstances such as prolonged starvation and chronic respiratory acidosis.

(3) BLOOD SUPPLY OF THE KIDNEY

(1) Renal Blood Vessels

(i) *Renal Artery*, a major branch of Aorta supplying each kidney separately.

↓

(ii) *Interlobar Arteries* which ascend between the medullary pyramids (Fig. 55.6). [In medulla]

↓

(iii) *Arcuate Arteries* which course between cortex and medulla parallel to the cortical surface.

↓

(iv) *Interlobular Arteries* which run towards the surface and give off straight branches.

↓

(v) *Afferent Arterioles* – short and thick walled, each divide into multiple capillary branches to form, glomerulus. *They form a major site of autoregulatory resistance* (page 511) and are also responsible for high hydrostatic pressure in the glomerular capillaries than in the other capillaries (page 518)

↓

(vi) *Glomerular Capillaries exhibit higher pressure* than that in other body capillary bed because afferent arterioles are short and thick walled.

↓ *join to form*

(vii) *Efferent Arterioles* – diameter smaller to afferent arterioles but possess a thinner wall due to relatively little smooth muscle in them. These vessels *have a relatively high resistance.*

↓ *they break up to form*

(i) in cortical nephrons – ***Peritubular Capillary Plexus*** which surrounds all the convoluted tubules in the cortex.

(ii) in JM nephrons – not only form peritubular capillary plexus but also subdivide into bands of straight vessels, called ***Vasa Recta***, which run parallel to the convoluted tubules into the medulla (Also see to page 545).

(i) and (ii) join to form

(viii) *Stellate Vein*

↓ *drains into*

(ix) *Interlobular Vein*

↓

(x) *Arcuate Vein*

↓

(xi) *Interlobar Vein*

↓

(xii) *Renal Vein* → Inferior vena cava

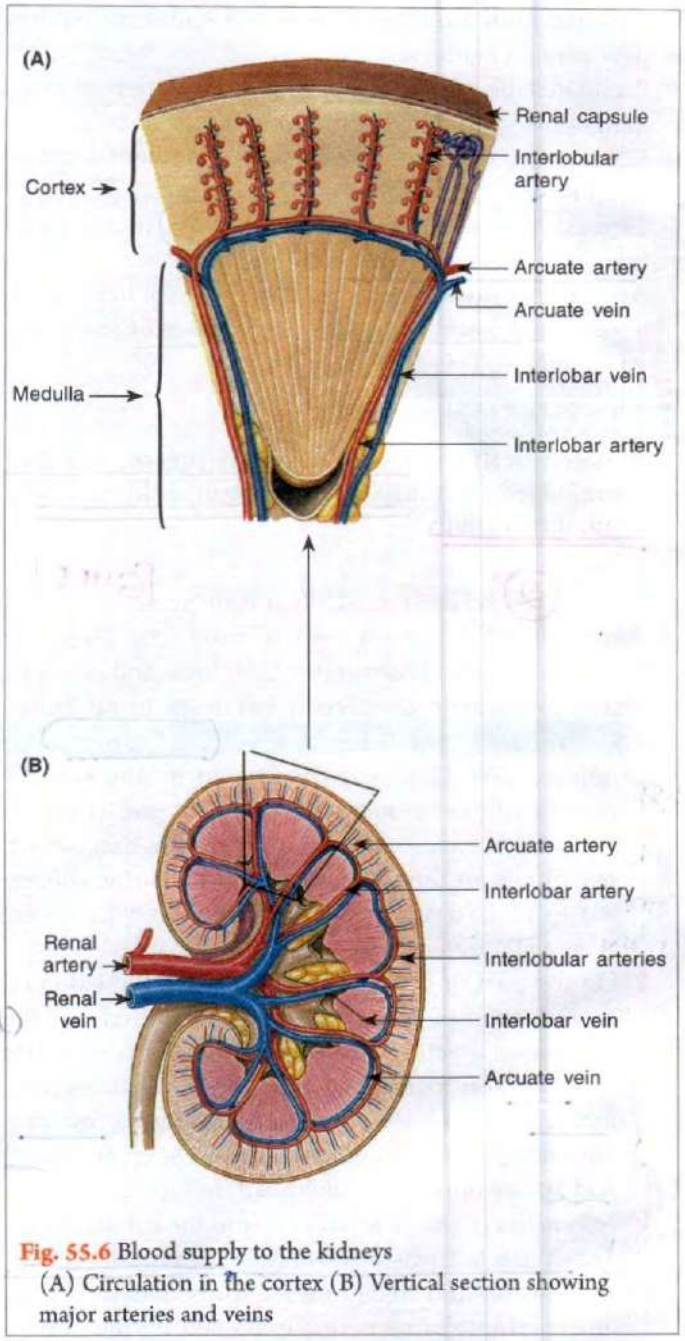


Fig. 55.6 Blood supply to the kidneys
(A) Circulation in the cortex (B) Vertical section showing major arteries and veins

Important Notes

Arteriole → Capil. → Arteriole → Capil.

1. The arterial segments between glomeruli and tubules are technically a 'portal system' (portal means gate), and *the glomerular capillaries are the only capillaries in the body that drain into arterioles.*
2. The efferent arteriole from each glomerulus breaks up into capillaries that supply a number of different nephrons. Thus, the tubule of each nephron does not necessarily receive blood from the efferent arteriole of that nephron.
3. In humans, total surface area of renal capillaries is equal to the total surface area of the tubules = 12 m^2.
4. The volume of blood in the renal capillaries at any given time is 30-40 mL.
5. The kidneys have an abundant lymphatic supply that drains via the thoracic duct into the venous circulation in the thorax.
6. *Pressure in renal vessels* (Fig. 55.7):
   (i) *In glomerular capillaries:* 45 mmHg
   (ii) *In peritubular capillaries:* 8 mmHg (due to drop in pressure in the efferent arterioles)
   (iii) *In renal vein:* 4 mmHg

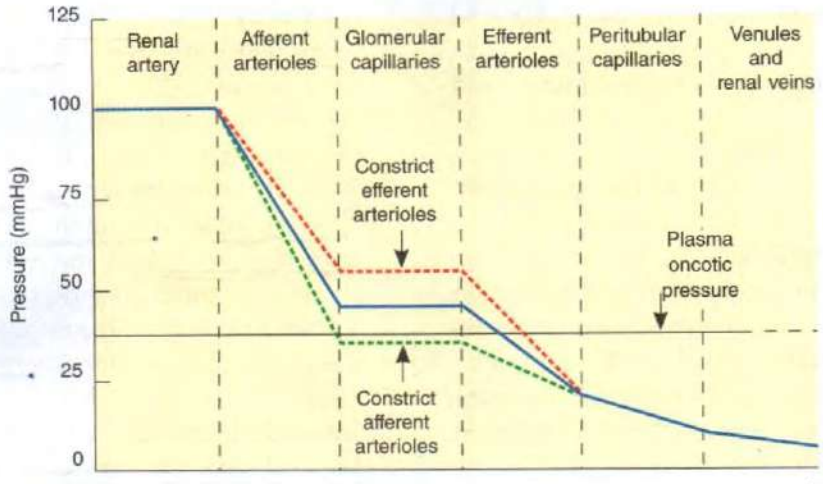


Fig. 55.7 The fall in mean BP across the renal circulation. **Note** that when the afferent arterioles constrict, the pressure in the glomerular capillaries falls and when the efferent arterioles constrict, the pressure in the glomerular capillaries rises.

Innervation of Renal Vessels Renal vessels are innervated by *Efferent and Afferent nerves.*

A. **Efferent nerve supply** is by two branches of autonomic nervous system:

1. *Parasympathetic Innervation* from vagus (X) nerve, function is uncertain.
2. *Sympathetic innervation* from T_{10} to L_2 intermediolateral grey segment of spinal cord through ***splanchnic nerves***. The sympathetic cell bodies are located in superior mesenteric ganglion and sympathetic fibres are distributed:
   (i) primarily to the afferent and efferent arterioles; PCT, DCT and thick ascending limb of loop of Henle; and
   (ii) also end in close proximity to the renal tubular cells and JG cells.

There is some tonic discharge in the renal nerves at rest. This is mediated in part by VMC (vasomotor centre) in the medulla and by parts of cerebral cortex.

Stimulation of renal sympathetic nerve produces:

(i) *Fall in RBF* (renal blood flow) due to both afferent and efferent arteriolar constriction which causes increase in renal vascular resistance, the effect is mediated by:
   (a) mainly by α_1–adrenergic receptors; and
   (b) to a lesser extent by post-synaptic α_2–adrenergic receptors

(ii) *Increased renin secretion* from JG cells via β_1-adrenergic receptors located on the membranes of these cells by a direct action of released NE.

(iii) *Increased reabsorption of Na^+ and water* in the tubules due to a direct action of catecholamines on the renal tubules. This effect may be mediated by both α or β-adrenergic receptors.

B. *Afferent Nerve Supply* to convey pain sensibility in kidney disease. The afferent fibers pass along the sympathetic afferents into the doral nerve roots of $L_{1,2}$ and lower thoracic segment.

Important Note

An increase in ureteral pressure in one kidney reflexly leads to a decrease in efferent nerve activity to the contralateral kidney to cause an increase in its excretion of Na^+ and water (called *Reno-renal reflex*; probably mediated by renal afferents).

Factors affecting blood supply to the kidneys

1. *Catecholamines,* specially nor-epinephrine (NE) constricts renal vessels, the greatest effect being exerted on the interlobular arteries, the afferent and efferent arterioles. However, dopamine causes renal vasodilatation and natriuresis.
2. *Angiotensin II:*
   (i) *In low* concentration causes selective constriction of efferent arterioles
   (ii) *In high* concentration causes constriction of both afferent and efferent arterioles.
3. *Prostaglandins (PG):* PGE_2 and PGI_2-vasodilate both afferent and efferent arterioles and cause increase in blood flow in renal cortex but decrease blood flow in the renal medulla.

4. *Exercise*, increases sympathetic activity thus markedly decreases RBF due to increased release of NE and Angiotensin II.
5. *Haemorrhage* by release of catecholamines and angiotensin II causes:
   (i) marked decrease in RBF
   (ii) Prostaglandin production locally in kidney, increases RBF

   Net effect is decrease in RBF.
6. *Change of posture* from supine to standing position causes BP to fall; this via baroreceptors, increases sympathetic activity and eventually RBF decreases.
7. *Increase in renal arterial B.P.* causes reflex decrease in sympathetic tone, this by renal arteriolar dilatation results in increase in RBF.
8. *Acetylcholine* increases RBF by producing renal vasodilatation.
9. *High protein diet* increases RBF by increasing the glomerular capillary pressure.
10. *Other agents.* Bradykinin, nitric oxide and dopamine by producing vasodilation of renal arterioles leads to an increase in RBF.

Peculiarities of Renal Circulation

1. Under basal conditions, renal blood flow (RBF) is 1.2 to 1.3 L/min. (300-400 mL/100 gm/min). This *RBF is very high* as compared to flow through other body organs. For example:

Coronary blood flow	:	60-80 mL/100 gm/min.
Brain blood flow	:	55 mL/100 gm/min.
Skeletal muscle blood flow	:	3–4 mL/100 gm/min.

A large RBF is required to produce a high GFR for the excretion of metabolic byproducts *e.g.* urea, uric acid, creatinine.

2. **Arterio-venous oxygen difference**
   ($A-V$ O_2 difference or oxygen extraction) across kidneys is *low*. Therefore, renal venous blood has high pO_2 and is 80-85% saturated with oxygen.

∴ Kidney consumes less of O_2.

Organ	Arterial O_2 content (mL/dL)	Venous O_2 content (mL/dL)	$(A-V)$ O_2 difference (mL/dL)
Kidney	19	17.5	1.5 (very low)
Coronary circulation	19	7-8	10-12
Brain	19	12	6-7
Whole body	19	14	5

From (1) and (2) above, oxygen consumption (V_{O_2}) of kidney is about 18-20 mL/min (6 mL/100 gm/min) which represents 8% of V_{O_2} of whole body at rest (normal: 250 mL/ min). Therefore, renal V_{O_2} is very high and ranked second to the heart (heart V_{O_2} at rest = 8-10 mL/100 gm/min).

3. Normal resting RBF amounts to ≥ 80% of the maximal flow possible; during maximum vasodilatation it increases to 1.5 times the resting level (as compared to skeletal muscle resting blood flow which is only 1/20th to 1/30th of the maximal).
4. Renal vascular circuit has very little basal tone.
5. $(A-V)$ O_2 difference in most body organs is inversely proportional to their blood flow, whereas $(A-V)$ O_2 difference in kidney does not change inspite of massive alteration in blood flow indicating that the V_{O_2} in kidneys varies in direct proportion with blood flow.
6. Blood flow and V_{O_2} within the kidneys is not homogenous because **cortical blood flow and V_{O_2} is more than that of medulla. (Table 55.2)**

Table 55.2 : Blood flow and oxygen consumption (Vo_2) within the kidney

	Cortex	Outer medulla	Inner medulla
1. Blood flow mg/gm of tissue/min	5	2.5	0.6
2. Percent of total RBF	90-92%	7-10%	1-2%
3. Vo_2 (mL/100 gm/min)	9	–	0.4
4. pO_2 (mmHg)	50	–	15

High cortical blood flow is due to the fact that it contains the major components of the nephron. **Low blood flow in inner medulla** is due to low velocity of blood traversing in the vasa recta which in turn is *due to*:

(i) Vasa recta length is 40 mm, this is about 40 times the length of an ideal systemic capillary which increases the resistance to blood flow.
(ii) Near hair pin bend in the inner most part of medulla, interstitium osmotic pressure is high (2000 mosm/L) which causes dehydration of blood, thus increases viscosity near the hair pin bend (for details, refer to page 544).
(iii) Low hydrostatic pressure head.

Slow medullary flow is of great *importance* for the development of hyperosmolarity of the inner medulla and hence for the production of hypertonic urine.

Important Note

The pO_2 of medulla is 15 mmHg, thus it is vulnerable to hypoxia if blood flow is further reduced. However, nitric oxide and prostaglandins (secreted by type I medullary interstitial cells)maintain the balance between low blood flow and metabolic needs.

7. *Autoregulation* of renal blood flow (RBF) and glomerular filtration rate (GFR) **(Fig. 55.8)**

(i) RBF shows ***efficient*** phenomenon of *autoregulation e.g.* in most organs increase in perfusion pressure is associated with a decrease in vascular resistance across the organ, therefore, blood flow increases; whereas in kidney there is an increase in resistance which parallels the increase in pressure and blood flow is unchanged.

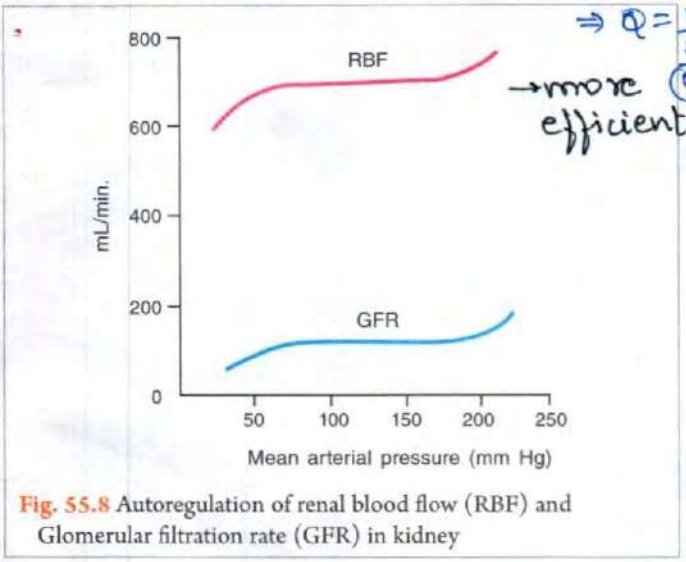


Fig. 55.8 Autoregulation of renal blood flow (RBF) and Glomerular filtration rate (GFR) in kidney

(ii) RBF and GFR remain remarkably constant despite the change in mean arterial BP between 90 and 220 mmHg (*Bayliss-Folkow*-1902).

(iii) *Autoregulation is a feature of only cortical blood flow*. Medullary blood flow does *not* show autoregulation. As medullary blood flow is only 8% of total RBF, the effect of BP on the RBF due to changes of medullary flow are marked.

(iv) At the perfusion pressure of 50 mmHg, RBF is 600 mL/min (200 mL/100 gm/min), *if perfusion pressure decrease < 50 mmHg the kidney* ***does not filter***. (Also refer to page 353).

(v) Renal autoregulation is present in denervated and in isolated perfused kidney; in the transplanted kidney after adrenal demedullation and in the absence of blood cells, but it is prevented by the administration of drugs that paralyse the vascular smooth muscle.

(vi) Autoregulation is *due to nitric oxide* and alteration in the *intrinsic myogenic tone* of afferent arteriolar vessels in response to changes in the perfusion pressure (page 315). How? The afferent arteriole has a larger diameter than the efferent arteriole and is major site of autoregulatory resistance. When resistance is altered in the afferent arterioles, RBF changes in the same direction, *e.g.* stretch of the afferent arterioles caused by a rise of pressure causes a contraction response of the smooth muscle of the arteriolar media and this reduction in calibre raises the resistance to blood flow.

(vii) At low perfusion pressure *angiotensin II* also plays a role by constricting the efferent arterioles, thus maintaining the GFR. That is why renal failure sometimes develops in patients with poor renal perfusion who are treated with drugs which inhibit angiotensin converting enzyme (page 506).

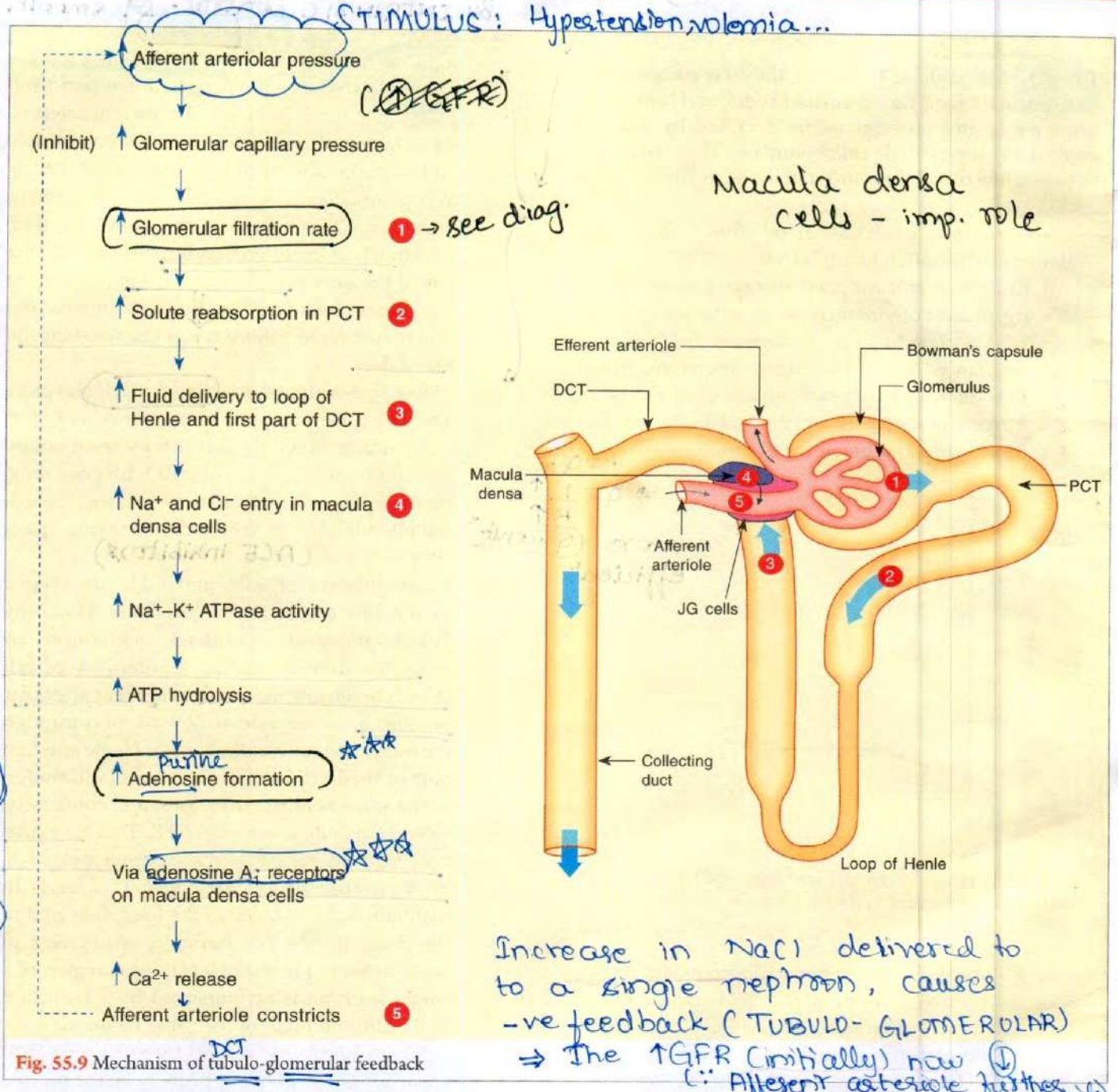
(viii) Autoregulation of RBF and GFR are related to the filtered load of salts, NaCl. How? By **Tubulo-glomerular Feedback** mechanism, *i.e.* a *negative feedback* relating the delivery of salt (NaCl) to the macula densa to afferent arteriolar resistance. As the rate of flow of fluid through the ascending limb of the loop of Henle and first part of the DCT increases, glomerular filtration in the same nephron decreases; and conversely, a decrease in flow increases GFR. *Thus the signals from the renal tubules in each nephron feedback to affect filtration in its glomerulus*. This tends to maintain the constancy of the load delivered to the distal tubule. For example, an increase in NaCl delivered to the macula densa region of a single nephron is accompanied by a reduction in the filtration rate of the same nephron.

Mechanism of Tubulo-glomerular Feedback **(Fig. 55.9)**

The ***sensor*** for the response appears to be the macula densa, and GFR is probably adjusted by constriction or dilatation of the afferent arteriole. How? The increased Na^+ (and Cl^-) that enters the macula densa cells in DCT casues increased Na^+–K^+ ATPase activity thereby resulting in increased ATP hydrolysis and finally more adenosine is formed. Adenosine acts via adenosine A_1 receptors on macula densa cells to increase the release of Ca^{2+}. This causes afferent arterioles vasoconstriction and a resultant decrease in GFR. Constriction may be mediated by thromboxane A_2, renin-angiotensin system, prostaglandin (PG) or cAMP. The component of the tubular fluid responsible for the feedback may be Cl^-, and the degree of constriction is

* Hormonal regulation by prostaglandins
* Posture

STIMULUS: Hypertension, volemia...



Increase in NaCl delivered to to a single nephron, causes -ve feedback (TUBULO-GLOMERULAR)
⇒ The ↑GFR (initially) now ↓ (∵ Afferent arteriole further constricts)

Fig. 55.9 Mechanism of tubulo-glomerular feedback

probably proportionate to the rate of Cl^- reabsorption across the macula densa. The *sensitivity of the feedback is increased* when ECFV is decreased and decreased when ECFV is expanded. (Also refer to page 525, *Glomerulo-tubular balance*).

8. ***RBF, Renal V_{O_2} and tubular reabsorption of sodium***

The kidneys are unique in that changes in RBF are accompanied by parallel changes in renal V_{O_2}. The renal function with which the renal V_{O_2} correlates best is the rate of active transport of Na^+ because the largest chemical energy expenditure of the kidneys (renal V_{O_2}) is required to reabsorb the filtered load of sodium.

As the filtered load of sodium to be reabsorbed at any one $[Na^+]$ in the plasma is determined by the GFR, it follows that renal V_{O_2} *is linearly related to the active reabsorption of* Na^+. A decrease in RBF is usually associated with a decrease in GFR, which leads to a decrease in the filtered load of NaCl to be reabsorbed. Thus, there exists a linear relationship between renal V_{O_2} and RBF above perfusion pressure of 50 mmHg; this also holds true for GFR.

The kidney is ***unique*** in that the blood flow, hence GFR and hence filtered load of sodium determines renal metabolism (compared to skeletal or cardiac muscle, metabolism determines the blood flow).

• Extraction ratio; Eff. & Actual Renal plasma flow; (ER)

9. *RBF can be measured with:*
   (i) *flow meters e.g.* electromagnetic flow meter;
   (ii) *by applying the Fick's principle* to the kidneys *i.e.* by measuring the amount of a given substance taken up per unit of time and dividing it by the arterio-venous difference for the substance across the kidney.

• Para amino hippuric acid. → ER = 0.9
• Inulin → To measure Renal Plasma flow (NOT GFR)

* Effective Renal plasma Flow (ERPF)

= Urine conc. of PAHA (U) × Vol. of bl. flow (V)
Plasma conc. of PAHA (P)

* ARPF = 630 mL / 0.9 (E.R) = 700

* Renal Blood flow = Renal plasma flow × 1/(1-Hct)
= 1273 mL/min

$$ERPF = \frac{U \times V}{P}$$

$$C_x = \frac{U_x \times V}{P_x}$$

$$BV = \frac{PV}{(1-Hct)}$$

Study Questions

1. Write short notes on:
   (i) Juxta glomerular apparatus
   (ii) Renin angiotensin system
   (iii) Actions of angiotensin II
   (iv) Regulation of renin secretion
   (v) Functions of the kidney
   (vi) Peculiarities of renal circulation
   (vii) Autoregulation of renal blood flow
   (viii) Tubuloglomerular feedback.
   (ix) Two types of nephrons in the kidney
   (x) Peritubular capillary plexus and vasa recta
   (xi) Malpighian corpuscle
   (xii) Innervation of renal vessels
   (xiii) Factor affecting blood supply to kidneys
2. Draw well labelled diagram of:
   (i) Electron microscopic structure of glomerular membrane
   (ii) Juxtaglomerular apparatus.
3. Give and explain the perfusion pressure at which kidney does not filter?
4. Give physiological basis of:
   (i) PCT has a high rate of oxygen consumption
   (ii) High pressure in glomerular capillaries
   (iii) High resistance is offered to blood flow in afferent arteriole
   (iv) Medullary blood flow does not show autoregulation
   (v) Renal failure develops in persons with poor renal perfusion.
   (vi) Cortical blood flow and oxygen consumption is more than that of medulla.
5. Give physiological significance of
   (i) Ultrafiltrate
   (ii) P and I-cells in collecting tubules
   (iii) Macula dense
   (iv) Rena-renal reflex
   (v) Tubuloglomerular feedback
6. Justify, one major function of kidney is "Excretion of waste products".

MCQs

1. *True* about glomerular membrane:
   (a) A closely applied membrane to the loop of Henle
   (b) A closely applied membrane to Bowman's capsule proper
   (c) Comprises visceral and parietal layers of Bowman's capsule
   (d) Structures intervening the blood within the capillary loop and Bowman's space
2. Capillary pressure in renal glomeruli is:
   (a) Lower than pressure in efferent arterioles
   (b) Increases when afferent arterioles constricts
   (c) Higher than the most other capillaries in the body
   (d) Fall by approx. 10% when BP decreases by 10% from normal level
3. Normal kidney *does not* allow passage of:
   (a) Substances >8 nm in diameter
   (b) Lysozyme
   (c) IgG
   (d) Albumin

4. *Not* a feature of proximal convoluted tubule:
   (a) Tubular cells have brush border and are rich in mitochondria
   (b) Responsible for active reabsorption of 80% sodium filtered
   (c) Low rate of O_2 consumption
   (d) Cells at the apex are united by tight junction
5. Juxta glomerular cells are located in:
   (a) Afferent arteriole (b) Efferent arteriole
   (c) Distal convoluted tubule (d) Glomerular tuft
6. Cortical nephrons differ from juxtamedullary nephrons in all *except*:
   (a) Smaller size glomeruli (b) Rate of filtration is slow
   (c) Play a major role in excretion of waste (d) Filtration at glomeruli occurs under pressure
7. Which is *true* regarding renin secretion:
   (a) Increased K^+ in PCT increases renin secretion (b) Decreased Na^+ in DCT increases renin secretion
   (c) Inversely proportional to the potassium levels (d) Directly proportional to the ADH levels
8. Most potent endogenous vasopressor is:
   (a) Aldosterone (b) Nor-epinephrine (c) Angiotensin II (d) Cortisol
9. Kidneys *cannot* concentrate urine more than ..... times the plasma osmolar concentration:
   (a) 2-3 (b) 4-5 (c) 6-7 (d) 8-9
10. Glomerular capillaries exhibit higher pressure than that in other body capillary bed because:
   (a) Afferent arterioles are major site of autoregulatory resistance
   (b) Efferent arterioles have relatively high resistances
   (c) There are two sets of capillaries in the kidney
   (d) All of the above
11. *Not true* of blood supply to renal tubules:
   (a) Glomerular capillaries are the only capillaries in the body that drains into arterioles
   (b) Tubules of each nephron receive blood supply only from the efferent arteriole of that nephron
   (c) Total surface area of renal capillaries is equal to the total surface area of the tubules
   (d) Pressure in renal vein is about 4 mmHg
12. The volume of blood in the renal capillaries at any given time is:
   (a) 30-40 mL (b) 70-100 mL
   (c) 100-300 mL (d) 300-450 mL
13. Action of renal nerves on urine formation is limited to their effect on:
   (a) Release of angiotensin (b) Pressure and flow of blood through the kidney
   (c) Reabsorption of glucose (d) Reabsorption of sodium by the tubules
14. A-V O_2 difference in kidney is:
   (a) 1.5 mL/dL (b) 5 mL/dL
   (c) 10 mL/dL (d) 15 mL/dL
15. *True* statement regarding oxygen consumption in the kidney is:
   (a) Oxygen consumption increases as blood flow increases
   (b) O_2 consumption is maximum in renal medulla
   (c) O_2 consumption in mL/min is maximum as compared to any organ in the body
   (d) Consumption is equal in both cortex and medulla
16. Normal distribution of percentage of total renal blood flow to cortex, outer and inner medulla respectively is:
   (a) 90-92%; 7-10%; 1-2% (b) 80%; 18%; 2%
   (c) 80%; 1-2%; 17-18% (d) 7-10%; 80%; 1-2%
17. Kidney *does not* filter if the perfusion pressure falls to:
   (a) 100 mmHg (b) 90 mmHg
   (c) 70 mmHg (d) 50 mmHg
18. *Not* a *true* statement regarding autoregulation of renal blood flow:
   (a) Remarkably constant between mean BP of 90 to 220 mmHg
   (b) Feature of only cortical blood flow
   (c) Also present in denervated kidney
   (d) Angiotensin II plays a major role

19. *Not* a mechanism of tubulo-glomerular feedback:
    (a) Sensor for the response is macula densa
    (b) GFR is adjusted by alteration in diameter of efferent arteriole
    (c) Cl^- is probably responsible for the feedback
    (d) Sensitivity of feedback is increased when ECFV is decreased
20. Hydrostatic pressure is highest in capillaries of:
    (a) Brain (b) Kidney
    (c) Liver (d) Lungs
21. Normal glomerular capillary pressure is ..... mmHg:
    (a) 15 (b) 25
    (c) 35 (d) 45
22. Juxta glomerular apparatus (JGA) comprises of all of the following *except*:
    (a) Juxtaglomerular cells (b) Macula densa cells
    (c) Lacis cells (d) Principal cells
23. Juxta-medullary nephrons in kidney are what percentage of total nephrons?
    (a) 15 (b) 30
    (c) 60 (d) 80
24. Macula densa cells get stimulated by:
    (a) Hypovolemia (b) Decreased K^+
    (c) Decreased Na^+ (d) Alteration in transmural pressure
25. What percentage of glomerular filtrate is normally reabsorbed?
    (a) 1% (b) 10%
    (c) 80% (d) 99%
26. Increased amounts of erythropoietin might be released from the kidneys *except* when the:
    (a) Arterial pO_2 is normal and the arterial O_2 content is reduced
    (b) Arterial pO_2 and arterial O_2 content are reduced
    (c) Arterial pO_2 is low and the saturation of haemoglobin with oxygen is much reduced
    (d) Tissue pO_2 and renal blood flow are both increased
27. Normal peritubular capillaries pressure is:
    (a) 4 mmHg (b) 8 mmHg
    (c) 25 mmHg (d) 45 mmHg
28. The renal function with which the renal O_2 consumption correlates best is:
    (a) Rate of active transport of Na^+ (b) A-V O_2 difference across the kidney
    (c) Renal blood flow (d) GFR

Answers

1. (d)	2. (c)	3. (a)	4. (c)	5. (a)	6. (d)	7. (b)	8. (c)	9. (b)	10. (a)
11. (b)	12. (b)	13. (b)	14. (a)	15. (a)	16. (a)	17. (d)	18. (d)	19. (b)	20. (b)
21. (d)	22. (d)	23. (a)	24. (c)	25. (d)	26. (d)	27. (b)	28. (a)		

GFR: Rate at which plasma is filtered by all nephrons of BOTH KIDNEYS PER MINUTE

Chapter 56

Mechanism of Formation of Urine

I. Introduction
II. Glomerular Filtration
  A. Glomerular filtration Rate (GFR): Definition; Normal value; Mechanism and factors affecting.
  B. Glomerular filtration versus systemic filtration. C. Filtration Fraction (FF).
III. Reabsorption and secretion in renal tubules or Renal tubular transport
  A. General considerations.
  B. Overview of renal transport mechanisms throughout the tubular segments.
  C. Transport of individual substances in different segments of the renal tubule.

INTRODUCTION

The constancy of body internal environment is maintained, in large part, by the continuous functioning of its about 2.6 million nephrons. As blood passes through the kidneys, the nephrons clear the plasma of some substances *e.g.* urea, while simultaneously retaining other essential substances such as water.

1. Substances to be excreted are removed by ***Glomerular Filtration*** and ***Renal Tubular Secretion*** and passed into the urine (Fig. 56.1).
2. Substances that the body needs are retained by *Renal Tubular Absorption e.g.* Na^+, HCO_3^- and returned to the body by ***Reabsorptive Processes***.

(Also refer to pages 519–520)

Concentration of urine + Acidification — Plasma filtration

GLOMERULAR FILTRATION

Filtration is the bulk transport of a fluid with its dissolved small solutes across a membrane. ***Glomerular filtration*** is the initial step in urine formation, the plasma that traverses the glomerular capillaries is filtered by the highly permeable glomerular membrane and the resultant fluid, the *glomerular filtrate*, is passed into Bowman's capsule.

Normal Renal blood flow (RBF):
= 1.2 to 1.3 L/min (about 20-25% of resting cardiac output)

Approx. 1/10th of RBF is filtered off into Bowman's capsule as the blood passes through the glomeruli, the filtrate is identical with plasma in respect of following:

(1) osmolality, pH
(2) electrical conductivity
(3) concentration of electrolytes and of smaller organic molecules, *e.g.* glucose, urea and creatinine.

Therefore, *glomerular filtrate* is called the ***Ultrafiltrate of plasma*** (page 503). It practically contains no protein and no cells.

A. GLOMERULAR FILTRATION RATE (GFR)

Definition – GFR refers to the volume of the glomerular filtrate formed each minute by all the nephrons in both the kidneys.

Normal value: 125 mL/min = 170–180 L/day. GFR value in females is 10% lower than those in males.

At a rate of 125 mL/min the kidneys filter, in one day, the amount of fluid:

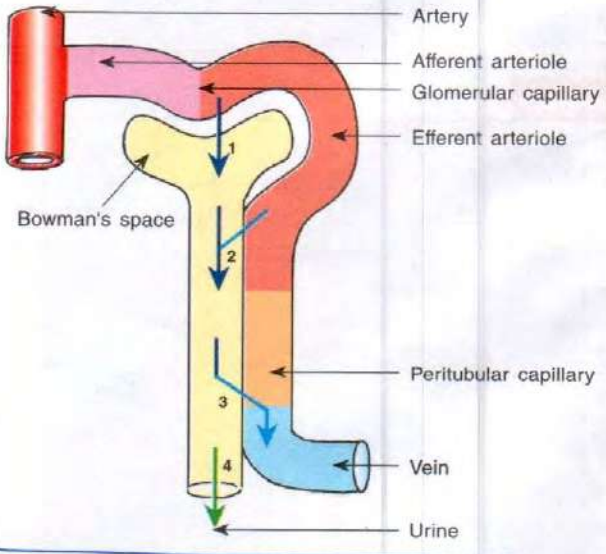


Fig. 56.1 Filtration, secretion and reabsorption in kidney
Amount Excreted (4) = Amount filtered (1) + Amount secreted (2) – Amount reabsorbed (3)

= 4 times the total body water
= 15 times ECFV
= 60 times the plasma volume

(III) Mechanism of glomerular filtration (Fig. 56.2)

The mechanism of filtration across the glomerular capillaries is the same as the mechanism governing the filtration across all other body capillaries (page 53) *i.e.*

(i) the hydrostatic pressure gradient across the capillary wall
(ii) the osmotic pressure gradient across the capillary wall
(iii) the permeability of the capillaries; and
(iv) the size of the capillary bed.

Therefore, for each nephron, *effective filtration pressure* (EFP):

$$EFP = (P_{Cap} - P_{Bow}) - (COP_{Cap} - COP_{Bow})$$

where,

P_{Cap} : Hydrostatic pressure in the glomerular capillaries = 45 mmHg. (Higher than that in other capillaries – Why? page 508). It constitutes a filtration force driving fluid out of the glomerular capillaries and into Bowman's capsule.

P_{Bow} : Hydrostatic pressure in the Bowman's capsule = 10 mmHg. It opposes filtration.

COP_{Cap} : Colloidal osmotic pressure (COP) of the plasma in the glomerular capillaries bringing fluid into glomerular capillaries = 25 mmHg.

COP_{Bow} : COP of the filtrate in the Bowman's capsule, normally it is 'zero', because glomerular filtrate contains no proteins.

$$GFR = K_f \times EFP = K_f \times ([P_{GL} - P_{BC}] - [\pi_{GL} - \pi_{BC}])$$

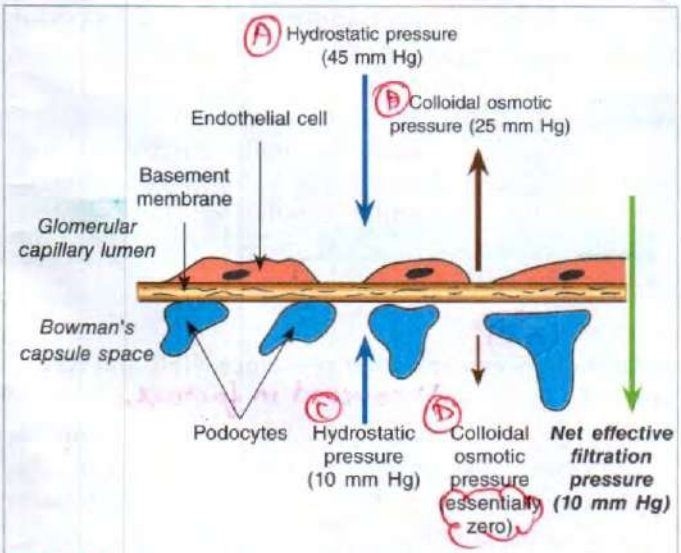


Fig. 56.2 A diagrammatic representation of mechanism of glomerular filtration.
(**Note**: Length of the arrows denotes magnitude and direction of the force.)

where,

K_f : *Filtration coefficient* of the glomerular membrane. The glomerular barrier consists the capillary endothelium, basement membrane and filtration slit of the podocytes. Thus 'kf' is a function of the capillary surface area and the membrane permeability. In both the kidneys GFR at 1 mmHg EFP is called 'K_f'. Normally K_f = 12.5 mL/min/mmHg.

Therefore, GFR = 12.5 [(45–10) – (25–0)]
= 12.5 × 10
= 125 mL/min.

(IV) Factors affecting glomerular filtration rate (GFR)

1. GFR decreases with *advancing age* due to decrease in renal plasma flow (RPF), cardiac output (CO) and renal tissue mass.
2. GFR changes in linearity with *changes in the RBF, i.e.* GFR is directly proportional to RBF.
3. *Changes in hydrostatic pressure in the glomerular capillaries* (P_{Cap}).
   (i) Changes in systemic BP
   P_{Cap} is directly proportional to systemic BP when mean systemic arterial BP falls below 90 mmHg or rises above 220 mmHg. (As between 90 to 220 mmHg MBP, changes in renal vascular resistance due to autoregulation tend to stabilize filtration pressure, page 511).
   (ii) *Afferent or efferent arteriolar constriction*. Constriction of afferent arteriole reduces both the RPF and GFR whereas efferent arteriole constriction reduces RPF but increases GFR (page 519). However, the GFR tends to be maintained when efferent arteriolar constriction is greater than afferent arteriolar constriction.
4. *Changes in hydrostatic pressure in Bowman's capsule* (P_{Bow}).
   (i) Ureteral obstruction (e.g. Renal stones) → increases P_{Bow} → decreases GFR.
   (ii) Oedema of kidney inside tight renal capsule → increases P_{Bow} → decreases GFR.
5. *Changes in the concentration of plasma proteins.*
   (i) Hypoproteinemia → decreases COP_{Cap} → increases GFR.
   (ii) Dehydration (or ↓ RBF) → increases COP_{Cap} → decreases GFR.
6. *State of glomerular membrane* (filtering membrane).
   The permeability of the glomerular membrane is approx. 50 times that of the capillaries in the skeletal muscle.
   Normally,
   (i) glomerular membrane is absolutely impermeable to molecules more than 4 nm in diameter or molecular weight (MW) above 70,000

(ii) ratio of concentration of crystalloid in the filtrate to their concentration in plasma is unity, whereas

(iii) ratio of concentration of haemoglobin (MW 68000) in the filtrate to their concentration in plasma is 0.03; and

(iv) ratio of concentration of albumin (MW 70000) in the filtrate to their concentrartion in plasma is <0.01.

Sialoproteins present in the wall of glomerular membrane are negatively charged with the result filtration of cationic substances is slightly greater than that of the neutral or anionic substances of the same size. *(Note: circulating albumin is negatively charged with an average diameter of about 7 nm).*

Important Note

In diseases, the negative charges in the glomerular membrane disappear and its permeability increases, this result in filtration of large amounts of albumin and other large molecules.

7. *Size of the capillary bed*

Contraction of mesangial cells (page 505) tends to distort and encroach on the capillary lumen thus, there is decrease in the area available for filtration **(Table 56.1)**. This decreases K_f (filtration co-efficient, page 517) which then decreases GFR.

Table 56.1: Agents causing contraction or relaxation of mesangial cells

Contraction → ↓ GFR	Relaxation → ↑ GFR
Angiotensin II (important regulator)	ANP (Atrial natriuretic peptide) (page 559)
ADH	Cyclic AMP
Endothelins	Dopamine
Histamine	PGE_2
Leukotrienes C and D	
Nor-epinephrine (NE)	
PGF_2 (prostaglandin)	
Platelet-activating factor	
Platelet-derived growth factor	
Thromboxane A_2	

↓: decrease; ↑: increase (**Note**: There are angiotensin II receptors in the glomeruli)

B. GLOMERULAR FILTRATION VERSUS SYSTEMIC FILTRATION

Refer to **Table 56.2**.

Table 56.2: glomerular filtration versus systemic filtration

Glomerular Filtration	Systemic Filtration
1. *Capillary exchange rate* Total glomerular capillary exchange area: Approximately 0.5–1.5 m^2/100gm of renal tissue, of which 2–3% is available for filtration. Therefore, filtration surface measures 320–480 cm^2.	Total exchange area of systemic capillary bed: 1000 m^2, if all the systemic capillaries are open. At rest only 25% of systemic capillaries are open at any time.
2. *Filtration rate* GFR *i.e.* fluid filtered from glomerular capillaries, is 180L/day which far exceeds the filtration from systemic capillaries. It has been found that filtration coefficient of the glomerulus is 50–100 times greater than that of a muscle capillary.	Fluid filtered from systemic capillaries: approx. 20L/day; of these 16-18L/day are reabsorbed and remaining 2-4 L/day represent lymph flow.

C. FILTRATION FRACTION: FF

It is the fraction of the plasma passing through kidneys which is filtered at the glomerulus *i.e.* the ratio of glomerular filtration rate (GFR) to renal plasma flow (RPF).

Therefore,

$$FF = \frac{GFR\ (mL/min)}{RPF\ (mL/min)} = \frac{125\ mL/min}{700\ mL/min}$$

$$= 0.16 - 0.20 \text{ } i.e. \text{ } 16\text{-}20\% \text{ of RPF}$$

Significance: Increase in FF produces increase in the protein concentration of peritubular capillaries blood which leads to increased reabsorption in the PCT (opposite happen when FF decrease)

Note

In healthy individuals, normally 16–20% of the plasma flowing through the kidneys gets filtered through the glomerular capillaries. The filtration fraction gets altered by any of the factors affecting either GFR or RPF or both)

Relation between arteriolar resistance, GFR and RPF (Table 56.3)

1. *Constriction of a vessel:* If flow is constant, vasoconstriction results in an increase in hydrostatic pressure proximally (P_1) and a fall in hydrostatic pressure distally (P_2).

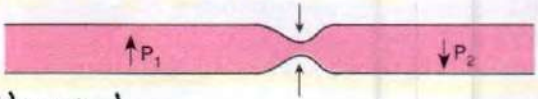
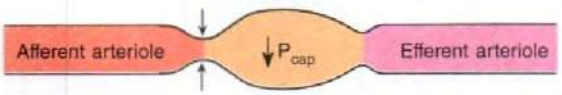


Table 56.3: Effects of changes in renal vascular resistance with constant renal perfusion

	Renal arteriolar vascular resistance		RPF (mL/min)	GFR (mL/min)	Filtration Fraction (GFR/RPF)
	Afferent	Efferent			
1.	↑	–	↓	↓	No change
2.	↓	–	↑	↑	No change
3.	–	↑	↓	↑	Increases (seen in glomerulonephritis; and hypotension which causes efferent arteriolar constriction)
4.	–	↓	↑	↓	Decreases (seen in essential hypertension)

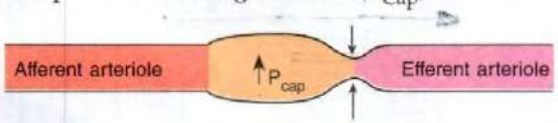
2. *Constriction of afferent arteriole* reduces both the RPF and GFR without affecting FF
   (i) ↓ RPF is due to ↑ resistance proximal to glomeruli, and
   (ii) ↓ GFR is due to ↓ hydrostatic pressure within capillaries of the glomeruli (P_{Cap}).



Note

Opposite happens when afferent arterioles dilates.

3. *Constriction of efferent arteriole* reduces RPF but increases GFR, leading to an increase in FF
   (i) ↓ RPF is due to ↑ resistance distal to glomeruli; and
   (ii) ↑ GFR is due to ↑ hydrostatic pressure within capillaries of the glomeruli (P_{Cap}).



Note

Opposite happens with dilatation of efferent arterioles.

3 REABSORPTION AND SECRETION IN RENAL TUBULES

A. GENERAL CONSIDERATIONS

1. The terms, *Renal tubular secretion* and *Renal tubular reabsorption* refer to the 'direction of transport', not to differences in the underlying mechanisms of transport, therefore,
   (i) *Secretion* refers to the transport of solutes (e.g. K^+, H^+) from the peritubular capillaries into the tubular lumen, *i.e.* it is the addition of a substance to the filtrate.
   (ii) *Reabsorption* denotes the active transport of solutes and the passive movement of water from the tubular lumen into the peritubular capillaries *i.e.* it is the removal of a substance from the filtrate (e.g. Na^+ reabsorption from PCT).
2. ***The Filtered Load*** *i.e.* the amount of solute transported across the glomerular membranes per unit time. Mathematically, filtered load
   = GFR × plasma concentration of the solute (P_x)
   = mL/min × mg/mL
   = mg/min.
3. ***The Excretion Rate.*** It is the amount of a substance that appears in the urine per unit time. Mathematically, excretion rate
   = urine flow rate (V) × urine concentration of the substance (U_x)
   = $V\ U_x$
   = mL/min × mg/mL
   = mg/min.
   (i) *Secretion:* if the excretion rate exceeds the filtered load, net tubular secretion of that substance has occurred.
   (ii) *Reabsorption:* if the filtered load exceeds the excretion rate, net reabsorption of that substance has occurred.
4. ***Renal Tubular Transport Maximum (T_m)***
   It refers to the maximal amount of a given solute that can be transported (reabsorbed or secreted) per minute by the renal tubules. The limit is due to saturation of the specific transport systems involved.
   (i) The highest attainable rate of *reabsorption* is called the ***maximum tubular reabsorptive capacity*** and is designated 'Tr' (or T_m). *Substances that are reabsorbed by an active carrier mediated process and that have a T_m* include: phosphate ion (HPO_4^{2-}), Sulphate (SO_4^{2-}), glucose, amino-acids, uric acid, albumin, acetoacetate, β-hydroxybutyrate and α-ketoglutarate.
   (ii) The highest attainable rate of *secretion* is called the maximum ***tubular secretory capacity*** and is

designated T_s (or T_m). *Substances that are secreted by the kidneys and have a T_m* include: penicillin, certain diuretics, salicylate, para-amino hippuric acid (PAH) and thiamine (Vitamin B_1).

Calculation of Tr or Ts: Transport maximum is the difference between filtered load and excretion rate.

(i) *Exceptions*

(a) Uric acid (organic substance) and K^+ (inorganic cation) are both reabsorbed and secreted by the kidney.

(b) Some solutes have no definite upper limit for unidirectional transport, and, hence, have no transport maximum because their rate of transport is determined by the electrochemical gradient and renal tubular flow rate. Examples:

(i) the reabsorption of Na^+ along the nephron has no transport maximum; and

(ii) the secretion of K^+ by the DCT has no transport maximum.

(c) *The threshold concentration* *i.e.* the plasma concentration at which a solute begins to appear in urine. It is characteristic for the substance.

5. Reabsorption and excretion of various substances by the kidney in a normal adult human (see to table below).

Substance	Plasma conc. (mg/dL)	Reabsorbed per day	Excreted per day
Na^+	330	98%	2%
K^+	17	94%	6%
Glucose	100	100%	Nil
HCO_3^-	150	100%	Nil
Cl^-	365	98%	2%
Urea	30	53%	47%
Uric acid	4	98%	2%
Ca^{2+}	10	98.8%	1.2%
Phosphate	3	76.5%	23.5%
Total solute	-	99%	1%
Water	-	88–90%	10–12%*

Note

* In the presence of maximal ADH effect, more than 99% of the water is reabsorbed.

B. OVERVIEW OF RENAL TRANSPORT MECHANISMS THROUGHOUT THE TUBULAR SEGMENTS OF THE NEPHRON (Table 56.4).

1. *Pumps* represent *'primary active transport processes'* (page 18). They directly use the energy obtained from the hydrolysis of adenosine triphosphate (ATP) to transport material against an energy (*e.g.* concentration, electrical) gradient. For example Na^+ transport through the basolateral membrane of PCT cells is mediated principally by the $Na^+ - K^+$ ATPase pump, which transports Na^+ against an electrochemical potential from the cell interior and maintains a low intracellular Na^+ concentration (Figs. 56.3 and 56.4).

2. *Carriers* represent *'facilitated diffusion'* (page 15), which involves the passive transport of a substance by a protein carrier from a region of higher concentration to a region of lower concentration. This provides energy for active (uphill) transport of other solutes (*e.g.* glucose, amino acids). Therefore, also called *carrier mediated (secondary active) transport* (page 19).

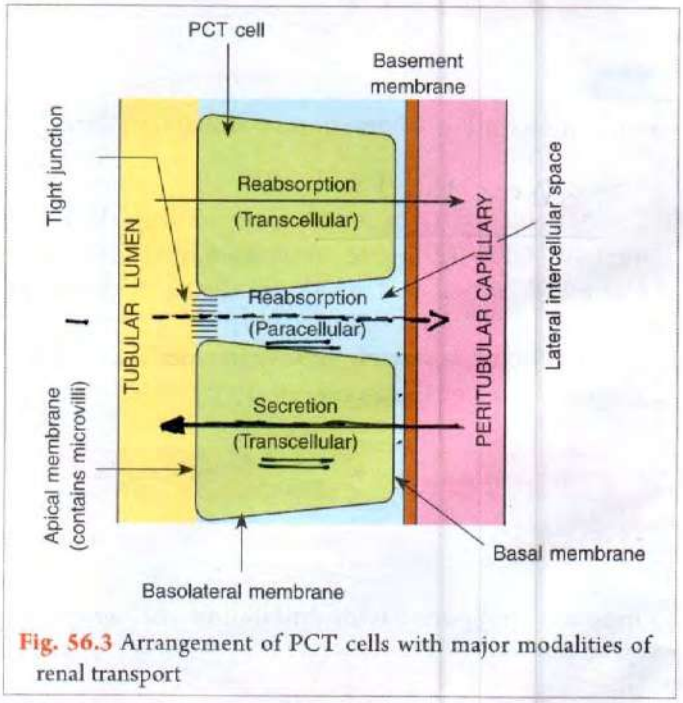


Fig. 56.3 Arrangement of PCT cells with major modalities of renal transport

Table 56.4: Various transport mechanisms that exist in a nephron

Pumps	Carriers		Channels
	Symporters	Antiporters	
$3Na^+ - 2K^+ - ATPase$	$Na^+ -$ Glucose	$Na^+ - H^+$	Na^+
$3H^+ - ATPase$	$Na^+ -$ Amino Acid	$Na^+ - NH_4^+$	K^+
$H^+ - K^+ - ATPase$	$2Na^+ - HPO_4^{2-}$	$Na^+ - Ca^{2+}$	Cl^-
$Ca^{2+} - ATPase$	$Na^+ - 3HCO_3^-$	$Cl^- - HCO_3^-$	Ca^{2+}
	$Na^+ - 2Cl^- - K^+$		
	$K^+ - Cl^-$		

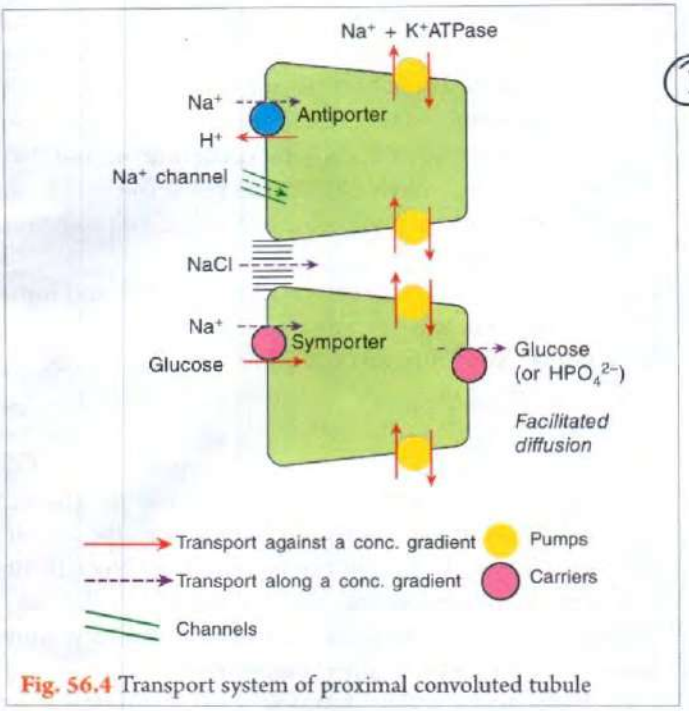


Fig. 56.4 Transport system of proximal convoluted tubule

Carrier types (**Fig. 2.6**, page 20)

(i) ***Uniporters*** are carriers that transport a single particle in one direction, *e.g.* facilitated diffusion of glucose.

(ii) ***Symporters (Cotransport)*** denotes the transport of two substances by a protein carrier in the 'same direction'.

(iii) ***Antiporters (Counter transport)*** defines the transport of two substances by a protein carrier in 'opposite directions'.

3. ***Channels*** represent *diffusion* (page 15). It is a passive process by which uncharged particles in solution flow down their concentration (chemical) gradient (*i.e.* particles move from areas of high concentration to areas of lower concentration) For example a diffusion mechanism is available for Na^+ to pass through the tight junction of PCT cells in association with Cl^-.

4. ***Transepithelial Transport*** (Refer to **Fig. 56.3**)

The differences between apical and basolateral membrane properties account for the transepithelial transport of all solutes, which can occur via two pathways:

(i) The ***Transcellular Pathway*** (*i.e.* across the cell), is used for *Active transepithelial transport*. Approx. 2/3rd of the Na^+ transport is active and transcellular.

(ii) The ***Paracellular (Intercellular) Pathway***, which follows the lateral intercellular spaces, is used for *passive transepithelial transport*. Approx. 1/3rd of NaCl transport is passive and paracellular.

C. TRANSPORT OF INDIVIDUAL SUBSTANCES IN DIFFERENT SEGMENTS OF THE RENAL TUBULE

General

1. *The PCT*

(i) *Reabsorbs:* 70% – 85% of the filtered Na^+; Cl^-; HCO_3^- and water; and almost 100% of the filtered K^+; HPO_4^{2-}; amino acids and glucose

Note

Reabsorption of water is passive, and reabsorption of solutes can be passive or active; solute reabsorption generates an osmotic gradient, which causes the passive reabsorption of water (osmosis).

(ii) *Secretes:* H^+, end products of metabolism (organic acids and bases such as PAH, bile salts, oxalate, urate, catecholamine) and potentially harmful drugs or toxins such as penicillin and salicylates.

2. *Loop of Henle*

(i) *Thin descending segment:* highly permeable to water but relatively impermeable to solids (specially Nacl).

(ii) *Thin ascending segment:* impermeable to water but permeable to Nacl and urea; causes passive reabsorption of Nacl.

(iii) *Thick ascending segment:* impermeable to water and solute but actively reabsorbs Nacl by $Na^+ - K^+$ pump and $Na^+ - 2Cl^- - K^+$ symporter (page 545)

In passing through the loop of Henle, of the total filtered load: 5–10% of water gets reabsorbed (mainly from the thin descending limb) and 20–25% of Na^+, Cl^- and K^+ are reabsorbed (mostly in the thick ascending limb).

3. *DCT and CT*

(i) *Reabsorbs:* 5–20% of the filtered Na^+, Cl^-, HCO_3^- and water

(ii) *Secretes:* K^+ or H^+ via competitive processes (this involves the exchange of Na^+ and HCO_3^- (pages 524 and 526)

Note

In the terminal DCT and CT, urea along with water is passively reabsorbed under the influence of ADH (page 545).

D. GLUCOSE REABSORPTION

1. Glucose is filtered at a rate of approx. 100 mg/min. (Filtered load of glucose) *How?*

Resting plasma level of glucose × GFR = 80 mg/dL × 125 mL/min. Essentially all (100%) of the glucose

is actively reabsorbed into the PCT provided its concentration in the plasma is normal.

2. The glucose reabsorption and excretion processes are function of the *plasma glucose concentration (P_G). How?* **(Fig. 56.5)**
   (i) Increasing the P_G results in progressive linear increase in the *filtered load* of glucose.
   (ii) At low P_G, the reabsorption of glucose is complete (100%), hence no glucose is excreted in urine.
   (iii) When P_G increases above 180-200 mg/ dL, the glucose reabsorption is not complete and it passes out in urine (***Glycosuria***). This plasma glucose level at which glucose first appears in urine is called *Renal Threshold for glucose*. The actual renal threshold is:
   * 200 mg/dL of arterial plasma, and
   * 180 mg/dL of venous plasma (because 20 mg/dL gets utilized while passing through tissues). (Also see to **Table 56.5**)

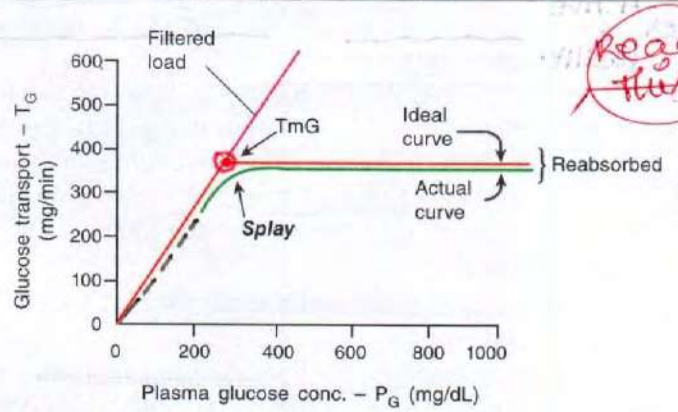
   (iv) As the *'reabsorptive limit of tubule' (Tr)* (page 519) is approached, the urinary excretion rate increases linearly with increasing P_G. *Why?*
      (a) When the Tr is reached, the amount of glucose reabsorbed per minute remains constant and is independent of P_G.
      (b) When the Tr is exceeded, the amount of glucose that passes in the urine increases linearly with P_G, the limit is referred to as *Tubular Maximum for Glucose (TmG)* and the reabsorption of glucose as a *Tm-limited process*.
   (v) The TmG is approx. 375 mg/min in men and 300 mg/min in women. Thus, the renal threshold *i.e.* P_G above which glycosuria occurs can be predicted from TmG. *How?*
   Normally, when glucose is filtered @ 100 mg/min, P_G is 80 mg/dL,
   if glucose is filtered @ 375 mg/min, P_G will be

$$\frac{80 \times 375}{100} = 300 \text{ mg/dL}$$

   Therefore, the ***predicted*** *renal threshold for glucose* would be approx. 300 mg/dL. However, the ***actual*** *renal threshold for glucose* is about 200 mg/dL of arterial plasma or 180 mg/dL of venous plasma.

This is so because the relation between P_G and T_G would have been obtained as ***ideal curve*** **(Fig. 56.5)**, if –
   (i) TmG in all the tubules were identical *i.e.* 375 mg/min, and
   (ii) all the glucose were removed from each tubule when the amount filtered was below the TmG.

However, ***actual curve*** obtained is rounded rather than sharply angulated and deviates considerably from the ideal curve. This deviation is called *'SPLAY'*.



IDEALLY:

P_G (mg/dL)	Glucose filtered (mg/min) (P_G × GFR/100)
80	100
100	125
200	250
300	375
400	500
500	625
600	750

Fig. 56.5 Relation between the plasma glucose concentration (P_G) and glucose transport

Table 56.5: Factors affecting renal threshold for glucose

Increases	Decreases
1. Old Age → Blood vessels sclerosis → ↓ s RBF → Mild ↓ GFR, therefore, less glucose is filtered.	Renal diabetes or Renal glycosuria. Here TmG is low (because of kidney diseases), therefore, even with low blood glucose, glycosuria occurs.
2. Extreme diabetes mellitus → ↑ glucose is filtered → (i) glycosuria (ii) osmotic diuresis → ↑ Na^+ excretion → ↑ Na^+ concentration within tubules → ↑ renal threshold for glucose.	

↑ : increase; ↓ : decrease

It represents the secretion of glucose in urine before saturation of reabsorption (TmG) is fully achieved. It is due to two reasons:

(1) Not all the 2.6 million nephrons in the kidney have exactly the same TmG or filtration rate. In some, TmG is exceeded at low level of P_G.

(2) Some glucose escapes reabsorption when the amount filtered is below the TmG because the reactions involved in glucose transport are not completely irreversible.

The degree of rounding is inversely proportional to the efficacy with which the transport mechanism binds the substance it transports.

Important Note

The plasma glucose of a healthy subject almost never becomes high enough (even after a meal) to cause glucose excretion in the urine. However, in uncontrolled diabetes mellitus plasma glucose rise to high levels, causing the filtered load of glucose to exceed the TmG; Thus resulting in glycosuria.

Mechanism of glucose reabsorption

Glucose reabsorption in the kidneys is similar to its absorption as in the GIT (page 260). Glucose and Na^+ bind to a common carrier (or symport *i.e. SGLT*-2) in the luminal membrane, and glucose is carried into the cell as Na^+ moves down its electrical and chemical gradient (Fig. 56.6). The Na^+ is then pumped out of the cell into the lateral intercellular spaces, and the glucose moves into the peritubular capillaries via GLUT-2 by simple diffusion. Thus, glucose transport across PCT cells is an example of *secondary active transport* (page 18); the energy for the active transport is provided by the $Na^+ - K^+$ ATPase that pumps the Na^+ out of the cell. 100% of the filtered glucose is reabsorbed in the PCT.

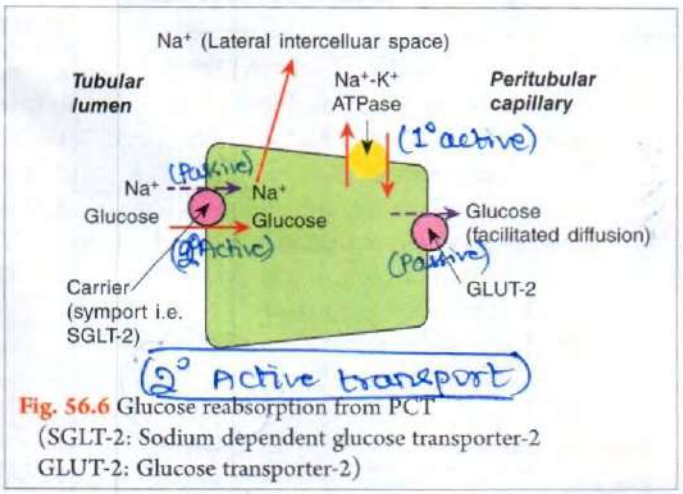


Fig. 56.6 Glucose reabsorption from PCT
(SGLT-2: Sodium dependent glucose transporter-2
GLUT-2: Glucose transporter-2)

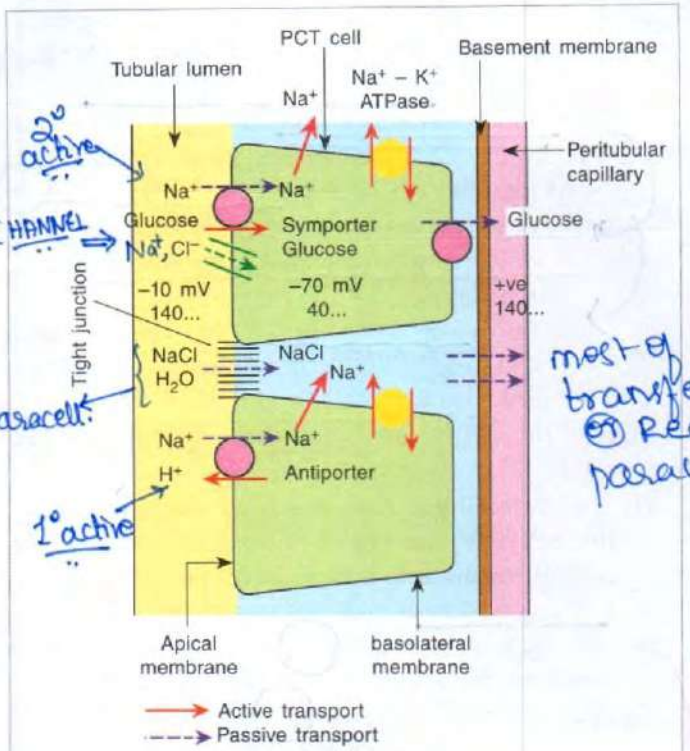


Fig. 56.7 Sodium reabsorption from PCT
(.. Na^+ conc. mM/kg water)

Na⁺ REABSORPTION

Filtered load of sodium is 575-580 gm/day; approx. 98% of which is reabsorbed: 60% by PCT, 20% by thick ascending limb of loop of Henle, 15% gets reabsorbed by DCT, 2-3% by CT and the remaining 2% is excreted in urine.

A. Reabsorption from PCT

Na^+ reabsorption across the PCT occurs in 2 steps: (Fig. 56.7)

Step 1: *Through apical (luminal) membrane i.e. from the tubular* lumen into the tubular epithelial cells. Na^+ is transported through apical membrane *passively* down an electrical and chemical gradient by facilitated diffusion.

	Tubular lumen	PCT cells interior
Concentration (mmol/kg water)	140	40
Membrane potential (mV)	–10	–70*

(*positively charged Na^+ attracted by negative interior)

This step involves *two processes*:

(1) First, Na^+ undergoes *'passive cotransport'* with the secondary active transport of glucose or amino acid via symporters (page 20).

(2) Next, Na^+ reabsorption occurs coupled with H^+ secretion (by Na^+-H^+ exchange). The transport of Na^+ in the PCT is not associated with K^+ secretion.

To maintain electroneutrality the entry of Na^+ into the tubular cell is balanced by 2 mechanisms:

(i) (mainly) by passive inward diffusion of Cl^- down its concentration gradient (Cl^- is the only quantitatively reabsorbable anion in the filtrate), or

(ii) by regeneration of HCO_3^- from cellular CO_2 and water in the presence of carbonic anhydrase.

Step 2: Through the basolateral membrane, involves two processes:

(1) The intracellular Na^+ is *actively* pumped out of the cell into the lateral intercellular space. The energy for the active transport is provided by the $Na^+ - K^+$ ATPase that pumps the Na^+ out of the cell. Approx. 2/3rd *i.e.* 67% of Na^+ reabsorption is based on this active transport process, and

(2) The remaining 1/3rd *i.e.* 33% of Na^+ reabsorption occurs '*passively*' and paracellularly by *Solvent Drag* (page 18). How?

(i) The efflux of Na^+ into intercellular space creates an electrical gradient for the efflux of Cl^- and HCO_3^-, which, in turn, generates an osmotic gradient for water transport into the intercellular spaces.

(ii) The osmotic movement of water generates a small increment in hydrostatic pressure, and some bulk flow of water containing Na^+, Cl^- and HCO_3^- proceeds into the peritubular capillaries.

Note

Normally 75% of the reabsorbed Na^+ is accompanied by Cl^- (*chloride driven sodium transport*) and 25% by HCO_3^-.

75% Cl^- : 25% HCO_3^-

B. Reabsorption from loop of Henle

In the **thin segment** of the ascending limb, NaCl is *passively* reabsorbed. In the **thick segment** the reabsorption of Na^+ is maintained by a primary *active process* dependent on $Na^+ - K^+$ ATPase pumps. The luminal entry of Na^+ into epithelial cells of this segment is by cotransport with K^+ and Cl^- via the $Na^+ - 2Cl^- - K^+$ symporter (for details refer to page 545).

C. Reabsorption from DCT and CT

Only 18-20% of the filtered Na^+ is reabsorbed at the apical membrane in DCT and CT. This involves 2 processes:

(1) 3/4th *i.e.* 75% of the Na^+ presented to the DCT and CT gets reabsorbed by *chloride driven sodium transport* (see above).

(2) The remaining 1/4th *i.e.* 25% of Na^+ is reabsorbed by two electrically linked *cation-exchange processes:*

(i) $Na^+ - H^+$ exchange – this process involves Na^+ reabsorption and H^+ secretion;

(ii) $Na^+ - K^+$ exchange, involves Na^+ reabsorption and K^+ secretion.

These cation-exchange processes are competitive (*i.e.* K^+ competes with H^+ for Na^+) and are enhanced by aldosterone (see below).

D. Factors affecting Na^+ Reabsorption

1. ***Peritubular capillary hydrostatic pressure (CHP) or colloidal osmotic pressure (COP) of plasma proteins***

↑ CHP or ↓ COP → ↓ transport of solute and water into the capillaries → expansion of lateral intercellular spaces → leakage of some salt and water across the tight junctions back into the tubular lumen → ↓s the net Na^+ reabsorption. *For example:*

(i) *if GFR increases, it leads to:*

(a) ↑ oncotic pressure of plasma by the time it reaches the efferent arteriole → ↑ Na^+ reabsorption

(b) ↑s Na^+ filtered load → ↑s Na^+ excretion which exceeds Na^+ reabsorption.

(a) and (b) net result would be an increase in Na^+ excretion (***Natriuresis***).

(ii) *Change in ECFV*

(a) Intravenous administration of saline → ↑ circulating ANP (atrial natriuretic peptide secreted by atrial muscle cells) → relaxes mesangial cells → ↑ GFR (page 518) → *Natriuresis.*

(b) ↓ in ECFV → renal vasoconstriction → ↓ hydrostatic pressure in peritubular capillaries → ↑s Na^+ reabsorption from PCT.

2. ***Effect of hormones***

(i) *Aldosterone* → ↑ $Na^+ - K^+$ ATPase in basolateral membrane of DCT and primarily of CT (by increasing number of Na^+ channels page 724) → ↑ Na^+ reabsorption with secretion of K^+ and H^+. Potent stimulant for aldosterone secretion is the activation of renin-angiotensin mechanism with production of angiotensin II (page 506).

(ii) *Glucocorticoids e.g.* cortisol →

(a) ↑ Na^+ reabsorption from DCT and CT

(b) ↑ GFR

Net effect is *Natriuresis*

3. ***Effect of other ions***

For every Na^+ reabsorption in DCT, one K^+ is secreted into the lumen which competes with H^+, therefore, any

factor which affects K^+ or H^+ secretion will affect the Na^+ reabsorption.

4. *Other factors producing Natriuresis*
   (i) Increased salt intake by decreasing aldosterone secretion
   (ii) PGE_2: by inhibiting Na^+–K^+ ATPase, and by increasing intracellular Ca^{2+}, which inhibits Na^+ transport (via inhibiting number of Na^+ channels)
   (iii) Endothelin and IL-1: Stimulate PGE_2 formation
   (iv) ANP: by increasing intracellular cGMP. It also produces diuresis (page 559).

Important Note

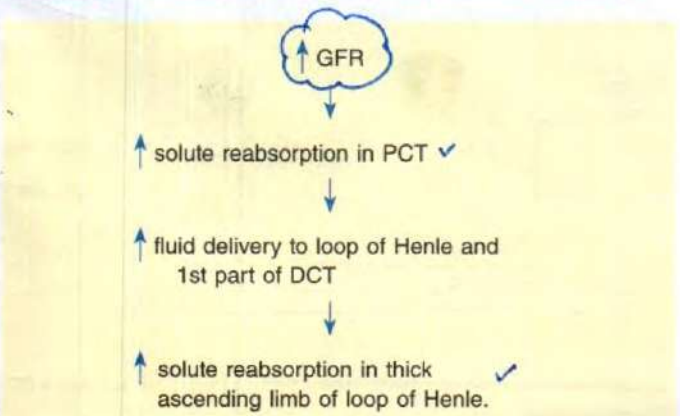
Stimulation of sympathetic renal nerves increases Na^+ and water reabsorption (Mechanism page 505). It also increases renin release thus activating angiotensin II formation (page 506)

Regulation of Na^+ excretion

The amount of Na^+ filtered is so large that if total amount of Na^+ reabsorbed stayed constant, a small rise in GFR of only 2 mL per min would result in Na^+ excretion more than double. Conversely, a small fall in GFR would reduce Na^+ excretion to zero. However, total amount of Na^+ reabsorption is directly proportional to GFR *i.e.* total amount of Na^+ reabsorbed rises when GFR rises and vice versa. Most of this change occur in PCT. Thus *"the tubule tends to reabsorb a constant fraction of the amount filtered rather than a constant amount"*. This proportionality is referred to as *Glomerulo-tubular Balance*. It is true for a number of other substances also, but is especially apparent in the case of Na^+ reabsorption mechanism of *glomerulo-tubular balance* (for details refer to page 512). Exact mechanism of this is not known, however,

High GFR → ↑ oncotic pressure of plasma by the time it reaches the efferent arteriole → ↑ sodium reabsorption from the renal tubule.

Summary: Mechanism of glomerulo-tubular balance



Applied Aspect

1. Patients with kidney disease retain excessive amount of Na^+ and become oedematous due to:
   (i) *Acute glomerulonephritis* → decrease in amount of Na^+ filtered,
   (ii) (a) *Nephrotic syndrome* → increase in aldosterone secretion; and
   (b) decrease in plasma protein → oedema → ↓ plasma volume → ↑ aldosterone secretion via renin-angiotensin system.
2. In hypokalemic alkalosis (induced by chronic vomiting or hyperaldosteronemia), the exchange of H^+ for Na^+ is greater than the exchange of K^+ for Na^+, the urine becomes more acidic and plasma becomes more alkaline.
3. In hypoaldosteronism → ↓ Na^+ reabsorption from DCT → ↓ secretion of K^+ and H^+ causing hyperkalemia and acidosis.

K^+ TRANSPORT

More than 98% of the total body potassium is contained in the cells and only 2% is in the ECF (*Normal: ECF* $[K^+]$ *is 4 mEq/L*). This precise control of ECF $[K^+]$ necessary for normal cell function as hypo or hyperkalemia results in intracellular acidosis or alkalosis respectively (page 528; also refer to page 178). The maintenance of ECF $[K^+]$ depends primarily on kidneys to adjust their K^+ transport.

1. K^+ is completely filtered at the glomerulus and it is the only plasma electrolyte that is both reabsorbed and secreted into the renal tubules.
2. K^+ *reabsorption* occurs by *active transport* in the PCT, ascending limb of loop of Henle, DCT and CT.
   (i) Almost all the filtered K^+ is actively reabsorbed by the PCT and not more than 10% of the filtered load is delivered to the DCT.
   (ii) Most of the K^+ produced in the urine is due to secretion of the K^+ in the DCT which contributes approx. 75% of the excreted K^+. (Fig. 56.8)

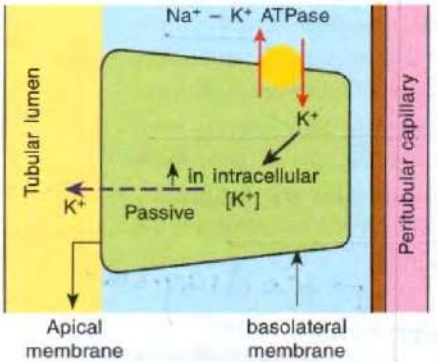


Fig. 56.8 Potassium transport in distal convoluted tubules

3. K^+ secretion involves an *active and a passive process*. It is largely a function of the DCT and is influenced by aldosterone.
   (i) The critical step is the active transport of K^+ from the interstitial fluid (blood) across the basolateral membrane into the DCT. This active transport step is associated with active Na^+ efflux via the $Na^+ - K^+$ ATPase system.
   (ii) The elevated intracellular K^+ so achieved favours the net K^+ diffusion at the apical membrane down a concentration gradient into the tubular lumen. The transcellular transport of K^+ is passive (DCT cells are freely permeable to K^+).

Table 56.6: Factors producing hypo and hyperkalemia

Hypokalemia	Hyperkalemia
Mechanism: By shifting K^+ into cells, therefore, ECF $[K^+]$ decreases	By shifting K^+ out of the cells, therefore, ECF $[K^+]$ increases
Major causes	
1. Insulin (page 748)	1. Diabetes mellitus due to insulin deficiency (page 752)
2. Aldosterone (page 725)	2. Addison's disease due to aldosterone deficiency.
3. Catecholamines by activating β_2-adrenergic receptors (page 739)	3. Tissue damage (specially skeletal muscle or RBC lysis)
4. Alkalosis (page 528)	4. Acidosis (page 528)
	5. After heavy exercise due to K^+ loss from the skeletal muscle
	6. Increase plasma osmolality due to cellular dehydration.

Factors affecting K^+ transport

1. Tubular secretion of K^+ is markedly decreased in **K^+ and Na^+ depletion**.
2. In DCT, **K^+ and H^+** are both exchanged for Na^+ which is reabsorbed and the two ions compete for the exchange mechanism. Therefore, K^+ excretion is decreased when H^+ secretion is increased. (Also refer to page 527).
3. The rate of K^+ secretion is proportionate to the **rate of flow of the tubular fluid** through the distal portion of the nephron, because with rapid flow (*as occurs with high sodium intake*) there is less opportunity for the tubular K^+ concentration to rise to a value that stops further secretion.
4. **Mineralocorticoids** *e.g.* aldosterone, increases K^+ secretion by:
   (i) increasing the activity of basolateral $Na^+ - K^+$ ATPase pump, which increases K^+ entry into the DCT and the $[K^+]$ gradient from cell to lumen,
   (ii) increasing the permeability of the luminal membrane to K^+, further increasing K^+ diffusion (secretion). (Refer to **Table 56.6** for factors producing hypo and hyperkalemia).

HCO_3^- REABSORPTION

It is important to note reabsorption of HCO_3^- alone cannot maintain normal acid base balance, therefore, kidney is capable of generating new HCO_3^- as well. HCO_3^- reabsorption occurs throughout the nephron, except in the descending limb of loop of Henle.

A. Reabsorption from PCT

1. Approx. **90%** of the filtered HCO_3^- is reabsorbed into the PCT by *secondary active transport* (antiport) via the $Na^+ - H^+$ exchanger, this represents more than 4000 mEq/day.
2. The secreted H^+ reacts with filtered HCO_3^- to form carbonic acid (H_2CO_3). The presence of carbonic anhydrase (CA) on the microvilli of the luminal border of the PCT catalyzes the rapid dehydration of H_2CO_3 to form CO_2 and water **(Fig. 56.9)**.
3. Both CO_2 and H_2O diffuse back into the PCT cells which together with CO_2 derived from cell metabolism

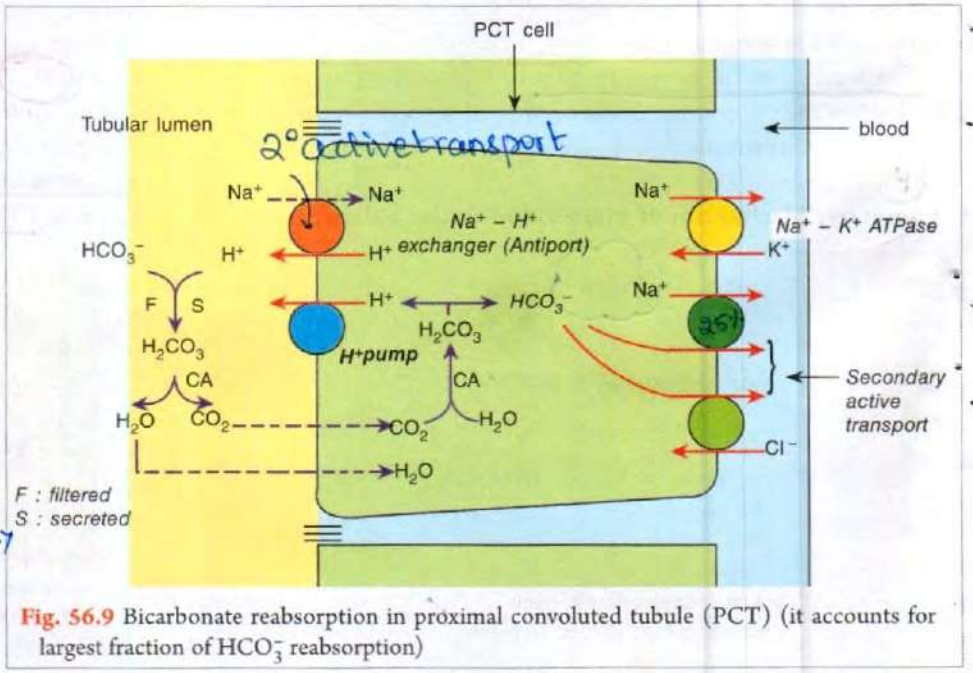


Fig. 56.9 Bicarbonate reabsorption in proximal convoluted tubule (PCT) (it accounts for largest fraction of HCO_3^- reabsorption)

is rehydrated by intracellular 'CA' into H_2CO_3; which dissociates to form H^+ and HCO_3^-. This HCO_3^- is termed as ***newly synthesized HCO_3^-*** by renal tubular cells.

(i) This *newly synthesized HCO_3^-* is reabsorbed across the basolateral membrane by a 'secondary active transport system' into the peritubular blood along with equimolar amounts of Na^+, and

(ii) the H^+ is secreted again by H^+ pump into the lumen.

Important Notes

1. The Na^+ delivered to the peritubular capillaries come from the filtrate in the lumen whereas the HCO_3^- transported into the capillaries is synthesized within the PCT cell and is *not* the same HCO_3^- that was filtered.
2. For each mole of HCO_3^- removed from the tubular fluid, one mole of HCO_3^- diffuses from the tubular cells into the blood, even though it is not the same mole that disappeared from the tubular fluid.
3. Buffering of secreted H^+ by filtered HCO_3^- *does not contribute to the urinary excretion of H^+*. The CO_2 formed in the lumen from secreted H^+ returns to the tubular cell to form another H^+ and, therefore, NO NET H^+ SECRETION occurs.

B. Reabsorption from the DCT and CT

1. The remaining 10-15% of the filtered HCO_3^- is reabsorbed by the DCT and CT via a mechanism that involves the exchange of Na^+ for K^+ or H^+ (as in the PCT).
2. Unlike the PCT, the DCT and CT have $H^+ - K^+ - ATPase$ (or $Na^+ - K^+ - ATPase$ pumps) at the luminal membrane and the lack of 'CA' from that site (Fig. 56.10).

Important Notes

1. The HCO_3^- that is generated within the tubular cell does not represent filtered HCO_3^- and the quantity of HCO_3^- in the renal vein exceeds the amount that entered the kidneys. Thus, the *kidneys besides conserving the filtered HCO_3^- also add newly synthesized HCO_3^- to the plasma.*
2. The renal contribution of new HCO_3^- is accompanied by the excretion of an equivalent amount of acid in the urine in the form of *Titratable Acid*, NH_4^+ or both (page 553).
3. The amount of new *HCO_3^-* formed/day (approx. 70-100 mEq/day) is much less than the quantity of filtered HCO_3^- reabsorbed/day (> 4300 mEq/day).

H^+ SECRETION

1. H^+ secretion is a process by which the filtered HCO_3^- is reabsorbed and the tubular fluid becomes acidic. The process of H^+ secretion, like the HCO_3^- reabsorption, occurs throughout the nephron, except in the descending limb of the loop of Henle. H^+ secretion permits conservation of HCO_3^-.
2. H^+ secretion into the tubular lumen occurs by active transport and is coupled to Na^+ reabsorption. Therefore, *for each mole of H^+ secreted, one mole of Na^+ and one mole of HCO_3^- are reabsorbed into the peritubular capillaries.* [Figs. 56.9, 59.1 (page 553) and 59.2 (page 554)] The renal epithelium secretes approx. 4300 mEq (mmol) of H^+ daily.
3. Approx. 85% of the total H^+ secretion occurs in the PCT. PCT cells are very rich in enzyme carbonic anhydrase (CA).
4. Approx. 10% of the total H^+ secretion occurs in DCT, and
5. Approx. 5% of the total H^+ secretion occurs in CT by the I-cells (page 504).

(I) H^+ Secretion in PCT, DCT and CT

(See under HCO_3^- reabsorption, page 500)

Important Note

In the DCT and CT, H^+ secretion is relatively independent of Na^+ and most of H^+ is secreted by an *ATP-driven* proton pump.

(II) Factors Affecting H^+ Secretion and HCO_3^- Reabsorption

1. *CO_2 content of the tubular cell*

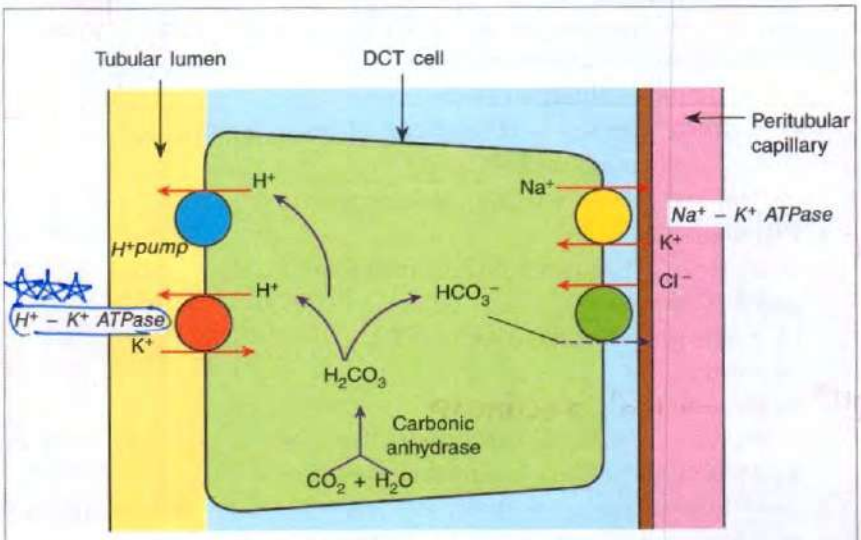


Fig. 56.10 Bicarbonate reabsorption in distal convoluted tubule

(i) **In hypoventilation** (*e.g.* emphysema, respiratory depression, CO_2 inhalation or respiratory acidosis) → ↓ respiration → less washing out of CO_2 → ↑ CO_2 in tubular cells → ↑ intracellular HCO_3^- → ↑ H^+ secretion and ↑ HCO_3^- reabsorption.

(ii) **In hyperventilation** (*e.g.* high altitude, voluntary hyperventilation, systemic alkalosis) → ↑ respiration → ↑ washing out of CO_2 from the body → ↓ CO_2 in the cells → ↓ H^+ formation within tubular cells →

(a) ↓ H^+ secretion causes ↑ K^+ secretion by DCT (since K^+ and H^+ competitively occupy a common transport mechanism) → ↑ K^+ excretion.

(b) ↓ HCO_3^- reabsorption → ↓ Na^+ reabsorption → ↑ $NaHCO_3$ excretion in urine *(alkaline urine)*.

2. ***Carbonic Anhydrase Inhibitors (CAI)*** *e.g.* Acetazolamide (diamox) → ↓ 'CA' → ↓ intracellular H_2CO_3 formation → ↓ H^+ formation within tubular cells →
   (i) ↓ H^+ secretion
   (ii) ↑ K^+ secretion and
   (iii) ↓ HCO_3^- reabsorption

3. ***Plasma [K^+].*** There is an inverse relationship between plasma [K^+] and proximal HCO_3^- reabsorption **(Fig. 56.11)**.
   (i) **Hypokalemia** → ↑ in HCO_3^- reabsorption because ↓ plasma [K^+] →
   (a) intracellular acidosis
   (b) provides a concentration gradient for K^+ efflux, therefore, both H^+ and Na^+ enter the cell to maintain electroneutrality.

   Increase in intracellular [H^+] favours HCO_3^- reabsorption because HCO_3^- reabsorption depends on H^+ secretion.

   (ii) **Hyperkalemia** → ↓ in HCO_3^- reabsorption, because ↑ in plasma [K^+] →
   (a) intracellular alkalosis
   (b) K^+ influx → H^+ and Na^+ efflux to maintain electroneutrality.

   (a) and (b) → ↓ HCO_3^- reabsorption.

4. ***Plasma [Cl^-]***
   if decreases, it causes ↑ HCO_3^- reabsorption and ↑ H^+ secretion.
   Opposite is seen when plasma [Cl^-] increases.

5. ***ECFV***
   if decreases → Renal vasoconstriction → ↓ peritubular capillary hydrostatic pressure → ↑ Renin release → stimulate renin-angiotensin mechanism → ↑ aldosterone secretion → ↑ Na^+ Reabsorption → ↑ H^+ and K^+ secretion and ↑ HCO_3^- reabsorption. Opposite happens when ECFV expands.

6. ***Aldosterone***
   ↑ aldosterone secretion by its 'direct action' on tubular cells and by increased Na^+ reabsorption causes ↑ H^+ and K^+ secretion and ↑ HCO_3^- reabsorption.

Summary

In general, conditions which increases the H^+ secretion are associated with increase HCO_3^- reabsorption. This is seen in:
(i) hypoventilation
(ii) hypokalemia
(iii) hypovolemia
(iv) decrease in plasma [Cl^-]; and
(v) increased aldosterone secretion.

(I) Cl^- TRANSPORT

1. Cl^- reabsorption occurs at a rate of 20,000 mEq/day and is *inversely related to HCO_3^- reabsorption*. Therefore, plasma [Cl^-] varies inversely with the HCO_3^- reabsorption rate.
2. Cl^- is the chief anion to accompany Na^+ through the renal tubular epithelium. As Na^+ reabsorption is under aldosterone control, the plasma [Cl^-] is secondarily influenced by aldosterone.
3. ***Passive transport*** (**Fig. 56.4**, page 521)
   (i) As water moves out of the tubule secondary to Na^+ reabsorption, the increase in luminal [Cl^-] acts as a driving force for paracellular Cl^- reabsorption by diffusion.
   (ii) Active transport of Na^+ leaves the luminal membrane negatively charged with reference to the interstitial fluid. This transtubular voltage gradient constitutes a second driving force for paracellular Cl^- reabsorption by diffusion.

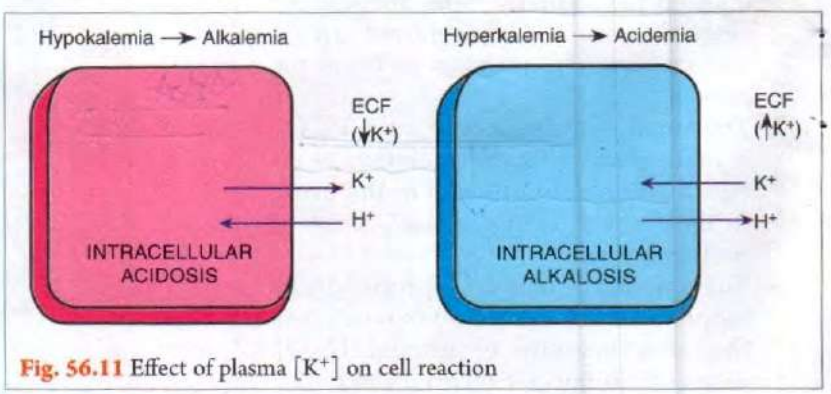


Fig. 56.11 Effect of plasma [K^+] on cell reaction

2. **Give physiological basis of:**
   (i) Albumin is absent from the urine of normal individuals
   (ii) Filtration of cationic substances is greater than that of anionic and neutral substances of same diameter.
   (iii) Predicted renal threshold for glucose is more than the actual value
   (iv) Chloride driven Na^+ transport in PCT
   (v) Hyperventilation is associated with alkaline urine
   (vi) Patients with kidney disease become oedematous
   (vii) Acidosis is a common complication in chronic renal disease.
3. **Write short notes on:**
   (i) Mechanism of glomerular filtration and Factors affecting GFR
   (ii) Filtration fraction and factors affecting it.
   (iii) Maximum tubular reabsorptive and secretory capacity
   (iv) Renal transport mechanisms
   (v) Mechanism of glucose reabsorption in the kidney
   (vi) Tubular handling of Na^+ and water
   (vii) Renal tubular reabsorption and secretion of HCO_3^- and H^+
   (viii) Effect of plasma $[K^+]$ on cell reaction
   (ix) Factors affecting renal threshold for glucose
   (x) 'Splay' in TmG
   (xi) Na^+ reabsorption from PCT and factors affecting it
   (xi) Factors affecting H^+ secretion and HCO_3^- reabsorption
   (xii) Aquaporins.
   (xiii) Glomerular versus systemic filtration
   (xiv) Obligatory and facultative reabsorption of water
   (xv) Agents causing contraction or relaxation of mesangial cells
   (xvi) Transepithelial transport
   (xvii) Factors producing Natriuresis

MCQs

1. **Glomerular filtrate is identical with plasma in respect of the following *except*:**
   (a) Osmolality (b) pH (c) Concentration of electrolytes (d) Organic molecules
2. **Factor *not* affecting the glomerular filtration:**
   (a) Size of capillary bed (b) Permeability of the capillaries
   (c) Hydrostatic and osmotic gradient across capillary wall (d) Change in posture of the individual
3. ***Not a true* statement regarding filtration coefficient of the glomerular membrane:**
   (a) Normally is 10.5 mL/min/mmHg
   (b) Is a function of capillary surface area
   (c) Is defined as GFR at 1 mmHg effective filtration pressure
   (d) Is determined by permeability of glomerular membrane
4. **What happens to GFR if efferent arteriolar constriction is greater than afferent arteriolar constriction?**
   (a) Increases (b) Decreases
   (c) Tends to be maintained (d) First increases then decreases
5. **Filtration of cationic substances is greater than that of anionic substances because:**
   (a) Sialoprotein present in the wall of filtering membrane are negatively charged
   (b) Cations are smaller in size
   (c) Cations have lower molecular weight
   (d) Cations concentration is greater
6. **Glomerular filtration differs from systemic filtration in:**
   (a) Mechanism of filtration is different
   (b) Amount of filtered fluid is more
   (c) Capillary exchange area available for filtration at rest is more
   (d) All of the above

7. **Decrease in renal filtration fraction is seen in:**
   (a) Glomerulonephritis
   (b) Essential hypertension
   (c) Constriction of afferent arterioles
   (d) Constriction of efferent arterioles

8. **T_m limited reabsorption implies that:**
   (a) Reabsorption is passive
   (b) Amount of reabsorption depends critically on the length of time the substance is present in the tubules
   (c) Below a threshold tubular load, the substance is completely reabsorbed
   (d) Renal clearance of substance reabsorption decreases as its plasma concentration increases until its T_m is reached

9. **Which substance is actively transported by the tubular cells but has *not* been shown to have a T_m?**
   (a) Allbumin
   (b) Haemoglobin
   (c) Glucose
   (d) Sodium ions

10. **Na^+–K^+ ATPase pump in renal tubules helps in:**
   (a) Absorption of sodium
   (b) Excretion of sodium
   (c) Absorption of sodium and excretion of potassium
   (d) Absorption of potassium

11. **A major process involved in transport of substance in the kidney:**
   (a) Transepithelial transport
   (b) Transcellular transport
   (c) Paracellular transport
   (d) Intercellular transport

12. **The substance poorly reabsorbed in the nephron:**
   (a) Creatinine
   (b) Urea
   (c) Uric acid
   (d) All of the above

13. **Predicted : actual renal threshold for glucose is ..... mg/dL of arterial plasma:**
   (a) 300 : 200
   (b) 200 : 300
   (c) 200 : 180
   (d) 180 : 200

14. **Percentage reabsorption of total sodium filtered in different parts of a nephron:**
   (a) PCT (80%); DCT (15%); CT (2-3%)
   (b) PCT (80%); DCT (20%)
   (c) PCT (60%); DCT (30%); CT (10%)
   (d) PCT (20%); DCT (80%)

15. ***Not true* about chloride driven sodium transport in PCT:**
   (a) Na^+ efflux is associated with efflux of Cl^- and HCO^-_3
   (b) 75% of Na^+ reabsorption is accompanied by Cl^-
   (c) 25% of Na^+ reabsorption is accompanied by HCO^-_3
   (d) Is an active process

16. **$Na^+ - 2\ Cl^- - K^+$ cotransporter is active in:**
   (a) PCT
   (b) Descending limb of loop of Henle
   (c) Thin ascending limb of loop of Henle
   (d) Thick ascending limb of loop of Henle

17. **Aldosterone:**
   (a) Produced in the juxtaglomerular apparatus
   (b) Increases sodium reabsorption by the nephron
   (c) Increases potassium reabsorption by the nephron
   (d) Tends to increase the hydrogen ion concentration in the blood

18. ***Not* a cause of natriuresis:**
   (a) Increase in GFR
   (b) Cortisol
   (c) Aldosterone
   (d) Overhydration

19. ***True* about glomerulo-tubular balance:**
   (a) Renal tubules tend to reabsorb a constant fraction of Na^+ filtered rather than a constant amount
   (b) Total amount of Na^+ reabsorbed by renal tubules stayed constant
   (c) True only for sodium ions
   (d) GFR has no role to play

20. **What is *not true* for the potassium?**
   (a) Secreted in the distal convoluted tubule (DCT)
   (b) Reabsorbed in proximal convoluted tubule
   (c) Competes with hydrogen ions for secretion by DCT in exchange for sodium ions
   (d) Excretion in urine is decreased by mineralocorticoids

21. **Which part of nephron is essential for maintaining plasma potassium homeostasis?**
   (a) PCT
   (b) Loop of Henle
   (c) DCT and CT
   (d) All of the above

22. **Potassium secretion is controlled by all *except*:**
   (a) Glucocorticoids
   (b) Na^+–K^+ ATPase
   (c) Electrochemical gradient between tubular lumen and blood
   (d) Permeability of luminal membrane to K^+

23. **Bulk of H^+ secretion along the length of renal nephron appears in the tubular fluid at the level of:**
(a) PCT (b) Descending loop of Henle (c) Ascending loop of Henle (d) DCT

24. **Percentage of water reabsorbed in different segment of the nephron:**
(a) PCT (80%); Loop of Henle (10%); DCT and CT (Nil) (b) PCT (80%); Loop of Henle (10%); DCT and CT (10-12%)
(c) PCT (88%); DCT and CT (12%) (d) PCT (99%); DCT and CT (1%)

25. **Percentage of filtered water reabsorbed by the kidney in the absence of ADH:**
(a) 10-12% (b) 50% (c) 75-80% (d) 88%

26. **In the kidney, obligatory reabsorption of water *differs* from facultative water reabsorption:**
(a) Occurs by active processes (b) Responsible for 75-80% of filtered water reabsorption
(c) Influenced by ADH (d) Occurs in the terminal DCT and CT

27. **Obligatory volume of urine:**
(a) Cannot be less than 500 mL/day (b) ADH is secreted in high concentration
(c) Necessary to excrete waste products from the body (d) All of the above are true

28. **Glomerular filtration per day is:**
(a) 40-50 litres (b) 90-100 litres (c) 140-150 litres (d) 170-180 litres

29. **Hydrostatic and colloidal osmotic pressure in glomerular capillaries respectively is:**
(a) 45 and 25 mmHg (b) 25 and 45 mmHg (c) 10 and 25 mmHg (d) 25 and 10 mmHg

30. **Net filtration pressure in the kidney is ..... mmHg:**
(a) 5 (b) 10 (c) 15 (d) 20

31. **Normal renal filtration fraction is .....%**
(a) 12-16 (b) 16-20 (c) 20-25 (d) 25-30

32. **Which of the following is *not* normally filtered or reabsorbed to a significant degree by the tubules of kidney?**
(a) Inulin (b) Plasma proteins (c) Uric acid (d) Para-aminohippuric acid

33. **The primary active transporter in the kidney includes:**
(a) Na^+–K^+ ATPase (b) H^+–K^+ ATPase (c) Ca^{2+} ATPase (d) All of the above

34. ***Not* a symporter carrier transport mechanism in a nephron:**
(a) Na^+ – glucose carrier (b) Na^+ – amino acid carrier (c) $Na^+ – H^+$ carrier (d) $Na^+ – 2\ Cl^- – K^+$ carrier

35. **The proximal convoluted tubules (PCT):**
(a) Reabsorb most of the water and salts of the glomerular filtrate
(b) Reabsorb half the glucose in the glomerular filtrate
(c) Contain juxtaglomerular cells which secrete renin
(d) Are the main target cells for antidiuretic hormone

36. **In renal glycosuria, the renal threshold for glucose is:**
(a) low (b) high (c) same (d) greatly increased

37. **Glucose is transported through:**
(a) Na^+ antiport (b) Na^+ cotransport (c) K^+ antiport (d) K^+ symport

38. **All of the following are reabsorbed in DCT *except*:**
(a) Na^+ (b) K^+ (c) HCO_3^- (d) H^+

39. **True statement regarding sodium reabsorption from DCT and CT:**
(a) 75% gets absorbed by cation exchange process (b) 25% gets absorbed by chloride driven sodium transport
(c) K^+ competes with H^+ for Na^+ reabsorption (d) Stimulated by ADH

Answers

1. (d)	2. (d)	3. (a)	4. (c)	5. (a)	6. (b)	7. (b)	8. (c)	9. (d)	10. (c)	11. (a)	12. (d)	13. (a)	14. (a)	15. (d)
16. (d)	17. (b)	18. (c)	19. (a)	20. (d)	21. (c)	22. (a)	23. (a)	24. (b)	25. (d)	26. (b)	27. (d)	28. (d)	29. (a)	30. (b)
31. (b)	32. (b)	33. (d)	34. (c)	35. (a)	36. (a)	37. (b)	38. (d)	39. (c)						

Chapter 57

Renal Clearance

GFR = 125 mL/min
RPF = 700 mL/min
RBF = 1250 mL/min

I. Introduction
II. Significance of Renal Clearance
III. Applications
A. As a measure of GFR
B. As a measure to estimate tubular secretory capacity
C. As a measure of RPF and RBF
D. As a measure of 'osmotic' and 'free-water' clearances
E. As a measure of excretion of waste products
IV. Applied Aspect: Uremia; Dialysis therapy

1 INTRODUCTION

go with Eg.

The clearance value (C) of a substance (or plasma constituent) *is the volume of plasma (in mL)* that contains the amount of the substance (or constituent) which is excreted in the urine, in one minute. Thus, *clearance is measure of the volume of plasma completely freed of a given substance per minute by the kidneys.* (through excretion in urine)

Renal clearance of a given substance (C*x*) is calculated from the *urinary excretion rate* of that substance (U*x*.V page 519) divided by its plasma concentration (P*x*) i.e.

$$C = \frac{U_x \times V}{P_x}$$

$$Cx = \frac{\text{renal 'excretion rate' of the substance } (Ux.V)}{\text{concentration of the substance in the arterial plasma } (Px)}$$

#: Clearance < GFR

Important Note

Arterial plasma concentration of a substance is same in all parts of the arterial circulation. However, if the substance is not metabolized in the tissues, its level in peripheral venous plasma can be substituted for the arterial plasma level.

Systemic

For example: ***clearance value for urea*** (in mL/min) *i.e.* C_{urea}

$$= \frac{\text{Amount of urea excreted in urine in one minute}}{\text{plasma concentration of urea}}$$

$$= \frac{U_{urea}\, V}{P_{urea}}$$

choice of subs.

Normally:

U_{urea} : Urinary concentration of Urea = 20 mg/mL

V : Urine flow per minute = 1 mL/min (***) Std. value

P_{urea} : plasma concentration of Urea = 30 mg/dL

$$= \frac{20 \times 100}{30}$$

$$= 67 \text{ mL/min}$$

2 SIGNIFICANCE OF RENAL CLEARANCE

Renal clearance provides a useful way of assessing the excretory functions of the kidneys such as: glomerular filtration, tubular reabsorption and secretion; and RBF. How?

1. The equation $C_x = U_x V / P_x$, can be used to measure the clearance of any substance that is present in the plasma and excreted by the kidneys.
   Plasma concentration rather than whole blood concentration is used in calculation of renal clearance, because only the plasma is cleared by filtration.
2. By a simple rearrangement of the renal clearance ratio: $C_x \cdot P_x = U_x \cdot V$; the *clearance* is a comparison of the amount of substance removed from plasma per unit time ($C_x \cdot P_x$) with the amount of substance excreted in the urine per unit time ($U_x \cdot V$).

3 APPLICATIONS

A. AS A MEASURE OF GLOMERULAR FILTRATION RATE (GFR)

A substance which is freely filtered out from the glomeruli in the same concentration as in plasma and in the tubules,

and should be neither reabsorbed nor secreted will measure the GFR.

The amount of such a substance in the urine per unit of time must have been produced by filtering exactly the number of 'milliliters' of plasma that contains that amount.

Salient features of a substance suitable for measuring the GFR by determining its clearance are:

1. Freely filtered *i.e.* not bound to protein in plasma or separated in the process of ultra filtration.
2. Not reabsorbed or secreted by tubules.
3. Not metabolized to another substance.
4. Not stored in kidney.
5. Not toxic and biologically inert.
6. Has no effect on filtration rate.
7. Preferably easy to measure in plasma and urine.

Some *examples* of such a substance (marker) are:

(i) *Inulin* – a polyasaccharide, polymer of fructose with MW 5200; it does not occur naturally in the body

(ii) *Creatinine* (Cr)

(iii) Sodium ferrocyanides

(iv) Mannitol

(v) Sorbitol

(vi) Sucrose

(vii) Radioactive cobalt labelled Vit. B_{12}

(viii) ^{51}Cr-labelled EDTA

(ix) Radio iodine labelled hypaque

Clearance value of any of these substances will be equal to GFR.

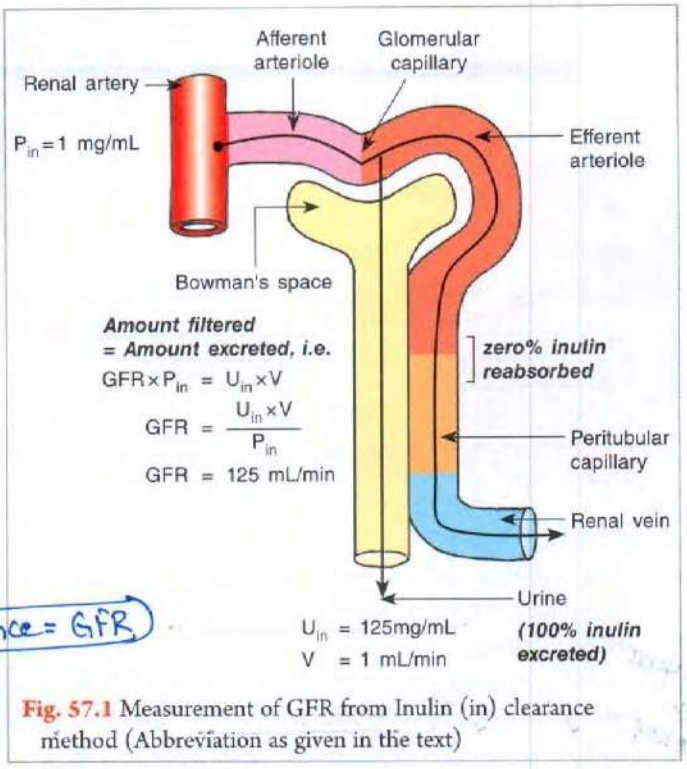


Fig. 57.1 Measurement of GFR from Inulin (in) clearance method (Abbreviation as given in the text)

Inulin Clearance: Method (Fig. 57.1)

To determine the GFR in man a large initial dose of inulin is injected intravenously (I.V.) which is followed by a constant inulin infusion at a rate which compensates for its loss in urine. A reasonably constant arterial plasma level is thereby maintained. The inulin concentration of plasma of venous blood (P_{in}), the urinary concentration (U_{in}) and the volume of urine excreted per minute (V) are determined and the clearance calculated, for example:

Let P_{in} = 1 mg/mL
U_{in} = 125 mg/mL
V = 1 mL/min

then $$C_{in} = \frac{U_{in} \cdot V}{P_{in}} = \frac{125 \times 1}{1}$$
$$= 125 \text{ mL/min}$$

Note

If a substance is not metabolized to any extent in the tissues, its level in the venous plasma can be substituted for the arterial plasma level.

Creatinine Clearance

Although inulin clearance can be used to measure GFR, in 'clinical' practice it is more common to determine the 24-hour ***endogenous 'creatinine' clearance*** as estimate of GFR. Its determination does not require administration of exogenous creatinine, as creatinine is a product of muscle metabolism. Normally production and breakdown rates of phosphocreatine are relatively constant, and the plasma concentration of creatinine does not vary much. *Endogenous creatinine clearance* is easy to measure and hence ***used clinically*** as an estimate of GFR.

Normal range for creatinine clearance: 80-110 mL/min. However, some creatinine is reabsorbed by the tubules and some may be secreted. Therefore, *when precise measurement of GFR is required, inulin clearance (C_{in}) is preferred.*

Significance: Inulin clearance is used as *'indicator of plasma clearance mechanism'*. How?

A comparison of the *clearance of a given substance (C_x) with the clearance of inulin (C_{in})* provides information about the renal mechanisms used to remove the substance from plasma **(Fig. 57.2)**.

1. ***When C_x equals C_{in} (i.e. GFR), excretion is by filtration alone.*** Therefore, the mass of the substance excreted in urine per unit time ($U_x \cdot V$) equals the mass of the substance filtered during the same time ($GFR \cdot P_x$) *i.e.* $U_x \cdot V = C_{in} \cdot P_x$ (as C_{in} = GFR).

   Here, the clearance ratio (ratio of clearance of any substance 'C_x' to the clearance of inulin 'C_{in}' *i.e.* $C_x/C_{in} = 1$.

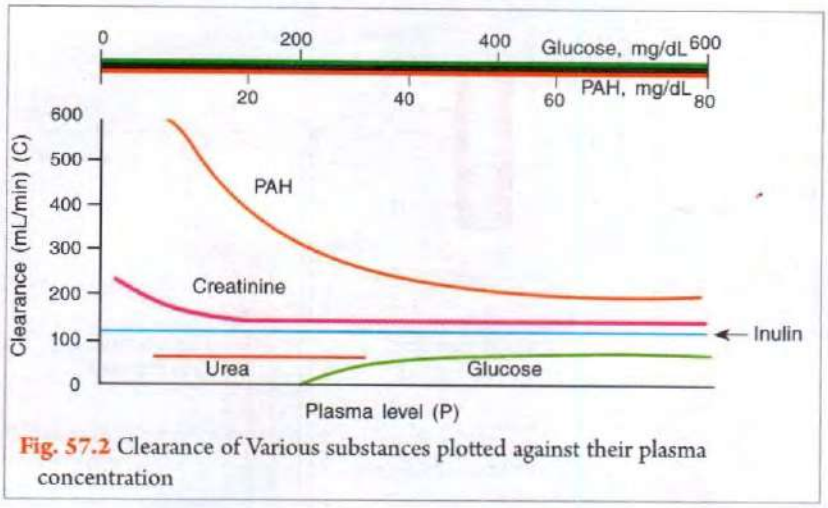


Fig. 57.2 Clearance of Various substances plotted against their plasma concentration

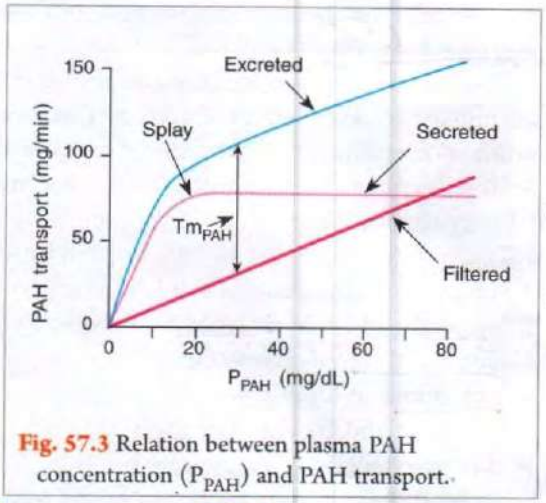


Fig. 57.3 Relation between plasma PAH concentration (P_{PAH}) and PAH transport.

A clearance ratio 1 indicates, on a net basis, the substance is *neither reabsorbed nor secreted and, therefore, is only filtered.*

Substances with clearance ratio close to 1 include: *mannitol, sorbitol, ferricyanide, Vitamin* B_{12} *and sucrose (I.V.).*

2. **When C_x is less than C_{in}, *excretion is by filtration and reabsorption.***
   (i) Here $U_x \cdot V < C_{in} \cdot P_x$ (excretion rate of substance < filtration load)
   (ii) $C_x/C_{in} < 1$
      (a) A clearance ratio <1 indicates, *on a net basis, the substance undergoes reabsorption.*
      (b) Substances with clearance ratio <1 include: *Glucose, xylose and fructose.*
3. **When C_x is greater than C_{in}, *excretion is by filtration and secretion.*** Here,
   (i) $U_x \cdot V > C_{in} \cdot P_x$ (excretion rate of substance > filtration load)
   (ii) $C_x/C_{in} > 1$.
      (a) A clearance ratio of more than 1 indicates the net secretion of the substance into the lumen, therefore, *the substance is cleared by filtration and secretion.*
      (b) Substances with clearance ratio greater than 1 include: *para-aminohippuric acid (PAH), phenol red, iodopyracet, certain penicillins and creatinine.*

B. AS A MEASURE TO ESTIMATE TUBULAR SECRETORY CAPACITY (T_S):

PAH Clearance

Para-aminohippuric acid (PAH) is a weak organic acid that is actively secreted into the PCT by a transport maximum limited process.

As approx. 10% of the plasma PAH is bound to plasma proteins, PAH is not entirely freely filtrable and plasma PAH concentration (P_{PAH}) is greater than that in the glomerular filtrate. However, PAH binding does not significantly diminish the effectiveness of tubular secretion.

The *filtered load* (page 519) of PAH is a linear function of plasma PAH (P_{PAH}), but PAH secretion increases as P_{PAH} increases only until a Tm_{PAH} is reached **(Fig. 57.3)**. The Tm_{PAH} is about 80 mg/min, therefore,

1. **When P_{PAH} is low**, practically all of the PAH that is not filtered is secreted, and PAH is almost completely cleared from the plasma by the combined processes of glomerular filtration and tubular secretion **(Fig. 57.3)**.
2. **When P_{PAH} is above 20 mg/dL**, the transepithelial secretory mechanism becomes saturated, and the Tm_{PAH} is reached. At this the quantity of PAH secreted per minute remains constant and is independent of P_{PAH}.
3. **When the P_{PAH} increases above Tm_{PAH}**, clearance of PAH (C_{PAH}) falls progressively and it becomes more a function of glomerular filtration (because the amount of PAH secreted becomes a smaller and smaller fraction of total amount excreted). Finally the C_{PAH} approaches clearance of inulin (X_{in}).
4. *Because the TmPAH is nearly constant, it is used clinically to estimate tubular secretory capacity (T_s).*

$$\text{Clearance} = \frac{(T_{PAH} + \text{Fluid filtered}) \cdot V}{P_{PAH}}$$

C. AS A MEASURE OF RENAL PLASMA FLOW (RPF) AND RENAL BLOOD FLOW (RBF)

Principle

RBF can be measured by applying the *Fick's principle* to the kidneys, *i.e.* the amount of a substance taken up by any organ per unit time is equal to the arterio-venous difference for the substance across the organ times the blood flow.

Amount of substance taken/min = (A–V) difference of substance × Blood flow per minute

i.e. Blood flow per minute = $\frac{\text{Amount of substance taken/min}}{\text{(A–V) difference of the substance}}$

Since the kidney filters plasma, the RPF equals the amount of a substance excreted per unit time divided by the renal arterio-venous difference of the substance.

Criteria of the substance used

A substance

(1) which is almost completely extracted from the blood during each passage through the kidney,
(2) which is neither metabolized, stored, nor produced by the kidney,
(3) which does not itself affect RBF,
(4) if its concentration in arterial and renal venous plasma can be measured easily, and
(5) which is actively secreted by the tubules from the blood into their lumen.

The clearance value of such a substance gives a measure of the RPF.

A few such substances are:

(1) ***Diodrast***-organic iodine compound, often have unpleasant side-effects when injected,
(2) ***Para-amino hippuric acid*** (PAH) – Preferred, because its ***extraction ratio*** (E) (page 514) is high *i.e.*

$$E = \frac{\text{(Arterial PAH Concentration minus Renal Venous PAH Concentration)}}{\text{Arterial PAH Concentration}} = 1 - \frac{V_{PAH}}{A_{PAH}}$$

More than 90% of PAH in arterial blood is removed in a single circulation in the kidney. RPF can be measured by modifying the Fick's principle. Therefore,

$$RPF = \frac{\text{Amount of PAH excreted in urine/min: } U_{PAH} \cdot V}{A_{PAH} - V_{PAH}} = \frac{U_{PAH} \cdot V}{A_{PAH} - V_{PAH}}$$

where,

U_{PAH} – urinary concentration of PAH: mg/mL.
V – rate of urine flow: mL/min.
A_{PAH} or V_{PAH} – Concentration of PAH (in mg/mL) in renal artery and renal vein respectively.

At low concentration of PAH in arterial plasma, the renal clearance is nearly complete. Therefore, the PAH concentration in renal venous plasma may be taken to be zero. Thus,

$$RPF = \frac{U_{PAH} \cdot V}{A_{PAH}} = C_{PAH}$$

1. Approx. 10-15% of the total renal plasma flow perfuses non-excretory (non-tubular) portion of the kidney, *e.g.* the renal capsule, perirenal fat, renal medulla and renal pelvis; therefore, this plasma cannot be completely cleared of PAH by filtration and secretion (Thus C_{PAH} does *not* measure RPF to this region of the kidney).
2. As about 10% of the PAH remains in the renal venous plasma, the RPF calculated from C_{PAH} underestimates the actual flow by about 10%. Therefore, C_{PAH} actually measures the ***effective renal plasma flow*** (ERPF), *i.e.*

$$ERPF = \frac{U_{PAH} \cdot V}{A_{PAH}} = C_{PAH}$$

3. Because PAH is not metabolized or excreted by any organ other than the kidney, a sample from peripheral vein can be used to measure arterial plasma PAH concentration. Therefore,

$$ERPF = \frac{U_{PAH} \cdot V}{P_{PAH}} = C_{PAH}$$

Normally U_{PAH} = 14 mg/mL
V = 1 mL/min
P_{PAH} = 0.02 mg/mL

Therefore,

$$ERPF = \frac{14 \times 1}{0.02} = 700 \text{ mL/min}$$

Thus RBF can be determined from ERPF as follows:

When plasma flow is 55 mL per min (100 – Haematocrit: 45%), total RBF = 100 mL per min.

As normal plasma flow is 700 mL/min, then

$$\text{Total RBF} = \frac{100 \times 700}{55} = \text{approx. 1273 mL/min.}$$

D. AS A MEASURE OF "OSMOTIC" AND 'FREE WATER' CLEARANCES

Osmotic clearance (C_{osm}) is the amount of water necessary to excrete the osmotic load in a urine that is isotonic with plasma. It measures the rate at which plasma is cleared of osmotic particles and is calculated as:

$$C_{osm} = \frac{U_{osm} \cdot V}{P_{osm}}$$

where V = rate of urine flow
U_{osm} = urinary osmolality
P_{osm} = plasma osmolality

Normally, C_{osm} is about 3 mL/min. It is:
(1) increased in osmotic diuresis (page 547), and
(2) decreased in fasting or diet deficient in proteins.

(1) In order to quantitate the gain or loss of water by excretion of a concentrated or dilute urine, the *"free water" clearance* (C_{H_2O}) is calculated:

$$C_{H_2O} = V - C_{osm}$$

From the above equation,

$$C_{H_2O} = V - \frac{U_{osm} \cdot V}{P_{osm}}$$

$$= V\left(1 - \frac{U_{osm}}{P_{osm}}\right)$$

Normally, U_{osm} is more than P_{osm}, therefore, C_{H_2O} is usually *negative*. (Table 57.1) This means that the volume of urine excreted is less than the osmolar clearance *i.e.* the urine is more concentrated (hypertonic) than plasma. Thus C_{H_2O} measures the rate of clearance of 'free water' that is not bound to the osmotic particles. Similarly, during antidiuresis (*e.g.* in dehydrated state and in the presence of ADH), U_{osm} is more than P_{osm}, therefore C_{H_2O} is negative, indicating that water is being conserved by the excretion of a small volume of concentrated urine.

In water diuresis (seen in the absence of ADH or following water ingestion), free water clearance (C_{H_2O}) becomes ***positive*** *i.e.* urine becomes hypotonic (U_{osm} is less than P_{osm}). Maximum 'free water' clearance is 15-20 L/day (10-15 mL/min).

E. AS A MEASURE OF EXCRETION OF WASTE PRODUCT

C_{urea} measures the rate of excretion of waste products (page 528). It can be calculated as follows:

$$C_{urea} = \frac{\text{Amount of urea excreted in urine (U) } \times \text{ rate of urine flow (V)}}{\text{plasma concentration of urea } (P_{urea})}$$

(i) When rate of urine flow is less than 2 mL/ min, clearance of urea is called *Standard Urea Clearance* ($C_{urea\text{-}s}$). It is calculated as UV/P_{urea}. *Normally* it is 54 mL/min (range : 40-65 mL/min).

(ii) When rate of urine flow is more than 2 mL/min, excretion of urea is maximal, called the *Maximum Urea Clearance* ($C_{urea\text{-}m}$). Normally it is 75 mL/min (range : 60-90 mL/min).

C_{urea} *measures the rate of excretion of waste products.* Normally, one half of urea filtered is returned to the blood from the tubular fluid; when the tubules are damaged their relative impermeability to urea is decreased and more urea correspondingly returns to the blood. Hence, during the early stages of polyuria in renal failure, C_{urea} remains low and blood urea concentration is correspondingly raised.

Because normal range of C_{urea} is wide and it does not give much information, therefore, currently it is an *obsolete test* and is *not used clinically.*

(4) APPLIED ASPECT: UREMIA

In chronic renal failure, (i.e., *progressive and irreversible loss of nephrons function*) breakdown products of protein metabolism (urea, creatinine, organic acid and phenol etc., page 621) accumulate in the blood, resulting in a clinical syndrome called ***Uremia***.

Characteristic features

1. *Early:* Nausea, vomiting, anorexia, lethargy, anaemia, frequent fractures.
2. *Late:*
   (i) Impairment of higher mental functions resulting in drowsiness, impaired judgement, dull pain sensibility, headache, loss of self control, disorientation, confusion etc.,

Table 57.1: Alteration in water metabolism produced by ADH

	GFR (mL/min)	% of filtered water reabsorbed	Urine volume (mL/day)	Urine concentration (mosm/kg water)	Gain or loss of water in excess of solute mL/day
Urine isotonic with plasma	125	98.7	2400	290	. . .
ADH-maximum antidiuresis	125	99.7	500	1400	1900 gain *i.e.* C_{H_2O} : *Negative*
No ADH → diabetes insipidus	125	87.1	23300	30	20900 loss *i.e.* C_{H_2O} : *Positive*

(ii) Muscle twitching, convulsions, coma and finally death.

(For other features refer to summary **(Fig. 57.4)**)

Treatment

Failing kidneys reach a point when they can no longer excrete water and ions at rates that mantain body balance of these substances. Moreover, they cannot excrete waste products as fast as they are produced. The techniques used to replace the kidney's excretory functions is ***Dialysis therapy***. *Dialysis* literally means to separate substances using a membrane.

DIALYSIS

1. *Definition:* It is a process of separating the soluble crystalloids from the colloid in a mixture by means of a *Dialyser* (*i.e. a semipermeable membrane* generally made of cellulose or cellophane that is highly permeable to most solutes but relatively impermeable to protein and completely impermeable to blood cells.)

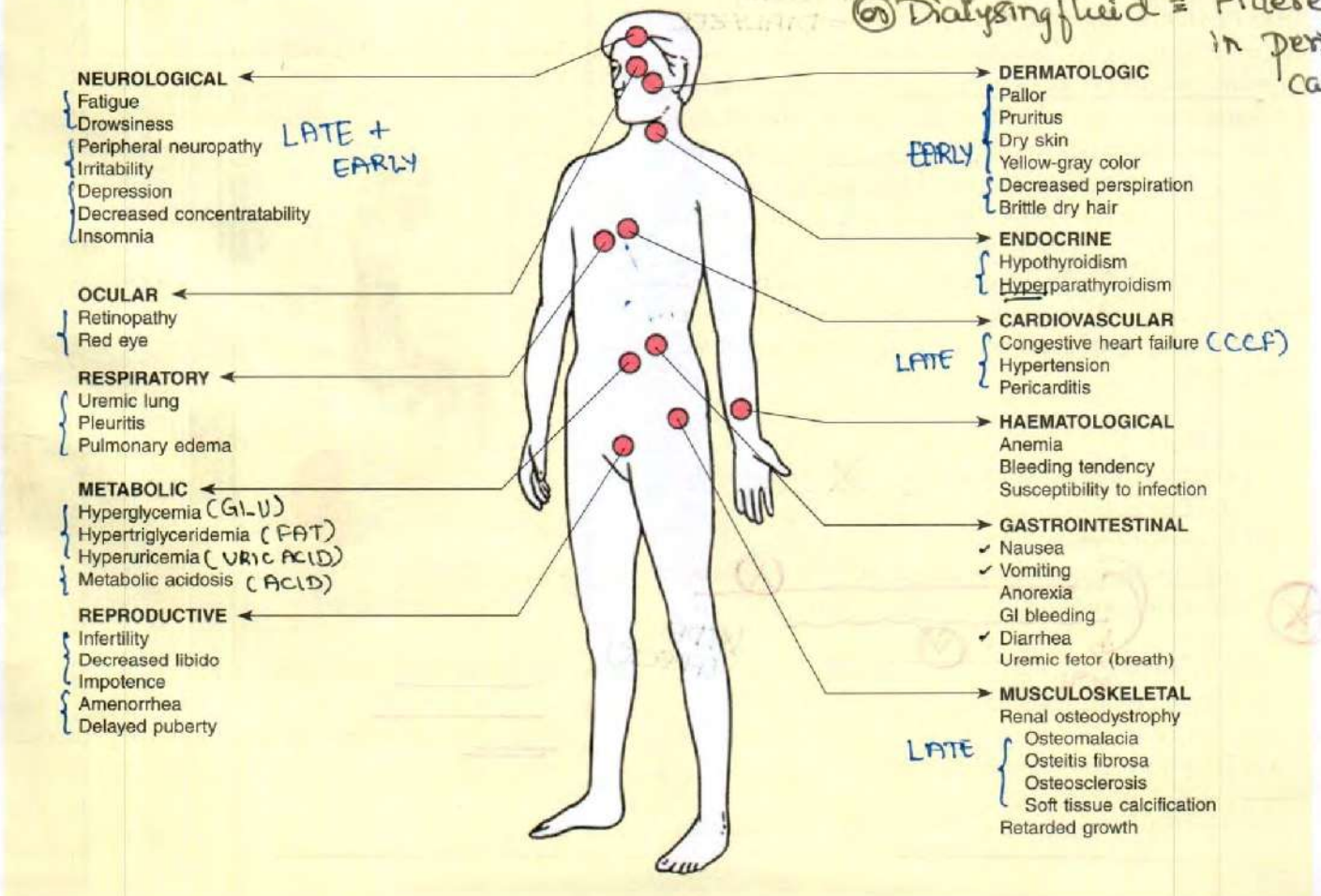
2. *Aim*
   (i) to remove waste products and excess of water and ions from the blood stream.
   (ii) to maintain proper clinical balance of the blood.

3. *Principle/Physiological basis*

Dialysis is based on the principle of ***Diffusion Equilibirum***. The blood is made to pass continuously between two thin membranes of cellophane; on the other side of the membrane is a *Dialyzing fluid* to allow all the constituents of plasma except proteins to diffuse in either direction. Therefore, if the plasma $[K^+]$ of the patient is above normal, K^+ diffuses out of the blood across the cellophane tubing and into the dialyzing fluid. Similarly, waste products and excess of other substances also diffuse into the dialyzing fluid and thus are removed from the body.

The *dialyzing fluid (the Dialysate)* is salt solution with ionic composition similar to or lower than those in normal plasma. In general, it contains Na^+, K^+ and HCO_3^- in a higher concentration than in normal plasma; and urea, urates, creatinine, phosphate, sulphate are absent.

Summary: Clinical features of uremia.



4. *Indications*
   (i) Patients with acute reversible renal failure (may require treatment only for days *or* week.
   (ii) Patients with chronic irreversible renal failure (may require treatment several times a week for the rest of their lives, unless they receive a ***renal transplant***.)
   (iii) Patients suffering from poisoning *or* drug overdose.

5. *Types/Techniques*

(A) Hemodialysis (or Artificial Kidney) **(Fig. 57.5)**

Here heparinized (anticoagulated) blood is pumped from one of the patient's arteries through cellophane tubing that is surrounded by a large volume of dialyzing fluid. Blood flows in one direction and dialyzing fluid in the other across the tubing. The tubing then conducts the purified blood back into the patient by way of a vein. As blood flows through the tubing, the concentrations of non protein plasma solutes tend to reach *diffusion equilibrium* with those of the solutes in the dialyzing fluid. Dialysate flowing out of the machine is collected outside in a bottle and discarded. The treatment requires 4–6 hours dialysis run.

(B) Peritoneal dialysis → Peritoneal cavity = DIALYZER (Memb.)

It uses the lining of the person's own abdominal cavity *(peritoneum)* as a dialysis membrane. Dialyzing fluid is injected via a needle inserted through the abdominal wall, into the cavity and allowed to remain there for hours, during which solutes diffuse into the fluid from the patient's blood. The dialyzing fluid is then removed by reinserting the needle and is replaced with new fluid. This procedure can be performed several times daily (anywhere *or* at home) by a patient who is simultaneously doing normal activities **(Fig. 57.6)**.

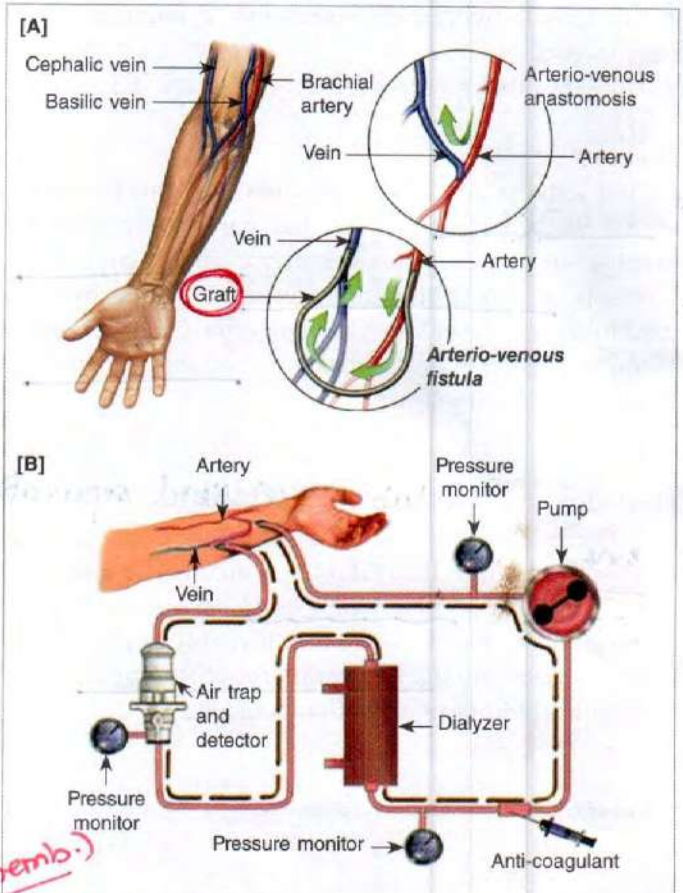


Fig. 57.5 Hemodialysis: (A) Arteriovenous fistula and (B) Technique

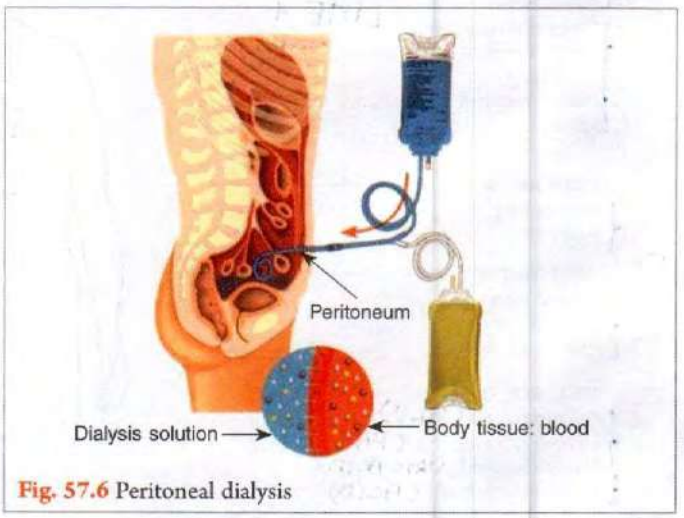


Fig. 57.6 Peritoneal dialysis

5. *Limitations*
   (i) Dialysis cannot maintain completely normal body fluid composition as kidney does.
   (ii) It cannot replace all the kidney functions which are affected, specially secretion of erythropoietin; therefore, anaemia following a dialysis is the rule.

7. *Complications* (SIDE EFFECTS)
   (i) Hypotension; anaemia
   (ii) Nausea
   (iii) Dyspnoea, chest and back pain
   (iv) Hypersensitivity reactions
   (v) Hypoproteinaemia
   (vi) Peritonitis, a major complication of peritoneal dialysis.

Study Questions

1. **Write short notes on:**
   (i) Significance and application of renal clearance
   (ii) Characeristics of a substance for measuring GFR
   (iii) Physiological significance of inulin clearance
   (iv) PAH clearance
   (v) Osmotic and free water clearance
   (vi) Urea clearance
   (vii) Clinical features of uremia
   (viii) Dialylsis therapy
   (ix) Artificial kidney
2. **Why is PAH used to measure tubular secretory capacity?**
3. **Give advantages and disadvantages of inulin and creatinine clearance to measure GFR.**
4. **By giving suitable examples: How would you determine that excretion is**
   (i) Filtration alone
   (ii) Filtration and reabsorption
   (iii) Filtration and secretion.
5. **Give physiological basis of:**
   (i) Renal clearance of a substance
   (ii) Clinically creatinine clearance is used to estimate GFR
6. **Name tests separately to measure each of them and describe any one them in detail.**
   (i) GFR
   (ii) Tubular secretory capacity
   (iii) RPF and RBF
   (iv) Water clearance
   (v) Excretion of waste products

MCQs

1. ***Not true* of renal clearance of a given substance is:**
   (a) Expressed in mL/min
   (b) Excretion rate of substance to its concentration in plasma
   (c) Measure of volume of plasma completely freed of a given substance per minute
   (d) Can be calculated by considering concentration of substance in the whole blood
2. **Renal clearance can be used to measure all *except*:**
   (a) GFR
   (b) Tubular secretory capacity
   (c) Tubular reabsorptive capacity
   (d) Renal blood flow
3. **Which of the following is filtered but *not* reabsorbed by the renal tubules?**
   (a) Para-aminohippuric acid
   (b) Inulin
   (c) Plasma proteins
   (d) Glucose
4. **Following is least absorbable in tubules:**
   (a) Creatinine
   (b) Glucose
   (c) Urea
   (d) Sucrose
5. **Widely used clinical test for estimation of GFR is:**
   (a) Inulin clearance
   (b) Creatinine clearance
   (c) Sucrose clearance
   (d) Radioactive cobalt labelled vitamin B_{12}
6. **Creatinine is *not* ideal to measure GFR in humans because?**
   (a) It is toxic
   (b) Some of it is reabsorbed by tubules and some may be secreted
   (c) Not freely filtered
   (d) Affects filtration rate
7. **When clearance of a given substance equals inulin clearance, excretion is by:**
   (a) Filtration and reabsorption
   (b) Filtration alone
   (c) Filtration and secretion
   (d) Synthesis of a substance in tubules and secreted
8. **When a substance is excreted via kidneys by filtration and secretion, it is most likely to be:**
   (a) Mannitol
   (b) Glucose
   (c) PAH
   (d) Vitamin B_{12}
9. **Two substances that can probably be used to determine filtration fraction are:**
   (a) Inulin and mannitol
   (b) Urea and diodrast
   (c) PAH and phenol red
   (d) Inulin and PAH
10. **Effective renal plasma flow is lower than actual renal plasma flow by:**
    (a) 5%
    (b) 10%
    (c) 15%
    (d) 20%

11. **Amount of water necessary to excrete the osmotic load in urine that is isotonic with plasma is called:**
    (a) Free water clearance
    (b) Osmotic clearance
    (c) Negative water clearance
    (d) Positive water clearance
12. **Amount of inulin reabsorbed after filtration is:**
    (a) Zero
    (b) 1 mg
    (c) 2 mg
    (d) 3 mg
13. **Substance *not* excreted via kidney by filtration alone is:**
    (a) Glucose
    (b) Vitamin B_{12}
    (c) Mannitol
    (d) Sucrose
14. **For which of the following substances would you expect the renal clearance to be the lowest, under normal conditions?**
    (a) Vitamin B_{12}
    (b) Creatinine
    (c) PAH
    (d) Glucose
15. **A substance which has a renal clearance more than that of inulin is probably:**
    (a) Only filtered at the glomeruli
    (b) Only secreted by the tubules
    (c) Filtered and reabsorbed
    (d) Synthesized in tubules and secreted
16. **Substance clinically used to measure renal plasma flow (RPF) is:**
    (a) Para-aminohippuric acid (PAH)
    (b) Diodrast
    (c) Cr-labelled EDTA
    (d) Radioactive labelled iodine

Answers

1. (d) 2. (c) 3. (b) 4. (a) 5. (b) 6. (b) 7. (b) 8. (c) 9. (d) 10. (b)
11. (b) 12. (a) 13. (a) 14. (d) 15. (d) 16. (a)

Chapter 58

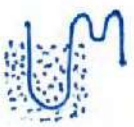
Mechanism of Concentration and Dilution of Urine — The Counter Current System

I. General Considerations
II. Mechanism of Concentration and Dilution
A. Overview
B. Counter Current Multipliers
C. Counter Current Exchangers
D. Role of Urea
E. Other examples of Counter Current System
F. Summary
III. Diuresis—water versus osmotic diuresis
IV. Diuretics

1 GENERAL CONSIDERATIONS

A. The kidney forms urine which varies widely in its solute concentration according to the need of the body, e.g. Plasma osmolarity = 300 mosm/L
   (i) *In overhydration,* kidney can produce urine of 50 mosm/L *i.e.* 1/6th the osmolar concentration of plasma.
   (ii) *During dehydration* kidney can produce urine of 1200 mosm/L *i.e.* 4 times the osmolar concentration of plasma.

B. **Components of the concentrating and diluting system**
   1. The formation of urine that is dilute (hyposmotic to plasma) or concentrated (hyperosmotic to plasma) is achieved by the **Counter Current System.** (CCS)
   2. This system consists of the
      (i) Descending limb of the loop of Henle (DLH)
      (ii) Thin and thick segments of ascending loop of Henle (ALH)
      (iii) Medullary interstitium
      (iv) Distal convoluted tubule
      (v) Collecting duct, and
      (vi) Vasa recta.



C. ***A counter current system*** is a system in which the inflow runs parallel to, counter to and in close proximity to the outflow for some distance. The operation of such a system in increasing the heating at the apex of a loop of pipe raises the temperature of water by 5°C, but the heated water flowing away from the heater warms the inflow **(Fig. 58.1)**. A gradient of temperature is thus set up along the pipe, so that at the bend the temperature is raised not from 15 to 20°C but from 45 to 50°C.

Refer diag:

2 MECHANISM OF CONCENTRATION AND DILUTION

A. OVERVIEW

1. The fundamental processes involved in the excretion of a concentrated or diluted urine include:

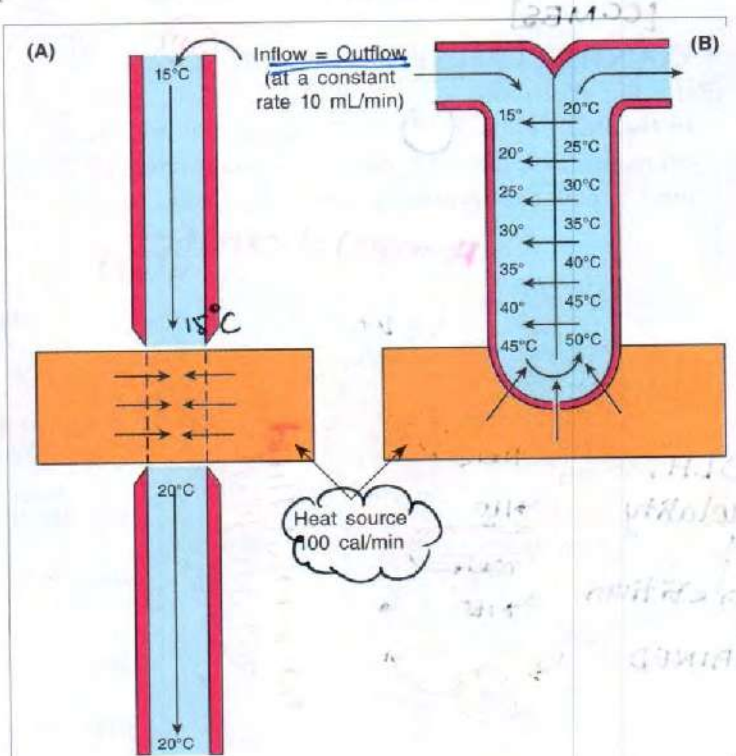


Fig. 58.1 The operation of a thermal counter current exchange system (A) Heater surrounds a pipe and raises the temperature of water flowing through the pipe by 5°C. (B) Pipe is bent into a tight U-shape. The same heater applied at the base of the U sets up a temperature gradient throughout the length of the pipe, so that at the bend the temperature is raised from 45 to 50°C.

(i) *Variable permeability of the nephron* to the passive reabsorption of water along an osmotic gradient and passive diffusion of urea along its concentration gradient.

(ii) *Passive reabsorption* of NaCl by the thin segment of ALH.

(iii) *Active reabsorption* of Na^+ by the thick segment of ALH via the $Na^+ - 2Cl^- - K^+$ symporter and the $Na^+ - K^+ -$ ATPase pump.

2. The concentration of the tubular fluid occurs mainly in the juxta medullary nephron alone, therefore *counter current system is the feature of juxta medullary nephron.* However, similar events also occur in the cortical nephron.

3. The concentrating mechanism depends upon the existance of a gradient of increasing osmolality along the medullary pyramids. This gradient is:

(i) *produced by* operation of the loop of Henle and collecting duct as **Counter Current Multipliers,** and

(ii) *maintained by* Vasa Recta as **Counter Current Exchangers.**

(i) and (ii) together called as *Counter Current Multiplier Exchange System or Counter Current System.*

B. COUNTER CURRENT MULTIPLIERS

(Refer Fig. 58.2)

1. *In the PCT* as about 70% of filtered solutes and water get reabsorbed, therefore, osmolar concentration of PCT fluid is equal to osmolar concentration of plasma *i.e.* 300 mosm/kg water. (Also refer to page 550)

2. *The Loop of Henle*

A. *DLH* – it is the *'concentrating' segment* of the nephron. How?

DLH is
- highly permeable to water due to presence of Aquaporin–1, and
- relatively impermeable (low permeability) to solute.

Therefore,

(i) solute-free water moves into the interstitium,

(ii) solute concentration in DLH increases; the predominant solute is NaCl with relatively small amounts of urea.

(i) and (ii) result in:

(a) fluid in the DLH to become concentrated;

(b) fluid in the DLH nearly attains the osmolality of adjacent medullary interstitium which is about 1000–1200 mosmol/L.

Important Note

* The interstitial osmolality is maintained by solvent-free solute (NaCl) that is transported out of ALH.

B. *ALH* – The thick and thin segments of ALH constitute the *'Diluting' segment* of the nephron. How? (Fig. 58.2)

(i) The *thin segment* is
- impermeable to water, and
- permeable to NaCl and urea.

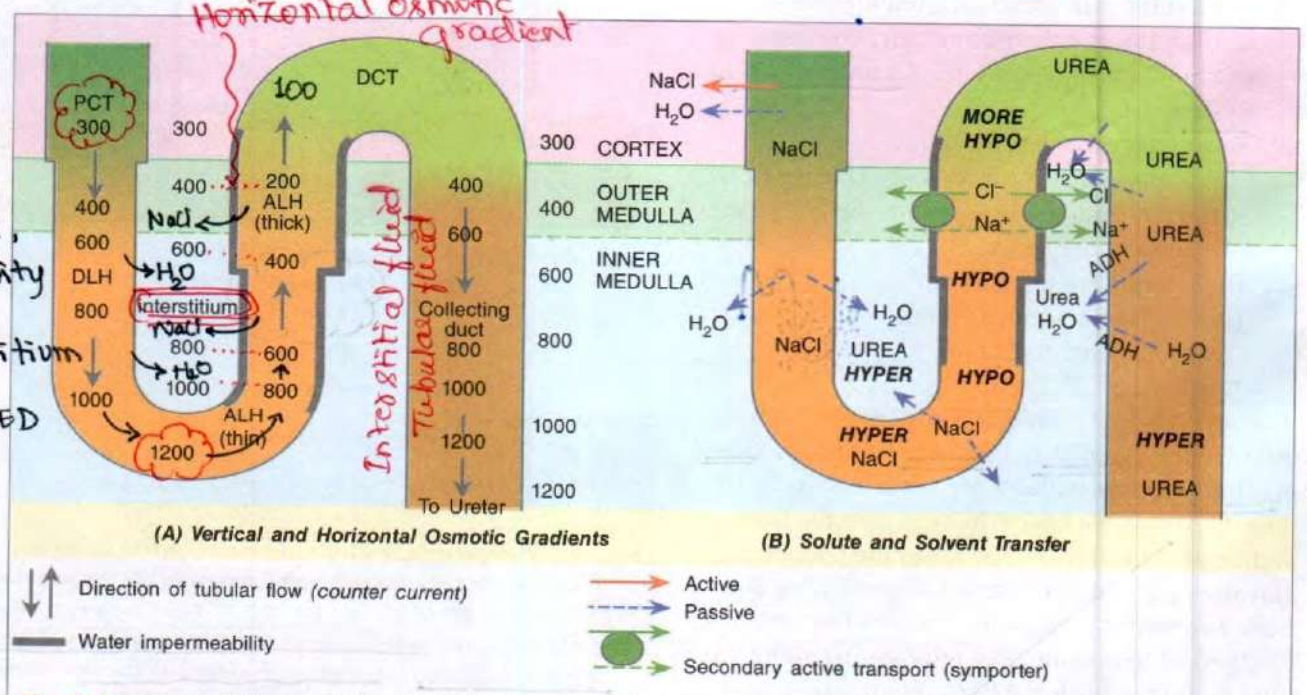


Fig. 58.2 Summary of changes in the Osmolality (in mOsm/L) of tubular fluid in various parts of the Juxtamedullary nephron
ALH and DLH: Ascending and descending loop of Henle

(ii) The *thick segment* is
- impermeable to water and solute, but
- actively transports NaCl out of the lumen into interstitium by $Na^+ - K^+ - ATPase$ and $Na^+ - 2Cl^- - K^+$ symporter. *(Secondary active transport)*

Therefore, as fluid passes through the ALH, NaCl diffuses out into the interstitium causing:

(a) interstitial fluid to become 'hyperosmotic' and

(b) tubular fluid becomes more and more 'hyposmotic', rich in urea, which flows into DCT and collecting ducts.

Active and passive transport of NaCl from ALH (thick and thin segments respectively) to the interstitium forms a *horizontal* osmotic gradient of upto 200 mosm/kg water between the tubular fluid of the ALH and the combined fluid of the interstitium and the DLH.

3. ***DCT and collecting duct***

A. *Cortical and outer medullary portion of DCT and collecting duct.*

The early part of DCT (in effect an extension of the thick segment of ALH) is relatively *impermeable to water*. However, the collecting duct segment is *impermeable to urea and NaCl, but permeable to water*. Therefore, as the fluid passes through this segment

(a) increased medullary interstitium osmolality causes urea-free water reabsorption from this segment into it, and

(b) urea concentration of tubular fluid increases markedly.

*Thus **urea** becomes the principal solute in tubular fluid entering the inner medullary collecting duct.*

B. *Inner medullary portion of collecting duct.*

In this portion *ADH increases both water and urea* permeabilities, therefore,

(a) Urea along with water moves out *passively* along its concentration gradient into medullary interstitium where it is trapped by counter current exchange in vasa recta, thus maintaining the high osmolality of the medullary pyramids.

(b) Removal of additional water under the influence of ADH results in the excretion of a low volume, hypertonic urine. (Also refer to page 529).

(c) *In the ADH absence*, the dilute tubular fluid entering the DCT and collecting tubule remains hypotonic and is excreted as a high volume, hypotonic urine.

In the renal medulla all the tubular structures (except the ALH) are in osmotic equilibrium with interstitium. Therfore, DLH acquires the increased osmolality of the surrounding interstitium. This effort is multiplied as new iso-osmolar filtrate arrives at the DLH and forces the concentrated tubular contents towards the tip of the loop of Henle.

Important Note

The greater the length of the loop of Henle, the greater the osmolality that can be reached at the tip of the pyramid.

Concentration of the fluid in this segment is further increased by the forceful active transport of Na^+ from the adjacent ALH. Therefore, *Counter Current System operates:*

(1) to establish an osmotic concentration gradient in the interstitium, increasing from the corticomedullary junction to the tip of the loop of Henle;

(2) to concentrate the urine by the passive extraction of water and urea through the collecting ducts.

C. COUNTER CURRENT EXCHANGERS

(Refer to **Fig. 58.3**) The osmotic gradient in the medullary pyramids would not last long if the NaCl and urea in the interstitium were removed by circulation. The concentrations of Na^+ and urea in the medullary interstitium are kept high by the slow blood flow in the vasa recta (page 510). These solutes remain in the pyramids to maintain hyperosmolality of the medullary interstitium primarily because the ***vasa recta** operates as counter current exchanger.* The arrangement of the descending and ascending limbs of the vasa recta in close proximity to each other functions to **maintain the hyperosmolality of the medullary interstitium**. How?

1. The *descending* vasa recta have a ***non-fenestrated*** endothelium that contains a facilitated transporter for urea; and the *ascending* vasa recta have a ***fenestrated*** endothelium that helps to conserve solutes.
2. NaCl and urea that have been passively reabsorbed from the loop of Henle and collecting tubule accumulate in the medullary interstitium, where they are absorbed by the descending limb of vasa recta and returned to the interstitium by the ascending limb of the vasa recta.
3. This counter current exchange 'traps' the solutes in the medullary interstitium and increases the medullary osmolality.
4. At the same time water diffuses from the descending limb of vasa recta into the interstitium and thus plasma

Blood flow ↓ ⇒ water uptake by ascending limb of vasa recta ↓

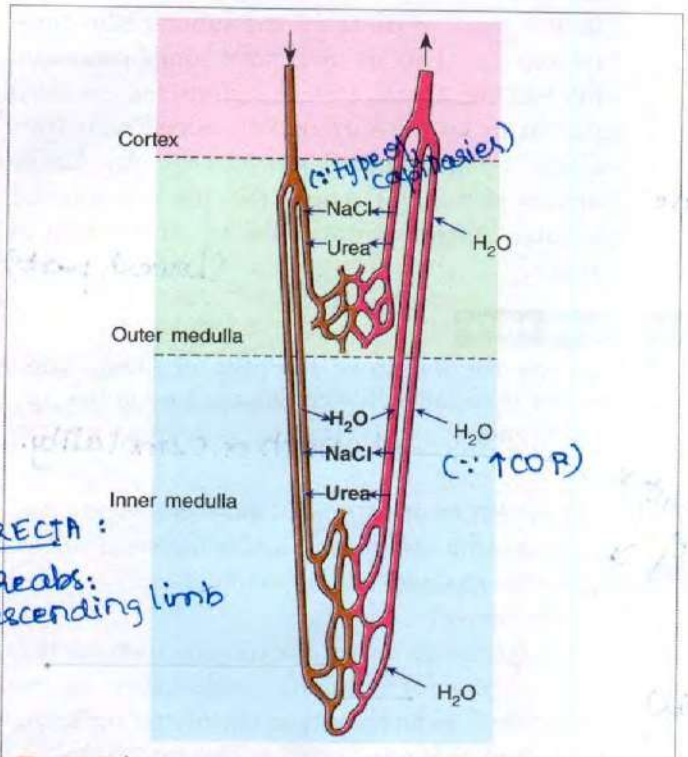


Fig. 58.3 The vasa recta function to maintain the hyperosmolality of medullary interstitium

protein concentration in the descending limb of vasa recta increases. In the ascending limb of vasa recta, the increase in oncotic pressure causes the capillaries to take in fluid. (H_2O)

In this manner, water reabsorbed by the nephron is removed from the interstitium and returned to the general circulation. Therefore, *the solutes tend to recirculate in the medulla and water tends to bypass it* so that medullary interstitium hyperosmolality is maintained. The faster is the flow the less effective is the bypass.

5. *The medullary osmolality is inversely proportional to medullary blood flow.* Therefore, if medullary blood flow increases, e.g. due to hemodilution or increase in ECFV, it
   (i) decreases medullary osmolality, and
   (ii) decreases water reabsorption.
   (i) and (ii) result in production of large volume of diluted urine. (DIURESIS)
   Conversely, decrease in medullary blood flow causes production of small volume of concentrated urine.

Important Note

Counter current exchange is a 'Passive Process'. It depends upon the diffusion of water and solutes in both directions across the permeable walls of the vasa recta. It could not maintain the osmotic gradient along the pyramids if the process of counter current multiplication in the loop of Henle were to cease.

D. ROLE OF UREA

1. The **main functions** of urea in the counter current system are:
   (i) to exert an osmotic effect on the DLH (descending limb of the loop of Henle);
   (ii) promoting the extraction of water, and
   (iii) raising the intraluminal concentration of NaCl.
2. *Urea increases urine osmolality* as follows **(Fig. 58.4)**:
   (i) The urea concentration of the tubular fluid is high when the fluid enters the inner medullary portion of the collecting ducts and urea passively diffuses into the *medullary interstitium* along with water where it is trapped by counter current exchangers in the vasa recta.

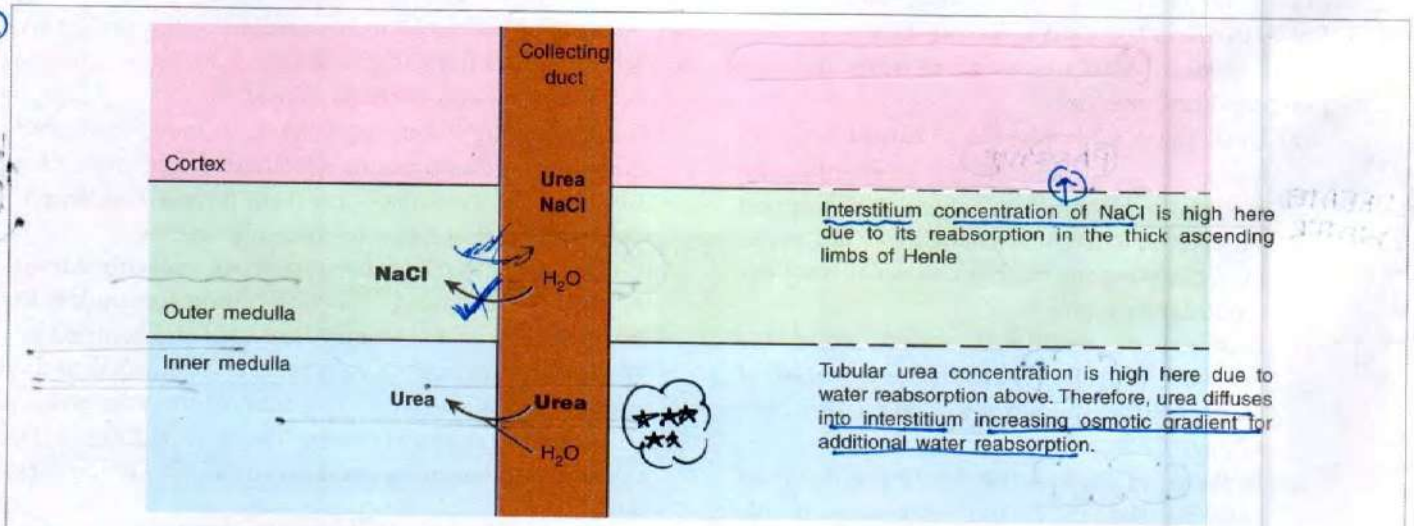


Fig. 58.4 The differences between the relative NaCl and urea concentrations in tubular fluid and the interstitium are represented by '**Type size**'

What is the driving force for NaCl reabs. in CT when although NaCl interstitium (medullary) is already high.

Note

Urea transport is mediated by four Urea transporter: UT–A1 to UT–A4, and all are regulated by ADH.

(ii) Increased urea concentration in the inner medulla exerts an osmotic effect on DLH causing *water to move out of DLH*. This increases NaCl concentration in DLH above that in the interstitium thus favouring passive reabsorption of NaCl from the ALH into the interstitium.

(iii) As urea is the principal solute in the tubular fluid of inner medullary collecting duct, this *establishes the gradient for urea and NaCl in the opposite direction i.e.* urea concentration in inner medullary collecting tubular fluid is higher than that in the interstitium and NaCl concentration in the collecting duct is lower than that in the outer medullary interstitium. (The high NaCl concentration in outer medullary interstitium is due to NaCl reabsorption from the thick segment of ALH).

(iv) Thus, despite equal osmolalities on both sides of the collecting tubules at the inner-outer medullary junction, the effective driving force for water transport favours water reabsorption.

Therefore, *urea adds to the effects of NaCl in creating a hyperosmolal state in the medullary interstitium.*

3. The amount of urea in the medullary interstitium and finally in the urine varies with the amount of urea filtered. Therefore, a *high protein diet* by increasing the filtered load of urea increases the ability of the kidney to concentrate the urine.
4. At low urine flow rates, only 20-30% of the filtered urea is excreted and at high urine flow rates approx. 50-70% of the filtered urea is excreted.

E. OTHER EXAMPLES OF COUNTER CURRENT SYSTEM

1. Heat exchanges between the arteries and venae comitantes of the limb (more in mammals living in cold water), therefore, heat is transferred from the arterial blood flowing into the limbs to the adjacent veins draining blood back into the body, making the tips of the limbs cold while conserving body heat (also see to page 586) **(Fig. 58.5)**.
2. There is 'counter current system' of villous blood vessels in the small intestine (page 380).

F. SUMMARY: IMPORTANT POINTS TO REMEMBER

1. The concentration ability of the kidney is directly related to the length of loop of Henle. The DLH is the concentrating segment of the nephron whereas the ALH is the diluting segment.
2. When *hypotonic urine* is produced, the hypotonicity of tubular fluid persists throughout the length of DCT. However, when *isotonic or hypertonic urine* is produced, the tubular fluid regains isotonicity by the last 3rd part of the DCT. Therefore, *tubular fluid at the end of DCT is either hypotonic or isotonic, but it is never hypertonic*. This shows that dilution of urine occurs before the DCT is reached while its concentration is achieved by the reabsorption of water from the tubular fluid as it traverses the collecting duct.
3. The osmolality of the interstitium increases down the cortico-medullary junction.
4. The medullary osmotic gradient decreases in *water diuresis* and increases when ADH is administered.
5. The osmolality of the urine produced is usually equal to the osmolality of the tissue at the tip of the renal papilla.
6. The production of concentrated urine depends on the relatively low renal medullary blood flow. Increase in medullary blood flow produces dilute urine.
7. The amount of water reabsorbed in the collecting duct depends on:
   (i) *Osmolality of the medullary interstitium:* This determines the magnitude of the force available for translocating water.
   (ii) *Circulating level of ADH:* The higher the plasma ADH level, the greater would be the concentration of urine formed and less its volume.

Note

Loss of functioning of nephrons or disruption of counter current mechanism result in fixed low specific gravity of urine (page 568)

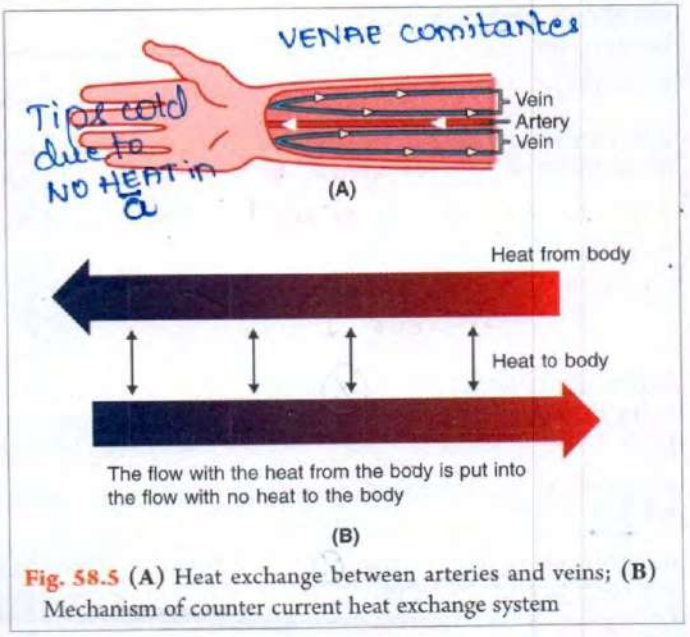


Fig. 58.5 **(A)** Heat exchange between arteries and veins; **(B)** Mechanism of counter current heat exchange system

(3) DIURESIS: WATER VERSUS OSMOTIC DIURESIS

Diuresis means increase in flow of urine (also refer to page 529). Differences between water and osmotic diuresis are given in Table 58.1 (also see to page 538).

(4) DIURETICS

Agents that cause increase in urinary flow are called *Diuretics*. (Normal urine flow is 1 mL/min or 1.5 L/day). These are often administered in clinical conditions such as oedema and hypertension in which ECFV is expanded. Some of the important features of various diuretics are given in Table 58.2.

Table 58.1: Water and Osmotic Diuresis compared

Water diuresis	Osmotic diuresis
1. It is *produced by* drinking large amounts (about 2% of body weight ≡ IL) of water or hypotonic fluid. It begins about 15 minutes after ingestion of a water load and reaches its maximum in 40 minutes.	1. It is *produced due to* presence of large quantities of unabsorbed solutes such as Na^+, glucose, urea etc. in the renal tubules when the filtered load exceeds the maximum capacity of the tubule to reabsorb them.
2. It is *characterized by* diuresis of dilute urine (50 mosmol/L) upto a volume of 20L/day.	2. It is *characterized by* diuresis in which concentration of urine is approximately that of plasma (300 mosmol/L) in spite of maximal ADH secretion and very large urine flow rates (>20L/day) can be produced.
Note The maximal urine flow that can be produced during water diuresis is 15 mL/min (page 538). If water is ingested at a higher rate than this, ECF become hypotonic and may lead to water intoxication (page 720).	
3. It is *produced due to* inhibition of ADH secretion secondary to decrease in plasma osmolality after water is absorbed. Therefore, amount of **water reabsorbed in the PCT of the nephron is normal**.	3. It is *produced due to* **decreased water reabsorption in the PCT and loop of Henle** secondary to: (i) administration of large amount of sodium, urea or mannitol. (ii) Substances present in amounts exceeding the capacity of tubules to reabsorb them *e.g.* glucose in DM.

Table 58.2: Diuretics—Salient features

Mode of action	Name of the agent (Trade name)	Remarks
1. Inhibition of ADH secretion	Water; Ethanol	• Produces water diuresis (see Table above)
2. Secretion of large quantities of osmotically active substances within renal tubules	Glucose; Mannitol	• Produces ***osmotic diuresis*** (see table above) • Clinical use—limited
3. Inhibition of action of ADH	V_2 (Vasopressin) receptors antagonist	• Used for research purposes
4. Inhibition/decreased renal tubular reabsorption of Na^+	Xanthines (Caffeine, theophylline)	• Clinically not used
5. Acidifying the tubular fluid	Acidifying salts ($CaCl_2$, NH_4Cl)	• Clinically not used
6. Carbonic anhydrase inhibitors causing decrease H^+ secretion	Acetazolamide (Diamox)	• Also produces increase in Na^+ and K^+ excretion and decrease HCO_3^- reabsorption (page 528) • *Extensively used clinically* • Hypokalemia is a major side effect
7. Inhibition of Na^+-K^+-$2Cl^-$ cotransport in the thick ascending limb of loop of Henle	• Furosemide (Lasix) • Ethacrynic acid (Edecrin) • Bumetanide (Bumet)	• Popularly called *loop Diuretics*. • *Extensively used clinically* • Hypokalemia, a major side effect
8. Inhibition of Na^+–Cl^- co-transport in the DCT	• Thiazides (Diuril, Esidrex)	• *Extensively used clinically* • Hypokalemia, a major side effect
9. Inhibition of Na^+-K^+ exchange in collecting ducts by inhibiting aldosterone or Na^+ channels (ENaCs)	• Spironolactone (Aldactone) • Amiloride (Midamor, Biduret)	• *Most commonly used diuretic in clinical practice* • K^+ sparing (retaining) diuretics, do not produce hypokalemia.

Study Questions

1. **Give physiological basis of:**
   (i) Countercurrent multipliers and exchangers
   (ii) Water and osmotic diuresis
   (iii) High protein diet increases the ability of kidney to concentrate the urine
   (iv) Renal medulla is sensitive to hypoxia damage
2. **Write short notes on:**
   (i) Counter current system in the body
   (ii) Role of urea in counter current system
   (iii) Loop diuretics
   (iv) Counter current exchange system
3. **How is hyperosmolality of the medullary interstitium is maintained?**
4. **What determines the amount of water reabsorption in the collecting tubules?**
5. **What determines the production of concentrated and diluted urine?**
6. **What will happen and why?**
   (i) If blood flow in the vasa recta increases
   (ii) to urine solutes concentration in overhydration and dehydration
   (iii) if medullary osmolality decreases
7. **Define counter current system. Give physio-clinical significance of its operation.**
8. **List the major classes of diuretics used clinically. Give their mechanism of action.**
9. **Draw diagram:**
   (i) Operation of a thermal counter current exchange system
   (ii) Urea increases urine osmolality

For water, in counter current exch. Recretion Reabs. into vasarecta occurs in its desc limb while, Reabs. → ascend limb. VICE-versa for solutes ⇒ Solutes remain in medullary interstit ⇒ water enters gen. circ

MCQs

1. **Kidney is capable of altering the urine osmolarity from ..... to ..... times the osmolar concentration of plasma:**
   (a) 1/8 to 2-3 (b) 1/6 to 4-5
   (c) 1/4 to 6-7 (d) 1/2 to 8-9
2. **Concentrating or diluting ability of urine depends upon functioning of all *except*:**
   (a) Loop of Henle (b) PCT
   (c) DCT (d) Collecting duct
3. **The fundamental process involved in excretion of a concentrated or diluted urine include all *except*:**
   (a) Variable permeability of nephron to water
   (b) Passive diffusion of urea into the medullary interstitium
   (c) Active reabsorption of NaCl by thin ascending limb of Henle
   (d) Active reabsorption of Na^+ by thick ascending limb of Henle
4. **Water permeability is maximum in which portion of the nephron:**
   (a) Descending limb of Henle (b) Thin ascending limb of Henle
   (c) Thick ascending limb of Henle (d) DCT
5. **The counter current multiplier system that maintain medullary interstitial osmolality is largely dependent upon NaCl to move out of:**
   (a) Thin descending limb of Henle (b) Thin and thick ascending limb of Henle (ALH)
   (c) Distal convoluted tubule (d) Collecting tubule
6. **Which of the following statement is *incorrect*?**
   (a) Countercurrent flow in the vasa recta minimizes solute loss from the medulla of the kidney
   (b) There is a net movement of water out of the descending loop of Henle
   (c) The thick ascending loop of henle is highly permeable to water
   (d) Blood flow through the vasa recta is very low
7. **The production of concentrated urine from glomerular filtrate:**
   (a) Is due to active reabsorption of water by the tubular epithelium
   (b) Is completed in the loop of Henle
   (c) Is dependent on anti-diuretic hormone
   (d) Increases progressively along the renal tubule

8. **In the nephron all is true, *except*:**
   (a) Fluid in the tip of the loop of Henle is hypertonic with respect to glomerular filtrate
   (b) Glomerular filtrate is hypertonic with respect to the fluid in the distal convoluted tubule
   (c) Antidiuretic hormone causes the fluid in the collecting ducts to be hypertonic with respect to that in the proximal convoluted tubule
   (d) The fluid at the end of proximal convoluted tubule is hypertonic with respect to glomerular filtrate
9. ***True* about counter current exchange process in the kidney:**
   (a) A passive process
   (b) Could maintain osmotic gradient across the pyramids alone
   (c) Depends upon diffusion of water out of vasa recta
   (d) Depends upon diffusion of solutes into the vasa recta
10. **Water diuresis differs from osmotic diuresis in that:**
   (a) It is produced due to presence of large quantities of unabsorbed solutes in renal tubules
   (b) Characterized by production of large volume of urine
   (c) Produced due to decreased reabsorption of water in PCT and loop of Henle
   (d) Charactrerized by diuresis of dilute urine
11. **Counter current system is mainly feature of:**
   (a) Cortical nephron
   (b) Juxtamedullary nephron
   (c) (a) and (b) both
   (d) Vasa recta
12. **Osmolar concentration of PCT fluid is:**
   (a) 300 mOsm/L
   (b) Less than osmolar concentration of plasma
   (c) More than osmolar concentration of plasma
   (d) Isosmotic
13. **Diluting segment of the nephron is:**
   (a) PCT
   (b) Descending segment of loop of Henle
   (c) Thick ascending segment of loop of Henle
   (d) Thin and thick ascending segment of loop of Henle
14. **Which of the following ion is *not* handled at loop:**
   (a) Na^+
   (b) K^+
   (c) Cl^-
   (d) Urea
15. **Normally a horizontal osmotic gradient of ..... mosmol/L exists between the ascending segment of loop of Henle and medullary interstitium:**
   (a) 100
   (b) 200
   (c) 300
   (d) 400
16. ***Not a true* statement about counter current system, it operates:**
   (a) To establish an osmotic concentration gradient in the interstitium
   (b) To concentrate the urine
   (c) To passively extract water through collecting ducts
   (d) To actively extract urea out of the tubules
17. **The hyperosmolarity of the renal medulla is due to increased content of:**
   (a) K^+
   (b) Na^+ and urea
   (c) Glucose
   (d) Na^+
18. **At high urine flow rate, what percentage of filtered urea is excreted?**
   (a) 20-30%
   (b) 40-50%
   (c) 50-70%
   (d) Above 70%

Answers

1. (b)	2. (b)	3. (c)	4. (a)	5. (b)	6. (c)	7. (c)	8. (b)	9. (a)	10. (d)
11. (b)	12. (a)	13. (d)	14. (b)	15. (b)	16. (d)	17. (b)	18. (c)		

Chapter

59

Acidification of Urine

I. Introduction
II. Renal Regulation of Acid-Base Balance
A. Introduction
B. Role of the Kidneys
C. Buffer Systems in the Kidney
D. Titratable Acidity
E. Excretion of H^+

1) Volatile acid = carbonic acid ≡ Taken care by LUNGS

2) Non-volatile = fixed = Non-carbonic ≡ KIDNEYS

INTRODUCTION

1. An ***Acid*** is a substance that acts as a proton (*i.e.* H^+) donor. The acids (such as hydrochloric and sulphuric acids) that are 100% ionized in solution are known as *strong acids*; (Eg: HCl) while carbonic and lactic acids, which do not completely ionize in solution, are ***weak acids***. The *acidity* of a solution refers to the free (unbound) H^+ concentration in the solution.
2. A ***Base*** is a substance that accepts protons (*i.e.* H^+) in solution e.g. bicarbonate ion (HCO_3^-), phosphate ion (HPO_4^{2-}), acetate ion (CH_3COO^-), proteinate$^-$, HbO_2^- and Hb^-. *Strong bases* are 100% ionized in solution while *weak bases* are only partially ionized.
3. The body produces large amounts of *acid* in two *forms*:
   (i) *Carbonic acid* (H_2CO_3) is called ***volatile acid*** because CO_2 can be formed from H_2CO_3 and, in turn can be eliminiated by the lungs, and
   (ii) *Non-carbonic acids* cannot be converted to CO_2 and, therefore, are called ***Non-volatile*** or ***fixed acids*** e.g H_2SO_4 (a product of protein catabolism), HCl, phosphoric acid (H_3PO_4, a product of phospholipid metabolism), ketoacids (aceto-acetic acid and β-hydroxy-butyric acid) and lactic acid.

(NH_4^+, HCl)

However, body fluids are maintained in an alkaline state (pH = 7.4).

4. *Sources of H^+* [LUNGS + KIDNEY] → Produc. → Removal
   (i) *As an end product of metabolism:* the greatest source of H^+ is CO_2 produced as end product of cellular metabolism, the amount of CO_2 produced during a day in a normal individual is capable of forming 20-40 mEq of H^+. ($Glu + O_2 \rightarrow H_2O + CO_2 \leftrightarrows H^+ + HCO_3^-$)
   (ii) *Diets* which are high in protein, generate between 50-100 mEq of H^+ per day.
   (iii) Renal tubular generation of H^+.
   (iv) Other sources of H^+ are the same as that of sources of non-carbonic acid (see below).
5. There are three *processes available for acid removal i.e.* for maintaining the concentration of H^+ within normal limits:
   (i) Combination of H^+ with:
      (a) a blood buffer e.g. HCO_3^-, haemoglobin; or
      (b) an intracellular buffer e.g. organic or inorganic phosphate
   (ii) Reduction of Carbonic acid (H_2CO_3) by elimination of CO_2 via respiration. ($H^+ + HCO_3^- \rightarrow H_2O + CO_2\uparrow$)
   (iii) Reduction of non-carbonic acid by renal elimination of H^+
6. ***Concept of pH, H^+ concentration and buffer system*** ***(page 31)***

RENAL REGULATION OF ACID-BASE BALANCE

A. INTRODUCTION

1. The kidneys are responsible for clearing the body of metabolically produced non-carbonic acids (see above). Therefore, normal urine reaction is acidic in nature.
2. ***Sources of non-carbonic acid:*** (also the sources of H^+, see above)
   (i) **Cellular metabolism** produces CO_2, a large part of which is expelled from the lungs and remaining gets hydrated with body fluids to form carbonic acid (H_2CO_3).
   (ii) **High protein diets** contain large amounts of phosphorus and sulphur; oxidation of these complexes forms anions of phosphate (PO_4^{3-}) and sulphate (SO_4^{2-}) which lead to formation of non-carbonic acid e.g. H_2SO_4 and H_3PO_4.

Respiratory { Acidosis: pH < 7.4 ; Alkalosis: pH > 7.4 } Range of change: 0.08

Henderson's eqn: $pH = pK + \log \frac{[Anion]}{[Salt]}$

(iii) **Waste products** of metabolism form acid phosphate ($H_2PO_4^-$). (Phosphate acidosis)

(iv) **Strenuous exercise** leads to formation of lactic acid from skeletal muscles. (Lactosis) [Cahill's cycle]

(v) Under some pathological conditions such as **starvation or uncontrolled diabetes mellitus** accumulation of organic acids such as acetoacetic acid and β-hydroxybutyric acid occurs. (Ketoacidosis)

(vi) **Ingestion of acidifying salts** e.g. NH_4Cl and $CaCl_2$ which in effect add HCl to the body.

(vii) **Renal tubular generation of H^+.** Therefore, in renal failure, failure to excrete normal acid load leads to acidosis.

3. In all body fluids, electrical neutrality must be maintained *i.e.* anions like phosphate or sulphate must be 'covered' by an equal amount of cations. This can only be achieved by daily excretion of the excess of anion (formed whether normally or otherwise) in the urine. If these anions which are excreted in the urine are fully 'covered' by an equivalent amount of cations (mainly Na^+), this will produce serious consequences. However, this situation is prevented due to manufacture of two important cations, H^+ and NH_4^+ by the kidneys which 'cover' the excreted anions.

B. ROLE OF THE KIDNEYS

1. The kidneys perform *two major functions:*
   (i) The kidneys stabilize the standard HCO_3^- pool by obligatory reabsorption (mainly by PCT) and by controlled reabsorption of filtered HCO_3^- (by the DCT and CT) (page 526).
   (ii) The kidneys excrete a daily load of 50–100 mEq of metabolically produced non-carbonic acid, which represents a H^+ excretion of 1 mEq/kg of body weight/day. (50kg person, 100 kg person)
2. *The major sites of urine acidification are the DCT and CT* since most of secreted H^+ in the PCT form CO_2 and H_2O from H_2CO_3. Essentially, all of the H^+ within the tubular lumen is from the tubular secretion of H^+ generated by metabolism. There is *no* significant contribution of H^+ from the glomerular filtrate, which accounts for <0.1 nmol of H^+ per day. (Buffer systems act at DCT & CT)
3. The amount of acid secreted depends upon the subsequent events in the tubular urine. The maximal H^+ gradient against which the transport mechanism can secrete H^+ (in humans) corresponds to a urine pH of about 4.4 *i.e.* a $[H^+]$ of 40×10^{-6} Eq/L. Since the plasma $[H^+]$ is 40×10^{-9} Eq/L, *the kidney can cause a 1000 fold $[H^+]$ gradient between the plasma and urine. Because the lowest pH attainable in urine* is 4.4, which is thus called the ***Limiting pH of Urine*** (*Davenport, H.W.*-1969). (Max. gradient for H^+ secretion corresponds to pH=4.4) (H^+ secretion-PCT)
4. If there were no buffers that 'tied up' H^+ in the urine, this pH would be reached rapidly and H^+ secretion would stop. However, three important reactions ***(Buffer Systems)*** in the tubular fluid remove free H^+, permitting more acid to be secreted.
5. The *major buffer systems* present in kidneys are:
   (i) *Bicarbonate system*
   (ii) *Dibasic phosphate system*, and
   (iii) *Ammonia system*

(URINARY) (Intracellular) * Plasma prot. ↳ * Hb.

C. BUFFER SYSTEMS IN THE KIDNEY

General (Also see page 31)

1. At normal blood pH of 7.4 (approx. that of the glomerular filtrate), since $pKH_2CO_3 = 6.1$, the ratio of $[HCO_3^-]/[H_2CO_3]$ is 20/1 for $pH = pk + \log [HCO_3^-]/[H_2CO_3]$...(page 29) (For carbonate buffer system)
   i.e. $7.4 = 6.1 + 1.3$
   $7.4 = 6.1 + \log 20$ (as $\log 20 = 1.3$)
   Thus, at a urinary pH of 6.1,
   $\log [HCO_3^-]/[H_2CO_3]$ is zero,
   and hence $[HCO_3^-] = [H_2CO_3]$
2. Similarly for $pK_{phos} = 6.8$
   i.e. $7.4 = 6.8 + \log [HPO_4^{2-}]/[H_2PO^{4-}]$ (For phosphate buffers)
   $7.4 = 6.8 + 0.6$
   $7.4 = 6.8 + \log 4$ (as $\log 4 = 0.6$)
   Correspondingly, the phosphate equilibrium in plasma is such that *four* parts of basic phosphate (HPO_4^{2-}) exist to *one* part of 'acidic' phosphate ($H_2PO_4^-$) at a pH of 7.4. Thus at a urinary pH of 5.8, ten parts of phosphate are in the *acidic form and one part in the basic form.*
   $(5.8 = 6.8 - 1)$ (as $\log 1/10 = -1$)
   For each phosphate ion excreted as $H_2PO_4^-$ one Na^+ saved and one H^+ is excreted.

#: Ideally, pH should be equal to pK

Important Note

The phosphate are by far most important buffer in the urine, although in acidic urine the formation of NH_4^+ from NH_3 and H^+ helps to keep the $[H^+]$ lower than it would otherwise be (see below).

A change of pH in the tubular fluid is achieved by a tubular transport system, described as under:

→ see 556.

I. ***Bicarbonate System:*** *Reaction with bicarbonate ions* (HCO_3^-) (**Fig. 56.9** page 526) ($pK = 6.1$)

1. The concentration of HCO_3^- in plasma and consequently in glomerular filtrate is (24 mEq/L whereas that of phosphate is only 1.5 mEq/L. The majority of secreted H^+ in the PCT reacts with HCO_3^- to form H_2CO_3 and is used to bring about

PCT: • Excretion of H^+ • Reabsorp of HCO_3^- : REABSORP.

HCO_3^- reabsorption. The CO_2 formed in the lumen from secreted H^+ returns to the tubular cell to form another H^+ and *no net* H^+ *secretion occurs.* (See chap 56)

2. Since most of the secreted H^+ is removed from the tubule, the pH of the tubular fluid is changed very little; as a result it does not contribute to the urinary excretion of acid.
3. ***The H^+ secreted in excess*** of those required for HCO_3^- reabsorption are excreted in the urine as *titratable acid*. These H^+ are buffered in the tubular fluid by the remaining other two buffer systems (see below).

II. ***Dibasic Phosphate System:*** *Reaction with Dibasic phosphate ions (HPO_4^{2-})* **(Fig. 59.1)**

1. Besides HCO_3^-, HPO_4^{2-} represents a major filtered conjugate base. Approx. 75% of the filtered HPO_4^{2-} is reabsorbed by the PCT, therefore, only 25% of the filtered HPO_4^{2-} is available for buffering in DCT and CT (because it is here that the phosphate which escapes reabsorption in the PCT gets concentrated by the reabsorption of water).
2. The H^+ secreted into the tubules, therefore, can react with filtered HPO_4^{2-} rather than the filtered HCO_3^-. Approx. 10-30 mEq (mmol) of H^+ per day are buffered by HPO_4^{2-}.
3. The exchange of H^+ for Na^+ converts *dibasic sodium phosphate* (Na_2HPO_4) in the glomerular filtrate into *acidic sodium dihydrogen phosphate* (NaH_2PO_4) and is excreted in the urine as ***Titratable Acid***.
4. *The PCT is the major nephron site where titratable acid is formed.* Additional titratable acid is generated along the collecting duct by a H^+-ATPase pump.

III. ***Ammonia System:*** *Reaction with Ammonia (NH_3)*

A. Secretion of Ammonia (NH_3)

1. Ammonia (NH_3) enters the tubular lumen NOT BY FILTRATION but by tubular synthesis and secretion, which normally is confined to the DCT and CT.
2. The **sources of NH_3** in renal tubular cells are:
   (i) 60% produced by deamination of amino acid 'glutamine', which comes mainly from the metabolism of amino acids in the liver.

$$\text{Glutamine} + H_2O \xrightarrow[\text{(abundant in renal tubular cells)}]{\text{Glutaminase}} \text{Glutamic acid} + NH_3$$

   (ii) 30% produced by deamination of glutamic acid

$$\text{Glutamic acid} \xrightarrow[\text{(GDH)}]{\text{Glutamic dehydrogenase}} \alpha\text{-ketoglutaric acid} + NH_3$$

   (iii) 10% produced by
   (a) deamination of other amino acids e.g. Asparagine, glycine, alanine etc. into α-ketoacids and NH_3.
   (b) Some NH_3 comes directly from arterial blood.
3. Most of the ammonium ion (NH_4^+) excreted in urine is produced in the PCT cells from amino acids, primarily glutamine. → Glutamate + NH_4^+ (PCT)

B. Formation of Ammonium ion (NH_4^+)

NH_3 passively diffuses out of tubular cells easily (because it is lipid soluble) along its concentration

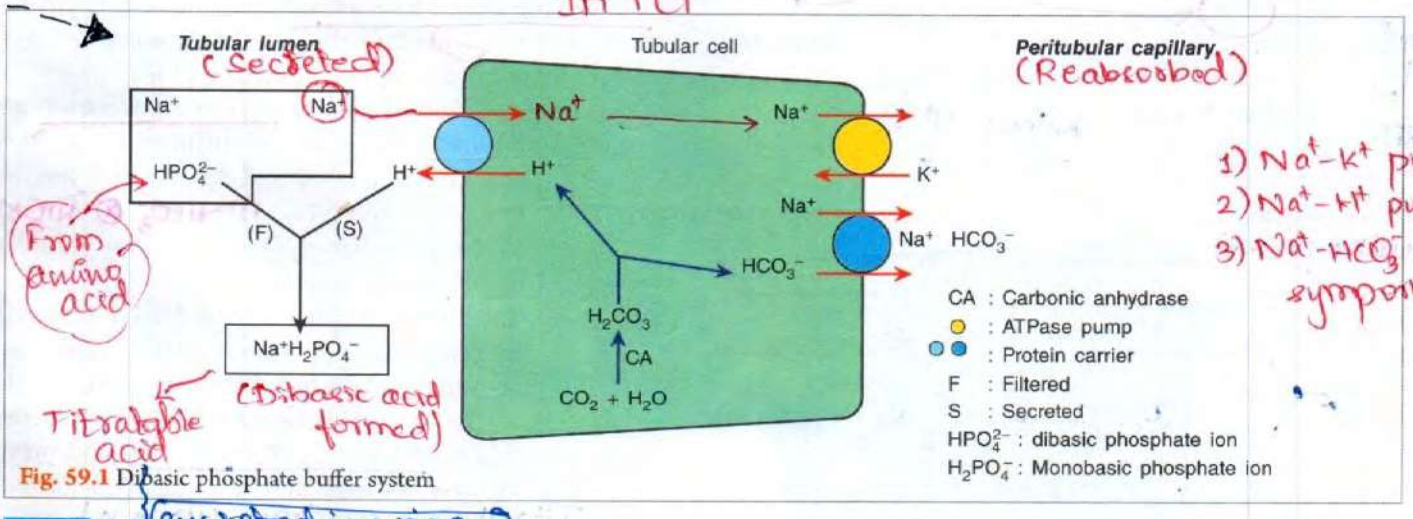


Fig. 59.1 Dibasic phosphate buffer system

Note

The HCO_3^- that is generated in the tubular cells and enters the peritubular capillary blood represents a *Net gain* of HCO_3^- by the blood, rather than merely a replacement of filtered HCO_3^- (compare with Fig 56.9 on page 526).

gradient, called ***non-ionic diffusion*** (page 16) of NH_3. In the lumen NH_3 combines with H^+ to form ammonium ion (NH_4^+). NH_4^+ is relatively lipid insoluble, therefore, stays in the lumen.

C. *Excretion of Ammonium ion (NH_4^+)*

1. The important physiologic characteristic of the ammonia system (NH_3/NH_4^+) is that, as H^+ is combined with intra-luminal buffer (NH_3), H^+ is excreted in urine as NH_4^+, a substance that *does not* cause the pH of urine to fall.
2. Ammonia system has a very high pK (about 9), which means that, at the usual urine pH of 6.0, practically all of the nonpolar NH_3 that enters the DCT lumen immediately combines with H^+ to form NH_4^+. Therefore, when urine pH is more than 6.0, NH_3 being lipid soluble and in gaseous form, diffuses to the other side of the membrane *i.e.* absorbed into the peritubular capillary. Thus, ammonium content of the urine is negligible until pH falls below 6.0. Ammonium excretion then increases linearly as the urinary pH falls below this value. *The renal excretion of NH_4^+ causes the net addition of HCO_3^- to the plasma.*
3. The amount of NH_4^+ formed depends upon the pH of the tubular fluid and the rate of NH_3 production. At any given rate of NH_3 production, the amount of NH_4^+ formed is proportionate to the amount of H^+ available and, therefore, to the rate of H^+ secretion. Thus, *the NH_4^+ content of an alkaline urine (pH more than 6.0) is nil whereas that of a maximally acid urine is high.*
4. *The Ammonium system assists the conservation of Na^+ and HCO_3^- by the body.*
5. Approx. 30-50 mEq (mmol) of H^+ per day are buffered by NH_3 and excreted as NH_4^+.
6. **Applied Aspect**
   In chronic severe *metabolic acidosis* (page 565), NH_3 serves as the major urinary buffer, and NH_4^+ excretion can increase from a normal value of 30 mEq per day to 500 mEq per day. How?
   During metabolic acidosis (*i.e.* accumulation of acid other than H_2CO_3 because CO_2 of H_2CO_3 can be breathed out) the NH_3 formation in kidney increases causing further removal of H^+ from tubular fluid and consequently further increase in H^+ secretion.

Causes of metabolic acidosis

(i) Diabetic acidosis leads to accumulation of acetoacetic acid and β-hydroxybutyric acid.
(ii) Exercise results in accumulation of lactic acid.
(iii) Renal failure
(iv) Starvation
(Also refer to **Table 60.3**, page 565)

D. TITRATABLE ACIDITY

1. H^+ secreted into the lumen that reacts with HCO_3^- is not excreted, whereas H^+ secreted into the lumen that reacts with a non-bicarbonate buffer remains in the tubular fluid and is excreted. (Also refer to page 552).
2. The renal contribution of *newly synthesized HCO_3^-* (page 526) is accompanied by the excretion of an equivalent amount of acid in the urine in the form of ***Titratable Acid***, NH_4^+ or both. Therefore, each H^+ that reacts with buffer organic anions other than HCO_3^- contributes to the urinary *Titratable Acidity*. It is measured by determining *the amount of alkali (in mmol/L) that must be added to the urine to return its pH to 7.4 i.e.* the normal pH of the glomerular filtrate.
3. Titratable acid is largely attributed to the conversion of dibasic phosphate ions (HPO_4^{2-}) to acidic phosphate ion ($H_2PO_4^-$). Therefore, this is a poor measure of the total amount of H^+ secreted by the tubules.
4. The titratable acidity obviously measures only a fraction of the acid secreted since it does not measure the H^+ that combines with HCO_3^- nor that which combines with NH_3.
5. *PCT is the major nephron site where titratable acid is formed.*
6. The titratable acidity (in mmol/L) multiplied by the urine volume (L/day) gives the amount of sodium conserved by the renal mechanism of H^+ secretion. In healthy man this is normally 20-30 mmol/day and may increase to 5 folds in severe diabetic acidosis.

E. EXCRETION OF H^+

The total amount of H^+ excreted daily by an individual with a normal diet equals the sum of titratable acid and NH_4^+ excreted *i.e.* approx.

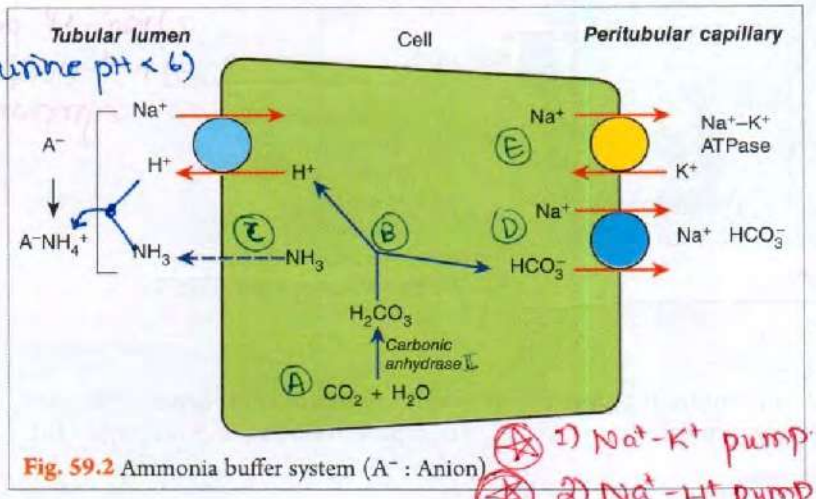


Fig. 59.2 Ammonia buffer system (A^- : Anion)

40-80 mEq (mmol) of H^+ per day. It is only a minute concentration of free H^+ which normally exists in the final urine inspite of 4300 mEq (mmol) of H^+ secreted daily.

The normal urinary ratio of NH_4^+ to titratable acid is between 2.5 and 1. In diabetic ketoacidosis, this ratio, however, remains within the normal range. **(Table 59.1)**

Table 59.1: Urinary Acid Excretion in Health and disease (in mEq or mmol H^+/day)

Urinary Acid		Normal Excretion	Excretion in Diabetic ketoacidosis
Titratable Acid	(25%)	10-30	75-250
Ammonium ion	(75%)	30-50	300-500
Total		40-80	375-750

Study Questions

1. **Write short notes on:**
   (i) Limiting pH of urine
   (ii) Titratable acidity and its significance
   (iii) Major buffer systems in kidney
   (iv) Volatile and non-volatile acid.
   (v) Renal regulation of acid base balance
   (vi) Buffer system in the kidney
2. **Give the sources of production of:**
   (i) H^+ in the body
   (ii) NH_3 in the renal tubular cells.
3. **Outline the process involved in the secretion of H^+ into the renal tubules.**
4. **Name the processes by which acid is removed from the body.**
5. **Give physiological basis of:**
   (i) Urine reaction is acidic in nature
   (ii) Major sites of urine acidification are the DCT and CT
   (iii) PCT is the major site where titratable acid is formed.
6. **Draw diagrams:**
   (i) Dibasic phosphate system
   (ii) Ammonia buffer system

MCQs

1. ***False*** **about volatile acid:**
   (a) Forms carbonic acid from CO_2
   (b) Forms CO_2 from carbonic acid
   (c) H_2CO_3 is an example
   (d) Can be eliminated by the lungs
2. **Greatest source of H^+ in the body is:**
   (a) End product of cellular metabolism
   (b) High protein diet
   (c) Synthesized by renal tubular cells
   (d) Waste product of metabolism
3. **Process *not* involved in maintaining normal H^+ concentration of the body:**
   (a) Elimination of CO_2 via respiration
   (b) Reduction of non-carbonic acid by renal elimination of H^+
   (c) Blood buffer e.g. organic phosphates
   (d) Intracellular buffers
4. **Cations manufactured by the kidney to cover the excreted anions in urine are:**
   (a) H^+ and Na^+ (b) NH_4^+ and H^+ (c) Na^+ and H^+ (d) K^+ and H^+
5. **The maximal H^+ gradient against which transport mechanism can secrete H^+ corresponds to a urine pH of:**
   (a) 4.4 (b) 4.8 (c) 5.2 (d) 6.0
6. ***Not true*** **statement regarding titratable acid:**
   (a) PCT is the major nephron site of its formation
   (b) Some of it is also generated in collecting tubules by H^+-ATPase pump
   (c) Measured by determining the amount of alkali that must be added to urine to return its pH to pH of glomerular filtrate
   (d) Each H^+ that reacts with HCO_3^- contributes to its formation

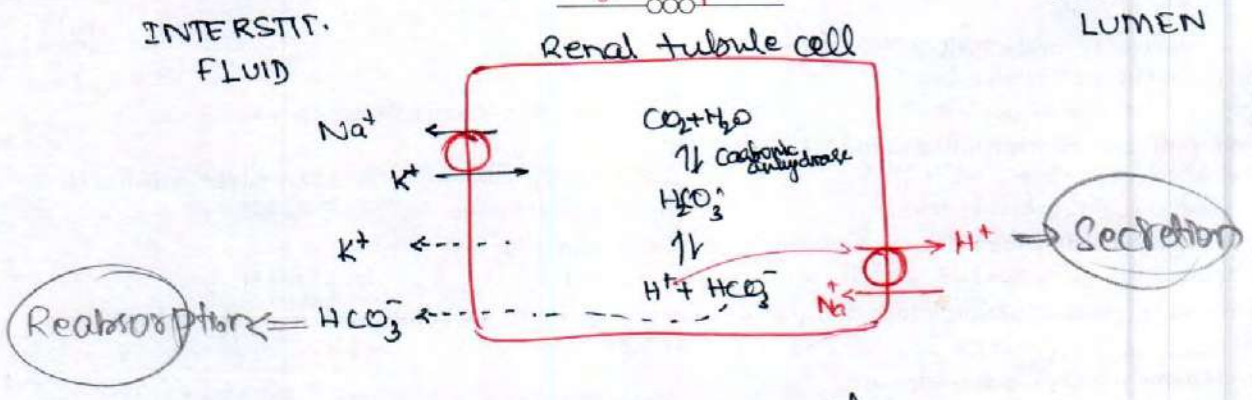
7. **Major source of ammonia production in renal tubular cells is by:**
   (a) Deamination of glutamine
   (b) Deamination of glutamic acid
   (c) Deamination of other amino acids: glycine, asparagine, alanine
   (d) Comes directly from arterial blood
8. **Body fluid pH depends primarily on the renal control of:**
   (a) Reabsorption of sodium in exchange for potassium
   (b) Secretion of H^+ in exchange for ammonia
   (c) Secretion of H^+ in exchange for potassium
   (d) Secretion of H^+ in exchange for sodium
9. ***Not a true* statement regarding fixed acids:**
   (a) Also called non-volatile acids or non-carbonic acids
   (b) Cannot be converted to CO_2
   (c) Can be formed from H_2CO_3
   (d) Ketoacids is an example
10. **Which is *not* a source of production of non-volatile acid in the body?**
   (a) Ingestion of acidifying salts
   (b) Starvation
   (c) High carbohydrate diet
   (d) Renal tubular generation of H^+
11. **Renal failure results in:**
   (a) Acidosis
   (b) Alkalosis
   (c) Dehydration
   (d) Overhydration
12. **Kidney can cause ..... fold $[H^+]$ gradient between the plasma and urine:**
   (a) 10
   (b) 100
   (c) 1000
   (d) 10000
13. **The buffer system in the kidney to excrete H^+ is all *except*:**
   (a) Bicarbonate
   (b) Dibasic phosphate
   (c) Ammonia
   (d) Urate
14. **What percentage of filtered dibasic phosphate ions (HPO_4^{2-}) is available for buffering?**
   (a) 25%
   (b) 50%
   (c) 75%
   (d) 100%
15. **The pK of ammonia buffer system is:**
   (a) 6.1
   (b) 6.8
   (c) 7.4
   (d) 9.0

Answers

1. (a)	2. (a)	3. (c)	4. (b)	5. (a)	6. (d)	7. (a)	8. (d)	9. (c)	10. (c)
11. (a)	12. (c)	13. (d)	14. (a)	15. (d)					

HCO_3^- buffer system:

- $Na^+ - K^+$ pump
- $Na^+ - H^+$ pump
- HCO_3^- uniporter



* Secretion of acid by PCT

#: In DCT & CT, Intercalated cells

#: K^+ balance → (3rd)
Na^+ balance → (5th)

Chapter 60

Regulation of Volume and Concentration of Body Fluids

(Kidney homeostasis)

TBW : ICF : ECF = 3 : 2 : 1 = 42 : 28 : 14

(3L) Plasma
(1L) Transcellular
(very less) Interstit.

I. Regulatory Mechanisms
A. Introduction — B. Defence of Tonicity
C. Defence of Volume — D. Defence of H^+ Concentration
II. Disturbance of Volume and Concentration of Body Fluids - dehydration, overhydration
III. Acid-base Abnormalities
A. Respiratory Acidosis and Alkalosis — B. Metabolic Acidosis and Alkalosis
IV. Anion Gap

Cholera → Hypovolemic shock → death

H_2O input: Food, Intake directly, Chem. reac. — This can change!!

Tbw maintenance by

REGULATORY MECHANISMS

A. INTRODUCTION

The details of 'body fluid compartments' (ECF, ICF etc.) are discussed in Unit I, *Body Water and Body Fluids* (page 27).

1. The compositions of the ECF and ICF differ from each other and are maintained in a steady-state condition by a variety of regulatory processes called ***Homeostatic Mechanisms*** *e.g.* the buffering properties of the body fluids and the renal and respiratory adjustments to the presence of excess acid or alkali are examples of homeostatic mechanisms. (Also refer to pages 4, 507)
2. The composition of the ECF is maintained by the CVS, respiratory, renal, gastrointestinal, endocrine and nervous systems acting in a coordinated fashion.
3. The composition of the ICF is maintained by the cell membrane which mediates the transport of material between the ICF and ECF.

Given here are the major homeostatic mechanisms that operate, primarily through the kidneys and the lungs, to maintain the *tonicity*, the *volume* and the specific ionic composition, particularly the H^+ *concentration of the ECF.*

H_2O output: → Insensible loss (Skin, Lungs) (1000 mL) → Sweat (Few mL - 1 L) → Faeces, URINE (1-2 L) — This can change!

B. DEFENCE OF TONICITY

108-200 mL

Normal plasma osmolality is: 280-295 mosm/ L (Average 290 mosm/L) (page 17). Defence of tonicity is primarily the function of
(1) ADH (vasopressin, page 673), and
(2) Thirst Mechanism (page 1008).

direction of movem.

$$\text{Total body osmolality } \alpha \frac{\text{Total body } Na^+ \textit{ plus } \text{Total body } K^+}{\text{Total body water (TBW)}}$$

Therefore, total body osmolality changes if there is a disproportion between the amount of these electrolytes and the amount of water ingested or lost from the body. Mechanisms defending ECF tonicity are summarized in **Fig. 60.1.**

The intensity of thirst and ADH secretion is directly proportional to the plasma osmolality. ∝ Thirst, ADH

Significant changes in ADH secretion occur when plasma osmolality is changed as little as 1%. Therefore, the osmolality of the plasma in normal individuals is maintained very close to 290 mosm/L.

C. DEFENCE OF VOLUME [Na^+ Balance]

Volume of the ECF is determined by two mechanisms:
1. by the plasma osmolality primarily, and
2. by control of water excretion through:
   (i) ADH; GFR; Thirst mechanism; Urine output;
   (ii) Angiotensin II, and
   (iii) Atrial Natriuretic Peptide (ANP).

1. ***Plasma Osmolality*** *i.e.* the total amount of osmotically active solute in the ECF (page 17). The Na^+ and Cl^- are predominant osmotically active substances in the ECF. Since changes in Cl^- are mainly secondary to changes in Na^+, therefore, the amount of Na^+ in ECF is the most important determinant of ECFV. Thus, the mechanisms that control Na^+ balance are the major mechanisms defending ECFV (Refer to page 524).

2. ***Control of Water Excretion***

(i) *ROLE OF ADH*

In general, increase in ECFV inhibits ADH secretion whereas decrease in ECFV stimulates it. However, *major stimuli for ADH secretions are:* plasma hyperosmolality & Hypovolemia.

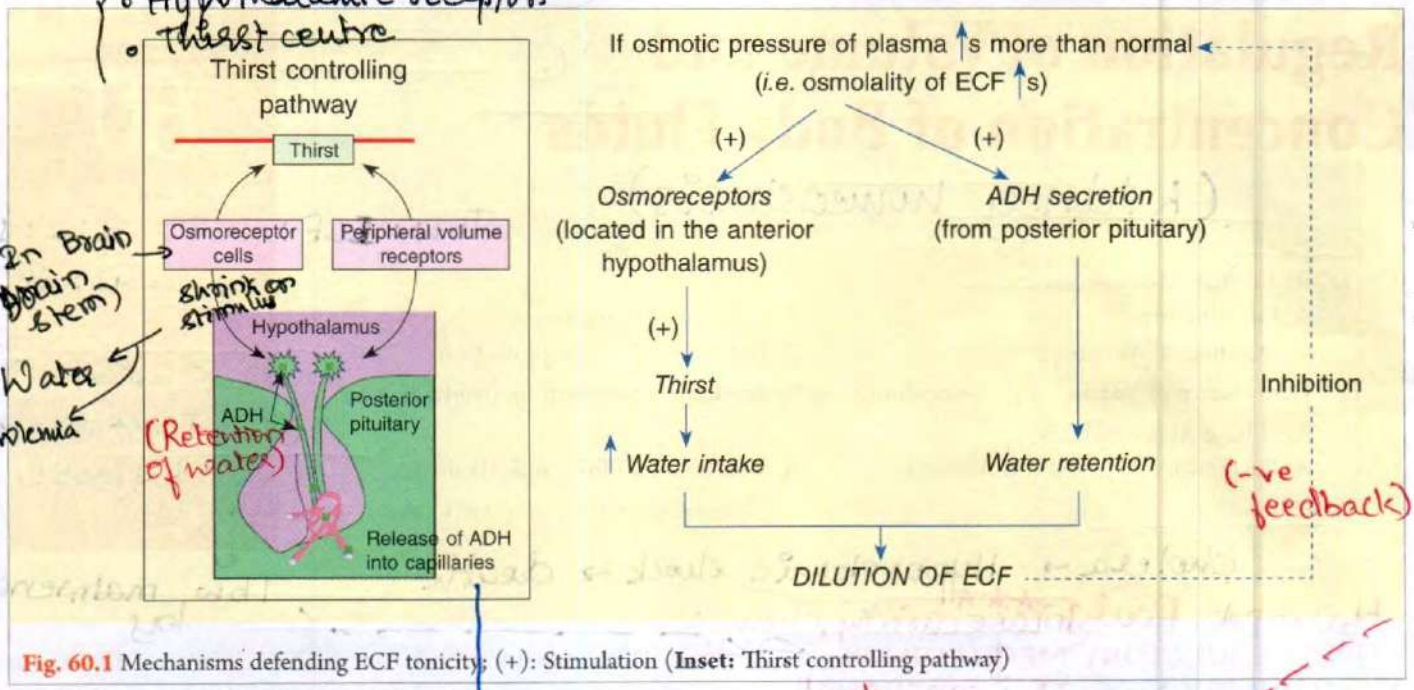


Fig. 60.1 Mechanisms defending ECF tonicity; (+): Stimulation (**Inset:** Thirst controlling pathway)

and hypovolemia. Therefore,

(a) 1% to 2% *increase in plasma osmolality* → directly stimulate *Osmoreceptors* (located in the anterior hypothalamus); and

(b) 10% *decrease in effective circulating blood volume* → decreases firing from *Baroreceptors* (venous and arterial).

(a) and (b) → ↑ ADH secretion

↓

↑ plasma ADH

↓

↑ permeability of DCT and collecting tubules to water

↓

↑ *solute free* water reabsorption

↓

↓ water excretion.

(For details of ADH actions, refer to page 673)

Important Note

Since the plasma [Na^+] accounts for 95% of the effective osmotic pressure, therefore, in general, the plasma [Na^+] is the primary determinant of ADH secretion. In states of hypovolemia, however, the *volume stimuli* become dominant over the osmotic regulation of ADH secretion; because, decrease in blood volume without an alteration in the tonicity of body fluids may cause ADH release.

(ii) *ROLE OF ANGIOTENSIN II* (FIG. 60.2)

Haemorrhage, diuretic administration, salt depletion, acute hypotension; chronic disorders associated with oedema *e.g.* cirrhosis with ascites, CCF (congestive cardiac failure) and nephrotic syndrome

↓

↓ in Effective circulating blood volume

↓

↑ Renin secretion from JGA

Renin-Angiotensin System

↑ Angiotensin II formation (page 506)

↓

(i) Generalised vasoconstrictor effect

(ii) Stimulate secretion and synthesis of Aldosterone from the adrenal cortex → ↑ Na^+ reabsorption in DCT and CT

(iii) Stimulate ADH secretion → ↑ solute-free water reabsorption (see above)

(iv) Stimulate thirst mechanism → ↑ water intake

(ii), (iii) and (iv) help to increase ECFV, therefore, Angiotensin II plays a key role in the body response to hypovolemia

Important Note

Difference between Aldosterone and ADH in regulation of ECFV is that:

By restricting the renal excretion of Na^+, which is the main determinant of plasma osmolality, aldosterone regulates the ECFV. Therefore, *Aldosterone regulates the total body Na+ content; while ADH regulates the plasma Na+ concentration by conserving total body water.*

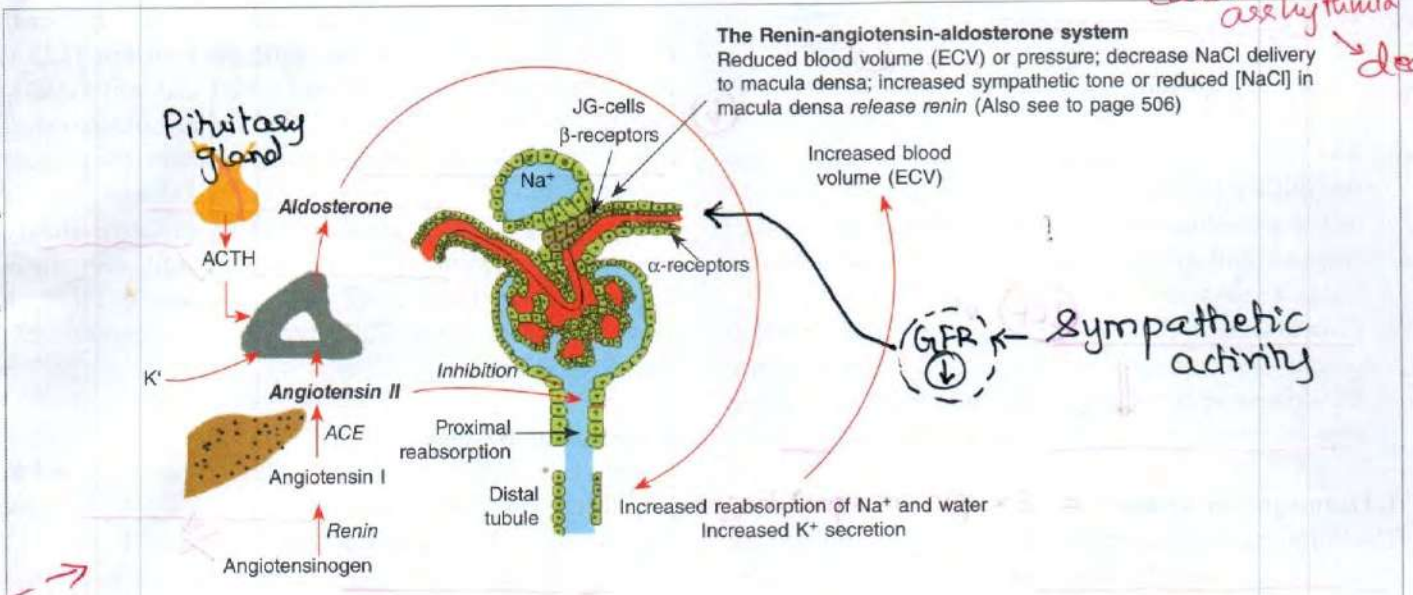


Fig. 60.2 Renin-angiotensin-aldosterone system (ACE: Angiotensin converting enzyme)

(iii) *ROLE OF ATRIAL NATRIURETIC PEPTIDE (ANP)*

ANP refers to a group of polypeptides produced by the atrial muscle cells that increases the urinary excretion of sodium. ANP is secreted when NaCl intake is increased and/or increase in ECFV.

↑ ECFV → stimulate atrial stretch receptors in the right atrium → ↑ Secretion of ANP → (a) Natriuresis and (b) Diuresis. How? ↑ ANP →

(a) Efferent arteriolar constriction → ↑ Glomerular capillary pressure → ↑ GFR → *Natriuresis* (Page 517).

(b) Afferent arteriolar relaxation →
- ↑ Hydrostatic pressure at JGA
- ↑ in NaCl delivery to macula densa
- also ↑ glomerular capillary pressure

(i) and (ii) → ↓ Renin Secretion → ↓ Angiotensin II →
- ↓ Aldosterone → ↓ Na^+ reabsorption → *Natriuresis*.
- ↓ ADH → Diuresis

Note

ANP inhibits renin secretion. It also antagonizes the action of many vasoconstrictor agents and thus decreases arterial B.P.

D. DEFENCE OF H^+ CONCENTRATION

Functioning of a cell is very sensitive to changes in its H^+ concentration. Intracellular H^+ concentration is different from extracellular pH which in turn is dependent upon ECF H^+ concentration (Also refer to page 31).

The intra and extracellular pH are generally maintained at very constant level *e.g.* the pH of the ECF is 7.4 and in health, this value varies less than ± 0.05 pH unit. Body pH is stabilized by the *Buffering Capacity* of the body fluids. The principal buffers in the body fluids and ***body buffer systems*** are given in **Table 60.1**.

Table 60.1: The principal buffers in the body fluids i.e. 'Body Buffer Systems'

1. Whole Blood	(i) Haemoglobin system ($H Hb \rightleftharpoons H^+ + Hb^-$)
	(ii) Protein System ($H Prot \rightleftharpoons H^+ + Prot^-$)
	(iii) Carbonic Acid-Bicarbonate System ($H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$)
2. Interstitial Fluid	(i) Carbonic Acid-Bicarbonate System ($H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$)
3. Intracellular Fluid (ICF)	(i) Protein system
	(ii) Phosphate system ($H_2PO_4^- \rightleftharpoons H^+ + HPO_4^{2-}$)

Important Concepts

1. The bicarbonate buffer system does not function as a buffer for carbonic acid.
2. The non-bicarbonate buffer systems (haemoglobin, protein and phosphate) can buffer both non-carbonic and carbonic acids. Therefore,
   (i) Whole blood is an excellent buffering system because of its non-bicarbonate and bicarbonate buffer systems. *More than* 90% of the blood's capacity to buffer carbonic acid is attributed to the haemoglobin buffer system.

(ii) Plasma which lacks haemoglobin has a considerable capacity for buffering non-carbonic acids but a much smaller capacity for buffering carbonic acid.

3. The $[HCO_3^-]$ of the interstitial fluid is higher than the $[HCO_3^-]$ of plasma. Therefore, the total capacity of the interstitial fluid to buffer non-carbonic acid is considerably greater than that of the total blood to buffer these acids.
4. Protein and organic phosphate compounds exist in quantitatively significant amounts in the ICF, giving this compartment the capacity to effectively buffer both non-carbonic and carbonic acids as well as alkali.

1. Haemoglobin System

The buffering action of haemoglobin is due mainly to the imidazole groups of the histidine residues. Haemoglobin molecule contains 38 histidine, plus it is present in large amounts; haemoglobin in blood has *'six' times the buffering capacity of the plasma proteins*. In addition, imidazole groups of deoxy-haemoglobin *i.e.* reduced haemoglobin (Hb^-) dissociate less than those of oxyhaemoglobin (HbO_2); therefore, Hb^- produces less H^+ at a given pH than does oxyhaemoglobin (HbO_2), making it a weaker acid and thus Hb^- becomes a more effective buffer when CO_2 (and hence H^+) are added from the tissues.

2. Protein System

Plasma proteins are effective buffers, because both their free carboxyl (COOH) and their free amino (NH_3^+) groups dissociate (page 53), therefore,

$$RCOOH \rightleftharpoons RCOO^- + H^+$$

$$\text{Therefore, } pH = pK_{RCOOH} + \text{Log } \frac{[RCOO^-]}{[RCOOH]}$$

$$\text{and } RNH_3^+ \rightleftharpoons RNH_2 + H^+$$

$$\text{Therefore, } pH = pK_{RNH_3^+} + \text{Log } \frac{[RNH_2]}{[RNH_3^+]}$$

3. Carbonic Acid-Bicarbonate System ($H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$)

This system consists of H_2CO_3 (weak acid) and HCO_3^-. Carbonic acid (H_2CO_3) is only partially dissociated into H^+ and HCO_3^-. Therefore,

(i) if H^+ is added to a solution of H_2CO_3, the equilibrium shifts to the left and most of the added H^+ is removed from solution, and
(ii) if OH^- is added, H^+ and OH^- combine, taking H^+ out of solution.

However, the decrease is countered by more dissociation of H_2CO_3, and the decline in H^+ concentration is minimized.

Carbonic acid-bicarbonate system is one of the ***most effective buffer systems in the body,*** because the H_2CO_3 level in plasma is in equilibrium with the dissolved CO_2 ($H_2CO_3 \rightleftharpoons CO_2 + H_2O$); and the amount of dissolved CO_2 is controlled by respiration. In addition, the plasma concentration of HCO_3^- is regulated by kidneys.

The reaction $CO_2 + H_2O \rightleftharpoons H_2CO_3$ proceeds slowly in either direction unless the enzyme carbonic anhydrase (CA) is present. There is no "CA" in plasma, but there is an abundant supply in RBC, gastric acid secreting cells and renal tubular cells.

4. Phosphate System

The system $H_2PO_4^- \rightleftharpoons H^+ + HPO_4^{2-}$ has a pK of 6.8. In plasma, the phosphate concentration is too low for this system to be a quantitatively important buffer, but it is important intracellularly, and it frequently plays a significant role in the urine (page 553).

DISTURBANCE OF VOLUME AND CONCENTRATION OF BODY FLUIDS

GENERAL CONCEPTS

1. The clinical terms for volume abnormalities are dehydration and overhydration associated with decrease or increase in ECFV respectively.
2. ***Tonicity*** refers to the osmolality of a solution relative to plasma.
   (i) ***Isotonic solution*** means solution having the same osmolality as that of plasma. Such solutions do not change the volume of the cell. *Examples* of isotonic solutions are: 0.9% NaCl; 5% glucose; 20% urea; 10% mannitol.
   (ii) ***Hypotonic solution*** means osmolality of solution less than that of plasma. It causes a cell to swell, and if sufficiently dilute, to burst (lyse).
   (iii) ***Hypertonic solution*** means osmolality of solution more than that of plasma. It causes a cell to shrink (undergo crenation).
3. The ***isosmotic, hyperosmotic and hyposmotic*** refer to the osmolar concentration of the ECF in its new steady-state and are used to describe changes in volumes (*i.e.* dehydration and overhydration).
4. Both water loss and Na^+ loss are associated with a decrease in ECFV, which is determined by the amount of Na^+ in the body *i.e.* Na^+ content, *not by the Na^+ concentration* in the plasma.
   (i) A simple water deficit reduces the ECF and ICF proportionately;
   (ii) A NaCl deficit always decreases the ECFV.

Disturbances of volume and concentration of body fluids in dehydration and overhydration is given in **Fig. 60.3** and **Table 60.2.**

EFFECT OF HYDRATION & DEHYDRATION

Table 60.2: Disturbance of volume and concentration of body fluids in dehydration and overhydration (Also refer to Fig. 60.3)

Type of Change	Volume (L) ICF	Volume (L) ECF	Osmolality (mosm/L) ICF	Osmolality (mosm/L) ECF	Causes	Description
I. Dehydration						
1. *Isosmotic*	0	↓	0	0	• Haemorrhage • Burns → plasma loss • Vomiting • Diarrhoea	Initially loss of fluid from plasma get replaced from I.S. → No major change in ECF osmolality; therefore, *no change in ICFV.*
2. *Hyperosmotic*	↓	↓	↑	↑	Water deficit due to • ↓ intake • Diabetes mellitus • Diabetes insipidus • Alcoholism • Excessive sweating	Fluid loss from plasma → ↑ plasma osmolality → plasma draw fluid from I.S. → ↑ osmolality of I.S. → I.S. draws the fluid from ICF compartment, therefore, finally *both ICFV and ECFV decreases with ↑ osmolality.*
3. *Hyposmotic* (More danger)	↑	↓	↓	↓	Addison's disease → renal loss of NaCl due to adrenal insufficiency	– In. diarrhoea, water-borne disease Vomiting.
II. Overhydration						
1. *Isosmotic*	0	↑	0	0	• Oedema • ↑ I.V. Administration of isotonic NaCl	ECF: volume increases with normal osmolality ICF: both volume and osmolality normal
2. *Hyperosmotic*	↓	↑	↑	↑	• ↑ Administration of hypertonic fluids	↑ plasma osmolality → (i) water shifts from I.S. into plasma → ↑ plasma volume; (ii) NaCl to diffuse into I.S. → ↑ osmolality of ECF → water to move out of ICF (↓ ICFV) → ↑ ECFV Finally, therefore, ↑ *osmolality of both ICF and ECF*
3. *Hyposmotic*	↑	↑	↓	↓	• Ingestion of large volume of water • SIADH (syndrome of inappropriate ADH secretion, page 673)	Initially, ↑ plasma volume → ↓ plasma osmolality → Shift of water to I.S. → I.S. osmolality ↓s → Water enters ICF compartment Finally, *both ECFV and ICFV increases with ↓ osmolality*

I.S. : Interstitial space; ↑ : Increase; ↓ : Decrease; 0 : No change

ACID-BASE ABNORMALITIES

GENERAL

1. ***Acidosis (Acidemia)*** is an abnormal clinical condition caused by accumulation of acid (or loss of base) sufficient to decrease pH below 7.35 (or to increase the $[H^+]$) of blood in the absence of compensatory changes.
2. ***Alkalosis (Alkalemia)*** *i.e.* an abnormal clinical condition caused by the accumulation of base (or the loss of acid) sufficient to raise pH above 7.45 (or to decrease $[H^+]$) of blood in the absence of compensatory changes.
3. ***Disturbances of Acid-Base balance*** are given in **Table 60.3.**
4. Acid base disturbances of ***Respiratory Origin*** lead to secondary changes in blood $[HCO_3^-]$ by appropriate adjustment of the rate of H^+ secretion/excretion from the renal tubular cell into the tubular lumen.
5. Acid-base disturbances of ***Metabolic Origin*** lead to secondary adjustment of the CO_2 tension (arterial pCO_2) by changes in the rate of alveolar ventilation.

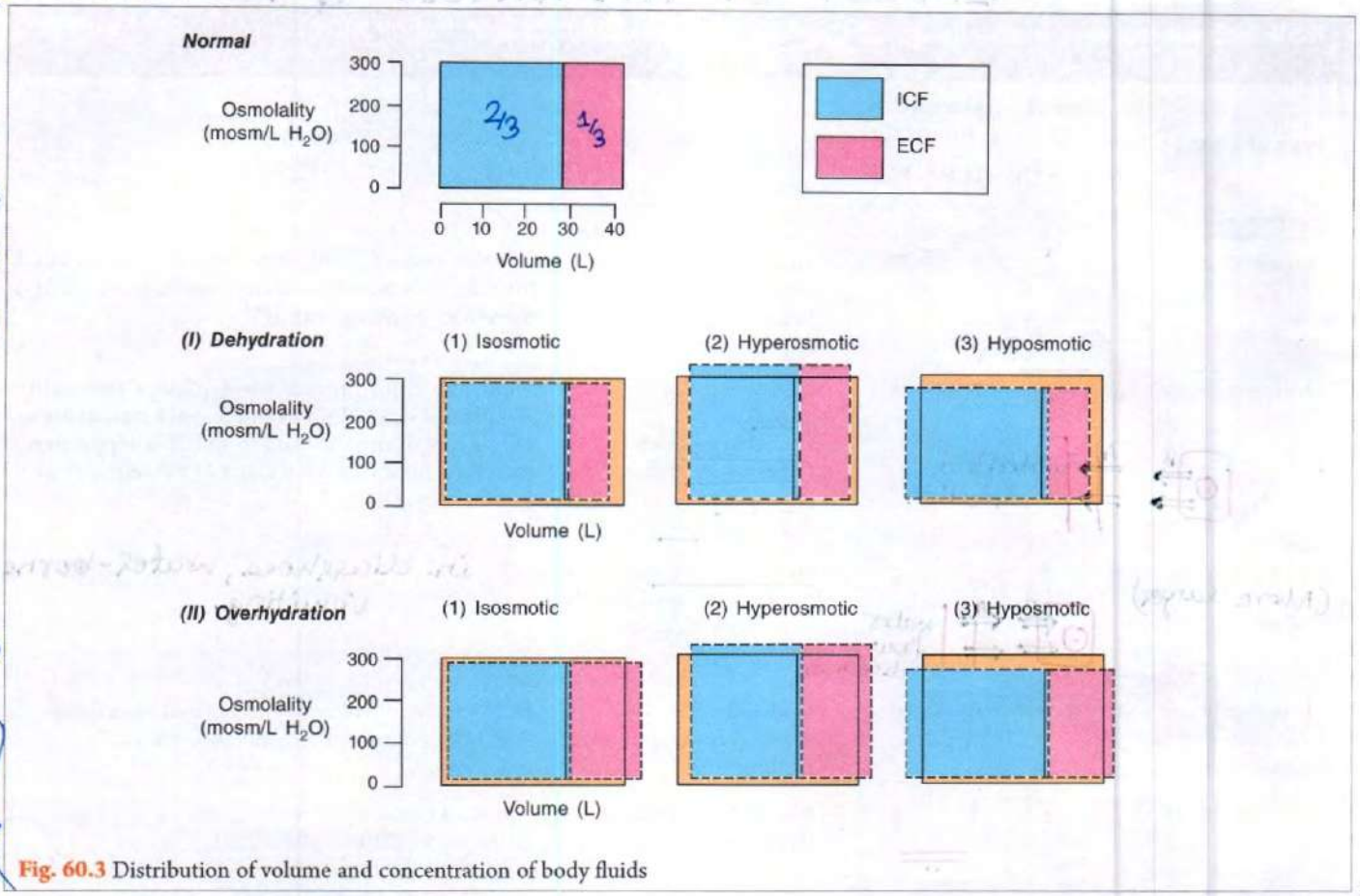


Fig. 60.3 Distribution of volume and concentration of body fluids

A. RESPIRATORY ACIDOSIS AND ALKALOSIS

Overall View

Respiratory disorder

↓

Changes arterial pCO_2

From Henderson-Hasselbalch equation for the bicarbonate system (pages 29 and 581)

↓

Changes $[HCO_3^-]$ / $[CO_2]$ ratio

↓

Changes arterial pH

Acid-Base Disturbances: Refer to Table 60.4

Renal Compensation: Refer to Fig. 60.4 and Table 60.5

1. HCO_3^- reabsorption in the renal tubules depends on
   (i) Filtered load of HCO_3^- (*i.e.* GFR × plasma HCO_3^- level)
   (ii) Rate of H^+ secretion by renal tubular cells.
2. Since HCO_3^- is reabsorbed by exchange for H^+, therefore, rate of H^+ secretion (and hence rate of HCO_3^- reabsorption) is directly proportional to arterial pCO_2 *i.e.* greater the amount of CO_2 that is available to form H_2CO_3 in the cells, the greater the amount of H^+ that can be secreted.

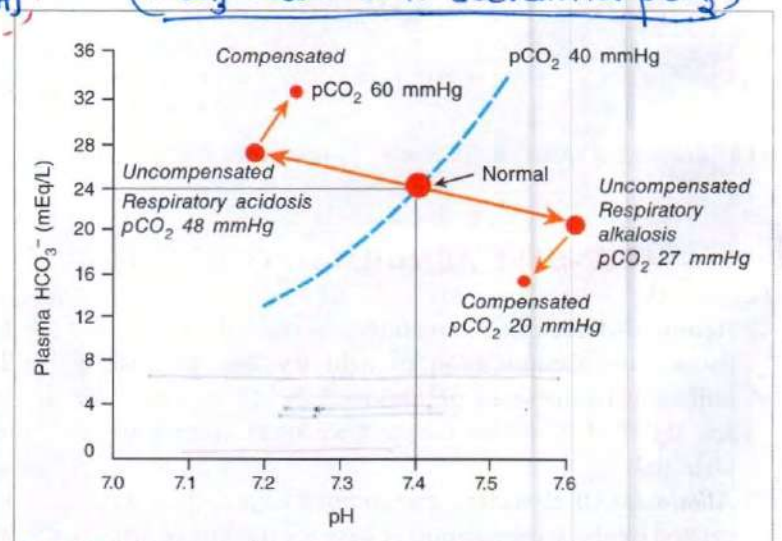


Fig. 60.4 Changes in plasma pH, HCO_3^- and pCO_2 in respiratory acidosis and alkalosis

Table 60.3: Disturbances of acid-base balance ↓: decrease; ↑: increase

Condition	Definition	Arterial plasma			Causes
		pH	HCO_3^- (mEq/L)	pCO_2 (mmHg)	
Normal	–	7.40	24	40	–
(1) *Respiratory Acidosis*	A disorder characterized by a reduced arterial pH (↑ $[H^+]$), ↑ CO_2 tension (*Hypercapnia*), and a variable ↑ in the plasma $[HCO_3^-]$	7.34	25	48	↓ in alveolar ventilation due to: • COPD (chronic obstructive pulmonary disease) • overdose of respiratory depressants • Emphysema • Breathing 7% CO_2
(2) *Respiratory Alkalosis*	A disorder characterized by an elevated arterial pH (↓$[H^+]$), a low CO_2 tension (*Hypocapnia*), and a variable ↓ in plasma $[HCO_3^-]$	7.53	22	27	↑ in alveolar ventilation due to: • Hyperventilation (Hyperpnoea)–voluntary or at high altitude • Anxiety, hysteria • Salicylate overdosage
(3) *Metabolic Acidosis*	A disorder characterized by a low arterial pH (↑ $[H^+]$) or a reduced plasma $[HCO_3^-]$	7.28	18	40	• Accumulation of Ketoacids (diabetes mellitus) or Lactic acid (Exercise) • Ingestion of - alcohol, NH_4Cl, salicylates • Loss of HCO_3^- : severe Diarrhoea, Fistula
(4) *Metabolic Alkalosis*	A disorder characterized by an elevated arterial pH (↓$[H^+]$) or an increased plasma $[HCO_3^-]$	7.50	30	40	• ↓ production or loss of acid via - kidneys or GIT (vomiting) • Ingestion of HCO_3^- or other base • Excessive renal reabsorption of HCO_3^- • Overtreatment with HCO_3^- or Lactate
	← *In uncompensated state* →				

Table 60.4: Acid-Base disturbances in respiratory acidosis and alkalosis compared

Respiratory acidosis	Respiratory alkalosis
↓ alveolar ventilation	↑ alveolar ventilation
↑ arterial pCO_2 (due to CO_2 retention)	↓ arterial pCO_2 (due to CO_2 washout)
↑ H_2CO_3	↓ H_2CO_3
(i) ↑ $[H^+]$ → ↓ pH (*e.g.* ≤ 7.2) (ii) ↑ $[HCO_3^-]$	(i) ↓ $[H^+]$ → ↑ pH (*e.g.* ≥ 7.6) (ii) ↓ $[HCO_3^-]$

Table 60.5: Renal compensatory mechanisms in respiratory acidosis and alkalosis

In Respiratory acidosis	In Respiratory alkalosis
(1) Renal tubular secretion of H^+ ↑ s, → removal of H^+ from the body → rise of pH *i.e.* pH corrected to normal	(1) Renal tubular secretion of H^+ ↓ s, → retention of H^+ in the body → fall in pH *i.e.* pH corrected to normal.
(2) ↑ HCO_3^- reabsorption in spite of ↑ plasma HCO_3^- → further ↑ in plasma HCO_3^- → ↑ Cl^- excretion → ↓ plasma Cl^-	(2) ↓ HCO_3^- reabsorption → ↑ HCO_3^- excretion → further ↓ s already low plasma HCO_3^-.

B. METABOLIC ACIDOSIS AND ALKALOSIS

Acid-Base disturbances and body compensation (respiratory and renal) during metabolic acidosis and alkalosis are summarised in Table 60.6 and Fig. 60.5.

ANION GAP

The concentration of anions and cations in plasma, must be equal to maintain electrical neutrality (page 29). Thus there is no 'anion gap' in the plasma. However, in clinical practice, only certain cations (Na^+) and anions (Cl^- and HCO_3^-) are normally measured. This results in the *'anion gap'*. Its normal value is: 12 mEq/L. How?

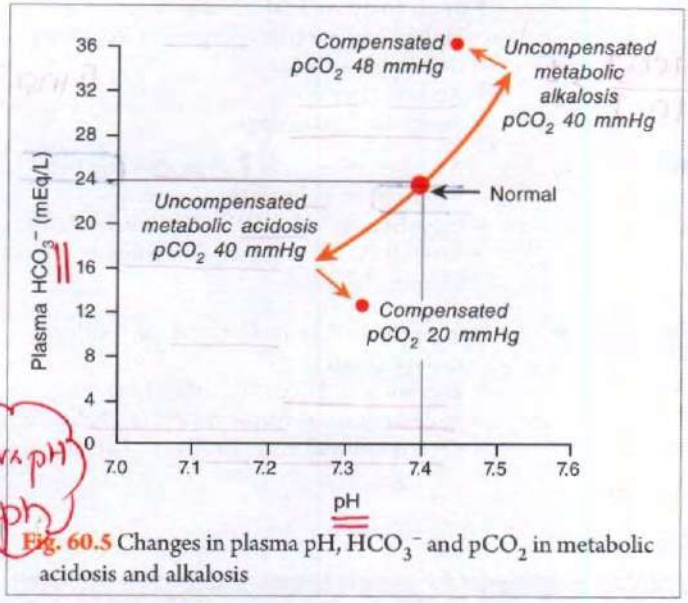


Fig. 60.5 Changes in plasma pH, HCO_3^- and pCO_2 in metabolic acidosis and alkalosis

Plasma Anion gap = $[Na^+] - [HCO_3^-] - [Cl^-]$
= 145 – 24 – 109 = 12 mEq/L

Clinically acid-base status (*specially metabolic acidosis* see above) can be diagnosed by assessing the difference between the concentration of cations (other than Na^+) and the concentration of anions (other than Cl^- and HCO_3^-) in the plasma (*Anion Gap*) (Fig. 60.6).

It includes proteins in the anionic form (dibasic phosphate ions: HPO_4^{2-}, sulphate ions: SO_4^{2-}) and organic acids.

The anion gap thus gets altered with the alteration in the concentration of *unmeasured anions* (such as albumin, phosphate, sulphate and other organic acids) and concentration of *unmeasured cations* (such as Ca^{2+}, Mg^{2+} and K^+). Conditions that can *alter* the anion gap are given in the Table below.

Anion gap: Increased in	Anion gap: Decreased in
1. Decrease in plasma concentration of K^+, Ca^{2+} or Mg^{2+}	1. Increase in cations
2. Increase in concentration of unmeasured anions essentially the plasma proteins.	2. Decrease in plasma albumin.
3. Accumulation of organic anions such as lactic acid, ketoacids This results in *metabolic acidosis*. (Page 565)	

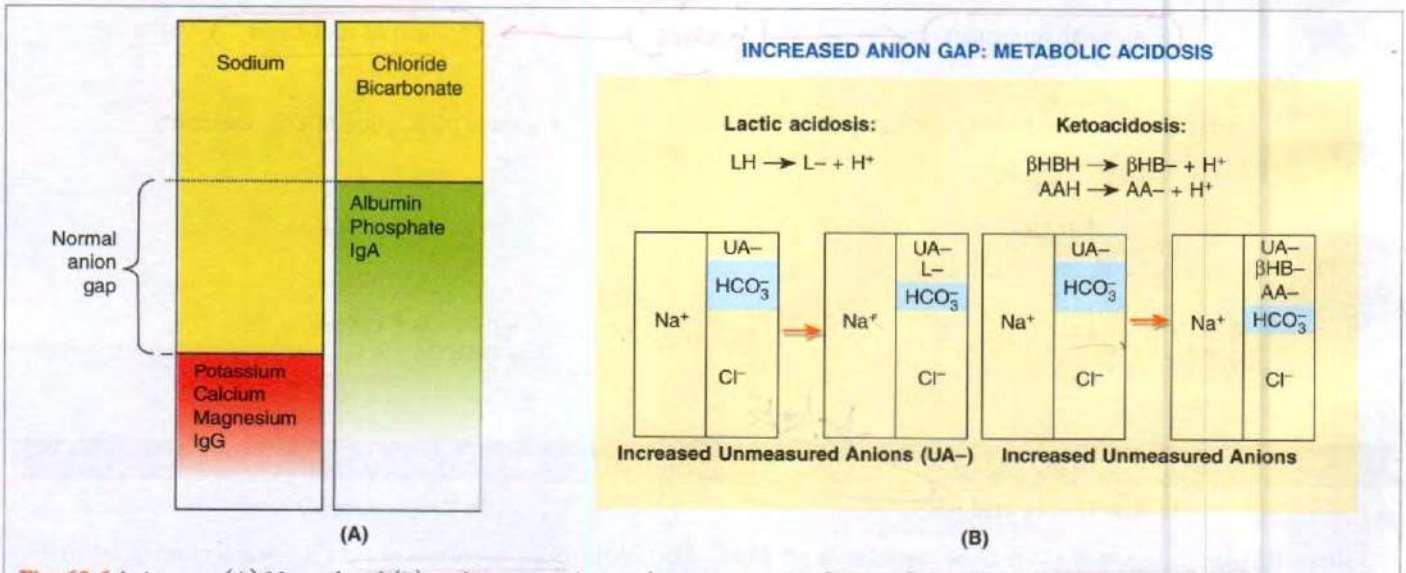


Fig. 60.6 Anion gap (A) Normal and (B) in disease conditions (LH: Lactic acid; βHBH: βhydrooxybutyric acid–ketoacid)

Table 60.6: Metabolic acidosis and alkalosis compared

Metabolic Acidosis	Metabolic Alkalosis
Changes Addition of acid or removal of base → ↑ free H^+ → ↓ pH → (1) H^+ is buffered forming H_2CO_3, and (2) Hb^-, $prot^-$, HCO_3^- level in plasma falls.	↑s plasma HCO_3^- level and ↑s pH
Compensation *A. Respiratory Compensation* ↑ in plasma $[H^+]$ → stimulate respiration → ↓ arterial pCO_2 → ↑ pH to normal.	*A. Respiratory Compensation* ↓ in plasma $[H^+]$ → ↓ in pulmonary ventilation → ↑ arterial pCO_2 → ↑ $H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$, bringing pH back to normal and elevating plasma HCO_3^- level further. The magnitude of this compensation is limited by the carotid and aortic chemoreceptor mechanisms, which drive the respiratory centre if there is any appreciable fall in the arterial pCO_2.
B. Renal Compensation It causes excretion of extra H^+. How? (1) The anions that replace HCO_3^- in the plasma are filtered, each with a cation (mainly Na^+), thus maintaining electrical neutrality. (2) The renal tubular cells secrete H^+ into the tubular fluid in exchange for Na^+; and for each H^+ secreted, one Na^+ and one HCO_3^- are added to blood. However, secreted H^+ reacts with buffer systems in the kidney (page 552), therefore, large amounts of H^+ can be secreted, permitting large amounts of HCO_3^- to be reabsorbed. (3) In chronic acidosis, glutamine synthesis in liver is increased and provides the kidneys with an additional source of NH_4^+. The respiratory compensation tends to inhibit the renal response, because decrease in pCO_2 hinders acid secretion, but it also decreases the filtered load of HCO_3^-, therefore, its net inhibitory effect is slight.	*B. Renal Compensation* It causes ↑ in HCO_3^- excretion. How? (1) More renal H^+ secretion is expended in reabsorbing the increased filtered load of HCO_3^- → acidic urine, however, if the HCO_3^- level in plasma exceeds 28 mEq/L, HCO_3^- appears in the urine (page 527). (2) The rise in pCO_2 inhibits the renal compensation by facilitating acid secretion, but its effect is relatively slight.

Study Questions

1. **Write short notes on:**
   - (i) Mechanisms maintaining: ECF tonicity, Volume and H^+ concentration
   - (ii) Body buffer system
   - (iii) Major stimuli for ADH secretion
   - (iv) Anion gap
   - (v) Principal buffers in the blood
   - (vi) Unique features of bicarbonate buffer system
   - (vii) Major sources of acid loads presented to the body in daily living.
   - (viii) ANP (Atrial Natriuretic peptide)
   - (ix) Function of angiotensin II
   - (x) Role of aldosterone and ADH in regulation of ECFV
   - (xi) Compensation in respiratory acidosis and alkalosis
   - (xii) Compensation in metabolic acidosis and alkalosis.
2. **Give physiological basis of:**
   - (i) Plasma Na^+ concentration is the primary determinant of ADH secretion
   - (ii) Angiotensin II plays important role in the body response to hypovolemia
   - (iii) Increase in ANP secretion leads to natriuresis
   - (iv) Haemoglobin is the main buffer system in the body.
3. **Describe briefs disturbances of volume and osmolality of body fluids in:**
   - (i) Isosmotic dehydration, and
   - (ii) Isosmotic overhydration.
4. **Give examples of homeostatic mechanisms.**
5. **Give the conditions producing disturbance in the acid base balance. Also give their biochemical picture.**
6. **Depict diagrammatically:**
   - (i) Renin angiotensin-aldosterone system
   - (ii) Changes in plasma pH, HCO_3^- and pCO_2 in respiratory acidosis and alkalism
   - (iii) Changes in plasma pH, HCO_3^- and pCO_2 in metabolic acidosis and alkalism

MCQs

1. **Changes in ADH secretion occurs significantly when plasma osmolality is changed as little as:**
   (a) 0.5% (b) 1.0%
   (c) 1.5% (d) 2.0%
2. **The most powerful feedback system for controlling plasma osmolality and sodium concentration is:**
   (a) ADH and thirst (b) Salt
   (c) Angiotensin-renin system (d) Countercurrent system
3. **Which of the following changes would *not* occur as a result of dehydration (loss of water, but *not* solute)?**
   (a) Increased secretion of antidiuretic hormone (b) Increased plasma sodium concentration
   (c) Decreased permeability of the collecting ducts to water (d) Increased solute concentration in the renal medulla
4. **Which of the following plays a key role in the body response to hypovolemia?**
   (a) ADH (b) Angiotensin II
   (c) Atrial natriuretic peptide (d) Aldosterone
5. **Atrial natriuretic peptide (ANP) causes:**
   (a) Increase in cardiac output (b) Increase in blood volume
   (c) Increase in urine output (d) Increase in renin secretion
6. **The principal buffers in the ECF are the following *except*:**
   (a) Haemoglobin (b) Protein
   (c) Phosphate (d) H_2CO_3
7. **Which of the following acts as an intracellular buffer?**
   (a) HCO_3^- (b) Cl^-
   (c) PO_4^{2-} (d) SO_4^{2-}
8. **Which is *not* an isotonic solution?**
   (a) 0.9% NaCl (b) 20% glucose
   (c) 20% urea (d) 10% mannitol
9. **Respiratory acidosis is characterized by:**
   (a) Decreased pCO_2 and decreased pH (b) Increased pCO_2 and decreased pH
   (c) Increased pCO_2 and increased pH (d) Decreased pCO_2 and increased pH
10. **Severe anxiety may cause:**
    (a) Respiratory acidosis (b) Respiratory alkalosis
    (c) Metabolic acidosis (d) Metabolic alkalosis
11. **If arterial plasma parameters read: pH 7.34, HCO_3^- 25 mEq/L and pCO_2 48 mmHg, the person is suffering from:**
    (a) Respiratory acidosis (b) Respiratory alkalosis
    (c) Metabolic acidosis (d) Metabolic alkalosis
12. **Severe diarrhoea may lead to:**
    (a) Metabolic acidosis (b) Metabolic alkalosis
    (c) Respiratory acidosis (d) Respiratory alkalosis
13. **Which is *not* a cause of isosmotic dehydration?**
    (a) Haemorrhage (b) Diarrhoea
    (c) Alcoholism (d) Burns
14. **Metabolic alkalosis associated with prolonged vomiting is primarily due to loss of:**
    (a) Sodium (b) Potassium
    (c) Chloride (d) Hydrogen ion
15. **Most important renal defence against respiratory alkalosis is:**
    (a) Increased excretion of HCO_3^- (b) Increased excretion of H^+
    (c) Decreased production of ammonia (d) Increased secretion of K^+
16. **Renal compensation for metabolic acidosis involves all of the following *except*:**
    (a) Increased tubular secretion of hydrogen ions (b) Activation of the tubular ammonia buffer system
    (c) Increased filtration of bicarbonate ions (d) Increased excretion of hydrogen ions in the urine
17. **Normal plasma osmolality is:**
    (a) 260 mosm/L (b) 270 mosm/L
    (c) 280 mosm/L (d) 290 mosm/L

18. **Which of the following generate thirst?**
    (a) Increased plasma osmolality (b) Increased tonicity of intracellular fluid
    (c) Increase in cell size (d) Increase in ECFV

19. **The acute control response to increased extracellular fluid volume involves all of the following *except*:**
    (a) Increased urine osmolarity (b) Decreased sympathetic stimulation of kidneys
    (c) Decreased secretion of ADH (d) Increased urine output

20. ***Not* an action of atrial natriuretic peptide:**
    (a) Natriuresis (b) Diuresis
    (c) Increases GFR (d) Decreases glomerular capillary pressure

21. **Buffering action of haemoglobin is mainly due to:**
    (a) Heme (Haem) (b) Porphyrin
    (c) Imidazole group of histidine (d) Histidine group of imidazole

22. **Hypertonic contraction of fluid volume is caused by:**
    (a) Addison's disease (b) Cushing's disease
    (c) Salt losing nephropathy (d) Diabetes insipidus

23. **What happens to plasma HCO_3^- in metabolic acidosis?**
    (a) Increased (b) Decreased
    (c) Unchanged (d) Variable

Answers

1. (b)	2. (a)	3. (c)	4. (b)	5. (c)	6. (c)	7. (c)	8. (b)	9. (b)	10. (b)
11. (a)	12. (a)	13. (c)	14. (d)	15. (a)	16. (c)	17. (d)	18. (a)	19. (a)	20. (d)
21. (c)	22. (d)	23. (b)							

Chapter 61

Kidney (Renal) Function Tests

- Analysis of urine + blood.
- Renal clearance
- Renal radiology
- ⊕ Renal biopsy.

I. Urine examination
II. Blood examination
III. Renal clearance tests
  A. Tests for Glomerular functions
  B. Tests for Tubular functions
IV. Miscellaneous Tests

⇒ At least 2/3rd of kidney must be damaged. ⊕ Diab. Nephropathy.

Renal function tests are done:

1. To assess the functional capacity of the kidney.
2. To detect the renal impairment as early as possible. Therefore, these tests may detect kidney disease well before the symptoms develop and determine the etiology of renal disease.

Renal function tests are **divided into** the following:

I. *Urine Examination*
II. *Blood Examination*
III. *Renal Clearance Tests*
  (i) Tests for glomerular functions
  (ii) Tests for tubular functions
IV. *Miscellaneous Tests.*

⇒ General ANASARCA (Accumul. of fluids into extracell space (Extreme edema)

Most common DEHYDRATION (Excess H2O → physiol.)

Oliguria – Urine output less than the minimum normal volume of 400 mL/day (called ***Obligatory Urine Volume***).
Seen in • Dehydration
(i) Acute glomerulonephritis
(ii) Terminal stages of renal failure

Polyuria – Urine output > 2.5 L/day
Seen in Diab. insipidus, mellitus
(i) Chronic glomerulonephritis
(ii) Renal failure associated with nephrosclerosis

Anuria – Urine output < 100 mL/day
Seen in
Renal failure • Renal shutdown • Severe dehydr. • Urinary tract obstruction

URINE EXAMINATION

1. Colour

Normal – pale lemon yellow in colour due to presence of pigments:
(i) urochrome (metabolic cell product)
(ii) urobilin
(iii) uroerythrin

Brownish = Jaundice
Frothy = proteinuria
Red/dark brown = Porphyria

2. Composition

Inorganic :	Na^+	6 g/day
	K^+	2 g/day
	Ca^{2+}	0.2 g/day
	phosphate	1.7 g/day
Organic :	Urea	20-30 g/day
	Uric acid	0.6 g/day
	Creatinine	1.2 g/day

3. Volume

Normal – 1-2.5 L/day
(Average 1.5 L/day)

4. Specific Gravity (S.G.)

1001 – 1040 (determined by Urinometer)
1001 – with maximum diluted urine 1.003-1.030
1040 – with maximum concentration power of kidneys

In general, S.G. is inversely proportional to quantity of urine. It is contributed by electrolytes Na^+, K^+, Cl^- and urea.

S.G. is affected by the nature as well as by the number of osmotically active particles in the solution. Approximate correlation between S.G. and urine osmolality is as follows:

S.G.		*Osmolality (mosm/kg)*
1001	–	100
1010	–	300
1020	–	800
1025	–	1000
1030	–	1200
1040	–	1400

3.5 pH = 4.5-8.0 ⇒ Tubular acidification (H^+) impairment

⇒ Urea splitting org. ⊕

⊛ Osmolality – 50-1200 mosm/kg

Applied

(i) 12 hours water deprivation results in S.G. of urine to become 1025 with osmolal concentration 1000 mosm/L. Failure to do this indicates abnormal renal functioning.

(ii) The persistent production of urine which is isosmolar with plasma (300 mosm/L) with S.G. 1010 despite variation in water intake is called *Isosthenuria (fixed low S.G. urine*, seen in renal failure - also see to page 547). It indicate that the diluting and concentrating functions of the kidneys have been lost. This is due to loss of functioning of nephrons or disruption of the counter current mechanism.

(iii) *Increase in S.G.* is seen in:
(a) low water intake
(b) diabetes mellitus due to presence of glucose
(c) albuminuria
(d) acute nephritis

(iv) *Decrease in S.G.* is seen in:
(a) kidney damage (tubular damage resulting in loss of concentration power of kidney tubules)
(b) absence of ADH.

5. **Reaction** *i.e.* urine pH ranges from 4.5 to 8.0 (average 6 – 6.5; *slight acidic*, therefore, turns blue litmus red) After meals it may become alkaline (turns red litmus blue).
On exposure to atmosphere, urea in urine splits causing NH_4^+ release resulting in alkaline reaction.

6. **Microscopic Examination.** It is done for *urinary sediments* obtained after centrifuging the freshly voided urine sample at a rate of 3000 r.p.m. in centrifuge machine for 15 minutes. It normally contains **(Fig. 61.1)**:
(i) *1-2 W.B.C. or pus cells/HPF.* They appear slightly larger than the normal WBCs, round shape with lobed nuclei and refractile granular cytoplasm.
(ii) *Non-squamous epithelial cells*, called ***Hyaline Cast*** - occasionally seen. They are clear, colourless cast of the tubule made up of high molecular weight mucoprotein substances formed in the DCT and CT epithelium. They are seen as small cylinders with rounded ends, pale, transparent and homogenous bodies.

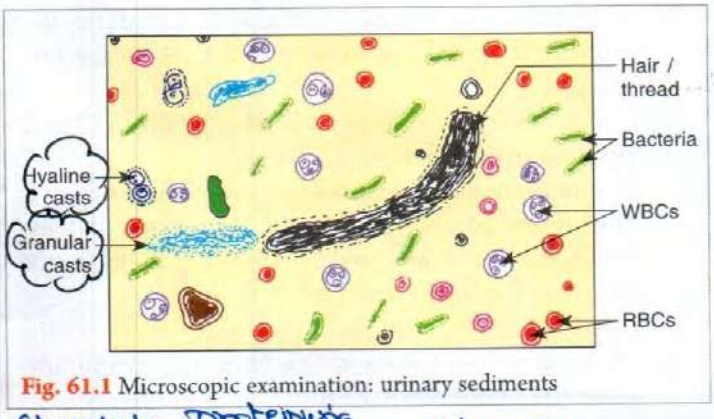


Fig. 61.1 Microscopic examination: urinary sediments

Presence of Granular Casts, RBC, bacteria, Glucose, Albumin and Ketone bodies is abnormal.

Granular casts are the hyaline casts embedded with RBCs or WBCs or these are degenerated glomerular cells or tubular *epithelial cells* in large number. Their presence indicates renal destruction of glomeruli or tubular cells.

'RBCs' are seen as roughly circular with clear yellow centres; whereas 'Epithelial cells' are seen as large oval cells with a single nucleus.

Albuminuria *i.e.* excretion of albumin in urine in amount above 150 mg/day.

Note

The amount of protein in the urine is normally less than 100 mg/day, most of which come from shed tubular cells.

Causes

(1) Change in the glomerular capillary permeability due to hypoxia, nephritis (inflammation *e.g.* TB) and carcinoma.
(2) Failure of reabsorptive process in the tubules.
(3) In some of normal individuals because of unknown cause, albumin appears in the urine when they are in standing position, called ***Orthostatic Albuminuria***.

BLOOD EXAMINATION

It is done to measure the substances in blood that are normally excreted by kidney. In general, their level in blood increases in kidney dysfunction.

1. ***Blood Urea:*** 20-40 mg/dL
2. ***S. (serum) creatinine:*** 0.6-1.5 mg/dL
These are useful indices of glomerular filtration. *The blood urea is nearly doubled when the GFR is halved.* S. creatinine level also increases with failure of glomerular filtration.
3. ***S. Electrolytes***
(i) $S.K^+$: 5 mEq/L; increases in severe oliguria or anuria
(ii) S. Na^+ : 152 mEq/L (3-3.5 mg/mL)
(iii) S. Ca^{2+} : 9-11 mg/dL
(iv) S. PO_4^{3-} : 3-4.5 mg/dL
(v) S. SO_4^{2-} : 0.5-1.5 mEq/L
(vi) S. Mg^{2+} : 1.5-2.5 mEq/L (1-2 mg/dL)

4. *S. Proteins*
   Total : 6.4-8.3 gm/dL
   Albumin : 3-5 gm/dL
   Globulin : 2-3 gm/dL
   A/G ratio : 1.7 : 1
   In nephrotic syndrome, S. albumin decreases and S. globulin increases, thus causing reversal of A/G ratio.
5. *S. cholesterol:* 150-240 mg/dL; increases in nephrotic syndrome.
6. *S. uric acid:* 2-4 mg/dL.

RENAL CLEARANCE TESTS

These tests are done to assess the functions of different parts of nephrons.

A. TESTS FOR GLOMERULAR FUNCTIONS

1. *Inulin clearance*
2. *Creatinine clearance*
   These are used to measure the GFR and are indicators of plasma clearance mechanisms (page 535).
3. Others
   (i) measurement of *renal plasma flow (RPF)* and *renal blood flow (RBF) (page 537)*
   (ii) measurement of *filtration fraction (FF)*

B. TESTS FOR TUBULAR FUNCTIONS

1. PAH clearance

As the Tm_{PAH} (maximum secretory capacity of tubules for PAH) is nearly constant at about 80 mg/min, it is used clinically to estimate tubular secretory capacity (page 537).

Measurement of power of tubular transport can also be assessed by determining TmG. Decrease in Tm_{PAH} and TmG values indicate tubular damage.

Normal average plasma PAH (P_{PAH}) value is 20 mg/dL; it may increase to 50 mg/dL where tubular secretory function is at maximum.

2. Urea Clearance (C_{urea}) (Refer page 535)

3. Phenol Sulphonephthalein (PSP) Excretion Test

A known amount of PSP administered I.M. or I.V. PSP is eliminated by kidneys only and its concentration in urine can be measured easily. The time of its first appearance in urine and quantity eliminated within a definite period are taken as a measure of the functional capacity of the kidneys.

In normal individuals, ≥ 25% of the injected dye is excreted in urine in the first 15 minutes of administration; and at least 70% of injected dye is excreted in 2 hours.

Decrease in PSP excretion indicates a general loss of nephron function.

Increase in PSP excretion indicates:
(i) early stage of renal inflammation;
(ii) hyperthyroidism.

4. Measurement of Water Reabsorption

(i) Normally concentration of creatinine in urine (U) and plasma (P) *i.e.* U/P for creatinine is 100/1; if ratio decreases, it indicates decreased water reabsorption; if ratio becomes 1/1, it indicates no water reabsorption.

(ii) *Water concentration test or water deprivation test*
Withdrawal of oral fluids for 12-18 hours in a healthy individual results in concentration of urine.
(a) If S.G. is > 1020 indicates *no gross* abnormality *i.e.* kidney's concentration power is normal.
(b) S.G. 1008 to 1010 is isotonic with plasma. This S.G. requires no work to be done for excretion *i.e.* loss of concentration power of kidneys.

(iii) *Water dilution test or water excretion test*
Water intake of about 2% of body weight (approx. 1 L) in a normal individual results in excretion of 70% of the water intake within 5 hours with S.G. 1005.

5. Others: Methods of Study of Tubular Functions

(i) *Micropuncturing* the various parts of tubules and analysing fluid with reference to volume and composition.

(ii) *Stop Flow Technique*
Ureteral obstruction for 1–1½ minutes, prevents further filtration (back pressure effect). After 4-8 minutes, take samples (0.5 mL each) of tubular fluid (30 samples) from ureter. First 5-6 samples will be representing fluid from CT; next few from DCT; another few from loop of Henle and last 5-6 samples from PCT; analyse each sample. One minute before releasing the obstruction, inject Inulin or ferrocyanide I.V. (as an indicator). Therefore, the moment it appears in urine it will indicate the last sample.

(iii) *Microcryoscopic studies i.e.* studying slices of renal tissue at different depths
Osmotic pressure of various slices increases as one goes from cortex to medulla. Therefore, kidney functions can be assessed by studying the changes which take place from 'outside to inside'.

(iv) *Microelectrode Studies*
These have been done to measure the membrane potential of the tubular cells.

(a)	pot. diff. b/w tubular lumen and interior of PCT cells	–70 mV; former –ve
(b)	pot diff. b/w tubular cells and ECF	–70 mV; former –ve
(c)	pot. diff. b/w lumen of first 1/4 PCT cells and ECF	–2 mV; former –ve
(d)	pot diff. b/w lumen of last 3/4 PCT cells and ECF	+2 mV; former +ve
(e)	pot diff. b/w lumen of descending loop of Henle and ECF	–7 mV; former –ve
(f)	pot. diff. b/w lumen of ascending loop of Henle and ECF	+7 mV; former +ve
(g)	pot. diff. b/w lumen of DCT and ECF	–45 mV; former –ve
(h)	pot. diff. b/w lumen of CT and ECF	–35 mV; former –ve

(pot. diff. : potential difference; b/w : between; –ve : negative; +ve : positive)

MISCELLANEOUS TESTS

1. ***Intravenous Pyelography (I.V.P.)*** → Gives (Shape, Size, All details)
The agents *Diodrast or Iopax* are rapidly excreted by the kidney and are opaque to X-rays. About 15 gm of the substance in 20 mL of a 10% glucose solution is given I.V. and X-ray is taken. In a normal healthy person, a distinct *Pyelogram* (*i.e.* size, shape and detail anatomy of kidneys) will be obtained in 2-10 minutes. In renal insufficiency, it may be delayed for hours. This method is also useful in demonstrating stone, growth etc. in renal passage.
2. Measurement of alkaline reserve; pH of the blood; total and free acidity of urine; ammonia in urine; systemic arterial blood pressure, gives idea about regulation of these functions.
3. ***Arteriography***
After administration of radio-opaque dyes in any convenient artery, usually in the femoral artery, X-ray is taken. This gives an idea of renal vasculature and any obstruction therein can be visualised.
4. ***Ultrasonography*** (Most modern)
The shape and size of the kidneys, the presence of any stone/cyst/tumour can be diagnosed from this test. Currently it is very popular.
5. ***Isotope Perfusion Studies*** are done for academic/research interest only
6. ***Renal Biopsy***
By means of a special needle inserted into the back, biopsy specimen of the kidney may be obtained for histological and cytological examination. The whole procedure is referred as *fine needle aspiration cytology (FNAC)*. The method is of a great clinical importance in the diagnosis and assessment of kidney dysfunctions.

Study Questions

1. What gives colour to normal urine?
2. Define and explain:
   (i) Oliguria, polyuria and anuria (ii) Obligatory urine volume (iii) Isosthenuria
   (iv) Hyaline and granular casts (v) Albuminuria (vi) FNAC.
3. Mention the finding of normal urine examination.
4. Write briefly about:
   (i) Tests for glomerular function (ii) Tests for tubular function
   (iii) Water concentration and dilution test (iv) I.V.P.
   (v) Tests for excretion of waste products by the kidney.
5. Give an account of various tests done to assess the functional capacity of the kidney.
6. What tests will you prescribe to detect an early impairment of renal function.

MCQs

1. Which of the following is minimally excreted in urine?
   (a) Urea (b) Creatinine (c) Uric acid (d) Phosphate
2. Specific gravity (SG) with maximum diluted and concentrated urine respectively is:
   (a) 1001; 1040 (b) 1010; 1020 (c) 1020; 1030 (d) 1030; 1040

3. *False* **statement with reference to specific gravity of urine:**
   (a) Inversely proportional to quantity of urine
   (b) Increases in kidney damage
   (c) Affected by number of osmotically active particles
   (d) In renal failure it becomes fixed and low despite variation in water intake
4. **Urine normally contains all of the following *except*:**
   (a) 1-2 pus cells/HPF
   (b) Hyaline casts
   (c) Granular casts
   (d) Non-squamous epithelial cells
5. **Granular casts in urine:**
   (a) Are regarded as normal
   (b) Are hyaline casts embedded with RBCs
   (c) Made up of mucoprotein substances
   (d) Formed in the DCT
6. **Excretion of proteins in urine upto ..... mg/day is regarded as normal:**
   (a) 50-150
   (b) 150-250
   (c) 250-500
   (d) Zero
7. **Test to assess renal tubular functions include all *except*:**
   (a) PAH clearance
   (b) Urea clearance
   (c) Intravenous pyelography (IVP)
   (d) Measurement of water reabsorption
8. *Not a true* **statement about urea clearance:**
   (a) Measure the rate of excretion of waste products
   (b) Obsolete test, not used clinically
   (c) Normal standard urea clearance is 54 mL/min
   (d) When rate of urine flow is below 2 mL/ min, excretion of urea is maximal
9. **Withdrawal of fluids for 12-18 hours indicates kidney concentration power is normal if specific gravity of urine is:**
   (a) 1001 to 1007
   (b) 1008 to 1010
   (c) 1010 to 1020
   (d) Above 1020
10. **Isosthenuria is:**
    (a) Low fixed specific gravity of urine (1010) despite variation in water intake
    (b) Dilute urine of specific gravity (1001)
    (c) Concentrated urine of specific gravity (1040)
    (d) None of the above
11. *Not true* **of urinary hyaline casts:**
    (a) Presence is abnormal
    (b) They are non-squamous epithelial tubular cells
    (c) They are clear, colourless casts of renal tubules
    (d) Seen as small cylinders with rounded ends
12. **In water dilution test for tubular function, the patient can excrete at least ....% of volume ingested within 5 hours:**
    (a) 40
    (b) 50
    (c) 70
    (d) 90

Answers

1. (c) 2. (a) 3. (b) 4. (c) 5. (b) 6. (a) 7. (c) 8. (d) 9. (d) 10. (a)
11. (a) 12. (c)

Chapter

62

Physiology of Micturition

I. Definition
II. Physiological anatomy of the urinary bladder
III. Nerve supply of the urinary bladder
IV. Postural activity of the urinary bladder
V. The micturition reflex
VI. Mechanism of voluntary micturition and its reflex control
VII. Applied aspects: Deafferentation; Denervation

1 DEFINITION

Micturition is a process by which the urinary bladder empties itself when it becomes filled.

2 PHYSIOLOGICAL ANATOMY OF THE URINARY BLADDER

Urinary bladder is mainly a smooth muscle hollow vesicle. The fibers are arranged in spiral, longitudinal and circular bundles. Urinary bladder is composed of the following:

1. *The Body* which is comprised of *Detrusor Muscle*. It is mainly responsible for emptying of the bladder during micturition.
2. *The Trigone*, a small triangular area near the mouth of urinary bladder, through which both ureters and urethra pass.
3. *The Internal Sphincter*. The trigonal muscle fibers get interlaced around the opening of urethra forming internal sphincter. Its *main functions* are:
   (i) to maintain tonic closure of the urethral opening; and
   (ii) prevents reflux of semen into bladder during ejaculation.
4. *The External Sphincter*. It is a voluntary skeletal muscle. Normally this sphincter remains tonically contracted which prevents constant dribbling of urine; but it can be reflexly or voluntarily relaxed at the time of micturition.

The *physiological capacity* of the urinary bladder varies with age.

At birth	:	20-50 mL
At 1 year	:	200 mL
Adults	:	600 mL

The *anatomical capacity* of bladder is 1 L. It is the capacity just before bladder rupture occurs. However, it is never approached under physiological conditions. The urine stored in the bladder remains unchanged in chemical composition since the luminal surface of the transitional epithelium (***urothelium***) forms a complete barrier to the passage of water and solutes.

3 NERVE SUPPLY OF THE URINARY BLADDER

Urinary bladder is innervated by (Fig. 62.1):

A. *Efferent* nerve supply:
   1. Sympathetic
   2. Parasympathetic
   3. Somatic
B. *Afferent* nerve supply

A. EFFERENT NERVE SUPPLY (Table 62.1)

B. AFFERENT NERVE SUPPLY

1. From the external sphincter and posterior urethra, afferent fibres pass along the *Pudendal Nerves* into the dorsal nerve roots of $S_{2,3,4}$.
2. From the body, trigone and internal sphincter, afferent fibers take a double route:
   (i) along the sympathetic nerve into the dorsal nerve roots of $L_{1,2}$ and the lower thoracic segment;
   (ii) along the sacral parasympathetic nerves into the sacral dorsal nerve roots.

Functions of Afferents:

1. Indicate the *degree of distension* (stretch) of urinary bladder. This sensation of stretch is mainly carried by afferent nerves which run along sacral parasympathetic nerves.
2. To convey *pain sensibility*. This is mainly carried by afferent nerves which run along sympathetic nerve.

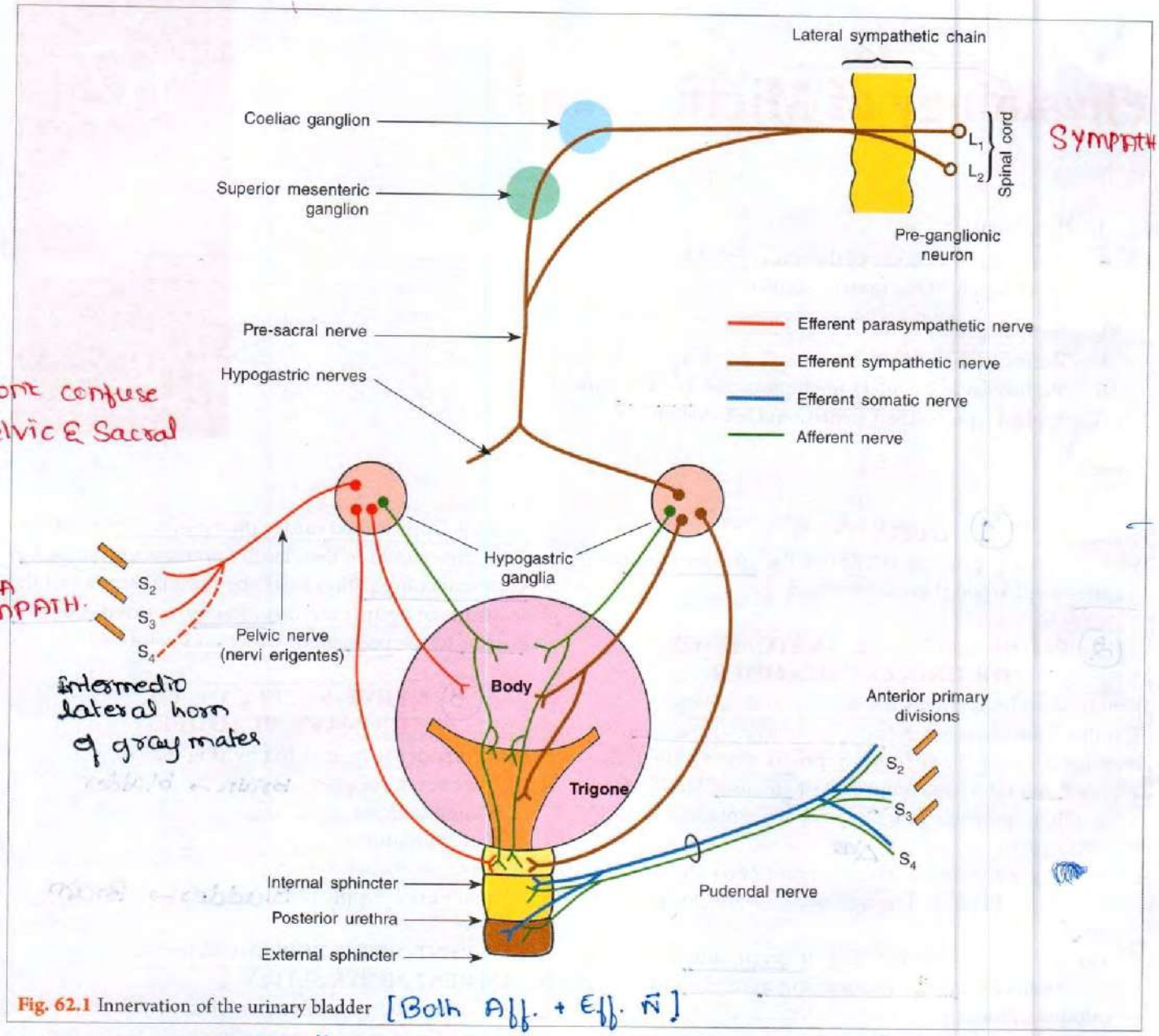


Fig. 62.1 Innervation of the urinary bladder

Therefore, traction on presacral nerves causes crushing kind of pain which is localized in the urinary bladder itself and is not referred to the skin.

(4) POSTURAL ACTIVITY OF THE URINARY BLADDER

Normal urinary bladder muscle shows property of *plasticity* i.e. when it is stretched the tension initially produced is not maintained. Therefore, no constant relationship can be assigned between the fiber length and the tension (page 188).

The relationship between intra-vesical pressure and volume can be studied by inserting a catheter into the bladder and emptying it; then recording the intra-vesical pressure while the bladder is filled with 50 mL increment of water (*Cystometry*).

A plot of intra-vesical pressure against the volume of fluid in the urinary bladder is called *Cystometrogram*. The curve shows *three components* (Fig. 62.2):

1. An initial slight rise in pressure, say upto 10 cm H_2O when the first increment in volume, upto 100 mL is produced.
2. A long nearly flat segment as further increment upto 300-400 mL is produced. Why so? Due to *intrinsic tone* of urinary bladder wall itself.
   (It manifests *Law of Laplace*, which states, distending pressure (P) in a spherical hollow viscus is equal to twice the wall tension (T) divided by the radius (R) *i.e.* $P = 2T/R$ (page 318). Thus, the tension increases as the organ fills, but so does the radius. Therefore, the pressure increase is slight until the organ is relatively full.)

Table 62.1: Features of efferent nerve supply of the urinary bladder (Refer to Fig. 62.1)

Origin	Course	Parts innervated	Effect of stimulation
1. Sympathetic			
From pre-ganglionic neurons which lie in the intermediolateral grey matter of $L_{1,2}$ segments of spinal cord	• Pre-synaptic fibers descend down through lateral sympathetic chain, coeliac and superior mesenteric ganglia. • Both fibers join to form *Pre-Sacral Nerve* in front of sacrum, which divides into two *Hypogastric Nerves* to end in hypogastric ganglia. • Post ganglionic fibers from here supply the bladder	• The body (detrusor) • Trigone and • Internal sphincter	Relaxation of detrusor muscle; Contraction of trigone and internal sphincter resulting in *retention of urine*
2. Parasympathetic			
From pre-gangalionic cells in $S_{2,3,4}$ (occ) intermediolateral grey matter of spinal cord	• Pre-synaptic fibers pass in *Pelvic Nerves (Nervi Erigentes)* to end in *Hypogastric Ganglia* (which serves as the ganglionic relay station for both sets of autonomic fibers) • Parasympathetic post-ganglionic fibers from hypogastric ganglia pass to innervate the bladder	• The body (detrusor) • Trigone • Internal sphincter	Contraction of detrusor muscle; relaxation of trigone and internal sphincter resulting in *emptying of bladder*
3. Somatic			
Anterior horn cells (anterior primary division) of $S_{2,3,4}$	*Pudendal (Pudic) Nerve*	• Prostatic (posterior) urethra and • External sphincter	parts innervated are under higher control, therefore, they can be reflexly and voluntarily relaxed at the time of micturition

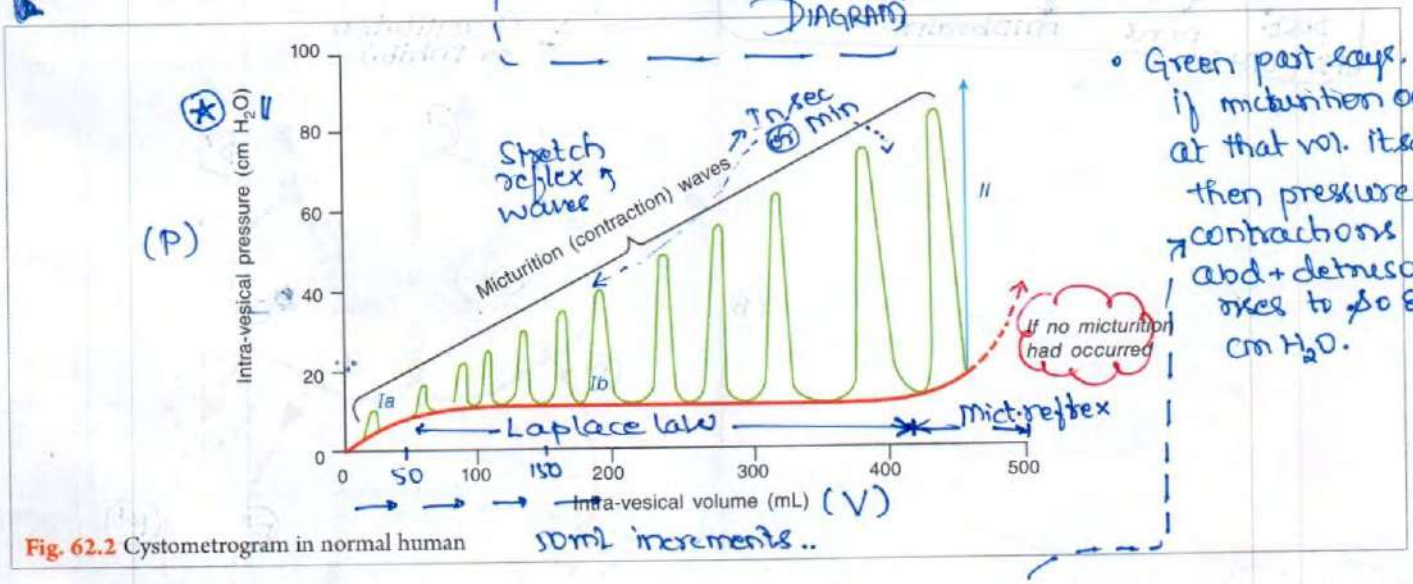


Fig. 62.2 Cystometrogram in normal human

3. Beyond this point, collection of more fluid results in a sudden sharp rise in pressure as the **Micturition Reflex** is triggered (see below).
The *three components* of *Cystometrogram* are called the segments *Ia, Ib and II*.
In cystometrogram, superimposed on the tonic pressure changes during filling of urinary bladder are periodic acute rise in the pressure, called *Micturition (Contraction) Waves*, which last from few seconds to more than a minute. These are the result of *stretch reflex* initiated by stretch receptors in the urinary bladder and proximal urethra.

Important Note

Stretch receptors have a lower threshold than the inherent contractile response of the smooth muscle.

This pressure can rise only few cm of water or it can rise above 100 cm H_2O at which a nervous reflex, called *Micturition Reflex* occurs, which either causes *Micturition* or a constant desire to urinate.

5 THE MICTURITION REFLEX: REFLEX CONTROL OF MICTURITION

As the urinary bladder fills with urine, the wall stretches, impulses are initiated by stretch receptors in the bladder wall causing sensory signals to convey to the dorsal nerve root $S_{2,3,4}$ segments of spinal cord through pelvic nerves and then back again to the urinary bladder through parasympathetic fibers in the same nerve. This whole constitutes the ***Micturition Reflex***. During this reflex, sympathetic efferents are reflexly inhibited.

Threshold for micturition reflex is adjusted by the activity of the *'facilitatory'* and *'inhibitory'* centres in the brain stem.

Higher Control of Micturition Reflex

It is in the brain stem by two areas:

1. ***Facilitatory Area*** is located in the **pontine region and posterior hypothalamus**. Transection of brain stem just above the pons causes lowering of threshold of micturition reflex. Therefore, less urinary bladder filling is required to trigger micturition reflex.
2. ***Inhibitory Area*** is located in the **mid brain**. Following transection at the top of mid brain, threshold for micturition reflex is essentially normal.

*Therefore, fundamentally 'micturition reflex' is a **Spinal Reflex**, facilitated and inhibited by higher brain centres.*

Note

Lesions in the superior frontal gyrus reduces desire to urinate and difficulty in stopping micturition once it has started.

6 MECHANISM OF VOLUNTARY MICTURITION AND ITS REFLEX CONTROL

1. ***Afferent pathway***

When the urinary bladder is sufficiently filled, intra-vesical pressure rises causing stimulation of stretch (pressure) receptors in the muscle coat and sends off impulses through pelvic nerves along sacral parasympathetic nerves into the sacral dorsal nerve roots ($S_{2,3,4}$); then via dorsal columns to brain, post-central gyrus (a sensory area in cerebral cortex) which gives desire to micturate.

Path of afferent fibers within CNS is not known.

2. ***Efferent pathway***

In response to desire to micturate, urinary bladder can be voluntarily emptied. Impulses from the top of motor area in cerebral cortex pass down to sacral segments causing stimulation of efferent parasympathetic fibers; then impulses pass along pelvic nerves (nervi erigentes) to cause contraction of body of urinary bladder wall and relaxation of trigone and internal sphincter. The centre in the brain controlling the external sphincter is reflexly inhibited, finally resulting in ***Emptying of Bladder*** (Fig. 62.3).

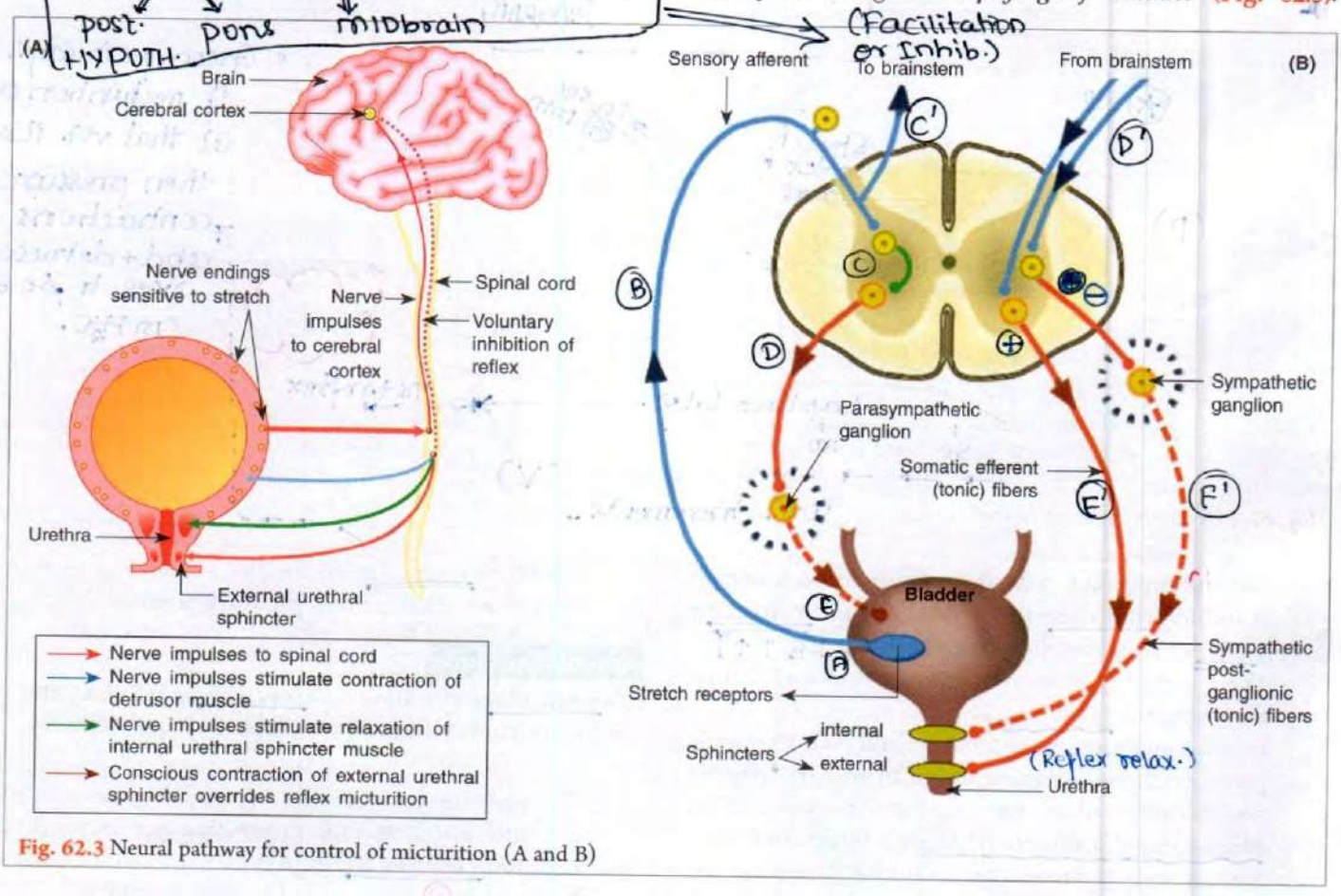


Fig. 62.3 Neural pathway for control of micturition (A and B)

The normal bladder is completely empty at the end of micturition; and the intra-vesical pressure is equal to the intra-abdominal pressure.

3. ***Accessory muscles involved during micturition*** are:
   (i) perineal muscles are relaxed
   (ii) abdominal wall muscles contracts
   (iii) diaphragm descends
   (iv) breathing is held with glottis closed.
   (Glottis is a triangular space between thyroid and arytenoid cartilage which is covered by vocal cords, page 1032.)
   (i) to (iv) cause increase in intra-abdominal pressure, therefore, compress bladder from without. This results in sharp increase in intra-vesical pressure and finally bladder emptying.
4. *After urination*, the female urethra empties by gravity while in males the urine remaining in the urethra is expelled by several contractions of the bulbo-cavernosus muscle.
5. *First urge to pass urine* is felt at a urinary bladder volume of approx 150 mL.
6. *A marked sense of fullness or discomfort* is felt at about 400 mL which normally results in initiation of micturition reflex.
7. *If inconvenient to micturate or desire to hold urine*, impulses from cerebral cortex via
   (i) inhibiting sacral parasympathetic efferent excitatory activity, and
   (ii) sympathetic efferent nerves stimulation, which relaxes detrusor muscles.
   (i) and (ii) causes inhibition and elongation of urinary bladder wall which decreases intra-vesical pressure and micturition contractions passes off wholly or partially. Therefore, desire to micturition passes off. This is only a temporary phase, as micturition contractions reappear after some time and when pressure increases to more than 100 cm H_2O, it results in acute discomfort and subject begs to be allowed to empty his bladder.
8. *By constant practice* urinary bladder can be trained to accommodate very large volume of urine before uncontrollable and unbearable rise of intra-vesical pressure occurs.
9. *Even moderate filling of urinary bladder* upto 200 mL, desire to micturate results in sharp increase in pressure, initiating micturition reflex and emptying of bladder.
10. *In young children*, postural activity of urinary bladder is less perfect and even small quantities of urine causes increase in pressure sufficient to send afferent impulses upto the spinal cord. Higher centres are not involved and micturition occurs, entirely reflexly *i.e.* at spinal level from stimulation of pelvic nerves (nervi erigentes).
11. *Postural behaviour* of urinary bladder can be *influenced by*:
    (i) **Emotional stress** *e.g.* examination, anxiety, the power of urinary bladder to elongate its fibers as its contents increase is in suspension. Therefore, results in constant desire to micturate with passage of only small quantities of urine on each occasion.
    (ii) **Cystitis** (inflammation) or urinary bladder stones cause increase in frequency of micturition (mechanism as above).
    (iii) **Enuresis** of children, mechanism not clear; probably it is psychological or as mentioned above.

APPLIED ASPECTS

In all types of urinary bladder dysfunctions, bladder contracts but contractions are insufficient to empty the bladder completely, therefore, some urine is left in the urinary bladder, called ***Residual Urine***.

A. **Deafferentation** *i.e.* effect of injury to afferent nerves seen in ***Tabes dorsalis*** (syphilis) which causes lesion of lumbo-sacral dorsal nerve roots. It is **characterised by**:
   1. Patient is unaware of state of distension of bladder.
   2. Voluntary micturition is possible.
   3. If patients fails to micturate at regular intervals, it causes accumulation of urine in urinary bladder, which leads to either
      (i) precipitation of involuntary automatic evacuation by contraction of detrusor muscle due to stretch, or
      (ii) increase in intra-vesical pressure till resistance of sphincters is passively overcome resulting in dribbling of urine.
   In either condition urinary bladder is called ***Automatic Bladder*** as a result of this it becomes distended, thin walled and hypotonic.

B. **Denervation** *i.e.* effect of injury to both afferent and efferent nerves. This is seen in tumour or injury to cauda equina and is **characterised by**:
   1. Complete loss of voluntary micturition. Therefore, difficulty in initiation of the act and patient cannot hold the urine at will.
   2. Urinary bladder becomes flaccid and distended, called ***Isolated Bladder or Decentralized Bladder***.
   3. Urinary bladder wall shows *property of plasticity i.e.* postural activity of urinary bladder become peculiar. Therefore,
      (i) ***Initially***, urinary bladder responds at irregular intervals to adequate internal distension by contraction of detrusor muscle, but these

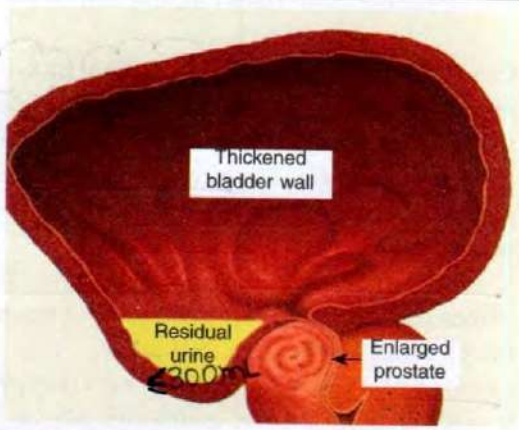


Fig. 62.4 Residual urine

contractions are not powerful enough or well coordinated resulting in incomplete micturition. Thus, a large volume of ***residual urine*** (upto 300 mL) may be left in the bladder **(Fig. 62.4)**.

(ii) *Later*, urinary bladder shows periodic automatic emptying through the intervention of local peripheral neuro-muscular mechanisms.

C. Effect of Section of Facilitatory and Inhibitory pathways descending from the brain.

As long as one pyramidal tract is functioning perfectly, control of urinary bladder remains normal. Therefore, urinary bladder dysfunction will appear only when the function of both pyramidal tracts is impaired by a spinal lesion above the lumbar region. It is seen in:

1. *Acute transection of spinal cord.*
2. *Syringomyelia.*
3. *Early compression of spinal cord.*
4. *Disseminated sclerosis* (scattered fibrosis): Here nerve cells get replaced by neuroglia cells due to their proliferation. How? Viral disease leads to multiple inflammatory foci, disseminated irregularly throughout the length of the cerebrospinal axis. Grey and white matter both get involved and show demyelination.

1. ***Early Stage:*** During spinal shock. ***Characteristic features:***

(i) Voluntary micturition is completely lost, therefore, patient finds difficulty in initiation of the act and cannot hold the urine at will.

(ii) Urinary bladder becomes flaccid, distended and unresponsive.

(iii) Activity in detrusor muscle remains in suspension for long period but activity in sphincters tone returns soon. *Why? Not known.* Therefore, bladder responds purely passively to distension with urine like an elastic bag or dead organ resulting in ***Retention of Urine.***

(iv) As a result of retention of urine, bladder becomes increasingly over-stretched, sphincters are finally forced open by high intra-vesical pressure with escape of small amount of urine at frequent intervals, called ***Retention with Overflow or Passive Incontinence or Overflow Incontinence.***

2. ***Late Stage:*** Seen when early stage is over.

(i) Micturition reflex returns although there is no voluntary control and no facilitation or inhibition from the higher centres. Therefore, micturition can be initiated by pinching or stroking the thigh, provoking a mild *mass reflex* (page 883).

(ii) Overstretching of urinary bladder wall decreases its nutrition, predisposes to infection, eventually producing *Cystitis.*

(iii) Repeated infections of urinary bladder cause hypertrophy of its wall, bladder 'shrinks' causing irreversible damage to bladder *i.e.* bladder never again responds normally to internal distension. Therefore, micturition reflex becomes hyperactive, probably due to denervation hypersensitization (page 189). As a result, bladder contracts at irregular intervals and even with small amounts of urine, called ***Spastic (or Contracted) Neurogenic Bladder*** **Fig. 62.5**. *This bladder dysfunction can be prevented by catheterizing the urinary bladder within 24 hours of spinal injury.*

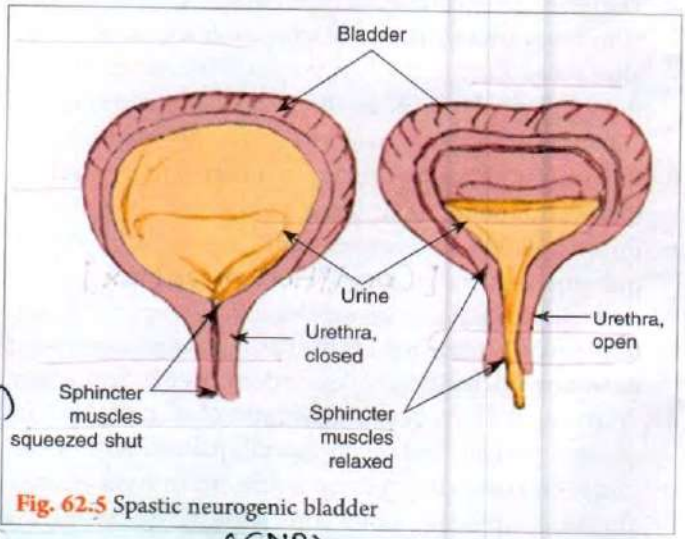


Fig. 62.5 Spastic neurogenic bladder

Study Questions

1. **Draw well labelled diagram to show:**
   (i) Innervation of the urinary bladder
   (ii) Cystometro gram
   (iii) Neural pathway for control of micturition
2. **Write short notes on:**
   (i) Cystometrogram and its physiological significance
   (ii) Functions of nerve supply to urinary bladder
   (iii) Postural activity of urinary bladder
   (iv) Micturition reflex and its higher control
   (v) Reflex control of micturition
   (vi) Factors influencing postural behaviour of urinary bladder
   (vii) Automatic bladder
   (viii) Spastic (contracted) neurogenic bladder.
   (ix) Residual urine
3. **Mention physiological capacity of urinary bladder at different ages. What is anatomical capacity of the bladder?**
4. **Give physiological basis of:**
   (i) Micturition waves
   (ii) Frequency of micturition during nervousness
   (iii) Chemical composition of urine stored in the urinary bladder remains unchanged.
5. **What will happen and why? If:**
   (i) Afferent nerve to urinary bladder is cut
   (ii) Urinary bladder is completely denervated
   (iii) Higher control of urinary bladder is lost.

MCQs

1. **During first year of life, urinary bladder physiological capacity is increased by .... times:**
   (a) 2 (b) 3 (c) 4 (d) 5
2. **The difference between physiological and anatomical capacity of urinary bladder in adults is ..... mL:**
   (a) 200 (b) 300 (c) 400 (d) Zero
3. **Pudendal (pudic) nerve controls:**
   (a) External sphincter of rectum (b) External sphincter of urinary bladder
   (c) Size of pupil (d) Gastroesophageal reflex
4. ***Not* a component of micturition reflex:**
   (a) Stretch receptors in the bladder wall (b) Dorsal nerve root $S_{2,3,4}$
   (c) Parasympathetic efferent nerve (d) Sympathetic efferent nerve
5. ***False* statement with reference to micturition reflex:**
   (a) Initiated at a urinary bladder volume of approx. 150 mL.
   (b) Has a fixed threshold
   (c) Can be inhibited or facilitated by centres in the brain stem
   (d) Cannot be inhibited, once intra-vesical pressure rises above 100 cm H_2O
6. **Amount of urine left in the urinary bladder at the end of micturition is .... mL:**
   (a) 50 (b) 100 (c) 150 (d) Zero
7. **In young children micturition is:**
   (a) Controlled by higher centres (b) a spinal reflex
   (c) Voluntarily controlled (d) A perfectly controlled activity
8. **Residual urine is:**
   (a) Urine left in the urinary bladder after micturition (b) A normal phenomenon
   (c) Never seen with urinary bladder dysfunction (d) A common finding of examination anxiety
9. **Deafferentation of urinary bladder is characterized by all *except*:**
   (a) Voluntary micturition not possible (b) Person unaware of state of distension of bladder
   (c) Automatic bladder (d) Residual urine

10. **Repeated infection of urinary bladder may lead to:**
    (a) Flaccid distended and unresponsive bladder
    (b) Retention with overflow
    (c) Isolated bladder
    (d) Spastic neurogenic bladder
11. **Filling of urinary bladder upto 300-400 mL fluid increases the intravesicle pressure to:**
    (a) 10 cm H_2O
    (b) 30 cm H_2O
    (c) 50 cm H_2O
    (d) Above 100 cm H_2O
12. **Micturition waves:**
    (a) Initiated by stretch receptors in the urinary bladder
    (b) Are periodic acute rise in intra-vesical pressure during filling of bladder
    (c) Are superimposed on the tonic pressure changes
    (d) All of the above are true
13. **Facilitatory and inhibitory areas for micturition reflex are mainly located in the:**
    (a) Medulla
    (b) Pons
    (d) Midbrain
    (d) Brain stem
14. **The first urge to pass urine is felt at a urinary bladder volume of about .... mL:**
    (a) 50
    (b) 100
    (c) 150
    (d) 250
15. **In a normal adult the sense of fullness or discomfort is felt when urinary bladder volume is about .... mL:**
    (a) 200
    (b) 400
    (c) 600
    (d) 800
16. **Denervation of urinary bladder may result in:**
    (a) Complete loss of voluntary micturition
    (b) Isolated bladder
    (c) Insufficient emptying
    (d) All of the above

Answers

1. (c)	2. (c)	3. (b)	4. (d)	5. (b)	6. (d)	7. (b)	8. (a)	9. (a)	10. (d)
11. (a)	12. (d)	13. (d)	14. (c)	15. (b)	16. (d)				

Regulation of Body Temperature in Humans

Chapter 63

I. Introduction
II. Normal Body Temperature
III. Factors Affecting Body Temperature
IV. Body Heat Production and Heat Loss
V. Temperature Regulating Mechanisms
VI. Applied Aspects
A. Fever B. Hypothermia

INTRODUCTION

1. In *'cold' blooded* animals *(Poikilothermic)*, temperature adjusting mechanisms are relatively rudimentary. Therefore, their body temperature fluctuates over a considerable range. The examples include: reptiles, amphibians, fish.
2. In *'warm' blooded* animals *(Homeothermic)*, body temperature is maintained constant within a narrow range inspite of wide variations in environmental (ambient) temperature. The examples include: man, other mammals, birds.
3. Human beings are ***Homeotherms***. Although the *'core' temperature (i.e.* internal body temperature) is maintained fairly constant in man through the regulation of heat balance in the body, the skin temperature can show considerable variation.
4. *Neutral (or Comfortable) Zone Temperature or Zone of Thermal Neutrality or Critical (Ambient) Air Temperature* – *Normally is* 27 ± 2°C.
Definition: The zone of ambient temperature at which warming and cooling of the body is least difficult *i.e.* the ambient temperature at which there is no active heat loss or heat gain mechanisms operated by the body.
Thus, it is the lowest ambient temperature at which mammals can maintain its body temperature at the basal metabolic rate. Any deviation from this temperature causes initiation of heat production or heat loss mechanisms.
5. *Why regulation of body temperature is required?*
(i) because the speed of chemical reactions varies with the temperature, and
(ii) because the enzyme systems of the body have narrow temperature range in which their function is optimal. Therefore, normal body functions depend on a relatively constant body temperature.

NORMAL BODY TEMPERATURE

1. ORAL TEMPERATURE

Average: 37°C (98.6°F)
Range: 36.3 – 37.1°C (97.3 – 98.8°F)

(To convert °C to °F, multiply by 9/5 and add 32; and to convert °F to °C, subtract 32 and multiply by 5/9).

Oral temperature is affected by many factors *i.e.* hot or cold drinks, chewing gum, smoking, mouth breathing etc. It is closely correlated with rise of temperature in subclavian artery.

Various parts of the body are at different temperatures, extremities are generally cooler than the rest of the body. *The temperature of the scrotum is carefully regulated at* 32°C.

2. CORE TEMPERATURE or INTERNAL BODY TEMPERATURE

It has been widely assumed to be an accurate index of the temperature of the blood to which the hypothalamic *thermoregulatory receptors* (page 586) are exposed. It is *0.5–1°C more* than the oral temperature. It varies least with changes in environmental temperature, therefore, it gives a poor reflection of rapid changes in blood temperature, but for slower changes it is less subjected to technical errors **(Fig. 63.1)**.

Sites for recording core temperature: rectum, vagina, oesophagus, tympanic membrane.

Lower lethal core temperature is 26°C, this leads to death due to cardiac failure.

Upper lethal core temperature is 43.5°C, this leads to death due to heat stroke.

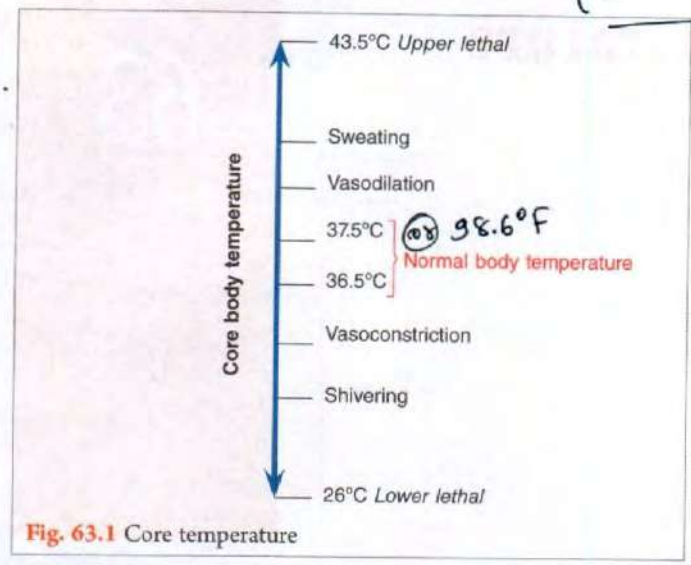


Fig. 63.1 Core temperature

Note

Core temperature of 41°C for prolonged periods produces irreversible brain damage.

FACTORS AFFECTING BODY TEMPERATURE

1. *Age*
   (i) Infants show wide range of variations in body temperature. It is generally more by 0.5°C above the established norms for adults due to:
      (a) irregular activities
      (b) brown fat (see below), and
      (c) thermoregulatory mechanisms are not fully developed.
   (ii) In old age, temperature is '*subnormal*' due to:
      (a) decrease activity
      (b) feeble circulation (because BMR is low)
      (c) thermoregulatory mechanisms are weak, that is why they are intolerant to extremes of external temperature.
2. *Sex*
   (i) In females body temperature is slightly low due to:
      (a) Low BMR
      (b) More subcutaneous fat (non-conductor)
   (ii) Menstruation: At the time of menstruation average temperature is at a minimum and increased by 0.5°C at the time of ovulation (progesteronal effect, page 800).
3. ***Constitutional Hyperthermia*** *i.e.* normal healthy adults with body temperature above normal upto 100°F.
4. ***Diurnal variation*** (circadian fluctuation) of upto 1.5°C may occur in normal person.
   Lowest: in early morning (after night rest) and maximum: in the evening. (Rhythm is reversed in night workers, which occurs after a few days.)
5. *Diseases*
   (i) Hyperthyroidism → ↑ BMR → body temperature is chronically elevated by 0.5°C.
   (ii) Hypothyroidism (Myxoedema) – opposite occurs.
6. *Exercise.* If marked, may cause increase in temperature upto 40–41°C (104–106°F) due to:
   (i) Inability of "heat dissipating mechanisms" to handle the greatly increased amount of heat produced.
   (ii) There is elevation of body temperature at which the heat dissipating mechanisms are activated during exercise *i.e.* ***resetting of the thermostat***.
7. *Emotional factors* can increase the body temperature by as much as 2°C due to unconscious tensing of the muscles. This may account for some unexplained fevers.

BODY HEAT PRODUCTION AND HEAT LOSS

In health, body temperature is always kept fairly close to normal level by maintaining a balance between heat production and heat loss (Fig. 63.2).

A. HEAT PRODUCTION or THERMOGENESIS

1. By metabolic activities of the body specially in:
   (i) Liver
   (ii) Heart
      (i) and (ii) cause relatively constant heat production.
   (iii) Skeletal muscle generates variable amount of heat, but a major source of heat production in the body:
      At rest: very little; and
      In exercise: a great deal.

   Heat production under basal (resting) conditions, called ***basal metabolic rate*** *(BMR)* is 1 kcal/kg/hour or 37–40 kcal/m^2 hour. This output works out at about 1500 kcal/day in females and 1700 kcal/day in males. (Also refer to page 652).
2. By assimilation of food *i.e.* *specific dynamic action* (SDA) of food. The SDA of a food is the obligatory energy expenditure that occurs during its assimilation into the body. Maximum heat production is seen after ingestion of proteins.
3. Heat gained by body *from the environment* from objects hotter than itself:
   (i) by direct radiation from the sun or heated ground
   (ii) by reflected radiation from the sky.
4. By *endocrine mechanisms*
   (i) Epinephrine and nor-epinephrine produce a rapid but short lived increase in heat production.

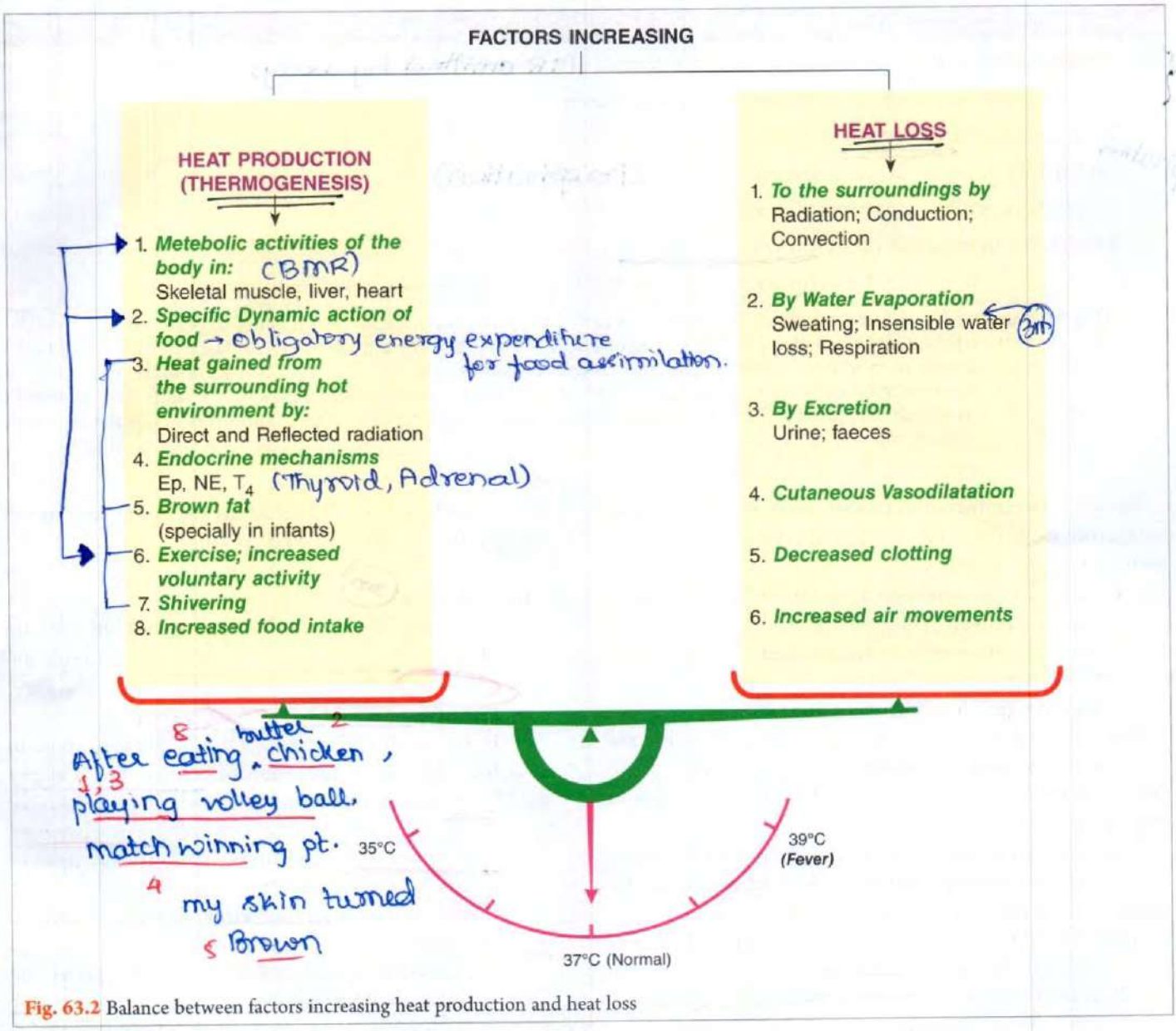


Fig. 63.2 Balance between factors increasing heat production and heat loss

Note

Sympathetic discharge is increased by feeding.

(ii) Thyroid hormones produce a slowly developing but prolonged increase in heat production.

5. A source of considerable heat production is a special type of fat, called **Brown Fat**, which makes up a small percentage of total body fat. It is more abundant in infants but is present in adults also. As brown fat cells contain several small droplets of fat rich in mitochondria, increased fatty acids oxidation in the mitochondria increases heat production (*i.e.* has a high rate of metabolism).

Sites of brown fat: Between the scapula; at the nape of neck, along the great vessels in the thorax and abdomen (for details, refer to page 611).

Important Notes

1. The standard unit of heat energy is the *'calorie' (cal)*, defined as the amount of heat energy necessary to raise the temperature of 1 gm of water by 1°C. This unit is also called the *Gram calorie, small calorie or standard calorie.* The unit commonly used in physiology and medicine is the Calorie (kilo-calorie, kcal), which equals 1000 calories.
2. One kg of body weight requires 0.8 kcal of heat to raise its temperature by 1°C. Therefore a person weighing 70 kg requires 56 (70 × 0.8) kcals of heat to raise its body temperature by 1°C.

B. HEAT LOSS

The processes by which heat is lost from the body when the environmental temperature is below body temperature are given in **Table 63.1**.

Table 63.1: Processes by which heat is lost from the body

1.	By *Radiation** from the body to cooler objects at a distance	50%	70%
2.	By *Conduction*** and Convection*** to the surrounding atmosphere	20%	
3.	By Vaporization (evaporation) of water		
	(i) Vaporization of sweat and insensible water loss		27%
	(ii) Warming and humidifying of inspired air		2%
4.	By excretion in urine and faeces (urination and defecation)		1%

1, 2 and 3(i) mechanisms are directly under the physiological control.

* It is the transfer of heat from one object to another at a different temperature with which it is not in contact.
** It is the heat exchange between objects at different temperatures that are in contact with one another. The amount of heat transferred is directly proportional to the temperature difference between the two objects in contact (thermal gradient).
*** It is the movement of the molecules of a gas or a liquid at one temperature to another location that is at a different temperature. It is greatly increased if the object moves about in the medium *e.g.* if a subject swims through water or a fan blows air through a room.

The relative contribution of each process that transfers heat away from the body varies with the environmental temperature, *for example,*

1. At 21°C, vaporization is a minor component in humans at rest;
2. As environmental temperature approaches body temperature, radiation losses decline and vaporization loss increases.

Since the conduction occurs from the surface of one object to the surface of another, the temperature of the skin determines to a large extent the degree to which body heat is lost or gained.

The amount of heat reaching the skin from the deep tissues can be varied by changing the blood flow to the skin, for example,

(1) when the cutaneous vessels are dilated, warm blood flows into the skin, and
(2) in the maximally vasoconstricted state, heat is held centrally in the body.

The rate at which heat is transferred from the deep tissues to the skin is called the ***Tissue Conductance.***

In humans, the other major process transferring heat from the body is by the way of **Vaporization of Water** from the skin and mucous membrane and respiratory passage.

Vaporization of 1 gm (approx. 1 mL) of water removes about 0.6 kcal of heat. A constant amount of water is vaporized all the time as follows:

A. ***Through Lungs***

On an average the water loss from the lungs is approx. 300 mL/day, equivalent to a heat loss of nearly 200 kcal. Some body heat is also lost via the lungs by raising the temperature of inspired air to body temperature, therefore, an increase in pulmonary ventilation (specially when the air is dry and cool as in winters) increases heat loss. This explains the dry feeling in the respiratory passage in cold weather.

B. ***Through Skin***

1. ***Insensible water loss (perspiration).*** It consists of the passage of water by diffusion through the epidermis which cannot be seen or felt. The fluid lost is not formed by sweat glands.
   It amounts to 50 mL/hour and is equivalent to heat loss by evaporation of approx. 30 kcal per hour. It is produced over the whole body surface at a fairly uniform rate and is largely independent of environmental conditions. It is always present unless humidity is 100%.
2. ***Sweating.*** There are two kinds of sweat glands in the body:
   (i) **Eccrine Glands** are *densest on the palms and soles;* next dense on the head, and much less dense on the trunk and extremities. They are supplied by *cholinergic fibers* present in sympathetic nerves and secrete a dilute solution containing NaCl, urea and lactic acid. Atropine inhibits its secretion.
   Eccrine glands are responsible for ***Thermal Sweating*** because their secretion increases due to:
   (a) rise of external or internal body temperature
   (b) emotional states resulting in sweating over palm, sole and axilla
   (c) exercise
   (d) vomiting, and
   (e) after ingestion of spicy food.
   (ii) **Apocrine Glands** develop from hair follicles. Found mainly in the axilla and around the nipples and in females in labia majora and