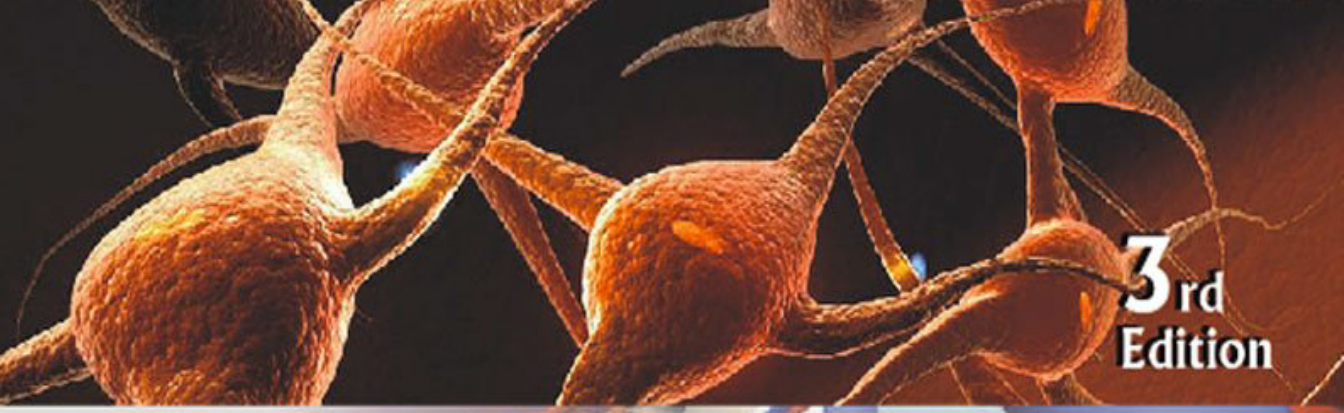

3rd Edition

Textbook of PHYSIOLOGY

R Chandramouli

JAYPEE

Textbook of Physiology

Textbook of Physiology

Third Edition

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Textbook of Physiology

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Preface to the Third Edition

What is new in the third edition?

The third edition has been revised with the inclusion of current developments in the field of Physiology. More illustrated figures, flow charts, tables and text boxes have been added in all the organ systems. The reader will definitely feel that they are simple, comprehensive and easy to understand with just a glance. The present edition includes self-study questions comprising, multiple choice questions and short answer questions at the end of each organ system. It is hoped that answering them would strengthen the knowledge acquired after the topics have been learned.

The author gratefully acknowledges the contribution of Ms Abida Parveen, Gulf Medical University, who has painstakingly spent a lot of time in going through the text and helped in the correction of mistakes.

The author also acknowledges the service rendered by Mr Sajjan, who executed computer assisted diagrams which are included in this edition. The author would like to express the deep sense of appreciation for Shri Jitendar P Vij (Chairman and Managing Director) of M/s Jaypee Brothers Medical Publishers (P) Ltd. for his commitment and support to bring out the third edition.

R Chandramouli

Preface to the First Edition

Textbook of Physiology has been primarily prepared to cater to the requirements of medical students, who have to follow the revised medical curriculum. Keeping in mind the syllabus recommended for their study, a text which is tailor-made for their use, has been presented in this book. A quick glance at the text will inspire anyone to learn more, even the topics, which are considered tough. The topics such as, cell physiology, biophysics, bioelectric properties of nerve, mechanics of muscle contraction, smooth muscle contraction, recent advances in GI hormones, transduction of sensory receptors, recent developments in hormone action, functions of nitric oxide, growth factors, regulation of cardiac function and arterial blood pressure, fluid and electrolyte balance have been deftly handled to give greater clarity and understanding. The diagrams included in the text are line drawings. The reader will find them more informative and easy to reproduce them in the examination.

The restructured curriculum lays emphasis on the objective type of questions in the examinations which include MCQs and short answer questions. The objective type of questions can be correctly answered by the student, only if he knows the subject precisely. This text contains more than 500 self-study questions, covering all the topics in physiology. The hallmark of this presentation is, the answers are given in the form of explanatory notes, which themselves form short answers for many questions, that are faced by the students in the examination. The author is confident that the reader, after learning the text, will find these questions highly rewarding in terms of knowledge. The topics covered in the text will be very much useful for medical, dental postgraduate students of physiology and also PG entrance preparation.

The author sincerely expresses his appreciation and thanks to Shri Jitendar P Vij, Managing Director of Jaypee Brothers Medical Publishers, Mr RK Yadav, Editorial Consultant for readily agreeing to publish this book within a short period. The author is extremely grateful to Dr HH Sudhakar, Department of Physiology, KIMS, for his painstaking efforts in doing the corrections of manuscripts. He has been a source of help and support in completing this work in time. The author sincerely acknowledges the help and service rendered by the artist Mr S Shekar, in drawing the illustrations for the text. The formatting of the text was done by Mr V Pavan, who has shown real professionalism in his work. The author gratefully acknowledges his help in completing the work in time.

R Chandramouli

Contents

1 Cell Physiology and Biophysics

CELL MEMBRANE

Cell forms the building block of life. They are highly specialized in various organs to carry out specific functions, but the structures present inside, namely the organelles are identical in all types of cells (Fig. 1.1). With the advent of recent techniques in the cell biology, it is now possible to separate organelles by ultracentrifugation.

Each cell has a boundary called plasma membrane, which has a diameter of 7.5 nm or 75 A°. The membrane is made up of phospholipids and proteins. The Lipid is arranged as ***bimolecular layer,*** with the hydrophilic polar groups facing the aqueous medium on both sides of the membrane, while the nonpolar hydrophobic groups extend into the interior of the membrane. The phosphate in the lipids is hydrophilic and attached to the head, whereas, the lipid is the nonpolar and attached to the tail. Two types of proteins are present in the cell membrane namely, peripheral and structural proteins. The proteins which are attached to the polar groups of the cell membrane are globular type and they are the ***peripheral proteins***. Peripheral proteins are present on both sides of the membrane and more on the exterior. Proteins also extend through the cell membrane forming ***structural***

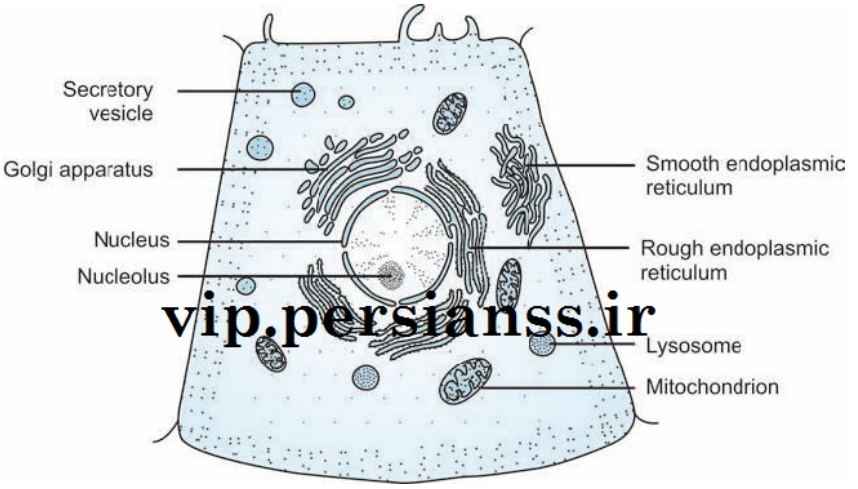


Fig. 1.1: Structure of a cell

proteins or integral proteins (Fig. 1.2). Attached to the peripheral proteins on the exterior, mucopolysaccharides are present. The important constituent of this is sialic acid and its presence on the cell membrane gives negative electrical charge to the cell surface. These glycoproteins are also responsible for the antigenicity of the cell membrane.

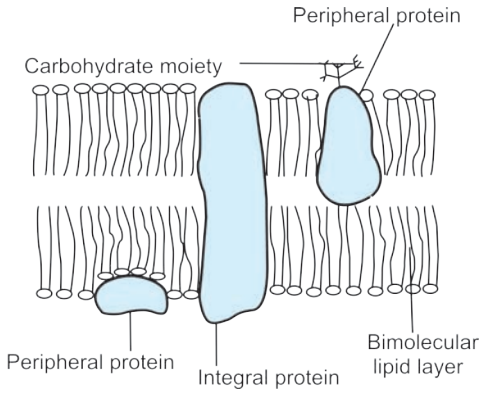


Fig. 1.2: Fluid mosaic model of cell membrane

The carbohydrate group is attached to the peripheral proteins on the outside of the membrane and form glycoproteins.They function as receptors and antigens. They also form glycocalyx which helps in intercellular connections

Fluid mosaic model

The lipids and proteins on the cell membrane are in a dynamic state, in the sense, that they are in a fluid mobile state and can change their shape and position. In addition, there is also turnover of membrane lipids and proteins.

Functions of cell membrane proteins

- The proteins serve as structural support to the membrane
- Act as antigen on the surface of the cell
- As channels or pores for the movement of ions and water
- Act as carrier proteins for the transport of solutes across the membrane
- Proteins on the surface of the cell membrane act as enzymes catalyzing chemical reactions
- Proteins also act as receptors for the hormones and neurotransmitters.

CELL ORGANELLES

They are the structures present inside the cell. It includes nucleus, endoplasmic reticulum, Golgi apparatus, mitochondria, lysosomes, microsomes, microtubules and microfilaments. The detailed descriptions of these structures are given below.

Nucleus

Nucleus is surrounded by a double layered membrane with pores 70 nm size.These pores serve as transport channels for RNA to migrate to cytoplasm. Nucleus contains chromatin and one or more nucleoli. The nucleoli contain RNA, while, the chromatin has the DNA, which carries the genetic information. The structure of DNA shows two strands, which are folded into a double helical structure called chromosomes. The number of chromosomes present in the nucleus of human being is 46, which includes 22 pairs of autosomes and one pair of sex chromosomes.

DNA is about 10 to 20 nm in size and chemically made up of four nucleotides namely, ***adenosine, thymidine, guanosine and cystidine.*** Each nucleotide consists of a pentose sugar deoxy ribose, phosphoric acid residue and a side chain consisting of bases namely, adenine, thymine, guanine, and cytosine.

The sequence with which the bases are arranged forms the ***genetic code.***

RNA is a single strand structure, which contains ribose sugar instead of deoxyribose and uracil instead of thymine base.

Protein synthesis depends on the genetic code present in DNA. The transfer of genetic information from DNA to the ribosomes occurs through mRNA and is called ***transcription.*** The mRNA binds to polyribosomes in the cytoplasm and assemble the amino acids with the help of tRNA. This process is called ***translation.*** The rough endoplasmic reticulum contains ribosomes on its surface and protein synthesis takes place on it. The synthesized protein undergoes post-translational modification, with cleavage of

bonds and side chain changes. It moves to Golgi apparatus for packaging and forms secretory vesicle.

Endoplasmic reticulum

It is one of the constituents of microsomes. It is of two types namely, *smooth endoplasmic reticulum* **(SER)** and *rough endoplasmic reticulum* **(RER**). The rough endoplasmic reticulum consists of flat vesicles which are interconnected to form a network of channels throughout the cell. The surface of the RER contains ribosomes on which the proteins are synthesized.

The SER has no ribosomes on the surface and is mainly concerned with the synthesis of lipids and steroids.

Golgi apparatus

It consists of stacks of flat vesicles and is closely associated with RER. Golgi apparatus receives the synthesized protein and modifies it by concentration, processing and packaging. The concentration of the synthesized molecule occurs by the removal of water, while processing involves cleavage of the precursor molecule and addition of carbohydrate moiety. The packaging of the synthesized molecule forms the secretory vesicle. The secretory vesicle is transported to the surface of the cell guided by the microtubules and released from the cell by exocytosis.

Mitochondria

It is oval shaped and has double layered cell membrane.The inner membrane has been thrown into folds called cristae which increase the surface area. The cristae includes the matrix (Fig. 1.3). The respiratory enzymes are present in the cristae while the matrix contains the nuclear material DNA and RNA. The RNA is involved in the synthesis of enzymes of oxidative phosphorylation, while the DNA facilitates mitochondrial replication. The mitochondria is called the power house of the cell, as the energy rich compound ATP is synthesized from them.

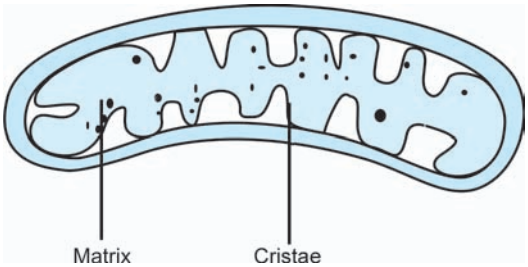


Fig. 1.3: Structure of a mitochondrion

The number of mitochondria in a cell varies in relation to its metabolic activity. The cells that are concerned with the secretion and those that are metabolically active, such as liver cells, will have more number of mitochondria.

Lysosomes

They are the vesicles containing hydrolytic enzymes. They are formed from endoplasmic reticulum and Golgi apparatus. The main function of lysosomes is in the digestion of the engulfed material. The substances that are engulfed into the cell by pinocytosis or phagocytosis, fuse with the lysosomes and form the digestive vacuole. The lysosomes which are not attached to the phagocytic vacuole are known as primary lysosomes and those which are attached are called secondary lysosomes. The acid hydrolases in lysosomes digest the engulfed material and the indigestible ones are in the residual body. It is released from the cell by exocytosis. The lysosomes also can digest the breakdown products of cell's own organelles. In certain pathological conditions such as gout, rheumatoid arthritis, the release of lysosomal enzymes is considered to be the causative factor in the pathogenesis of the disease.

Lysosomal storage diseases such as, Gaucher's disease, Fabry disease and Tay- Sachs disease are due to congenital absence of lysosomal enzymes. The absence of β-galactocerbrosidase causes Gaucher disease while lack of α-galactocerebrosidase results in Fabry's disease.

Peroxisomes

It is one of the constituents of microsomes and can be obtained by ultracentrifugation of the cell organelles. The peroxisomes are more in number in liver and kidney. They contain an enzyme called catalase which causes breakdown (detoxification) of hydrogen peroxide.They are also involved in the β oxidation of lipids and thereby regulate their level in the plasma.

Cytoskeleton

The cell contains fibers which include microtubules, intermediate filaments and microfilaments. These are known as cytoskeletal structures. They give structural stability to the cell and produce cell movement.

Microtubules

The microtubules give shape to the cell. They facilitate the transport of secretory vesicles and organelles to the cell surface. The microtubules are in a dynamic state as they can assemble and disintegrate in various conditions.

Microtubules are considered as cytoskeletal structures and they are made of 2 globular proteins, *α and β tubulin*. During cell division, the centrosomes produce a third subunit *γ tubulin*, which helps in the movement of chromosomes. These subunits of tubulin combine with proteins like *dynein*, *kinesin* and *dynamin* to give various functions.The movements of chromosomes during cell division and anterograde transport in the axon involve the tubulin interaction with dynein. The retrograde transport in the axon is due to the tubulin interacting with kinesin, while the sliding of microtubules with one another is by the interaction of dynamin with tubulin.

Within the cell, the proteins and cell organelles move with the help of molecular motors, which include **kinesin, dyneins** and **myosins**.

Microfilaments

Microfilaments are made of actin and their interaction with myosin I cause cell movement. Most of the cells contain actin at the cell surface. The interaction of actin with myosin II gives muscle contraction.

Cilia

Cilia and flagella are the cell processes surrounded by the extensions of plasma membrane. The intercellular structure consists of microtubules. There is an outer 9 units of microtubules and an inner pair present in the center. The movement of cilia or flagella is caused by the sliding of microtubules.

Cell adhesion molecules

Cell adhesion molecules(CAMs) are proteins, which facilitate attachment of the cell to basal lamina and between cells. Some of these molecules also project into the cell and are attached to the cytoskeleton. CAMs are broadly divided into **integrins, IgG superfamily** of immunoglobulins, cadherins and selectins.

These protein molecules assist intercellular connections, cell movement and transfer information between cells. The integrin molecules help in the attachment of cell to the extracellular matrix and in the absence of this molecule the cells tend to show death (apoptosis) earlier. CAMs are also involved in the pathophysiology of inflammation, wound healing and in the metastasis of tumors.

INTERCELLULAR CONNECTIONS

The cells are interconnected mainly in three ways namely tight junction, gap junction and desmosome (Fig. 1.4).

Gap Junction

It provides gaps or channels from one cell to another and allows the passage of small

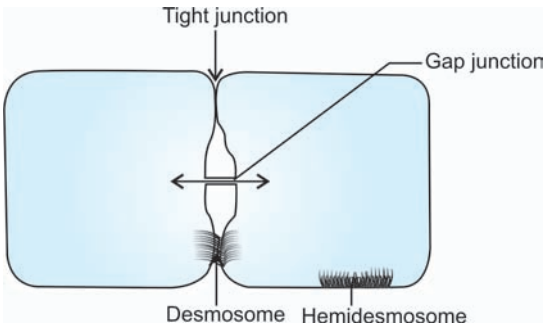


Fig. 1.4: Intercellular connections

molecules and ions. In smooth muscles and cardiac muscle, the electrical excitation spreads to adjacent cells by the movement of ions through the gap junctions.

Tight junction

It is a connection between two adjacent cells at the apical surface.The fusion of the lateral borders at the apical surface forms a barrier against the entry of substances. This kind of intercellular connection is seen in the intestinal epithelial cells, renal tubular cells and choroid plexus.

Desmosome

Two adjacent cells are held together by the electron dense fibrous substance in the extracellular space. There are intermediate filaments projecting from each cell, at the point of cell adhesion. The usual width of this connection is 20 nm. This type of connection between two cells helps to overcome mechanical stretching that occurs in the tissues like muscle and skin.

TRANSPORT MECHANISMS

The plasma membrane forms the boundary between the cell interior and exterior. The membrane shows transport of substances across the membrane in both ways.

Substances and ions move into the cell for metabolism, to give excitability of tissues and finally to maintain cell volume. There is also movement of substances from the cell into the exterior, for the sole purpose of maintaining homeostasis (*constancy of internal environment).*

Endocytosis

The uptake of molecules into the cell occurs by a process called ***endocytosis.*** The molecule at first fuses with the cell membrane and invaginates.The invagination is separated from the cell membrane and becomes a vesicle.

If fluid with the dissolved substances are engulfed into the cell, it is ***pinocytosis*** and the vesicle inside the cell is called pinocytic vesicle.

If particulate matter and bacteria are engulfed into the cell, it is known as ***phagocytosis*** (Fig. 1.5). The vesicle inside the cell forms the ***phagosome.***

In pinocytosis, the substances that are taken in to the cell include insulin, viruses, growth factors and low density lipoproteins. The process is mediated through receptors present on the cell surface. They are also other proteins namely

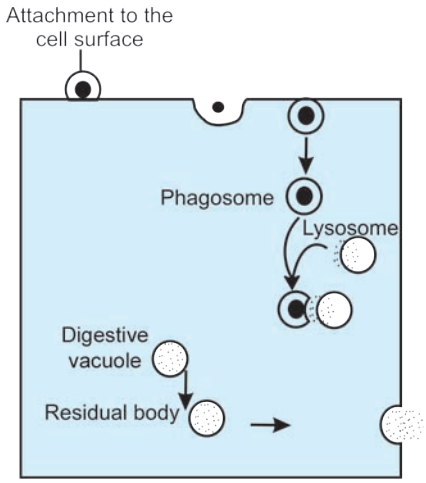


Fig. 1.5: Phagocytosis

clathrin, actin and myocin present beneath the receptors, which help the pinocytic vesicle to pinch off from the cell membrane. The process of endocytosis requires the supply of ATP as it is an active process.

During endocytosis process a part of the cell membrane is removed which is compensated by the addition of membrane to the cell surface during exocytosis when the secretory vesicle fuses with the cell membrane. This explains how the surface area of the cell membrane remains constant when these processes occur.

Exocytosis

Exocytosis refers to the release of substances from the cell. It could be the proteins synthesized by the RER or the cell organelles that are disintegrated. The synthesized proteins form the secretory vesicle and are guided by microtubules to the cell surface. The membrane of the vesicle and plasma membrane fuse and the contents of the vesicle are extruded out (Fig. 1.6). This process requires Ca^{++} entry into the cell and supply of energy.

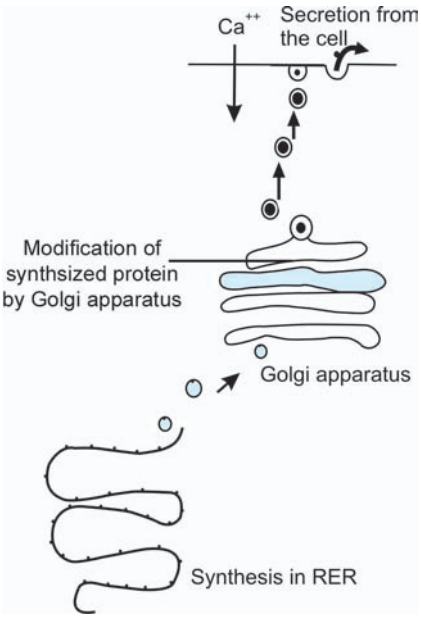


Fig. 1.6: Exocytosis

The Golgi apparatus takes up proteins synthesized from RER, where modification occurs, by concentration, processing and packaging. The modified protein becomes a secretory vesicle, which reaches the cell surface, guided by the microtubules

Transcytosis

Transcytosis also can be observed in the transport across the membrane. It refers to the movement of macromolecules (protein hormones) from one side of the cell to the other side unchanged. This kind of transport is seen in endothelia.

Passive transport

When there is a concentration gradient across the membrane, diffusion of water and solutes occur. The diffusion will be from a region of higher concentration to a region of lower concentration (Fig. 1.7).

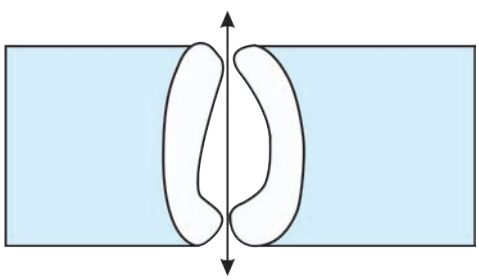


Fig. 1.7: Diffusion of solutes through leaky channels

Diffusion of solutes through leaky channels occurs on either side of the membrane depending on the concentration gradient

The process of diffusion depends on:

- Membrane permeability for the diffusing substance
- Concentration gradient
- Size of the molecule in relation to the pores
- Cross section of the diffusing area
- Temperature
- Distance involved during diffusion.

Diffusion of solute

Diffusion of solutes across the membrane follows **Fick's law**.It states that the rate of

diffusion (J) is directly related to the cross sectional area (A) through which diffusion takes place and concentration gradient (C/X)

$$J = -DA \frac{C}{X}$$

D = diffusion coefficient and – sign denotes the direction.

Diffusion of solvent

Osmosis

Water molecules move down along the concentration gradient through a semipermeable membrane and this process is called **osmosis** (Fig. 1.8). It is the movement of solvent into a region of greater concentration of solute to which the membrane is not permeable. Examples of water diffusion by osmosis are seen in renal tubules, intestinal mucosa, between cells and exterior, between capillaries and interstitial fluid. The process of osmosis creates hydrostatic pressure in the region to which the solvent diffuses. The movement of solvent can be prevented by applying pressure over the region of higher solute concentration. This pressure is known as **osmotic pressure.** It depends on the number of solute particles and in an ideal solution, the osmotic pressure (p) is related to:

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

n = number of solute particles
R = gas constant
T = absolute temperature
V = Volume

In our body such an ideal solution does not exist. Hence the osmotic pressure in the body fluids depends on the number of solute particles per unit volume of fluid.

Passive diffusion of water occurs whenever there is active solute movement. The concentration of solute in a fluid gives the tonicity. If the solute concentration is more, the tonicity of the fluid is referred as **hypertonic** and conversely, decreased solute concentration in a fluid gives rise to less tonicity and it is known as **hypotonic.**

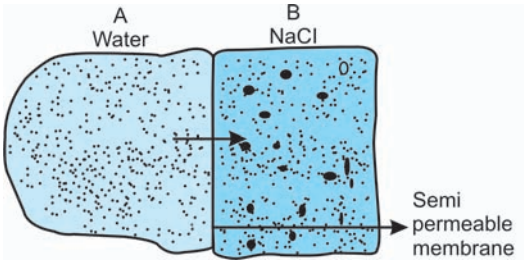


Fig. 1.8: Osmosis

Movement of water from A to B occurs through the semipermeable membrane as the solute (NaCl) concentration in B is greater

If fluids from two different compartments show the same tonicity, it is said to be **isotonic.** In isotonic state, there is no movement of water on either side of the cell membrane due to the osmotic equilibrium.

In mammals, solutions of 0.9% NaCl are isotonic to plasma.The osmotic concentration of solutes in plasma is **290 mEq/L**. This is equal to the freezing point of plasma –0.54°C. The osmotic concentration of solutes in plasma is known as **osmolality** and is due to the presence of osmotically active solutes like Na^+, Cl^-, HCO^-_3 urea and other solutes. Plasma proteins contribute very little to plasma osmolality, inspite of being present in greater amounts.

Plasma osmolality is altered when there is electrolyte and fluid disturbance in the body. In severe dehydration, hyperosmolality results. In renal failure, the retention of urea in plasma leads to hyperosmolar state, which results in coma.

Solvent drag

Whenever solvents diffuse, they tend to drag some solute with it. This is called solvent drag, and can be observed in capillaries and renal tubules.

Carrier mediated transport

A substance that cannot diffuse through the pores or leaky channels in the membrane can be transported by a carrier across the membrane.

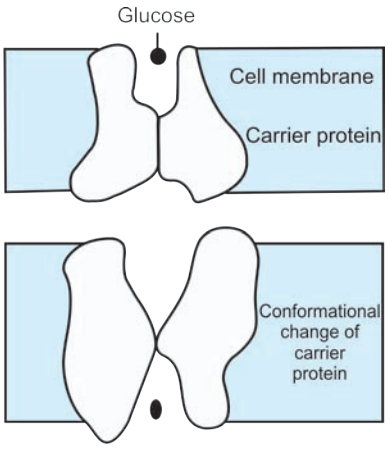


Fig. 1.9: Facilitated diffusion of glucose

Facilitated diffusion of solutes like glucose occurs by the conformational change of carrier protein in the cell membrane

Lipid soluble substances and respiratory gases can easily diffuse through the membrane. There are also substances of low molecular size, which can pass through the leaky channels. The solutes that cannot pass through these pores depend on the carrier proteins to transport them. If the transport occurs along the concentration gradient, it becomes a **facilitated diffusion**. It is a **downhill transport,** because, the transport does not require energy.

Facilitated diffusion is determined by:
- Concentration gradient
- Saturation of carrier protein.

The **carrier protein** shows:
- Specificity
- Competition
- Inhibition.

Facilitated diffusion can be seen in the transport of glucose and amino acids from the lumen of the renal tubule to the PCT cell and also from the lumen of intestine to the mucosal cell (Fig. 1.9). The carrier protein which transports glucose is called **glucose transporter.**

Cotransport

There are instances where two solutes are transported together by the carrier and such transport is called **symport** or **cotransport**. Examples of this transport are glucose transporter (**SGLUTI)** which transports both glucose and Na^+ from the lumen of intestine to the mucosal cell (Fig. 1.10). Amino acids are also transported as symport with Na^+ in the intestine. Similar cotransport also exists in the renal tubules.

In cotransport, the movement of solutes occurs by facilitated diffusion.

Active transport

Active transport of solutes occurs from a region of lower concentration to a region of higher concentration. This requires a carrier protein and the supply of energy, as it is an **uphill transport** (Fig. 1.11). Examples of active transport include Na^+ pump, secretion of H^+ from the parietal cells of gastric glands, iodide transport in the thyroid gland acinar cells, etc.

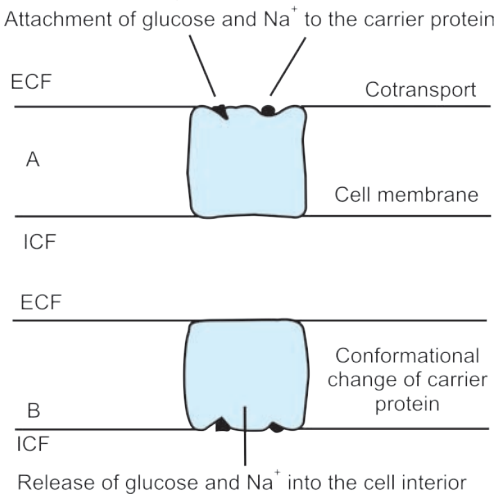


Fig. 1.10: Cotransport (symport)

In intestinal mucosa and renal tubules glucose enters the cell by cotransport with Na^+. The carrier protein is common to both.The attachment of both the solutes to the carrier molecule brings about conformational change, which causes the release of glucose and Na^+ into the cell interior

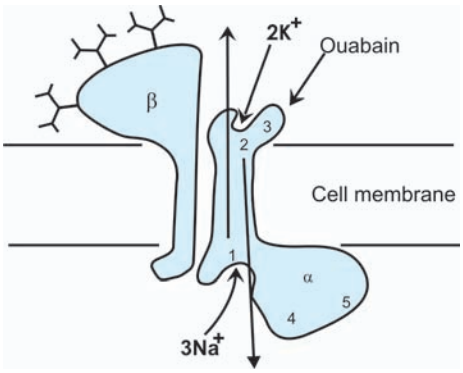


Fig. 1.11: Active transport mechanism by Na^+ - K^+ ATPase. The unit of the carrier protein has five binding sites. 1. Na^+ binding site; 2. K^+ binding site; 3. Ouabain binding site; 4. Phosphorylation site; 5. ATP binding site.

Sodium is actively transported from the interior of the cell to the exterior by the enzyme **Na^+-K^+ATPase**. The attachment of sodium and potassium occurs to the α subunit of the carrier molecule which is a larger globular protein spanning through the cell membrane. On the inner side of the α subunit, 3 binding sites for Na^+ and on the outer side 2 binding sites for K^+ are present. The α subunit also functions as ATPase on the inside of the protein, close to the sodium binding site. The function of the other smaller globular protein β is not clear.

At the intracellular surface, the carrier protein is phosphorylated causing ATP to hydrolyse into ADP and Pi. This causes attachment of 3 molecules of sodium and the conformational changes bring sodium molecules to the exterior of the cell membrane. Dephosphorylation of the carrier protein causes attachment of 2 molecules of K^+ and the conformational change of the carrier protein brings the K^+ molecules to the cell interior. Sodium pump is also known as electrogenic pump, as it causes more positivity on the outside of the membrane.Cardiac glycoside ouabain inhibits sodium pump by acting on the carrier molecule on the outside of the membrane. Sodium pump is also inhibited by metabolic poisons like dinitrophenol.

Secondary active transport

Active transport of Na^+ by the Na^+-K^+ ATPase carrier across the basolateral border of the intestinal mucosal cells and renal tubules, results in entry of glucose into the cell. As said above, glucose and Na^+ are cotransported from the lumen into the cell.The active transport of Na^+ in the basolateral surface provides the chemical gradient for further movement of Na^+ and

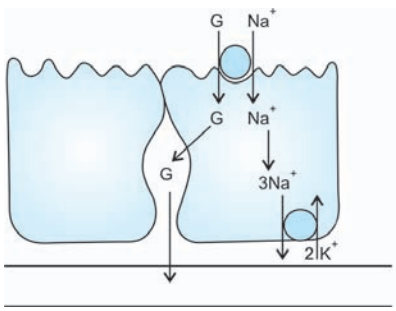


Fig. 1.12: Secondary active transport

The entry of glucose into the cell depends on the primary active transport of Na^+ at the basolateral border

glucose into the cell. Glucose transport into the cell forms the secondary active transport, as it depends on the active transport of Na^+ at the basolateral surface (Fig. 1.12).

Counter transport (Antiport)

Antiport

The solutes, when exchanged in the opposite direction by the carrier protein, form the **counter transport or antiport** (Fig. 1.13). Examples are:

- Na^+– H^+exchange in the renal tubes
- Na^+– K^+exchange (Na^+ pump)
- HCO_3^- – Cl^- exchange (chloride shift)
- Na^+– Ca^{++} exchange (heart and brain)

Gibbs Donnan's equilibrium

Donnan and Gibbs have shown that the sum of anions and cations on either side of the cell membrane are equal, eventhough their distribution across the membrane varies due to the selective permeability of the membrane.It is known that unequal distribution of ions gives electrical potential. The cations and anions on either side of the membrane are distributed in such a way that in each compartment their total number will be equal.This is called **Donnan's electrochemical equilibrium.**

$$\frac{\text{Diffusible cations}}{\text{Diffusible cations}} = \frac{\text{Diffusible anions}}{\text{Diffusible anions}}$$

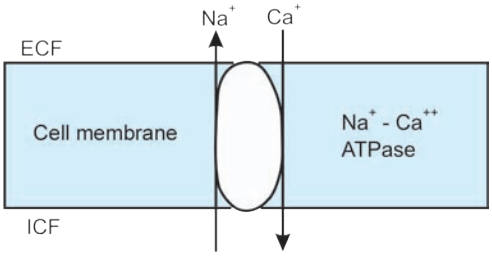


Fig. 1.13: Antiport: Na^+ and Ca^{++} exchange in the cardiac muscle

Examples of Donnan's ionic equilibrium can be observed in chloride shift which occur in the transport of CO_2. The exit of anion HCO^-_3 from RBC disturbs the electrochemical equilibrium. But, it is set right by the movement of another anion Cl^- from plasma into the RBC.

The entry of Na^+ into the cell through leaky channels of the membrane will also disturb Donnan's ionic equilibrium. But, the Na^+ pump mechanism does not allow this to happen. In addition to maintaining the electrochemical equilibrium, the Na^+ pump maintains the cell osmolality and cell shape.

Membrane potential

The unequal distribution of ions across the cell membrane, selective permeability of the cell membrane for K^+ and the electrogenic $Na^+ - K^+$ pump creates potential difference between inside and outside of the cell membrane. In excitable tissues namely nerve and muscle this electrical potential is significant for excitation of the cell. In these tissues the electrical potential does not alter the Donnan's ionic equilibrium as only a small number of ions are involved in the genesis of membrane potential.

Carrier mediated transport

It includes facilitated diffusion and active transport. The following properties are characteristics of carrier transport.

Contd....

Contd....

Saturation kinetics Increasing the concentration of the transported substance leads to no further transport, if the carrier protein is already saturated with the substance.

Specificity There is a chemical specificity existing between the carrier and the transported substance. A carrier specific for a substance cannot take up another type of substance.

Competitive inhibition If two substances show similarity in their structure, they compete with the carrier protein for binding. The transport of one of the substances will be decreased due to this.

Inhibition by metabolic poisons In active transport mechanism where energy supply is required, administration of metabolic poisons like phlorhizin inhibits transport, as ATP is not generated.

Counter transport

It is the exchange of solutes in the opposite directions across the cell membrane. This transport helps to maintain:
Cell volume
pH of cytosol
Membrane potential.

Counter transports can be energy dependent or a simple exchange of ions without depending on energy.

Osmotic pressure and oncotic pressure

Osmotic pressure refers to the presence of various osmotically active solutes like Na^+, Cl, urea, glucose, etc.The oncotic pressure on the other hand refers to the osmotic pressure produced by plasma proteins, especially albumin. The osmotic pressure produced by albumin is also known as colloidal osmotic pressure.

Osmolality and Osmolarity

Osmolality is the number of osmoles per kilogram of water.

Osmolarity is number of osmoles per liter of solution.

One osmole is 1 gram molecular weight of undissociated solute.

Examples: 180 grams of glucose will give 1 gram molecular weight and is equal to 1 osmole. If the solute dissociates into two ions, the number of osmoles for that solute will be double. For example, 58.5 grams of NaCl gives 1 gram molecular weight, which gives 2 osmoles, as NaCl dissociates into Na^+ and Cl^-.

The osmotic pressure of a solution depends on the number of osmotically active solutes (number of osmoles) and not to the mass of the solute.

CHEMICAL MESSENGERS

Communication from one cell to another usually takes place by the chemical messengers. These substances can be proteins, amino acids or lipids. Chemical messengers enter the cell through *gap junctions*, *paracrine*, *endocrine* and *neural* communications. There is also cell's own secretion that can act on itself which forms the *autocrine secretion*.

Chemical messengers produce their effects by acting on specific receptors situated on the surface of the cell. They form the **first messengers or ligands**. The intracellular effects are mediated through the release of other chemical substances known as **second messengers** and **third messengers.**

The examples of **second messengers** are:

- Cyclic AMP
- Inositol tri phosphate (IP_3)
- Diacyl glycerol (DAG)
- Ca^{++}

How are second messengers formed?

Second messengers are formed through a nucleotide regulatory protein called G protein. When a first messenger (ligand) binds to the receptor on the cell membrane, the ligand receptor (Gs) complex converts GTP to GDP and activates adenylyl cyclase, if cyclic AMP is formed as second messenger. The binding of the ligand with the Gi inhibits the adenylyl cyclase activity and prevents the formation of cyclic AMP. Cyclic AMP activates protein kinase A, which phosphorylates proteins, leading to the physiological effects (Fig. 1.14). The cyclic AMP is inactivated by the enzyme phosphodiesterase, which converts 3′ 5′ AMP to 5′ AMP and it is inactive. If this enzyme is inhibited, the activity of cyclic AMP can be prolonged. Substances like caffeine, theophylline show such effect when administered.

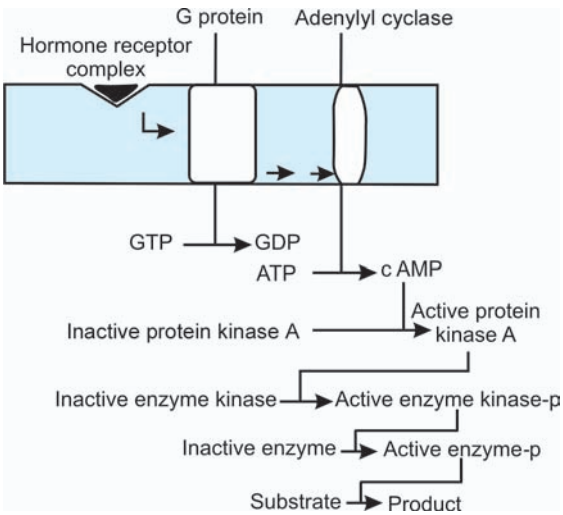


Fig. 1.14: Formation of cAMP and its action as second messenger

Activation of protein kinase A by cAMP causes phosphorylation of enzymes leading to its activation. The effect of the hormone occurs through the metabolic regulation of these enzymes or the activation of protein kinase A can lead to phosphorylation of protein, leading to protein synthesis

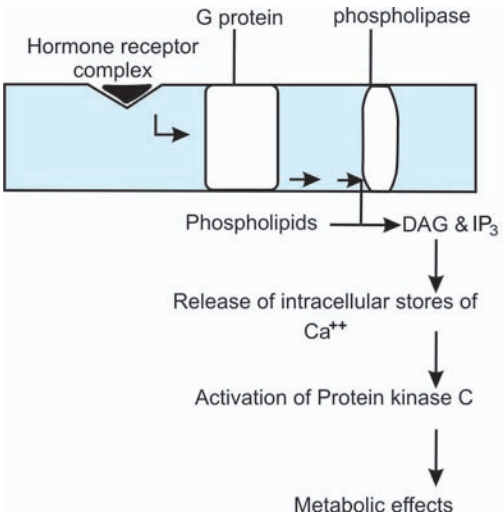


Fig. 1.15: Mechanism of action of DAG and IP_3 as second messenger

The membrane phospholipids release Diacylglycerol and Inositol tri phosphate by the action of phospholipase. The DAG and IP_3 that are formed release Ca^{++} from the intracellular stores such as mitochondria and endoplasmic reticulum.The rise in intracellular Ca^{++} and DAG activates Protein kinase C enzyme, which phosphorylates enzymes and regulates metabolic effects

Cyclic GMP

Similar to cyclic AMP, there is another nucleotide known as guanosine mono phosphate (cyclic GMP), which functions as the second messenger. Atrial natriuretic peptide (ANP) and nitric oxide (NO) mediate their effects through cyclic GMP. In the retina, during excitation of rods, GMP is converted to 5 GMP which is responsible for the photochemical changes.

IP_3 and DAG

In the formation of IP_3 and DAG as second messengers, the ligand receptor (Gs) complex converts GTP to GDP and activates phospholipase enzyme. This facilitates the formation of IP_3 and DAG from the phosphorylation of phosphatidyl inositol released from cell membrane.

The second messengers such as IP_3 and DAG cause rise in the intracellular Ca^{++} by promoting its release from the Ca^{++} stores such as endoplasmic reticulum, mitochondria and also by facilitating its entry into the cell from ECF, through the opening of Ca^{++} channels (Fig. 1.15). The intracellular Ca^{++} binds to the calcium binding protein called calmodulin, which activates various kinases such as myosin light chain kinase, phosphorylase kinase, etc. These kinases produce the physiological effects.The rise of Ca^{++} within the cell mediates such effects and hence, Ca^{++} forms the **third chemical messenger.** The rise in intracellular Ca^{++} also activates **protein kinase C,** which leads to the activation of many enzymes in the cell. Through this, the actions of the ligand can also occur.

Ca^{++} as second messenger

The binding of ligand with the receptor on the cell surface, opens up the Ca^{++} channels, facilitating its entry into the cell. Inside the cell, the calcium binds to a protein **calmodulin** and this complex causes activation or inactivation of enzymes. Through these changes, the physiological effects of the ligand can be observed (Fig. 1.16).

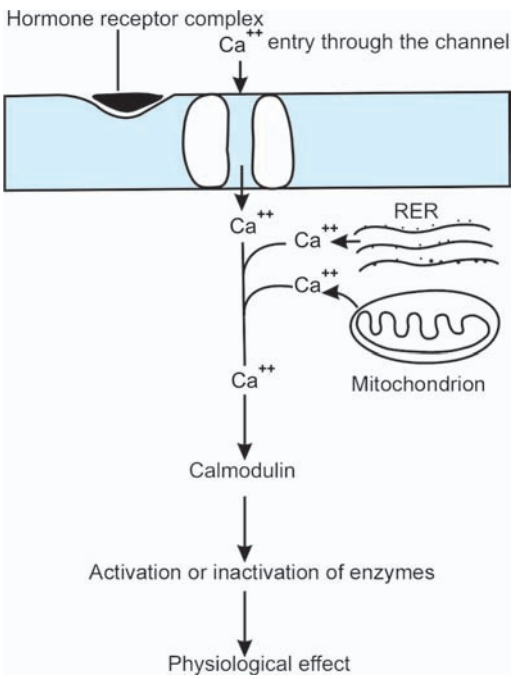


Fig. 1.16: Calcium as second messenger

Ligands mediating their effects through Cytoplasmic and nuclear receptors

Hormones such as T_3 (tri iodo thyronine) has receptors in the nucleus. The hormone after binding to the receptor in the nucleus, stimulates transcription of DNA in the chromatin. This leads to mRNA formation, which leaves nucleus and enters the endoplasmic reticulum. Here, new proteins are synthesized by translation. The synthesized protein can be an enzyme, secretory protein, structural protein, or receptor protein. The biological effects of the ligand are mediated through these new proteins (Fig. 1.17).

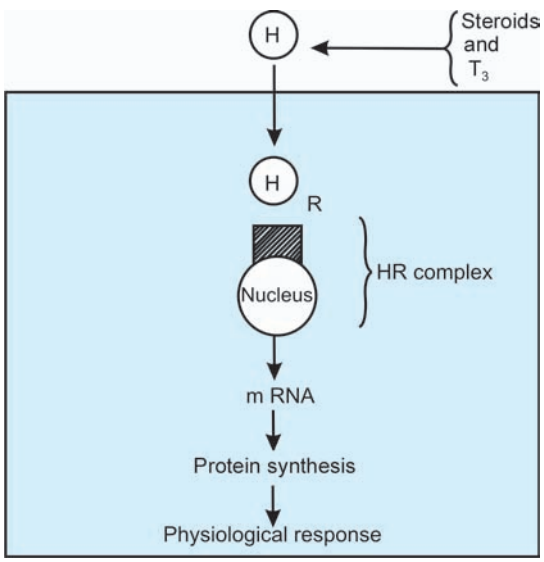


Fig. 1.17: Ligands mediating their effects through intracellular and nuclear receptors

Steroids have their receptors situated in the nucleus. The receptors are attached to a protein called Heat shock protein (HSP). This prevents the receptors getting attached to DNA and promote transcription, when the hormone is not present. When the hormone is attached to the receptor, the hormone receptor complex releases the heat shock protein.The release of HSP increases the affinity of the receptor for DNA and they are called HRE. (Hormone Response Elements). Thyroid hormones have their receptors in the nucleus.There is no HSP in the thyroid homones but the HRE are formed after the hormone binds to the receptor. This causes transcription and then translation leading to synthesis of proteins. Steroids have generally receptors in the cytosol or nucleus. The hormone receptor complex move to the DNA and produce changes as described for T_3

Examples of ligands mediating their effects through cyclic AMP

- Norepinephrine β_1 receptor
- Parathyroid hormone
- Glucagon
- Adrenocorticotrophic hormone
- Thyroid stimulating hormone

Receptors and its Regulation

As described earlier, the cell membrane has structural proteins, which project on the outer and inner surface of the membrane. It is the peripheral proteins present on the outer surface of the membrane which function as receptors for ligands and neurotransmitters. These receptors are not static structures, as they are dynamic and mobile on the cell membrane. Their number is also not constant, as there is addition and removal to their numbers during the process of regulation.

Examples of ligands mediating their effects through IP_3 and DAG

- Angiotensin
- Norepinephrine α_1 receptor
- Oxytocin
- Serotonin
- Gastrin
- Acetylcholine
- Vasopressin V_1 receptor

The regulation of receptors number is done by the ligand concentration in the ECF. If more amount of ligand is present, the number of receptors decreases which is called **down regulation**. On the other hand if the ligand concentration is less in the ECF, the receptor number increases on the cell membrane and it is known as **up regulation.**

Whenever a ligand binds to a receptor, ligand receptor complex is formed, which moves laterally on the membrane and form coated pits. These coated pits are pinched off from the membrane and taken inside the cell by endocytosis. This process is called **internalization** of receptors.

Lysosomes fuse with the endocytosed vesicle and release carbohydrate, amino acids and lipids into the cytoplasm, which are utilized for cell metabolism.

ION CHANNELS IN THE CELL MEMBRANE

Ion channels are formed in the cell membrane by the integral proteins.These channels are guarded by gates, which open and close, regulating the movement of ions. The gates are regulated either by voltage or chemical ligands. Accordingly, the channels are called voltage gated and ligand gated channels respectively.

Study of channels

Ion channels can be studied by **patch clamp technique** (Fig. 1.18). A microelectrode is kept on the surface of the membrane and a high resistance seal is applied around the tip of the microelectrode. By a suction force, a patch of cell membrane containing a few ion channels is taken inside the seal. The activity of the ion channels can be studied *in situ* or the small part of the membrane is removed and kept in solutions of known ion concentration to study the channel activity.

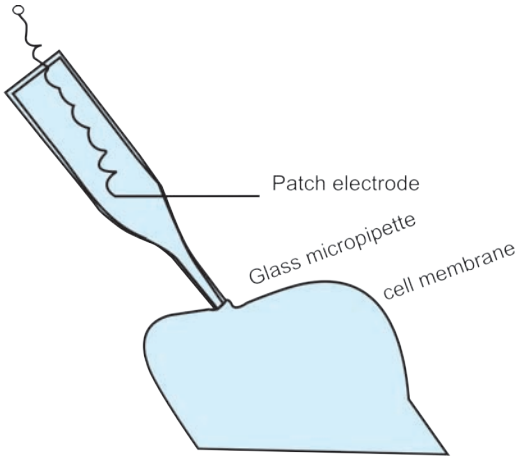
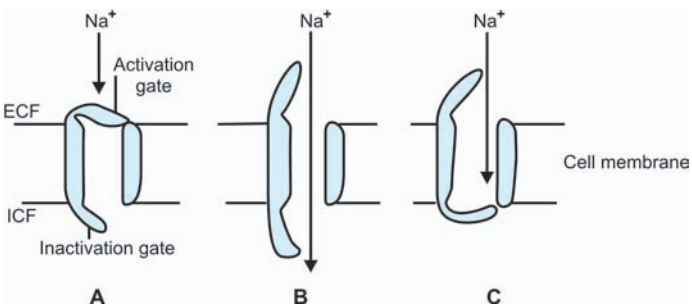


Fig. 1.18: Diagram to show patch clamp of membrane channels

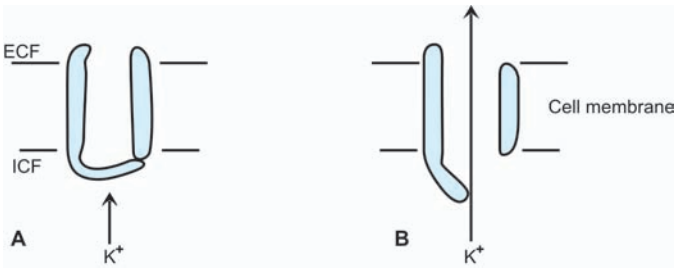
A glass micropipette is kept on the cell membrane and a piece of membrane containing a few channels is sucked into the micropipette and the edges of the pipette form the seal from the rest of the membrane.The electrode measures the current flow through the ion channels present in the patch of the membrane sucked into the glass micropipette

Sodium channel

It is a tetramer with four units.Each unit crosses the membrane six times. The pores with 0.5 nm diameter is present surrounding the subunits. The channel is guarded by gates, which are present in both inner and outer sides of the membrane. On the ECF side, there is activation gate. On the inner side of the membrane, there is an inactivation gate. For sodium to enter inside, both the gates should be in the open state (Fig. 1.19). The sodium channels are blocked by poisons like tetrodotoxin and saxitoxin.



Figs 1.19A to C: Voltage gated Na^+ channel
A. Resting (– 90 mv) B. Activated (– 90 mv to + 40 mv) C. Inactivated (+ 40 mv to – 90 mv)



Figs 1.20A and B: Voltage gated K^+ channel A. Resting (– 90 mv) B. Activated (+ 40 mv to – 90 mv)

Potassium channels

The structure of the potassium channel is similar to the sodium channel and they are the **voltage gated channels**. There is another type of K^+ channel which has only two subunits and crosses the membrane twice. These are called **inward rectifier potassium channels** which allow K^+ entry and not its exit. Unlike Na^+ channel, K^+ channel (voltaged gated channel) has only one gate and it is present on the inside of the membrane. There is no inactivation gate for the potassium channel. The gate should open for K^+ movement (Fig. 1.20). The voltage gated potassium channels can be blocked by chemicals like TEA (tetra ethyl ammonium) and 4 - amino pyridine. Ligand gated potassium channels also exist and activated by:

- Acetylcholine
- Ca^{++}
- *Arachidonic acid*
- ATP

Calcium channels

Calcium and chloride channels are similar to sodium channel in their structure. The ligand gated Ca^{++} channel is activated by hormones and neurotransmitters. The voltage gated Ca^{++} channel are of three types namely:

- Long lasting (L)
- Transient (T)
- Neuronal (N)

The long lasting voltage gated Ca^{++} channel is significant in the cardiac muscle and it is blocked by verapamil, nifedipine and diltiazem drugs. These drugs are extensively used in the treatment of cardiovascular diseases.

Self-study Questions

Multiple Choice Questions

Choose the single best answer

1. **The cell membrane proteins function as all of the following *except:***
   - **A.** Transporter
   - **B.** Ligand
   - **C.** Receptor
   - **D.** Channels

2. **The carbohydrate attachment to the peripheral proteins on the outer surface of the plasma membrane shows all of the following *except:***
   - **A.** Antigenicity
   - **B.** Surface electrical charge
   - **C.** Intercellular connections
   - **D.** Carrier

3. **Endoplasmic reticulum in a cell perform all of the following functions *except:***
   - **A.** Granular endoplasmic reticulum shows protein synthesis
   - **B.** In the skeletal and cardiac muscle granular endoplasmic reticulum forms sarcoplasmic reticulum
   - **C.** Lipid and steroid synthesis occur in the smooth endoplasmic reticulum
   - **D.** In the granular type endoplasmic reticulum translation of mRNA occurs on the ribosomes.

4. **Peroxisomes functions include:**
   - **A.** Transport
   - **B.** Packaging
   - **C.** Detoxification
   - **D.** Lipid synthesis

5. **Molecular motors include all of the following *except*:**
   - **A.** Dynein
   - **B.** Centrosome
   - **C.** Actin
   - **D.** Myosin

6. **The protein which is attached to the basal lamina and between cells include:**
   - **A.** Microfilaments
   - **B.** Cell adhesion molecules
   - **C.** Microtubules
   - **D.** Molecular motors

7. **The process of pinocytosis does not include the entry of which of the following into the cell?**
   - **A.** Viruses
   - **B.** Growth factors
   - **C.** Bacteria
   - **D.** Insulin

8. **Which of the following does not require a channel protein for its transport?**
   - **A.** Oxygen
   - **B.** Sodium
   - **C.** Water
   - **D.** Chloride

9. **Which of the following would decrease the rate of diffusion of substance across cell membrane?**
   - **A.** Increase in temperature
   - **B.** Increase in membrane permeability
   - **C.** Decrease in molecular size
   - **D.** Decrease in concentration gradient

10. **The function of tRNA in the cell include:**
    - **A.** Transcription
    - **B.** Translation
    - **C.** Replication
    - **D.** Transduction

11. **In cotransport, the solutes are transported by:**
    - **A.** Diffusion
    - **B.** Facilitated diffusion
    - **C.** Active transport
    - **D.** Counter transport

12. Which one of the following is an example of transport against electrochemical gradient?
- **A.** Diffusion
- **B.** Facilitated diffusion
- **C.** Active transport
- **D.** Secondary active transport

13. Channels in the cell membrane are formed from:
- **A.** Peripheral protein
- **B.** Integral protein
- **C.** Glycoprotein
- **D.** Bilipid layer

14. The example of secondary active transport includes:
- **A.** Sodium
- **B.** Water
- **C.** Chloride
- **D.** Glucose

15. The plasma oncotic pressure is due to the presence of:
- **A.** Sodium chloride
- **B.** Glucose
- **C.** Albumin
- **D.** Fibrinogen

16. The plasma osmolality is due to all of the following *except*:
- **A.** Urea
- **B.** Glucose
- **C.** NaCl
- **D.** Albumin

17. Which one of the following is not an example of counter transport?
- **A.** Na^+- Glucose
- **B.** Na^+ - Ca^{++}
- **C.** HCO_3^- - Cl^-
- **D.** Na^+- K^+

18. The hormone which mediates its effects through cGMP is:
- **A.** Vasopressin
- **B.** Oxytocin
- **C.** Atrial natriuretic peptide
- **D.** Angiotensin II

19. Voltage gated sodium channels can be blocked by:
- **A.** Tetrodotoxin
- **B.** α bungarotoxin
- **C.** Tetraethyl ammonium
- **D.** δ-tubocurare

20. The increase in the intracellular Ca^{++} level can occur due to release of Ca^{++} from all of the following *except:*
- **A.** Sarcoplasmic reticulum
- **B.** Mitochondria
- **C.** Calmodulin
- **B.** Endoplasmic reticulum

ANSWER KEYS

1. (B)	**2. (D)**	**3. (B)**	**4. (C)**	**5. (B)**	**6. (B)**	**7. (C)**	**8. (A)**	**9. (D)**	**10. (B)**
11. (B)	**12. (C)**	**13. (B)**	**14. (D)**	**15. (C)**	**16. (D)**	**17. (A)**	**18. (C)**	**19. (A)**	**20. (C)**

Short Answer Questions

1. List the functions of cell membrane proteins.
2. Describe the process of protein synthesis and its secretion from the cell.
3. Describe the function of lysosomes and explain its pathophysiology.
4. List the cytoskeletal proteins and their functions.
5. Describe the functions of cell adhesion molecules.
6. Describe the process of pinocytosis and phagocytosis with examples.
7. List the factors that influence diffusion.
8. List the differences between facilitated diffusion and active transport.
9. Describe carrier mediated transports with examples.
10. Define uniport, symport, and antiport processes in the cell.
11. What is the normal osmotic concentration of plasma? .
12. Describe Donnan's electrochemical equilibrium.
13. What are second messengers ? Describe how are they produced.
14. Name the hormones that mediate their effects through c AMP and cGMP.
15. Name the ligands that mediate their effects through IP3 and DAG.
16. Explain the process of down regulation and up regulation of receptors.
17. Name the agents that block the voltage gated channels of sodium, potassium and calcium

2 Nerve

NERVE BIOPHYSICS

In this section, the biophysical processes, which are involved in the excitation and conduction of impulses in a nerve fiber will be discussed.

Bioelectric potentials

Excitability

Any stimulus, whether it is mechanical, chemical or thermal, causes physico-chemical changes in the cell membrane, which ultimately gives rise to an impulse or action potential. The impulse generation in excitable tissues like nerve and muscle is possible due to the existence of an electrical potential. The electrical potential is established due to the potential difference between inside and outside of the cell. The cell interior shows electronegativity, with the exterior being electropositive. The discussion given below explains the reasons for such potential differences in the resting state.

Resting membrane potential

As said earlier, all living cells show potential difference between outside and inside of the cell membrane, with inside of the membrane being electronegative. This electrical potential varies from – 6 mv to – 90 mv depending on the type of cell. In excitable tissues, namely nerve and muscle, the membrane potential in the resting state varies from – 65 mv to – 90 mv. This is known as **resting membrane potential (RMP)** (Fig. 2.1). There are three important mechanisms, which contribute to the existence of membrane potential and they are described below.

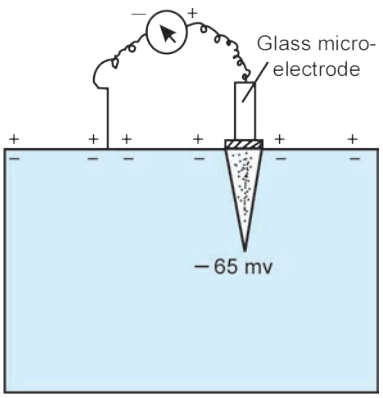


Fig. 2.1: Recording of resting membrane potential in a nerve cell. A glass micropipette filled with KCl solution and connected to a microelectrode is kept inside the cell and another electrode is kept on the surface of the cell membrane.The microelectrode that is kept inside the cell records the potential difference between inside and outside of the cell membrane which forms the resting membrane potential

I. Selective permeability of the membrane as the cause of membrane potential

The cell membrane is selectively permeable to ions. The distribution of ions across the membrane shows that Na^+ is present more outside (145 mEq /L), whereas, the cell interior contains

more of K^+ ion (150 mEq/L). There is also more of Cl^- present outside.

In the resting state, the cell membrane is not permeable to Na^+ but it is relatively more permeable to K^+. The K^+ ions move through the leaky channels. The K^+ ion leaves the cell along the concentration gradient. The exit of K^+ creates electronegativity on the inner side of the membrane. On the outer side of the membrane, there is a positive charge due to Na^+ ions being present in greater concentration. Each of the three ions namely Na^+, K^+ and Cl^- develops equilibrium potentials (diffusion potential), due to the chemical and electrical gradients on both sides of the membrane.

Potassium diffusion potential

Potassium ion shows chemical gradient favouring its exit. There is an electrical gradient for K^+ to enter the cell from outside. At a particular point the influx and efflux will exactly balance each other and no further movement of K^+ occurs (Fig. 2.2). The potential at this point is called equilibrium potential and it can be determined by the **Nernst equation**.

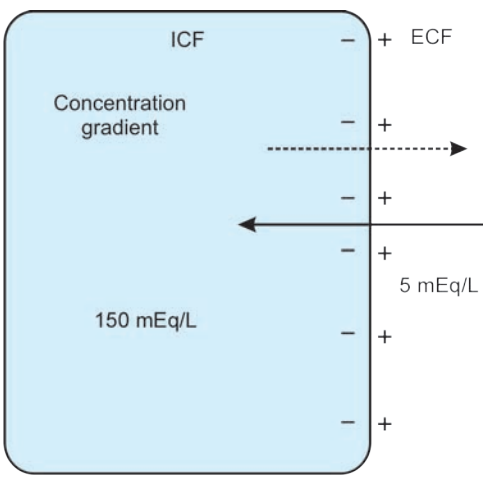


Fig. 2.2: Potassium diffusion potential

Potassium shows concentration gradient from inside to outside of the cell and the electrical gradient from outside to inside of the cell. At the membrane potential of – 90 mv, the two gradients equal each other and no net flow of K^+ occurs. This is called potassium equilibrium potential.

$$\text{Eq. pot } (E_K) = \frac{RT}{zF} \ln \frac{\text{Conc. of ion inside}}{\text{Conc. of ion outside}}$$

R = Gas constant
T = Absolute temperature
F = Faraday constant
z = Valency of ion
ln = Natural log

If the equation is simplified, it will be

$$E_K = -61 \log \frac{150\ \text{mEq/L}}{5\ \text{mEq/L}}$$

$$= -90\ \text{mv}$$

Similar to potassium ion, diffusion potentials for sodium and chloride are also present.

Diffusion potential of Na^+

It is different from that of K^+. The sodium has both electrical and chemical gradients favoring its entry into the cell. But the cell membrane is not permeable to Na^+ ions. Nevertheless, there is some amount of Na^+ entry into the cell, through the leaky channels in the membrane (Fig. 2.3). The sodium that leaks into the cell is actively pumped out by the sodium pump present in the membrane.

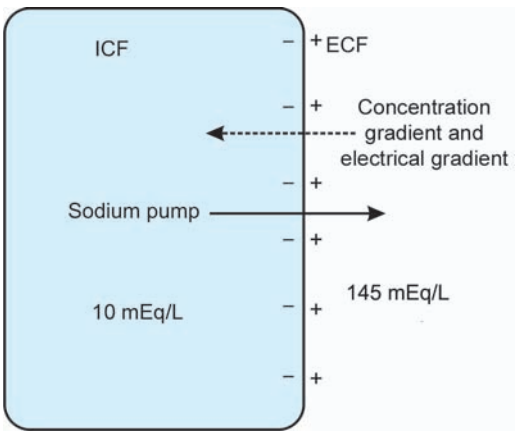


Fig. 2.3: Diffusion potential of sodium

Sodium has both concentration and electrical gradients from outside to inside of the cell. The selective permeability of the membrane restricts the entry of sodium. The sodium pump will also keep the concentration of sodium more outside of the cell. At the potential of +65 mv, there will be no movement of sodium which forms its equilibrium potential.

Diffusion potential of chloride

E_{Cl} is very close to potassium. Chloride is more in extracellular fluid (120 mEq/L). There is a concentration gradient favouring its entry into the cell and the membrane is also freely permeable to it. The presence of negative charge on the inner side of the membrane opposes the negatively charged chloride ion entering the cell (Fig. 2.4). This establishes the equilibrium potential for chloride (E_{Cl}) = – 85 mv.

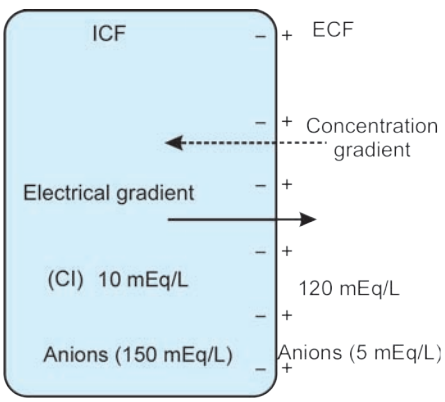


Fig. 2.4: Diffusion potential of chloride

The chloride ion has concentration gradient from ECF to ICF and the electrical gradient from ICF to ECF. The anions which are in greater amounts in ICF are negatively charged and repel the entry of negatively charged Cl ion. At the potential of – 85 mv, there is no net movement of chloride ions. This potential is called chloride equilibrium potential

If we consider the equilibrium potential of Na^+, K^+ and Cl^-, it can be understood, that the membrane potential is developed mainly as a result of K^+ exit, due to selective permeability of the membrane. Thus, the resting membrane potential is closer to the equilibrium potential of K^+ and not to Na^+ or Cl^-. Finally, the magnitude of membrane potential will depend upon the distribution of these three ions across the membrane. This is developed from the concentration gradient for the ion and the membrane permeability for its movement. Combined expression of both is given in **Goldman constant field equation.**

$$V = \frac{RT}{F} \log \frac{P_K (K^+)_o + P_{Na} (Na^+)_o + P_{cl} (Cl^-)_i}{P_K (K^+)_i + P_{Na} (Na^+)_i + P_{cl} (Cl^-)_o}$$

V = Potential difference
R = Gas constant
T = Absolute temperature
F = Faraday constant
P = Permeability of the membrane
o = Ion concentration outside
i = Ion concentration inside

From the above discussion, it can be summerized that the membrane potential exists due to the unequal distribution of ions across the membrane, caused by the selective permeability of the membrane.

II. Electrogenic pump as the cause of membrane potential

Sodium pump that exists in the cell membrane is known as **electrogenic pump,** as it causes more positive charge on the outside of the membrane by actively pumping Na^+ out of the cell. The Na^+ - K^+ ATPase is the enzyme that pumps 3 molecules of Na^+ outside and brings in only 2 molecules of K^+. This unequal ion distribution makes outside more positive than inside (Figs 1.11 and 2.5). Thus, the Na^+ pump helps to maintain electrical potential **(electrogenic pump).**

As said earlier, the same mechanism maintains the cell size and volume. The inhibition of

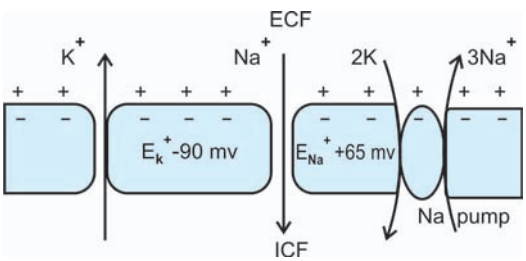


Fig. 2.5: Electrogenic pump of Na^+

The sodium pump keeps more of positive charges on the outside of the membrane as compared to inside, which produced potential difference across the cell membrane

sodium pump abolishes the electrical potential and the cell swells in size due to water entry caused by the osmotic gradient.

III. Presence of anions as the cause of membrane potential

The interior of the cell also contains impermeable anions namely proteins, phosphates and sulphates. These anions are negatively charged and they also contribute to the electronegativity of the interior, which also contribute to the existence of resting membrane potential.

Equilibrium potentials and conductance of the membrane

When the membrane potential and the equilibrium potentials are same for an ion, then, there will be no movement of ion. That is, there is no driving force available for its movement. On the other hand, if the equilibrium potential and membrane potential for an ion are different, the difference between these two will be the driving force for the ion movement, provided, the permeability of the membrane for the ion is present.

The equilibrium potentials of K^+, Na^+, Cl^- ions across the membrane and conductance (g) of ions through the membrane can be combined to determine the resting membrane potential which is called **Hodgkin- Huxley equivalent electrical circuit.**

$$\text{emf} = \frac{E_K(g_k)+E_{Na}(g_{Na})+E_{Cl}(g_{Cl})}{g_K + g_{Na} + g_{Cl}}$$

E = Equilibrium potential
g = Conductance

Changes in ion concentration and Its effect on the membrane potential

Increase in ECF potassium concentration, will cause the membrane potential to decrease, as there is a concentration gradient for K^+ to move into the cell.

Decrease in ECF concentration of K^+ leads to a rise in the membrane potential (more negative). It is because of concentration gradient existing from the cell to the ECF.

Changes in ECF sodium concentration do not affect significantly the membrane potential due to the fact that the membrane is not freely permeable to it.

Changes in the ECF calcium level affect the membrane potential significantly. Decrease in plasma Ca^{++} causes increased excitability of nerve and muscle. It is due to decreased calcium causing greater depolarization. Rise in plasma calcium causes stabilization of the membrane, which increases the threshold of excitation.

Action potential

The resting membrane potential develops into an action potential on application of an adequate strength of stimulus (Fig. 2.6). The sequence of events that occurs during the generation of action potential is described below.

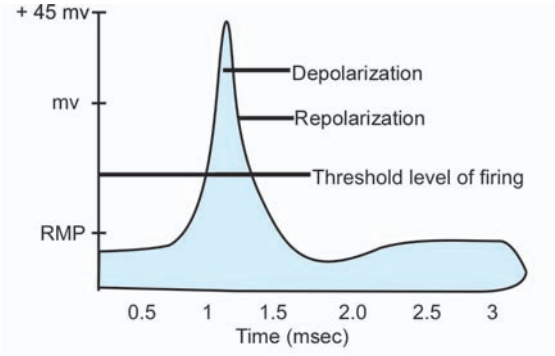


Fig. 2.6: Action potential from an axon

Depolarization

The opening of voltage gated Na^+ channels leads to a decrease in the membrane potential from the resting level. The fall in membrane potential opens more Na^+ channels, as the conductance of the membrane for sodium is increased (Fig. 2.7). This is a **positive feedback cycle**, wherein, decrease in the membrane potential causes more Na^+ entry, which in turn leads to further fall in the membrane potential. This is repeated until the inactivation of Na^+channel

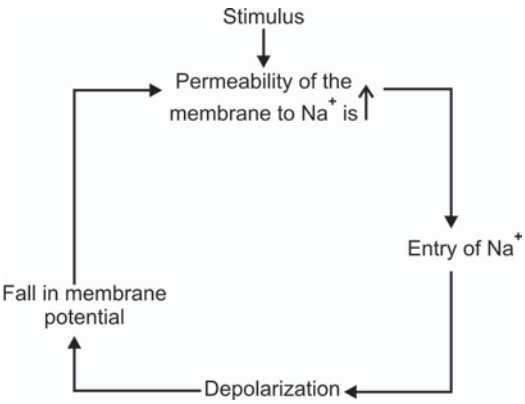


Fig. 2.7: Positive feedback mechanism in sodium conductance through the cell membrane during depolarization

occurs.The result is, the potential reaches +45 mv and the inner side of the membrane becomes electropositive. At this point, the action potential is fully formed.

In the recording of action potential, the depolarization is marked as upward stroke. It can be learnt from the positive feedback cycle which occurs during depolarization, that increase in ion conductance reduces the membrane potential (Fig. 2.8). This leads to further ion entry and a greater fall in membrane potential which leads to the development of action potential. Depolarization ends with the inactivation of Na^+ channels.

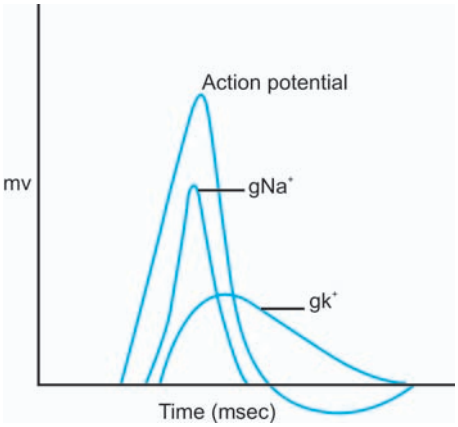


Fig. 2.8: Relationship between action potential and conductance of ions. Note the increased conductance of Na^+ during the upstroke of the action potential which corresponds to depolarization. The conductance of K^+ is increased during the down stroke of the action potential which corresponds to repolarization

Repolarization

It begins with the opening of K^+ channels causing its exit. The membrane potential increases towards the negative side. The recording shows repolarization as downward deflection. During repolarization, the membrane conductance for K^+ is increased.

After potentials called **after depolarization** occurs at the end of repolarization.This is due to slow K^+ exit. This phase is followed by **after hyperpolarization**, caused by the slow closure of K^+ channels and the potential becomes more negative. With the closure of K^+ channels, the membrane potential reaches the resting level.

Channel activity during action potential

The channel activity is studied by patch clamp technique (See Fig. 1.18).

At the resting membrane potential level The Na^+ channels show closure of activation gate and inactivation gates being in open state. *These voltage gated channels are activated by the voltage stimulus*. Now, *activation* gates open, allowing Na^+ influx and depolarization. At the end of depolarization, the inactivation gates close, stopping the Na^+ entry into the cell. *Repolarization begins* with opening of gates of K^+ channel, causing K^+ exit. At the *end of repolarization*, gates of K^+ channel close, which stop K^+ exit. This leads to the membrane potential reaching the resting level.

Time dependence

The time dependence in the opening and closing of channels is important for the action potential to fully develop. For example, if inactivation gates of Na^+ channels close, when activation gates open, depolarization will not reach the threshold level for the action potential to develop. In this situation, repolarization starts before the completion of depolarization.

Methods to study Ionic current during action potential

Voltage Clamp Technique

It has been said earlier that changes in voltage can bring about changes in membrane potential, which inturn, alters the voltage. There is a mutual relationship between these two. To study the current flow and ionic flow independently without the influence of one on the other, an experiment was conducted by Hodgkin and Huxley in 1952 called **voltage clamp technique**. In this the membrane potential is clamped at a desired level to record the movement of ions and current flow. It will be seen that, when voltage is clamped at 0 volts, (membrane is depolarised from –70 mv to 0 volts) there is an inward current flow, due to the influx of Na^+ ions (Fig. 2.9).

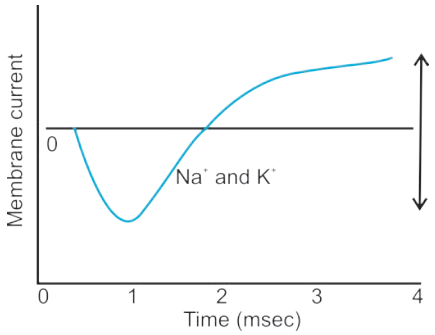


Fig. 2.9: Voltage clamp technique showing sodium and potassium current

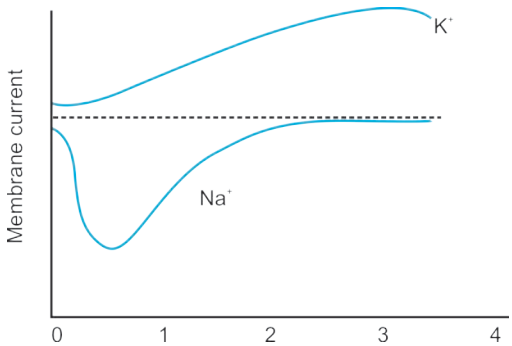


Fig. 2.10: Voltage clamp technique showing sodium current and potassium current separately

Note the sodium current which is inward and its decline over time when the clamp is maintained at the 0 voltage. The K^+ current does not decline over time and exists as long as the voltage is maintained

However, the inward Na^+ current declines over a period of time.It is because of clamping of membrane potential. If the clamp is fixed at a different level now, say, at +15 mv, again inward current of Na^+ can be recorded. In the case of K^+ current, it is the outward flow, due to the exit of K^+ occurs. This current flow does not decline over a period of time and can be recorded, as long as the clamp is maintained (Fig. 2.10). Like this, the membrane potential can be clamped, at various levels and at each level, the flow of current and ion movement can be studied.

Properties of Action Potential

All or None Law

Action potential developed in an excitable tissue obeys all or none law. It states that with a threshold strength of stimulus, the response obtained is maximum, or not at all if it is below threshold.

Accommodation

In excitable tissue, the intensity of stimulus that is applied should be of threshold value and it should be quickly rising. Threshold strength of current, if it is slowly rising, will not give any response, as the tissue is accommodated to this kind of current. With slowly rising current, the membrane potential will not reach the threshold level of firing to form the action potential. The number of Na^+ channels activated are not many, due to the fact that the K^+ channels also open, which allows repolarization to occur. Hence action potential cannot develop.

Relationship between excitability and action potential

There is a period during action potential, in which a second stimulus no matter how strong it may be, cannot produce a response.This period is known as **absolute refractory period** (Fig. 2.11). It corresponds to the entire depolarization and one third of repolarization. With the

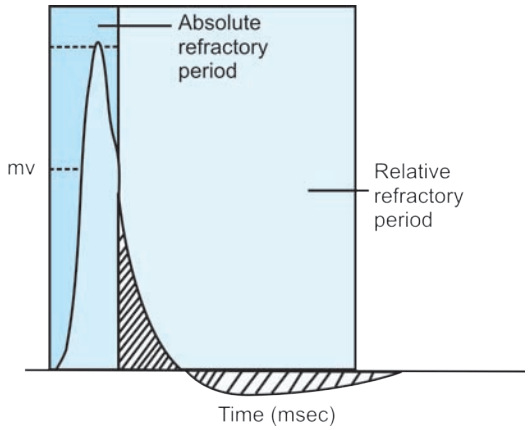


Fig. 2.11: Relationship between action potential and excitability

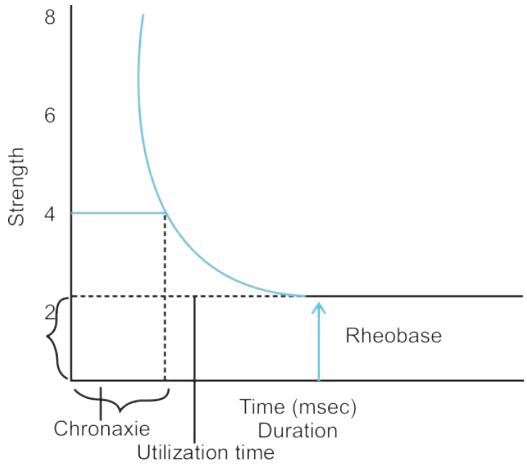


Fig. 2.12: Strength duration relationship in an excitable tissue

beginning of repolarization, there will be only a few Na^+ channels, which are in a state where activation gates close and inactivation gates open. These channels can be activated by a stronger intensity of stimulus and the excitation produced during this phase is called **relative refractory period**. This is followed by a period, which corresponds to the delayed K^+ exit known as after depolarization. The excitability in this period is more and known as super normal phase. It leads to a period called after hyperpolarization, in which the excitability becomes less (subnormal phase). This is caused by increased K^+ exit due to slow closure of potassium channels. The membrane potential reaches the resting level and the excitability returns to the normal state after the subnormal phase.

Strength-duration relationship

It is known that threshold strength of current, if it is quickly rising will give a response. The minimum strength of stimulus that is required to elicit a response is called **rheobase**. The time required to produce this response is the utilization time. If the intensity of stimulus is kept twice the rheobase, the time taken to excite can be determined. This is **chronaxie** and it is defined as the duration at twice the rheobase strength (Fig. 2.12). Chronaxie values are useful in determining the excitability pattern of tissues. Greater the chronaxie, lesser the excitability of the tissue.

Electrotonic potentials (Local response)

They are the graded, nonpropagated potentials and help to develop the propagated action potential. When an action potential is developed at a point in the nerve fiber, there will be a current flow to the adjacent region. This is known as local response or electrotonic depolarization. They are graded, that is, their size depends on the intensity of stimulus and it is nonpropagatory (Fig. 2.13) However, when the local response attains the threshold level, a propagated action potential is developed. The electrotonic potential, which spreads from the point of action potential, shows two features namely, space constant and time constant.

Space constant

It refers to the minimum distance over which the size of the electrotonic potential falls to 37% of maximum value (Fig. 2.14). That is, when a point on the nerve fiber develops an action potential, the electrotonic depolarization along the length of membrane declines exponentially as the distance is increased. In mammalian nerve fibers, the space constant is 1 to 3 mm.

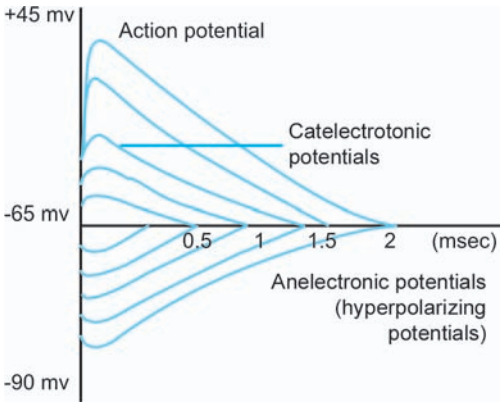


Fig. 2.13: Electrotonic potentials (depolarizing potentials)

Action potential develops when the local depolarizing potentials at the cathodal current reaches threshold level of firing. Note the potential at the anode, which shows linear relationship with the intensity of stimulation

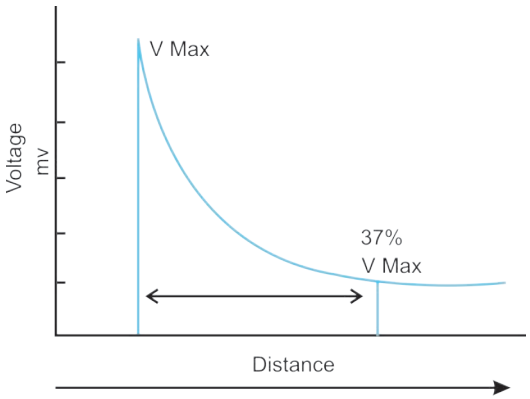


Fig. 2.14: Space constant during electrotonic depolarization

Space constant refers to the distance from the point of stimulus to the fall of 37% of potential. Greater the space constant, greater the spread of passive current along the axon

Space constant depends on

- Membrane resistance
- Axoplasmic resistance

If **membrane resistance** is increased, the space constant will become more. In otherwords, the electrotonic depolarization will spread to a greater distance.

There is presence of **axoplasmic resistance** for the spread of electrotonic potential and it is called longitudinal resistance. Increase in the axoplasmic resistance will cause decrease in the space constant. In larger size nerve fibers, the cross sectional area is increased and this lowers the longitudinal axoplasmic resistance. Hence, the speed of conduction in larger nerve fibers is greater.

Time constant

It is the time that is taken for the electrotonic depolarization to decline to 63% of the maximum (Fig. 2.15). More the time constant, lesser the excitation. This will also decrease the velocity of conduction. In diseases affecting the myelination of neuron, as in multiple sclerosis, there is increased capacitance and decreased resistance of the membrane. This increases the time constant and conduction becomes slower. This is the reason for the occurrence of sensory and motor deficits in such disorders.

As mentioned earlier, subthreshold stimulus fails to reach the threshold level of firing. It gives rise to a nonpropagated, but graded potential

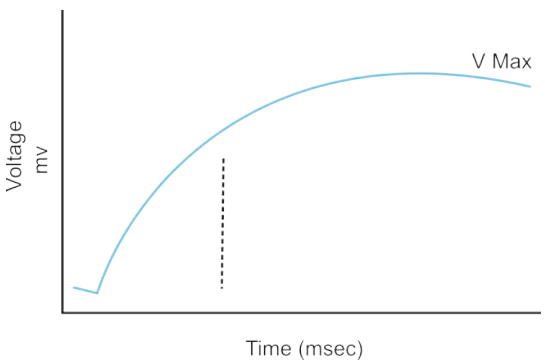


Fig. 2.15: Time constant during electrotonic depolarization

Time constant is the time taken for the electrotonic depolarization to fall at 63% of the maximum potential (Vmax). Greater the time constant, more the time taken for the passive spread of current along the axon and the velocity of propagation of impulse will also be slow

called *electrotonic potentials*. When the intensity of stimulus is raised, the size of the potential rises to the threshold level and an action potential can be formed. Electrical potential developed at the cathodal current and at the anodal current is not similar.

Effect of cathodal current

Application of cathodal current causes depolarizing potential called catelectrotonic potential. At the cathode, the current flows outward and depolarizes the membrane. It is a nonpropagated, graded local response. However, depending on the intensity of stimulus, the potential reaches the threshold level and an action potential can be formed.

Effect of anodal current

Anodal current causes hyperpolarization of the membrane, as the current flows inward.The potential recorded at the anodal current is called anelectrotonic potentials. There is a linear rise in the membrane potential, as the intensity of anodal current is increased. This is in comparison to cathodal stimulation, where, the depolarizing potential on reaching the threshold level, gives rise to an action potential.

NERVE CONDUCTION

Conduction of action potential along the nerve fiber is similar to the current flowing through a cable. The plasma membrane acts as an insulator, preventing the spread of current to the surrounding structures. The membrane also shows high capacitance, which becomes charged, when there is excitation. The spread of impulse is not same as the conduction in a cable. In a cable, the inner metal offers less resistance and current can spread over a long distance. Since the insulation in a cable is perfect, there is no lateral spread of current. In the case of nerve fiber, there is axoplasm that offers high longitudinal resistance and the insulation of the membrane is not totally perfect, as in a cable. Hence, the impulse from one point can not spread to a long distance as it happens in the case of a cable.The following mechanisms explain the conduction in both myelinated and unmyelinated nerve fibers.

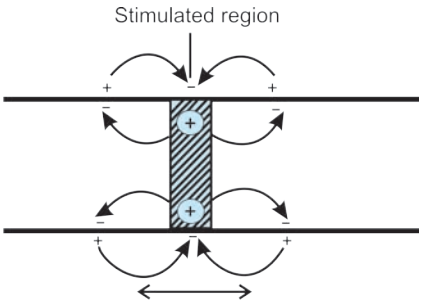
Conduction in an unmyelinated nerve fiber

In an unmyelinated nerve fiber, the action potential spreads by **self excitation and self propagation.** As said earlier, action potential formed at a point in the membrane, results in a local current spreading to adjacent regions. The region of the membrane, which is closer to the action potential, will have greater current flow and there will be a local circuit formed between the positive and negative charges on both sides of the membrane.This is called **local response.** Local response causes electrotonic depolarization of the membrane. When depolarization reaches the threshold level, an action potential is developed. Like this, at each successive point along the membrane, the process is repeated until the conduction is completed along the length of the fiber (Fig. 2.16).

In unmyelinated nerve fiber, conduction is directly proportional to the square root of diameter of the fiber. The reason is, that in larger size nerve fibers, there is lesser longitudinal axoplasmic resistance.

Conduction in a myelinated nerve fiber

In the case of myelinated nerve fiber, the current flow and ionic flow occur at the nodes of Ranvier. In this region, the myelin sheath is absent. The myelin sheath gives less capacitance and greater resistance, whereas, at the nodes of Ranvier, there is greater capacitance and less resistance. When one node of Ranvier is excited, the next node of Ranvier shows local response. That is, a local current circuit is formed between the positive and negative charges through the axoplasm and ECF. This causes electrotonic depolarization of the next node of Ranvier



Current circuit is established between the stimulated and unstimulated regions.When the potential in the adjacent region reaches the threshold level, it gets stimulated.Like this the successive points along the axon are excited

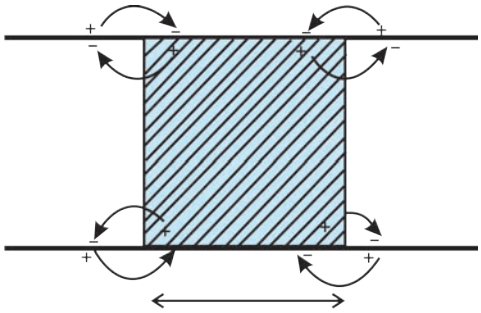


Fig. 2.16: Conduction of impulses in an unmyelinated nerve fiber

The impulse spreads through electrotonic depolarisation of the successive points on the axonal membrane

ahead. When depolarization reaches the threshold level, an action potential is developed at this node of Ranvier, bypassing the internode. This process is repeated at each node of Ranvier. Thus the impulse in a myelinated nerve fiber, jumps from one node of Ranvier to another node. This method of conduction is called **Saltatory conduction** (Fig. 2.17). The velocity of conduction is greater in myelinated nerve fibers due to this phenomenon.

Metabolic efficiency in a myelinated nerve fiber is high, because the impulses need to be conducted from node to node only. Energy is required for pumping ions only at the nodes of Ranvier. Secondly, at the nodes of Ranvier, there is a maximum number of Na^+ channels per square micron metres of area. These two factors help in the efficient conduction of nerve impulse in myelinated nerve fibers.

NERVE FIBER TYPES

Action potential recorded from a single axon shows a monophasic potential, whereas, spikes from a mixed nerve gives a **compound action potential** (Fig. 2.18). It consists of action potentials from different fiber types present in

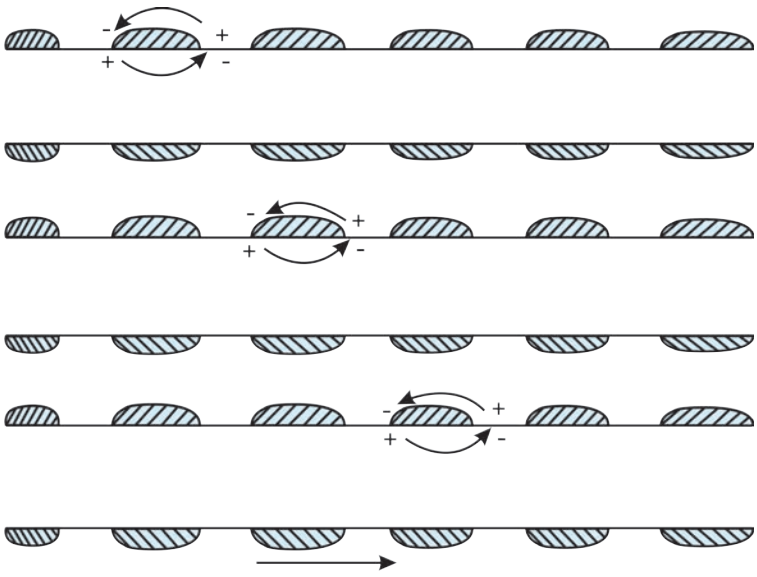


Fig. 2.17: Saltatory conduction in myelinated nerve fiber

The impulse travels from one node of Ranvier to next node of Ranvier, through the current circuit established between the stimulated nodes of Ranvier and the unstimulated adjacent node.The circuit is made through the ECF and axoplasm, leaving the internode. When the fall in membrane potential reaches the threshold level, a propagated action potential is developed at the adjacent node

a mixed nerve. **Erlanger and Gasser** (1937) studied the spikes from mixed nerve and classified the nerve according to the fiber diameter. According to this classification, there are three types namely: A, B and C

A, it is sub divided into α, β, γ, δ

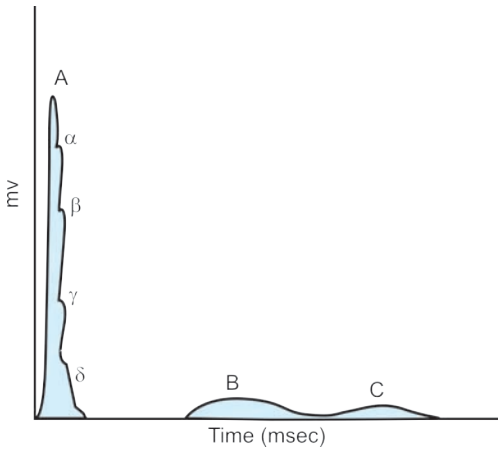


Fig. 2.18: Compound action potential showing the various types of nerve fibers

A mixed nerve will not have all the fiber types. When an action potential is recorded, by stimulating the nerve trunk, it can be seen that the spike from the largest fiber size appears first. The last spike comes from the fiber which is the smallest in diameter. If the recording electrode is placed close to the stimulating electrode, the action potential of larger size fiber only appears. As the recording electrode is moved farther from the stimulating electrode, the action potentials from all fiber types appear. This is due to the different conduction velocities of fiber types present in the mixed nerve. Table 2.1 gives the types of nerve fibers, their characteristics and their function.

Energy metabolism in nerve

The energy requirement of nerve is much lower than muscle. It is only for the ion pump, energy is required. Nerve excitation and conduction can occur without ATP supply. If there is a prolonged excitation, then only ATP is required. If there is maximum stimulation of nerve, the metabolism increases by two times, whereas, in muscle a similar state would cause increase by 100 times.

Table 2.1: Types of nerve fibers, characteristics and functions

Fiber type	size(mm)	Velocity (m/sec)	Functions
A α	12-20	60-120	Somatic, motor and proprioceptive
A β	6-12	30-60	Touch,kinesthetic and pressure
A γ	3-5	15-30	Muscle spindles, touch, pressure
A δ	1-5	12-30	Pain,temperature and pressure
B	1-3	3-15	ANS preganglionic fibers
C (unmyeli-nated)	0.5-1.5	0.5-2	Pain, postganglionic fibers of ANS

Transmembrane potential in an excitable tissue can be measured by introducing a glass capillary electrode filled with sodium chloride or potassium chloride into the interior of the cell. There is another indifferent electrode, which is kept on the surface of the cell. The electrodes are connected to a voltmeter, which would record the potential difference between these two electrodes. There will be a negative deflection (– 70 mv) in the voltmeter indicating the resting membrane potential.

The activity of nervous tissue develops electrical current, which is very minute (mv) and lasts for only a few milli seconds. To record such electrical activity, we need a system to amplify the signal and display the events on the screen. **Cathode ray oscilloscope** fulfills these requirements. Most of the observations of electrical activity of nervous tissue are from a **giant squid axon,** which is larger in size (up to 1 mm diameter). Recording from a single axon gives a monophasic actionpotential. If

Contd...

Contd....

two recording electrodes are kept at a distance on the surface and stimulated, a biphasic action potential is recorded. Recording of action potential from the cathode ray oscilloscope shows that there is a marking of stimulus artefact, which indicates the point of stimulation. Stimulus artefact is due to the leakage of current from the stimulating electrode, spreading towards the recording electrode. The period between stimulus artefact and the point of beginning of action potential, is called latent period. The duration of action potential in an axon is 0.5 msec. The overshoot and downstroke lasts for 0.12 msec.The remaining duration is occupied by the undershoot phase of the action potential.

STRUCTURE OF NEURON

Nerve and muscle are excitable tissues. The properties of nerve are excitability and conductivity. **Neurons** form the structural and functional unit of the nervous tissue (Fig. 2.19). There are 10^{12} neurons and more than this number (10 to 15 times), the supporting cells called **glia** are present in the nervous system (Fig. 2.20).

Glial cells

The glial cells are of three types in the central nervous system. They are **microglia**, **oligodendroglia** and **astrocyte.** The function of microglia is phagocytic and also considered as tissue macrophage. The oligodendroglia is responsible for the formation of myelin sheath in the central nervous system. The myelin sheath

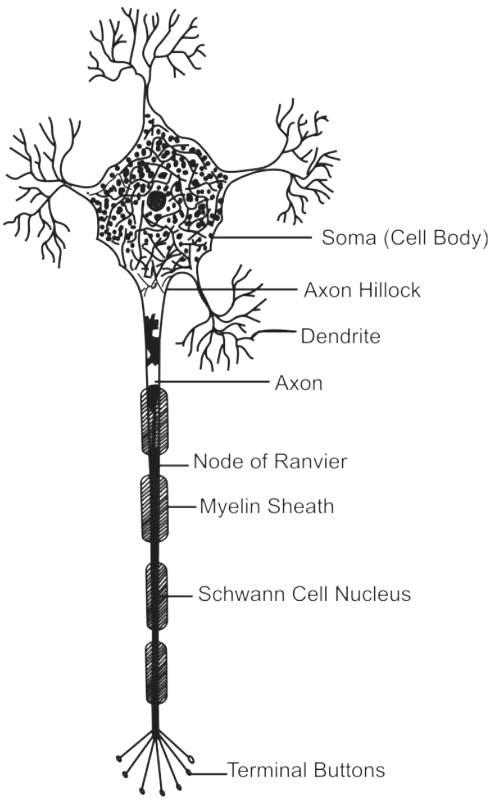


Fig. 2.19: Structure of a neuron

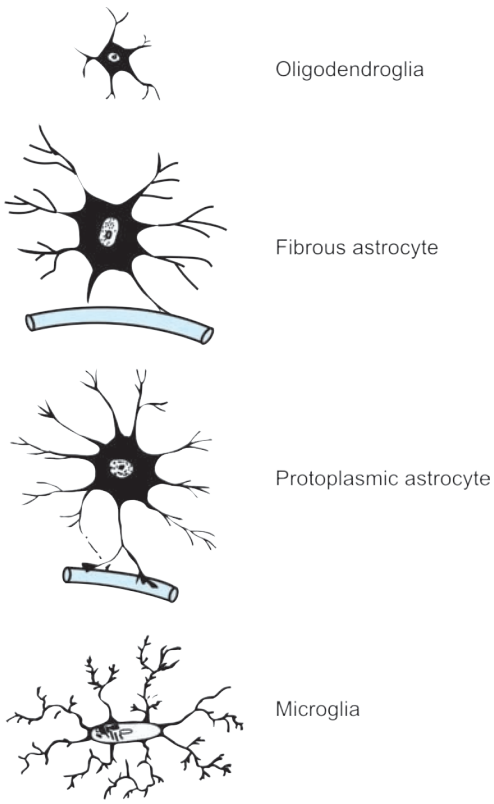


Fig. 2.20: Glial cells in the central nervous system

of the peripheral nerves is formed by the Schwann cell. The astrocytes give a proper milieu for the neuronal function. It removes K^+ from the interstitial space and also takes up neurotransmitters. The other function of astrocyte is to form blood brain barrier, by its end feet connecting with the brain capillaries.

A neuron, depending upon the position of the dendrites and axon on the cell body, can be classified into *unipolar, bipolar and multipolar*.

Structure of neuron

A typical neuron contains a cell body or soma. It receives many branched net work of fibers called dendrites. It is considered as the receptive region, where the impulses are received from other neurons. From the cell body, a single long process arises, which is called axon. It arises from an enlargement in the soma known as axon hillock. The axon at its end shows branches called terminal buttons or axon telodendria. There are also collaterals, which can arise from the axon. The cell body shows cell organelles like in any other cell, but the *distinguishing features are:*

Presence of Nissl granules, which is absent in axon hillock. Nissl granules disintegrate when nerve fiber degenerates **(chromatolysis).**

Absence of centrosome and hence the power of cell division is lost.

Neurofilaments and microtubules function as cytoskeleton and transport medium respectively.

Axoplasmic transport

The cytoplasm from the soma flows into the axon as axoplasm. In the axoplasm, the neurofilaments and microtubules are present, which help in the transport of amino acids, proteins and neurotransmitters from cell body to the axon terminal. This is called axoplasmic transport **(anterograde transport).** There is also transport from the axonal end to the cell body, known as **retrograde axonal transport**. Transport of nerve growth factor, viruses and neurotransmitters to the cell body are examples of this transport.

The axon is covered by a membrane called neurilemma. In addition to the neurilemma, there could be another layer called myelin sheath and such fibers are called **myelinated fibers**. Myelin sheath is interrupted at regular intervals of 1.5 mm. These interruptions, where myelin sheath is absent is called **Node of Ranvier**.

Myelin formation

Myelin sheath is formed by the Schwann cells in peripheral nerves and by the oligodendroglia in the central nervous system. Both Schwann cell and oligodendroglia are grouped under glial cells. Each Schwann cell gives myelin sheath to a single axon, whereas, each oligodendroglia in the central nervous system, forms myelin sheath for 4 or 5 axons. The myelin formation in a peripheral nerve fiber involves Schwann cell repeatedly dividing and rotating around the central axon forming several layers of myelin. The chemical composition of myelin shows that it is made of phospholipids and cholesterol. Hence, it gives the best insulation for the nerve conduction, but has poor capacitance. In myelinated fibers, the velocity of conduction is high, as there is a saltatory type of conduction taking place.

Axons can also exist without the myelin sheath and such fibers are called **nonmyelinated fibers**. In this, the neurilemmal sheath surrounding the axon is continuous. These fibers are lesser in diameter as compared to myelinated fibers. The velocity of conduction is also lower.

DEGENERATION AND REGENERATION IN PERIPHERAL NERVE FIBERS

Wallerian degeneration

Peripheral nerve fibers, when damaged or crushed, degenerates and subsequently, it shows regeneration. This restores the functional ability of the damaged fiber. In central nervous system, once the nerve fiber is cut, it degenerates and no regeneration occurs.

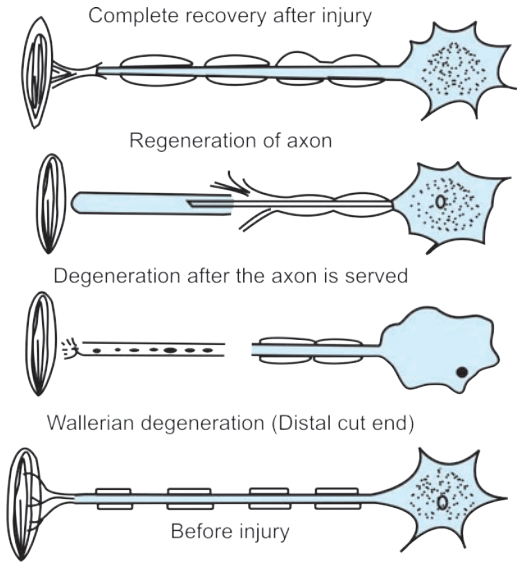


Fig. 2.21: Wallerian degeneration and regeneration in a peripheral nerve fiber

When an axon is crushed, the distal part (periphery) degenerates. This is called Wallerian degeneration, named after Waller (1850) who described the features of degeneration in the distal cut end (Fig. 2.21). Conduction of impulses to the distal end can be recorded up to 48 hours and thereafter conduction stops. The axoplasm breaks up into bits and myelin sheath disintegrates. There is proliferation of Schwann cells, macrophages, which clear the debris and finally an empty neurilemmal tube only is present in the distal end. Proximal cut end also shows degenerative changes such as swelling of cell body, nucleus taking up eccentric position and chromatolysis (disintegration of Nissl granules). Degenerative changes seen in the cell body are due to the retrograde axoplasmic transport of substances released from the site of injury to the cell body.

Regeneration

Regeneration depends on the extent of injury. If there is separation of cut ends extending more than 3 mm, then, the best way would be to suture the cut ends, so that, proper connections can take place between them.

From the proximal cut end many axonal sprouts develop and grow towards the distal cut end. They grow at the rate of 1 to 2 mm per day. One of the axonal sprouts successfully enters the distal neurilemmal tube and grows upto the terminal. Schwann cell multiplies and myelin sheath is formed thereafter. After establishing the connection the fiber reinnervates the target tissue.

Sometimes, the distance between cut ends becomes longer, then, in such cases, the axonal sprouts can become attached to neighbouring fibers and develop into a neuroma. If sensory fibers are involved in this, it will result in severe pain. This can be seen as a complication of amputation of limbs. Suturing the cut ends will reduce the distance between the cut ends, if it is longer.

Regeneration is facilitated by various growth factors which include NGF (nerve growth factor), and neurotrophin 3.

When an axon is cut or damaged the structure innervated by it namely the muscle shows increased sensitivity to the released neurotransmitter from the cut end. This is called **denervation hypersensitivity**. This phenomenon is also believed to be responsible for the increased excitability of structures below the damaged or lesioned part of the central nervous system.

Self-study Questions

Multiple Choice Questions

Choose the single best answer

1. **In the resting state the conductance of membrane is more for which of the ions?**
   A. Na^+ **B.** Ca^{++}
   C. K^+ **D.** Cl^-

2. **After hyperpolarization in the action potential is caused by:**
   A. Increased exit of K^+ from the cell
   B. Entry of Cl^- into the cell
   C. Delay in sodium pump
   D. Increased exit of Na^+ from the cell

3. **Which of the following would carry the membrane potential closer to firing level?**
   A. Anodal current
   B. Exit of K^+
   C. Cathodal current
   D. Accommodation

4. **Repolarization of the membrane begins when:**
   A. Sodium channels are inactivated
   B. Potassium channels close
   C. Sodium entry slows down
   D. Sodium channels are activated

5. **The number of Na^+ channels per square micrometer of membrane in a myelinated mammalian neuron would be greatest at the:**
   A. Cell body
   B. Dendrite
   C. Nodes of Ranvier
   D. Axon terminal

6. **What would be the effect of decrease in ECF Na^+ on the resting membrane potential?**
   A. Not much effect
   B. Decrease in membrane potential
   C. Increase in membrane potential
   D. Accommodation

7. **Action potential propagation velocity increases with an increase in axon's:**
   A. Time constant
   B. Capacitance
   C. Myelination
   D. Axoplasmic resistance

8. **The excitability of the tissue would be greater in the tissue showing:**
   A. Lesser chronaxie
   B. Refractory period
   C. More chronaxie
   D. Accommodation

9. **Anodal current in the excitable tissue causes:**
   A. Depolarization
   B. Hyperpolarization
   C. Action potential
   D. Current sink

10. **Myelin sheath in an axon increases:**
    A. space constant
    B. Axoplasmic resistance
    C. Capacitance
    D. Time constant

11. **Voltage gated Na^+ channels are blocked by:**
    A. Ouabain
    B. Tetrodotoxin
    C. Tetraethyl ammonium
    D. Verapamil

12. Local potentials in the excitable tissue:
- **A**. Are non-propagatory
- **B**. Can be graded
- **C**. Show temporal and spatial summations
- **D**. Show all of the above

13. Conductance of membrane for chloride ion in the excitable tissue indicates which of the following?
- **A.** Hyperpolarization
- **B**. Depolarization
- **C**. No effect in the membrane potential
- **D**. Repolarization

14. Which one of the following nerve fibers is more susceptible to mechanical pressure?
- **A.** A α
- **B.** Aγ
- **C.** B
- **D.** C

15. In ECF, the rise in the concentration of which of the ion can cause decrease in membrane potential?
- **A.** Ca^{++}
- **B.** K^{+}
- **C.** Na^{+}
- **D.** Cl^{-}

ANSWER KEYS

1. (C) 2. (A) 3. (C) 4. (A) 5. (C) 6. (A) 7. (C) 8. (A) 9. (B) 10. (A)

11. (B) 12. (D) 13. (A) 14. (A) 15. (B)

Short Answer Questions

1. List the causes of resting membrane potential.
2. Explain why sodium pump is known as electrogenic pump.
3. State what happens to resting membrane potential when ECF concentration of K^+ and Ca^{++} is decreased.
4. Describe the ionic basis of action potential.
5. Describe the channel activity during action potential.
6. Describe refractory period in the excitable tissue.
7. Describe strength – duration relationship in the excitable tissue.
8. Describe the mechanism of conduction in the myelinated and nonmyelinated nerve fibers.
9. State the advantages of salutatory conduction.
10. List the factors that affect nerve conduction.
11. Describe the classification of nerve fibers based on its size.
12. List the glial cells and their functions.
13. Describe how myelin sheath is formed in the peripheral nervous system.
14. Describe Wallerian degeneration and regeneration.

3 Muscle

Skeletal, cardiac and smooth muscles are the types of muscles present. In many respects, the three types differ from one another, yet, they exhibit the basic properties characteristic of muscles, namely, excitation and contraction (Table 3.1).

SKELETAL MUSCLE

It is a voluntary muscle and its attachment to the skeleton enables us to do voluntary movements. Skeletal muscle is covered by a connective tissue called **epimysium**. The muscle contains a number of fascicles, which are covered by a connective tissue **perimysium**. Each fascicle (bundle) contains a large number of muscle fibers and they are covered by **endomysium**. Each muscle fiber can be called as a muscle cell and the membrane lining it is called **sarcolemma**. The muscle fiber is a multinucleated cell and the nuclei are found beneath the sarcolemma. The sarcoplasm contains cell organelles such as Golgi apparatus, mitochondria, myoglobin, sarcoplasmic reticulum, enzymes, lipids, proteins and glycogen, etc. The muscle fiber consists of a number of myofibrils, which contain the contractile proteins. Each myofibril is further sub-divided into sarcomeres.

Sarcomere

Sarcomere is the structural and functional unit of the muscle, which extends between two adjacent Z lines. A sarcomere includes a complete A band (darker zone) and one half of I band (lighter zone) on either side of A band. The A band contains the thick myosin filaments, whereas the I band consists of the thin filaments, which include actin, tropomyosin and troponin. The lighter H band in the center of A band could be seen when the muscle is relaxed. A transverse section of A band will show that each myosin filament is surrounded by six actin filaments arranged in a hexagonal fashion.

In resting state, sarcomere width is 2.2 µ and when it shortens is reduced to 1.6 µ (Fig. 3.1). The changes observed in sarcomere during muscle shortening are given below;

Decrease in the width of sarcomere, I band and H zone occurs. The length of the contractile filaments namely actin and myosin and width of A band shows no change during muscle contraction.

Contractile proteins

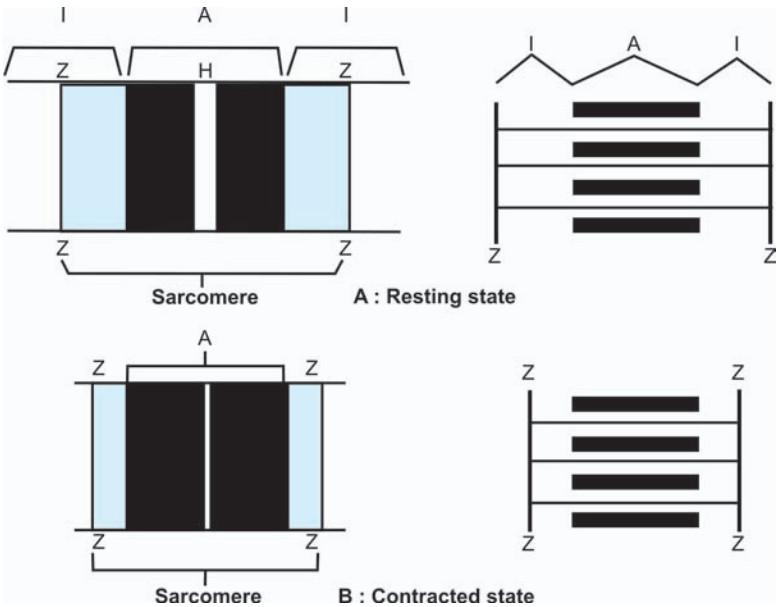
They are actin and myosin.

Myosin

It is a thick filament present in the **A band** and each thick filament contain 200 to 300 myosin molecules. The type of myosin present in the muscle is myosin II. There are two heads for myosin molecule, which are formed by 2 heavy chains and 4 light chains. The N terminal of the

Table 3.1: Structure and functions of the three types of muscles compared

STRUCTURE			
Characteristcs	***Skeletal muscle***	***Cardiac muscle***	***Smooth muscle***
Motor end plate	Present	None	None
Fiber	Cylindrical and long	Branched	Fusiform and short
Mitochondria	Few	Many	Few
Sarcomere	Present	Present	None
Syncytium	None	Functional	Present (bridges)
Sarcoplasmic reticulum	Very well developed	Well developed	Poorly developed
ATPase	Greater	Average	Little
FUNCTION			
Response	Graded	All or none law	Graded
Refractory	Short period	Longer (.25 sec)	Short
Pacemaker	None	Present	Present
Tetanus	Yes	No	Yes
Nerve stimulation for contraction	Necessary	Not required	Not required
All or none law	No	Yes	No
Length-tension relationship	Sarcomere less than 2.2 μ shows the relationship	Depends on preload	Tension developed is variable
Force-velocity relationship	Inversely related	Directly related within physiological limits	Inversely related



Figs 3.1A and B: Diagram of a sarcomere during resting and contracted states

heavy chain and 4 light chains form the two heads of myosin (Fig. 3.2). Each head has a binding site for actin and another site acts as ATPase. Myosin molecules are arranged symmetrically on either side of the center of sarcomere. The orientation of myosin molecule in one half of sarcomere is opposite to the orientation in the other half of sarcomere (Fig. 3.3). The heads project from the molecule at an angle to form cross bridges with actin.

Actin

It is present in the thin filament. Each filament contains 300 to 400 actin molecules. The structure shows that it is made of globular proteins arranged in a double helical fashion. Between the actin heads, in the groove, the binding site for myosin is present (Fig. 3.5). Situated within the actin are the two regulatory proteins called tropomyosin and troponin (Fig. 3.4).

The actin is connected to the muscle membrane proteins α and β dystroglycans through dystrophin molecules. The dystroglycans are further extended to the extracellular matrix and as well as to the sarcolemma glycoproteins. These connections give strength to the muscle.

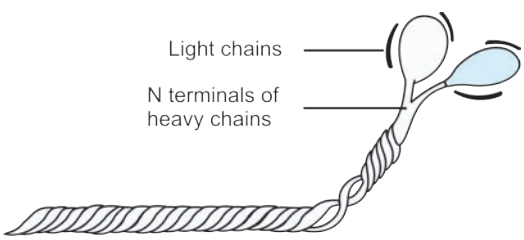


Fig. 3.2: Structure of myosin

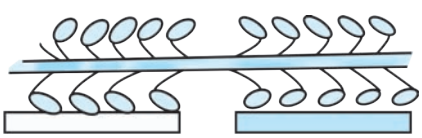


Fig. 3.3: Orientation of myosin molecules in A band

The figure 3.2 shows the formation of two heads of myosin from the N terminals of two heavy chains and four light chains. The figure 3.3 shows the arrangement of myosin molecules where the orientation of the head in one half is opposite to the orientation in the other half of A band

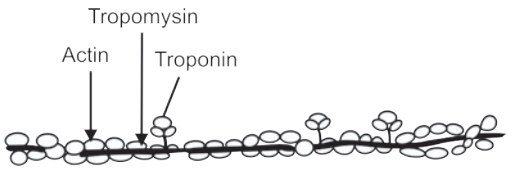


Fig. 3.4: Structure of actin with the two regulatory proteins

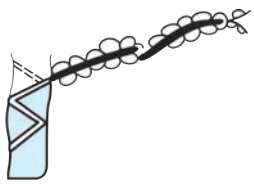


Fig. 3.5: Actin with the binding sites uncovered

Tropomyosin

It is in a filamentous form, present between the two chains of actin. It covers the binding sites of myosin in actin. Attached to tropomyosin, at regular intervals, troponin is present. It has three sub units namely,

Troponin C—Gives attachment to Ca^{++}

Troponin T—Gives attachment to tropomyosin

Troponin I—Inhibits the interaction between actin and myosin.

Sarcotubular system

From sarcolemma, there is a well developed sarcotubular system, surrounding the myofibrils and appear as vesicles and tubules. It consists of;

Transverse tubular system (T system)

Sarcoplasmic reticulum (longitudinal tubular system) (Fig. 3.6).

T system is an inward extension of the plasma membrane, whereas, the sarcoplasmic

reticulum surrounds the myofibrils and at A-I band junction, it forms cisternae. This terminal cisternae contains Ca^{++}, which is released, when action potential reaches the triad. The function of T system is to transmit action potential from the sarcolemma to the fibrils in the muscle.

Triad

In skeletal muscle, at the A-I band junction, a **triad** is formed by these sarcotubular structures, with a centrally placed T system and cisternae of sarcoplasmic reticulum being present on either side of it (Fig. 3.6). There are end feet projections from the T system to the cisternae. This enables the action potential from T system to reach the terminal cisternae.

The T tubule membrane has voltage gated dihydropyridine receptors, which are activated by depolarization of the T tubule system. In skeletal muscle, the depolarization of the T tubule membrane activates dihydropyridine receptors to cause the release of Ca^{++} from the cisternae of sarcoplasmic reticulum at the triad. In cardiac muscle, the dihydropyridine receptor activation will cause influx of Ca^{++} into the T system from ECF. This in turn leads to the release of Ca^{++} from cisternae of the sarcoplasmic reticulum at the triad.

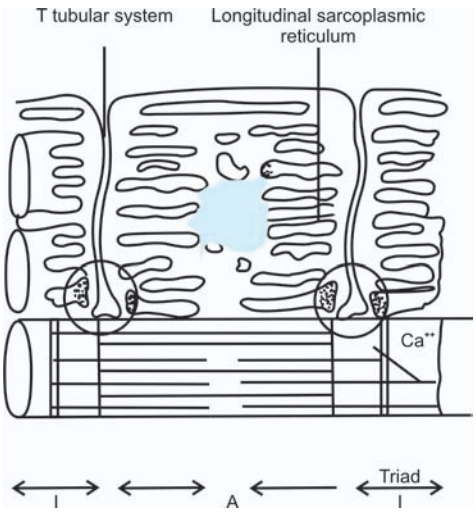


Fig. 3.6: Structure of sarcoplasmic reticulum and triad in a skeletal muscle

MOLECULAR EVENTS DURING MUSCULAR CONTRACTION

Skeletal muscle contraction is preceded by the excitation of the muscle by the nerve fiber. The events that occur from the point of excitation of the muscle to its contraction is called **excitation-contraction coupling.**

Recent concepts about the molecular basis of muscle contraction, is explained on the basis of cross bridge mechanism, which also includes sliding filament process.

Role of Ca^{++} in the activation of contraction

The arrival of action potential at the triad, releases Ca^{++} from cisternae, which increases its concentration in the cytosol. Calcium is the ion which is responsible for initiating the muscle contraction. Calcium from cytosol is attached to troponin C, which brings about conformational changes in troponin molecule, leading to sinking of tropomyosin in the groove of actin. This uncovers the binding sites in actin.

Cross-bridge mechanism

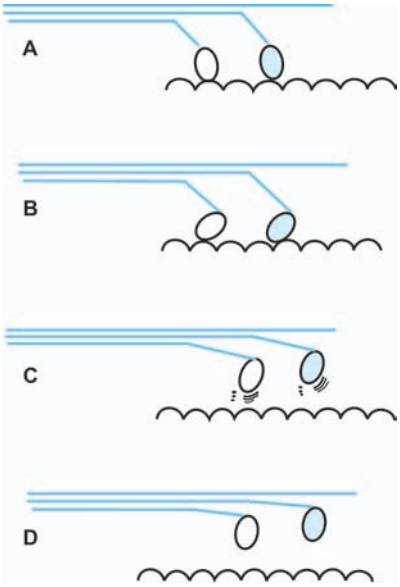
Myosin head is tilted at an angle towards the actin. There is a cleft in the head in which ATP fits in. The myosin head has ATPase, which causes hydrolysis of ATP and gives ADP, inorganic phosphate and energy. The hydrolysis of ATP in the head of myosin, leads to its distortion and straightens out towards actin (90^o orientation) to get attached to it. This process completes the cross bridge formation, which is followed by sliding filament mechanism.

Sliding filament mechanism

The head of myosin produces a swiveling movement, which is also called **power stroke** mechanism. The movement occurs due to the result of change in the orientation of myosin head. That is, from 90^o orientation, it is changed

to 45° and then the movement is produced (Fig. 3.7). This change in orientation of myosin head is brought about by the release of products of hydrolysis of ATP namely ADP, and Pi.

The thin filament moves towards the center of sarcomere by the power stroke movement. It is believed that each power stroke produces shortening of muscle by 1%. In order to achieve complete contraction of muscle, the head of myosin is attached to a new binding site in actin, and repeates power stroke movement. The attachment, power stroke and detachment are repeated over and again, until final shortening is produced. Myosin,after the release of the products of ATP hydrolysis, shows less affinity to actin and it is ready to accept a new ATP. The attachment of ATP to myosin, causes detachment of myosin from actin, leading to relaxation (Fig. 3.8).



Figs 3.7A to D: Mechanism of power stroke during muscle contraction is diagrammatically explained (A) The head of myosin stretches towards actin at 90° and form cross-bridges (B) The myosin head produces swiveling movement (power stroke) by bending at 45° which causes actin to move towards the center of A band (C) The head of myosin is detached from actin (D) Myosin head is stretched at 90° for attachment to a new binding site in actin

Steps in skeletal muscle contraction

Excitation of motor neuron
↓
Release of acetylcholine at the motor end plate
↓
Formation of endplate potential at the endplate membrane
↓
Formation of action potential and
Spread of depolarization inwards along the "T" tubules
↓
Release of Ca^{++} from the cisternae of the sarcoplasmic reticulum
↓
Attachment of Ca^{++} to troponin C and uncovering of myosin
binding sites in actin
↓
Cross bridge formation between actin and myosin and sliding of actin on myosin by power stroke movement of head of myosin
↓
Movement of actin filaments and repeat of power stroke movement on new binding sites
↓
Muscle shortening

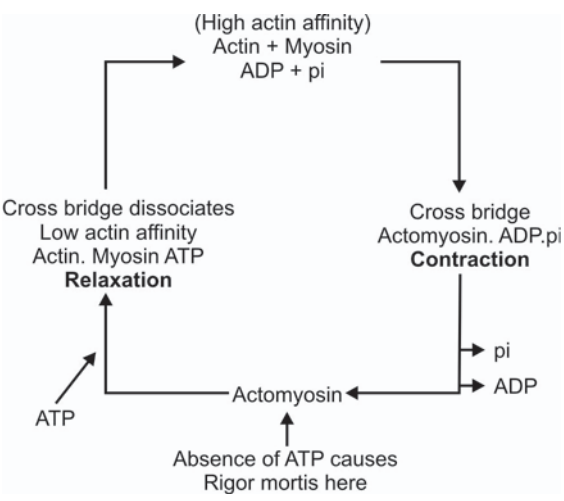


Fig. 3.8: Role of ATP in muscle contraction and relaxation

Relaxation

Relaxation of muscle depends on the active pumping of Ca^{++} back into the terminal cisternae. The receptor which opens up Ca^{++} channels for uptake in the cisternae of the sarcoplasmic reticulum is voltage gated and known as Ryanodine receptors. The enzyme that is involved in the active transport is Ca^{++}-Mg^{++} ATPase. The Ca^{++} pump into the cisternae, results in the removal of Ca^{++} from troponin C. This causes conformational changes in troponin molecule, which makes tropomyosin to be lifted and rest in the groove of actin. These changes will cover the binding sites in actin. The interaction between actin and myosin gets inhibited by troponin I,resulting in muscle relaxation.

Steps in skeletal muscle relaxation

Active transport of Ca^{++} into cisternae
↓
Removal of Ca^{++} from troponin C
↓
Inhibition of interaction between actin and myosin
↓
ATP binds to myosin, cross bridges dissociates
↓
Relaxation of muscle

Rigor mortis

Absence of ATP causes myosin remaining attached to actin, as the cross bridge is not broken It leads to stiffening of the muscle called rigor mortis, which is observed after death.

MECHANICS OF MUSCLE CONTRACTION

There are two types of muscle contractions, namely **isotonic** and **isometric**.

Isotonic contraction

In isotonic contraction, the muscle is allowed to shorten, but the tension in the muscle remains constant. Force developed from contractile elements is transmitted to the series elastic component **(SEC),** which comprises, tendon and other elastic tissues (Fig. 3.9). The stretching of SEC transmits force to the skeleton for movement. In isotonic contraction, the muscle lifts a load and there is external work done. The load acts on the muscle during shortening and hence it is called afterload. *All isotonic contractions occur in afterloaded conditions only.* The lifting of load by the muscle stretches series elastic component and the force developed will be equal to the afterload. As the muscle shortens, its length varies, but the length of SEC remains constant.

Isometric contraction

In this type, the tension developed in muscle is variable, but the length of muscle remains constant. This type of contraction is seen in postural muscles, which maintain muscle tone. Experimentally, isometric contraction can be recorded by attaching a force transducer to one end of the muscle and the other end is fixed firmly.The recording will show the force developed in the muscle.The muscle is not allowed to shorten, due to the fact, that the SEC stretches simultaneously, as the muscle develops tension.This prevents the muscle shortening.

Length-tension relationship

The muscle which is attached to the skeleton shows sarcomere width as 2.2 μ. This length is

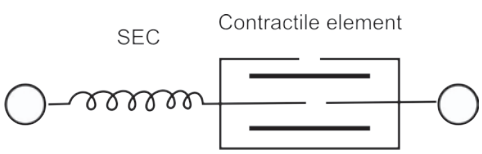


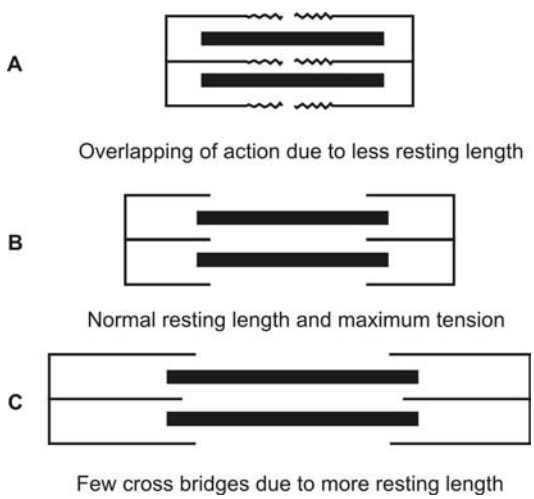
Fig. 3.9: Diagrammatic representation of series elastic component (SEC) and contractile elements in an isotonic muscle contraction

The tendons and elastic tissues in muscle form the SEC.The lifting of load by the muscle during shortening (after load), stretches the SEC and the force from it is transmitted to the skeleton for lifting the load. During shortening of the muscle, the length of SEC remains same, but not so with the muscle

the **resting length** and the tension developed for this length is maximum, due to stretching. At this length of sarcomere, there are maximum number of cross bridges, hence there is maximum tension. This is called **resting tension**. If the length of muscle is shorter than the resting length, the thin filaments begin to overlap and the number of cross bridges become less. If the length of muscle is longer than the resting length, only a few cross bridges will form and the tension declines (Fig. 3.10).

The relationship between length and tension can be studied in an isometric contraction. Experimentally, it is possible to measure the tension developed at various lengths of muscle. We can observe that when the muscle is allowed to shorten isometrically, the tension developed is over and above the resting tension and is called *total tension*. The difference between total tension and resting tension gives *active tension*.

In experimental setup, where isotonic type of contractions are recorded, it can be observed that the sarcomere length of muscle is less than 2.2 μ when isolated from skeleton. Stretching by load (preload) will cause greater tension and gives greater force of contraction (Starling's phenomenon). If stretching of sarcomere takes place beyond 2.2 μ, the force developed is decreased and greater force of contraction can not occur. It should be remembered that the muscle which is attached to the skeleton in our body, has a sarcomere length 2.2 μ (optimium length) and the tension developed is maximum (Fig. 3.11).



Figs 3.10A to C: Length-tension relationships in different lengths of sarcomere: (A) Resting length is shorter and hence the thin filaments overlap, giving less number of cross bridges. (B) Normal resting length (2.2 μ), with maximum number of cross bridges, giving maximum tension. (C) Resting length is greater, and thin filaments are wide apart with less number of cross bridges. In A and C the tension developed is less

Force-velocity relationship

There is an inverse relationship between force and velocity (Fig. 3.12). As the load on the muscle is increased, the force (tension) developed is decreased and consequently, the velocity of shortening becomes less. The velocity of shortening is maximum when after load on the muscle is nil. That is, at the resting length, the velocity of muscle shortening is at its best when there is no load. It is our common experience that whenever we lift a light weight object, it is done faster, while it is not so, when we try to lift a heavy weight object. It is only in isotonic type of contraction, force velocity relationship can be observed.

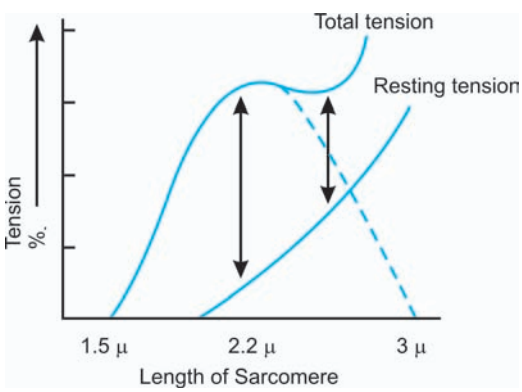


Fig. 3.11: Tension developed at various lengths of sarcomere in the contraction of skeletal muscle. Maximum tension is present at the resting length. Increasing or decreasing the resting length lowers the tension

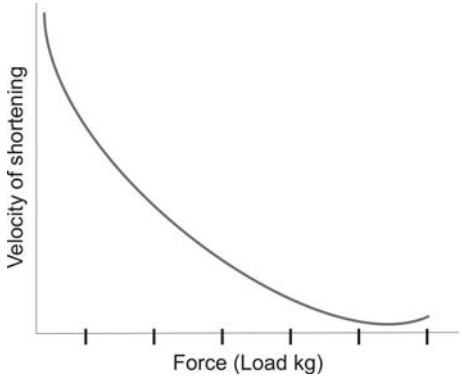


Fig. 3.12: Graph showing the force-velocity relationship in skeletal muscle contraction. As the load is increased on the muscle, the tension developed is less and hence the decreased velocity of shortening occurs

Summation of contractions and tetanus

Application of several successive stimuli on skeletal muscle leads to summation of contractions, in which the tension developed is much greater than in a single twitch and this is known as **tetanus**. If the frequency of stimuli given is lower than required for complete tetanus, then, it will lead to incomplete relaxation of successive contractions. This is known as incomplete tetanus or **clonus**. In our body, all our voluntary contractions involve tetanic contractions of muscles.

In cardiac muscle, because of longer refractory period, summation of contractions and tetanus are not possible.

Fatigue

When there is repeated contractions of muscle, the muscle loses its elasticity after sometime and fails to contract. This is called fatigue and it is a reversible effect. In an isolated muscle nerve preparation, the seat of fatigue is at the neuromuscular junction, where the exhaustion of acetylcholine occurs for repeated stimulation of the motor neuron.

In the intact body, the fatigue occurs after a severe muscular work. Here, the seat of fatigue is at the muscle and synapse. The accumulation of acid metabolite such as lactic acid is said to be responsible for fatigue, where the muscle is involved. The fatigue also results due to diminished neuromuscular transmission and depletion of muscle glycogen. After a period of rest, the acid metabolite is washed away in the circulation and muscle can contract again.

ENERGY MECHANISM DURING MUSCLE CONTRACTION

Muscle shortening requires energy and this comes from the hydrolysis of ATP. The hydrolysis of ATP yields ADP, inorganic phosphate, and energy. The energy released is utilized for cross bridge formation and power stroke mechanism. The muscle sarcoplasm contains a high energy compound called **creatine phosphate**, which contains one high energy phosphate. The ADP, which is formed by ATP hydrolysis, is rephosphorylated into ATP by creatine phosphate to enable continuous supply of ATP during muscle contraction.

The chemical reaction proceeds as follows

ADP + Creatine phosphate →Creatine + ATP

The energy rich compound creatine phosphate is readily used for the regeneration of ATP anaerobically. This process of regeneration will help muscle shortening only for a brief period. If muscular effort is marked, then the other mechanisms of regeneration of ATP will occur. They are:

- *Anaerobic glycolysis*
- *Aerobic oxidation of glucose*
- *Oxidation of fatty acids*.

The ATP formed is utilized for rephosphorylating creatine to creatine phosphate, which acts as energy source for muscle contraction.

Aerobic glycolysis leads to the formation of pyruvate, which enters tri carboxylic acid cycle (TCA) where, in the presence of oxygen, oxidation occurs, yielding 38 molecules of ATP

Anaerobic glycolysis occurs, if the muscular work is severe, the oxygen supplied will not be sufficient and hence anaerobic glycolysis takes

place to give lactic acid instead of pyruvate. Lactic acid enters the **Cori Cycle** to form glycogen in the liver. The breakdown of glycogen releases glucose into the blood.

The oxidation of fatty acids (β oxidation) yields much greater amount of ATP. It has been observed that during rest and moderate muscular activity, oxidation of fatty acids forms the important source of energy. However, during marked muscular work, glucose oxidation becomes significant, as the lipid oxidation can not quickly supply the energy.

Oxygen debt

Prolonged muscle shortening leads to oxygen deficit, as the supply does not match with the demand. In these situations, anaerobic glycolysis occurs as said earlier. After the muscular work is over, there is increased oxygen consumption, which is proportional to the oxygen deficit. The increased oxygen consumption noticed after the muscular work is called oxygen debt. The excess oxygen consumed is utilised for the oxidation of as lactic acid and to replenish ATP and creatine phosphate stores.

Heat production in muscle

When a muscle is allowed to shorten, it does external work and it releases heat. It is only in isotonic type of muscle contraction there is external work done and heat is liberated. In isometric type, no external work occurs, but the varying tension in the muscle causes internal work to take place. With the help of thermocouples, it is possible to record the temperature changes in the muscle during muscle contraction. The muscle releases the following types of heat.

a. **Resting heat:** It is the basal heat released from the muscle and it reflects the BMR
b. **Initial heat:** It consists of two types namely the *activation heat* and *shortening heat*

   Activation heat is related to heat production from cross bridge formation and the **shortening heat** is as a result of heat release from power stroke mechanism. The activation heat and shortening heat are together known as initial heat.
c. **Relaxation heat:** It is the heat release from the process which bring the muscle length to normal resting level.
d. **Recovery heat:** It is also known as delayed heat and it is equal to initial heat. It restores the muscle to the precontraction state.

In isometric type of contraction, there is only resting heat that can be measured for the reasons mentioned earlier.

MOTOR UNIT

A motor neuron supplying a group of muscle fibers forms the motor unit (Fig. 3.13). The number of muscle fibers innervated by the motor neuron varies depending upon the kind of muscles and nature of contractions produced by them. Fine and precise movements, like visual fixation of objects by the ocular muscles will involve only a few muscle fibers being supplied by a motor neuron. Whereas, gross movements produced by limb muscles will have a motor neuron innervating many muscle fibers.

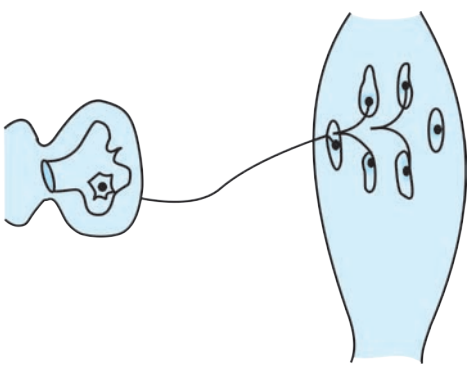


Fig. 3.13: Diagram of a motor unit

Motor units are of two types

a. Fast twitch
b. Slow twitch

Fast twitch motor units are seen in muscles, which are involved in rapid movements. They are called **white muscles**. They undergo fatigue easily.

Slow twitch motor units are in **red muscles** and are fatigue resistant, has more myoglobin and capillary density. These type of muscles are involved in maintaining posture and can be seen in antigravity muscles.

Motor units show recruitment and as a result, the muscle contractions are graded. The increase in recruitment, increases the tension in the muscle. The preference of recruitment, whether slow or fast twitch motor units, depends on the nature of motor activities (phasic or static).

The motor units during voluntary action do not fire, all at the same time. That is, they fire asynchronously, so that, motor activity of individual units can merge to give a smooth contraction. If all the units fire synchronously, then a spasmodic type of contraction will occur.

EMG

Electromyography is used to record the electrical activity of motor units. The instrument primarily consists of recording electrodes, which may be surface disc or metal electrodes or needle electrodes or fine wire electrodes. Needle and fine wire electrodes are used to record directly the activity of individual muscle fibers. The surface electrodes will only record the surface potentials of muscle from the skin covering it.

There is also an amplifier to amplify the signal and a display unit, which could be a cathode ray oscilloscope or a recording unit.

EMG record will show a basal electrical activity of the units, which correspond to the muscle tone and firing of motor units when the muscle contracts. Greater the force of contraction, greater the recruitment of motor units and hence greater the frequency and amplitude of firing of motor units.

Normal EMG recording will reveal the following characteristics.

The firing of motor units are termed motor unit potentials (MUP) and its features are given below.

Amplitude	0.5 to 2 mv
Frequency	up to 500 Hz
Duration	2 to 12 msec

Uses of EMG

EMG recording is of value in clinical and electrophysiological studies of muscle diseases (Myopathies).

Abnormal electrical activity of motor units can be seen as fibrillation potentials and fasciculations.

Fibrillation potentials

It is the firing of single motor units and cannot be seen in naked eye. They are due to the hypersensitivity to acetylcholine released from the cut or damaged nerve ending (**denervation hypersensitivity)**.

Fasciculations

It is the activity of a group of motor units and can be seen visually. It occurs due to the abnormal firing of spinal motor neuron as observed in anterior horn disease (poliomyelitis).

Fibrillation potentials and fasciculations are characteristics of lower motor neuron lesions.

NEUROMUSCULAR TRANSMISSION

Muscle fibers are innervated by the axons of motor neuron. Each axon supplies a single muscle fiber, forming one to one relationship called neuromuscular junction. The axon loses its myelin sheath before ending on the muscle fiber. The axon terminal dips into the groove or depression formed by the end plate of the muscle membrane. Between the end plate of muscle membrane and axon terminal, there is a synaptic cleft, which contains basal lamina. It contains

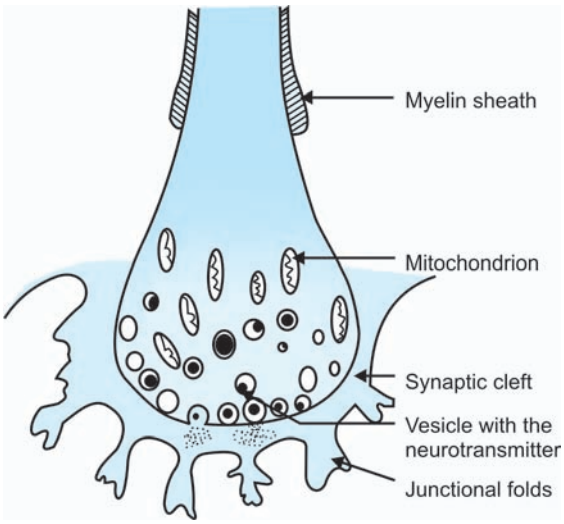


Fig. 3.14: Neuromuscular junction

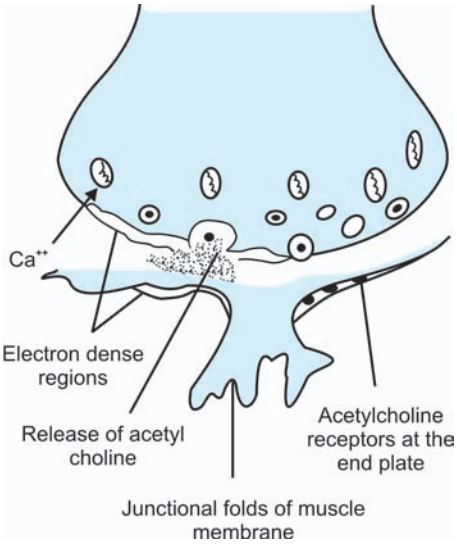


Fig. 3.15: Neuromuscular transmission

an enzyme **acetylcholine esterase**, which hydrolyses acetylcholine.

The axon terminal shows the presence of mitochondria, and a large number of vesicles containing acetylcholine. The muscle membrane facing the axon terminal shows invaginations forming folds called junctional folds (Fig. 3.14). In these folds, acetylcholine receptors are concentrated.

Synthesis of acetylcholine takes place at the axon terminal with the help of the enzyme **choline acetyltransferase**. The reaction is as follows

Acetyl CoA + choline $\xrightarrow{\textbf{choline acetyl transferase}}$ Acetyl choline

Release of acetylcholine

The arrival of action potential at the axon terminal, opens up voltage gated Ca^{++}channels, causing entry of Ca^{++} into the axon terminal. This triggers the subsequent steps in the exocytosis process, which include vesicles moving towards the axon terminal and fusion of the vesicle membrane with the axon terminal membrane to release the transmitter (Fig. 3.15).

Action on end plate

Acetylcholine receptors are found more at the junctional folds of the end plate. They are the **nicotinic receptors** and have five sub units. Two alpha sub units of the receptor protein has the binding sites for acetylcholine. The receptors contain channels for both Na^+ and K^+ and open, when acetylcholine binds to the receptor. Since these channels are activated by the transmitter, they are called ligand gated channels. They do not open by voltage or depolarization of the membrane. The opening of both the channels in the receptor, causes movement of ions. The electrochemical gradient for Na^+ to enter is far greater than the gradient for K^+ ion to leave and hence depolarization of muscle membrane occurs.

The membrane potential falls from - 90 mv to - 60 mv (towards threshold level). This is a graded, nonpropagatory electrical potential known as **end plate potential (EPP)** (Fig. 3.16). When action potential arrives at the axon terminal, there will be release of acetylcholine

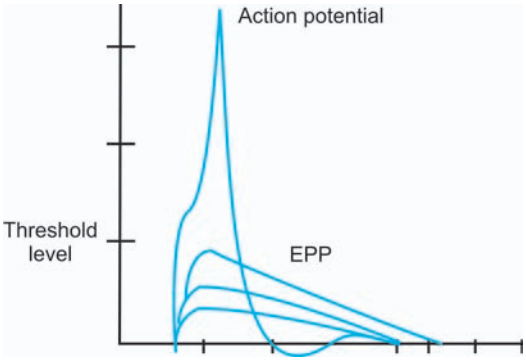


Fig. 3.16: End plate potential and the development of action potential when EPP reaches the threshold level of firing

from 200 to 300 vesicles and the depolarization of the end plate (EPP) is sufficient to reach the threshold level of firing forming a propagated action potential. The action potential spreads to the contiguous end plate membrane.

It has been found that each vesicle, when fuses with the axon terminal, releases approximately 5000 to 10,000 acetylcholine molecules. Even when there is no nerve stimulation, there is a spontaneous release of acetylcholine called **quantal release**. This release occurs from a single vesicle. The quantal release of acetylcholine can cause depolarization of the end plate membrane to only 1 mv. These potentials cannot reach the threshold level of firing and they are called **miniature end plate potentials (MEPP).** Quantal release of acetylcholine helps to maintain the integrity of muscle fiber.

Acetylcholine inactivation

The enzyme **acetylcholine esterase,** present in the basal lamina, hydrolyzes acetyl choline into choline and acetic acid. The reaction proceeds as follows

$$\text{Acetylcholine} \xrightarrow{\text{Acetylcholine esterase}} \text{Acetic acid} + \text{Choline}$$

Inactivation of acetylcholine at the neuromuscular junction is necessary to prevent continuous stimulation of muscle contraction. Choline uptake at the axon terminal is significant, as the choline, after its uptake is reutilized for the synthesis of acetylcholine. Agents, which block the uptake of choline at the axon terminal will result in reduced synthesis of acetylcholine and this mechanism can be used to minimise the action of acetylcholine at the end plate.

Blocking of neuromuscular transmission

Neuromuscular transmission is blocked by drugs, poisons and toxins.

Curare: It is an alkaloid and a poisonous substance. When injected into the body, it binds to acetylcholine receptors by displacing the transmitter (competitive inhibition). Although, curare binds to the acetylcholine receptors, it does not cause depolarization of the end plate. (non depolarizing type). If sufficient number of receptors are occupied by curare, it will lead to muscular paralysis including respiratory muscles and results in death.

Succinyl choline: It is also a neuromuscular blocking agent. It causes depolarization of the end plate initially, but, later inactivates Na^+channels and blocks the neuromuscular transmission. This depolarizing type of agent is used to cause muscle relaxation during surgery.

Botulinum toxin: It inhibits the release of acetylcholine from vesicles and thereby prevents transmission.

Alpha bungarotoxin: It is a snake venom obtained from cobra. It binds irreversibly to the nicotinic receptors of the acetylcholine at the end plate. When the venom enters the body, it will get attached to the end plate acetylcholine receptors and causes paralysis of muscles, including respiratory muscles and results in death.

Acetylcholine esterase inhibitors

They are two types namely reversible and irreversible. They help to increase the concentration of acetylcholine at the end plate. **Reversible inhibitors** include neostigmine and physostigmine.

The **irreversible group** of agents include insecticide and nerve gas poisons.

Diseases affecting neuromuscular transmission

Myasthenia gravis

It is an autoimmune disease, in which the body produces antibodies against its own acetylcholine receptor protein. When acetylcholine receptors are blocked by autoimmune antibodies, the released actylcholine cannot act on the receptors. This results in blocking of transmission and leads to muscle weakness and paralysis. It has been observed that thymus gets enlarged, showing its link in the pathophysiology of myasthenia gravis. The disease is treated by the administration of anticholine esterases like neostigmine and physostigmine, which increase the concentration of acetylcholine at the end plate. Increased concentrations of acetylcholine at the end plate, will help to displace the autoantibodies from the receptors.

CARDIAC MUSCLE

It is striated like skeletal muscle, but, involuntary in nature. The muscle fiber has branches and interdigitate with each other (Fig. 3.17). Each fiber forms a separate cell, separated from others by intercalated disc, which occurs at the Z lines. There are gap junctions, where cell membranes of two adjacent cells join. These gap junctions form low electrical resistance bridges for the conduction of action potentials from one cell to another. In this way, cardiac impulse spreads to the entire myocardium through this functional syncytium. There is a sarcoplasmic tubular system with T system facing Z line, instead of at the AI band junction as seen in skeletal muscle.

Contraction in cardiac muscle

Contraction of cardiac muscle shows that Ca^{+}entry into the cell is vital for excitation contraction coupling. The Ca^{++}release from cisternae is not sufficient to activate troponin C. Hence Ca^{++} enters from extracellular fluid when the cell is depolarized. The Ca^{++} influx occurs through dihydroxypyridine channels (Cardiac cell has four types of Ca^{++} channels) and after entering the T system, it causes Ca^{++} release from the terminal cisternae. This is known as calcium induced calcium release. The Ca^{++} concentration in the cardiac cell is maintained by Na^{+}-Ca^{++} exchange transport.

The action potential recorded from myocardium shows (Figs 3.18 and 3.23).

Depolarization marked as overshoot, caused by opening of voltage gated Na^{+} channels.

Repolarization begins, but lasts only for a very brief period (**initial repolarization**). This

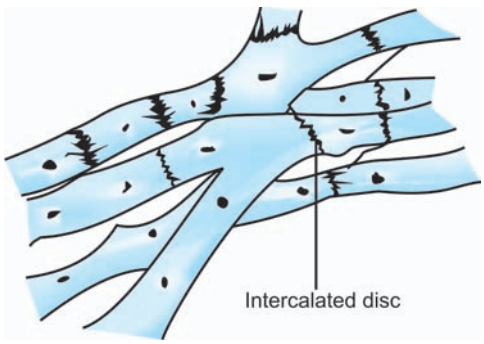


Fig. 3.17: Structure of a cardiac muscle

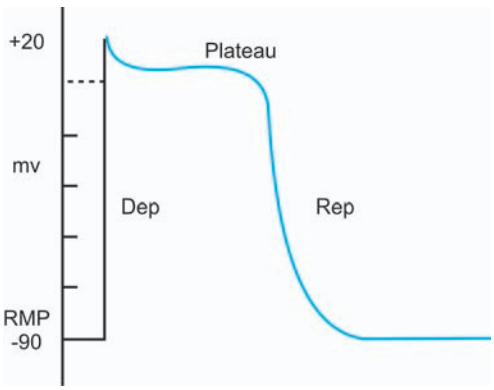


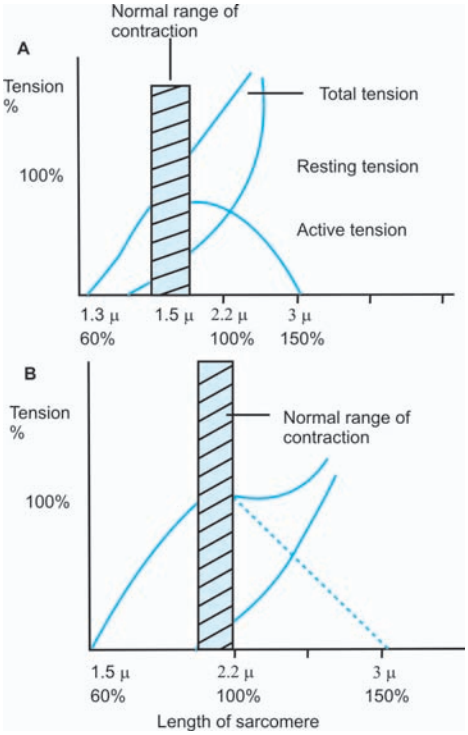
Fig. 3.18: Action potential from the ventricular myocardium

is followed by a **prolonged plateau**, due to Ca^{++} influx through voltage gated slow channels. After the plateau, repolarization is completed and is caused by K^{+} exit. The phenomenon of plateau in cardiac cell gives a longer refractory period (0.25 sec), which prevents tetanization of the muscle.

Length-tension relationship in cardiac muscle

In cardiac muscle, the sarcomere length prior to contraction depends on the preload. That is, the amount of blood entering the ventricle during diastole (end diastolic volume) will determine the length of sarcomere.

The resting length of sarcomere is 60%, which allows room for stretching up to 100%, to attain maximum tension (ventricular pressure) (Fig. 3.19). Increasing the sarcomere length stretches the initial length of the fiber, which in turn increases tension, causing greater force of contraction **(Starling's law of the heart).** The force of contraction is also regulated by the circulating hormones like catecholamines and Ca^{++} ions.



Figs 3.19A and B: (A) Length tension relationship in cardiac muscle (B) Compared with that of skeletal muscle

Note the resting sarcomere length is only 1.5 μ cardiac muscle and can stretch up to 2.2 μ, to give maximum tension. In skeletal muscle, the resting length is already 2.2 μ which develops maximum tension

SMOOTH MUSCLE

It is a plain muscle or unstriated muscle and involuntary in nature. They are spindle shaped, 2.5 μ in diameter and 20 to 500 μ in length. Smooth muscle consists of two types, namely **visceral** and **multi unit** types.

Multi unit type is present in iris and ciliary muscle. This type shows individual, discrete fibers and hence gives graded response, as in skeletal muscle.

Visceral smooth muscles can be seen in visceral structures like urinary bladder, wall of the blood vessels, GI tract, uterus, etc.

Visceral smooth muscle has gap junctions for the passage of action potentials.The structure of the muscle fiber shows the presence of contractile proteins, but they are not organized in the form of sarcomeres. They are freely dispersed in the sarcoplasm. Actin is attached to the dense bodies, which either freely float or attached to the cell membrane (Fig. 3.20).

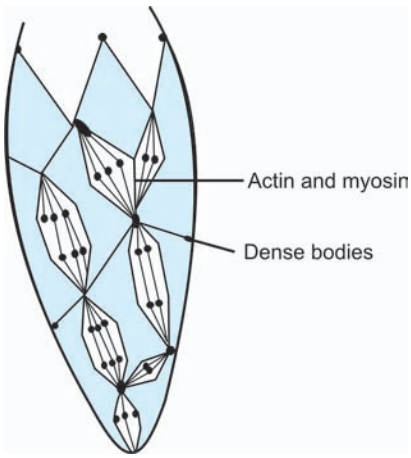


Fig. 3.20: Structure of a visceral smooth muscle

Note the absence of sarcoplasmic reticulum and the attachment of contractile proteins to the dense bodies in the sarcoplasm

Sarcoplasmic reticulum is poorly developed and the notable feature is the presence of only a few mitochondria in the cell. This indicates energy is obtained mostly from glycolysis and moreover the energy requirement in smooth muscle is very less. It can contract tonically for a long period of time.

The visceral smooth muscle is innervated by autonomic fibers, but they are present to regulate the contractions and not necessary to initiate it. The stimulus for the smooth muscle contraction is distention of the wall. There are nerve plexuses in the wall **(Meissner's plexus**), which can give slow wave potentials. These on attaining the threshold value give rise to a propagated action potential. The smooth muscle has an unstable membrane potential, due to which it can generate the impulse on its own with just a small fall in membrane potential. The resting membrane potential is around – 50 to – 60 mv and it is not a true RMP as it is unstable.

The action potentials formed is called spikes and it shows rhythmic changes. Spikes may occur in the rising phase of action potential or the action potential may have a prolonged plateau (Fig. 3.21). The excitation contraction coupling shows that the contraction and relaxation occur slowly, lasting for several milli seconds. The contractions may begin 0.2 msec after the spike is formed and the peak occuring after 0.5 msec.

Role of Ca^{++}

In smooth muscle, Ca^{++} entry through the voltage gated channels is necessary for contraction, as there is no well developed sarcoplasmic reticulum for Ca^{++} release. Calcium after its entry, binds to the protein *calmodulin* in the sarcoplasm. This inturn activates **myosin light chain kinase** enzyme. In smooth muscle, myosin head has to be phosphorylated prior to its contraction, which is done by the enzyme myosin light chain kinase.The phosphorylation of myosin head, leads to sliding of actin over myosin and results in muscle shortening (Fig. 3.22). Relaxation of the muscle occurs by dephosphorylation of myosin head, but it is not complete. There is a mechanism called ***latch bridge***, in which actin and myosin cross bridge remain attached even after dephosphorylation of myosin head. Latch bridge mechanism causes a sustained contraction with less expenditure of energy. This is called tonic contraction of smooth muscle. Complete relaxation of smooth muscle probably occurs when Ca^{++}totally dissociates from calmodulin.

Length-tension relationship

Visceral smooth muscle shows, that as the length of the fiber is increased by stretching, the tension decreases. This is due to the property of smooth muscle called **plasticity**. The tension developed is variable and difficult to correlate with the muscle at different lengths.

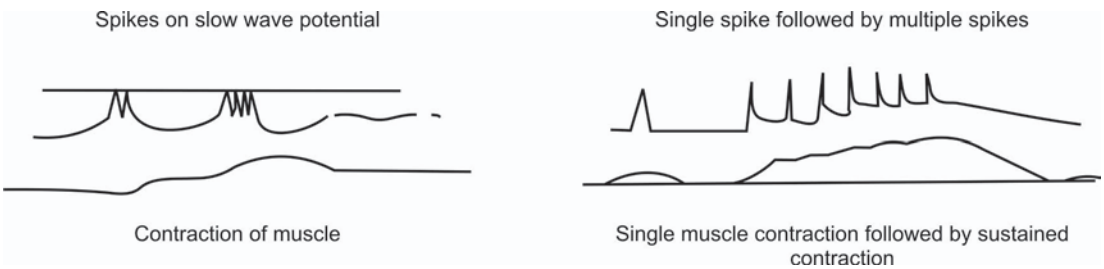


Fig. 3.21: Relationship between spike potentials and contraction in smooth muscle

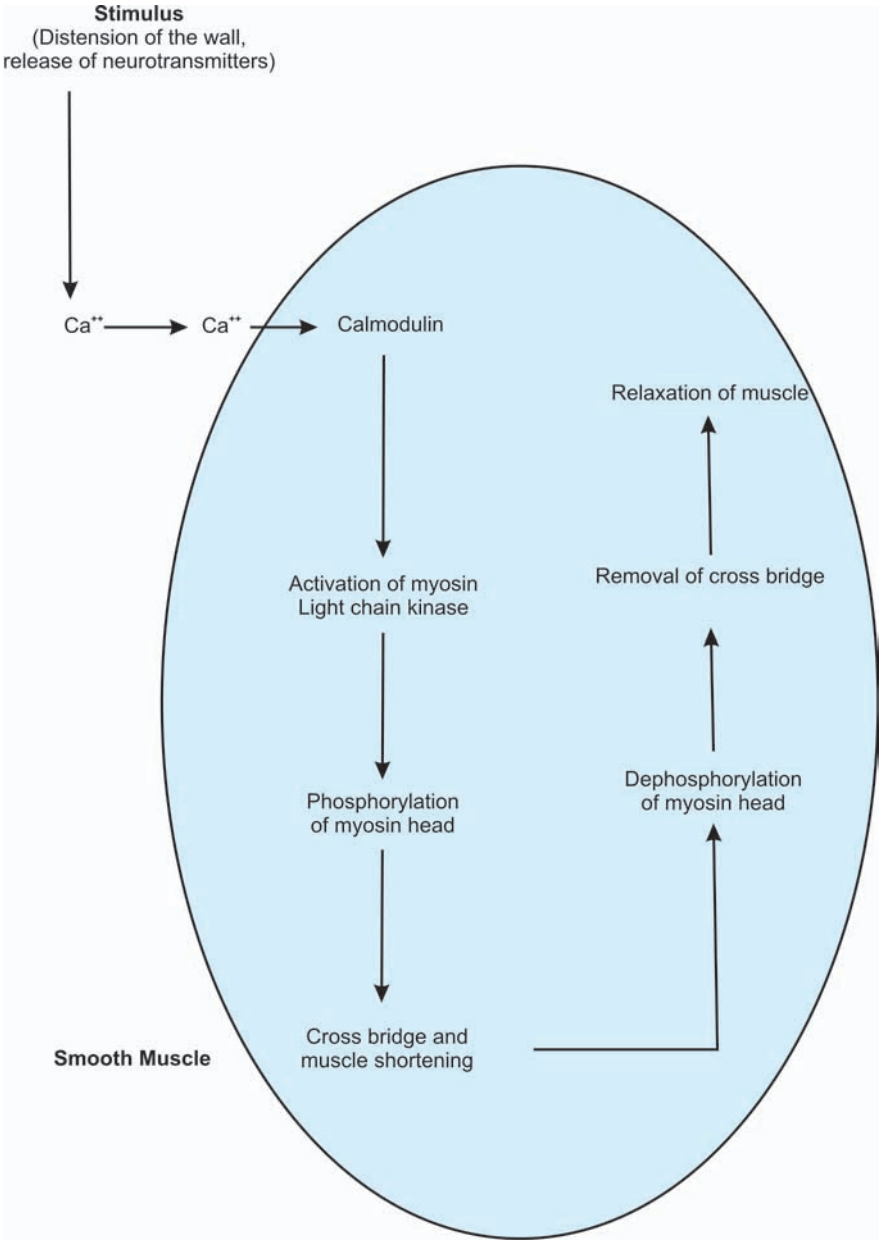


Fig. 3.22: Diagrammatic representation of mechanism of smooth muscle contraction and relaxation

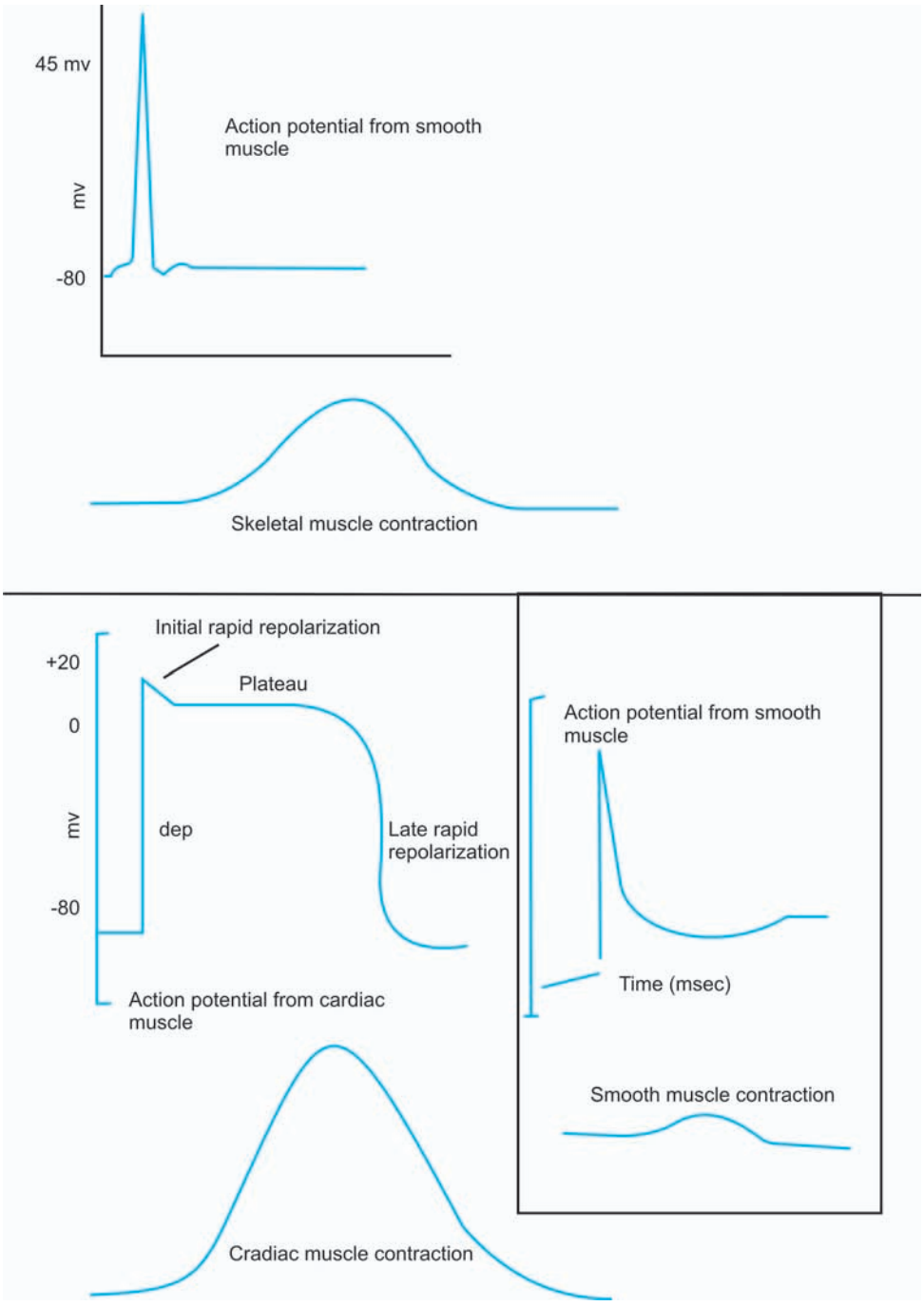


Fig. 3.23: Action potentials of the three types of muscles are correlated with contraction

Self-study Questions

Multiple Choice Questions

Choose the single best answer

1. **The ATPase activity to cleave ATP and utilize energy for the contraction of muscle is present in the:**
   A. Actin
   B. Myosin
   C. Troponin
   D. Tropomyosin

2. **The cross bridge between actin and myosin can be prevented by the binding of:**
   A. Troponin I with myosin
   B. Troponin C with Ca^{++}
   C. Troponin I with tropomyosin
   D. Troponin T with tropomyosin

3. **After a prolonged work, fatigue occurs in which of the following?**
   A. Cardiac muscle
   B. Visceral smooth muscle
   C. Slow twitch skeletal muscle
   D. Fast twitch skeletal muscle

4. **Regarding isotonic muscle contraction all of the following are true *except*:**
   A. Muscle is allowed to shorten
   B. Tension remains constant
   C. Length of the muscle remains constant
   D. External work is done

5. **The protein that is involved in skeletal muscle contraction but not in smooth muscle contraction is:**
   A. Troponin
   B. Actin
   C. Myosin
   D. ATPase

6. **D tubocurare inhibits neuromuscular transmission as it is a:**
   A. Cholinesterase inhibitor
   B. Nicotinic antagonist
   C. Nicotinic agonist
   D. Muscarinic agonist

7. **In myasthenia gravis antibodies are produced against:**
   A. Acetylcholine
   B. Acetylcholine esterase
   C. Acetylcholine receptors
   D. Ion channels on the endplate

8. **Relaxation of muscle occurs by involving all of the following mechanisms *except*:**
   A. Hydrolysis of ATP in the myosin
   B. Removal of Ca^{++} from troponin C
   C. Attachment of ATP to myosin head
   D. Active pumping of Ca^{++} into the cisternae

9. **All of the following would lead to contraction of skeletal muscle *except:***
   A. Depolarization of T tubules
   B. Ca^{++} release from troponin C
   C. Ca^{++} release from cisternae
   D. Depolarization of sarcolemma

10. **Which of the following would be useful in the treatment of curare poisoning?**
    A. Neostigmine
    B. Nifedepine
    C. Succinylcholine
    D. Tetrodotoxin

11. **During muscle shortening decrease in width of all of the following occurs *except:***
    A. A band
    B. I band
    C. H zone
    D. Sarcomere

12. Which one of the following would increase acetylcholine concentration at the neuromuscular junction when administered?

A. Curare
B. Neostigmine
C. Botulinum toxin
D. Succinylcholine

13. During muscle shortening power stroke movement is produced by:

A. Actin
B. Myosin
C. Troponin
D. Tropomyosin

ANSWER KEYS

1. (B) 2. (A) 3. (D) 4. (C) 5. (A) 6. (B) 7. (C) 8. (A) 9. (B) 10. (A)
11. (A) 12. (B) 13. (B)

Short Answer Questions

1. Desribe the changes that occur in a sarcomere during muscle contraction.
2. List the functional differences between skeletal muscle and cardiac muscle.
3. Enumerate the steps involved in skeletal muscle contraction.
4. Explain the role of Ca^{++} in muscle contraction and relaxation.
5. Define rigor mortis. Explain its mechanism.
6. Enumerate the steps involved in smooth muscle contraction.
7. List the causes of fatigue.
8. Define motor unit and describe how its activity can be studied.
9. Compare the length tension relationship between skeletal muscle and cardiac muscle.
10. Explain why cardiac muscle can not be tetanized.
11. List the differences between isotonic and isometric contractions.
12. List the uses of EMG.
13. State the differences between fast twitch and slow twitch muscle fibers.
14. Enumerate the steps involved in the neuromuscular transmission.
15. State the differences between EPP and action potential.
16. List the factors that affect neuromuscular transmission and state how the block can be reversed.
17. Describe how neuromuscular transmission is blocked in myasthenia gravis.
18. Compare and contrast the action potentials recorded from the skeletal muscle and cardiac ventricular muscle fibers.

4 Central Nervous System

Introduction

Central and peripheral are the two divisions of the nervous system (Fig. 4.1). Central nervous system (CNS) includes the spinal cord and the brain. It receives information about changes in the environment through the peripheral nervous system. The CNS processes the information and integrates them. This helps in the perception of sensations at the conscious level and to give appropriate motor response in the form of behavior. In humans, central nervous system is very well developed with the neocortex showing the highest specialization for higher functions such as cognition, learning, memory, speech and emotions.

Peripheral nervous system includes spinal nerves, cranial nerves and autonomic fibers. The peripheral nervous system forms the link between the environment and the CNS. Its primary function is to detect changes in the environment, for which the sensory receptors are involved. The first step in achieving this function involves transduction at the receptors, which is followed by the communication between neurons. In the mammalian nervous system, the communication between two neurons is commonly effected, by the release of chemical transmitters, which is known as synaptic transmission.

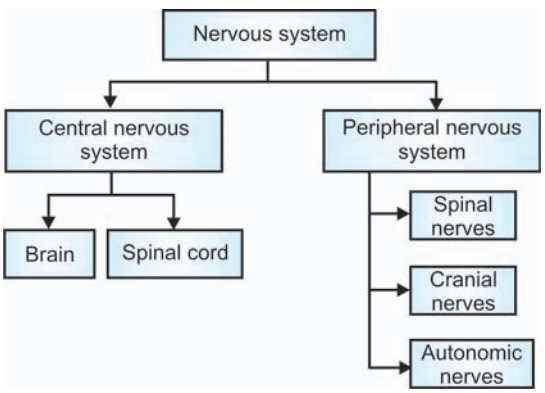


Fig. 4.1: Divisions of the nervous system

SYNAPTIC TRANSMISSION

Synapse is a junction between two neurons, where functional continuity is established through chemical transmission. These synapses are called chemical synapses which form the most common mode of transmission for impulses in the CNS. In invertebrates, there are electrical synapses, where impulses are transmitted through the gap junctions between neurons and low electrical resistance in the synapse.

Structure of synapse

Synapses are formed between an axon and a dendrite **(axodendritic)**, an axon with a cell body (**axo somatic**) and an axon with another axon **(axo axonic)** (Fig. 4.2). The neuron that carries the impulse towards the synapse is called the presynaptic neuron and the neuron that receives the impulse is known as postsynaptic neuron

(Fig. 4.3). Between these two neurons, there is a synaptic cleft with 20-40 nm width. The presynaptic and postsynaptic regions in the synaptic cleft show electron dense appearance, which characterizes the presence of synaptic vesicles and receptors respectively. The vesicles contain the neurotransmitters, which can be either excitatory or inhibitory. There is also presence of a number of mitochondria in the presynaptic terminal. The postsynaptic membrane contains receptors, specific for the transmitter that is released from the presynaptic terminal.

Mechanism of synaptic transmission

When an action potential reaches the presynaptic terminal, there is entry of Ca^{++} from ECF into the terminal. This causes the vesicle membrane to fuse with the presynaptic membrane and release the contents (**exocytosis**) (Fig. 4.4). The synaptic vesicle membrane protein **Synaptobrevin** combines with the cell membrane protein **syntaxin** to facilitate fusion. The released transmitter is attached to the receptors, present on the postsynaptic membrane and produces the electrical response. If there is a release of excitatory transmitter, an excitatory postsynaptic potential (**EPSP**) is developed or inhibitory postsynaptic potential **(IPSP**), if the transmitter released is inhibitory in nature. The released transmitter is either inactivated by the enzymes present in the synaptic cleft or taken up (reuptake) by the presynaptic neuron.

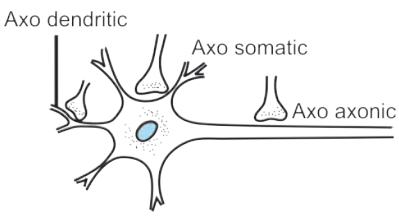


Fig. 4.2: Three types of synapses

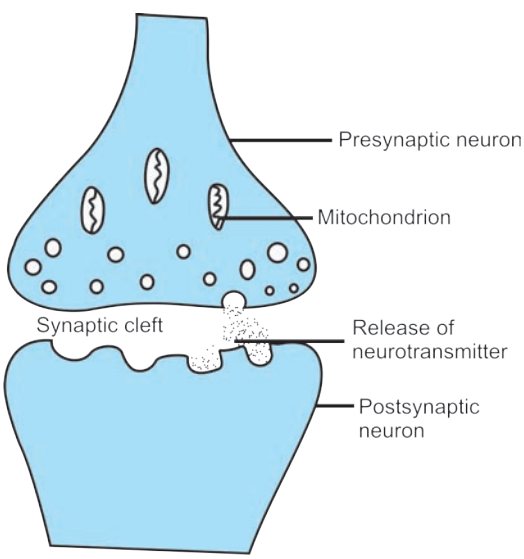


Fig. 4.3: Structure of a synapse

Properties of synapse

Oneway conduction

Transmission occurs from presynaptic to postsynaptic neuron and not vice versa, since only in the presynaptic neuron, there is presence of mechanism for the chemical transmission.

Synaptic delay

The minimum time required for transmission in a single synapse is 0.5 msec. The delay will be more if many synapses are involved between two neurons.

Fatigue

Repeated stimulation of a neuron leads to the exhaustion of neurotransmitters which results in fatigue.

Synapses are also sensitive to hypoxia and metabolic poisons.

Summation

Synaptic transmission shows temporal and spatial summations. If two or more stimuli from a single presynaptic neuron reaches the synapse successively at quicker intervals of time, **temporal summation** occurs causing postsynaptic neuron to fire (Fig. 4.5). Conversely, if stimuli from many presynaptic neurons reach the synapse

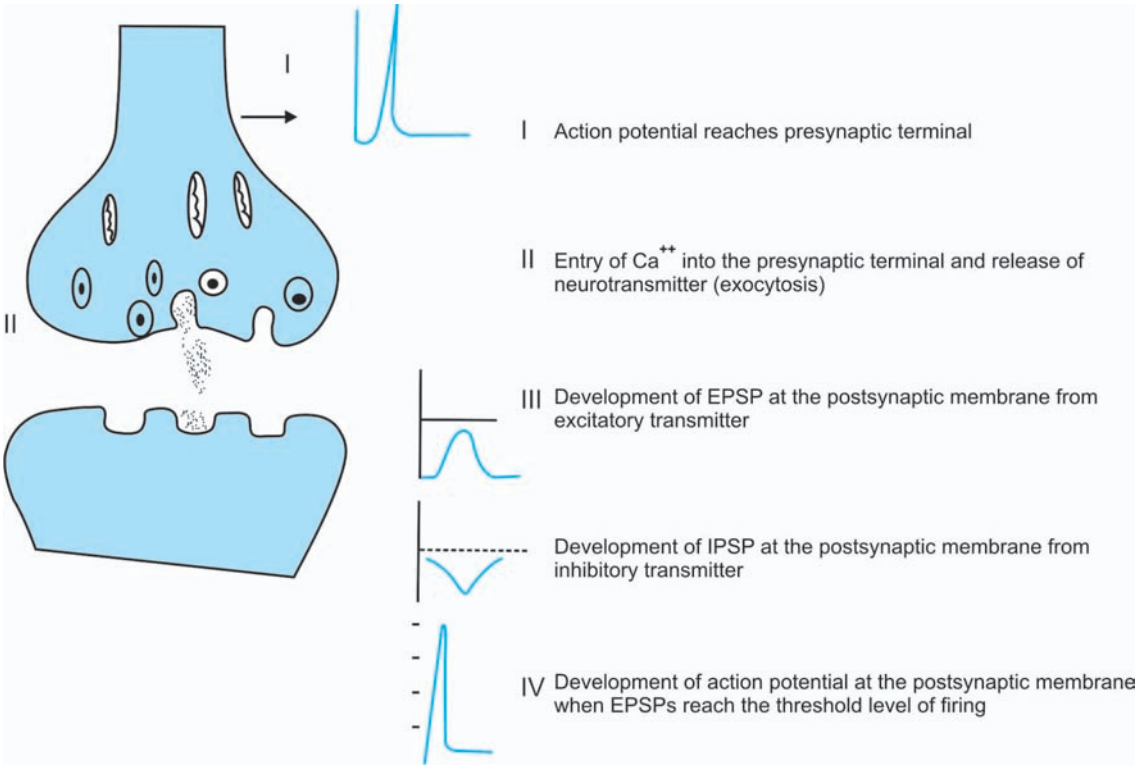
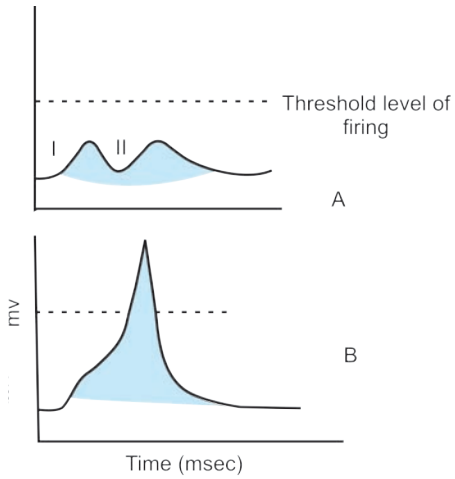
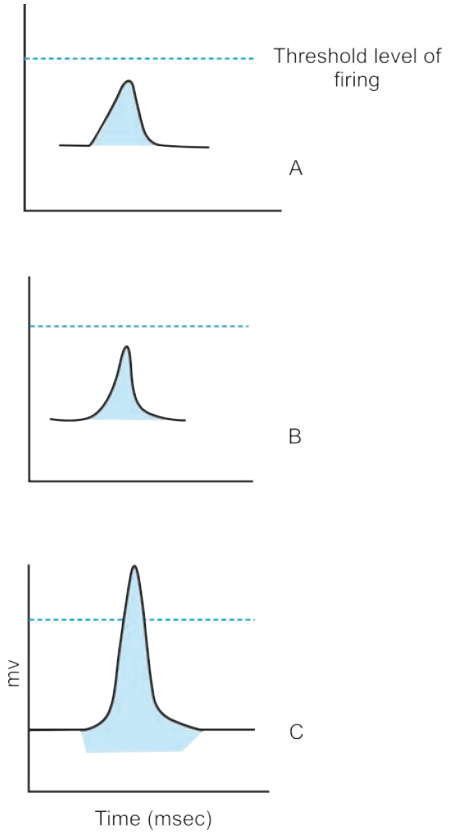


Fig. 4.4: Steps in the mechanism of synaptic transmission



Figs 4.5A and B: Temporal summation in the excitation of postsynaptic neuron

A and B. Two or more stimuli when applied one after another at quicker intervals of time, the EPSPs summate and reach the threshold level of firing to produce an action potential



Figs 4.6A to C: Spatial summation in the excitation of postsynaptic neuron

A and B. Two or more stimuli when simultaneously given, the EPSPs summate at the postsynaptic membrane, causing the membrane potential to reach the threshold level of firing.

C. Action potential development from such summations

simultaneously, **spatial summation** at the post synaptic neuron causes it to fire (Fig. 4.6).

Convergence and divergence

Many neurons can converge on a single neuron forming convergence or a neuron can divide and end on many neurons to form divergence (Fig. 4.7).

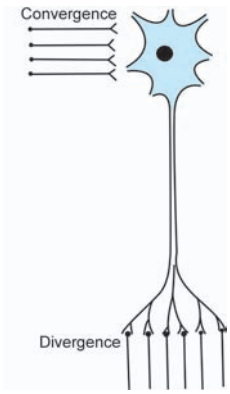


Fig. 4.7: Convergence and divergence of neurons

Subliminal fringe

The stimulation of a neuron can lead to the firing of postsynaptic neurons, which are in direct in contact with it and these neurons are said to be in the **discharge zone**. There will be other neighbouring neurons, which receive impulses, but do not fire, as the change in the membrane potential has not declined to the firing level. These neurons which develop only EPSPs and not the action potential are in a state of subliminal fringe (Fig. 4.8). These neurons can develop action potentials by the summation of EPSPs.

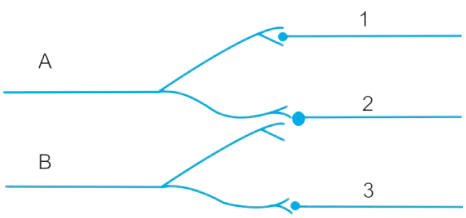


Fig. 4.8: Stimulation of presynaptic neuron A causes firing of postsynaptic neuron 1 and the postsynaptic neuron 2 shows increased excitability by developing EPSP. Stimulation of presynaptic neuron B would cause firing of postsynaptic neuron 3 and development of EPSP in neuron 2. When both A and B are stimulated together all the three postsynaptic neurons (1, 2 and 3) would fire giving a greater response. It is because the postsynaptic neuron 2, which has been developing only EPSP for each of the presynaptic neuron's individual stimulation, now summate and forms action potential when both of them are stimulated together. The neuron 2, is said to be in the subliminal fringe

Occlusion

If presynaptic neurons share a common post-synaptic neuron, then the response obtained by stimulating them together will be lesser than the response observed, when the afferents are stimulated separately. It is due to the occlusion of response, as the afferents share a common postsynaptic neuron (Fig. 4.9).

Electrical properties

EPSP

The release of excitatory neurotransmitter causes a localized depolarization at the post synaptic membrane. This reduces the membrane potential by 10 to 15 mv. The local depolarization thus developed is nonpropagatory and called as **excitatory postsynaptic potential (EPSP)** (Fig. 4.10). The ionic basis for this is due to the entry of Na^+ ions or Ca^{++} and the EPSPs that are developed can summate to form an action potential. The EPSP does not show all or none character, but it gives temporal and spatial summations.

IPSP

The electrical response at the postsynaptic membrane caused by the release of inhibitory transmitter is known as **inhibitory postsynaptic potential (IPSP).** It is a hyperpolarizing current, which causes the membrane potential to increase (Fig. 4.11). This inhibits the membrane excitation. The ionic basis of IPSP is due to the influx of Cl^- or exit of K^+ ions. Either of these mechanisms can cause increased negativity inside. The IPSP also shows temporal and spatial summations.

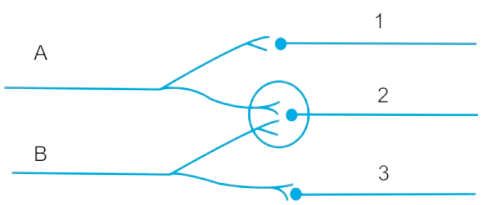


Fig. 4.9: Occlusion in synapse

Stimulation of presynaptic neuron A causes firing of postsynaptic neurons 1 and 2. Stimulation of presynaptic neuron B would cause firing of post synaptic neurons 2 and 3. But when both A and B are excited together, the firing would occur only in 1 and 2, thus giving a lesser response. It is because the neuron 2 has been the common motor neuron for the presynaptic neurons A and B and hence the response gets occluded when they are excited together

Types of inhibition in CNS

Direct inhibition

It is the inhibition caused by the release of inhibitory transmitter. In the CNS, the inhibition of a neuron usually occurs through an inhibitory interneuron **(Golgi bottle interneuron)** (Fig. 4.12). In the spinal cord, brainstem and retina, the inhibitory neurotransmitter is **glycine** and in the

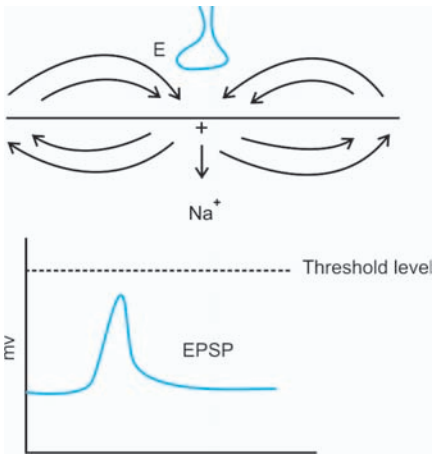


Fig. 4.10: Excitatory postsynaptic potential (EPSP)

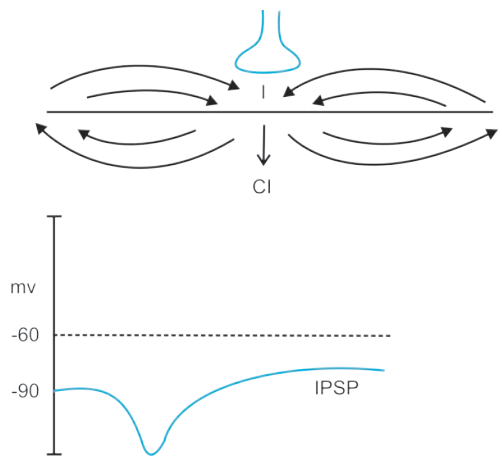


Fig. 4.11: Inhibitory postsynaptic potential

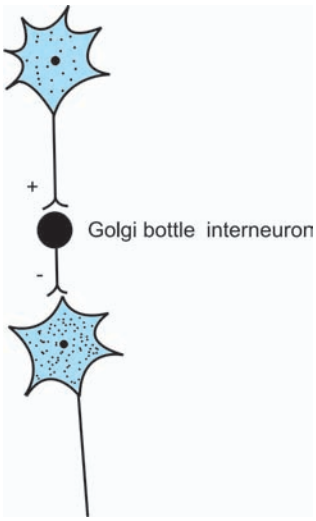


Fig. 4.12: Inhibition of post synaptic neuron through Golgi bottle interneuron. The inhibitory interneurons secrete either GABA or Glycine

In the spinal cord, brainstem and retina, glycine is the inhibitory transmitter, while in the brain and retina, GABA is the inhibitory transmitter. The excitatory presynaptic neuron ends on the inhibitory interneuron and excites it. The interneuron releases the inhibitory transmitter and inhibits the postsynaptic neuron

brain and retina it is **GABA**. The direct inhibition is sensitive to strychnine poisoning.

Indirect inhibition

In excitatory synapse, repeated stimulation leads to refractoriness of the postsynaptic neuron. Hence, there will be no response to subsequent stimuli. This is called indirect inhibition.

Presynaptic inhibition

In the excitatory synapse, the presynaptic neuron receives an inhibitory interneuron at the terminal or anywhere in the axon, forming axoaxonic synaptic connection (Fig. 4.13). The inhibitory interneuron releases **GABA**. It reduces the neurotransmitter release from the presynaptic terminal and hence the inhibition of postsynaptic neuron. Presynaptic inhibition persists for a longer duration and hence it is not sensitive to strychnine poisoning.

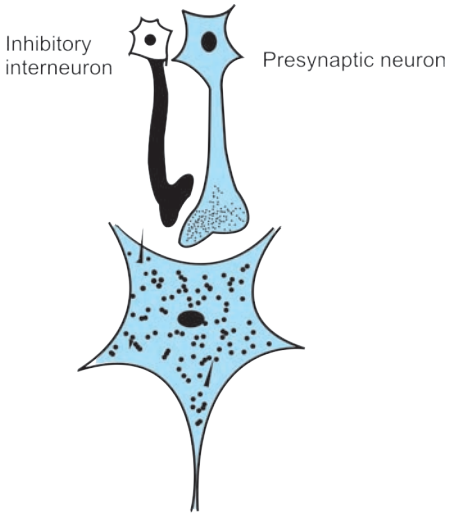


Fig. 4.13: Presynaptic inhibition

Renshaw cell inhibition

It is a feed back inhibition of ventral motor neuron of the spinal cord. A collateral from the ventral nerve ends on an inhibitory interneuron called Renshaw cell and excites it (Fig. 4.14). This inturn inhibits the ventral motor neuron by releasing **glycine.**

There are also other types of inhibitions in the CNS such as *feed forward inhibition (cerebellum), reciprocal innervation in the flexor reflex and autogenic inhibition in inverse stretch reflex.*

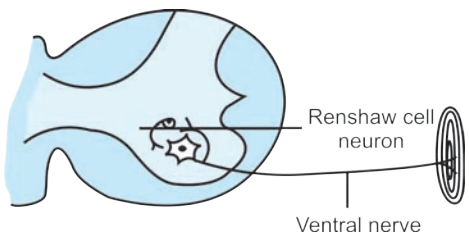


Fig. 4.14: Renshaw cell inhibition in spinal cord

Synaptic plasticity

The repeated stimulation of neurons gives rise to morphological and functional changes in the synapse known as **plasticity**. It includes **presynaptic facilitation, post tetanic potentiation, long term potentiation, habituation and sensitization.**

In **presynaptic facilitation**, there is an excitatory interneuron ending on the presynaptic neuron. The release of serotonin from the interneuron causes increased Ca^{++} influx into the presynaptic terminal to result in greater release of neurotransmitter. This leads to increased excitability of postsynaptic neuron.

In **post tetanic potentiation,** the tetanic stimulation of presynaptic neuron increases the excitability of postsynaptic neuron after a pause, following the tetanic stimulation. It is due to the accumulation of Ca^{++} into the presynaptic neuron.

Habituation occurs when certain stimuli, which are not significant, applied repeatedly, fails to give response in the neurons.

In **long term potentiation (LTP),** the mechanism is similar to the post tetanic potentiation, but changes occur in the postsynaptic neuron. Both LTP and post tetanic potentiation are involved in learning and memory processes.

Long term depression (LTD) is opposite to that of LTP and occurs in all the regions of the brain. It occurs when there is weak stimulation of presynaptic neurons, which reduces Ca^{++} influx into the presynaptic terminal. LTD is said to be associated with learning process similar to LTP.

NEUROTRANSMITTERS

A substance is called a neurotransmitter, when it fulfills the following criteria.

The substance should be synthesized and released from the presynaptic neuron when stimulated by an appropriate stimulus.

Microapplication of the substance to the postsynaptic membrane should mimic the effects of stimulation of presynaptic neuron.

Pharmacological agents should be able to modify the effects of presynaptic neuron stimulation.

Types of neurotransmitters

Neurotransmitters have been broadly divided into the following types based on their chemical structure. They include amines, amino acids, polypeptides, purines, pyrimidine and gases (CO and NO).

Acetylcholine

It is synthesised from the reaction involving choline and acetate in the presence of enzyme choline acetyltransferase. Acetylcholine is the neurotransmitter, which is secreted from all the motor neurons that come out of the spinal cord (Fig. 4.15). It is released in the ganglion of the autonomic nervous system and at the post ganglionic nerve endings of parasympathetic neurons. In some of the sympathetic post ganglionic neurons also (sweat glands, skeletal muscle) acetylcholine is secreted. The transmitter at the neuromuscular junction is acetylcholine. In addition to these, it also forms the transmitter

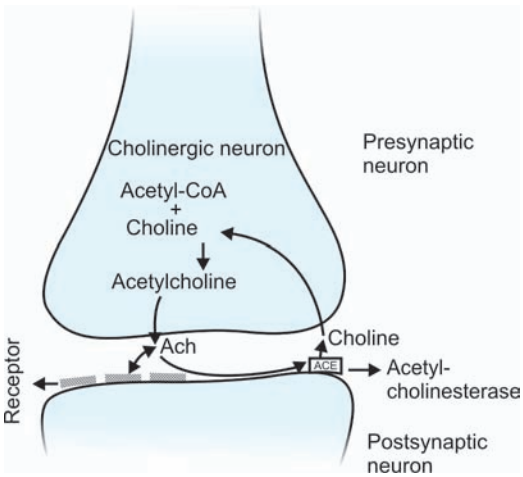


Fig. 4.15: Events at cholinergic synaptic transmission

in a number of pathways in the central nervous system. The inactivation of acetylcholine is catalyzed by acetylcholinesterase.

Acetylcholine receptors

There are two types of receptors present for acetylcholine known as **muscarinic** and **nicotinic**. The actions of acetylcholine on smooth muscle glands mimic the actions of muscarinic alkaloid and the actions are blocked by atropine. The actions on the autonomic ganglion mimic the effects of nicotine which are not blocked by atropine. These nicotinic receptors are also present in the neurons of CNS and in neuromuscular junction. In the brain, both nicotinic and muscarinic receptors for acetylcholine are present.

Nicotinic receptors

Nicotinic acetylcholine receptors show the presence of 5 subunits around a channel in the cell membrane. There are two identical αsubunits to which acetylcholine binds, resulting in the configurational change of the receptor. This leads to the opening of Na^+ channel to produce depolarization. This type of receptor is seen in the ganglia and neuronal membrane. In the ganglia and neuromuscular junction, the receptor is arranged in a symmetrical fashion, whereas in the neuron, it is arranged in a pentagonal array. The nicotinic receptor at the neuromuscular junction can be blocked by the snake venom α-bangarotoxin and the nicotinic receptors present in the brain show no such blockade.

Muscarinic receptors

Muscarinic acetylcholine receptors are of 5 types encoded by five different genes. These receptors are coupled to G proteins or K^+ channels or phospholipase C. In the brain M_1 receptors are more and M_2 is concentrated in the heart. The pancreatic islets and acinar tissue contain M_4 type. M_3 and M_4 receptors are present in the smooth muscle.

Biogenic amines

It includes epinephrine, norepinephrine, dopamine, serotonin and histamine. Epinephrine, norepinephrine and dopamine are the catecholamines, which show a common biosynthetic pathway from the amino acid tyrosine. Norepinephrine is the neurotransmitter in most of the postganglionic sympathetic neurons. It is also found in the neurons of the brain. Epinephrine secreting neurons are also present in the brain. Dopamine secreting neurons are found more in the mid brain and striatum.

Catecholamines are inactivated either by reuptake into the presynaptic neuron or enzymatically metabolized by **MAO** (monoamine oxidase) and **COMT** (catechol-ortho-methytransferase).

Reuptake of neurotransmitters into the presynaptic terminal can cause cessation of action of the neurotransmitter. Example of neurotransmitters showing reuptake include, monoamines, glutamate, GABA, glycine, taurine, proline and choline. Agents, which inhibit neurotransmitter reuptake can prolong the action of neurotransmitter. The therapeutic administration of antidepressant drugs produce their effect by inhibiting the reuptake of monoamines.

Adrenergic receptors

Catecholamines act on the adrenergic receptors namely αand β. Epinephrine and norepinephrine act both on α and β receptors. Epinephrine has greater affinity for β adrenergic receptors while norepinephrine has greater affinity for α adrenergic receptors. With regard to dopamine, five different types of receptors have been identified. D_1 and D_5 receptors increase cyclic AMP, while D_2, D_3 and D_4 receptors decrease cyclic AMP when mediating the effects. D_4 receptor is believed to be involved in the pathogenesis of schizophrenia, as seen from its increased affinity to the antipsychotic drug clozapine.

Serotonin (5 hydroxy tryptamine)

Serotonergic neurons are abundant in the brain stem. It is synthesized from the amino acid tryptophan. The transmitter is inactivated either by reuptake or enzymatic breakdown by MAO. The metabolite that is formed is 5-HIAA, which is excreted in the urine. Serotonin is also secreted from platelets and enterochromaffin cells of the intestine.

There are 7 types of serotonin receptors, which have been identified. 5-HT_2 and 5-HT_3 receptor types are involved in brain functions.

Histamine

Histaminergic neurons are present in the hypothalamus and pituitary gland. Histamine is synthesized by the decarboxylation of amino acid histidine. There are three types of receptors present namely, H_1, H_2 and H_3. H_1 receptors activate phospholipase C and H_2 receptors increase intracellular cyclic AMP.

Amino acid transmitters

Glutamate and **aspartate** are the most commonly distributed **excitatory neurotransmitters** in the brain. Glutamate receptors include **metabotropic** and **ionotropic** types.

Metabotropic receptors are G-protein coupled serpentine receptors present in the presynaptic and postsynaptic membranes in the brain. These receptors are said to be involved in synaptic plasticity especially in the hippocampus and cerebellum.

Ionotropic receptors are ligand gated ion channels and include three types:

1. Kainate
2. AMPA (α Amino-3-hydroxy-5- methylisoxazole-4- propionate)
3. NMDA (N- methyl- D-aspartate).

Kainate receptors allows movement of Na^+ and K^+. They are found presynaptically on GABA secreting neurons. They are also present postsynaptically in many regions of the brain.

AMPA receptors causes movement of Na^+ and Ca^{++}. They are present in the same neurons which has NMDA receptors.

NMDA receptors permits entry of Ca^{++}. Glutamate and aspartate act on NMDA receptors. Both the amino acids are excitatory transmitters in the brain and spinal cord. The excitation of the brain is mostly due to the release of these amino acids. Glutamate from the interstitial fluid is taken up by the glutaminergic neurons and stored in the synaptic vesicles. NMDA receptors require activation by glycine to respond to glutamate. The activation of NMDA opens up the Ca^{++} channels but entry of the ion is blocked by Mg^{++} ion. The block is removed when partial depolarization of the neuron occurs which allows Ca^{++} influx. Glutamate can bind to both AMPA and NMDA receptors.

In hippocampus, the NMDA receptors are more and blockade of these receptors affects memory and learning by preventing long term potentiation.

Interestingly, the same neurons is also believed to destroy the cell bodies of neurons due to intense stimulation. That is why the agents that act on the NMDA receptors are also called ***excitotoxins.*** The excitotoxin effect of glutamate is implicated in the brain cell death after a stroke.

Inhibitory transmitters

Glycine

It is released from the spinal interneurons and brain stem. It also acts on the NMDA receptors in the brain and functions as excitatory transmitter in this region. The inhibitory effect of glycine in the spinal cord and brain stem is by direct inhibition, which causes opening of Cl^- channel and increased conductance of the ion. The action is antagonized by strychnine, which results in convulsions.

GABA

It is produced from glutamate by decarboxylation. The enzyme, which catalyses is GAD (glutamic acid decarboxylase). GABA is secreted from the neurons present in the brain and retina. GABA is also secreted in the presynaptic inhibition. The transmitter is inactivated by the enzyme GABA transaminase and also by the active reuptake in

the presynaptic neurons. The receptors for GABA are divided into three types called $GABA_A$, $GABA_B$ and $GABA_C$, which belong to metabotropic and ionotropic categories. $GABA_B$ receptor mediates the effect, through G protein and increases K^+ conductance. $GABA_A$ receptor causes increased conductance of Cl^- ion (Fig. 4.16). $GABA_C$ receptors are found in the retina in adults. Benzodiazapine group of drugs produces their antianxiety effects by acting through $GABA_A$ receptor. The actions of barbiturate, alcohol, anesthetics are also mediated by $GABA_A$ receptor. The actions of $GABA_A$ receptor are blocked by bicuculline while picrotoxin blocks $GABA_B$ receptor.

Peptides

There are approximately 25 neuropeptides that have been identified so far. Neuropeptides are released in low concentrations and the effect lasts longer on the target neurons. These substances also act as hormones and neuromodulators. In some places, neuro peptides coexist with neurotransmitters and their secretion occurs depending on the stimulus. Opioid peptides, purine and nucleotides act as neurotransmitters as well as neuromodulators. Neuropeptides are synthesized in the cell body and transported through the axonal transport to the axonal terminal. This is in contrast to other neurotransmitters which are synthesized at the nerve terminal itself.

Opioid peptides

These peptides bind to the opioid receptors. The effects produced by them are analgesia and behavioral changes. There are three types of opioid peptides namely **enkephalin, endorphin** and **dynorphin.** Enkephalin is a penta peptide, ie, Met-enkephalin and Leu-enkephalin. The other two types are longer peptides.

β endorphins are synthesized from a large precursor molecule **pro-opiomelanocortin,** secreted from pituitary and adrenal medulla. This peptide molecule contains β endorphin, enkephalin, ACTH, MSH, etc. There is a separate enkephalin and endorphin secreting neurons exist in the brain. Opioid receptors are of three types namely μ, κ, δ. The μ receptors when activated increase the K^+ conductance and hyperpolarise the neurons. Stimulation of k and δ receptors close the Ca^{++} channels. β endorphin, which is endogenously produced binds to the μ receptors. Dynorphins are the ligands for k receptors.

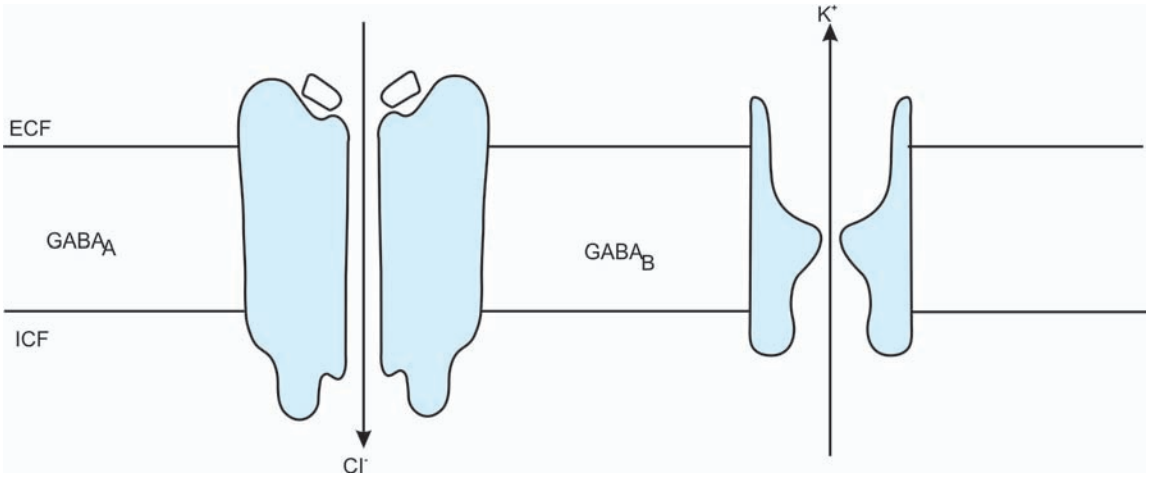


Fig. 4.16: $GABA_A$ and $GABA_B$ receptors
$GABA_A$ Increases conductance for Cl^-
$GABA_B$ Increases conductance for K^+

Other peptides

Substance P

It contains 11 amino acid residues. It is present in the intestine, peripheral nerves and brain. Substance P belongs to a group of neuropeptides called tachykinins. The release of this transmitter is seen in the synapse between primary sensory afferent and interneuron in the dorsal horn of the spinal cord and is responsible for pain stimulation. Enkephalins inhibit the release of substance P from these neurons and inhibit pain. In the intestine the release of substance P is involved in peristalsis.

VIP

It is distributed in the GI tract and CNS. It is an inhibitory transmitter in the vascular and non-vascular smooth muscle. It is an excitatory transmitter in the glandular epithelial cells.

VIP coexists with acetylcholine at the parasympathetic postganglionic terminals that innervate the pancreatic acinar cells. In the brain, VIP is an excitatory transmitter. Secretin, glucagon, CCK and GIP are also found in the neurons of the CNS. Their role in the nervous system is not known.

NO (Nitric oxide)

Nitric oxide gas, which is released by the endothelium of blood vessels, is also secreted as a neurotransmitter in the regions of the brain concerned with long term behavior and memory. It is synthesized and secreted from the presynaptic terminals instantly whenever required. The NO gas diffuses into the postsynaptic membrane and alters neuronal excitability. It mediates its action through **cGMP.**

Other functions of NO

Nitric oxide is also involved besides brain functions and relaxation of smooth muscle of vascular endothelium, in cytotoxic activity of macrophages (inflammatory reaction), development of atherosclerosis, angiogenesis, penile erection and relaxation of lower esophageal sphincter.

SENSORY SYSTEM

Sensory receptors

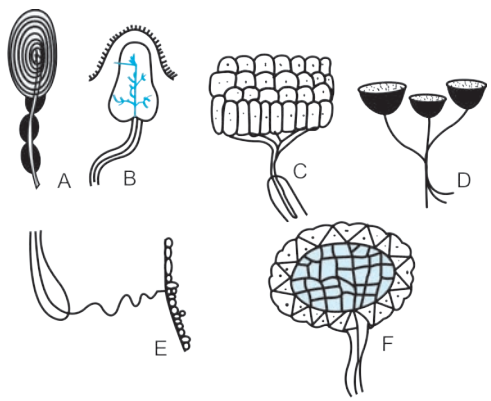
Receptor is a modified sensory end organ which functions as a transducer. It transforms the stimulus energy into an electrical energy which is propagated as an action potential.

Types of sensory receptors

Depending upon the type of stimulus energy acting on the receptors, they are classified as **telereceptors** (rods and cones), **exteroceptors** (cutaneous receptors), **interoceptors** (visceral receptors) and proprioceptors (muscle spindle, tendon and joints).

Cutaneous receptors

These receptors are present in the skin and subcutaneous tissue (Fig. 4.17). They convey the various types of sensations such as touch, pressure, pain, warm and cold to the CNS. There are three types of cutaneous receptors present namely, **mechanoreceptors** (*touch and pressure*), **thermoreceptors** *(warm and cold)* and **nociceptors** (*pain*).



Figs 4.17A to F: Cutaneous sensory receptors

A: Pacinian corpuscle; B: Meissner corpuscle
C: Free nerve ending; D: Krause end bulb
E: Ruffini end organ; F: Merkel disc

Histological study of cutaneous receptors has shown that these receptors are either expanded endings or encapsulated endings of sensory afferents. Ruffini end organ, Merkel disc (expanded endings), Meissner's corpuscle, Pacinian corpuscle' Krause's end bulb (encapulated endings) are examples of mechanoreceptors. They respond to touch and pressure. The bending of hairs in the skin forms the best stimulus for the tactile sensation. The thermoreceptors and nociceptors are bare nerve endings.

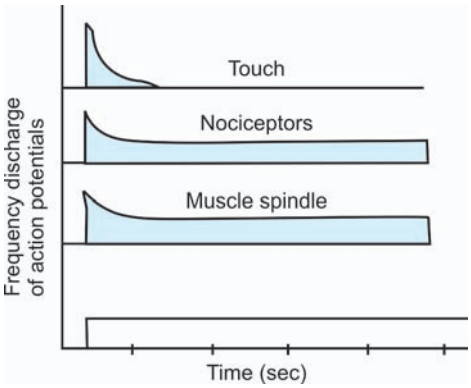


Fig. 4.18: Adaptation of sensory receptors

Properties of receptors

Specificity

Receptors are specific to a particular stimulus energy. Each sensory modality is carried by a specific pathway and there is a topographical representation of the body in the sensory cortex. There are discrete pathways, which project sensations to the sensory cortex. A particular sensation is felt due to the activity of a specific receptor, which sends impulses in a specific pathway to the cortex. This forms the doctrine of **Muller's law of specific nerve energy**, which is responsible for the recognition of each type of sensory modality.

Adequate stimulus

The stimulus energy to which the receptor is most sensitive is called the adequate stimulus.

Adaptation (Fig. 4.18)

Sensory receptors show stimulus response in two ways. Rapidly adapting receptors (**phasic receptors**) like touch, pressure, show decline in the frequency of action potentials over time, with the rate of stimulus application remaining same. But, when there is a change in the rate of stimulus application, the discharge rate of action potentials is increased. In this way, the phasic receptors encode information about the rate of change of stimulus application.

Tonic receptors such as muscle spindle, nociceptors, thermoreceptors, do not show adaptation. The discharge of impulses (firing of action potentials) does not show decline over time, when the stimulus is continuously applied. The tonic receptors show functional significance, as for example, the information carried by the muscle spindle helps to maintain muscle tone and posture. Likewise, the nociceptors and thermoreceptors convey about the tissue damage occurring as a result of their stimulation.

Sensory coding

The receptor potential shows a linear relationship to the stimulus intensity. After attaining the action potential level, further increase in the intensity of stimulus leads to the increased frequency of firing of action potentials (Fig. 4.19). The rate of firing of action potentials from the sensory nerve will correspond to the intensity of stimuli.

The sensory neurons are able to encode stimuli based on the type of receptor that is stimulated, the nature of response shown by the receptor to the stimulus, and information processed in the sensory pathway. The information that is encoded by the sensory neurons will include the following.

Sensory modality, spatial location, threshold, intensity, frequency and duration.

Sensory modality

A particular pathway carrying a specific stimulus will form a **labeled line** and the particular sensation which is perceived will be the sensory modality.

Spatial location

Activation of a particular group of sensory units containing the receptive fields is responsible for spatial location of sensory stimulus.

Threshold

If a stimulus has to be perceived as a sensation, the intensity of stimulus should be at threshold level or more than threshold.

Intensity of stimulus

The intensity of stimuli is encoded as the mean frequency of discharge of action potentials from sensory neurons. In addition, the number of sensory receptors that are activated will also contribute to the encoding of stimulus intensity.

Generator potential (Receptor potential)

It is an electrical potential which is developed, when a receptor is stimulated with a specific stimulus energy. The generator potential has been studied in detail in a pacinian corpuscle (Fig. 4.20). The sensory afferent ends naked within the receptor. The myelination begins within the receptor and the first of node of Ranvier starts from the receptor. The application of sustained touch gives pressure stimulus, which causes stimulation of Pacinian corpuscle. At first, there is a localized depolarization caused by the Na^+ entry. The membrane potential falls by 10 to 15 mv. This nonpropagatory local potential is similar to the EPSP. As the intensity of stimulus is increased, the magnitude of generator potential is also increased. The attainment of threshold level of firing leads to the action potential formation. The naked unmyelinated sensory nerve ending within the receptor is the region where generator potential is produced. There is a current sink between this region and the Ist node of Ranvier, leading to the development of action potential in the latter.

Intensity discrimination

Information regarding the intensity of stimulus is transmitted due to the rate of change of frequency of action potentials and the number of receptors that are activated. The intensity discrimination of a sensory stimulus is related to the magnitude of stimulus which is explained by the power function **(Steven's Power law function).**

Earlier, the **Weber Fechner law** has been applied for the intensity discrimination, which stated that the magnitude of sensation felt is proportional to the log of intensity of stimulus. The power function law is expressed as

$$\mathbf{V = k \times I^n \text{ or } R = kS^A}$$

R is the sensation felt and k and A are constants. S = intensity of stimulus.

The power function states that as the stimulus intensity is increased, the firing frequency is not linearly increased. However, it will be related to the intensity of the stimulus by a power function.

Sensory unit

A single sensory axon with its branches forms the sensory unit. When a stimulus is applied, a response is produced from the region of the area that is stimulated. This is called **receptive field**. As the stimulus intensity is increased, more and more sensory units are activated and this is called **recruitment of sensory units.** It should be remembered that a sensory unit of one type of receptor could overlap with the sensory units of other types of receptors in the skin. This overlapping of sensory units from other receptors will also be stimulated when the intensity of stimulus is increased.

Physiology of cutaneous receptors

Mechanoreceptors

The mechanoreceptors are stimulated by touch, and pressure stimuli and they are carried by the

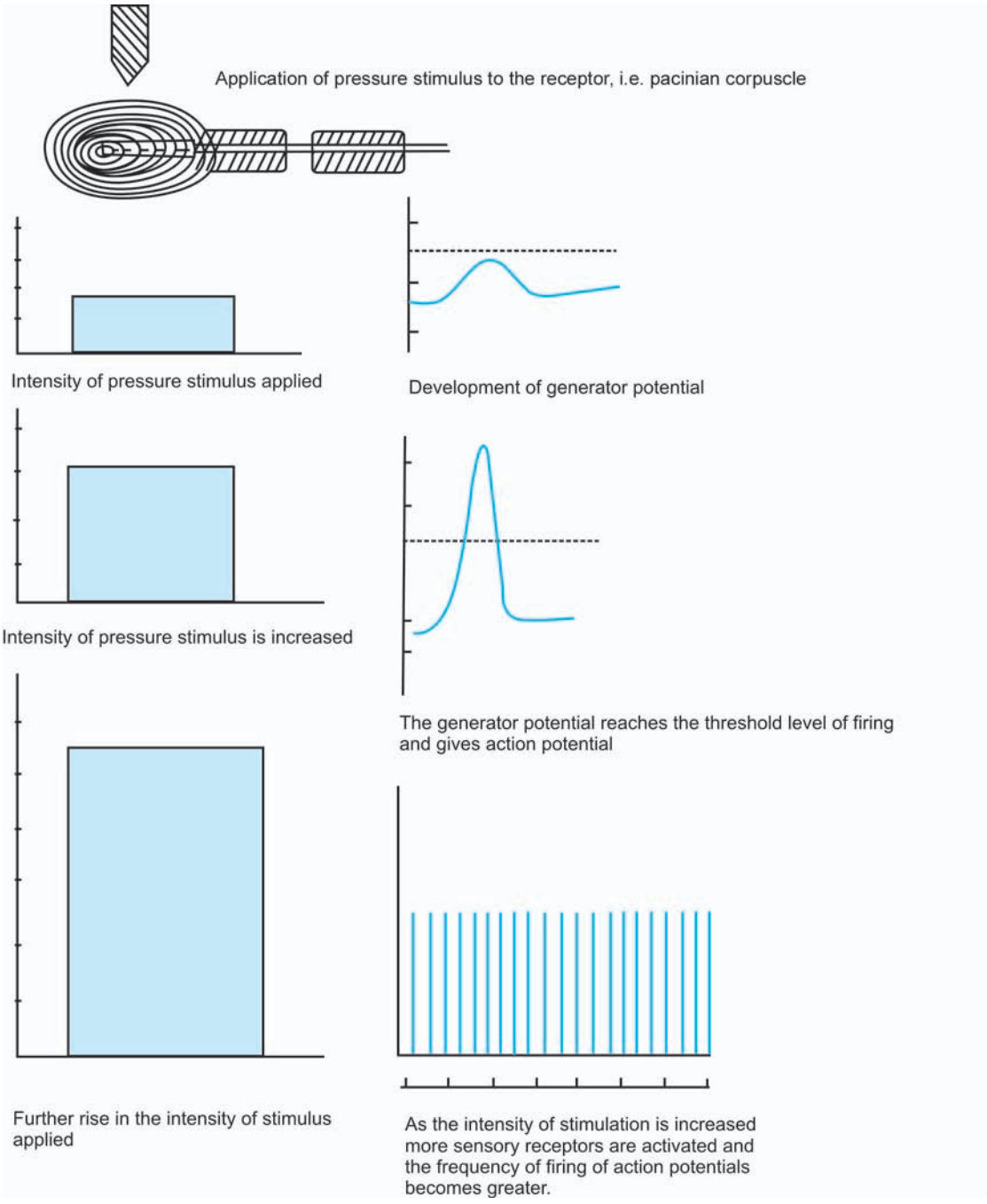


Fig. 4.19: Sensory coding of intensity of stimulus

Aβ and C type of fibers. Although, the stimuli from the mechanoreceptors are normally carried by the myelinated Aβ fibers, there are also slowly adapting mechanoreceptors, activated by the stimuli such as stroking of the skin (slowly moving mechanical stimuli), that are carried by the C fibers. Their existence has been identified in humans recently. The receptors for touch are more numerous in fingertips, lips and less in the trunk. These receptors include both rapidly adapting Meissner's corpuscle, pacinian corpuscles and more slowly adapting Merkel's disc, Ruffini's end organ. The receptors around the hair follicles are stimulated by the fine touch stimulation. The hair follicle acts as a lever and the bending of the hair forms the best stimulus.

Thermoreceptors

The thermoreceptors are sensitive to cold and warm. They are free nerve endings present in the skin. The cold sensation is carried by the Aδ and C, whereas, the warm is carried by only C fibers. Both the types of (cold and warm) receptors are stimulated when the body temperature is between 31 to 38° C. This range of temperature is called **neutral zone,** where adaptation is present (Fig. 4.21). Rise in body temperature above 38° C stimulates only the warm receptors while the cold receptors remain silent. This will be seen until the temperature reaches 45° C. Temperature beyond this will not stimulate the thermoreceptors, but stimulates the nociceptors which give pain sensation. The fall in body temperature below 30° C stimulates only the cold receptors and the warm receptors remain inactive. Temperature below 10° C will stimulate the nociceptors and give pain sensation.

Nociceptors

Nociceptors are receptors which give the sensation of pain. They are stimulated, when the tissue damage occurs, caused by the mechanical, chemical and thermal stimuli. Based on the type of afferents that carry pain impulses, the nociceptors are classified as **Aδ mechanical nociceptors** and **C polynodal** nociceptors. The Aδ mechanical nociceptors stimulation is carried by the myelinated Aδ fibers while the C polynodal nociceptor stimulation is carried by the unmyelinated C fibers. The polynodal **nociceptors** respond to mechanical, thermal and chemical noxious stimuli, whereas, the Aδ mechanical nociceptors respond only to mechanical noxious stimuli.

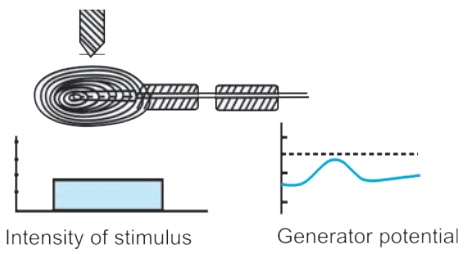


Fig. 4.20: Development of generator potential in a pacinian corpuscle to the pressure stimulus. Generator potential is a receptor potential and refers to the local depolarization. They are nonpropagatory. These potentials can summate and reach the threshold level of firing to form the action potential

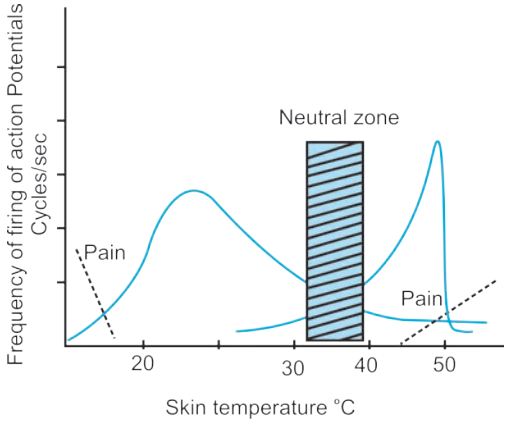


Fig. 4.21: Cold and warm receptor activity at different skin temperatures

Proprioceptors

Proprioceptors are the receptors present in muscle spindles, tendon and joints. The receptor types that are included here, are the muscle spindles and Golgi tendon organs. They are involved in proprioception and control of motor action. The mechanoreceptors namely, pacinian corpuscle and Ruffini's end organ are also present in the muscles and joints, conveying the mechanical stimulation such as pressure, vibration and joint movement. The nociceptors which are present in these regions respond to pressure, extreme movements of joints and chemical stimuli from muscle ischemia.

SENSORY PATHWAYS

Organization

The pathways carrying sensory modalities will have serially arranged neurons such as **first** order, **second** order, **third** order and **fourth** order, which transmit the sensory information. There are three levels of tracts, which are formed between these four orders of neurons.

I Order neuron

The primary afferent with the receptor at its ending form the I order neuron. It is responsible for responding to the specific stimulus and sending the encoded information to the spinal cord.

II Order neuron

It is present either in the spinal cord or in the brain stem. It receives encoded sensory information from the I order neuron and transmits them to the thalamus. While ascending, the II order neuron crosses to the opposite side and end in the thalamus.

III Order neuron

It is present in the thalamus sensory relay neuclei. From here, specific projections go to the sensory cortex which contains the IV order neurons. The processing of information which begins at the II and III order neurons reaches the peak in the IV order neurons, present in the somesthetic area of cerebral cortex. This facilitates the perception of sensations at the conscious level.

Dorsal column

The sensations of **fine touch, localization, two point discrimination, vibration, pressure, kinesthetic and stereognosis** are carried by the fasciculus gracilis and fasciculus cuneatus, which occupy the dorsal column of the spinal cord (Fig. 4.22). From lower half of the body, the impulses are carried by the fasciculus gracilis and from the upper half of the body, the impulses travel through the fasciculus cuneatus. The dorsal column is an uncrossed tract in the spinal cord and form the I order pathway. The sensations of fine touch, pressure, vibration, etc. from the

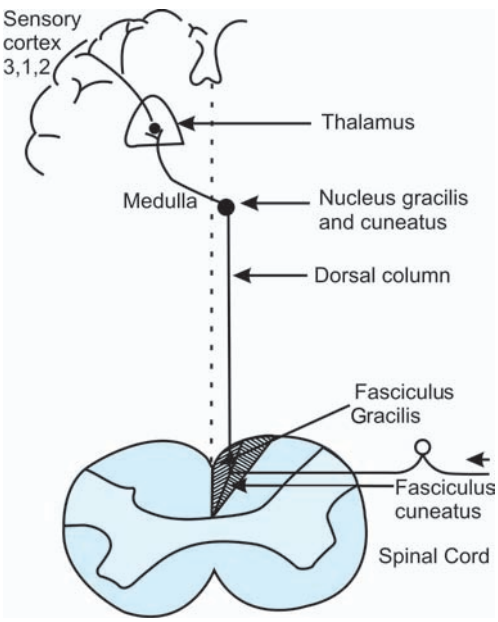


Fig. 4.22: Dorsal column (lemniscal pathway). Fine touch, two point discrimination, vibration, pressure, joint and position sense and stereognosis are carried by this tract

face region travel through the trigeminal nerve and enter the brain stem in the medulla. The dorsal column on entering the medulla ends in nucleus gracilis and nucleus cuneatus. The II order neurons decussate immediately to the opposite side. The crossed internal arcuate fibers form the medial lemniscus and together with spinal and trigeminal lemniscus, enter the thalamus. The medial and spinal white columns end in the **postero ventro lateral nucleus**, while, the trigeminal lemniscus end in **postero ventral medial nucleus** of thalamus. The third order pathway from these thalamic nuclei projects to the sensory cortex and end in areas 3, 1 and 2.

Anterolateral system

Pathway of pain, temperature and crude touch

It comprises lateral and ventral spinothalamic tracts. It carries **crude touch, pain and temperature**. It is a crossed tract in the spinal cord (Fig. 4.23). The fibers carrying these sensations from the periphery travel in the lateral division of the dorsal nerve and end in the dorsal horn laminae I-V in the spinal cord. The second order pathway is formed from here after crossing over to the opposite side of the spinal cord. The crossed fibers travel in the anterior and lateral lemnisci and together constitute the anterolateral system. In the medulla, the second order pathway continues as the spinal lemniscus. It projects to the thalamus and end in postero venterolateral nucleus. In the brain stem, projections from the spinal lemniscus are also present to the areas like reticular formation, hypothalamus and limbic system. Third order pathway from the thalamus goes to the somesthetic area of the cerebral cortex.

The sensations of pain, temperature, and touch from the face region are carried by the trigeminal nerve. The sensory division of trigeminal nerve enters the brainstem and together with the spinal and medial lemnisci goes to the thalamus. In the thalamus the trigeminal afferents end in the ventero postero medial nucleus and not postero ventero lateral (PVL) nucleus. The third order afferents reach the somesthetic area of the cerebral cortex.

Touch pathway (Figs 4.22 and 4.23)

Touch has two **pathways** in the spinal cord. The fine touch is carried by the uncrossed dorsal column, whereas, the crude touch is carried by the anterolateral system which is a crossed tract. In the hemisection of the spinal cord, the touch is not totally abolished as there are two pathways. Hence it is blunted in such conditions.

The spinothalamic tract neurons which carry touch, temperature and noxious stimuli have different types of neurons responding to different kind of stimuli as mentioned above. The neuron, which responds to the noxious stimulus, also responds to the tactile stimulus but the latter is ignored at the cortical level. These neurons

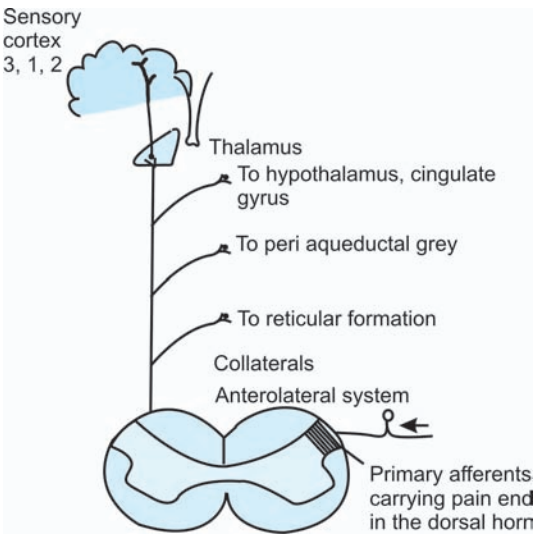


Fig. 4.23: Pathway of pain. The primary afferents, which carry pain end in the dorsal horn. The fibers (Aδ) which carry fast pain release glutamate and the fibers (C) carrying slow pain release substance P at the nerve endings in the dorsal horn. These transmitters are responsible for the activation of pain pathways. Anterolateral system also carries crude touch and temperature sensations

which are activated by both noxious and mechanical stimuli are called **wide dynamic range neurons.**

Spinocerebellar tracts

The proprioceptive impulses which convey muscle sense, joint sense and movement are carried by the dorsal and ventral spinocerebellar tracts. They are uncrossed in the spinal cord. The proprioceptive sensations carried by them do not reach the conscious level of perception. The pathway is considered significant, as it is involved in the control of motor activity which is regulated by the cerebellum.

PHYSIOLOGY OF PAIN

The interpretation of pain involves physical, psychological and physiological factors. The pain sensation is a protective mechanism which informs the organism about the damage occurring in the tissues. The receptors for pain are nociceptors, which are the free nerve endings. They are excited by the noxious stimuli. The release of chemical substance like histamine at the tissues causes the excitation of nociceptors, which results in the generation of action potentials in the nerve fibers that carry pain. Pain impulses are carried by two types of fibers, depending upon the nature of stimuli. Pain arising due to mechanical stimuli is carried by the myelinated Aδ fibers, while the pain resulting from chemical and thermal stimuli is carried by the unmyelinated C fiber.

Pain pathway

The free nerve endings carrying pain stimuli enter the dorsal nerve root ganglia in spinal nerves and cranial nerve ganglia in cranial nerves. It has been shown that these afferents secrete **substance P** for slow pain and **glutamate** for fast pain at the first synapse in the spinal cord. These chemical mediators are responsible for the stimulation of the pain pathways. The first order neuron enters dorsal horn of the spinal cord to end in the laminae I, II and V. The II and III laminae form the gelatinosa of Rolando (Fig. 4.24). There are descending pathways from the raphe nucleus in the brain stem which end presynaptically on the afferents carrying pain in the dorsal horn. The larger myelinated medial division of the dorsal nerve, which carry tactile, pressure, vibration, etc. also sends collaterals and end presynaptically on the afferents carrying pain in the dorsal horn. These presynaptic endings on the afferents carrying pain help in the inhibition of pain transmission.

The specific ascending pathway carrying pain in the spinal cord is the anterolateral system. The sensation of pain from the face is carried by the trigeminal nerve which enters the brain stem. The anterolateral and trigeminal pathways carrying pain impulses send projections to the brain stem, midline intralaminar nuclei of the thalamus, and reticular formation. There are also projections going to the hypothalamus and cingulate gyrus from these areas.

The second order neuron carrying pain impulses end in the thalamus ventral posterior lateral nucleus(VPL)and from the face, the trigeminal afferents carry the pain and end in the ventral posterior medial nucleus (VPM). The third order neurons from the thalamus go to the sensory cortex S I and S II. The lower center for pain is the thalamus. The interpretation of pain, emotional and affective reactions to pain are due to the activity of S I, S II and cingulate gyrus.

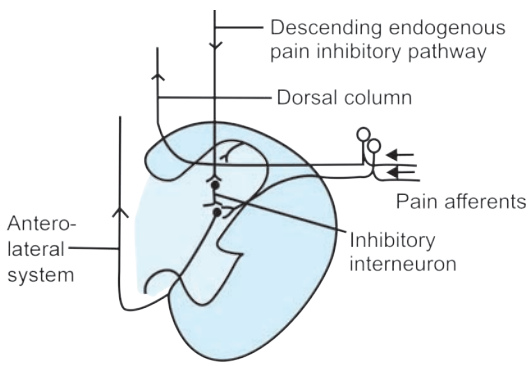


Fig. 4.24: Gate control mechanism in the dorsal horn showing inhibition of pain transmission

Types of pain

Fast and slow pain

The application of noxious stimuli in the skin gives first a sharp, pricking pain which is better localized. This is carried by the Aδ fibers and called **fast pain**. This is followed by a dull, poorly localized pain which is carried by C type fibers. This is called **slow pain.**

Deep pain

Pain from deeper structures in the periphery like periosteum, bones, tendons and joints give pain, which are dull and diffuse in their localization. It is also accompanied by autonomic effects such as nausea, vomiting, sweating and fall in blood pressure.

Muscle pain

The pain from muscles is due to the ischemia caused by the reduced blood flow as occurs in severe muscular work. The pain in **intermittent claudication** is due to the ischemia of the limb and accumulation of K^+. In such conditions, the removal of metabolites by the blood circulation relieves the pain.

Headache

The causes of headache involve many factors. The irritation of meninges, dural sheath, rise in intracranial pressure, sinuses inflammation, etc can cause headache. The most important is the vascular effect, where the distension of cerebral blood vessels results in headache. The headache in migraine is due to this factor.

Visceral pain

The distension of viscera, inflammation or ischaemia are the major factors that cause visceral pain. The autonomic fibers comprising both sympathetic and parasympathetic nerves, carry pain impulses from the viscera and enter the dorsal root ganglion. The pathway in the spinal cord is through the anterolateral system as in somatic pain.

Referred pain

It is often experienced that visceral pain is referred to the periphery. The ischaemic pain of the heart is referred to the left arm and left shoulder. The pain due to the inflammation of diaphragm is referred to the tip of shoulder. The pain due to the inflammation of appendix is referred to umbilicus.These are some of the examples of referred pain. There are several theories to explain the mechanism of referred pain.

In **dermatomal theory**, the viscera and the somatic structure arise from a common dermatome during embryonic development. Later in life, when pain comes from the viscera, it is referred to the periphery which has the same dermatomal origin during embryonic development.

In **facilitation mechanism**, the arrival of pain impulses from the viscera facilitates the sub threshold stimuli in the spinal cord coming from the periphery (Fig. 4.25).

In **convergence mechanism**, the arrival of pain impulses from the viscera and periphery in the spinal cord, results in the pain being referred to the periphery, as the spinal cord normally used to receive pain impulses from the periphery (Fig. 4.26).

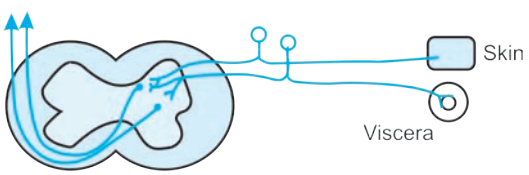


Fig. 4.25: Facilitation of visceral pain in the spinal cord

The pain impulses coming from viscera facilitate the sensory afferents coming from the periphery

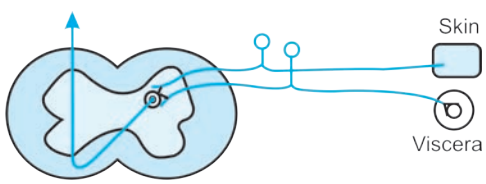


Fig. 4.26: Convergence mechanism of referred pain

The visceral fibers carrying pain and somatic fibers carrying touch and pressure converge in the spinal cord. This leads to the projection of visceral pain to the periphery, as the spinal cord is normally used to receive pain impulses from it

Phantom pain

It is the pain sensation experienced from the limb, which has been amputated surgically. The reason for this phantom pain is the fibers from the proximal stump send impulses due to irritation. Recently, it has been shown that the cortical representation of the amputated limb remains active, receiving sensory impulses, as a result of the new connections from other areas of the sensory cortex like face region. This is a kind of plasticity in the sensory cortex, which is believed to be responsible for the phantom pain.

The sensory cortical neurons exhibit plasticity as in other parts of the brain depending on its activation or use. If a digit or arm is amputated, the sensory area representing them will be occupied by neighbouring cortical neurons.

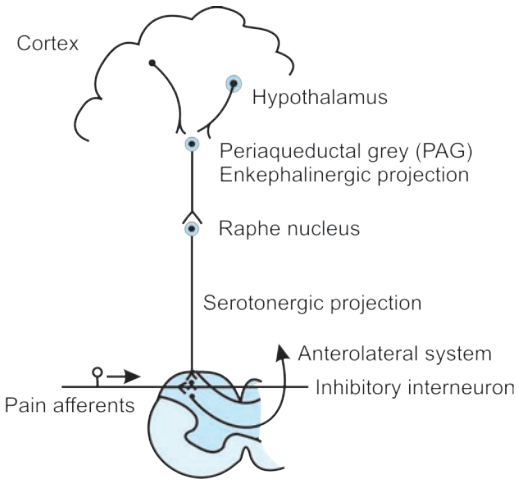


Fig. 4.27: Descending endogenous inhibitory pathway for pain

Pain inhibition

There are endogenous opioid and GABAergic mechanisms, besides the neural control that are involved in the pain inhibition.

Gate control mechanism

The dorsal horn of spinal cord functions as the gate, which modifies the transmission of pain impulses in the spinal cord. There are collaterals from the large myelinated fibers which end presynaptically on the smaller unmyelinated pain carrying fibers coming to the dorsal horn (Fig. 4.24). The transmission of impulses in the large myelinated fibers causes inhibition of transmission in the smaller unmyelinated fibers, which carry pain.

Central opioid system includes enkephalins and endorphins. The periaqueductal grey (PAG) in the brain stem receives projections from the ascending tracts carrying pain. From the PAG, enkephalinergic neurons go to the raphe nucleus, which send serotonergic neurons to the spinal cord. This descending tract ends in the dorsal horn and inhibits the pain transmission in the afferents coming from periphery (Fig. 4.27).

Stress analgesia

Pain inhibition also results from stress exposure. It is probably mediated by the endogenous endorphin secretion, as the effect is blocked by the antagonist naloxone. It is now believed that other inhibitory transmitters such as GABA are also involved in the stress induced analgesia.

Pain inhibition can also be produced by various procedures such as **anterolateral cordotomy**, **prefrontal lobotomy**, **cingulate gyrectomy**, **counter irritants, acupuncture**, **etc.**

Synthetic senses

The combination of two sensations gives synthetic sense like vibration, stereognosis, etc. It is produced by the stimulation of two cutaneous sensory receptors simultaneously. The stimulation of both touch and pressure receptors are responsible for the sense of vibration and stereognosis.

SOMATOTOPIC ORGANIZATION

The somesthetic area in the cortex is present in the postcentral gyrus (Fig. 4.28). It contains the primary sensory area SI and represented by Brodmann's areas 3,1 and 2. In the post central gyrus, the body is represented upside down with the face region being present in the lateral and lower part of SI. The lower limb is represented towards the medial side of the cortex. The somatotopic representation of the contralateral half of the body is distorted, in the sense, that the part of the body, which has dense sensory receptors will have wider representation in the cortex. The somatotopic representation of one half of the body is called **sensory homunculus** (Figs 4.29 and 4.30). There is also sensory processing in another area called SII, which is present in the superior wall of the lateral fissure. The somatotopic representation is also present in SII, but it contains the representation of both sides of the body. The function of SII is also in the processing of sensory stimuli based on the previous experience. The processing of sensory information in SII is in series with SI and not parallel. Projections from SI and SII go to the association cortex in the parietal lobe, frontal lobe and motor cortex. This helps to give proper motor action in response to the sensory stimuli that is perceived by the sensory cortex.

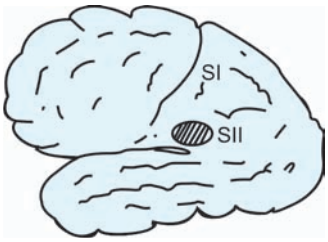


Fig. 4.28: Sensory cortex showing primary and secondary sensory areas. SI is the primary sensory area situated in the postcentral gyrus. SII is the secondary sensory area situated in the superior wall of the lateral sulcus

The processing of sensory stimuli in SI is based on the presence of neurons arranged in columns present in the layer IV of sensory cortex. There are columns specific for each modality of sensation. That is, there will be columns specific for touch, pressure, temperature, etc. Similar columns are present in the visual cortex. Lesion of the sensory cortex causes loss of tactile discrimination, position sense, stereognosis and vibratory sensations.

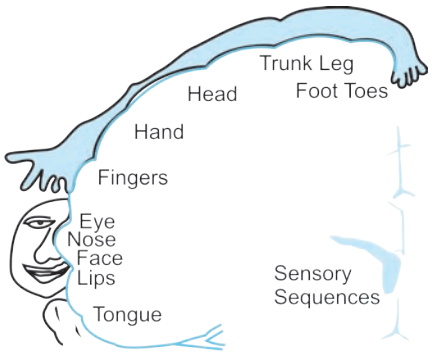


Fig. 4.29: Sensory homunculus

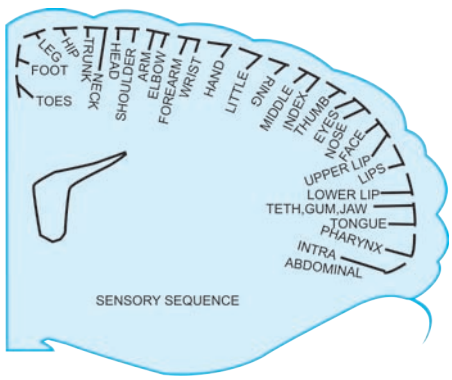


Fig. 4.30: Sensory sequence of contralateral half of the body in the postcentral gyrus. Note the black bars representing the regions of the body, the length of which is proportionate to their involvement in the sensory functions

Effects of sensory cortex lesions

Damage or lesion of primary sensory cortex **(SI)** causes loss of position and discriminative senses. The discriminative sense include tactile localization and two point discrimination. There will also be astereognosis as projection from SI to posterior parietal cortex is affected. The cutaneous sensations such as temperature, crude touch, pain are not affected by cortical lesion. These sensations can be perceived even after the removal of sensory cortex. The sensory processing in SII is affected when lesion of SI occurs. But the lesion of SII does not cause loss of this function in SI.

MOTOR ORGANIZATION AND CONTROL

Motor organization at the spinal level

In humans, motor activity is organized and controlled at various levels in the nervous system. The lowest organization is in the spinal cord and the motor cortex forms the highest level of organization. The descending motor pathways are controlled by basal ganglia and cerebellum. These controls make the motor activity smooth and purposeful.

Reflex action

At the spinal cord level, the automatic motor response to a sensory stimulus, called reflex action can be noticed. The reflex action has neural components called **reflex arc**, which consists of the receptor, the afferent nerve, the center, the efferent nerve and the effector organ (muscle or glands) (Fig. 4.31). This reflex action exists, even if the spinal cord does not receive the higher centers influence, thereby showing that there are reflexes which are integrated at the spinal cord level itself.

Types of reflexes

The clinical examination of reflexes involves superficial, deep and pathological reflexes. Physiologically, reflexes are studied under flexor and stretch reflexes.

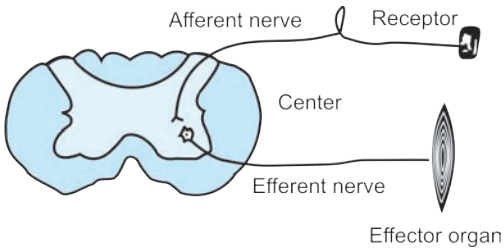


Fig. 4.31: Reflex arc

Flexor reflex

It is a **polysynaptic reflex**. The presence of more than one interneuron between the afferent nerve and the efferent motor neuron is the characteristic feature of polysynaptic pathway. Flexor reflex can be seen when noxious stimulus is applied to the skin. The reflex response shown is the withdrawal of the limb. Hence, flexor reflex is also called withdrawal reflex. The flexor reflex exhibits certain characteristics, which are grouped under properties of reflexes.

Properties of reflexes

Local sign

In flexor reflex the response depends on which part of the limb is stimulated. This is called local sign.

CES and CIS

The presence of polysynaptic pathway and the existence of connections between several interneurons present above and below the spinal cord segments give rise to **central excitatory state** (CES) and **central inhibitory state** (CIS) depending upon the neurotransmitters released at the synapse. In central excitatory state, the excitatory influence overbalances inhibitory influence and in the central inhibitory state, the opposite effect is seen.

After discharge

The background activity produced by CES and CIS, enables the nervous system to respond to stimuli more effectively. It is also observed that

the neuronal circuits established with the interneurons form the **reverberating circuit** and is responsible for the discharge of impulses, even after the stimulus is no longer present (Fig. 4.32). Thus, we see that even though the stimulus is withdrawn, the motor neuron continues to show activity due to the reverberating neural circuit. This repeated firing of motor neurons after the stimulus is withdrawn is called **after discharge**.

Irradiation and recruitment of motor units

In polysynaptic reflex, increasing the intensity of stimulus leads to the spread of impulses to more segments of spinal cord known as **irradiation of stimulus**. This is followed by an increase in the number of motor units activity called **recruitment of motor units**. The irradiation of stimulus and recruitment of motor units help to give a greater reflex response as the intensity of stimulus becomes stronger.

Reciprocal innervation

When flexor reflex occurs, there is a **reciprocal innervation,** which causes the extensors to be inhibited (Fig. 4.33). Reciprocal innervation is also present in stretch reflex.

Crossed extensor reflex

There is another characteristic phenomenon in flexor reflex known as **crossed extensor reflex**. The flexion of one limb causes extension of the opposite limb and it helps to maintain posture.

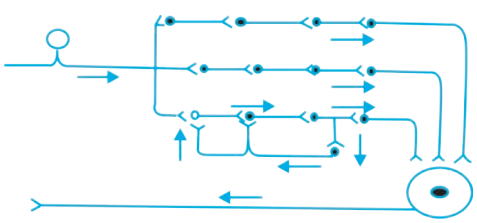


Fig. 4.32: Diagram of neuronal connections to show reverberating circuit. The arrangement of neuronal connections is such that re-excitation can occur. These circuits are responsible for afterdischarge of impulses

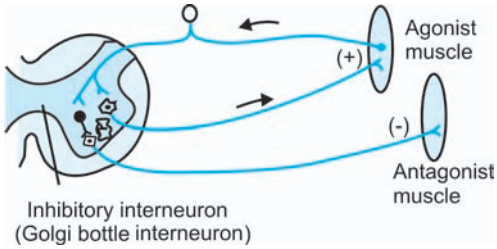


Fig. 4.33: Reciprocal innervation

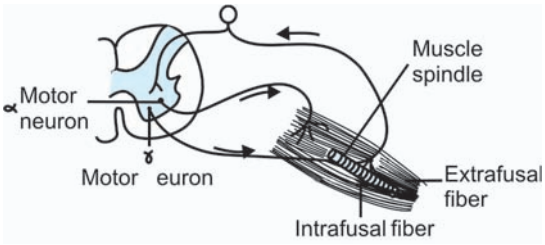


Fig. 4.34: Stretch reflex

Stretch reflex

Muscle tone

The reflex contraction that occurs when a muscle is stretched forms the stretch reflex. It is a **monosynaptic reflex** as there is only a single synapse between the afferent nerve and the efferent motor neuron (Fig. 4.34). The stretch reflex is the basis for the existence of muscle tone and posture. The regulation of posture is due to adjustment of muscle tone. The receptor for the stretch reflex is the muscle spindle present in the muscle. Each muscle spindle contains 8 to 10 intrafusal fibers. They are present parallel to extrafusal fibers which contain the contractile units.

The intrafusal fibers are of two types namely **nuclear bag fiber** and **nuclear chain fiber**. The ends of both types of intrafusal fibers contain end plates and hence they are contractile in nature. The central region does not have such features but contains sensory endings (Fig. 4.35). The sensory innervation consists of primary and secondary endings. The primary innervation is given by the group **Ia** fibers, which end in the nuclear bag and nuclear chain fibers forming

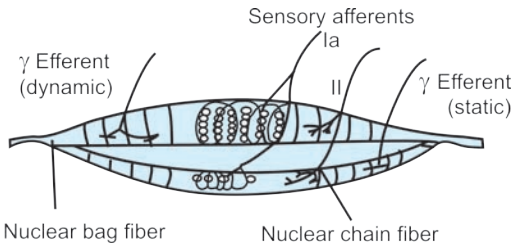


Fig. 4.35: Diagram of intrafusal fibers and their innervation

annulospiral endings. The secondary sensory innervation is provided by the group II fibers, to which supply only the nuclear chain fibers to form **flower spray endings**.

The primary endings in the nuclear bag fiber convey the dynamic (phasic) response from the spindle, which consists of sensing muscle length and rate of change of length. The primary endings on the nuclear chain fiber convey the static response, which includes detection of muscle length only.

Motor innervation of spindles

The spindle also receives motor innervation through γ motor neurons. There is also innervation by Aβ fibers to the intrafusal and extrafusal fibers. It forms plate endings in the nuclear bag fibers and trail endings (network) in the nuclear chain fibers. The γ neurons are of two types, namely γ dynamic and γ static neurons. The γ dynamic neurons mainly excites the nuclear bag fibers and γ static neurons activate nuclear chain fibers.

The sensory afferents in the spindle are stimulated by:

Stretching of Muscle

Stimulation of γ motor neurons.

Gamma motor neuron discharge

The γ motor neurons are under the influence of higher centers namely the reticular formation. The activity of γ efferents to the spindle causes the contractile parts of the intrafusal fiber to shorten. This inturn causes deformation of sensory endings in the central region and excites them. The stimulation of sensory afferents causes reflex contraction of extrafusal fibers, through α motor neuron. The presence of γ motor neuron innervation in the spindle helps to increase the spindle sensitivity to stretch and maintain muscle length.

If γ motor neuron innervation had been absent, then the spindle would unload and shorten, as the extrafusal fibers contract. This will not give tone to the muscle. For tone to be present in the muscle, there should be a sustained discharge of spindle activity, so that, when extrafusal fibers shorten, the muscle length is maintained, through the γ motor firing to the spindles. Infact, there is a coactivation of α and γ motor neurons, which maintain the muscle length (Fig. 4.36). In this way, the muscle spindle helps to maintain the length and tone of the muscle.

The γ motor neurons are under the influence of descending motor tracts especially the reticulospinal tract. The activation of α motor neuron during voluntary action coactivates γ efferents also, inorder to maintain muscle length when extrafusal fibers contract.

Increased activity of γ motor neurons causes increased muscle tone resulting in rigidity. Reduced discharge of γ motor neurons causes hypotonia of the muscle and flaccidity. γ activity is increased in anxiety, unexpected movement, stimulation of skin by noxious agents, Jendrassik's maneuver.

Golgi tendon organ reflex

The tendons also have receptors called **Golgi tendon organ**. This is supplied by group **Ib** afferents and responds to excessive stretch of muscle. The afferents from the tendon reach the spinal cord and end on an inhibitory interneuron (Fig. 4.37). The stimulation of this inhibits the motor neuron supplying the muscle and the muscle relaxes. This is a feed back mechanism to prevent excessive stretch or force being developed in the muscle.

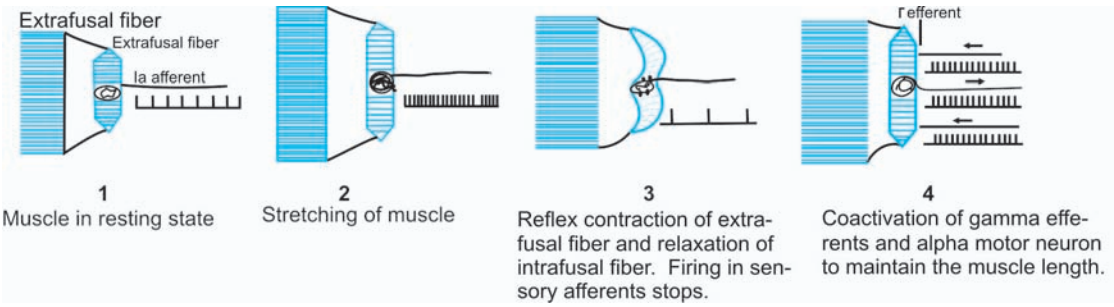


Fig. 4.36: Diagrammatic representation to show the mechanism of stimulation of intrafusal fibers

Inverse stretch reflex

The inhibition of stretch reflex when the muscle is excessively stretched is called inverse stretch reflex (autogenic inhibition). The receptors are the Golgi tendon organs present in the tendon of the muscle. Inverse stretch reflex can be demonstrated in the **lengthening reaction** or **clasp knife rigidity**. In a spastic muscle, when an attempt is made to flex the arm, it meets with resistance. But when more force is used, the stretch reflex becomes inhibited by the stimulation of Golgi tendon organ receptors. The muscle gives way suddenly and the arm is flexed. This feedback control maintains the force developed in a muscle.

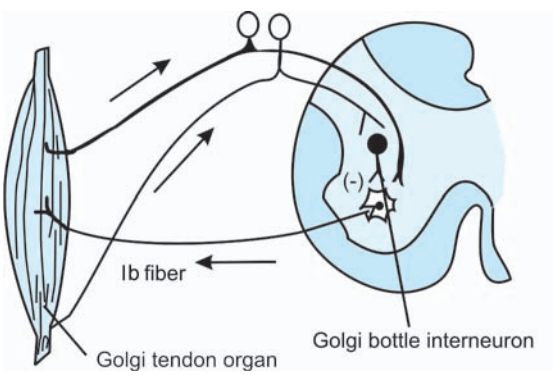


Fig. 4.37: Inverse stretch reflex

The excessive stretch of muscle stimulates Golgi tendon organ receptors and reflex inhibition of muscle occurs through Golgi bottle interneuron. The inverse stretch reflex helps to maintain muscle tension

SPINAL CORD

Spinal cord is situated in the vertebral canal. It extends from the first cervical vertebra to the upper border of second lumbar vertebra. Rostrally, it is continuous with the medulla oblongata and caudally, it ends in filum terminale, which is attached to the coccyx. There are 31 segments in the spinal cord, which are distributed as, cervical 8, thoracic 12, lumbar 5, sacral 5 and coccyx 1. Each segment gives off a pair of spinal nerve (Fig. 4.38).

Each segment shows certain common features, which can be observed in the cross section of the spinal cord. The cross section of the spinal cord shows an outer white matter and an inner grey matter. The white matter contains nerve tracts, in which the myelinated fibers are predominant.

The central **grey matter** contains nerve cells and glia. The center of the grey matter shows a canal, which is lined by ependymal cells and contains cerebrospinal fluid. The central canal

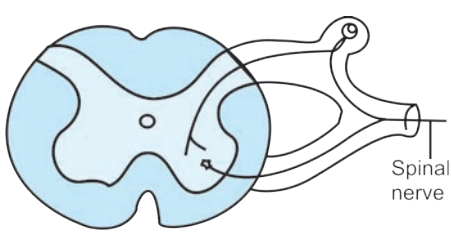


Fig. 4.38: Formation of spinal nerve

divides the transverse commissure into anterior and posterior grey commissures. The lateral aspect of the grey matter extends into dorsal horn and ventral horn. In the thoracolumbar segments, there is also presence of intermedio lateral cell column which gives rise to preganglionic sympathetic fibers.

Ventral horn of spinal cord

Ventral horn contains motor neurons (Fig. 4.39). These nerve cells give rise to ventral nerve, which forms the final common pathway.

There are two types of motor neurons in the ventral horn. They are **α** and **γ** motor neurons. The alpha motor neurons are larger in size, with the diameter showing up to 12-20 μm. The corticospinal tracts end on the alpha motor neurons, either directly or through the interneurons. The axons of the alpha motor neurons supply the skeletal muscle and produce voluntary movement. The gamma motor neurons are smaller in size and give axons to the muscle spindles. This innervation is useful in maintaining the muscle tone. The motor neurons in the ventral horn which executes the motor command of the descending motor tract forms **the final common pathway**. The ventral horn of the spinal cord shows recruitment of motor units depending on the motor tasks to be performed. This aspect of deciding which motor units to be activated resides in the spinal cord itself, so that the motor cortex can be left free for motor planning and execution.

Situated medially in the ventral horn, is another motor neuron which is actually an interneuron, called **Renshaw cell**. This is an inhibitory interneuron, which receives collateral from the ventral nerve. The stimulation of Renshaw cell inhibits the alpha motor neuron, forming the feedback control. On the basal part of ventral horn is the Clarke's column which is also a group of nerve cells, sending projections to the cerebellum. The intermediolateral cell column in the thoracolumbar segments, gives rise to preganglionic sympathetic fibers.

The **dorsal horn** of the grey matter has seven laminae. The laminae II and III form the substantia gelatinosa of Rolando. The nociceptive afferents coming from the periphery synapse here. The dorsal horn acts as a gate for the sensory afferents in regulating the sensory input reaching the nervous system. The descending fibers which inhibit pain afferents, end in the dorsal horn through an interneuron. In the dorsal horn, the larger size afferent fibers carrying touch and pressure inhibit smaller fibers which carry pain.

The **white matter** of the spinal cord invests the grey matter and contains dorsal, lateral and anterior (ventral) funiculi. The presence of more number of myelinated fibers gives white appearance. These tracts include both ascending and descending fibers. The ascending fibers, not only connect the different segments of the spinal cord, but also the various regions of the brain.

The major **ascending tracts** of spinal cord are (Fig. 4.40).

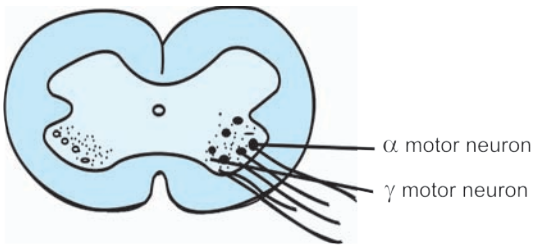


Fig. 4.39: Motor neurons of spinal cord

The larger sized ones are α motor neurons and the smaller ones are γ motor neurons. Alpha motor neurons that are situated medially supply proximal musculature, while the laterally situated neurons supply distal musculature

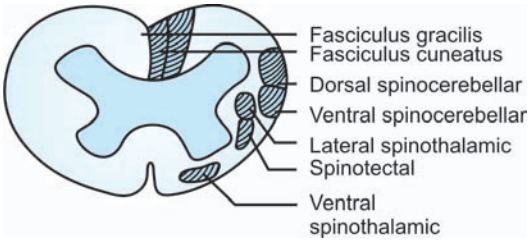


Fig. 4.40: Transverse section of spinal cord showing ascending tracts

Dorsal column (fasciculus gracilis and fasciculus cuneatus). These uncrossed fibers carry touch, two point discrimination, vibration, kinesthetic, pressure and stereognosis.

Anterolateral system comprises lateral spinothalamic and ventral spinothalamic tracts. These crossed fibers carry pain, temperature and crude touch.

Spinocerebellar afferents consist of dorsal and ventral spino cerebellar tracts. The dorsal tract goes ipsilaterally to the cerebellum, while the ventral tract goes to the ipsilateral and contralateral cerebellum. They carry proprioceptive impulses (unconscious) and help to coordinate voluntary movements.

Spinotectal tract carries fibers to superior colliculus and help to coordinate visuospinal reflexes.

The **principal descending tracts** in the spinal cord belong to two divisions, consisting of tracts descending in the lateral and ventral funiculi (Fig. 4.41).

Tracts in the lateral funiculus

Lateral corticospinal tract rubrospinal tract

Lateral corticospinal tract is recent in the evolutionary development and is concerned with the execution of fine, skilled voluntary movements from the distal musculature, especially with the digits.

The rubrospinal tract, although occupies the lateral funiculus, is concerned with the voluntary movements of proximal and distal muscles.

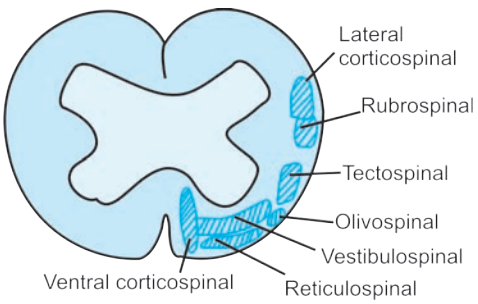


Fig. 4.41: Descending pathways in the spinal cord

The **ventral funiculus** carries the following tracts:

- Ventral corticospinal
- Tectospinal
- Vestibulospinal
- Reticulospinal
- Olivospinal.

The ventral corticospinal tract is part of the pyramidal tract. It causes contraction of proximal group of muscles. The remaining tracts in the ventral funiculus are all extrapyramidal fibers, concerned with the regulation of muscle tone, posture, and equilibrium and control the voluntary movements.

Interneurons of spinal cord

There are two groups of interneurons present namely, lateral and medial (Fig. 4.42). The descending motor tracts, usually end on the α and γ motor neurons, through these interneurons. The lateral group receives fibers from lateral corticospinal tract (Fig. 4.43), while

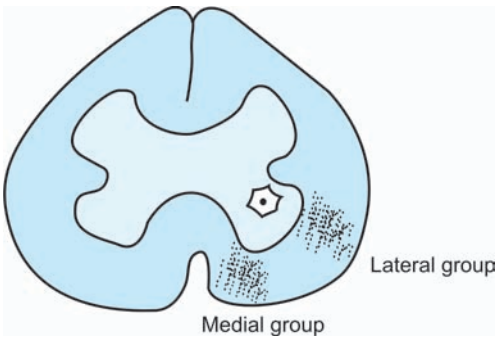


Fig. 4.42: Interneurons in the white matter of spinal cord

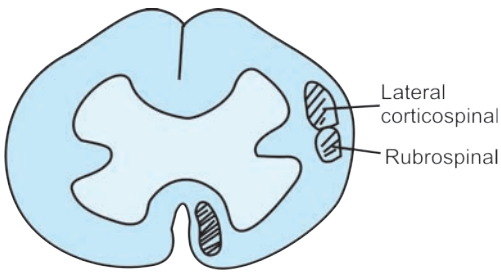


Fig. 4.43: Lateral descending pathways in the spinal cord

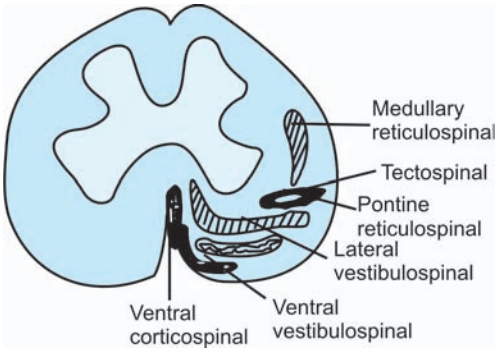


Fig. 4.44: Medial system pathways
The tracts other than the ventral corticospinal form the extrapyramidal tracts and they end on the medial group of interneurons

the medial group interneurons receive the descending extrapyramidal fibers and ventral corticospinal tract. The interneurons projecting the lateral and ventral corticospinal tracts end on the α motor neurons, while the extrapyramidal tracts end on the γ motor neurons through the medial group interneurons (Fig. 4.44).

MOTOR ORGANIZATION IN THE BRAINSTEM

Extrapyramidal pathways

Brainstem contains nerve cells such as red nucleus, superior colliculus, vestibular nucleus and reticular formation. They receive connections from cortex, basal ganglia and cerebellum. The descending pathways from these nuclei form the extrapyramidal tracts (Fig. 4.45). They descend in the spinal cord mixed with the pyramidal tract. The extrapyramidal tracts descend in the ventral funiculus of spinal cord and end in medial interneurons. They are concerned with the regulation of muscle tone, posture and control of voluntary movement These extrapyramidal tracts are also active during voluntary action, in giving the background tone and posture.

The important *extrapyramidal tracts* are:

- Rubrospinal
- Vestibulospinal

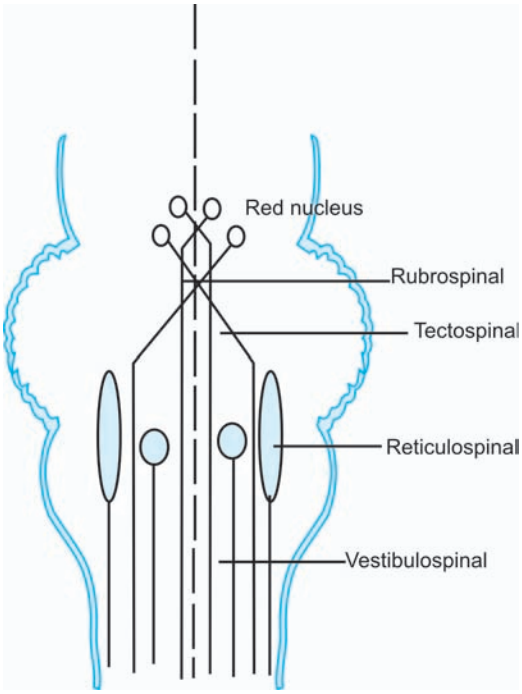


Fig. 4.45: Extrapyramidal tracts

- Reticulospinal
- Olivospinal
- Tectospinal

Rubrospinal tract

It arises from the red nucleus situated in the midbrain. The fibers after leaving the nucleus, immediately cross over (floral decussation) and descend in the lateral funiculus to reach the spinal cord. The fibers end on the spinal motor neurons through the lateral interneurons. Corticorubrospinal tract is more associated with the corticospinal tract and controls distal muscles up to the wrist.

Tectospinal tract

The fibers arise from the superior colliculus of the mid brain. Fibers cross over, travel in the anterior funiculus of the spinal cord, and end in the cervical and upper thoracic segments. These fibers through medial interneurons end in the

spinal motor neurons. Tectospinal tract is involved in the execution of visuospinal reflexes.

Vestibulospinal tract

There are two divisions namely lateral and medial vestibulospinal tracts present in the ventral funiculus of the spinal cord. The former arises from lateral vestibular nucleus (Deiter's nucleus), while the latter arises from medial vestibular nucleus. In the spinal cord they end on the alpha and gamma motor neurons through medial interneurons. Vestibulospinal tract is considered important for maintaining muscle tone, posture and balance. The regulation of these functions is possible, due to the impulses reaching the vestibular nuclei from the vestibular apparatus and cerebellum.

Reticulospinal tract

In the brainstem, there are ill defined groups of nerve cells, which form the reticular formation. The pontine reticular formation gives rise to medial reticulospinal tract and the lateral reticulospinal tract arises from the medullary reticular formation. They travel ipsilaterlly in the anterior funiculus and end in gamma motor neurons through medial interneurons. These extrapyramidal fibers are involved in gamma efferent discharge to the muscle spindles, which help to maintain muscle tone.

Olivospinal tract

The inferior olivary nucleus in the midbrain sends fibers to the cervical segments of spinal cord ipsilaterally as olivospinal tract. The function of this extrapyrmidal tract is not known clearly.

Summary of the functions of extrapyramidal tracts:

- Regulate muscle tone
- Maintain posture
- Maintain equilibrium
- Control voluntary movements.

From brainstem there are also postural reflexes, such as righting and labyrinthine reflexes, which are described under postural reflexes.

MOTOR CONTROL FROM BASAL GANGLIA

There are three nuclear masses present underlying the cortex. These structures are grouped under basal ganglia and includes **caudate nucleus, putamen** and **globus pallidus**. These nuclei are also functionally related to the subthalamic nucleus, red nucleus, thalamus, substantia nigra. The caudate and putamen together form the **striatum**, while the putamen and globus pallidus constitute **lenticular nucleus**.

The activity of basal ganglia's nuclei depends on the type of transmitters released from the nerve terminals. There are four types of neurotransmitter systems that can be seen in the basal ganglia (Fig. 4.46).

1. **Glutaminergic:** They are present in the cortico striatal neurons and in neurons from subthalamic nucles to globus pallidus
2. **Cholinergic:** Intrastriatal projections contain these fibers
3. **GABAergic:** Connections from striatum to substantia nigra and globus pallidus and from globus pallidus to thalamus show GABAergic neurons.
4. **Dopaminergic:** The projections from substantia nigra to striatum include these neurons.

Cholinergic neurons are excitatory, while GABA-ergic and Dopaminergic neurons are inhibitory in function.

Connections of basal ganglia

There are three important neural circuits in basal ganglia, which help in the control of motor activity.

1. The caudate nucleus and putamen receives connections from all over the cerebral cortex (Figs 4.47 and 4.48). The caudate nucleus sends projections to the cortex through thalamus. The putaman sends its connections to cortex through globus pallidus and thalamus.

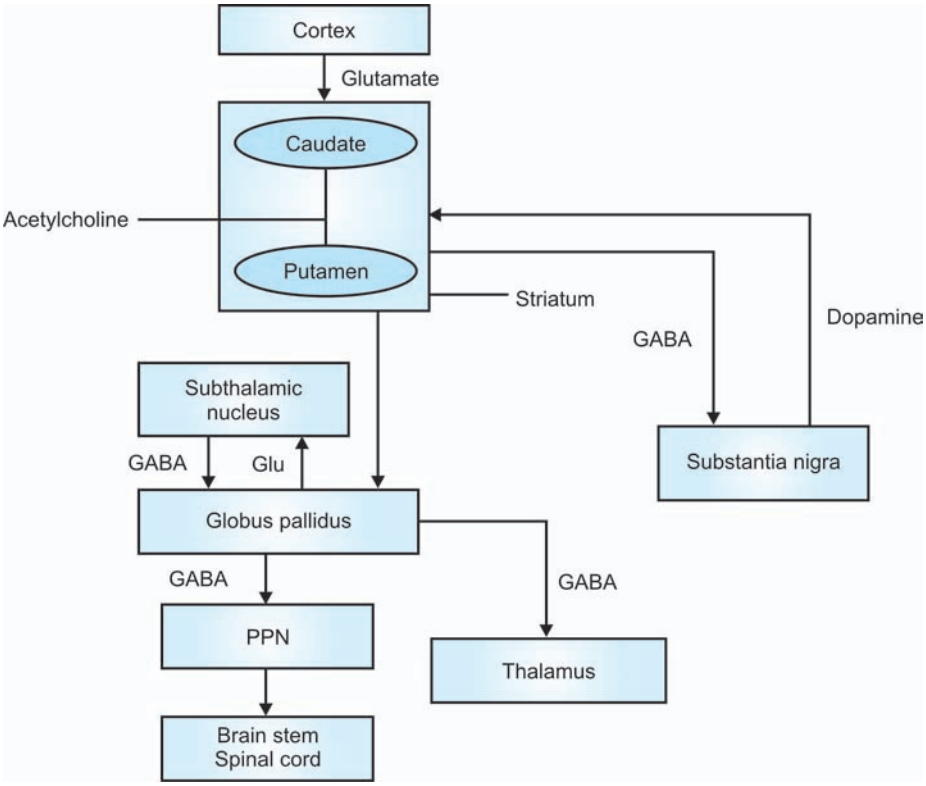


Fig. 4.46: Connections of basal ganglia and its neurotransmitter systems
PPN: Pedunculo pontine nuclei
GABA and Dopamine: Inhibitory
Glutamate and Acetylcholine: Excitatory

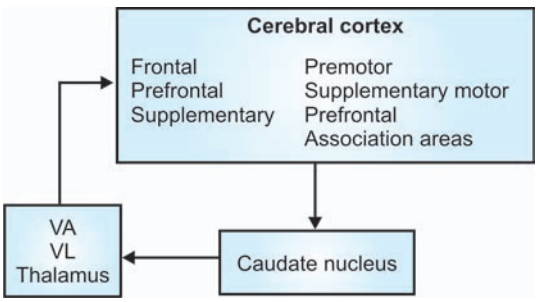


Fig. 4.47: Caudate neural circuit
VA: Ventroanterior nucleus
VL: Ventrolateral nucleus

2. To and fro connections exist between the striatum and substantia nigra (Figs 4.46 and 4.49). The projection from striatum to nigra is GABA ergic, while the projection from nigra to striatum is dopaminergic.
3. Globus pallidus is the nucleus from where efferent fibers of basal ganglia leave. It is connected to the subthalamic nucleus and substantia nigra. From these nuclei, projections go to red nucleus and reticular formation (Fig. 4.46). The descending rubrospinal and reticulospinal tracts help in the regulation of tone and posture.

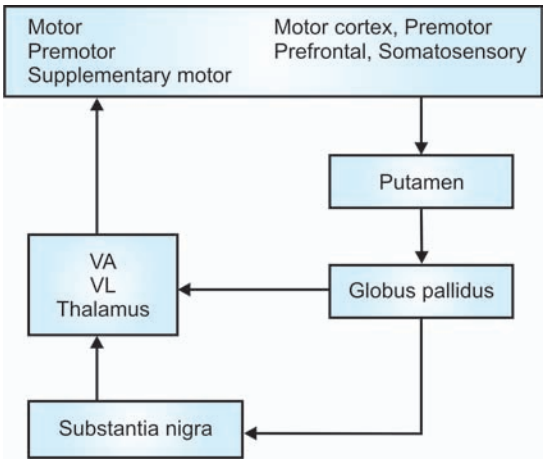


Fig. 4.48: Putamen neural circuit

VA: Ventroanterior nucleus
VL: Ventrolateral nucleus

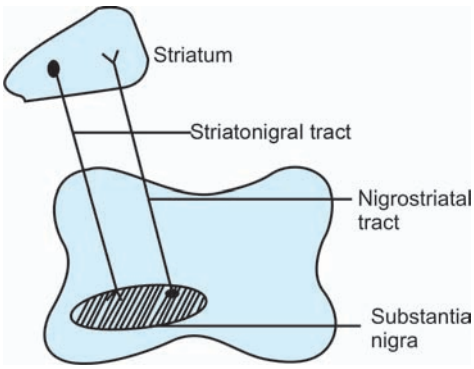


Fig. 4.49: Neuronal connections between striatum and substantia nigra

Functions of basal ganglia (Table 4.1)

Functions of basal ganglia are known from the effects of lesion of its nuclei in experimental animals and diseases produced by its damage in humans. It has been shown that basal ganglia nuclei discharge even before the motor movement begins. This indicates its involvement in motor planning and programming. The study of neural connections between basal ganglia nuclei to the motor cortex, pre motor areas and cortical association areas revealed the role of basal ganglia in the control of learned motor movement and control of cognitive planning of motor activity. The connections of globus pallidus with the brain stem and from there to the spinal cord facilitate the control of muscle tone and posture.

The functions of basal ganglia can be summarized as follows:

- Planning and programming of motor activity
- Control of learned motor activity—**Putamen neural circuit** is responsible for the control of learned motor actions which are routinely performed in our daily life.
- Control of cognitive planning of motor movement—**Caudate neural circuit** is involved for this function. The motor actions which require thinking and execution are controlled by the Caudate neural circuit.
- Control of muscle tone and posture is achieved by the descending extrapyramidal system.

Disorders of basal ganglia

Wilson's disease

It is a genetic disorder inherited as autosomal recessive character.

The gene for the synthesis of protein ceruloplasmin (copper carrying protein) is absent.

Excessive deposition of copper damages the lenticular nucleus.

Rigidity and involuntary tremors are characteristics of Wilson's disease.

Table 4.1: Neural circuits, their function and effects of lesion in basal ganglia

Basal ganglia neural circuit	*Function*	*Effects of lesion*
Caudate neural circuit	Cognitive control of motor activity	Putamen - **chorea** Damage to caudate & putamen - **Huntington's disease**
Putamen neural circuit	Control of learned patterns of movement	Lesion of subthalamic nucleus-hemibalismus Lesion of globus pallidus - **athetosis**
Nigro striatal circuit	Regulates balance between excitation and inhibition to maintain normal motor function	**Parkinson's disease** Bradykinesia Akinesia Rigidity (cogwheel or lead pipe) resting tremor

Huntington chorea

It is also a genetic disorder, inherited as autosomal dominant character.

Affects individuals in 35 to 50 years of age.

- The abnormal gene causes repeat of codon CAG (cytosine, adenine & guanosine) giving several times the synthesis of glutamine in the neuronal protein.
- The abnormal protein causes loss of GABA-ergic neurons from striatum to globus pallidus and cholinergic neurons within the striatum.
- The disorder includes hyperkinetic, choreiform movements.
- In advanced stage, dementia and difficulty in speech are also observed.

Parkinson's disease

It is a neurodegenerative disorder caused by many factors including old age. The nigrostriatal dopaminergic neurons degenerate, which affects putamen more than caudate.

The pathogenesis of Parkinson's disease can be explained by the imbalance between the excitatory and inhibitory outputs from putamen to globus pallidus and from there to thalamus and brainstem. In normal persons, the balance between the excitatory and inhibitory outputs from putamen to globus pallidus is controlled. In Parkinson's disease, the degeneration of dopaminergic neurons in putamen causes reduced inhibition on the subthalamic nucleus, which leads to its increased excitation on globus pallidus internal segment. Since the output from globus pallidus internal segment is inhibitory, the excitation of this will cause increased inhibitory output to thalamus and brain stem causing neurological symptoms of Parkinson's disease.

The neurological symptoms include hyperkinetic and hypokinetic movements. **Rigidity** (*lead pipe or cog wheal type*) and **resting tremors** are characteristic features of Parkinson's disease. The other symptoms which are also observed are, festinating gait, poverty of movements (hypokinesia or bradykinesia) and lack of emotional expression in the face.

L-Dopa which is a precursor of dopamine is effective in the treatment of this disease, as it can freely cross the blood brain barrier.

Chorea and athetosis

Damage to putamen causes chorea and lesion of globus pallidus results in athetosis. Chorea is characterized by involuntary dance like movements and athetosis shows slow writhing movments of limbs.

Ballism

Lesion of subthalamic nucleus causes flailing, violent, involuntary movements. If occurs on one side of the body, it is called **hemiballism**.

MOTOR CONTROL FROM CEREBELLUM

Cerebellum is present in the hindbrain and attached to the brainstem through cerebellar peduncles (Table 4.2). The chief functions include

- Regulation of tone, posture and equilibrium
- Coordination of voluntary movements
- Programming and planning of motor action
- Control of rate, range, force and direction of voluntary movements.

Physiologically the cerebellum is divided into three divisions (Fig. 4.50).

1. **Vestibulocerebellum:** It includes flocullonodular lobe. It is the oldest part phylogenetically and hence called archi cerebellum.
2. **Spinocerebellum:** The central vermal and paravermal parts are included in this part.
3. **Neocerebellum** The lateral cerebellar hemispheres form this division. It is well

Table 4.2: Divisions and functions of cerebellum

Region	*Function*
Vestibulocerebellum (Flocculonodular lobe)	Control of tone, posture and equilibrium
Spinocerebellum (Central vermis and adjacent medial part)	Coordination of movements and control of distal limb muscles
Neocerebellum (Lateral cerebellar hemispheres)	Planning and programming of movements

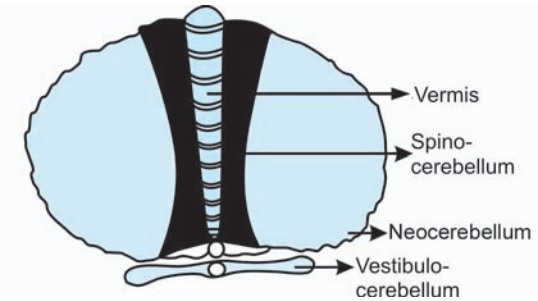


Fig. 4.50: Physiological subdivisions of cerebellum

developed in humans, as it is recent in the phylogenetic development.

The surface of cerebellum contains grey matter with foldings greater than cerebral cortex. The inner white matter contains tracts and four pairs of nuclei. The nuclei are **fastigii, interpositus** (consisting of *globosus* and *emboliformis*) and **dentatus.**

The cytoarchitecture of cerebellar cortex shows three layers which are uniform throughout unlike cerebral cortex (Fig. 4.51).

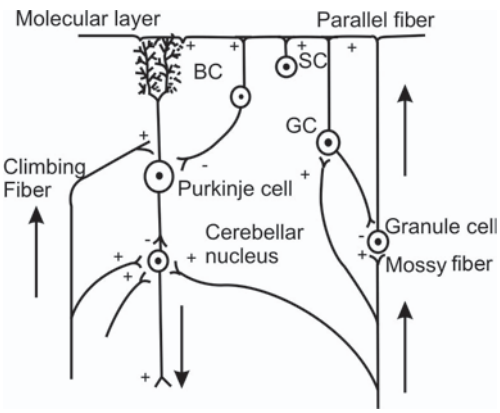


Fig. 4.51: Cytoarchitecture of cerebellar cortex

BC: Basket cell, SC: Stellate cell, GC: Golgi cell.

These are interneurons and inhibitory in nature.The neurotransmitter that is released from them is GABA and their activity helps to regulate the functions of Purkinje and granule cells

The three layers are:

- Molecular layer
- Purkinje layer
- Granule cell layer

The outermost layer is the molecular layer formed by the synapses between dendrites of Purkinje cells and axons of granule cell. The Purkinje cell sends its dendrites with profuse branches to the surface. A single axon from Purkinje cell ends on the cerebellar nuclei. The granule cell receives mossy fibers and the synaptic connection between them, forms the glomeruli. The axon of granule cell projects to the cortical surface and divides into a T shape to form the parallel fiber. It makes synaptic connection with the dendrites of several Purkinje cells. The cerebellar cortex, in addition to mossy fiber input, also receives climbing fiber to the Purkinje cell. Climbing fiber comes from the inferior olive and carries proprioceptive impulses. The climbing fiber makes one to one contact with the Purkinje cell, unlike mossy fiber which ends on several Purkinje cells.

There are three interneurons to control the activity of Purkinje cells and granule cells. The **stellate** and **basket** interneurons inhibit Purkinje cell, while **Golgi cell** inhibits granule cells. The Purkinje cell output to the cerebellar nuclei is inhibitory. But the output from cerebellar nuclei is excitatory, because, they receive excitatory afferents from other sources. In cerebellum the interneurons secrete **GABA** and the excitatory neurons secrete **glutamate** as the transmitter.

In cerebellum, the tactile tonotopic representation of the body is present. In the anterior and upper part of the posterior lobe, the body is represented upside down and in the lower part of posterior lobe, the body is represented upright. The axial parts of limbs and trunk are represented in the vermal and paravermal regions, while, the distal limbs are represented in the cerebellar hemispheres.

Connections of cerebellum

Afferents (Fig. 4.52)

- Dorsal spinocerebellar tract
- Ventral spinocerebellar tract
- Olivocerebellar tract
- Tectocerebellar tract
- Vestibulocerebellar tract
- Cuneocerebellar tract
- Pontocerebellar tract

Efferents (Fig. 4.53)

- Cerebellovestibular tract
- Cerebellopontine tract
- Cerebellothalamic tract
- Cerebelloreticular tract.

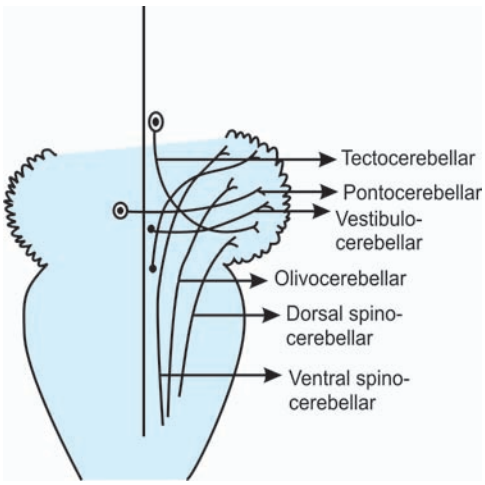


Fig. 4.52: Afferent connections of cerebellum

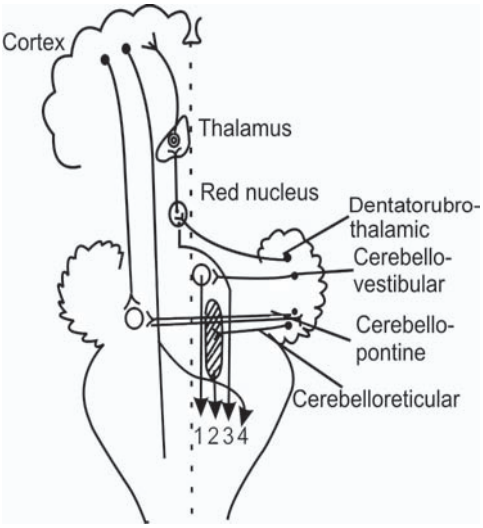


Fig. 4.53: Efferent connections of cerebellum

1. Vestibulospinal tract
2. Reticulospinal tract
3. Rubrospinal tract
4. Corticospinal tract

Cerebellum is able to control and co-ordinate the motor movements through these descending pathways

Functions

Vestibulocerebellum

The nucleus which sends the efferent connection is fastigium. The efferents go to lateral vestibular nucleus and reticular formation (Fig. 4.54). From there, the descending pathway reaches spinal cord in the ventral funiculus and end in the interneurons in the medial group. The activity of these two extrapyramidal tracts, helps to maintain muscle tone, posture and equilibrium.

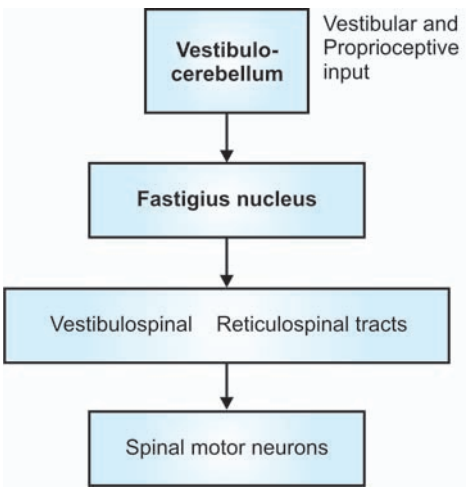


Fig. 4.54: Connections of vestibulocerebellum

Spinocerebellum

The nucleus interpositus from this part of the cerebellum sends efferent projections to the red nucleus of the opposite side. The red nucleus

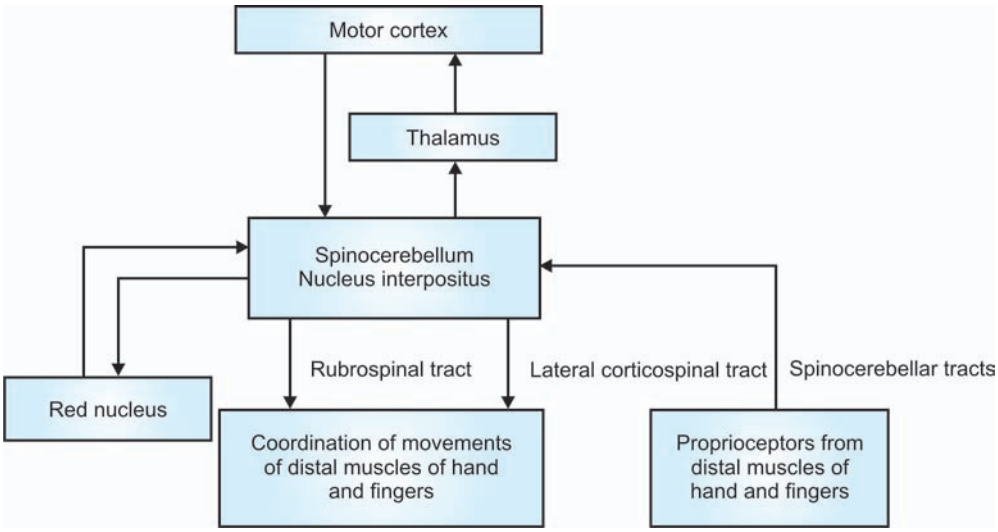


Fig. 4.55: Connections and functions of spinocerebellum

sends fibers, which cross to the opposite side in the brain stem and descend in the spinal cord as rubrospinal tract (Fig. 4.55). The rubrospinal tract together with lateral corticospinal tract controls the movement of distal muscles of hand and fingers. The spino cerebellum receives information from motor cortex and red nucleus about the intended plan of motor movement. As the movement is being performed, the spino cerebellum receives feed back information from proprioceptors of distal muscles of hand and fingers. Now the intermediate zone of spinocerebellum compares the actual movement with the intended plan. If there are deviations between the plan and the execution, the interpositus nucleus sends corrective signals to red nucleus and to motor cortex through thalamus. The rubrospinal and the lateral cortico spinal tracts give the required corrections in the coordination of movements of the agonist and antagonist muscles to make the movement purposeful.

Another important function of the cerebellum is to damp the movements to prevent overshoot. Loss of this function of cerebellum causes past pointing and intention tremor or action tremor.

Cerebrocerebellum (Neocerebellum)

It consists of lateral cerebellar hemispheres which are well developed in humans.

Its main functions are,

Planning of sequential movements

Timing of complex movements

The planning of sequential movements involve smooth progression of movement from one to next in orderly succession. This is possible by two way communication between premotor cortex and sensory cortex with the lateral cerebellar hemispheres. The plan of sequential movement first starts from the premotor and sensory cortex to cerebellum lateral hemispheres and the cerebellum too sends its plan to cerebral cortex (Fig. 4.56).

The dentate nucleus shows its activity for what the next sequential movement should be as the movement is being carried out.

The neocerebellum also provides the required timing for each succeeding movement. This is especially necessary when complex motor movements such as writing, running , speech,etc.

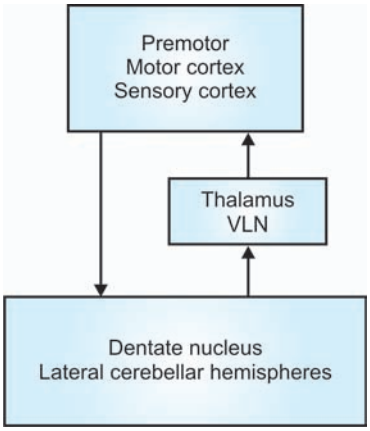


Fig 4.56: Two way communication between motor cortex and lateral cerebellar hemispheres. The connections help in planning and programming of sequential movements

VLN : Ventrolateral nucleus

are carried out. Damage to cerebellum often results in failure of smooth progression of movements. Clinically, dysdiadochokinesia and dysarthria are commonly observed in cerebellar damage.

Motor learning

Cerebellum is considered important in the learning of new motor tasks. This is seen from the activity of climbing fiber, which modifies the activity of mossy fiber. The climbing fiber activity gives complex electrical spikes, whereas, the mossy fiber activity gives a single action potential. This is believed to be inhibited or modified by the complex spikes coming from climbing fiber during motor learning. The result of which leads to the control of timing of discharge of impulses from cerebellar nuclei for learning the motor tasks without any difficulty.

Effects of lesion of cerebellum

Damage or lesion of cerebellum, affects only the motor functions. The sensory and intellectual functions are unaffected. The effects occur on the ipsilateral side of the lesion.

- ***Hypotonia and asthenia of muscles:*** The hypotonia is responsible for pendular knee jerk. In animals cerebellar lesion causes rigidity.
- ***Ataxia:*** It is in coordination of the moments of muscles and it includes several types which are described below:

1. ***Staggering gait:*** Incoordination in the posture and equilibrium.
2. ***Dysmetria (pastpointing):*** The incoordination of voluntary action in controlling rate, range, force and direction and this gives either undershooting or overshooting the target .
3. ***Asynergia:*** Incoordination of agonists and antagonists group of muscles and this causes **adiadochokinesia** (alternate movements cannot be performed). The adiadochokinesia is an example of failure of progression of sequential movements.

   Dysarthria is another example of failure of progression of muscles of speech. There is incoordination of muscles of speech.
4. ***Intention tremor*** (tremor of limbs when voluntary action is attempted). Incoordination of voluntary action is responsible for this and the intensity of tremor increases during voluntary action.
5. **Staccato speech** or scanning speech. This is due to incoordination of muscles of speech.

- **Nystagmus:** This occurs due to incoordination of eye muscles, which causes to and fro movement of eyeball.
- **Decomposition of movement:** This is the inability to perform movements involving more than one joint.

MOTOR CORTEX

The execution of voluntary movements is carried out by the descending corticospinal tract, which arise from motor cortex. The motor cortex is present in the precentral gyrus which includes the Brodmann's area 4 (Fig. 4.57). It contains the representation of the opposite half of the body called **motor homunculus**. The body is represented upside down with leg at the medial surface and face towards the lateral surface of the hemisphere (Fig. 4.58). Facial area is bilaterally represented in the cortex and the rest

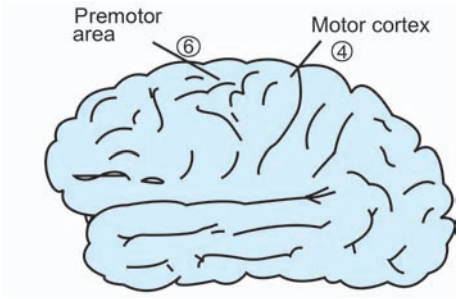


Fig. 4.57: Lateral view of cerebral hemisphere showing motor cortex situated in the precentral gyrus

Area 4 is the motor area; area 6 premotor area. Supplementary motor area is situated in the superior border of area 6

is generally unilateral. The part of the body, which is more involved in voluntary activity has a wider representation in cortex. This gives a disproportionate body representation, with the hand, feet, lips, having a wider representation and trunk and proximal part of limbs with a smaller representation in the motor cortex. Electrical stimulation of an area in the motor homunculus causes contraction of discrete group of muscles in the opposite side of the body. From motor cortex, the fibers going to corticospinal tract constitute 30%.

Supplementary motor area (Fig. 4.57)

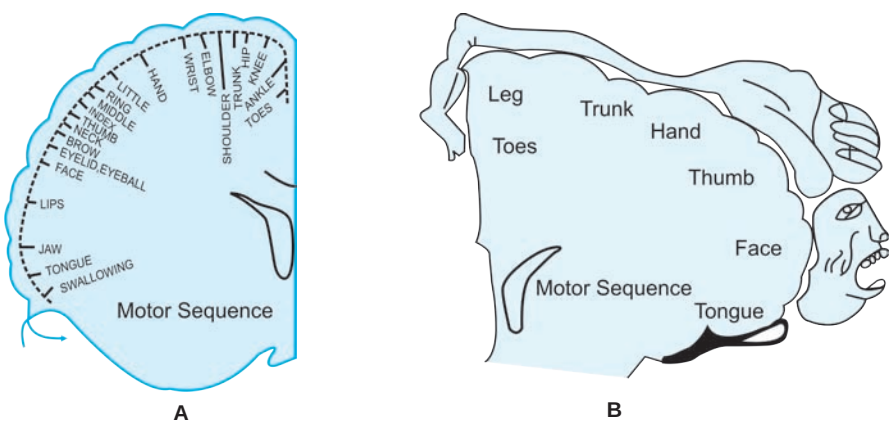
It is situated in the superior surface of the premotor area 6. It is concerned with motor planning and execution of complex voluntary movements. It also helps in the coordination of voluntary movements.

Premotor cortex

It is present in front of area 4 (Fig. 4.59). It includes areas 6, 8 and 44. Premotor areas receive major input from somesthetic areas of sensory cortex. It gives fibers (30%) to the corticospinal tract. This projection helps to regulate posture during voluntary movements, as it influences the medial descending pathways.

Somatosensory cortex and posterior parietal cortex (Fig. 4.59)

The corticospinal tract also receives fibers from sensory cortex (areas 3, 1 and 2) and sensory association areas (5 and 7), situated in the posterior



Figs 4.58A and B: (A) Motor sequence of the contralateral half of the body in the motor cortex, (B) Motor sequence and Motor homunculus in the motor cortex

The motor cortex shows upside down representation of opposite half of the body. The region of the body that is involved in motor activity has a greater representation in the cortex

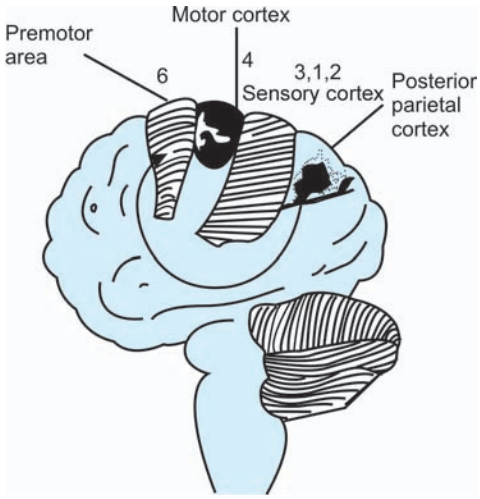


Fig. 4.59: Diagram of cerebral cortex to show motor cortex and its relation with the premotor, supplementary motor, somatosensory areas and posterior parietal cortex. The fibers for corticospinal tract arise from all these regions

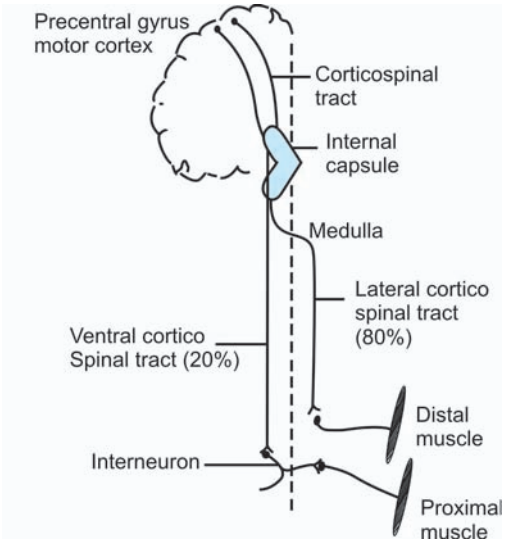


Fig. 4.60: Corticospinal tracts (Pyramidal tract)

parietal cortex. The projections from these areas to the corticospinal tract constitute 40% and help to execute learned sequence of motor activity.

Pyramidal tract

The descending motor tract,which arises from Betz cells of motor cortex, includes fibers from area 4, (30%), premotor area and supplementary motor area (30%), somatosensory and posterior parietal cortex (40%). The fibers after leaving the cortex, enter the internal capsule and occupy the genu and anterior two third of posterior limb. The fibers from internal capsule descend down in the brain stem and give projections to the opposite cranial nerve nuclei to form **corticobulbar tract.**

In the medulla, 80% of fibers cross over to the opposite side which forms a pyramid like appearance and hence the name pyramidal tract. The crossed fibers descend as **lateral cortico-spinal tract** (Fig. 4.60). The uncrossed 20% of fibers descend as **ventral corticospinal tract** which also becomes crossed to the opposite side of the spinal cord at the termination. Lateral cortico spinal tract ends directly on the motor neuron in the spinal cord whereas, the ventral corticospinal tract ends on the motor neuron through an interneuron. The motor tract, which descends from motor cortex and end on spinal motor neurons forms the **upper motor neuron**. The anterior horn cell containing the spinal motor nerve which supplies muscle is called **lower motor neuron**.

Functions of pyramidal tract

The lateral corticospinal tract is well developed in humans and controls the motor activity of muscles of distal limbs such as digits. These muscles are involved in the execution of smooth, skilled and purposeful voluntary movements.

The ventral corticospinal tract is phylogenetically older and is involved in the control of motor activity of proximal group of musculature in limbs.

Effects of lesion of corticospinal tract

The most common occurrence is a vascular lesion in the internal capsule which results in **hemiplegia.** It is the paralysis of one half of the body. The paralysis will be in the opposite side of the body in hemiplegia. The extensors of the lower limbs and flexors of the forelimbs show

spastic paralysis. The superficial reflexes are lost. The abnormal plantar reflex namely the **Babinski's sign appears**. The **ankle clonus** will also be present.

Effects of lesion of lower motor neuron

Injury or damage to spinal motor neuron causes **flaccid paralysis** of muscles in the affected segment of spinal cord. The superficial reflexes are also lost. The atrophy of muscles and abnormal electrical potentials, which are characteristics of lower motor neuron lesion are observed. The abnormal electrical potentials such as fasciculation and fibrillation potentials occur due to denervation hypersensitivity of the affected muscles. These changes also cause reaction of degeneration in the electrical excitation of the muscle. The Babinski's sign and ankle clonus are not present in lower motor neuron lesion.

Lower Motor Neuron Paralysis

Caused by lesion of anterior horn cells in the spinal cord and cranial nerve nuclei or the nerves arising from them

- Flaccid paralysis
- Hypotonia
- Atrophy of muscles
- Loss of superficial and deep reflexes
- Fibrillation and fasciculations from the affected muscles

Upper Motor Neuron Paralysis

Caused by the lesion of descending motor tract

- Spastic paralysis
- Hypertonia (clasp knife rigidity)
- Exaggeration of deep reflexes
- Loss of superficial reflexes
- Positive Babinski's sign
- No muscle atrophy

Overall motor organization and control

Motor action involves execution of simple reflex to a more complex type of motor sequence of movements, organized and controlled by spinal cord, brain stem, basal ganglia, cerebellum and motor cortex. In humans, the neocortex is highly developed and hence with greater encephalization, it is possible to produce a complex motor sequence of movements. To produce a voluntary movement, the idea is formed first in the association areas of frontal and parietal cortices. The motor activity that is executed is always in response to a sensory stimulus. The idea which is generated in the association areas is converted into motor planning and program in the supplementary motor cortex, basal ganglia and cerebellum. The motor action, which is to be performed is communicated to the motor cortex. This area executes the intended movement through the corticospinal tract. As the motor action is being executed, there are other regions of the brain like basal ganglia, cerebellum, reticular formation, vestibular nucleus and red nucleus, which help to maintain the background muscle tone and posture. The neocerebellum, through its feedback connection with the cerebral cortex (cerebro-cerebellar-cerebral circuit), is able to produce motor activity which is coordinated and error free.

SPINAL CORD LESIONS

Spinal shock

This is produced when an injury causes complete sectioning of spinal cord resulting in total paralysis below the level of lesion with loss of all reflexes and sensations. This state is called spinal shock and is due to the removal of higher center influence on the spinal cord. If the lesion occurs in the cervical segments, the effects are severe with profound fall in arterial blood pressure and paralysis of all four limbs **(quadriplegia).** The blood pressure fall is due to the removal of vasomotor tone. Lesion of the spinal cord in the lower thoracic and lumbar segments causes paralysis of lower limbs **(paraplegia)** and the fall in blood pressure is not severe.

Autonomic disturbances are also seen which include loss of sweating, loss of sphincter control of bladder and rectum. Inability to get erection and ejaculation in males are also observed.

Recovery from spinal shock

Lower animals recover quickly from spinal shock and duration of recovery becomes longer, if the organism is placed higher in the phylogenetic scale. In humans the spinal shock lasts minimum of 2 weeks in the absence of complications. Recovery also depends upon the management of the patient with good nursing and medical care.

The recovery from spinal shock can be explained based on denervation hypersensitivity to the transmitters released from neurons still remaining or sprout of collaterals from the existing neurons which make connections with the interneurons and motor neurons.

The first sign of recovery is flexor withdrawal reflex to a noxious stimulus. In some subjects the extensor reflex appears first, but in both the cases, the reflexes are exaggerated.

The return of flexor reflex also causes **mass reflex**. Mild stroking of medial part of thigh or genitalia results in flexor spasm of muscles of thigh and abdominal muscles. This is accompanied by autonomic disturbances like changes in arterial blood pressure, sweating, etc. The mass reflex is utilized by the subject for emptying the bladder and rectum.

The recovery from spinal shock also shows return of vasomotor tone and sweating. The erection and ejaculation can be possible in males.

Hemisection of spinal cord

Lesion of one half of spinal cord due to the injury affects sensory and motor functions. The symptoms are clinically known as **Brown-Sequard syndrome.** The effects, which are seen at the level of lesion, below the level on the same side and opposite side are described below.

At the level of lesion

Sensory loss: Loss of all sensations.
Few segments above the level of lesion;
Hyperaesthesia: Increased sensitivity to touch and pain sensations due to local irritation.
Motor loss: Loss of all reflexes and effects of lower motor neuron lesion occur.

Below the level of lesion

Same side

Sensory loss: Loss of dorsal column sensations.
motor loss: Upper motor neuron lesion effects such as spastic paralysis, Babinski's sign and ankle clonus are present.

Opposite side

sensory loss: Loss of pain and temperature sensations.

POSTURAL REFLEXES

Posture refers to the maintenance of upright position against gravity. The other function of postural reflex is to maintain balance and give background muscle tone during voluntary action. The mechanism in the maintenance of muscle tone forms the **static postural reflex** and the mechanism, which maintains balance during voluntary action forms the **phasic postural reflex.** These reflexes are integrated at various levels of nervous system starting from spinal cord to cerebral cortex, involving brain stem, basal ganglia, reticular formation and cerebellum. The importance of these regions in the regulation of posture can be known from the effects of experimental lesions in animals at various levels of nervous system.

Lesion of spinal cord and its effects on posture

The spinal shock that occurs in the complete section of spinal cord, results in total paralysis below the level of lesion and is due to the removal of higher center influence on the spinal cord. During recovery, the flexor withdrawal reflex appears for noxious stimuli. The stretch reflex returns later. In both the cases the recovery is not complete and the reflexes are exaggerated. The experimental study, nevertheless, reveals the importance of spinal cord in the integration of reflexes such as stretch reflex, crossed extensor reflex, positive and negative supporting reactions.

Stretch reflex: It is a *myotatic reflex* responsible for maintaining muscle tone. The muscle tone forms the basis for posture regulation. Though the spinal cord integrates stretch reflex, the higher centers, such as reticular formation and vestibular nuclei regulate it.

Crossed extensor reflex: Flexion of one limb causes extension of opposite limb to maintain posture.

Positive supporting reaction: The standing posture is due to this mechanism. The dorsiflexion of toes forms the stimulus and the leg is converted into a rigid pillar by the contraction of both flexors and extensors.

Negative supporting reaction: The lifting of leg away from the ground is due to this reflex. The plantar flexion of toes forms the stimulus and the extensors are inhibited. The alternating positive and negative supporting reactions produce walking movement.

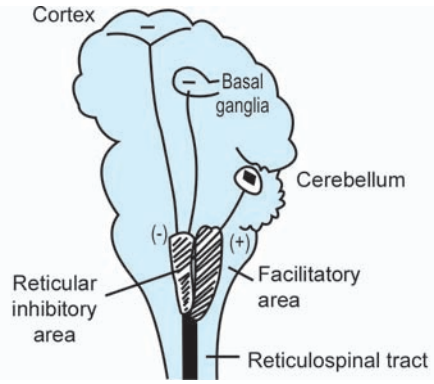


Fig. 4.61: Diagram to explain the mechanism of decerebrate rigidity. The inhibitory region of reticular formation receives input from cortex, basal ganglia and cerebellum. The activity of reticulospinal tract depends on the balance of activity between facilitatory and inhibitory regions of reticular formation. When decerebration is made, two of the three inhibitory inputs are removed and the facilitatory reticular formation will now become unopposed. Hence the rigidity of the muscle occurs

Lesion in the brainstem and its effects on postural reflexes

Decerebrate rigidity

Lesion at the upper border of pons *(mid collicular section)* results in **decerebrate rigidity**. The animal shows exaggerated extensor tone of all the four limbs and takes up a caricature of standing posture. It has no righting reflexes, but tonic labyrinthine and tonic neck reflexes are present.

The mechanism of decerebrate rigidity is due to the removal of inhibitory influences which go to medullary reticular formation. This causes the facilitatory region of reticular formation to become unopposed, resulting in increased firing of impulses in gamma motor neurons. The increased firing of γ motor neuron results in rigidity.

The medullary reticular formation receives three inhibitory inputs namely, cerebral cortex suppressor regions (2s, 4s, 8s 32), caudate nucleus of basal ganglia and anterior lobe of cerebellum (Fig. 4.61). When decerebration is done, two of the three inhibitory inputs are removed. The inhibitory influence coming from the anterior lobe of cerebellum will only be present. However, the medullary reticular facilitatory region becomes unopposed and this causes increased firing of γ motor neurons to the muscle spindles and hence the rigidity occurs. The role of γ motor neuron's increased discharge, as the cause for rigidity can be shown by sectioning the dorsal nerve and observing the absence of rigidity in the concerned spinal segments.

Decerebrate rigidity can also occur due to the removal of anterior lobe of cerebellum in animals. In this case, the rigidity is caused by the increased discharge of α motor neuron. The vestibular nuclei receives inhibitory influence from two sources. One is cerebral cortex and the other is anterior lobe of cerebellum. The removal of anterior lobe of cerebellum inhibitory influence on the vestibular nuclei, results in increased discharge in the vestibulospinal tract and increases the firing of αmotor neuron, giving rise to rigidity. The deafferentation in this case, does not abolish rigidity.

The decerebrate preparation gives us the understanding that the brainstem has centers, which influence the stretch reflex and also integrate righting reflexes.

Decorticate preparation

Removal of cerebral cortex causes rigidity of the antigravity muscles, due to the removal of inhibitory influence from cortex to the medullary reticular formation. In this preparation, the righting reflexes are present, but the visual righting reflex, placing and hopping reactions are absent, as they are integrated in the cortex. The decorticate animal shows tonic labyrinthine and tonic neck reflexes.

Tonic labyrinthine reflex is caused by the stimulation of utricle by gravity, due to the change in the position of head, which reflexly increases the tone of extensors of limbs.

Tonic neck reflex is caused by the stimulation of neck proprioceptors, due to the change in the position of the head and reflexly causes change in the tone of the limb muscles. If the animal is turned to one side, the extensor tone of the limbs on that side increases (jaw limbs) and in the opposite side the tone decreases. Bending the head forward, causes increase in the extensor tone in the hind limbs and decrease in the forelimbs, whereas, the bending of head backward, causes increase in the extensor tone of the fore limbs and decrease in hind limbs.

Midbrain preparation

Section at the superior border of the midbrain, results in midbrain animal. The animal shows rigidity only when placed on its back. Otherwise, it can do normal phasic postural reflexes, without any sign of rigidity. The righting reflexes are retained, barring visual righting reflex.

Righting reflexes

The righting reflexes are necessary to maintain balance and orient the head and body in relation to space.

There are five types of righting reflexes. The first four types are integrated in midbrain, while the fifth one (optical righting reflex) is integrated in the occipital cortex. The righting reflexes are:

1. Labyrinthine righting reflex
2. Neck righting reflex
3. Body righting reflex acting on the head
4. Body righting reflex acting on the body
5. Optical righting reflex.

Labyrinthine righting reflex: The stimulation of utricle by gravity due to movement of head results in reflex contraction of neck muscles and the head is held in position in relation to space.

Neck righting reflex acting on the body: The movement of head to a new position causes reflex contraction of muscles of the trunk and limbs to take up a position in relation to the change in position of head. These changes help the organism to orient itself and maintain balance.

Body righting reflex acting on the head: The movement of body also can cause reflex contraction of neck muscles, so that the head can be oriented to a new posture.

Body righting reflex acting on body: When one side of the body is stimulated by pressure and tactile receptors, the tone of the extensors on the opposite side is increased to maintain posture.

Optical righting reflex: Righting of the head also occurs, due to visual stimuli. The center which integrates this reflex is present in the occipital cortex. If labyrinthines are removed or diseased, underwater divers would find it difficult to orient themselves to reach the surface. This is especially significant when vision is impaired.

Placing and hopping reactions: A blindfolded animal which is suspended in air, when comes in contact with any hard surface, the extensor tone of limbs increases. This enables the animal to place itself on the surface. In hopping reaction, the maintenance of balance occurs, when the animal is laterally tilted. The placing and hopping reactions are integrated in the cerebral cortex.

The reflexes described above are seen, when the organism is in static position and during voluntary movement. They are grouped under static reflexes. The reflexes which occur to maintain balance during acceleration of the head are grouped under statokinetic reflexes.

Statokinetic reflexes

Posture maintenance becomes important when there is movement of head. When the head is turned to a new position in space, the tone of limb and body muscles are adjusted to maintain balance and also there is a reflex movement of eyes to fix objects in the visual field. There are two types of receptors responding to two types of acceleration. The vestibular apparatus which is also called labyrinthine apparatus consists of membranous labyrinth containing fluid filled semicircular canals and otolith organs. The semicircular canals respond to **angular acceleration** (rotational movement of head) and otolith organs respond to **linear acceleration** of head such as forward and vertical movement of head.

Semicircular canals

They are enclosed in a bony canal in the inner ear. There are three pairs, comprising *horizontal, superior* and *posterior canals* (Fig. 4.62).These membranous canals are filled with a fluid called endolymph. It has the composition similar to ICF. Each canal is at right angles to one another in each ear. The horizontal canal of one side is in the same plane to that of opposite side, whereas, the superior canal of one side is parallel to the posterior canal of opposite side. Each canal has a dilated part called ampulla which contains the receptors. The semicircular canals emerge from utricle which communicates with saccule. The saccule inturn is connected to cochlea.

In the ampulla, there is a mass of tissue called cristae in which the hair cells are present. The hair cells are the receptors and the hairs are embedded in a gelatinous substance called cupula, which completely blocks the roof of ampulla (Fig. 4.63).The base of hair cells are supplied by the axons of vestibular division of VIII cranial nerve. There is a single tallest hair called **kinocilium** at one end of the cell. The remaining hairs show a progressive decrease in height and these are called **stereocilia** (Fig. 4.64). The hairs are in a polarised state when not stimulated. The bending of stereocilia towards kinocilium causes depolarization of hair cells and

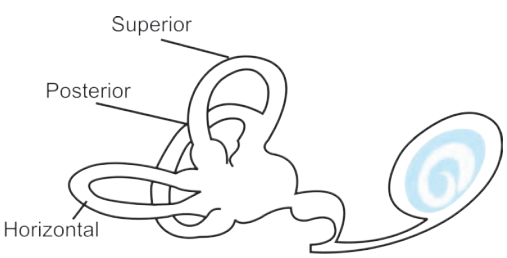


Fig. 4.62: Semicircular canals

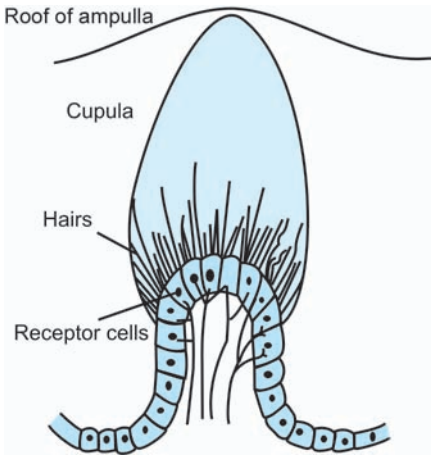


Fig. 4.63: Diagram of cristae present in the ampulla of semicircular canal

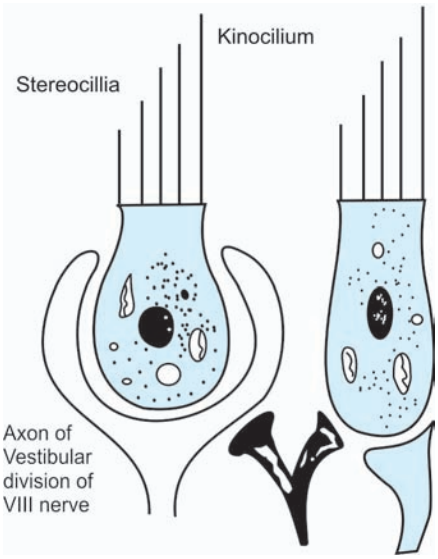


Fig. 4.64: Hair cells in cristae

stereocilia bending away from kinocilium leads to hyperpolarization (Fig. 4.66). The horizontal canal shows stimulation of hair cells as described above. The vertical canals show exactly the opposite mechanism. That is, the bending of stereocilia away from kinocilium causes depolarization of hair cells.

The otolith organs are stimulated by gravitational pull. The utricle responds to horizontal linear movement of head and the saccule is stimulated by vertical linear acceleration of head. They convey information about the static position of head and also changes in its position during linear acceleration. The hair cells are present in the macula and the hairs are covered by calcium carbonate crystals called **otokinia** (Fig. 4.65). Since the specific gravity of otokinia is higher than endolymph, the hairs sink within the otokinia and gravitational pull causes bending of hairs resulting in depolarization of hair cells. Lateral tilting of head would stimulate utricle, whereas, the saccule is stimulated by the linear acceleration of the head, such as, jumping, walking and forward movement of head. The otolith organs stimulation can be noticed during travel in train,car, bus and ship. The symptoms of motion sickness are due to the excessive stimulation of otolith organs.

Stimulation of semicircular canals: There is a tonic basal discharge of impulses from these canals even during absence of rotational movement. The stimulation of the canal will occur, when the rotation is in the same plane as that of the canal. For example, rotation of the head with forward bending of head at 30° would stimulate the horizontal canal. The rotation of head causes movement of endolymph within the canal. Because of the inertia of the fluid, it lags behind rotational movement and when it starts moving, it will be in the opposite direction of rotational movement (Figs 4.66 and 4.67). For example, if rotation of head from right to left occurs, the endolymph in the left canal would move in the opposite direction, but it causes the bending of cupula backward and the hair cells are stimulated in the left canal. This forms the leading canal. In the right canal, the movement of endolymph causes the bending of cupula forward and the hair cells are hyperpolarised. This canal is the trailing canal. When the speed of rotation of head is uniform, then the speed of fluid movement becomes identical to it, the cupula comes back to its normal position and stimulation stops. However, if the speed of head rotation is changed, the rate of change of speed is sensed by the stimulation of hair cells again. When rotation stops (deceleration), the endolymph moves in

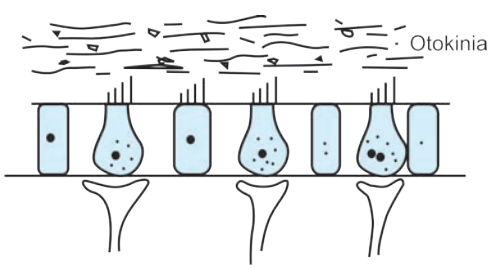


Fig. 4.65: Otolith organs

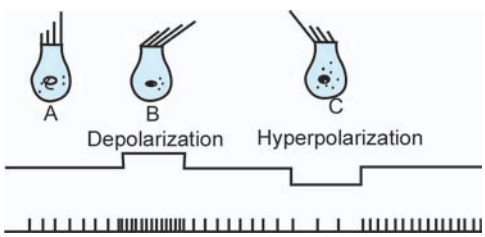


Fig. 4.66: Mechanism of stimulation of hair cells. In horizontal canals, the bending of stereocilia towards kinocilium will produce depolarization, while stereocilia bending away from kinocilium will cause hyperpolarization

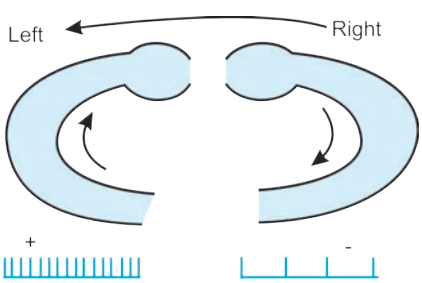


Fig. 4.67: Mechanism of stimulation in horizontal canal. The left canal is the leading canal and the right canal is the trailing canal

opposite direction in the canal, causing the stimulation in the trailing canal and inhibition in the leading canal. At the end of rotation, there is a feeling to fall towards the opposite direction of rotation. This is due to the changes described in the canal at the cessation of rotation. Thus, we observe that the semicircular canals send information to the brainstem about the beginning of rotation, rate of change of speed of rotation and end of rotation.

In the case of vertical canals, the stimulation of superior canal of one side will cause the inhibition of posterior canal of opposite side and vice versa.

Vestibulo ocular reflex

During rotational movement, the eyes move in the opposite direction for visual fixation of objects. This reflex occurs due to the stimulation of semicircular canals. The connection from vestibular nuclei in the medulla, to the III, IV and VI cranial nerve nuclei through medial longitudinal bundle is responsible for the reflex response.

Nystagmus

The jerky movement of the eye at the beginning and end of rotation is known as nystagmus. It is a reflex,which helps in the visual fixation of stationary objects when the body rotates At the start of rotation, the eyes move slowly in a direction opposite to the direction of rotation. Then the eyes quickly move to a new fixation point. This is followed by slow movement in the opposite direction. The quick component of eye movement during nystagmus gives the direction of rotation. When rotation stops, the quick component will be in the opposite direction of rotation (post-rotatory nystagmus). The slow component is due to the impulses coming from labyrinths and the quick component occurs due to the activity of brain stem. Lesion of labyrinths or cerebellum results in spontaneous nystagmus. That is, without any rotational movement the nystagmus occurs and is abnormal.

Central vestibular connections

The axons (vestibular division of VIII cranial nerve) coming from hair cells of membranous labyrinths and otolith organs, reach the brain stem and end in four groups of vestibular nuclei (superior, inferior, medial and lateral). The lateral nucleus **(Deiter's nucleus)** sends descending fibers to the spinal cord forming vestibulospinal tract (Fig. 4.68). The vestibular nuclei receive connections (inhibitory) from cortex and cerebellum. It also sends connections to vestibulo cerebellum. The medial longitudinal bundle from vestibular nuclei sends connections to 3rd, 4 th and 6 th cranial nerve nuclei. The vestibulo spinal tract activity helps to change the tone of extensors of limbs and body during change in position of head. The connections from vestibular nuclei to cranial nerve nuclei supplying ocular muscles help to give visual fixation of objects during movement of head.

Effects of excessive stimulation of vestibular apparatus

During motion in train, bus, ship, car, elevators, etc. the labyrinths are under constant stimulation and this leads to autonomic disturbances like headache, vertigo, nausea, vomiting, sweating and changes in blood pressure. These symptoms are called **motion sickness.**

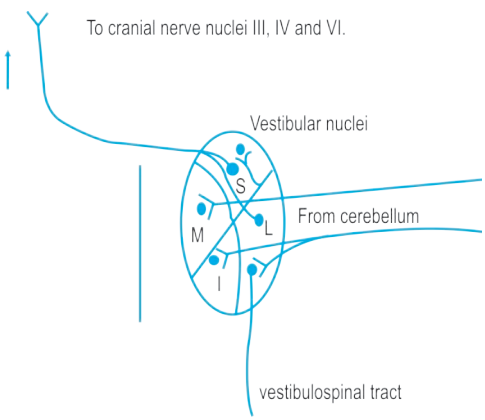


Fig. 4.68: Connections of vestibular nuclei

Vertigo: It is false sense of rotation when actual rotation is not present. This occurs in motion sickness, and clinical stimulation of semicircular canals.

Clinical stimulation of semicircular canals is done by irrigating the ear with cold or warm saline with the help of a syringe. The temperature difference between the saline and endolymph will cause convection currents, resulting in stimulation of the canal and symptoms of nystagmus, vertigo, nausea, etc, will occur. The canal is also stimulated clinically by using Barany chair and subjecting the individual for rotation in the horizontal direction. This stimulates the horizontal canal and the symptoms described above can be noticed.

THALAMUS

It is a mass of grey matter consisting of two thalami. The two thalami are connected by massa intermedia, which contain midline group of intrinsic nuclei.In each thalamus, there is a intralaminar group, which also contain intrinsic nuclei.

There are specific and nonspecific nuclei which are concerned with the regulation of cortical activity, visceral functions, emotion, sensory and motor functions.

The specific nuclei of thalamus are divided as follows (Fig. 4.69):

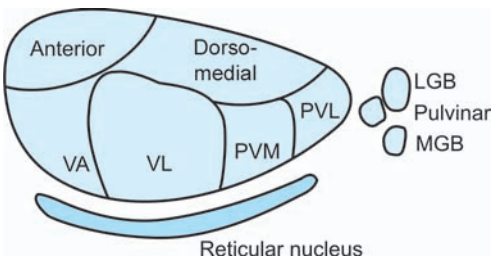


Fig. 4.69: Nuclei of thalamus

VA ventral anterior; VL ventrolateral;
PVM posteroventromedial; PVL posteroventrolateral;
LGB lateral geniculate body; MGB medial geniculate body

Anterior nucleus

Dorsal

Dorsomedial
Dorsolateral

Ventral

Anterior lateral
Posterior lateral
Medial

Pulvinar
Lateral geniculate body
Medial geniculate body
Reticular nucleus
Connections of thalamic nuclei

Thalamo-cortical-thalamic circuit

The thalamic nuclei, especially the midline group, intralaminar group, dorsomedial and lateral groups send projections to neocortex and from neocortex, there are connections coming back to thalamus forming a circuit (thalamo-cortical-thalamic circuit). This is responsible for both cortical and thalamic activity. The electrical activity (EEG)of cortical neurons is due to the activity of thalamocortical projections.

Anterior nucleus

The afferents come from the mammillary body of hypothalamus and the efferents go to cingulate gyrus. This forms part of the circuit for emotion.

Dorsomedial

It receives connections from striatum and hypothalamus and sends efferents to cortex.

Ventral anterior

Afferents from globus pallidus and substantia nigra go to this nucleus. The efferents are projected to the frontal cortex.

Ventrolateral

Fibers from dentate nucleus of cerebellum go to this nucleus directly or through red nucleus. Efferent connections from here go to frontal lobe.

Ventral posterior lateral (VPL)

It receives sensory projections from spinal and medial lemnisci, which carry protopathic and dorsal column sensations respectively. The VPL nucleus form the sensory relay center for these sensations coming from all parts of the body except face region.

Ventral posterior medial

It receives sensory projections from the face region through trigeminal lemniscus.

Pulvinar

It receives connections from midline and intralaminar groups of nuclei and sends its projections to association cortex.

Lateral geniculate body

The visual impulses carried by the optic tract are relayed here.

Medial geniculate body

It forms the auditory relay center.

Reticular nucleus

It receives afferents from intrinsic nuclei and sends projections to the cortex.

Intrinsic nuclei

The midline and intralaminar group of nuclei receive afferents from reticular formation. From reticular formation diffuse projections, which are nonspecific go to cortex directly and also through thalamic intrinsic nuclei.These nonspecific projections form **ARAS (ascending reticular activating system**) and is responsible for alertness and arousal reaction.

Functions of thalamus

- Thalamus forms the sensory relay center. The dorsal column sensations are particularly important, as the lesion of thalamus causes loss of touch, pressure, vibratory and kinaesthetic sensations. The appearance of astereognosis is not uncommon in thalamic lesions.
- Center for crude sensations. The medial group of nucleus receive fibers from VPL nucleus and perceive sensations of pain and temperature. In thalamic lesions which spare medial group of nuclei, there is increased pain sensation known as thalamic pain can be noticed.
- It regulates emotion and visceral functions. The neural circuit for emotion passes through the anterior nucleus of thalamus. Through its connections with limbic system, hypothalamus and frontal cortex, it helps in the regulation of emotion and visceral functions.
- Participation in ARAS: Intrinsic thalamic nuclei receive afferents from reticular formation which are nonspecific in nature. From here nonspecific diffuse projections go to the cortex. There is also nonspecific diffuse projections directly going to the cortex without passing through the thalamus. This ascending reticular activating system is responsible for arousal and alertness.
- Regulation of voluntary movements: The thalamus is strategically situated connecting neocerebellum and frontal motor cortex. The cerebro-cerebellar-cerebral neural circuit passes through ventrolateral nucleus. Lesions of thalamus give disturbance in voluntary actions such as ataxia and intention tremor.

Thalamic syndrome

- Thrombosis of posterolateral branch of posterior cerebral artery results in ischaemia of posteroventral thalamus. The symptoms observed in such lesions are:
- Increased pain (thalamic pain)
- Astereognosis
- Ataxia
- Intention tremor
- Emotional disturbances
- Thalamic hand (flexion of arm at the elbow, extension at the wrist and interphalangeal joints).
- Phantom limb (In amputated persons the feeling of limb being present will be there, even though it has been amputated)

RETICULAR FORMATION

Brainstem contains a number of ill-defined, non-specific network of nerve cells and nerve fibers which constitute the reticular formation. It contains both descending and ascending pathways.

Connections to reticular formation are formed from cerebral cortex, basal ganglia, cerebellum, thalamus and hypothalamus. The ascending specific sensory tracts before ending in thalamic nuclei give off collaterals to the brain stem reticular formation.

ARAS (ascending reticular activating system)

Reticular formation sends diffuse nonspecific projections to cortex directly and also through intralaminar nuclei (Fig. 4.70). This ascending nonspecific pathway is called ARAS, as it is responsible for the cortical activity, leading to arousal and alertness. The EEG waves become desynchronised fast activity giving β waves. The activity of ARAS to cortex depends upon the sensory input, through collaterals from ascending sensory tracts. Decrease in the activity of ARAS as a result of decreased sensory stimuli causes sleep, with EEG showing delta waves. The arousal reaction caused by the activity of ARAS, gives background activity in the cortex, for proper appreciation of sensory stimuli which are projected to it. The awareness of sensory stimuli, environment and formation of thought processes leads to consciousness. The ARAS activity is believed to be responsible for these functions. The secondary response of evoked potentials is due to the ARAS activity. The arousal state is enhanced by epinephrine or sympathetic stimulation, which increases the ARAS activity. The actions of anesthesia, tranquilizers are mediated through the suppression of ARAS activity.

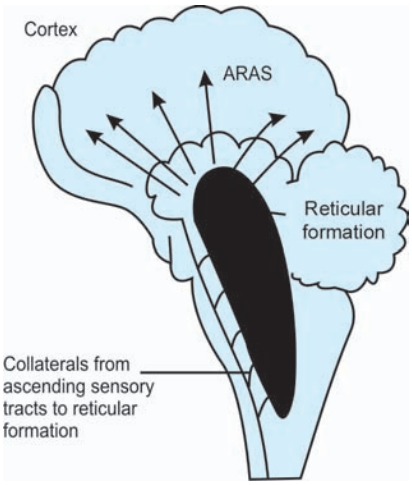


Fig. 4.70: Ascending reticular activating system (ARAS)

Descending reticulospinal pathway

The reticular formation in the medullary region receives inhibitory fibers from cerebral cortex caudate nucleus and anterior lobe of cerebellum (Fig. 4.61). The pontine reticular formation receives facilitatory influence from cortex. The activity of descending reticular formation depends upon the balance of activity between facilitatory and inhibitory regions of reticular formation. The descending reticulospinal tract influences γ motor neuron supplying the muscle spindles and maintains muscle tone. If facilitatory pontine reticular formation activity is unopposed, there will be increased firing of γ motor neurons resulting in rigidity.

If pontine reticular formation is suppressed or damaged, the muscle tone will be lost and posture cannot be maintained.

Functions of reticular formation

- Reticulospinal tract maintains muscle tone through its influence on γ motor neurons.
- The activity of ARAS gives rise to arousal and alertness. It is also responsible for sleep-wake cycle. Damage to the reticular activating system results in loss of consciousness (coma).
- Medullary reticular formation contains nerve cells, which are centers for regulating the activity of visceral organs such as heart, lungs, blood vessels, etc. Damage to medullary reticular formation suppresses the activity of these vital centers and results in death.

ELECTROENCEPHALOGRAM (EEG)

The thalamocortical nonspecific projections end on dendrites of cortical neurons and produce local hypo or hyperpolarizations, depending upon the type of transmitters released at the synapses. This electrical activity at the dendrites of cortical neurons causes current sink as the current goes in and out of dendrites (Fig. 4.71). These local, nonpropagatory synaptic potentials of dendrites of cortical neurons give oscillations or waves and can be recorded from the surface of the scalp. These waves are known as electroencephalogram. The current sink developed from dendrites leads to the formation of all or none law obeying propagatory action potential from the axon.

EEG recording can be done either by using bipolar or unipolar electrodes. The amplitude of the waves ranges from 5 μv to 100 μv.

Recording of EEG includes four types of waves.

Alpha wave

It has a frequency of 8 to 13/sec and amplitude ranges from 50 μv to 100 μv. It is a synchronized wave recorded from a quite brain without any mental activity with the eyes closed. It is present more in the parieto occipital cortex. Alpha wave rhythm can be recorded, if the eyes are closed. The alpha rhythm is also known as **Berger rhythm**. The alpha rhythm disappears when the eyes are open. The disappearance of alpha waves with the opening of eyes is called **alpha block**. The alpha wave is replaced by fast activity desynchronized beta waves.

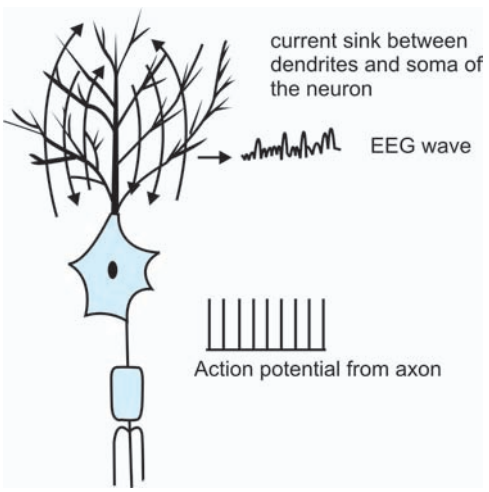


Fig. 4.71: Diagram to show how EEG waves are developed

The EEG wave is due to the dendritic activity. They are not the action potentials

Beta wave

The frequency is 13 to 30 cycles/sec and the amplitude is 5 to 10 μv. It is a desynchronized fast activity wave produced during alertness or arousal state.

Theta wave

It is usually seen during light sleep and not when awake. Its frequency is 4 to 7/sec. The amplitude is recorded upto 10 μv. The presence of theta wave in EEG recording of adults when awake indicates brain tumors.

Delta wave

It is a slow wave with a frequency of 1 to 4/sec and amplitude reaches upto 100 μv. It is produced during deep sleep in adults. It is not seen in adults when awake. If produced, it indicates brain lesions. Delta wave is normally seen in newborn infants.

γ oscillations: They are recorded when an individual is aroused and focussed attention on some thing. Its amplitude ranges from 30 to 80 μv.

Uses of EEG

Clinically EEG recording is useful to localize pathological conditions like subdural hematoma, where the EEG activity is damped. EEG recording is also useful to identify epileptogenic foci in the brain. During epileptic seizures the EEG shows high voltage waves.

SLEEP

Sleep-wake cycle follows circadian rhythm. In adults, the sleep period lasts for 6 to 7 hours and 16 to 18 hours in infants. The duration of sleep is gradually decreased during childhood. In old age, the duration is further reduced. During sleep, there is a temporary loss of consciousness with physiological changes, such as, fall in heart rate, blood pressure, respiratory rate and muscle tone.

There are two types of sleep which can be observed in an individual. They are **slow wave sleep (SWS)** and **REM sleep** (rapid eye movement sleep).

Slow wave sleep

It is a deep sleep and is interrupted 4 to 6 times in a day by REM sleep. SWS can be described with the help of EEG recording. There are four phases that can be noticed (Fig. 4.72).

I phase: The EEG shows fast activity beta waves (13 to 30 Hz) which are desynchronized. It indicates wakefulness.

II phase: The EEG shows synchronized alpha waves (8 to 13 Hz) as the person enters drowsiness.

III phase: The EEG records theta waves (4 to 7 Hz) and interspersed with spindles called sleep spindles. In this phase the individual enters light to moderate sleep.

IV phase: It is characterized by the presence of only slow waves delta (1 to 4 Hz). The sleep spindles disappear. The presence of delta wave during sleep indicates deep sleep state.

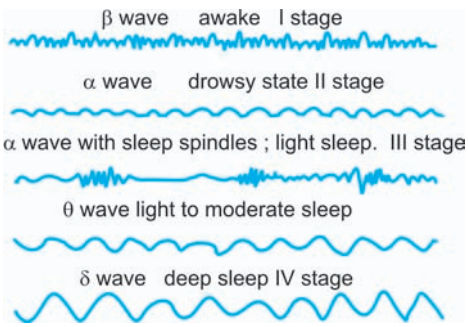


Fig. 4.72: Recording of EEG during slow wave sleep

REM sleep

During every 80 to 90 minutes of SWS, the pattern of sleep changes. The deep sleep changes into light sleep, which leads to REM sleep lasting for a few minutes. This period gives rise to once again SWS. It can be seen that with successive REM periods, the interval between them is shortened and the duration of REM is increased (Fig. 4.73). REM sleep is increased in infants, with a greater increase in premature infants.

Sleep disorders

- **Insomnia**: Lack of sleep. It occurs due to various medical and psychological conditions.
- **Norcolepsy:** It is the urge to go to sleep during day time. In this condition the REM sleep starts directly from light sleep with out going into deep sleep state.

REM sleep is characterized by rapid eye movements and it coincides with dream state. The EEG recording shows fast, desynchronized beta activity characteristic of arousal, eventhough, the threshold of awakening is increased. That is why, REM sleep is also called **paradoxical sleep**. During REM sleep, the irregularities in cardiac action, arterial pressure, respiration can be noticed. It is during REM period, occasionally awakening occurs during sleep.

Genesis of SWS

There are three subcortical regions namely diencephalic sleep zone, medullary reticular formation and basal as basal forebrain sleep zone

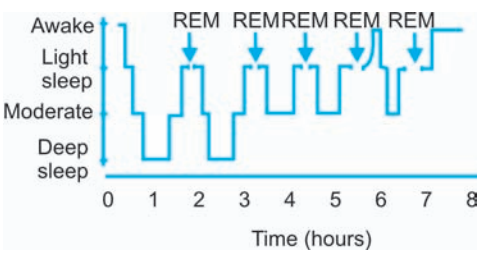


Fig. 4.73: REM sleep periods in an adult

involved in the slow wave sleep. Recent studies have shown that serotonin agonists suppress sleep and its antagonists increases slow wave sleep in humans.

Genesis of REM sleep

The center of REM sleep is situated in the pontine reticular formation. The ponto-geniculo-occipital spikes (PGO) develop due to cholinergic neuron activity, which causes REM sleep.

REM sleep is now considered significant in human beings for memory consolidation.

HYPOTHALAMUS

It is present in diencephalon and includes a number of nuclei. They are closely linked to limbic system, brainstem, cortex, and pituitary gland by their connections. These connections help the hypothalamus to control visceral, autonomic, endocrine and emotional responses. Hypothalamic neuronal activity gives appropriate behavioral responses to visceral and somatic sensory stimuli. The normal homeostasis in an organism depends upon the control of visceral and somatic functions, which are integrated in the hypothalamus.

Nuclei of hypothalamus

The nuclei can be included under four groups namely, periventricular, medial, lateral and posterior (Fig. 4.74).

The **periventricular group** includes the following nuclei:
Anterior
Arcuate
Median eminence
Suprachiasmatic
Supra optic
Para ventricular

Medial group contains

Dorso medial
Ventro medial
Medial preoptic

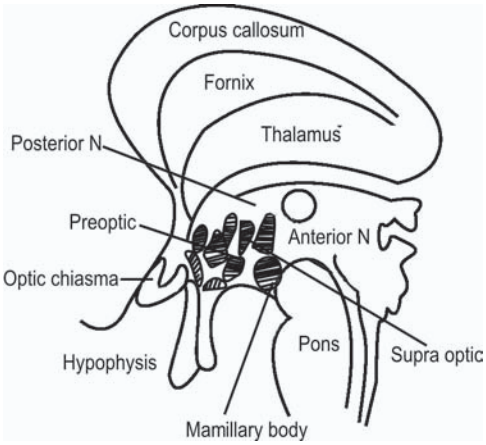


Fig. 4.74: Hypothalamic nuclei

Lateral group includes

Lateral preoptic
Lateral hypothalamus

Posterior group contains

Posterior nucleus
Mamillary body

Connections of hypothalamic nuclei

Afferent connections

Fornix connects hippocampus of limbic system to mamillary body.

Stria terminalis connects amygdala to lateral hypothalamus and preoptic area.

Medial forebrain bundle links septum to various hypothalamic nuclei.

Pallidothalamic fiber connects basal ganglia (lenticular nucleus) to the hypothalamus.

Noradrenergic bundle from locus ceruleus in the brainstem projects to the hypothalamus.

Serotonergic projections from Raphe nucleus go to the hypothalamus.

Efferent connections

Mamillo thalamic tract
Mamillo tegmental tract
Medial forebrain bundle from hypothalamus to the brainstem

Tuberoinfundibular tract
Hypothalamo hypophysial tract

Functions of hypothalamus

The major functions of hypothalamus are in the regulation of **visceral functions, behavior,** and **emotion.** They are achieved by the hypothalamic control of autonomic nervous system, endocrine glands, somatic and motor functions.

Control of autonomic nervous system

Hypothalamus has a number of areas where electrical stimulation causes effects of sympathetic stimulation which is part of fear and rage reaction. Electrical stimulation of lateral hypothalamus, and posterior hypothalamus results in increased heart rate, increase in arterial blood pressure, pupillary dilatation, piloerection, etc. They are the effects of sympathetic stimulation and from these studies, these areas are considered for controlling sympathetic activity. Electrical stimulation of anterior hypothalamus gives parasympathetic responses, such as, salivation, fall in heart rate, fall in blood pressure, increase in GI secretion and motility, micturition, etc. These effects are due to the activity of parasympathetic nervous system and this area has been considered for controlling parasympathetic activity. The autonomic responses mentioned above are necessary for the regulation of visceral function and maintenance of homeostasis.

Temperature regulation

Hypothalamus acts as a **thermostat,** regulating the body temperature. The anterior hypothalamus responds to the rise in body temperature. Lesion of this region causes hyperthermia and absence of cutaneous dilatation and sweating when body temperature rises. Anterior hypothalamus acts as **heat loss center**.

The posterior hypothalamus is stimulated by fall in body temperature (hypothermia). It causes cutaneous vasoconstriction, piloerection (goose bumps in humans) and shivering. These effects help to reduce the heat loss and increase heat production (shivering). If cold exposure is continued for a long time, thyroid and adrenal medulla are stimulated, as their secretion causes calorigenic action. The posterior hypothalamus is considered as **heat gain center**. The normal body temperature is due to the balance of activity between the heat loss and heat gain centers.

Regulation of food intake

Ventromedial nucleus of hypothalamus, when stimulated, causes stoppage of eating, while, the lesion of the same nucleus results in voracious eating (hyperphagia) leading to obesity. The ventromedial nucleus is the **satiety center**. The lateral hypothalamic nucleus is considered as **feeding center.** Electrical stimulation of this area causes increased appetite and feeding. Normal feeding depends upon the balance in the activity of satiety and feeding centers. The satiety center is tonically active as the glucoreceptors present around this nucleus show entry of glucose into it. The activity of VMN results in inhibition of feeding center. The satiety center is also influenced by distention of stomach and release of GI hormones like CCK, GRP, Somatostatin and glucagon.

The food intake regulation has also been explained by mechanisms, which include the suppression of appetite, and decreased food intake by the release of hormone **leptin** from adipose tissue, whenever fat deposition is increased. Leptin acts on the hypothalamus and decreases food intake by decreasing the activity of **neuropeptide Y**. The hormone neuropeptide Y when released causes increased food intake.

Regulation of body weight

Whenever the inhibition of satiety center occurs, it leads to increased food intake. The body weight increases and the satiety center is now reset for this increased body weight. After the attainment of increased body weight, the satiety center once again becomes active and inhibits the feeding center. This observation in animals led to the theory that VMN is responsible more for regulating the body weight, than regulating the feeding center.

Water balance

Lateral nuclei of hypothalamus, supraoptic nucleus, and regions around circumventricular organs near the third ventricle are considered centers for regulating water balance in the body. Increase in osmolality of plasma, as in loss of water or infusion of hypertonic saline causes the activity of osmoreceptors located in anterior hypothalamus. The rise in tonicity of blood stimulates osmoreceptors and causes thirst. There is also secretion of vasopressin from suprotic and paraventricular nuclei, due to the shrinking of osmoreceptors. The vasopressin hormone causes reabsorption of water in the distal segment of nephron. The fall in body water would also cause a fall in blood volume. This stimulates renal renin- angiotensin system. The angiotensin II that is formed enters the hypothalamus and stimulates the thirst center situated in the subfornical organ and OVLT. This causes drinking behavior.

Control of anterior pituitary

Periventricular nucleus and arcuate nucleus send projections to median eminence as tubero infundibular tract. These neurons secrete various peptides and monoamines, which are either excitatory or inhibitory on anterior pituitary hormone secretion. These hormones are called hypothalamic releasing and inhibiting hormones. From median eminence, they are transported to the anterior pituitary through hypophysial portal circulation. Some of the releasing hormones and their actions are as follows.

CRH- stimulates ACTH secretion

TRH- stimulates TSH,Growth hormone and prolactin secretion.

Somatostatin- inhibits growth hormone and TSH secretion.

GnRH- stimulates FSH and LH secretion.

Secretion of posterior pituitary hormones

The supraoptic and paraventricular nuclear groups secrete vasopressin and oxytocin, which are released from posterior pituitary. They are synthesized as large precursor molecule known as neurophysins I and II and transported along the axons of the hypothalamo-hypophysial tract to posterior pituitary.

Rage

Certain regions of hypothalamus like lateral hypothalamus and periventricular zone when stimulated show rage response. This aggressive behavior is due to hypothalamic connection with neocortex. If this connection is cut, the rage response can not be seen.

Sex behavior

Hypothalamus through its connection with the nuclei of limbic system, neocortex, endocrine glands and brainstem controls sex behavior. In humans, sex behavior is highly encephalized and hence the neocortex and association areas influence the sex behavior. In lower animals, oestrus, copulation are controlled by the hypothalamic nuclei, which include ventral and medial preoptic nuclei (Table 4.3).

Sleep

The diencephalic zone which includes posterior hypothalamus produces sleep when low frequency stimulation occurs. The basal forebrain sleep zone, which includes preoptic area is another region in the hypothalamus which also causes sleep by both low and high frequency stimulation. Damage to fibers coming from posterior hypothalamus results in sleep disorders.

Motivation

Medial forebrain bundle, which projects from limbic system to the brain stem through hypothalamic nuclei, is considered to be responsible for motivational behavior. It includes reward where the animal experiences pleasant effect when medial forebrain bundle or regions of hypothalamus and limbic system are stimulated electrically. The animal is motivated to press the

Tabel 4.3: Regions involved and their functions in hypothalamus

No	*Functions*	*Regions Involved*
1	Regulation of body temperature	Anterior hypothalamus (heat loss)Posterior hypothalamus (heat gain)
2	Regulation of autonomic nervous system	Anterior hypothalamus – parasympathetic activity Posterior hypothalamus – sympathetic activity
3	Regulation of body weight by feeding and satiety	Lateral nucleus – feeding Ventromedial nucleus – satiety
4	Regulation of plasma osmolality and ECF volume by thirst and secretion of ADH	Anterior hypothalamus (osmoreceptors)
5	Regulation of anterior pituitary hormone secretion	Median eminence
6	Posterior pituitary gland secretion	Supraoptic and paraventricular nuclei
7	Regulation of motivational behavior by reward and punishment	Medial forebrain bundle
8	Regulation of rage and sexual behavior	Lateral hypothalamus (Rage)Anterior and ventral hypothalamus (Sexual behavior)
9	Control of sleep	Preoptic (Basal forebrain sleep zone) Suprachiasmatic nucleus Diencephalic sleep zone (Posterior hypothalamus)
10	Control of body rhythm	Suprachiasmatic nucleus

bar for self-stimulation. When the animal experiences unpleasant effect caused by the foot shock, it is taken as punishment and the animal learns to avoid it. This reward and punishment are considered as motivation for the animal. Motivation is vital for the survival of the species.

Circadian rhythm

Suprachiasmatic nucleus is the center which controls circadian rhythm in the body such as secretion of adrenocortical hormones, body temperature, sleep-wake, etc. The input to the suprachiasmatic nucleus is the fiber coming from optic chiasma. The visual cues are essential for the diurnal rhythm.

Immunity

The hypothalamic regulation of sympathetic nervous system, adrenocortical and medullary secretions produce its effect on the immunity also. It is commonly observed that chronic exposure to psychological stress, which activates hypothalamo-pituitary-adrenal axis, results in suppression of immunity. Lesions of hypothalamic nuclei which are involved in the control of these functions, cause decreased immune response as evidenced by the reduced T cell lymphocytes in the blood.

LIMBIC SYSTEM

Limbic system is present in rhombencephalon and includes structures in allocortex, neocortex and subcortical areas.

The **allocortical** areas include

Hippocampus
Dentate gyrus
Olfactory area
Septum.

Areas of limbic system included in the cortex

Cingulate gyrus
subcallosal gyrus
Parieto-temporal cortex
Orbito-frontal lobe
Parahippocampal gyrus
Uncus

Subcortical areas

Anterior nucleus of thalamus
Amygdala
Basal ganglia
Hypothalamus

In lower animals, the limbic lobe serves largely for olfactory function, whereas, in humans, olfactory function of limbic lobe is reduced. It

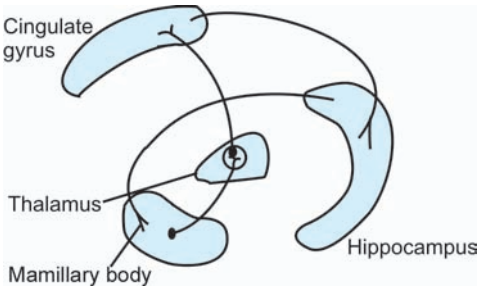


Fig. 4.75: Papez circuit for emotion

serves to control visceral function, emotion and behavior.These functions are achieved by its close connection with the hypothalamus.

Connections

- Through *fornix* hippocampus is connected to mamillary body of hypothalamus. From here fibers go to anterior nucleus of thalamus. The thalamic nuclei are connected to cingulate gyrus. The cingulate gyrus completes the circuit by sending fibers to hippocampus. This is the **Papez circuit** for emotion (Fig. 4.75).
- Stria terminalis connects amygdala to hypothalamus
- Medial forebrain bundle connects limbic system to brainstem through hypothalamus.

Functions of limbic lobe

Emotion

The **Papez circuit** described above is essential for emotion. Emotion consists of **cognition**, **affect** and **conation.** Cognition refers to the awareness of emotional stimuli. The affect will include the feelings like joy, sad, grief, love, hatred, anger, fear, etc. The conation helps to give appropriate motor response. There is always autonomic visceral activity, such as rise in heart rate, rise in blood pressure, respiratory changes, etc. and limbic system is responsible for emotion and behavioral response associated with it.

Regulation of visceral activity

The amygdala is considered to be involved in regulating visceral behavior through its connection with the hypothalamus. Electrical stimulation of amygdala causes aggressive behavior and absence of appetite, while the lesion of amygdala results in placidity, hyperphagia and hypersexuality.

Memory and learning

Hippocampus is involved in learning and formation of recent memory. The activity of **CA 1** neurons is especially believed to be involved in recent memory. The connection from hippocampus to neocortex consolidates recent memory into long-term memory. Moreover hippocampal neurons have **NMDA** receptors which show that glutamate and aspartate amino acids are released as neurotransmitters. These receptors are responsible for the **long-term potentiation (LTP).** In LTP, there is a long lasting facilitation of synaptic transmission in neurons following a brief period of high frequency stimulation. LTP is involved in learning and memory. The hippocampal neurons also show plasticity which is associated with learning.

Kindling effect

The hippocampus and olfactory cortex are susceptible to seizure induction by repeated weak electrical stimulation. Kindling effect gives us the understanding of learning process, as there is similar synaptic enhancement in both the conditions.

Motivation

Medial forebrain bundle which starts from limbic lobe projects to the brain stem through hypothalamus. Noradrenergic and dopaminergic neurons are said to be present in this tract. These monoamines are considered to be important for motivation and drive.

CEREBRAL CORTEX

Cytoarchitecture and higher functions

Cerebral hemisphere shows a number of foldings on the surface called gyri and grooves which

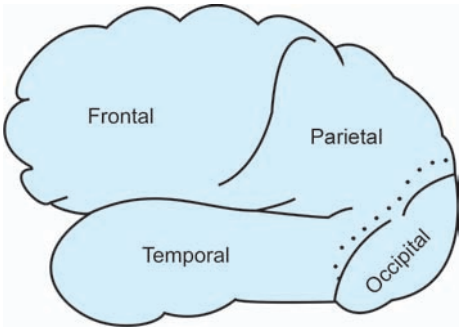


Fig. 4.76: Lateral surface of cerebral hemisphere to show the four lobes

separates them. There are two types of grooves namely sulci for shallow ones and fissures for deeper grooves. The surface of the cortex contains grey matter and inner to it is the white matter. The two hemispheres are connected by corpus callosum. Each hemisphere is divided into four lobes, present overlying the corresponding skull bones (Fig. 4.76).

The cytoarchitecture of cerebral cortex shows that 90% is made of neocortex and the remaining 10% includes allocortex and juxtallocortex. The neocortex is well developed in humans and it contains 6 layers. Allocortex has 3 layers and juxtallocortex contains 4 to 5 layers. The latter includes the structures between the allocortex and neocortex. Allocortex includes hippocampus, which is present on the medial surface of the cortex bordering brain stem. Part of hippocampus is folded into temporal lobe and cannot be seen directly unless dissected out.

Parahippocampal parts such as dentate gyrus and subiculum are considered as juxtallocortical regions.

Neocortex: The cytoarchitecture of neocortex shows the presence of 6 layers, which are described from above downwards as follows (Fig. 4.77).

I. **Molecular layer:** It is made up of synapses between the axon endings and dendrites.
II. **Outer granular layer:** It includes stellate cells which are interneurons. The cell body is small and dendrites are branched and present with

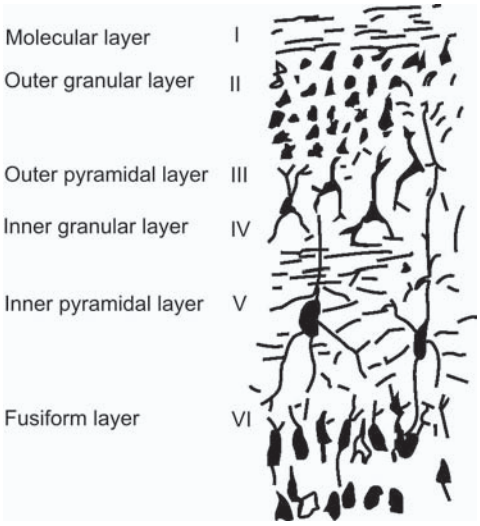


Fig. 4.77: Cytoarchitecture of cerebral cortex

spines (spiny stellate cells) and without spines (smooth stellate cells). The axon is short and projects upwards.

III. **Outer pyramidal layer:** The cells have small pyramidal cells. The axons coming from these cells are connected to other cortical areas.
IV. **Inner granular layer:** It consists of stellate cells especially the spiny types, which are excitatory in nature.
The layer IV is well developed in sensory cortex and visual cortex. The IV layer in these regions is called **outer lines of Baillarger** as the axons form a horizontal band. The cells in layer IV receive afferents from specific thalamic nuclei.
V. **Inner pyramidal layer:** It includes large pyramidal cells which are triangular in shape. There is a long dendrite going up with profuse branches (Fig. 4.78).

There are also dendrites around the basal part of the soma. A long axon from the soma goes downwards giving collaterals as they enter the brainstem. The axon projects upto spinal cord and forms corticospinal tract. Projections also go to thalamus nonspecific nuclei from the axon, which inturn sends fibers to cortex.

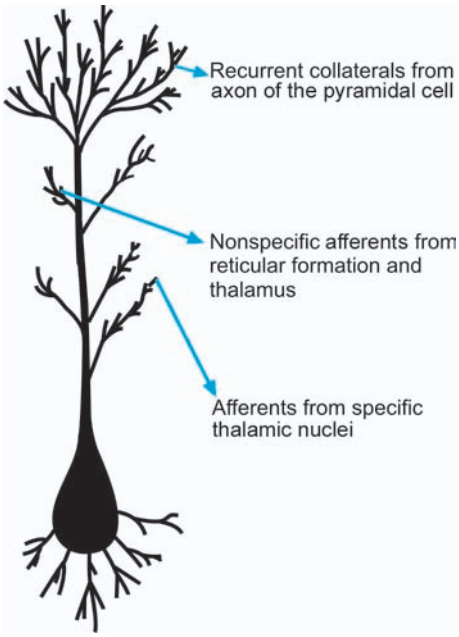


Fig. 4.78: Diagram of a pyramidal cell and the sites of important connections with other neurons

The inner pyramidal cell layer forms the **inner layer of Baillarger.**

VI. **Fusiform cell layer** (multiform cell layer): These cells are fusiform in shape and there are dendrites on both sides of soma. There is a short axon going downwards. Reciprocal connections between cortical and thalamic nuclei are present from this layer and these thalamocortical and corticothalamic projections are responsible for EEG activity.

In neocortex, all the six layers are not uniformly present in all regions. There are variations within the cortex. Totally there are 5 types of cytoarchitecture which can be seen. Type I is present in motor cortex where granular cells are few and pyramidal cells are more in number and they are called **agranular cortex**. In sensory areas of the cortex the granular cells predominate and they are called **granular cortex**. This forms the type V. In other types (II to IV) all the six layers are present forming **homotypical cortex.**

Methods of studying cortical function

Functions of cortex have been known from the effects of lesion, ablation and electrical stimulation of various areas of the cortex in animals. In humans, observations made on the effects of damage caused by brain tumors, effects noticed during surgical interventions to treat brain diseases, study of epilepsy, study of electrical activity of the brain by EEG recording, have also helped to understand the functions of cortex. More about cortical function can be obtained, especially to assess the activity of sensory pathway and its receiving area in the cortex by evoked potential recording.

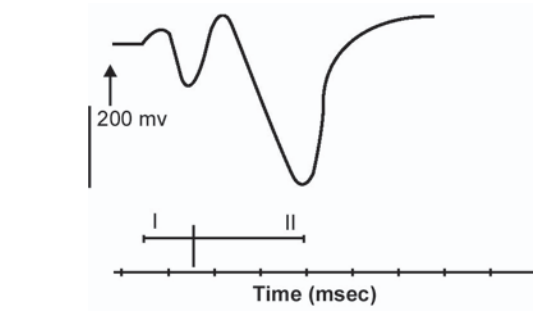
Evoked potential

If a sensory stimulus is given in the right toe of the foot, electrical potentials can be recorded from the left sensory cortex where right toe is represented. Similarly evoked potential can be obtained in the visual cortex and auditory cortex, for flash of light and click sound respectively.

Auditory evoked potential recording, helps to understand the brain stem activity and presence of any lesions in the auditory cortex can be detected.

Evoked potential recording shows surface positive and surface negative waves. That means, the deflections in the record would be in the opposite directions. The recording gives **primary evoked potential** and a **secondary diffuse response** (Fig. 4.79). The primary evoked potential comes after a short latency of 6 to 12 msec. It has a surface negative and positive waves (positive and negative deflections). The secondary diffuse response are recorded after a long latency of 30 to 40 msec and it has a large surface negative wave (positive deflection).

In **event related potentials (ERP)** recording, there is also another component called P_{300}. It is a long latency positive wave coming after 300 msec and it signifies the activity of the cortex, related to expectation, attention, psychological correlates and other higher neural functions.



I Primary evoked response

II Secondary evoked response

Arrow indicates the point of stimulation of peripheral nerve

Fig. 4.79: Evoked potential recorded from the contralateral sensory cortex by the stimulation of peripheral nerve in the limb

The cause of primary evoked potential is due to the activity of specific thalamocortical projection for the applied stimulus. That is why the response is localized in the receiving area of cortex.

The secondary diffuse response is due to the activity of nonspecific projections from thalamus to cortex. Hence the secondary response can be recorded from diffuse areas of the cortex.

Brain function can also be studied by **CT (computerized tomography), PET (positron emission tomography) and MRI (magnetic resonance imaging).** In PET scanning study, a radioactive isotope which emits positron is tagged to **2-deoxy glucose**. This compound is taken by the neurons but cannot be utilized by the cell. The concentration of deoxyglucose uptake by neurons is related to its activity. The PET scan study helps us to know the activity of different areas of the brain.

Functional areas of cerebral cortex (Figs 4.80 and 4.81)

Pioneering work in this field was done by Brodmann and Penfield. They were able to locate various functional areas in the cortex by observing the effects of electrical stimulation of different regions in the cortex in animals and similar studies in human subjects, who underwent neurosurgical operations. The functional areas have been given numerical numbers and called Brodmann's areas (see next page box).

Parietal lobe

It is present behind the post central gyrus and includes primary sensory area SI and posterior parietal cortex containing sensory association areas. The parietal cortex is concerned with perception of touch, two point discrimination, vibratory, pressure, kinesthetic, discriminatory temperature and stereognosis.The association areas help to interpret and give meaning to the sensory stimuli. Lesion of parietal cortex in the representational hemisphere results in disturbance of perception and behavior. The disorders include **astereognosis,** neglect (attention deficits), **anosognosia** (denial of one's

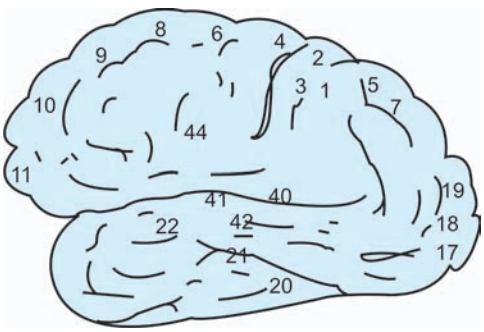


Fig. 4.80: Lateral surface of cerebral hemisphere showing Brodmann's areas

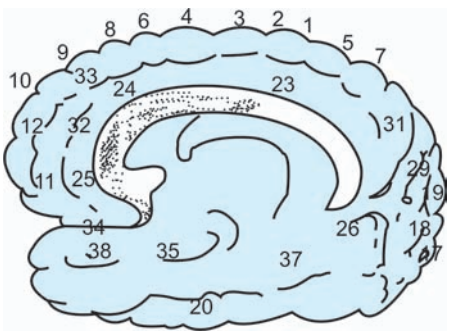


Fig. 4.81: Medial surface of cerebral hemisphere showing Brodmann's cortical areas

own parts of the body). Lesion of parietal lobe in the representational hemisphere also produces **Agnosia**. It is the inability to recognise objects by a sensory modality. Lesions of inferior parietal lobule in the posterior parietal lobe causes unilateral inattention and neglect.

Frontal lobe

It is present infront of precentral gyrus.It includes motor cortex and premotor area. They give fibers to corticospinal tract. The details of motor cortex have been dealt under motor system.

Temporal lobe

Temporal lobe includes auditory areas. It is intimately connected to limbic cortex and hence lesions of temporal lobe results in disorders that are similar to the effects of lesion of amygdala. The **temporal lobe syndrome** caused by the lesion of temporal lobe shows disturbances in visceral function and behavior. The symptoms include:

Placidity
Hyperphagia
Visual agnosia
Hypersexuality
Oral tendency while examining objects.

These symptoms are observed in animals with bilateral removal of temporal lobe and the animals are known as **Kluver-Bucy animals.**

In humans, temporal lobe syndrome can also give seizures (psychomotor epilepsy or temporal lobe epilepsy) which is characterized by emotional experiences and autonomic discharges. The subjects would also experience more often a situation as already seen **(deja vu)** or never seen when actually seen earlier **(samais vu).**

Association areas

Association areas are involved in understanding and interpretation of sensations (somatic, visual and auditory), elaboration of thoughts, speech and memory. The important association areas include **prefrontal-parieto-temporal-occipital.**

Functional areas of cerebral cortex

Brodmann's area no	*Location and function*
4	Precentral gyrus Motor cortex
3,1 and 2	Postcentral gyrus; Primary sensory area SI
5 and 7	Sensory association areas
6	Premotor area
8 to12, 13, 23, 24,32, 44 to 47	Prefrontal association areas.
44	Left lower frontal convolution. Broca's area. Motor area of speech.
41 and 42	Temporal cortex. auditory areas.
17, 18, and 19	Occipital lobe. visual areas.

The parieto temporal occipital association areas are concerned with somatosensory, visual and auditory processing, understanding and interpretation of sensory stimuli from visual objects.

Prefrontal cortex

It includes prefrontal asssociation areas, which are present infront of area 6. The prefrontal areas include Brodmann areas 8, 9, 10, 11, 12, 13, 23, 24, 32, 44 to 47 (Fig. 4.80).

Connections

Prefrontal lobe is connected to limbic cortex, hypothalamus, thalamus, striatum, brain stem, sensory and motor areas.

Afferent

Dorsomedial nucleus of thalamus sends fibers to prefrontal lobe.

Hippocampus projects to area 24 through mamillary body of hypothalamus (Papez circuit).

Area 18 of occipital cortex is connected to area 8 (frontal eye field). This is responsible for conjugate deviation of eye when the head turns.

Wernicke's area is connected to area 44.

Fibers projecting from sensory and motor cortex end in prefrontal areas.

Efferent connections

- Suppressor areas of prefrontal cortex (8s, 13s, 32s,) project to caudate nucleus and brain stem reticular formation.
- Cingulate gyrus (area 24) sends fibers to hippocampus.
- From prefrontal lobe fibers going to pons
- Fibers projecting to tegmentum of mid brain
- Fibers going to thalamus, striatum and hypothalamus.

Functions of prefrontal lobe

Planning of complex movements
Elaboration of thoughts, reasoning and memory.
Controls autonomic function and emotion by its connections with limbic lobe and hypothalamus.
Gives social and moral behavior.

Experimental neurosis

Animals conditioned to a stimulus, when presented with another identical stimulus, finds difficulty in responding to the correct one and this leads to experimental neurosis, characterized by frustration, depression, anxiety, etc. In such animals, if prefrontal lobectomy is done, the symptoms mentioned above disappear. This observation prompted psychiatrists and neuro-surgeons to try on human subjects, who showed similar symptoms in compulsive neurosis. The result of these trials showed that the patient's mental symtoms such as delusions, anxiety, depression, etc. did not bother them. However, they exhibited complete lack of moral and social behavior. Prefrontal lobectomy as well as prefrontal leucotomy in humans resulted in disappearance of suffering from pain, even though the subject perceives it.

Hemispheric specialization

Right hemisphere is specialized for:
Visuospatial relations
Understanding of spoken words
Reasoning
Recognition of faces and objects by their form
Understanding of musical themes
Recognition of emotion in other persons
Sustained attention to solving tasks involving more than one attributes.

Left hemisphere is known as dominant hemisphere for right handed persons as it is concerned with language functions. In 70% of left handers the left hemisphere itself is dominant.

Left hemisphere can perform language functions efficiently with the co-operation of right hemisphere. The right hemisphere understands and gives meaning to the left hemisphere's language functions.

Thus, we see, that actually there is no dominant and nondominant hemispheres existing. The left hemisphere is functioning as *categorical hemisphere* and the right hemisphere, which is complimentary to left hemisphere functions, as *representational hemisphere.*

Handedness and lateralization

In 96% of right handed persons, the left hemisphere is dominant and 4% shows right hemisphere dominance. In the case of left handed individuals, 70% shows left hemisphere dominance, 15% with right hemisphere dominance and another 15% with no clear lateralization.

Intracortical transfer of information

The transfer of information between the two hemispheres takes place through corpus callosum, optic chiasma, and anterior and posterior commissures.

If corpus callosum and optic chiasma were sectioned in animals, it was observed that learning from the right field of vision and right hand is correctly interpreted, as the information goes to the left hemisphere that is concerned with language functions. If learning takes place through left field vision and left hand, then, it is not interpreted correctly, as the information goes to the right hemisphere

which is not for language functions.This study clearly shows that each hemisphere sends information to the other for comparative and integrative purposes. It is likely that any ongoing learning experience crosses the midline through corpus callosum and sent to the contralateral hemisphere. Similar effects are observed in humans also, when corpus callosum has been sectioned, as in split brain cases, which was done in earlier days to treat epilepsy. Sperry found in the split brain cases, the difficulty in identifying and interpreting, when presented to the left hand for objects which are learnt through right hand. It is because of the absence of transfer of information from one hemisphere to the other.

Effects of damage to categorical hemisphere (left side)

Loss of language, intellectual functions associated with language, loss of reasoning power, inability to perform mathematical problems.

Effect of damage to representational hemisphere (right side)

Inability to understand and interpret the language functions given by the left hemisphere. Inability to understand musical themes, visual pattern and spatial relations between the subject and environment.

HIGHER FUNCTIONS OF CORTEX

Speech, learning and memory are considered as higher functions of cerebral cortex.

Speech

Speech development in humans depends on the ability to comprehend spoken and written words. Speech, reading and writing are all specialized functions of left cortex. The angular gyrus present in the posterior parietal cortex, behind areas 5 and 7 is responsible for understanding and interpretation of written words. There is an area in the left temporal lobe below and behind the superior temporal gyrus which is concerned with understanding and interpretation of spoken words (Fig. 4.82). These two regions project to **Wernicke's area** present in the left posterior part of superior temporal gyrus. This is the center for comprehension of both written and spoken words. The connection from angular gyrus to Wernicke's area is provided by arcuate fasciculus.The Wernicke's area converts comprehended written and spoken words into expressive form of speech. It is then sent to Broca's area, which is present on the same side (Fig. 4.83). It even prepares the words to be spoken and sends it to Broca's area.

Broca's area is present on the left lower frontal lobe and is the motor area for speech. Based on the information received from Wernicke's area,

Cortical region	*Location*	*Function*	*Effects of lesion*
Broca's area	Left lower frontal lobe	Motor area of speech	Motor aphasia (nonfluent)
Wernicke's area	Posterior lip of sylvian fissure	Language compre-hension	Fluent aphasia
Angular gyrus	Posterior parietal cortex (behind area 5 and 7)	Meaning and inter-pretation of written words	Word blindness
Parietal lobe	Behind central sulcus (3, 1 and 2)	Primary sensory area SI	Astereog-nosis

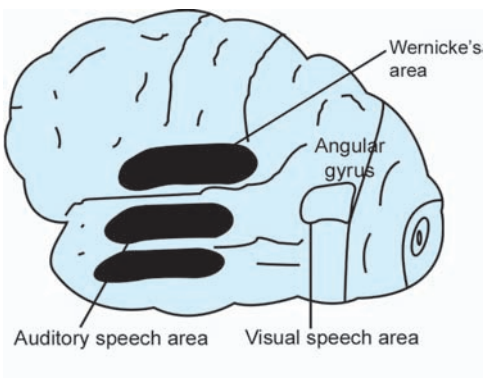


Fig. 4.82: Lateral surface of cerebral hemisphere to show area involved in language functions

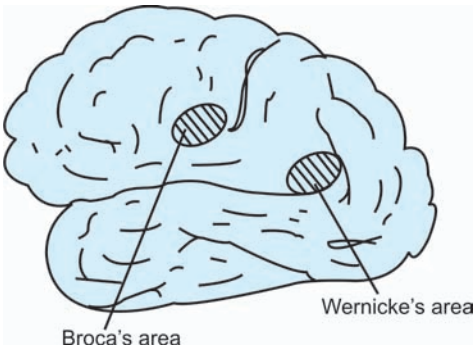


Fig. 4.83: Lateral surface of left cerebral hemisphere to show motor execution of speech.The Wernicke's area is responsible for comprehension of written and spoken words. Broca' area is the motor area, which produces expressive form of speech

Broca's area sends the pattern of vocalization to the motor cortex. The descending motor tract, which controls the muscles of speech is activated to produce speech.

Speech becomes meaningful only with the right hemisphere giving meaning to the left hemisphere language functions.

Speech disorders

Aphasia

It is the inability to understand written and spoken words caused by lesions of categorical hemisphere.

Types

Fluent aphasia

It involves both sensory and expressive parts of speech. It occurs in the lesion of Wernicke's area. The person cannot understand spoken and written words, but he can produce speech that has no sense. Hence this type is also called *fluent aphasia*.

Motor aphasia

The lesion of Broca's area (area 44) gives this type. It is a **nonfluent** type of aphasia. Speech is slow and there is inability to speak and write. However, the subjects with this aphasia can understand written and spoken words.

Word blindness (visual agnosia)

The person cannot understand and interpret written word or objects. Lesion of angular gyrus on the left side gives this disorder.

Word deafness (auditory agnosia)

The subject cannot understand spoken words, This occurs due to the lesion of auditory association area, situated on the left temporal lobe below and behind superior temporal gyrus.

Global aphasia

In this type there is loss of both receptive and expressive functions of speech. The speech is scant, and nonfluent.

Dysarthria

Difficulty in expression of speech. This is due to incoordination of speech muscles, which commonly occurs in cerebellar lesion.

Learning

Learning refers to the process of change in behavior as a consequence to experience. It is divided into nonassociative and associative types. In non-associative learning the organism learns about a single stimulus while in associate learning the animal learns the relation of one stimulus to another.

Nonassociative learning

The experiments conducted in marine mollusc *aplysia* revealed two important phenomena connected with learning. They are habituation and sensitization.

Habituation

Repeated stimuli, which are benign, will result in diminution of response gradually and after

some time the stimulus is ignored. The neural basis of this phenomenon is that there is low Ca^{++} availability in the neurons, as the transmitter released at the nerve endings decreases gradually with each repeated stimulus.

Sensitization

In this case the response to the repeated stimuli increases gradually, since the given stimulus has been perceived as threatening. A repeated stimulus gives a greater response if it is coupled one or more times with an unpleasant or pleasant stimulus. It has been shown that interneurons, which ends on the presynaptic terminals release serotonin. This chemical substance causes increased release of transmitter from presynaptic terminal through cyclic AMP mediated decrease in K^+ current, which leads to increase in magnitude of action potential. Both habituation and sensitization belong to nonassociative learning. Although, they have been observed in aplysia, similar nonassociative learning also occurs in humans.

Associative learning

Conditioned reflexes

In classical Pavlov's conditioned reflexes conditioned stimulus (CS) and unconditioned stimulus (US) are paired and it is always US preceding the CS. After a few trials only the CS (ringing of bell) is applied and the conditioned reflex response is observed, i.e. salivation.

The conditioned response disappears if only CS is given consistently. This is called **extinction.** However, if CS is now and then introduced following US, then conditioned response persists. This is called **reinforcement.**

In **operant conditioning** *(instrumental conditioning)* the learning process involves conditioned avoidance to a noxious stimulus which forms **negative reinforcement.** In **positive reinforcement** the animal experiences pleasant effect by repeated self stimulation of certain brain regions (medial forebrain bundle extending from limbic lobe to hypothalamus)or trained to receive food pellets by bar pressing.

Long-term potentiation (LTP) The learning process is also attributed to another neuronal activity called LTP, which occurs in the hippocampus and neocortex. LTP refers to the potentiation of response from neurons (CA 1 neurons in hippocampus) after repeated activation, caused by increased entry of Ca^{++}. The receptor involved is NMDA and the neurotransmitters are glutamate and aspartate. The presence of reverberating neuronal circuits facilitates LTP. Any new experience leads to changes in morphological, chemical and functional activity of synapses called **plasticity.** This is more common in hippocampus and is associated with learning.

Memory

The process of memory includes registration, retention and recall of events. There are different forms of memory, such as, short-term or recent memory, working memory, intermediate memory and long-term memory.

Short-term memory

It signifies the immediate recall of ongoing events and also events that occurred minutes, hours or days before. Short-term memory can be consolidated and converted into long-term memory. Short-term memory also includes working memory, which is a temporary storage of information for planning future action.

Long-term memory

It is the recall of events that occurred many years ago. Both short-term and long-term memories are considered as *declarative memory,* since it has been based on the actual recall of facts and events. The habits formed by repeated training and conditioned reflexes form reflexive or non-*declarative memory.* The memory can be divided into explicit which is declarative and implicit which is non-declarative.

Neurological basis of memory

Experiments conducted in aplysia demonstrated the presence of habituation and sensitization, which formed the basis of learning. Further studies in mammals showed that short-term and long-term potentiations in learning exist and they are characterized by synaptic facilitation in the neuronal circuits. Studies with PET scanning revealed that memory process involves many areas of cortex and hippocampus. The working memory which involves remembering numbers and letters for a short period, does not include hippocampus, but cerebral cortex neuronal activity.

Encoding of short-term memory involves hippocampus. If short-term memory is to be consolidated and converted into long-term memory, then, the adjacent portions of hippopcampus such as entorhinal, perirhinal and parahippocampal regions and dentate gyrus are also involved. During encoding of information in hippocampus, the CA 1 neurons are activated. The activity spreads to adjacent regions of hippocampus. From there the projections go to neocortex and activate the cortical neurons. This is how short-term memory is consolidated and converted into long-term memory in the cortex. If there is any damage to the connections from hippocampus to the neocortex going through the adjacent regions of hippocampus as mentioned above, encoding of long-term memory will not be possible. But the subject can recall old long-term memories. It has also been shown that connections of hippocampus to diencephalon (Papez circuit) are also involved in memory, which gives emotional color to it. The regions in the cortex, included for long term memory is visual, olfactory, auditory, and frontal cortices.

Cholinergic pathways in memory

The neuronal pathway for memory includes projections from hippocampus to mamillary body of hypothalamus and from there to anterior nucleus of thalamus. From thalamus projections go to prefrontal cortex and basal forebrain. This connection is said to be involved in recent memory. From basal forebrain , there is a diffuse cholinergic projection to neocortex and amygdala. The hippocampus also receives similar input from nucleus basalis Meynert. **Alzheimer's disease,** which causes senile dementia, shows degeneration of cholinergic neurons. The degeneration is possibly due to the changes in proteins associated with microtubules (*neurofibrillary tangles*) or formation of β-amyloid protein (*senile plaques*) around nerve cells.

Biochemical studies have shown that there is increased turnover of proteins in neurons during encoding of memory. Inhibition of protein synthesis by administering puromycin resulted in loss of memory.

Amnesia: Loss of memory is known as amnesia. Long-term memory is not formed in conditions such as

Brain concussion
Electroconvulsive shock (ECS)
Inhibition of protein synthesis

Retrograde amnesia: It is a condition in which there is inability to recall immediate events preceding brain concussion or ECS. However, the remote memories are intact.

Anterograde amnesia

Bilateral lesion of hippocampus results in this disorder. There is inability to learn and new memories can not be formed. The affected persons retains working memory.

CEREBROSPINAL FLUID

It is a colourless fluid present in the ventricles of brain, subarachnoid spaces of the brain and spinal cord. It has a volume of 150 ml. About 500 ml/day is formed and an equal amount is reabsorbed into the arachnoid villi, thus maintaining constant volume of fluid in CSF.

Formation: It is formed from choroid plexus in lateral ventricle (50 to 70%) and 30 to 50% from blood vessels lining the wall of the other ventricles of brain.

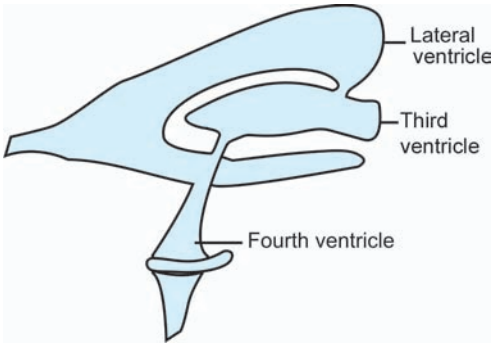


Fig. 4.84: CSF circulation in the ventricles of the brain

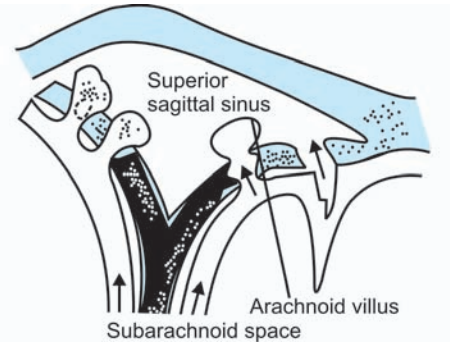


Fig. 4.85: CSF drainage into the arachnoid villi

CSF flows from lateral ventricle through foramen of Monro into third ventricle. From third ventricle, it flows into fourth ventricle, through aqueduct of Sylvius. The CSF from 4th ventricle enters subarachnoid spaces of brain and spinal cord, through foramen of Luschka and Magendie (Fig. 4.84).

Mechanism of formation: CSF formation occurs by ultrafiltration and secretion from the epithelium of choroid plexus.

Drainage: CSF is drained into arachnoid villi from where it enters subdural sinuses (Fig. 4.85). The reabsorption shows passive diffusion caused by hydrostatic pressure difference.

Composition: It is similar to brain ECF. When compared to plasma, the concentration of glucose and proteins are significantly lower.

CSF pressure: CSF pressure when measured from lumbar spinal segements (between L_3 and L_4), ranges from 70 to 150 mm of CSF. CSF pressure at above 115 mm CSF, filtration and absorption will be equal. Pressure below 65 mm of CSF, results in stoppage of absorption and fluid accumulates, giving rise to external hydrocephalus (communicating hydrocephalus). Blockade in ventricular system or foramen of Luschka and Magendie leads to accumulation of CSF proximal to block and results in internal hydrocephalus (noncommunicating hydrocephalus).

Functions of CSF

CSF gives buoyancy to the brain and cushions against any mechanical injury.

Maintain a constant fluid volume in the brain.

CSF is the route for the elimination of neurotransmitter metabolites.

Clinical importance CSF examination is a useful diagnostic procedure in neurological diseases such as meningitis, tumors of brain, intractable headache, etc. The appearance of fluid, concentrations of chloride, proteins, is specially investigated.

Blood brain barrier

Cerebral capillaries at birth lack blood brain barrier. There is a free movement of substances between blood and brain tissue and that is why, when severe jaundice occurs in newborn (hemolytic disease of newborn),the bile pigments cross the brain capillaries and damage the basal ganglia. This leads to extrapyramidal disorder and the condition is known as **kernicterus.**

In adults, the blood brain barrier is fully developed and hence only lipid soluble molecules and respiratory gases can pass through brain capillaries. There are two important factors which facilitate development of blood brain barrier. They are:

Presence of tight junctions between the endothelial cells of brain capillaries.

The end feet of astrocytes ending on the brain capillaries also contribute to the barrier.

Significance of blood brain barrier

Prevents entry of endogenous and exogenous toxic substances into the brain tissue. Maintains

steady state environment for neuronal activity. That is, it maintains the concentrations of Na^+, K^+, Ca^{++}, Mg^{++} etc., in ECF.

Prevents escape of neurotransmitters into general circulation.

Drugs selected for treatment of CNS diseases should show permeability across the barrier. The physician should be aware of this fact before selecting the drug.

Helps to identify the presence of tumors of brain, infection, irradiation, since the barrier breaks down in such conditions, the dye injected is taken freely *per se*.

Regions of brain outside blood brain barrier

There are regions of brain where the blood brain barrier is absent. It helps peptides and neurotransmitters to enter circulation and also secreted from it for regulating neuronal activity. These regions are situated around the wall of third ventricle. They are collectively known as **circumventricular organs** which include:

Neurohypophysis including ventral part of median eminence

Area postrema

Organum vasculosum of lamina terminalis

Subfornical organ.

AUTONOMIC NERVOUS SYSTEM

Autonomic nervous system (ANS) is concerned with the regulation of visceral activity and it is involuntary in nature. The afferent impulses from visceral sensory receptors travel in autonomic afferents, which have their cell bodies in dorsal root ganglia for spinal nerves and cranial nerve sensory ganglion for cranial nerves. The impulses go to central nervous system at various levels, i.e., spinal cord, brain stem, hypothalamus, limbic cortex and cerebral cortex. These impulses are integrated in these areas of CNS and appropriate visceral motor response is given by the efferents, which belong to ANS. In this way, the activity of ANS becomes important in maintaining steady state or homeostasis. *If CNS is important in giving steady state, by way of behavioral response to change in external environment, the ANS responds to changes in the internal environment by way of visceral motor response and contributes to homeostasis.* The activity of ANS depends on the visceral impulses reaching CNS which integrates them.

Divisions of autonomic nervous system

ANS is divided into sympathetic and parasympathetic nervous systems. In each division, there are two types of motor neurons present. They are preganglionic and postganglionic neurons. The former is myelinated belonging to B type nerve fiber, while, the latter is unmyelinated and belongs to C type nerve fiber. The involuntary nervous system, besides ANS, includes another type called enteric nervous system. This is the intrinsic nerve plexus present in the wall of the gut. The activity of enteric nervous system can be modified by ANS activity.

Sympathetic nervous system

The preganglionic fibers arise from intermediolateral cell column of thoracic and upper two or three lumbar segments of spinal cord. Hence this division is also known as thoracolumbar outflow. The fibers travel in white rami communicans and enter sympathetic ganglia, situated in paravertabral chain (Fig. 4.86). Since the preganglionic fibers travel up and down the sympathetic chain and end in many ganglia, all the spinal segments are represented by a sympathetic ganglion, eventhough the preganglionic fibers arise only from thoracic and first two lumbar segments. For example, the cervical segments are represented by *superior, middle and inferior cervical ganglia.* The C8 and T1 ganglia fuse to form stellate ganglion.

The postganglionic fibers of sympathetics arise from ganglia and go either directly to blood vessels, smooth muscles and glands or come out as grey rami communicans from ganglia and join spinal nerves. Sympathetic preganglionic fibers also can go directly to collateral ganglia situated close to the visceral organs. The postganglionic fibers arise from this ganglion and supply viseral organs.

Transmission in sympathetic nervous system

Sympathetic nervous system is called as **adrenergic system** as the transmitter released from postganglionic nerve endings is **norepinephrine**. Norepinephrine released from nerve endings can enter circulation and together with epinephrine and dopamine secreted from adrenal medulla, widespread effects in the body are produced. In some tissues, norepinephrine is released along with ATP and neuropeptide Y. They form co-transmitters and help in augmenting the action of norepinephrine in the target tissues. The sympathetic fibers to adrenal medulla is supplied by splanchnic nerve, which carry the preganglionic fibers. The adrenal medulla itself forms a ganglion. The release of acetylcholine at the preganglionic endings in the gland is necessary for catecholamine secretion. Norepinephrine, released from sympathetic post-ganglionic fibers and catecholamines, secreted from adrenal medulla, produce their effects by acting on the adrenergic receptors, which are α and β types. They are further divided into α_1, α_2, β_2 and β_3 subtypes.

Functions of sympathetic nervous system

Sympathetic nervous system is stimulated during stress and its activity helps to cope with the stress.

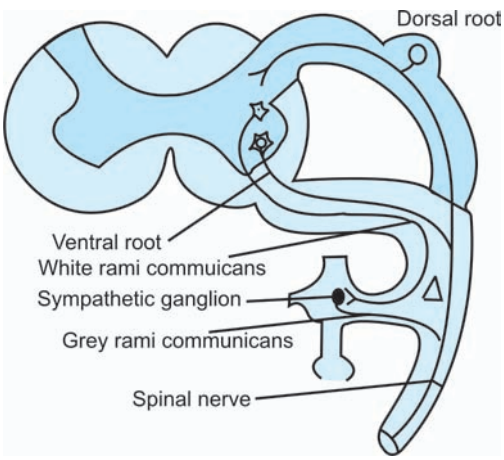


Fig. 4.86: Formation of sympathetic nervous system

It prepares the organism for fight or flight reactions. The activity of sympathetic system shows increase in heart rate, cardiac output, rise in arterial blood pressure, increase in respiration, dilatation of pupil, contraction of piloerector muscle, arousal, and increased glucose and fatty acids formation. Thus, we can observe that sympathetic activity is widespread and lasts for longer duration.

Parasympathetic nervous system

Parasympathetic division is called craniosacral outflow, since the preganglionic fibers arise from cranial motor nuclei of III, VII, IX and X nerves and from S2 to S4 spinal segments (pelvic nerve). The ganglion is situated close to the organs that they innervate.The ganglia also show release of peptides from preganglionic fibers besides acetylcholine. For example, the preganglionic fibers of sympathetics release substance P, neurotensin, enkephalin, etc. They function as **neuromodulators.**

Transmission in parasympathetic nervous system

Parasympathetic system is called **cholinergic system** as the transmitter released is acetylcholine at the postganglionic nerve endings. Acetylcholine is released as a transmitter from the following regions:

- Ganglia of both sympathetic and parasympathetic systems
- Parasympathetic postganglionic fibers
- Motor end plate
- Sympathetic postganglionic fibers in eccrine sweat glands and skeletal muscle arterioles (sympathetic cholinergic)
- Neurons of CNS.

Acetylcholine also coexists with VIP in the fibers supplying bronchiole smooth muscle. The co-transmitter potentiates the action of acetylcholine. Acetylcholine release occurs very near the target tissue and the action is also restricted to the target tissue. The action lasts for a short duration only, as the transmitter is quickly hydrolyzed by the acetylcholine esterase enzyme present at the site of action.

The actions of acetylcholine are due to two types of receptors namely, **nicotinic** and **muscarinic**. The action of acetylcholine at the ganglia and endplate is through nicotinic receptor. At the ganglia, the action of nicotine can be blocked by hexamethonium. At the endplate, nicotinic receptors are blocked by d-tubocurare and not by hexamethonium. Muscarinic receptors of acetylcholine are distributed in GI tract, heart, iris, sweat glands, bronchiole smooth muscle and the action is blocked by atropine.

Functions of parasympathetic system

In most of the visceral organs, there is a dual innervation supplied by the autonomic nervous system. If one division of ANS causes excitation, the other would cause inhibition, thus, a balance in the activity of both sympathetic and parasympathetic is maintained. There are tissues, where, only sympathetic activity is present. They are sweat glands, cutaneous blood vessels, piloerector muscle in the skin. The secretion of tears, pupil constriction, salivary secretion, dilatation of blood vessels, heart rate regulation by cardiac inhibition, GI tract secretion of digestive juices, gut motility, bladder contraction, erection of penis in males, are some of the important effects of parasympathetic nervous system.

Regulation of ANS activity

ANS activity is regulated by centres present in various parts of brain. Brain stem medullary reticular formation has centres regulating autonomic activity. These are cardiac, vasomotor, respiratory centres.The anterior and medial nuclei of hypothalamic regions regulate parasympathetic effects. The posterior and lateral nuclei of hypothalamus, controls sympathetic effects. The limbic cortex with its connections with hypothalamus also controls autonomic nervous system. Moreover, the limbic region is concerned with emotion and hence the visceral responses accompanying it are due to autonomic nervous system activity. The cerebral cortex also influences autonomic nervous system. The connections from hypothalamus to cortex could be responsible for it. The stress stimuli especially become important for the cortex to influence sympathetic response.

Effects of sympathetic stimulation

Heart	Increase in rate, force, conduction velocity (β_1)
Blood vessels	Vasoconstriction, rise in Blood pressure(α_1)
Iris of eye	Mydriasis (α_1)
Bronchiole	Relaxation of smooth muscle (β_2)
GI tract	Relaxation of stomach and intestine
Sphincter	Contraction (α)
Bladder	Relaxation of detrusor
sphincter	Contraction (α_1)
Liver	Glycogenolysis (β_2) lipolysis
Kidney	Renin release (β_1)
Uterus	Pregnant-Contraction (α_1) Nonpregnant-relaxation (β_2)
Skin	Pilomotor muscle contraction (α_1)

Effects of parasympathetic stimulation

Heart	Decrease in rate, force and conduction (vagal inhibition) (Muscarinic)
Blood vessels	Vasodilatation, fall in blood pressure (muscarinic)
Bronchiole	Contraction of smooth muscle
Iris of eye	Miosis (muscarinic)
Ciliary muscle	Contraction
Lacrimal	Secretion (tears)
Salivary glands	Secretion
GI tract	Secretion of digestive juices, increased motility
Sphincter	Relaxation
Bladder	Contraction of detrusor muscle
Sphincter	Relaxation
Male genitalia (penis)	Erection

Adrenergic receptors

Types	$\alpha_1, \alpha_2, \beta_1, \beta_2$.
Distribution	α_1 postsynaptic on effector organs
	α_2 presynaptic in nerve endings and postsynaptic in brain
	β_1 heart, JG cells of kidney
	β_2 bronchi, blood vessels uterus, GI tract.
Mechanisim of action	α_1 through IP_3/DAG
	α_2 decrease in cAMP increase in K^+ channel
	β_1 increase in cAMP through G protein
	β_2 increase in cAMP.
Antagonists	α_1 prozocin
	α_2 yohimbine
	β_1 atenolol, metoprolol
	β_2 butoxamine, α-methyl propranolol .

Cholinergic receptors

Types	**Muscarinic:** M_1, M_2 and M_3
	Nicotinic: N_M and N_N
Distribution	**M_1** CNS (cortex, hippo campus, corpus striatum)
	M_2 heart
	M_3 smooth muscles and glands
	N_M neuromuscular junction
	N_N autonomic ganglia adrenal medulla, CNS.
Mechanism of action:	**M_1** IP_3/DAG
	M_2 decrease in cAMP, K^+ channel opening.
	M_3 Increase in cytosolic Ca^{++} through IP_3/ DAG.
	N_M opening of cation channels (Na^+,K^+)
	N_N opening of Na^+ K^+, Ca^{++} channels
Antagonists: Atropine for all 3 types of muscarinic receptors.	
	N_M tubocurare
	α Bungarotoxin
	N_N Hexamethonium.

5 Special Senses

VISION

The eyeball consists of three layers, namely an outer sclera, a middle choroid and an inner retina (Fig. 5.1). The sclera anteriorly forms the transparent cornea. Choroid forms the middle layer and contains blood vessels that nourish the structure of the eyeball. The choroid anteriorly gives rise to ciliary body. From the ciliary body, zonule ligaments arise and hold the biconvex crystalline lens. Infront of the lens is the iris, which is attached to the ciliary body. The center of the iris contains an aperture called pupil, the diameter of which can be changed by the contraction of radial and circular muscles of iris. The space between cornea and iris, is the anterior chamber of eyeball and the space between iris and anterior surface of lens, forms the posterior chamber. In both the chambers, aqueous humor fluid is present. The space between the posterior surface of lens and retina contains the vitreous humor, which is a gelatinous body. Light rays pass through cornea, aqueous humor, lens and then strike the retina.

Anteriorly, the exposed part of the eyeball is covered by a mucous membrane called conjuctiva, which is reflected into the inner surface of the eyelids. The cornea and conjuctiva are

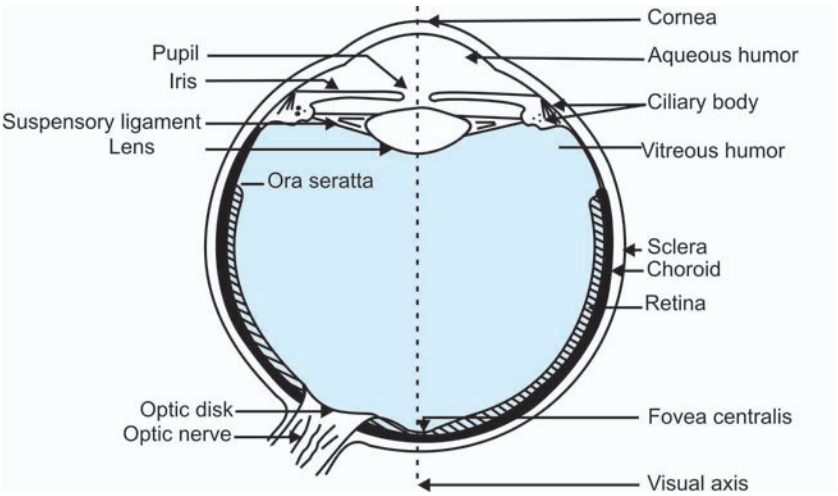


Fig. 5.1: Structure of eyeball

lubricated by the secretion from lacrimal glands. The secretion from this gland is known as tears. Lacrimal glands are situated at the upper and outer corner of eye socket. The eye blinking distributes the fluid in the anterior surface of the eye and drained into the nasal cavity through lacrimal canaliculi, lacrimal duct and nasolacrimal duct. Tears help to lubricate the eyelids and to wash away the dust particles and irritating substances in the eye. It also helps to correct the unevenness of corneal surface and prevents its drying. Tears also contain lysozyme and immunoglobulin A (IgA), which serve defense against infections. Finally, tears express emotion of an individual.

Retina

Retina forms the innermost layer of the eyeball. It contains visual receptors namely rods and cones. Retina contains 10 layers, which broadly can be organized as follows.

Receptor layer synapsing with bipolar cells.

Bipolar cells synapsing with ganglion cells.

The axons of ganglion cells form the optic nerve.

The receptors and bipolar cells are connected by a neuron called horizontal cells, while bipolar and ganglion cells are connected by amacrine neurons. The horizontal cells connect the receptor cells to one another in the outer plexiform layer. The amacrine cells connect ganglion cells to one another in the inner plexiform layer (Fig. 5.2).

Since the receptors are present adjacent to the pigment layer of choroid, the light rays have to pass through ganglion cell layer, bipolar cell layer and then strike the receptors. The layers of retina are bound by glial Müller's cells and from the processess of these cells, the outer and inner limiting membranes are formed. The center of retina contains a yellowish region called **macula lutea** and the depression in the center of lutea is called **fovea.** It is the visual center of the eye and contains only cones. The cones present here will have connection with a single bipolar and ganglion cells enhancing the visual acuity. From fovea, 3 mm medially, optic disc is present, which is called the **blind spot**. In this region the receptors are absent, as they are pushed to the side by the optic nerve which emerges from the eyeball.

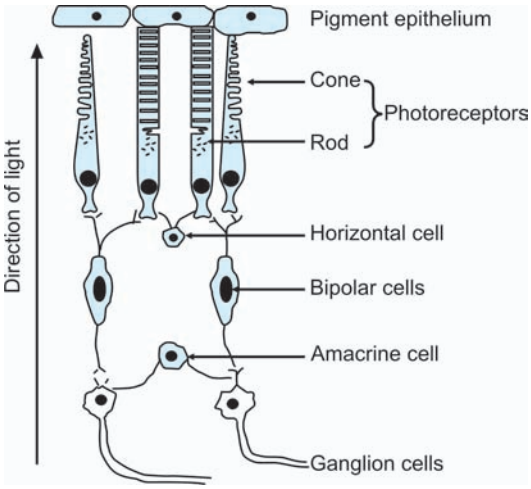


Fig. 5.2: Structure of retina showing principal layers

Visual receptors (Fig. 5.3)

Rods

Rods are predominant in the periphery of retina. Each rod shows an outer segment, an inner segment, nuclear region and a synaptic body. The outer segment contains a number of flattened disks. The disks contain photochemical pigment **rhodopsin.** The old disks are replaced by the new cells, which migrate from the basal segment. The inner segment contains mitochondria.

Rods are meant for dim light vision or **scotopic vision.** Rods show maximum absorption of light at **500 nm**. Bleaching of photochemical pigment (rhodopsin) occurs when light

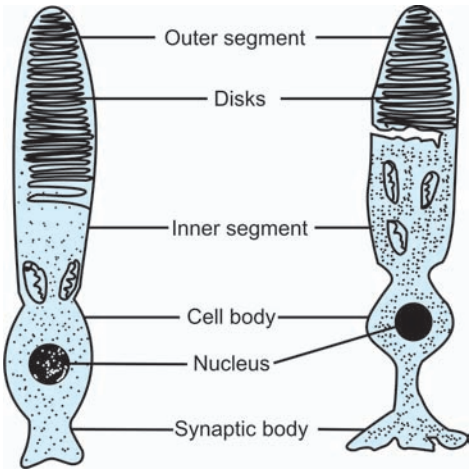


Fig. 5.3: Structure of rods and cones

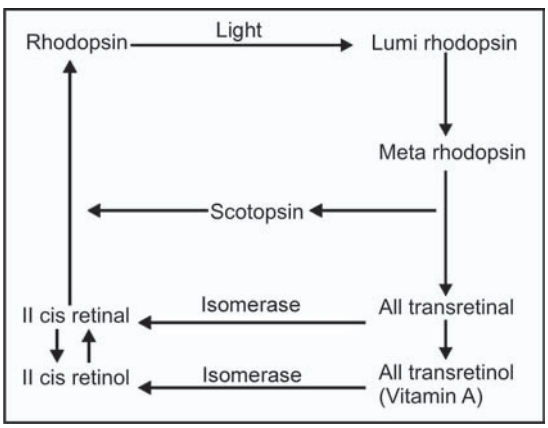


Fig. 5.4: Rhodopsin cycle. Rhodopsin bleaches when light strikes on it and form transretinal and scotopsin. Vitamin A converts trans form to cis form of retinal, which on combining with scotopsin, rhodopsin gets resynthesized

rays fall on rods. The regeneration of rhodopsin takes place from vit A in dark (Fig. 5.4). Deficiency of vit A causes **night blindness or nyctalopia**.

Cones

Cones are necessary for **photopic vision** or bright light vision. It is also responsible for *color vision and visual acuity*. The structure of cones shows conical outer segment and the saccules are formed due to the invagination of the cell membrane. This is in contrast to rods, where the disks are separated from cell membrane. In cones, the saccule renewal is a diffuse process, as it is formed from many areas of the outer segment. The outer segment contains the photochemical color pigment. Based on the different types of pigments, showing different wavelengths for the absorption of light, there are three types of cones that have been identified. **Red cones** absorb maximum light at **565 nm**, green **cones** absorb light at **550 nm** and **blue cones** show absorption of light at **440 nm.**

Electrophysiology of retina

Visual receptors and other neural structures show local graded potentials and action potentials develop only from ganglion cells. Secondly, rods, cones and horizontal cells give hyperpolarizing response, whereas, bipolar cells show either depolarization or hyperpolarization. In amacrine cells, depolarizing potentials, similar to generator potential can be observed.

When light does not fall on rods and cones (dark), the Na^+ conductance in the outer segment is increased in the receptors due to cGMP. When light strikes them, the Na^+ channel is closed and results in hyperpolarizing receptor potential. There is a activation of a G protein **transducin,** when rhodopsin is activated by light. This compound causes decrease in cGMP and hence closure of Na^+ channels in the outer segment. The amplitude of hyperpolarization, varies with the logarithm of light intensity. The onset of hyperpolarizing potentials inhibits the release of inhibitory synaptic transmitter from receptors and bipolar cells, leading to excitation of the ganglion cells.

In the absence of light rays (dark)

- Visual receptors are depolarized
- The receptor terminals release inhibitory transmitter
- The bipolar cells are hyperpolarized and the ganglion cell is not excited.

When light rays strike the retina

- Visual receptors are hyperpolarized.
- The inhibitory transmitter release from receptor terminals does not occur.
- The bipolar cells are depolarized and the ganglion cells are excited.

Organization of receptive fields in ganglion cells

As discussed earlier, many receptor cells converge on bipolar cells and many bipolar cells in turn converge inturn on ganglion cells. The receptors, which connect directly to ganglion cells, through bipolar cells form the **receptive field center** for that ganglion cell and also known as *central circuit*.

In dark, receptors are depolarized and hence, the release of inhibitory synaptic transmitter occurs from receptor terminals. This produces hyperpolarization of bipolar cells and as a result the ganglion cell is not excited.

When light strikes the receptor cells, it results in hyperpolarization, which decreases the release of inhibitory transmitter from receptor terminals. This will lead to depolarization of bipolar cells and consequently the ganglion cells are excited. Whenever light falls on any cell included in the receptive field, the excitation of that particular ganglion cell occurs.

Some of the receptor cells connected to bipolar cells are also influenced by horizontal cells. It is present at the place where receptors end on the bipolar cells. Thus, the receptive field center for these ganglion cells would be different. It will have a circular central field surrounded by an annulus or surround. If light falls on the center, the ganglion cell in the surround is inhibited through the horizontal-bipolar-ganglion cell pathway **(ON center and OFF surround).** That is, when a receptive area is stimulated, the inhibition of surround is similar to lateral inhibition. It helps to sharpen the visual stimuli and facilitate sensory discrimination.

If light falls on surround, the center of receptive field is inhibited and the surround is excited. This receptive field is called OFF center and ON surround. Both types of responses can be seen in the visual pathway and it is due to the nature of transmitter released from receptors.

Based on this, two types of bipolar cells are described. In 'ON' center and 'OFF' surround, the photoreceptors are hyperpolarized in the 'ON' center, causing depolarization of bipolar and ganglion cells. The receptive field in the OFF surround showed the opposite effects (Fig. 5.5).

Since the bipolar cells are connected to the ganglion cells, similar responses also can be observed in ganglion cells.

The significance of center-surround antagonism in the receptive fields is to transmit information only about changes in the levels of illumination within the visual field.

Amacrine cells influence bipolar cells connection with ganglion cells. Both horizontal and amacrine interneurons activity contributes to high contrast, sharp images throughout the wide range of intensities of incident light.

Organization of ganglion cell

There are two types of cells in the ganglion. One is larger and called M (magno). The other is smaller and called P (parvo). The M cells respond to cones activity and concerned with movement and stereopsis of visual image. The P cells respond to cones which carry information regarding color, texture and shape. The fibers from M cells and P cells project to magnocellular and parvocellular regions of lateral geniculate body respectively.

Organization in lateral geniculate body

LGB has six layers. The visual input coming from each eye is separated in the LGB. The uncrossed

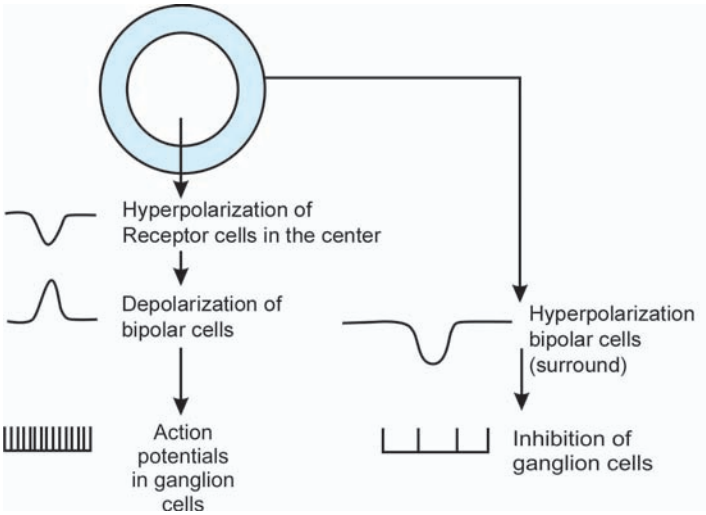


Fig. 5.5: Center–surround antagonism in the receptive field of retina. When light strikes the center, the surround is inhibited to enhance the contrast of the image

fibers (temporal fibers) go to layers 2, 3 and 5. The crossed fibers (nasal fibers) go to layers 1,4 and 6.

The cells in LGB also show On center–OFF surround and OFF center–ON surround organization similar to ganglion cells.

- Receptive field can be formed by the connections of both bipolar and horizontal cells with the receptors and ganglion cells
- In the receptive fields, center and surround neurons are present
- If light falls on the center the surround is inhibited and is known as ON center–OFF surround. If light falls on the surround, then it will be ON surround–OFF center receptive field
- The center–surround antagonism in the receptive field helps to sharpen the visual stimuli and facilitate sensory discrimination similar to lateral inhibition.

Visual cortex (striate cortex)

The axons of LGB carry optic radiations and project directly to visual cortex to end in area 17. The neurons in layer 4 of visual cortex show organization with ON center–OFF surround and OFF center–ON surround receptive fields. These neurons are also responsible for feature detection of visual input such as shape, orientation, etc. There are 3 types of cells responsible for this function. They are called simple cells, complex cells and hypercomplex cells.

Simple cells: They have elongated receptive fields instead of circular ones, as present in ganglion cells and LGB. These cells respond to dark and light bars, lines or edges of specific orientation.

Complex cells: Like simple cells, they respond to lightlines or dark bars which are moving.

Hypercomplex cells: These cells are stimulated by more complicated shapes with specific orientation moving across their respective fields. These cells are more in areas 18 and 19.

Columnar organization in visual cortex

As described above, the cortical cells respond to lines, bars and edges. The cells responding to this are arranged in columns, which are perpendicular to the cortex. Each column differs from the other in their orientation. With a difference of 10°, the columns are oriented to each other in a complete 360° circle. These columns are called **orientation columns** and they respond to visual stimuli of a particular orientation.

In each visual cortex, both the eyes are represented alternatingly in slabs called **ocular dominance** and they exist in the layer IV.

Other cortical areas contributing to vision

From the primary visual cortex, visual impulses also go to other regions and help in visual processing.

- Information about movement of visual image is processed in the parietal cortex.
- Information about color is processed in fusiform and lingual gyri of occipital lobe.
- The anterior inferior temporal cortex helps in recognition of visual objects.
- Parts of frontal lobe are also involved in the processing of visual information.
- The frontal eye field is responsible for conjugate movements of eye to the contralateral side.
- The prestriate area is involved in the integration of visual processing in the two hemispheres. Lesion of this region causes difficulty in object and pattern discrimination, visual spatial ability and visual learning.

Visual pathway (Fig. 5.6)

The optic nerves after emerging from the eyeball show decussation of nasal fibers at the optic chiasma. The uncrossed temporal fibers join with the crossed nasal fibers and form optic tract. From optic tract, collaterals go to suprachiasmatic nucleus of hypothalamus, which is responsible for circadian rhythm like, sleep wakefulness, secretion of melatonin, cortisol, etc.

The optic tract carrying visual impulses projects to thalamus and ends in the lateral geniculate body. The fibers carrying light reflexes do not go to thalamus. These fibers enter the midbrain and end in pretectal nucleus. The lateral geniculate body sends visual projections to occipital cortex to end in **area 17**, which is the **primary visual center**. The areas 18 and 19 form the association areas.

Visual field of the eye can be plotted using perimeter. The field of vision for each eye is separately determined and the normal range is as follows.

Superior 60°
Nasal 70°
Inferior 80°
Temporal 110°

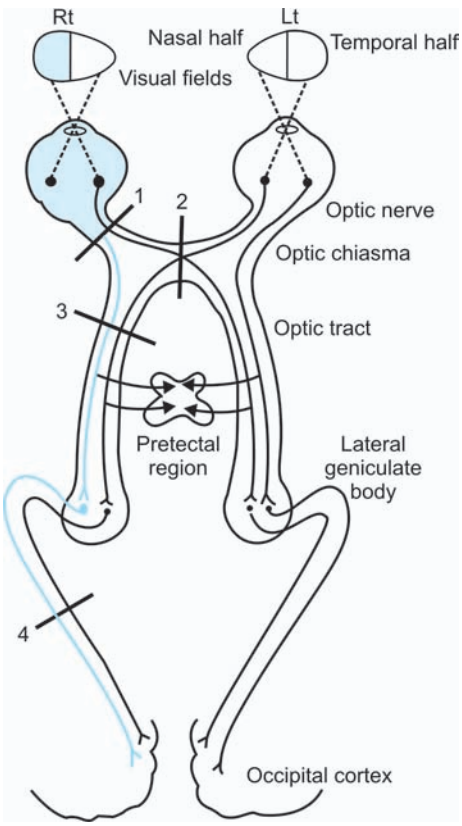


Fig. 5.6: Visual pathway

Each eye is divided into nasal half and temporal half. The light rays from nasal field of vision will strike the temporal half of retina, while those coming from temporal field of vision will strike nasal half of retina.The nasal fibers coming from retina carry temporal halves of field of vision and the temporal fibers carry nasal halves of field of vision. Hence, the optic tract which contains crossed nasal and uncrossed temporal fibers, will represent the opposite fields of vison. That is, right optic tract will carry field of vision from left halves and left optic tract carries right halves of field of vision.

Lesion of optic pathway

1. *Lesion of optic nerve*: Blindness of the affected eye.
2. *Lesion of middle part of optic chiasma*: Bitemporal heteronymous hemianopia.
   Lesion of lateral part of optic chiasma: Binasal heteronymous hemianopia.
3. *Lesion of right optic tract*: Left homonymous hemianopia.
4. *Lesion from LGB to Occipital lobe*: Opposite homonymous hemianopia with macular sparing.

Macular sparing: Macula of the eye is widely represented in the visual cortex in both halves of the occipital lobe (Fig. 5.7). Hence, lesion of optic tract on one side does not affect the macular vision. This is called macular sparing.

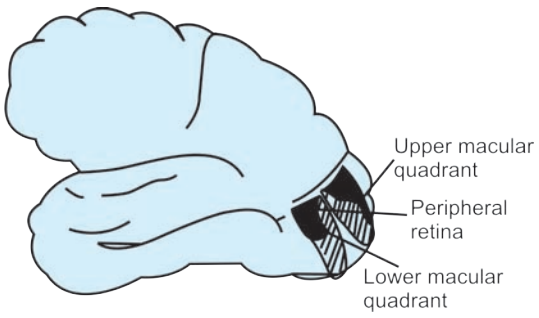


Fig. 5.7: Macular representation in occipital cortex
Note the large area of macular representation as compared to peripheral retina

Electroretinogram (ERG)

Electrical activity of retina can be studied by recording the sequence of potential changes, when light falls on retina. In humans, it can be recorded by keeping one electrode in cornea and another in the skin of scalp. The series of waves recorded are a, b, c and d (Fig. 5.8).

a wave is the initial negative deflection caused by receptor activity.
b wave is a positive deflection caused by bipolar and ganglion cell activity.
c wave is prolonged slow positive deflection due to hyperpolarization of pigmented epithelium.
d wave comes after switching off light, indicating the restoration of resting potential in the receptors.

ERG recording is useful in investigating retinal disorders.

Optics of eye

Parallel of light rays (6 meters distance), which strike the biconvex lens, gets refracted behind the lens. This point behind the lens is called the **principal focus.** The distance between the lens and principal focus forms **principal focal distance.** The refractive power of lens is measured as **diopters**. Greater the curvature of the lens, greater its refractive power. Diopter is the reciprocal of principal focal distance in meters (f).

$$P = 1/f$$

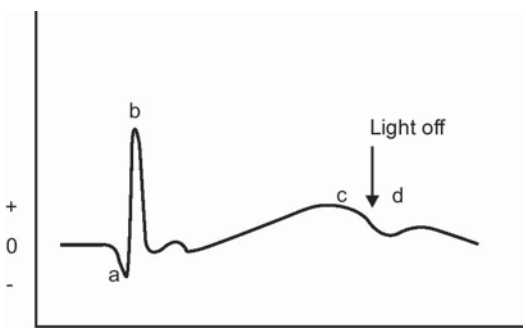


Fig. 5.8: Electroretinogram (ERG)

If concave lens is used, then the image cannot be formed, as the lens causes the refracted light rays away from the center of lens. In convergent lens (convex), the rays are bent towards the center of lens and hence the image is formed at principal focus. According to lens formula,

$$P = p\,\frac{1}{o} + \frac{1}{i} = \frac{1}{f}$$

o = object
i = image
f = focal distance in meters
p = D (lens power) diopters

Light rays pass through four refractive interfaces. They are anterior surface of cornea, posterior surface of cornea, anterior surface of lens and posterior surface of lens.

Cornea

Cornea is a transparent structure because of the absence of blood vessels. Hence, light rays can easily pass through it. It receives its oxygen and nutrients through diffusion from aqueous humor. Corneal transparency is affected in glaucoma. The maximum refraction of light occurs at the *anterior surface of cornea* because of greater difference between refractive indices of air and cornea. The contribution of refractive power by cornea is fixed (43D). It is about two-thirds of total refractory power of the eye.

Lens

The lens is also avascular. It consists of a capsule, a layer of epithelial cells and transparent fibers. The lens is suspended by zonule fibers, which stretch the lens and flatten it. The contraction of ciliary muscle releases the tension of zonule fibers, which leads to increase in anterior curvature of lens. This indicates that the refractive power of lens can be changed. The unaccommodated eye gives one-third of eye's refractive power. During accommodation, the lens adds another **+ 12D** power to the eye. Lens transparency is reduced by trauma, radioactive UV light and uncontrolled diabetes. The increased opacity of lens results in *cataract*. The diopteric power of human eye is **59D**. The cornea contributes **43D** and the remaining refractory power comes from lens.

The light rays pass through transparent media called **diopteric media**, which include *cornea, aqueous humor, lens* and *vitreous body*. The refraction to light occurs at two places, one in air corneal interface and another in lens. The maximum *refraction of* light occurs at the air corneal interface (42D). Lens shows diopters varying from 12D to 26D.

Reduced eye (Fig. 5.9)

It is the optical model of human eye, where the cornea and lens form a single refractive surface. The reduced eye has an axial length of 24 mm. The principal point is present at 17 mm distance from retina and the optical center (nodal point) is 7 mm from cornea. The light rays from the object which pass through this point do not refract. The 17 mm distance will be the focal length (1/f) . Therefore, the refractive power of such lens will be= 1000/17= 59D. This model helps to demonstrate the optics of the eye and the presence of any defects in it. The image that is formed on the retina is inverted. But the transmission of neural impulses from retina to the visual cortex leads to the perception of image upright.

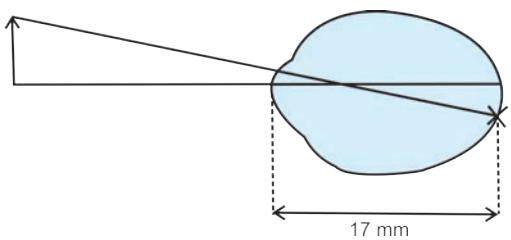


Fig. 5.9: Image formation in the reduced eye

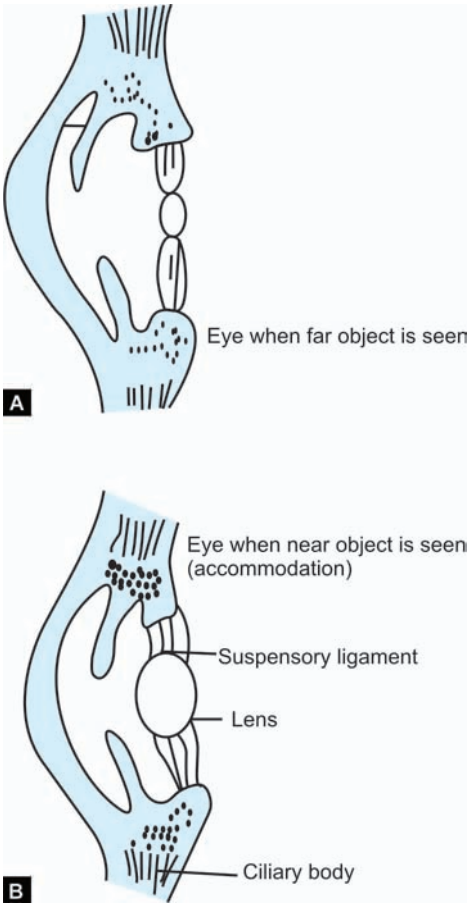
Accommodation

It is the ability of eye to see near vision. The near vision refers to the object kept at the distance lesser than 6 meters. There are three reflex

mechanisms during accommodation to near vision. They are:

- Increase in diopteric power of lens by 12D which is achieved by increase in the anterior curvature of lens
- Convergence of visual axis
- Pupillary constriction to increase the depth of focus.

The lens is held in position by the suspensory ligaments (zonule fibers). The reflex contraction of ciliary muscle by parasympathetic fibers in the 3rd cranial nerve releases the tension in the suspensory ligaments. This results in flattening of anterior curvature of lens (Fig. 5.10) (the lens becomes more convex). The total diopteric power of the eye during accommodation is 59 + 12= 71D (approximately 70 D).



Figs 5.10A and B: Accommodation of the eye to near vision

The convergence of visual axis is due to the contraction of medial rectus, brought by the 3rd nerve activity.

The pupillary constriction is also due to the activity of parasympathetic fibers in the 3rd nerve, which helps to reduce the spherical aberration and increase the depth of focus.

Accommodation pathway

The fibers carrying near vision go to occipital cortex and from here, the fibers go to frontal lobe. From here, projections reach Edinger Westphal nucleus through the midbrain pretectal nucleus. Preganglionic parasympathetic fibers go to ciliary ganglion and from here, postganglionic fibers travel in short ciliary nerve to supply the ciliary muscle. Since the near vision fibers go to occipital cortex, lesions of midbrain do not affect accommodation but light reflex is affected. This condition can be observed in **Argyll Robertson pupil.**

Purkinje sanson images

This is a simple experiment to show the changes occurring in the lens during accommodation. An object is focused into the eye of the subject, who is looking into a far distance. Three reflections are observed in the subject's eye. A small upright image reflected from cornea, larger faint upright image reflected from the anterior curvature of lens and a small inverted image reflected from the posterior curvature of lens. When the subject focuses on the near object, the larger upright image becomes smaller and moves towards the upright image. The images reflected from cornea and posterior curvature of lens remain in the same place. This demonstrates that only the anterior curvature of lens is increased during accommodation.

Near point of vision gradually recedes as the age advances. At 10 years of age, it is 8 cm and at 60 years it recedes to 80 cm. This increase in near point distance in old age is due to changes occurring in lens. In old age, the lens becomes hardened. The loss of accommodation in old age

is called **presbyopia** which is corrected by using converging lens (convex lens).

Dark adaptation

When a subject moves from bright light to dim light, there is a change of receptor activity. In bright light, cones are stimulated and at the same time rhodopsin in rods would be bleached. When the subject now moves suddenly to dim light the visual threshold decreases over time, which is called **dark adaptation**. The complete adaptation occurs by 20 minutes time for dark. The curve, when plotted with visual threshold verses time in the dark adaptation, it will show, first the cone acitivity, which is quick, but small in magnitude. This is followed by a greater magnitude of adaptation by rod activity, which is maximal by 20 minutes duration (Fig. 5.11). The duration of dark adaptation can be reduced by wearing red glasses, when exposed to bright light, as it prevents bleaching of rhodopsin in rods. Hence, moving suddenly to dim light will take less time for the fall in visual threshold, which helps aircraft pilots and radiologists.

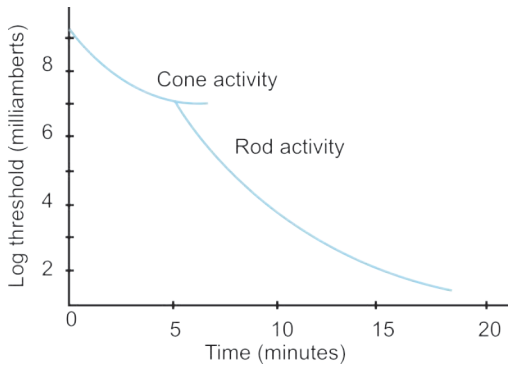


Fig. 5.11: Dark adaptation

During dark adaptation there is switch over of receptor activity from cones to rods. The visual threshold to low light intensities is decreased during dark adaptation.

Light adaptation

If the subject moves from dim light to bright light, the reverse changes occur. The visual threshold increases over time. The time taken for light adaptation is about 5 minutes.

Light reflex (pupillary reflexes)

Direct light reflex is observed when light is focused on one eye, the pupil of that eye constricts. The other unstimulated eye also shows pupillary constriction, which is called **consensual light reflex or indirect light reflex.** Pupillary constriction is also seen during accommodation to near vision.The constriction of the pupil (miosis) is a parasympathetic response mediated by the IIIrd cranial nerve. The fibers carrying light reflex travel in optic tract and go to pretectal nucleus in the midbrain. From pretectal nucleus, the fibers from each eye cross over and hence, the indirect light reflex response is seen. The pretectal nucleus sends fibers to Edinger Westphal nucleus of III nerve. The preganglionic parasympathetic fibers goes to ciliary ganglion. The post ganglionic fibers travel in short ciliary nerve and supply the circular muscle of iris (constrictor pupillae) (Fig. 5.12). The reflex contraction of this muscle causes the constriction of pupil. Pupillary constriction

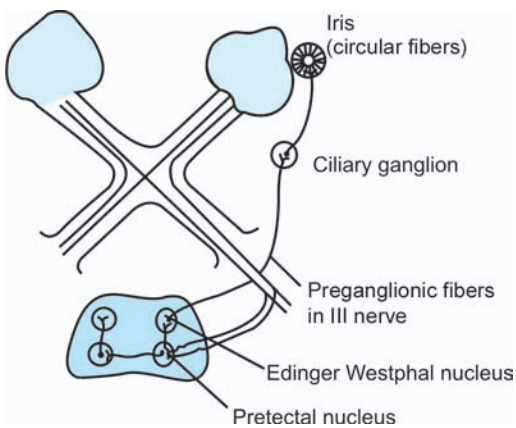


Fig. 5.12: Pathway of light reflex

can be abolished by administering parasympatholytic drugs like atropine or homoatropine, which will dilate the pupil. These drugs are used to examine the fundus of the eye. In a clinical condition such as **Argyll Robertson pupil**, the accommodation reflex is present, but, the light reflex is abolished. This condition occurs in neurosyphilis affecting the midbrain. With the effective treatment of syphilis with antibiotics, such observations are rarely made now.

Pupillary dilatation *(mydriasis)* is a sympathetic response. It is seen in sympathetic arousal and brought by the contraction of radial muscles of iris.

COLOR VISION

Color vision is a function of cones. Our eyes can perceive colors with wavelength of light ranging from 400 to 700 nm. Young & Helmholtz proposed **trichromatic theory of color vision** based on the existence of three types of cones, each responding to one of the primary colors namely, red, green and blue. Subsequent studies validated the theory proposed by Young & Helmholtz. The discovery of photochemical pigments for each type of cone indicated, that the color perception is due to the type of cone being stimulated and in what intensity. For example, if all the three types of cones are stimulated with equal intensity, white color is produced. If red cone and green cone are stimulated together, then a yellow color is obtained. Each cone shows maximum absorption of light at a particular wavelength (Fig. 5.13). The following chart explains the photochemical pigment in each cone and the absorption wavelength.

Various complimentary colors are produced by stimulating cones at different intensities of light. The color perception is the function of **occipital cortex** and the center is **lingual gyri** and **fusiform.** Neurons carrying color vision reach lateral geniculate body of thalamus to end in parvo cells. From here, the projections go to layer 4 of striate cortex **(blobs).** The blobs are the vertical arrangement of neurons and they respond to color vision. These cells also show *ON center–OFF surround* and OFF *center–ON surround* organization.

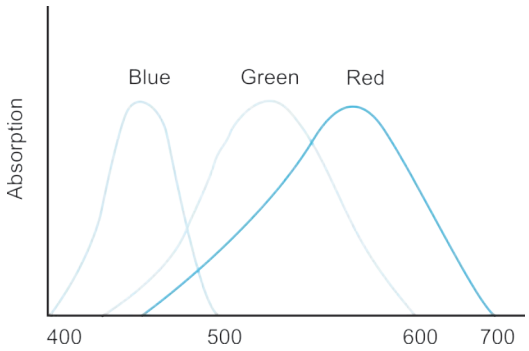


Fig. 5.13: Absorption spectra of the three types of cones

Type	Pigment	Wavelength
Red cone	Erythrolabe	565 nm
Green cone	Chlorolabe	550 nm
Blue cone	Cyanopsin	440 nm

Color blindness

Color blindness is genetically inherited as X linked recessive character. The females carry the defective gene and the males suffer from the disease. If one of the X chromosomes in female carries the defective gene X_1X (heterozygous), she will be the carrier of the disease. The male child born to her will have X_1Y chromosomes and suffer from color blindness. If both XX in female contain the defective gene, she will suffer from color blindness. Female children born to color blind male, will carry the defect and pass it to half of their male children.

Types of color blindness

There are three types namely monochromats, dichromats and trichromats.

Monochromats: It is the ability to see only one primary color. It is very rare.

Dichromats: The person can perceive two primary colors. Accordingly, protanopia, deuteroanopia and tritanopia are present.
Trichromats: This type is commonly observed. The subject is less sensitive to one of the primary colors. Hence, he will take more of that color, when asked to match with the given color spectra. Three types can be seen. In **protanomaly** less sensitivity to red is observed. Less sensitivity to green in **deuteranomaly** and less sensitivity to blue in **tritanomaly** are present.

Acuity of vision

The resolving power of eye is called visual acuity. It is the function of cones and maximum acuity is present in the fovea centralis. Test of acuity of vision can be done by Snellen's chart. It consists of rows of letters of different sizes with each row subtending an angle of 1 min at the nodal point at the distances specific for each row (60, 36, 24, 18, 12, 9, 6, and 5 meters). The subject is at a distance of 6 meters and each eye is tested separately. If the subject can read, say up to 7th row, his visual acuity for that eye is 6/6, which is normal. If he can read the letter upto 5th row his acuity is 6/12. In this way, it is possible to detect any defect in acuity of vision.

Binocular vision and stereopsis

WHen visual field is mapped for both the eyes in one chart, it will be observed that there is a central part, where fusion of visual field from both eyes occurs. The object viewed within this field will form binocular vision. Humans have binocular vision and it is the visual cortex which perceives the object as single image. The points on the retina on which the image of the object should fall to be seen binocularly as single object is termed as *corresponding points*. Double vision **(diplopia)** results when image on the retina does not fall within the corresponding points.

Depth perception (stereopsis)

Binocular vision gives better perception of depth, because the horizontal separation of images in two eyes produces retinal image disparity, which facilitates better depth perception. This is not possible with mono ocular vision where depth perception is possible by moving the eye from side-to-side.

Refractive errors of eye (Fig. 5.14)

Normally, parallel rays of light are brought to a sharp focus on the retina. The eye, which is receiving incident light from 6 meters distance (far vision) and focusing the image on the retina without accommodation is called **emmetropic eye** or normal eye.

Defects in refractive power of the eye results when the image is not focused on the retina due to, either the eye ball is too long or too short. The parallel rays of light fall in front and behind the retina respectively. Variations in refractive power of lens also can give defects.

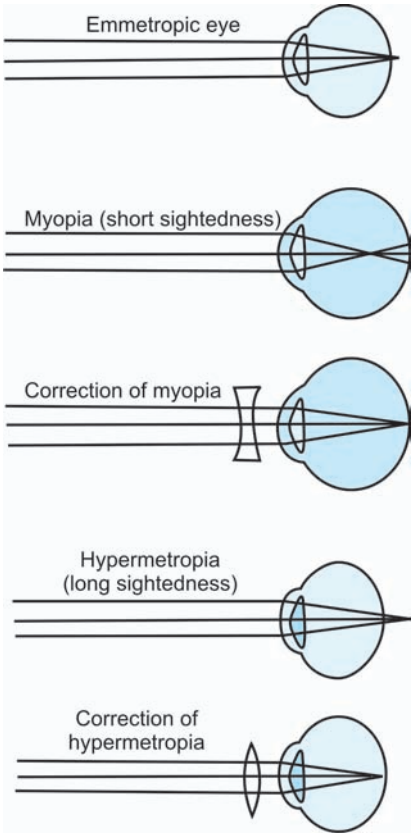


Fig. 5.14: Refractive errors of the eye and its correction

Myopia (short sightedness)

In this defect, the eyeball becomes too long and hence the parallel rays of light from infinite fall infront of retina, giving a blurred image. This is usually seen in children. Near vision is clearly seen and the far vision shows difficulty. The correction of this disorder can be done using concave lens (diverging lens). The power of the lens required is indicated by – sign .

Hypermetropia (long sightedness)

In this disorder the optical axis becomes too short and hence the image coming from parallel rays of light falls behind the retina and the image gets blurred. The subject can have a normal far vision, but his near vision is affected. This is corrected by using converging lens (convex). The power of lens is indicated by + sign.

Hypermetropia can also occur due to the hardening of lens, which changes its refractive power. This is observed in old age and the condition is called ***presbyopia***. This is corrected by convex lens.

Astigmatism

It is caused by changes in corneal surface. The light rays coming from vertical and horizontal curvatures of cornea are not focused sharply on the retina and hence blurred image is produced. It is corrected by using cylindrical lens.

Aqueous humor

It is a clear fluid present in the anterior and posterior chambers of the eye. It is formed in the posterior chamber from the epithelial cells lining the ciliary processes of the ciliary body. The formation involves secretion and filtration. The fluid from the posterior chamber enters the anterior chamber, through the pupil and is drained into the canal of Schlemm, at the corneoiridial angle. The volume and pressure of the intraocular fluid depends upon the balance between formation and drainage of fluid. Normal intraocular pressure ranges from 12 to 20 mm of Hg.

It has been observed that if intraocular pressure rises in Glaucoma the condition worsens resulting in blindness. In such cases it is necessary to reduce the intraocular pressure by β adrenergic blocking drugs or carbonic anhydrase inhibitors. It should be noted that Glaucoma is not caused by raised intraocular pressure. Intraocular pressure rises when obstruction of canal of Schlemm occurs due to prolonged dilatation of pupil. The iris is forced against the peripheral recess of the anterior chamber and hence the obstruction.

The composition of aqueous humor when compared with plasma shows that glucose, protein and urea are lower in aqueous humor, but the level of lactate, pyruvate, bicarbonate are greater. The reason is that glucose is utilised by the cornea and lens and hence lower concentration in aqueous humor. The anerobic glycolysis taking place in these tissues gives more amount of pyruvate and lactate.

The main function of this fluid is to provide nutrition to avascular structures like cornea and lens.

Ocular muscles and eye movements

There are six external ocular muscles which are responsible for eye movements. These includes **conjugate, vergence, saccades and pursuit.**

Conjugate movement of eye occurs, when both eyes move in the same direction.

Vergence movements include convergence and divergence movements. Far vision has divergence and near vision has convergence movements.

Saccades will have jerky movements of eye when focused on a fairly large object or during reading.

Pursuit movement is the movement of eye following the movement of the visual object. If the objects move faster as in traveling in a train or bus, optokinetic nystagmus occurs.

The ocular muscles producing these contractions are controlled by cranial nerves III, IV and VI. These cranial nerve nuclei receive projections from reticular formation, superior colliculus, pretectal region, cerebellum,

Brodmann's areas 17, 18 and 19 in occipital cortex and produce appropriate eye movements.

HEARING

Hearing helps man to know environmental cues for speech development and social communication. The stimulus for hearing is the sound, which travels in the conducting medium, as waves. Sound waves, on reaching the ear, stimulate the hearing apparatus and the sound is heard. Sound waves are produced due to pressure change caused by alternate compression and rarefaction of air molecules. The speed of sound wave in air medium is 330 meters/sec and in denser medium like water, the speed of travel would be greater.

Sound is transmitted through the external and middle ears to the inner ear. External ear consists of pinna, which in animals plays an important role in sound localization. In humans its role in sound localization is very localization much limited. The sound waves pass through the external auditory meatus to the tympanic membrane. The ceruminous glands and sebaceous glands lining the external auditory canal, secrete cerumen, which is a wax. It helps in preventing dust particles entering further into the ear. Excess secretion of cerumen and its accumulation leads to conduction deafness. The external auditory canal ends in tympanic membrane which shows its concavity protruding outwards.

Middle ear

It is present between tympanic membrane and oval window of inner ear (Fig. 5.15). It is an air filled cavity. The tympanic membrane vibrates in the same frequency as sound waves. When transmission of sound wave stops, the vibration of tympanic membrane also stops and hence the tympanic membrane is **critically damped**. The tympanic membrane is connected to the oval window by three ossicles namely **malleus, incus** and **stapes**. The handle of malleus is attached to the tympanic membrane and its head articulates with the head of incus. The handle of incus articulates with the head of stapes. The foot plate of stapes ends on the oval window.

The middle ear is connected to nasopharynx through the eustachian tube. Its opening in the nasophrynx is narrow and closed normally, except during swallowing, chewing, yawning, sneezing and vocalization. The opening of eustachian tube in nasopharynx facilitates equalisation of pressure on both sides of the tympanic membrane. This is disturbed during aeroplane ascent and descent, when the ambient pressure is different from atmospheric pressure. The unequal pressure on both sides of the tympanic membrane causes its bulging, producing pain. Relief from this can occur by swallowing fluid or chewing. The eustachian tube also helps to drain fluid into nasopharynx, which accumulates in the middle ear, as a

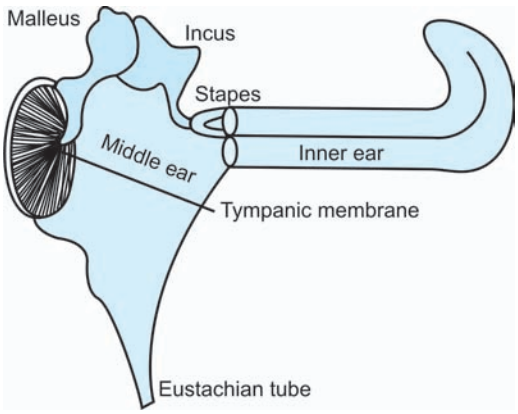


Fig. 5.15: Diagram of middle ear

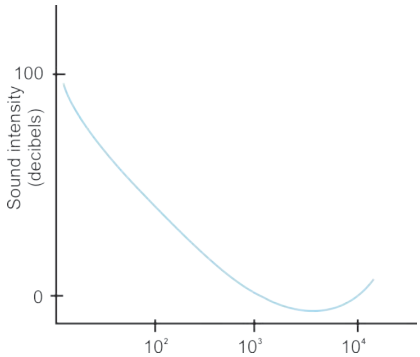


Fig. 5.16: Audibility curve of human ear

consequence of infection. It is common in children to observe throat infection spreading to middle ear through the eustachian tube. The bulging of eardrum due to the accumulation of fluid causes pain and sometimes it can cause due to tearing of eardrum also can occur.

Middle ear muscles (acoustic reflex)

There are two striated muscles in the middle ear namely tensor tympani and stapedius.These muscles are supplied by V and VII cranial nerves respectively. Whenever, there is a loud sound, these muscles reflexly contract and attenuate the sound intensity to protect the auditory receptors. The reflex contraction of middle ear muscles due to loud sound is called **acoustic reflex**. The contraction of tensor tympani pulls the tympanic membrane inwards and contraction of stapedius pulls foot plate of stapes out of the oval window. This mechanism is responsible for the attenuation of sound intensity. This protective reflex is not useful, when there is a loud sound instantly produced, as in gunshots. The reason is due to the time lag between the loud sound produced and reflex contraction of middle ear muscles. It has been observed that middle ear muscles contract to one's own loud speech. This reflex contraction helps the individual to hear his own speech and facilitates meaningful communication.

Qualities of sound

Sound has pitch (frequency) and intensity or loudness.

Pitch

Human ear can hear sound frequencies from 20 Hz to 20,000 Hz. But the maximum sensitivity is present between **1000 Hz to 4000** Hz frequencies, which is the range that our conversations normally occur (Fig. 5.16). As the age advances, there is hearing loss for higher frequencies of sound, which is called **presbycusis**. The speech that we produce is the mixture of pure tone, with various overtones superimposed on it. If this combination is periodically repeated, music is produced. But, if it is aperiodic, noise is heard.

Intensity of sound: Sound intensity or loudness is measured in **dB.** The **threshold of hearing in man is 0 dB** which has sound pressure of **0.0002 dynes/cm^2**. Sound intensity is determined as;

$$20 \log = \frac{\text{Intensity of sound}}{\text{Standard sound intensity}}$$

Humans can hear up to 140 dB sound intensity, but it results in pain. Our usual conversation will have sound pressure of 50 to 60 dB.

Impedance matching of middle ear

The main function of middle ear is impedance matching. When sound waves travel from air medium to fluid medium, part of the sound energy is lost, as the sound gets reflected in the fluid. This is the **acoustic impedance** present in the fluid. The middle ear should have a mechanism to overcome the acoustic impedance of the inner ear. Otherwise the sound that is reaching the receptors will have intensity lesser than the intensity of sound stimulus that is produced. It has been shown that there will be loss of 22 dB due to acoustic impedance of the inner ear if middle ear mechanism does not match it. The matching of inner ear impedance is achieved by the amplification of sound pressure in the middle ear. The amplification of sound is given by two ways. Firstly, the surface area ratio between tympanic membrane and oval window is 17:1, which causes sound amplification by 17 times. The transmission from a large surface area of tympanic membrane to a small surface area of oval window increases the pressure. The surface area of tympanic membrane is 55 cm^2 and that of oval window is 3 cm^2, which gives the ratio 17:1. Secondly, the mechanical lever like action of ossicles amplifies the sound intensity

by 1.3 times. The total amplification of sound pressure would be 17 × 1.3 = 22 times which exactly matches inner ear acoustic impedance.

Inner ear

The inner ear is a fluid filled cavity enclosed within the temporal bone. It contains vestibular and auditory receptor organs. The auditory receptors are present in the cochlea and vestibular organs contain the vestibular receptors.

Cochlea is divided into three compartments by two partitions namely Reissner's membrane and basilar membrane (Fig. 5.17).

Cochlea is wound round a central bony pillar modiolus by 2¾ turns.

The space above the Reissner's membrane is **scala vestibuli** to which the oval window opens. This space contains perilymph. Its composition is similar to ECF. The space below the basilar membrane is **scala tympani,** which opens into round window. It also contains perilymph. The space between Reissner's membrane and basilar membrane is **scala media** and is filled with endolymph. The composition of endolymph is similar to ICF with more K^+ (155 mEq/lit) and less Na^+ (5 mEq/ lit.). The scala vestibuli and scala tympani meet at the apex of cochlea (*helicotrema*), where the separation is not present.

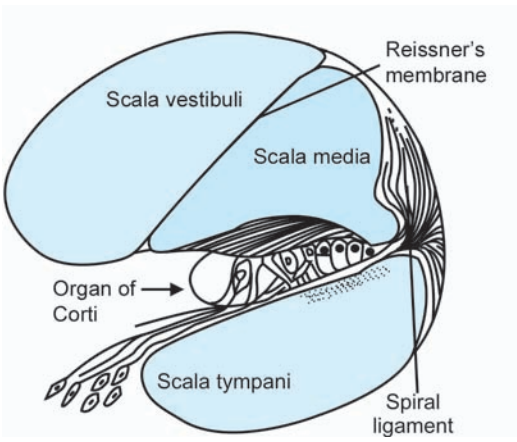


Fig. 5.17: Diagram of inner ear showing organ of Corti and the three scala

Organ of Corti (Fig. 5.18)

It contains receptors for hearing. It is present on the basilar membrane. There are two rods of Corti situated on the basilar membrane and between them is the tunnel of Corti. Outer to outer rod of Corti, are three rows of outer hair cells and inner to inner rod of Corti is a single row of inner hair cell present. There are also supporting cells in both types of hair cells present. The organ of Corti contains approximately 23,500 hair cells, out of which, 3500 are inner hair cells and 20,000 are outer hair cells. The hairs project to a gelatinous covering called tectorial membrane. At the apex, the tight junctions formed by the hair cells gives reticular lamina, through which the hairs pass through into tectorial membrane. The endolymph from scala media freely diffuses into tectorial membrane and bathes the hairs, while, the base of the cell is bathed by perilymph diffusing from scala tympani.

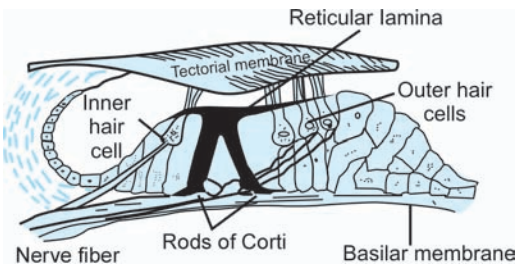


Fig. 5.18: Organ of Corti

Inner hair cell (Fig. 5.19)

It is flask shaped and contains hairs or **stereocilia** at the apex with gradations in their height. The base of the cell is supplied by the axons of cochlear division of VIII nerve. They are predominantly afferent. There are 33000 axons converging on the 12000 inner hair cells. There are also efferent fibers innervating the inner hair cells, but, they end presynaptically on the afferents.

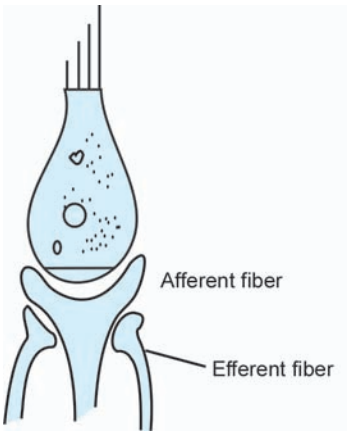


Fig. 5.19: Inner hair cell

Outer hair cells (Fig. 5.20)

It is cylindrical in shape and also contains stereocilia at the apex, similar to inner hair cell. The base of the cell receives mostly efferent innervation coming from superior olivary nucleus. The afferents supply is through collaterals coming from inner hair cells.

Mechanism of hearing

When sound wave strike the oval window, it sets up pressure change in the scala vestibuli as the oval window is pushed inwards. The pressure from scala vestibuli is transmitted to scala media, due to the vibrations of Reissner's membrane. The pressure change inturn causes the basilar membrane to vibrate, transmitting the pressure to scala tympani. This finally causes the round window to open outwards and releases the pressure. The vibrations of the basilar membrane, results in the stimulation of hair cells, which excites the nerve endings. Although it appears to be a simple mechanism for hearing, but actually the cochlea shows a complex organization for perceiving different frequencies of sound waves and a mechanism for coding of sound intensity. There is also tuning mechanism for selecting the correct frequencies of sound, so that the unwanted sounds are attenuated or inhibited.

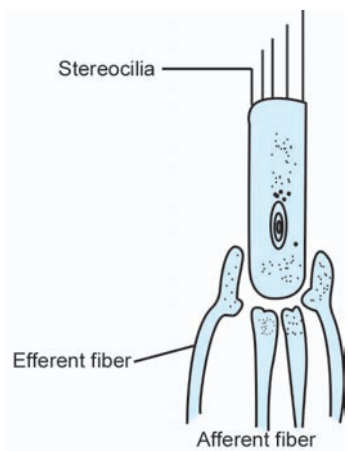


Fig. 5.20: Outer hair cell

Place theory of hearing

The mechanism of hearing has been explained by several theories. But place theory of hearing proposed by VonBekesy appears to be more relevant now, as the findings of the experimental studies confirm the existence of cochlear partition for sound frequencies which formed the basis of place theory of hearing.

The basilar membrane is not an uniform structure. It is compared to reeds present in a piano. The appearance of basilar membrane shows, that it is stiff and narrow at the base of cochlea, to enable it to vibrate for high frequencies of sound, but it is broader and compliant at the apex of cochlea, to resonate for low frequencies of sound. Between the base and apex of cochlea, the basilar membrane shows tuning for sound frequencies ranging from 20,000 to 20 Hz (Fig. 5.21).

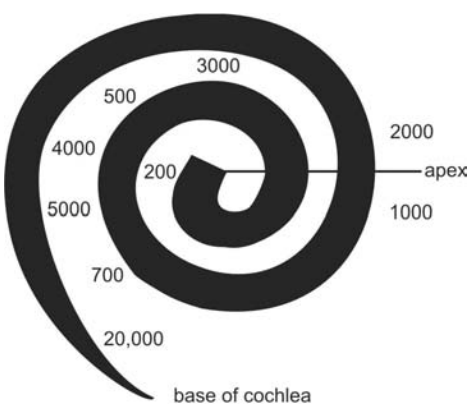


Fig. 5.21: Tonotopic map of sound frequencies on the basilar membrane

Tuning of inner hair cells

The inner hair cells receive mainly afferents and hence these cells respond directly to sound stimulus. The outer hair cells receive predominantly efferents and the activation of this tract leads to tuning of inner hair cells. The outer hair cells contain contractile proteins, which contract and shorten the cell when sound stimulus is present. This increases the sensitivity of inner hair cells for sound stimulus. Like the basilar membrane, the inner hair cells also show tuning for sound frequencies. Those present at the base of cochlea respond to high sound frequency and those at the apex of cochlea are stimulated by low frequency of sound.

Traveling wave

The vibration of basilar membrane produces wave, which travels towards the apex and dampens (Fig. 5.22). The whole basilar membrane moves to a given sound frequency, but the point on the basilar membrane, which coincides with the given sound frequency will produce maximum vibration. It will be observed that the amplitude of vibration of basilar membrane is inversely related to the distance from stapes (Fig. 5.23).

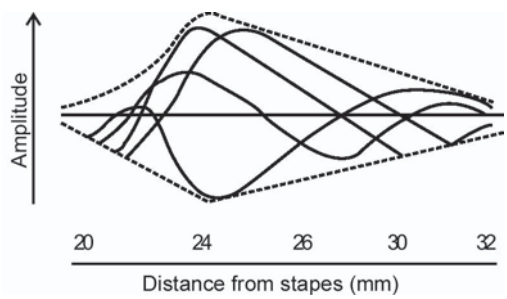


Fig. 5.22: Travelling waves

Note the relative amplitude of waves in relation to the distance from the stapes. The traveling wave, which shows maximum amplitude near the stapes, dampens as it moves towards apex of cochlea

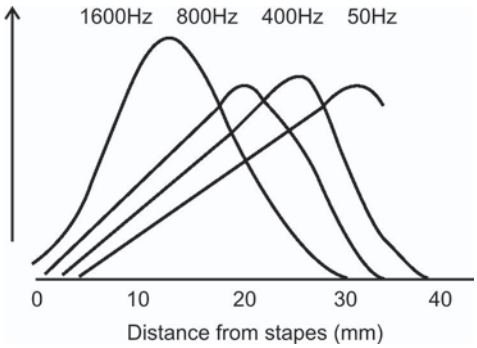


Fig. 5.23: Displacement of basilar membrane by different sound frequencies. The diagram shows the place theory of hearing as seen from the maximum displacement of basilar membrane in relation to the distance from stapes for high and low frequency of sound waves. The vertical arrow indicates the amplitude of displacement of basilar membrane

Coding of sound intensity

Sound intensity is coded by the number of hair cells stimulated and the frequency of firing of action potentials in the auditory nerve fiber. When the intensity of sound is greater, a large area of basilar membrane vibrates, exciting more hair cells, which produces increased rate of firing of action potentials. Greater the frequency of action potentials, greater the sound intensity perceived.

Transduction of receptors

The up and down movement of basilar membrane causes back and forth movement of hairs in the tectorial membrane. The hairs bend, due to the shearing force. The downward movement of basilar membrane causes the stereocilia to bend away from the tallest hair (towards the limbus) producing hyperpolarization of the hair cell (Fig. 5.24). When basilar membrane moves upwards, the stereocilia bend towards the tallest hair (away from limbus) producing depolarization of the hair cell (Fig. 5.24). Depolarization is caused as a result of influx of K^+ into the hair cell from endolymph. The endolymph in scala media has +80 mv potential,

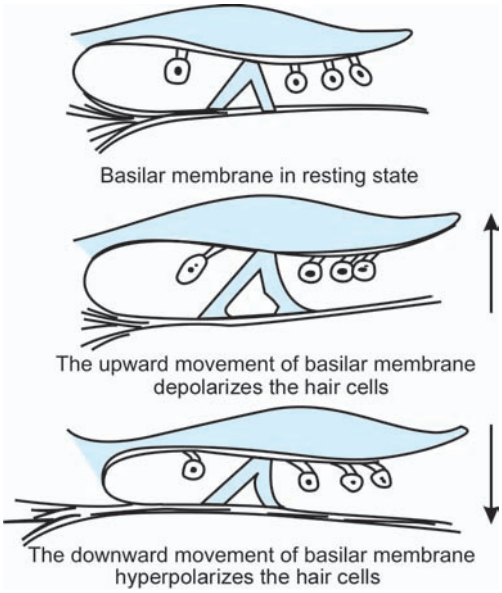


Fig. 5.24: Displacement of basilar membrane and its effect on the excitability of the hair cells

whereas, the potential of the hair cell is – 60 mv, so there is a potential gradient of 140 mv for K^+ to move into the cell. When stereocilia bends towards the tallest hair, the K^+ channels in the membrane of hair cells open, allowing its influx. When stereocilia bend away from the tallest hair, the K^+ channels are closed and hence hyperpolarization of hair cells occurs.

The receptor cells show at the apex receptor potentials when K^+ influx occurs. The receptor potentials recorded from the apex of hair cells are called **cochlear microphonics.** They faithfully reproduce the sound frequency. That is, at the base of cochlea, cochlear microphonics are produced for high frequency sound, while the apex of cochlea show cochlear microphonics for low frequency sound. Cochlear microphonics show no latent period, no refractive period, resistant to hypoxia, anesthesia and does not show all or none effect. In this way, the cochlear microphonics differ from action potentials. These receptor potentials developed at the apex of the hair cells leads to the development of generator potential from the base of the hair cell, which finally gives rise to action potential in the afferent nerve fiber.

It was suggested that for sound frequencies below 2,000 Hz, coding depended on the frequency of action potentials from auditory nerve fibers coming from the apex of cochlea. This was the *Volley effect* and together with the place theory, the **duplex theory of hearing** was proposed. But this could not be accepted due to the drawback in correlating frequency of sound to the frequency of action potentials from nerve fibers. As said above, the frequency of action potentials codes sound intensity and not the frequency of sound.

Auditory pathway (Fig. 5.25)

Auditory pathway in CNS is a complex one, due to the presence of feed forward and feed back loops, representing each ear bilaterally from cochlear nuclei to auditory cortex.

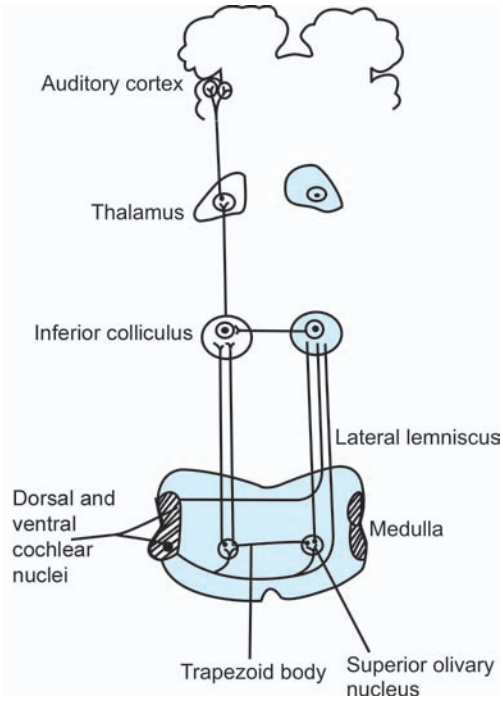


Fig. 5.25: Auditory pathway

The axons from the spiral ganglion go to medulla and end in dorsal and ventral cochlear nuclei. Dorsal cochlear nuclei send fibers mostly on the ipsilateral side in the lateral lemniscus to the midbrain and end in inferior colliculus. In the medulla itself, the two nuclei are connected reciprocally. The ventral cochlear nuclei send fibers in both ipsilateral and contra lateral pathways. It sends connections to the opposite superior olivary nucleus and trapezoid body. From superior olivary nucleus, fibers ascend in lateral lemniscus to inferior colliculus.

Inferior colliculus, which receive auditory fibers sends collaterals:

- To superior colliculus for integrating auditory and visual impulses
- To reticular formation for attention or arousal to auditory stimuli
- To cerebellum for servo control mechanism of voluntary movements.

Fibers from inferior colliculus go to thalamus to end in medial geniculate body, which is the auditory relay center. From thalamus, auditory projections are sent to temporal cortex to end in area 41, in the superior temporal gyrus. Areas 42 and 22 form the auditory association areas.

There is a tonotopic organization in the auditory cortex for sound frequency. The anterolateral part of superior temporal gyrus receives low frequency sound, while the posteromedial responds to high frequency sound. The function of cortex is not for perception of sound but for sound localization and understanding speech. Damage to auditory cortex on one side will not cause deafness, as both the ears are bilaterally represented.

Efferent supply to cochlea is from superior olive (olivocochlear bundle), which in turn receives central connections from cortex, thalamus and inferior colliculus. The efferent pathway is activated, whenever there is sound stimulus reaching the receptors. The efferent nerve activity helps to reduce or inhibit the unwanted sound and increase the sensitivity to only those sound stimuli to which attention is focused. Efferent nerve activity also helps to reduce the sound intensity reaching the central nervous system, when there is loud noise.

Sound Localization

Sound localization in humans depends on two mechanisms namely;

Time or phase difference in the arrival of sound stimulus between the two ears

Intensity difference between the two ears when sound is produced. The ear which is closer to the sound stimulus will be receiving greater intensity of sound.

Disorders of hearing

There are two types of deafness present. They are:

Conduction deafness

Nerve deafness (sensory neural)

Conduction deafness results in conditions like blockade of ear canal by wax, middle ear diseases like fixation of foot plate (otosclerosis) and inflammation of ossicles (otitis media). Nerve deafness occurs in prolonged administration of ototoxic drugs like streptomycin, gentamycin, etc., occupational hazards in workers exposed to loud sound and tumors of inner ear.

Conduction of sound to the inner ear occurs in two ways, i.e. **air conduction** (ossicular conduction) and **bone conduction.** The latter is given by transmitting sound through vibrations from mastoid process of temporal bone. This type of conduction requires greater intensity of sound.

Clinical audiometry

It is an objective test to assess the extent of hearing loss that is present and accordingly the hearing aid can be suggested for the patient. The threshold of hearing is determined for various frequencies of sound of pure tones from 125 Hz to 8000 Hz. The subject starts from 0 dB intensity for each tone and the intensity is increased by the subject himself from the control, until he hears the sound. It is the threshold of intensity for that frequency of sound. A graph is plotted with frequency verses intensity (Fig. 5.26).

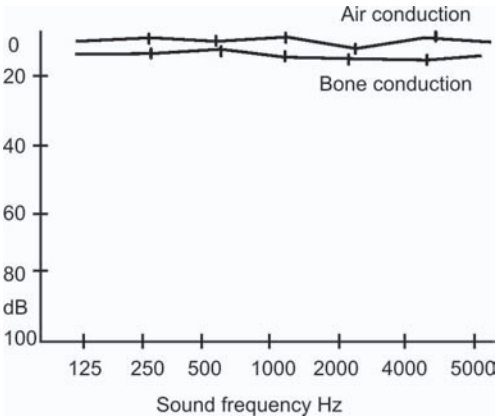


Fig. 5.26: Normal audiogram. In both air conduction and bone conduction, the threshold of hearing is determined for each frequency of sound given. The normal audiogram shows air conduction is better than bone conduction

The testing is done separately for both the ears and both air conduction and bone conduction testing is done for each ear. Air conduction is tested by using headphone and bone conduction is tested from vibrations of mastoid process of temporal bone. The graph that is obtained is compared with the normal standard graph for that age group. The difference in threshold intensity for each frequency will give the % of hearing loss.

Hearing tests: Tuning fork tests are done to find out the type of deafness. These tests are subjective and cannot tell the extent of hearing loss. The clinical audiometry is an objective test, which will give the extent of hearing loss present. In tuning fork tests, the frequency of the tuning fork is 256 cycles/sec. In three ways the tests can be conducted.

Weber test: The vibrating tuning fork is kept on the vertex of the skull. Normal subject would hear equally in both the ears. In conduction deafness, the sound is heard better in the affected ear because masking effect of environmental noise is absent. In nerve deafness, the normal ear hears better.

Rinne test: Vibrating tuning fork is kept on

Contd....

Contd....

the mastoid process of temporal bone until the subject no longer hears it, then the tuning fork is kept infront of the ear. In normal subject, the air conduction would be present after the bone conduction. In conduction deafness, the air conduction is not present, after the bone conduction. In nerve deafness, the air conduction is present after the bone conduction as long as nerve deafness is partial.

Schwabach test: The bone conduction is compared between the normal and the affected subject. In conduction deafness, the patient will hear in the affected ear better than normal subject. In nerve deafness, normal subject hears better than the patient.

CHEMICAL SENSES

Taste and smell are considered as chemical senses, since the stimulation of the receptors is by chemical substances. These two senses are essential for life survival in animals, whereas in man, they are not necessary for survival, but help in nutrition and digestion.

Taste (Gustation)

Gustatory receptors are present in taste buds. Taste buds are distributed in the papillae of tongue, oral cavity, epiglottis and pharynx. The papillae of tongue are of four types namely, *filiform, fungiform, foliate* and *circumvallate*. The filiform papillae do not contain taste buds. The fungiform and valate are distributed at the tip and sides of the tongue, while the circumvallate is arranged as V shaped at the back of the tongue. Each papilla contains 10 to 100 taste buds.

Taste buds (Fig. 5.27)

Taste bud structure shows it is oval shaped and contains receptor cells, supporting cells and basal cells. The receptor cells undergo degeneration in 7 to 10 days time and they are replaced by basal cells. The apex of receptor cell has cilia, which project into the taste pore. The

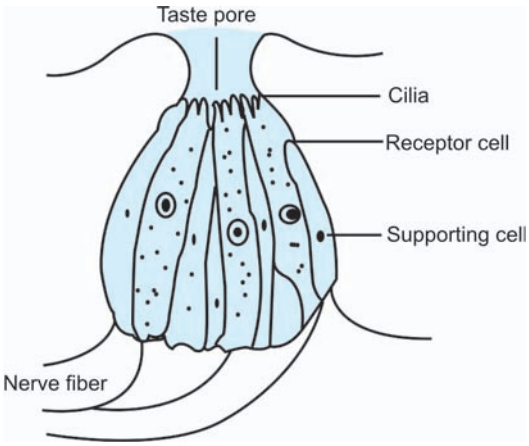


Fig. 5.27: Structure of a taste bud

dissolved sapid molecules come in contact with the receptor protein and produces receptor potential. The base of the receptor cell is supplied by afferents from **VII**, **IX** and **X** cranial nerves. The anterior two third of the tongue is supplied by VII nerve (facial), while the posterior third of the tongue is supplied by IX nerve (glossopharyngeal). The taste from pharyngeal region is carried by the X nerve (vagus). The somatosensory information from anterior two third is carried by trigeminal (V nerve) nerve. The general sensations like thermal from hot food and pain by irritants can affect the perception of taste. The general sensation from posterior third is carried by IX nerve itself.

There are four primary tastes namely sweet, sour, salt and bitter. It has been shown that basic taste sensations can be perceived from all areas of the tongue and not to a particular region in the tongue as believed earlier.

Taste receptors transduction

The salt taste is stimulated by NaCl which activates **ENaC** (Epithelial sodium channels) in the taste buds. The entry of Na^+ depolarizes the receptor cell and causes glutamate release which depolarizes the afferent neurons

The sour taste is caused by protons and bitter taste is produced by many compounds which include alkaloids. The protons act on the ENaC receptors in the taste buds and depolarize them. Also another receptor **HCN**(hyperpolarization activated cyclic nucleotide- gated cation channel) is involved for sour taste activation.

The bitter producing compounds activate G proteins in the receptors and depolarize them. Substances producing sweet taste also act via G proteins which include gustducin.

The fifth taste modality **umami** gives a pleasant and sweet taste but different from the standard sweet taste. It acts on the glutamate receptor in the taste buds and causes depolarization.

Taste coding

It depends on the pattern of receptor stimulated. The taste receptors are stimulated by all the four primary tastes. But the receptor sensitivity to one taste stimulus will be maximum. The nerve fibers coming from such receptors will be excited greatly than other nerve fibers. In this way, coding of taste is done at the periphery itself. The intensity depends on the frequency of action potentials in the afferents.

Transduction of receptors

Taste stimulation depends on the sapid molecules in dissolved medium, its concentration and the area of stimulation in the oral cavity. The attachment of gustatory molecules to the receptor protein causes formation of second messengers like cAMP, IP_3, through G protein activation. These second messengers cause change in the permeability of the receptor cell membrane, resulting in the opening of Na^+ channels and depolarization. The development of receptor potential at the apex of the receptor cell leads to the formation of generator potential at the base, which gives rise to propagated action potential from nerve fibers.

Adaptation

Taste receptors show adaptation, if the stimulation persists. The continued presence of gus-

tatory molecules results in decreased sensitivity of the receptors known as adaptation.

Taste pathway (Fig. 5.28)

The afferents carrying tastes (VII, IX and X) enter medulla and end in nucleus tractus solitarius. Second order neurons from this nucleus travel in medial lemniscus and end in thalamus. Third order neurons carrying tastes from thalamus go to sensory cortex (SI) and end in the face area, in the postcentral gyrus, which is the center for taste. Loss of taste sensation is called **ageusia** and decreased sensitivity to taste is called **hypogeusia.**

Smell (olfaction)

The sense of smell is highly developed in animals, where it helps in the survival of the animal. They are called **macrosmatic.** Humans are **microsmatic,** as the sense of smell is less developed in them.

Receptors for smell are situated in the olfactory mucosa present in the roof of the nose. The mucosa contains, besides the receptors,

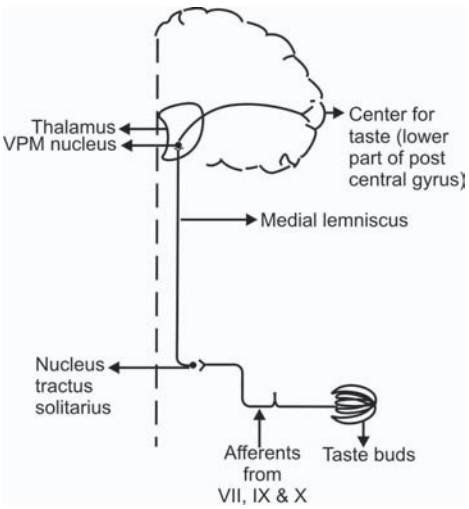


Fig. 5.28: Taste pathway

Bowman's glands, which secrete a mucus. Odour molecules first dissolve in the Bowman's glands secretion and then stimulate the receptors.

Olfactory receptors

Olfactory receptors are similar to a peripheral ganglion of central nervous system. Infact, the CNS is exposed to the external world through olfactory receptors. Like gustatory receptors, their life period is only for a few weeks and they are replaced by the basal cells. The receptor cells are also surrounded by the supporting cells. The apex of the receptor has cilia or dendritic rods to which the odor molecules are attached. The base of the cell is supplied by the axons of 1st cranial nerve (olfactory nerve).

Transduction of receptors

Stimulation of olfactory receptors requires the molecules to be volatile and should show water or lipid solubility. Flavour of food is due to the stimulation of both olfactory and gustatory receptors. Since the odorant molecules are carried in the inspired air, sniffing increases the concentration of molecules reaching the receptors. The transduction involves the opening of Ca^{++} channels in the receptor membrane, mediated by second messengers like cAMP and IP3. The development of receptor potential at the apex of the cell leads to the generator potential at the base, which finally causes action potential to develop from the nerve fibers.

Coding of odours

There are seven primary odours such as **floral, etheraeal, musky, camphor, putrid, pungent** and **peppermint**. The coding of odour is due to the pattern of action potentials in the nerve fibers. Normally, there is a spontaneous activity in the afferents coming from olfactory receptors. When stimulation occurs by odorant molecules, the frequency of discharge of impulses in the nerve fibers will become more. An olfactory

receptor can respond to more than one odorant. As said earlier, coding for an odorant depends on the pattern of nerve response, which inturn is related to the concentration of odorant. When an odour stimulates the receptors for a longer period of time, the sensitivity of the receptors for that odour decreases and the odorant is inhibited. This is known as **adaptation.**

Olfactory pathway (Fig. 5.29)

The axons coming from the receptors pierce the cribriform plate of ethmoid bone and go to olfactory bulb. Several thousand receptors converge on a single olfactory bulb. Here, they synapse with the dendrites of mitral and tufted cells and form olfactory glomeruli. The glomeruli also receive dendrites from granule cells and periglomerular cells, which connect mitral cells. The granule cell activity can modulate the output from olfactory bulb, whereas, the periglomerular cell activity modulates the input to the bulb. These interneurons give lateral inhibition and sharpening of the sensory stimulus. The activity of these cells also inhibits the afferent pathway, after sensory perception. This is possible, because of efferent connections coming from olfactory cortex to the bulb.

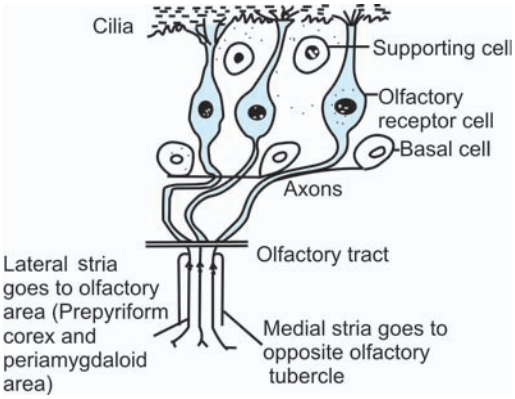


Fig. 5.29: Olfactory pathway

The axons of mitral cells form olfactory tract, which divides into lateral and medial divisions. The lateral division of olfactory tract goes ipsilaterally to olfactory lobe to end in **prepyrifrom cortex, amygdala** and **periamygdaloid areas**, which are the centers for olfaction. The medial division goes to the opposite olfactory tubercle. From here fibers go to dorsomedial nucleus of thalamus and orbitofrontal cortex. Thus, we observe that there are olfactory projections to thalamus and neocortex and not as believed earlier. Loss of smell is known as **anosmia.**

Self-study Questions

Multiple Choice Questions

Choose the single best answer

1. **The time taken for the transmission of impulses in monosynaptic pathway is:**
   A . 0.05 msec
   B. 0.05 sec
   C. 0.5 msec
   D. 0.1 sec

2. **Which of the following is present in all types of cutaneous sensory receptors?**
   A. Adaptation
   B. Encapsulated endings
   C. Specificity
   D. Encoding of multiple cutaneous sensations

3. **After discharge, irradiation and recruitment are characteristics of:**
   A. Extensor reflex
   B. Flexor reflex
   C. Crossed extensor reflex
   D. Golgi tendon reflex

4. **What activity should be taking place at the postsynaptic membrane, if its membrane potential changes from – 65 mv to – 50 mv?**
   A. EPSP **B.** IPSP
   C. EPP **D.** GP

5. **Prolongation of signal in a neuronal pool is called as:**
   A. Recruitment
   B. After discharge
   C. Facilitation
   D. Subliminal fringe

6. **Electrical response to subminimal stimuli in the excitable tissue would produce all of the following *except*:**
   A. Temporal summation
   B. Spatial summation
   C. Action potential
   D. Graded potentials

7. **Long term potentiation and presynaptic facilitation are associated with:**
   A. Learning
   B. Speech
   C. Sensory coding
   D. Wakefulness

8. **The release of excitatory transmitter from presynaptic terminal can be reduced by:**
   A. Renshaw cell inhibition
   B. Direct inhibition
   C. Presynaptic inhibition
   D. Indirect inhibition

9. **The glycine receptor contains which of the following ion channels?**
   A. K^+ **B.** Ca^{++}
   C. Na^+ **D.** Cl^-

10. **The cutaneous receptor stimulated by damage occurring in the tissues is:**
    A. Mechanoreceptor
    B. Nociceptor
    C. Thermoreceptor
    D. Proprioceptor

11. **The cutaneous receptor which shows decline of frequency of firing of action potentials over time for constant intensity of stimulation will be:**
    A. Pressure
    B. Pain
    C. Warm
    D. Touch

12. The local potential developed in a sensory receptor in response to a stimulus energy is known as:
- **A.** Action potential
- **B.** EPSP
- **C.** Generator potential
- **D.** EPP

13. Encoding of intensity of sensory stimulus include all of the following *except*:
- **A.** Number of receptors activated
- **B.** Frequency of firing of action potentials
- **C.** Rate of change of frequency of action potentials
- **D.** Spatial location of sensory stimulus

14. The nerve fibers which carry cutaneous sensory impulses include all of the following *except*:
- **A.** Aα
- **B.** Aβ
- **C.** Aδ
- **D.** C

15. The muscle spindle, Golgi tendon organ and joint receptors respond to:
- **A.** Joint movement
- **B.** Muscle contraction
- **C.** Muscle tension
- **D.** Vibration

16. The transmitter secreted at the primary afferent endings in the spinal cord for fast pain is:
- **A.** Glycine
- **B.** Substance P
- **C.** Glutamate
- **D.** GABA

17. Which is the region that gives descending pathway to the spinal cord to inhibit primary afferents carrying pain?
- **A.** Reticular formation
- **B.** Raphe nucleus
- **C.** Locus ceruleus
- **D.** Cingulate gyrus

18. The conscious component of proprioception is carried by:
- **A.** Spinocerebellar tracts
- **B.** Dorsal column
- **C.** Anterolateral column
- **D.** Spino olivary tract

19. A sharp pricking pain is carried by which of the following nerve fiber types?
- **A.** C
- **B.** Aα
- **C.** Aβ
- **D.** Aδ

20. Discriminative tactile sense is carried by:
- **A.** Anterolateral column
- **B.** Spinocerebellar tract
- **C.** Dorsal column
- **D.** A and C

21. Inhibition of pain can be produced by all of the following *except*:
- **A.** Lesion of thalamus
- **B.** Release of endogenous opioids
- **C.** Stimulation of touch fibers
- **D.** Stimulation of periaqueductal grey

22. The anterolateral column carries information about:
- **A.** Fine touch
- **B.** Pressure
- **C.** Vibration
- **D.** Temperature

23. The region which is least involved in receiving sensory processing is:
- **A.** Spinal cord
- **B.** Thalamus
- **C.** Basal ganglia
- **D.** Postcentral gyrus

24. Which of the following can occur in both flexor and extensor reflexes?
- **A.** After discharge
- **B.** Irradiation
- **C.** Reciprocal innervation
- **D.** Crossed extensor reflex

25. Golgi tendon organ receptors:
- **A.** Detect muscle length
- **B.** Detect muscle tension
- **C.** Are rapidly adapting
- D. Present in muscle

26. The second order neurons carrying vibratory sense synapse in:
- **A.** Spinal cord
- **B.** Medulla
- **C.** Thalamus
- **D.** Sensory cortex

27. Cell bodies for corticospinal tracts are present in:
- **A.** Motor cortex
- **B.** Premotor cortex
- **C.** Somatosensory cortex
- **D.** All of the above

28. Muscle tone will be abolished in all of the following conditions *except*:
- **A.** Damage to corticospinal tract
- **B.** Damage to anterior horn cell
- **C.** Lesion of dorsal nerve
- **D.** Lesion of ventral nerve

29. Muscle spindles show all of the following *except*:
- **A.** Contain both nuclear bag and nuclear chain fibers
- **B.** Innervated by Ia afferent fibers
- **C.** Innervated by alpha motor neurons
- **D.** Detect muscle length

30. Lateral corticospinal tract:
- **A.** Controls movements of proximal muscles of limbs
- **B.** Controls movements of distal muscles of limbs
- **C.** 80% of fibers descend as uncrossed
- **D.** ends on the anterior horn cell through interneuron

31. Total loss of muscle power is called:
- **A.** Paralysis
- **B.** Paresis
- **C.** Hemiparesis
- **D.** Atonia

32. The lesion of which of the following would produce aggravation of ataxia with the closure of eyes?
- **A.** Cerebellum
- **B.** Basal ganglia
- **C.** Thalamus
- **D.** Dorsal column

33. The reflex necessary for muscle tone is:
- **A.** Flexor reflex
- **B.** Stretch reflex
- **C.** Labyrinthine reflex
- **D.** Righting reflex

34. Lesion of Pyramidal tract would produce all of the following *except*:
- **A.** Spastic paralysis
- **B.** Muscle wasting
- **C.** Ankle clonus
- **D.** Babinski's sign

35. Muscle tone is increased in all of the following conditions *except*:
- **A.** Hemiplegia
- **B.** Cerebellar disease
- **C.** Parkinson's disease
- **D.** Wilson's disease

36. The primary afferent input to basal ganglia comes from:
- **A.** Cerebellum
- **B.** Reticular formation
- **C.** Cerebral cortex
- **D.** Thalamus

37. The extrapyramidal tract which controls posture and equilibrium is:
- **A.** Reticulospinal tract
- **B.** Tectospinal tract
- **C.** Vestibulospinal tract
- **D.** Olivospinal tract

38. The GABA ergic transmitter system is present in the following basal ganglia connections *except*:
- **A.** Striatopallidal
- **B.** Nigrostriatal
- **C.** Striatonigral
- **D.** Pallidothalamic

39. Degeneration of GABAergic neurons in the striatum causes:
- **A.** Parkinson's disease
- **B.** Athetosis
- **C.** Huntington's chorea
- **D.** Ballism

40. Resting tremor is characteristic of lesion of which of the following?
- **A.** Thalamus
- **B.** Basal ganglia
- **C.** Cerebellum
- **D.** Motor cortex

41. Cognitive control of voluntary movements is due to which of the following neural circuit activity?
A. Caudate
B. Putamen
C. Nigrostriatal
D. Pallidothalamic

42. The failure of progression of sequential movements is called:
A. Ataxia **B**. Asynergia
C. Atonia **D**. Asthenia

43. The neuron which sends output to the cerebellar nuclei is:
A. Pyramidal cell **B**. Basket cell
C. Stellate cell **D**. Purkinje cell

44. Bradykinesia is associated with the lesion of:
A. Cerebellum
B. Basal ganglia
C. Thalamus
D. Pontine reticular formation

45. Finger nose test in the neurological examination is done to assess:
A. Muscle tone
B. Muscle power
C. Coordination
D. Balance

46. Mental concentration is associated with which of the following waves of EEG?
A. α **B**. β
C. θ **D**. δ

47. Posture maintenance during linear acceleration of the head is due to the stimulation of:
A. Cerebellum
B. Vestibular nucleus
C. Otolith organs
D. Semicircular canals

48. Cerebellar damage causes all of the following *except*:
A. Dysarthria **B**. Dystonia
C. Dysmetria **D**. Asynergia

49. Ascending reticular activating system:
A. Sends specific sensory projections to cortex
B. Causes arousal on inhibition
C. Activity produces slow waves in EEG
D. Sends nonspecific sensory projections to cortex

50. Which of the following connects neocerebellum with motorcortex?
A. Thalamus
B. Basal ganglia
C. Medullary reticular formation
D. Pontine reticular formation

51. Right cerebral hemisphere functions include all of the following *except*:
A. Reasoning
B. Visuospatial relation
C. Understanding of speech
D. Language

52. Language comprehension is due to the activity of:
A. Auditory area
B. Wernicke's area
C. Broca's area
D. Sensory association areas

53. Which of the following would be affected when difficulty to produce speech after stroke occurs?
A. Prefrontal
B. Wernicke's area
C. Broca's area
D. Angular gyrus

54. Motivation or drive to do an act is due to the activity of which area of the brain?
A. Limbic lobe **B**. Basal ganglia
C. Cerebellum **D**. Motor cortex

55. The body circadian rhythms are controlled by:
A. Paraventricular nucleus
B. Supraoptic nucleus
C. Suprachiasmatic nucleus
D. Preoptic nucleus

56. Fast, desynchronized high frequency EEG waves can be recorded during:
- **A**. Awake
- **B**. Closure of eyes
- **C.** REM sleep
- **D.** A and C

57. Which of the following neural circuits are activated during emotion?
- **A.** Papez
- **B.** Putamen
- **C.** Caudate
- **D.** Thalamo cortical

58. Lesion of Wernicke's area would produce:
- **A**. Auditory agnosia
- **B**. Visual agnosia
- **C**. Motor aphasia
- **D**. Sensory aphasia

59. Which of the following neurotransmitter system is affected in Alzheimer's disease?
- **A**. Cholinergic
- **B**. Dopaminergic
- **C**. Adrenergic
- **D**. Serotonergic

60. Which part of the brain is activated during learning and short term memory?
- **A**. Cerebellum
- **B**. Hypothalamus
- **C**. Hippocampus
- **D**. Thalamus

61. Action potentials are produced from which of the retinal layers?
- **A**. Receptor cells
- **B**. Bipolar cells
- **C**. Amacrine cells
- **D**. Ganglion cells

62. When you are viewing a near object:
- **A.** The biconvex lens flattens
- **B.** The ciliary muscle relaxes
- **C.** The anterior curvature of lens increases
- **D.** The anterior curvature of cornea increases

63. Cones are responsible for all of the following *except*:
- **A.** Visual acuity
- **B.** Photopic vision
- **C.** Color vision
- **D.** Scotopic vision

64. When light strikes the photoreceptor:
- **A**. Depolarization occur
- **B**. Photochemical changes occur
- **C.** cGMP concentration increases
- **D.** Na^+ channels open

65. Rods are activated when:
- **A.** Scotopic vision changes to photopic vision
- **B.** Photopic vision changes to scotopic vision
- **C.** Resolving power of the eye is increased
- **D.** Blue color of the spectrum strikes the retina

66. In presbyopia :
- **A**. Near point increases
- **B**. Far point decreases
- **C**. Visual acuity increases
- **D**. Total refractive power increases

67. Bitemporal hemianopia is caused by the lesion of:
- **A.** Optic nerve
- **B.** Optic chiasma
- **C.** Optic tract
- **D.** Vsual cortex

68. Which one of the following will be the refractive power of the eye (diopters) when a subject views an object at 6 meters distance?
- **A.** 12
- **B.** 59
- **C.** 70
- **D.** 82

69. Localization of sound is the function of:
- **A**. Auditory cortex
- **B**. Outer hair cells
- **C**. Inner hair cells
- **D**. Olivocochlear bundle

70. Middle ear function includes all of the following *except*:
- **A**. Acoustic reflex
- **B**. Acoustic impedance
- **C**. Impedance matching
- **D**. Amplification of sound intensity

71. Depolarization of hair cells in the organ of corti is caused by:
- **A**. Entry of K^+
- **B**. Entry of Ca^{++}
- **C**. Entry of Na^+
- **B**. Exit of K^+

72. The auditory threshold in human is:
- **A.** 0 dB
- **B.** 0.1 dB
- **C.** 1 dB
- **D.** 10 dB

73. When a subject is unable to hear high frequency sound waves, the damage is likely to be in:
- **A**. Basilar membrane near the stapes
- **B**. Basilar membrane at the apex of cochlea
- **C**. Medial geniculate body in the thalamus
- **D**. Olivocochlear bundle

74. The normal range frequency of sound at which maximum sensitivity is present includes:
- **A.** 0- 140 Hz
- **B.** 20 – 20,000 Hz
- **C.** 1000- 4000Hz
- **D.** 5000- 8000 Hz

75. Action potentials from the axons of the auditory nerve are produced when:
- **A**. Basilar membrane moves upwards
- **B**. Basilar membrane moves downwards
- **C.** Na^+ channels open
- **D**. Stereocilia bends away from the Kinocilium

ANSWER KEYS

1. (C)	**2. (C)**	**3. (B)**	**4. (A)**	**5.(B)**	**6. (C)**	**7. (A)**	**8.(C)**	**9. (D)**	**10. (B)**
11. (D)	**12. (C)**	**13. (D)**	**14. (A)**	**15. (C)**	**16. (C)**	**17. (B)**	**18. (B)**	**19. (D)**	**20. (C)**
21. (A)	**22. (D)**	**23. (C)**	**24. (C)**	**25. (B)**	**26. (C)**	**27. (D)**	**28. (A)**	**29. (C)**	**30.(B)**
31. (A)	**32. (D)**	**33. (B)**	**34.(B)**	**35. (B)**	**36.(C)**	**37. (C)**	**38. (B)**	**39. (C)**	**40.(B)**
41. (A)	**42. (B)**	**43. (D)**	**44.(B)**	**45. (C)**	**46. (B)**	**47. (C)**	**48.(B)**	**49. (D)**	**50. (A)**
51. (D)	**52. (B)**	**53. (C)**	**54. (A)**	**55. (C)**	**56.(D)**	**57. (A)**	**58. (D)**	**59. (A)**	**60.(C)**
61. (D)	**62. (C)**	**63. (D)**	**64. (B)**	**65. (B)**	**66. (A)**	**67. (B)**	**68. (B)**	**69. (A)**	**70. (B)**
71. (A)	**72. (A)**	**73. (A)**	**74. (C)**	**75. (A)**					

Short Answer Questions

1. Enumerate the steps involved in synaptic transmission.
2. Describe the ionic basis for EPSP and IPSP.
3. Describe presynaptic inhibition and facilitation and state their significance.
4. List the inhibitory neurotransmitters and state where they are secreted in the nervous system.
5. Describe Renshaw cell inhibition and mention its significance.
6. Define synaptic plasticity and state its importance.
7. List the types of cutaneous sensory receptors.
8. Explain the property of adaptation in sensory receptors.
9. State the differences between generator potential and action potential.
10. Explain how the intensity of sensory stimuli is coded in the nervous system.
11. Define proprioceptors and mention their significance.
12. Enumerate the sensations carried by dorsal column.
13. List the sensations carried by anterolateral column.
14. State the neurotransmitters released at the primary sensory afferents for fast pain and slow pain.
15. Define referred pain and describe how is it produced.
16. Describe the endogenous pain inhibition.
17. Define stretch reflex and mention its significance.
18. List the areas in the cerebral cortex that contribute to the formation of pyramidal tract.
19. State the functions of lateral and ventral corticospinal tracts.
20. List the extrapyramidal tracts and state their functions.
21. Which is called the final common pathway and state what happens if it is damaged.
22. List the differences between the lesions of lower motor neuron and the upper motor neuron.
23. List the functions of basal ganglia and mention the neural circuit responsible for them.
24. Describe the effects of lesion of substantia nigra.
25. List the neurotransmitter systems in basal ganglia and state their functions.
26. List the functions of cerebellum and state the regions involved for them.
27. Describe the functions of nerocerebellum and state what happens when it is damaged.
28. State the effects of cerebellar disease.
29. List the functions of thalamus.
30. What clinical tests are performed to assess the cerebellar function.
31. Mention the effects of spinal shock.
32. List the effects of hemisection of spinal cord.
33. Describe ARAS and state its importance.
34. Describe the alpha wave of EEG and explain alpha block.
35. State the causes of REM sleep and slow wave sleep.
36. List the functions of hypothalamus.
37. State the functions of hippocampus.
39. State the function of the following Brodmann areas of cerebral cortex and mention the effects of damage to them Areas 4, 6, 3,1 and 2, 5 and 7, 44 40.
40 State the significance of association areas of cerebral cortex.

41. List the functions of prefrontal cortex.
42. State the functions of Wernicke's area, angular gyrus, Broca's area and mention the effects of lesion on them.
43. State the effects of vascular lesion in the internal capsule.
44. Describe how the short term =memory is encoded into a long term memory.
45. What are light receptors and describe their functions.
46. Describe Rhodopsin cycle.
47. State how excitation occurs when light rays strike the retina.
48. Describe visual pathway and state the effects of damage in optic chiasma and optic tract.
49. What is the normal refractive power of the eye? In what physiological condition it is increased?
50. List the mechanisms that occur during accommodation reflex of the eye.
51. State what happens during dark adaptation of the eye.
52. Describe light reflex and state its importance.
53. Explain the trichromatic theory of color vision.
54. Describe colour blindness.
55. Define visual acuity and state how it is tested clinically.
56. List the refractive errors of the eye and state how are they corrected.
57. List the functions of middle ear.
58. Explain impedance matching mechanism in the ear.
59. Define acoustic reflex.
60. In humans what is the normal range of maximum sensitivity for sound frequency exists and also state what is the normal threshold of hearing.
61. Describe the function of organ corti.
62. Describe how the basilar membrane is tuned for sound frequency and intensity.
63. Describe hearing tests.
64. Explain how sweet and sour taste sensations are perceived.
65. State the centers for taste and olfaction.

6 Blood

Blood is a fluid connective tissue, which circulates in the vascular channels to all tissues of the body. It constitutes about 6 to 7% of the body weight. It is also a complex fluid, where, in the aqueous medium, namely plasma, the three types of cells are suspended. The three types of cells are, erythrocytes, leucocytes and thrombocytes.

Blood performs various **functions,** which include,

Transport of respiratory gases.

Transport of nutrients and other substances like hormones, metals, drugs, dyes, etc.

Transport of waste products to the excretory organs.

Protection against infections.

Maintenance of body temperature by distributing heat.

Maintenance of acid base balance with the help of buffers.

The reaction of blood is slightly alkaline and its pH is 7.40 ± 0.02. The specific gravity of blood ranges from 1055 to 1060 and it is due to the solute concentration, especially the proteins. The viscosity of blood is primarily related to the red cell volume and to a lesser extent to the plasma proteins.

PLASMA

Plasma is the fluid part in the blood and it constitutes 55% by volume. It contains 92% water and 8% solids. The solids contain inorganic and organic substances. Organic constituents form the bulk of the solids present in plasma. It includes proteins, glucose, cholesterol, lipids, nonprotein nitrogenous substances (NPN), hormones, enzymes, etc.

Plasma proteins

Plasma proteins are **albumin, globulin** and **fibrinogen.**

Serum

It is obtained by allowing the blood to clot. The fluid that separates out from the clot is known as serum. The plasma without fibrinogen is serum.

Separation of plasma proteins

Plasma proteins are separated by electrophoresis, in which, the proteins, depending on their size separate on a strip of paper when electric current is passed through the electrolyte solution.

The total protein level in plasma is 6 to 7 gm%

Albumin	4 to 5 gm%
Globulin	2 to 3 gm%
Fibrinogen	0.25 to 0.3 gm%

Globulin is further subdivided into alpha, beta, gamma with 1 and 2 subfractions in each category.

Albumin is the major plasma protein and its molecular weight is 68,000. Globulin has many sub fractions and includes **prothrombin**, which is an important clotting factor. Globulin has a

molecular weight ranging from 90,000 to 1,55,000. Fibrinogen is the least in concentration, but its molecular weight is the highest, which is 3,40,000.

Functions of plasma proteins

- **Colloidal osmotic pressure**

Albumin is responsible for creating this pressure in plasma and it helps to oppose the filtration pressure. Normal colloidal osmotic pressure is 25 mmHg. Any decrease in this value, as seen in conditions like, malnutrition, excessive protein loss as in renal disease and reduced formation in liver disease, will lead to fluid loss from the capillaries and result in edema.

- **Transport of substances**

Albumin transports hormones such as thyroxine, dyes, enzymes, bile pigments and fatty acids. Globulin transports metals like iron **(transferrin**), copper (**ceruloplasmin)** and hormones such as, thyroxine (**TBG)** and cortisol (**transcortin).**

- **Coagulation of blood**

Fibrinogen, prothrombin and various other clotting factors help in the coagulation of blood.

- **Antibody formation**

Gamma globulins are immunoglobulins, which are produced against antigens. Immunoglobulins are of five types namely IgA, IgD, IgG, IgE and IgM.

- **Erythrocyte sedimentation rate**

Fibrinogen is the main plasma protein, which influences ESR by making red blood cells to form rouleaux. The next protein, which influences ESR is globulin.

- **Buffering action**

Plasma proteins are anions and exist in ionic form. This gives buffering capability for the plasma protein, which helps to maintain the pH of blood.

- **Protein reserve**

Plasma proteins act as protein reserve, which can be used by the tissues during starvation.

Formation of plasma proteins

Albumin, alpha, beta globulins and fibrinogen are produced in the liver, while gamma globulins are produced from the lymphoid tissue.

ESR

The rate of settling down of RBCs in a column of blood can be determined by Westergren and Wintrobe methods. In the Westergren's method, blood, after mixing with an anticoagulant, is drawn into the ESR tube upto O mark and fixed to the stand. At the end of one hour, the height of top clear plasma in mm, is noted as ESR reading. Normal values are; in males 3-5 mm, females 5-8 mm and infants 1-3 mm.

The rate of sedimentation of RBCs depends on the rouleaux formation, which is influenced by fibrinogen. Other factors, which also cause rouleaux formation of red cells can influence ESR.

ESR increases physiologically during menstruation and pregnancy. Pathological rise is seen in pulmonary tuberculosis, rheumatoid arthritis and other disease states, where tissue destruction occurs.

Clinically, ESR reading is useful as a prognostic value and has no diagnostic importance.

ERYTHROCYTES

Erythrocytes are biconcave, non-nucleated and disc shaped, with cytoplasm containing a red colour pigment hemoglobin. The cells have a mean diameter 7.5 µ and thickness 2 µ.The thickness is less at the center, which is 1.0 µ. In venous blood, they are slightly larger by about 0.5 µ, due to the osmotic changes. The cell membrane has a bimolecular lipid structure and the lipoprotein complex on the membrane contains, A, B and Rh antigens.

Metabolism in RBC

The cytoplasm has no mitochondria and hence no oxidative phosphorylation occurs in the red cell. There is anaerobic glycolysis leading to Embden Meyerhof pathway of glucose metabolism, which generates ATP and NADH. There is also formation of 2,3 DPG. To a little extent, there is also pentose phosphate pathway for glucose oxidation. Red cells, even through nonnucleated, are considered as living cells, since they carry out the above described metabolic reactions.

Red cell cytoplasm contains more potassium and also rich in carbonic anhydrase enzyme. The membrane has an active sodium pump mechanism to maintain a low level of its concentration inside the cell. This prevents any change in its size, due to osmotic changes.

The average surface area of a red cell is 120 μ^2 and the mean volume is 90 μ^3. The biconcave shape of the cell gives a greater surface area/ volume ratio and this is advantageous to the cell to increase its volume without rupture, if there is an osmotic change. Another advantage is that, there is a maximum surface area available per unit mass of hemoglobin for gas transport. The quantity of hemoglobin, in a single cell is about 33% of wet weight of the cell.

Red cells perform important *functions* such as transport of respiratory gases, and acts as a buffer(Hb).

Normal count of red cell in adult male ranges from 5 to 5.5 million per cubic millimeter of blood. In females, it is slightly lower due to the low basal metabolism and the range is 4 to 4.5 million cells per cubic millimeter of blood. In the new born, the count is more than adults. It ranges from 6.5 to 7 million per cubic millimeter of blood. The volume of red cells in 100 ml of blood forms the **hematocrit (Hct).** Its value in males is 47% and in females it is 43%.

Life span of RBC

Average life period of a red cell is 120 days and it can be determined, by tagging red cells to radio active isotopes P^{32} or Fe^{55} and reintroducing them into the circulation. The radio activity count is measured until 0 value. The number of days that takes to get 0 count gives the life period of red cell. The senile red cells are destroyed in the reticuloendothelial system (tissue macrophages) in spleen, liver and bone marrow.

Physiologically red cell count increases in the following

High attitude
Muscular exercise
Adrenaline secretion.

Polycythemia

The rise in RBC count is called polycythemia. Accompanying the rise in red cell count, there is also increase in Hb%. If the abnormal increase in the count is due to the tumor of the red bone marrow, it will be known as **polycythemia vera** and described under ***primary polycythemia.*** In this type, the erythropoietin level will be normal.

RBC number also increases in high altitude, pulmonary disorders, renal diseases and congenital cardiac defects. In all these conditions, there is presence of hypoxia, which stimulates the secretion of erythropoietin. The rise in RBC in such conditions is called ***secondary polycythemia.***

Polycythemia can also result due to the contraction of plasma volume and it is described as ***relative polycythemia.***

Anemia

Reduction in red cell count occurs in bone marrow suppression, increased destruction of red cells and nutritional deficiencies. The condition is known as anemia, which causes reduced

supply of oxygen to the tissues. Anemia could result from a decrease in hemoglobin concentration, or reduced red cell count, or both.

Polycythemia: Increased red cell number.

Primary polycythemia: Caused by tumor of red bone marrow, myeloproliferative disorders. It is a malignant condition and known as polycythemic rubra veera. In this type erythropoietin level is not elevated in plasma.

Secondary polycythemia: Caused by increased secretion of erythropoietin. It occurs in the following conditions:

High altitude

Pulmonary disorders

Congenital heart defects.

Relative polycythemia: Occurs due to reduction of plasma volume.

Erythropoiesis (Fig. 6.1)

During embryonic life, erythrocytes are formed from yolk sac. In early fetal life, liver and spleen take over the function of producing blood cells and the bone marrow joins the list of forming blood cells in the later months of fetal life. From birth until adulthood, the entire bone marrow is involved in blood cell production. In the adult, the shaft of long bones is occupied by the yellow fatty tissues. Red bone marrow at the ends of long bones, flat bones like skull, membranous bones like sternum, vertebrae, pelvis, and ribs are active sites of blood cell production in adult. Although, liver and spleen have lost the function of producing blood cells in adult, they are capable of forming cells when there is prolonged stimuli (extramedullary hemopoiesis).

Hematopoietic stem cells

It is pleuripotent and can give rise to all types of cells. They differentiate into committed stem cells,(progenitor cells) which form various types of blood cells. Hematopoietic stem cells are more in the umbilical cord blood. About 75% of bone

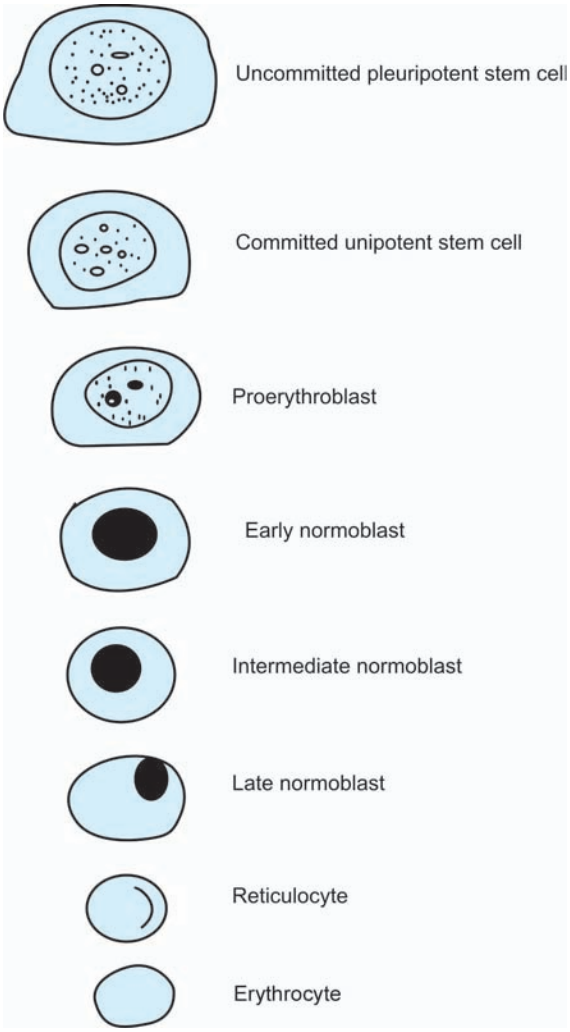


Fig. 6.1: Stages of erythropoiesis

marrow cells belong to myeloid series and the remaining 25% only is the erythroid series. The pleuripotent stem cells by dividing give rise to committed unipotent stem cells. Some of the new cells that are formed also become uncommitted stem cells, in order to maintain the pleuripotent stem cells number constant. In the red bone marrow, the white cells producing stem cells are more in number as their life period is short.

Hematopoietic stem cells themselves are formed from a stem cell, which is totipotent and can give rise to any type of cell in the body. These totipotent stem cells are a few in number in adults,

but available in plenty in blastocyst of the embryos. The stem cell research involves culturing these stem cells to obtain the required tissue for clinical application.

Committed and unipotent stem cell

The hemopoietic stem cell, which enters the erythroid series, becomes unipotent, committed stem cell, being facilitated by interleukins 1, 3 and 6 and granulocyte macrophage-colony stimulating factor (GM-CSF). The stem cell committed for the formation of erythrocytes is called **Colony forming Unit- Erythrocyte (CFU-E)**. The proliferation of further stages of red cell formation, depends on growth factors, colony stimulating factors (CSF), and the hormone erythropoietin. The **stages of erythropoiesis** are described below.

Proerythroblast

The precursor cell is large up to 20 microns size, nucleus is single and large, nucleolus is present. The cell shows active proliferation.

Early normoblast

The cell size is reduced. The nucleus condenses and chromatin threads appear. Nucleoli disappear and the cell continues to show active proliferation.

Intermediate normoblast

Nucleus further condenses. The cell size is also reduced. The cytoplasm shows the synthesis of hemoglobin and hence it takes both acidic and basic stains (polychromatophilic). The cell mitosis stops in this stage.

Late normoblast

There is reduction in the cell size. Nucleus is pushed to the periphery and at the end of this stage, it is extruded out by a process called pyknosis. The cytoplasm shows greater synthesis of hemoglobin.

Reticulocyte

After the extrusion of nucleus, the reticulocyte is formed and released into the circulation. The cell size becomes 7.2 µ. The cytoplasm has a fine reticulum, which are the nuclear remnants of RNA. Hemoglobin synthesis is completed and within 24 to 48 hours they mature into red cells in the circulation. Normal count of reticulocyte is 1% of red cell count. Reticulocytosis is the increase in reticulocyte count, which is seen during accelerated erythropoiesis from bone marrow stimulation. Treatment of pernicious anemia by vit B_{12} extract, shows the feature of reticulocytosis.

Erythrocyte

Both reticulocytes and erythrocytes are present in the peripheral circulation. The red cell, which is formed from reticulocyte, exhibits its normal morphology.

Regulation of erythropoiesis

Erythropoiesis is regulated by several factors and the important regulator is the red cell number, which provides sufficient amount of oxygen transported to the tissues. If transport of oxygen to the tissue is reduced as in anemia, the red cell production is stimulated. Likewise, hypoxia which occurs in high altitude and cardio respiratory diseases, the red cell production is increased. A number of erythrocytes are destroyed every day and equal number of cells is produced by the red bone marrow, so that, the total red cell number remains constant. To keep the red bone marrow in a steady state of erythropoietic activity, there should be availability of various factors, which are grouped as erythropoietic factors.

Hypoxia

Oxygen lack at the tissue level is the potent stimulus for erythropoiesis and it is brought about by the hormone erythropoietin, secreted from kidney. In high altitude, erythropoiesis is stimulated by hypoxia.

Erythropoietin

It is a glycoprotein hormone synthesised from kidney. The secretion of the hormone also occurs from the liver but in smaller amounts (15%). The stimulus for secretion is hypoxia and reduced red cell count. The reduced cell count itself gives hypoxia, which stimulates the hormone secretion. Erythropoietin acts on the bone marrow and causes proliferation of erythroid precursor cells. It facilitates the formation of unipotent, committed stem cell from the totipotent uncommitted stem cell. The proliferation of the precursor cells will accelerate erythropoiesis. Chronic renal disease usually is associated with anemia, as there is lack of erythropoietin.

Thyroxine, cortisol and growth hormone

These hormones stimulate red cell formation, due to their involvement in metabolism. They increase oxygen consumption by the cells and the resultant hypoxia stimulates erythropoiesis.

Androgens

They directly stimulate red bone marrow to produce red blood cells. Lack of androgens leads to anemia.

Vitamin B_{12} (cyanocobalamine)

It is extrinsic factor and for its absorption, intrinsic factor in the gastric juice should be present. The absorption of Vit B_{12} occurs from the terminal ileum. Vit B_{12} source comes from animal origin and bacterial flora. Vit B_{12} is necessary for DNA and RNA synthesis and due to this involvement, Vit B_{12} brings about proliferation and maturation of erythroid precursor cells. Lack of vit B_{12}, causes **pernicious anemia**, which is characterized by the presence of large immature cells called megaloblasts in the circulation.

Folic acid

It is necessary for the synthesis of RNA and through this involvement, it influences the proliferation and maturation of erythroid precursor cells. Deficiency of folic acid causes megaloblastic anemia, similar to Vit B_{12} deficiency, but without neurological symptoms.

Pyridoxine (vit B_6)

Pyridoxine is involved in hemoglobin synthesis.

Metals

Iron is necessary for hemoglobin synthesis. Deficiency of iron causes microcytic hypochromic anemia.

Iron is absorbed from the upper part of the small intestine. The absorption is regulated by apoferritin present in the mucosal cell (Fig. 6.2). When iron is bound to this compound, it is converted to ferritin. The saturation of apoferritin in the intestinal mucosal cell regulates iron absorption. The excess iron present in the diet is excreted in the feces. Iron is not only needed for hemoglobin synthesis, but also, required for myoglobin and enzymes like catalase synthesis. Iron is transported in the blood bound to *transferrin.* There is also reutilization or recycling of iron, released from the break down of red cell and hemoglobin. Excess iron, when released, is deposited in the tissues like spleen and the condition is called haemosiderosis. The iron from hemosiderin is not available for exchange, whereas, iron from ferritin is readily available for exchange. Normal requirement of iron in adult is 12 to 15 mg per day and the requirement is more in pregnant and reproductive women.

Other metals like zinc, copper, cobalt, manganese are also necessary for erythropoiesis.

LEUCOCYTES

Leucocytes are nucleated and larger in size than red blood cells. There are two types of leucocytes, namely, granulocytes and agranulocytes. Total count varies from 4,000, to 11,000 cells per cubic millimeter of blood. These white blood corpuscles are involved in protecting the body against infections and foreign bodies. A differential count of leucocytes reveals five types, which are described as follows:

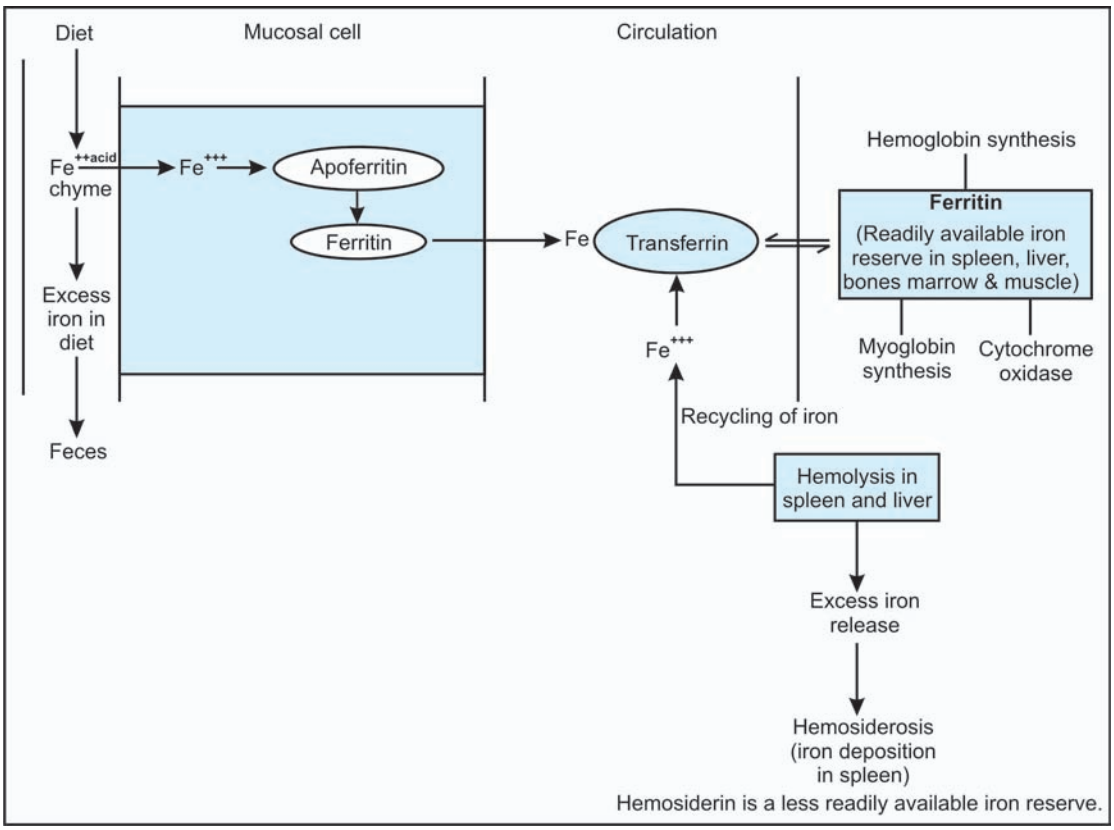


Fig. 6.2: Iron transport and metabolism in the body

Neutrophil	50% - 70%
Eosinophil	1% - 4%
Basophil	0% - 1%
Lymphocyte	25% - 40%
Monocyte	2% - 8%

Granulocytes

These cells have a nucleus, which is lobulated and the cytoplasm contains granules. These granules contain enzymes and proteins, which cause allergic and inflammatory reactions in the body when released. It includes three types, namely neutrophil, eosinophil and basophil.

Neutrophil

It constitutes 50% to 70% of the total leucocyte count. The cells are multilobed and usually ranges from 3 to 5. They are also known as polymorphonuclear leucocyte. Cell size is 10 microns in diameter and the cytoplasm contains fine granules, which appear purplish in a peripheral blood smear. The granules contain many enzymes, which include lysozymes and peroxidase. Lysozymal enzymes digest the bacteria and peroxidase reduces hydrogen peroxide.

The primary function of neutrophil is to provide defense against the invading micro organisms. This is achieved, by a process called **phagocytosis.** Neutrophils leave the circulation and enter the tissues where the micro organisms are present. They leave the capillary endothelial wall by binding to neutrophil adhesion molecule called integrin protein and through a process of diapedesis the neutrophils leave the capillary.

Phagocytic defence

Neutrophils and tissue macrophages produce phagocytic defence against invading bacteria, viruses and other foreign bodies. The entry of bacteria or foreign particulate matter into the body, triggers the bone marrow to release more neutrophils into the circulation, which forms the part of inflammatory response to the bacterial entry. The macrophages, lymphocytes, basophils, and mast cells release leucotrienes and complement proteins (C5a), which cause chemotaxis. That is, the neutrophils are attracted to migrate to the site of bacterial entry. The bacteria are now coated with the complement proteins and immunoglobulin IgG to be presented to the phagocytes. This is called **opsonization.** The opsonised bacteria bind to the receptors on the neutrophil membrane and by the process of endocytosis, the bacteria are ingested into the cell. The ingested bacteria are now enclosed in a vacuole called phagosome. Enzymes of the neutrophilic granules fuse with the phagosome and digest the bacteria.

Phagocytosis

- Neutrophils and macrophges give phagocytic function
- Migration of neutrophils to the site of bacterial entry, promoted by the release of leucotrienes and complement proteins
- Bacteria are presented to the neutrophil after coating with complement proteins (opsonization)
- Ingestion of bacteria by endocytosis and formation of a vacuole (phagosome)
- Release of bactericidal agents such as, free radicals and hydrogen peroxide from neutrophils
- Lysosomal enzymes digest the wall of the bacteria
- Enzymes in the neutrophilic granules digest the bacteria.

Release of bactericidal agents

There is increased respiratory burst in the neutrophil, where the cell shows accelerated oxygen uptake and metabolism leading to generation of **super oxide ion**, hydrogen peroxide and **free radical**. The free radical oxygen and **hydrogen peroxide** are **bactericidal agents**. The enzyme **myeloperoxidase** converts hydrogen peroxide and chloride ions to hypochlorite, which is also a bactericidal agent. These lysosomal enzymes digest the wall of the bacteria.

The process of phagocytosis, causes spillage of digestive enzymes into the surrounding tissues as part of the inflammatory reaction, causing destruction of the tissues.

Physiological rise in neutrophil count is observed in the following

Menstruation, pregnancy, muscular exercise, diurnal rise in the afternoon, and stress stimuli.

Pathological rise in neutrophil count can be seen in the following

Acute infections by pus forming organisms and myocardial infarction.

Neutrophils are removed through the excretion in gastrointestinal secretions. The average life of the cell in the circulation is 12 hours and when, it enters the extra vascular tissues, stays for 4 to 5 days. Neutrophils that leave the circulation will not come back. They are lost after their defense against the bacteria.

Eosinophil

These cells have large granules, which take up eosin stain and appear orange red. The cell is 10 microns in size and constitutes 1 - 4% of the total white cell count. The nucleus is bi lobed.

Eosinophils are concerned with the inhibition of allergic reactions. It also attacks parasites and larvae and kills them by their toxic chemicals released from them. The eosinophils kill parasites by releasing hydrolytic enzymes and a larvicidal peptide called major basic protein. Eosinophil number increases in allergic reactions and parasitic infestations. Allergic reaction is prevented by inhibiting histamine release.

Eosinophil also shows phagocytic action similar to neutrophil.

Eosinophilia indicates rise in eosinophil count and it is usually seen in the following:

- Allergic conditions like urticaria, hay fever and bronchial asthma,
- Parasitic infestations like hook worm, round worm, thread worm infestations.

Eosinophil count decreases after administration of glucocorticoid and adrenaline. The decrease is due to sequestration of eosinophils from the circulation to the organs like spleen and liver.

Basophil

Basophils are 10 microns in size and they usually have a bi lobed or sometimes three lobed nucleus. The cytoplasm contains coarse meta chromatic granules and appears as deep blue after staining the peripheral blood smear. Normal count of basophils is 0 to 1% of leucocyte count. Basophil cytoplasm granules contain heparin, histamine and serotonin.

Basophils mediate immediate hypersensitivity reactions including anaphylaxis. When IgE coated antigens bind to the Ig E receptors present on the basophils, the cells rupture and release histamine, bradykinin, serotonin, heparin, slow reacting substance of anaphylaxis, and other lysosomal enzymes. The released substances cause immediate hypersensitivity reactions which include urticaria, rhinnitis and anaphylaxis. The release of histamine from basophils is responsible for such reactions. Heparin release from basophils, activates lipoprotein lipase, which help triglyceride metabolism. Besides this function, heparin also acts as an anti coagulant.

Increase in basophils is seen in anaphylactic shock and other hypersensitivity reactions. Abnormal rise in basophil number is seen in leukemia.

Agranulocytes

These white cells have a single nucleus and the cytoplasm contains no granules. It consists of two types namely lymphocytes and monocytes.

Lymphocytes

The cells have a large nucleus and cytoplasm does not contain granules. Size varies from 8 to 15 microns. Its count is 2% to 40% of total white cell count.

The function of lymphocytes is in providing immunity to the body. Functionally, there are two types of lymphocytes, namely, T lymphocytes and B lymphocytes. T lymphocytes give cellular immunity and B lymphocytes are involved in humoral immunity. The detailed description of immunity has been dealt with separately.

Lymphocytes increase in number in chronic infections such as tuberculosis and the number decreases when cortisol is injected.

Monocytes

Monocytes are the largest size in white cell types. Its size ranges from 15 to 18 microns. The nucleus is kidney shaped and there is relatively more cytoplasm between nuclear membrane and cell membrane. The cell count is 2% to 8% of total leucocytes.

Monocytes, like neurtophil show **phagocytic function**. Monocytes leave the circulation and become tissue macrophage. Tissue macrophage lives for many months. Macrophages have many functions, which include their role in the processing of antigens and presenting them to T and B lymphocytes.

Monocytes count rises in kala azar, malaria, typhoid, infectious mononucleosis, bacterial endocarditis, protozoan disease, and Hodgkin's disease.

Tissue macrophage system

Macrophages are formed from monocytes present in the blood. Monocytes after leaving the circulation become tissue macrophages and assume different names in different tissues. They exist as Kupffer cells in the liver, histiocytes in the skin and subcutaneous tissue, alveolar macrophages in lungs, microglia in the brain and macrophages in the spleen and lymph nodes. These monocyte-macrophages were known as reticulo endothelial system earlier. The functions

of tissue macrophages include phagocytosis, destruction of old blood cells in the spleen, inflammation and immune mechanisms.

Leucopoiesis

The stem cell, which is unipotent and committed is present in the red bone marrow for the leucocytes. The stem cell for neutrophil and monocyte arise from a common precursor, whereas, eosinophil and basophil have different stem cells, as precursors for each type.

Regulation of leucopoiesis

The unipotent, committed stem cells of granulocytes proliferate by the presence of growth factors like:

- **GMCSF (granulocyte macrophage colony stimulating factor):** It is produced from macrophages, fibroblasts, T-lymphocytes, B-lymphocytes, natural killer cells and vascular endothelial cells. This growth factor is formed by the stimulation of interleukin I and tumor necrosis factor. GM -CSF is also required for the proliferation of erythroid precursors and development of macrophages.
- **Interleukin I:** This is also a growth factor for the proliferation of granulocytes precursors in the bone marrow. It is produced from macrophages.
- **Interleukin 3:** It is also a multi colony stimulating factor (multi-CS). IL-3 is produced from T-lymphocytes by the stimulatory effect of IL-I, IL-3, causes proliferation of all types of leucocyte precursors and also erythroid and T- lymphocytes.
- **Interleukin 5:** It is produced from T-lymphocytes and it is specifically required for the proliferation of eosinophil precursor.
- **Tumor necrosis factor:** Though its main action is to inhibit tumor growth, it also shows haemopoietic effect. It is produced from macrophages.

The stages of granulopoiesis in the red bone marrow, shows, the committed stem cell proliferating into myeloblast (Fig. 6.3). It is a large

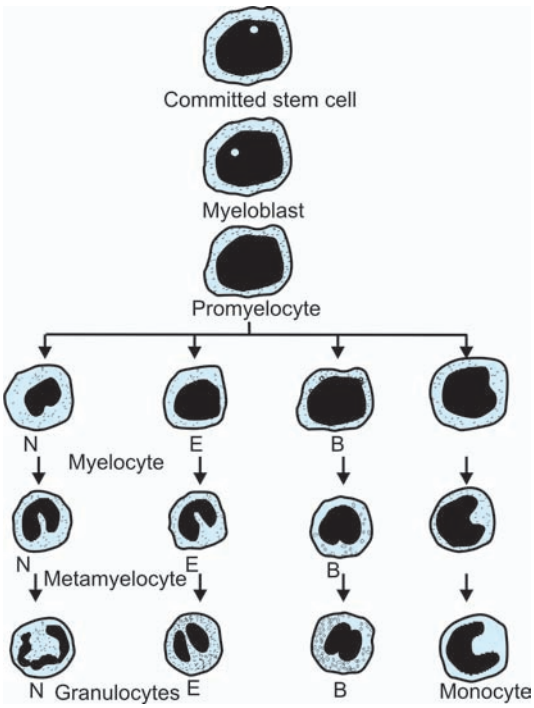


Fig. 6.3: Formation of granulocytes and monocyte

cell with 20 to 22 μ size and the nucleus is large, occupying the whole cell. There are also nucleoli present. The cell shows active mitosis. This stage gives rise to myelocyte, which is characterized by reduction of cell size and disappearance of nucleoli. The cell shows active mitosis and granules appear in the cytoplasm. The next stage is metamyelocyte, where the nucleus shows lobulation and the granules become more in cytoplasm. The cell size is further reduced and mitosis stops here.With the nucleus lobulation and specific granules filling the cytoplasm, the granulocytes are released into the circulation.

Neutrophils in the blood exist in two forms namely:

(a) Circulating granulocyte pool (CGP)
(b) Marginal granulocyte pool (MGP)

MGP refers to the neutrophils in the lining of small blood vessels like venules. This pool can readily enter the circulation, when there is a

demand. The rise in neutrophil, due to adrenaline and exercise, comes from the MGP.

Granulocytes live for 2 to 4 days. Some live for a few hours only. Those which leave the circulation and enter the tissues stay for longer days. Most of the white blood cells are lost in the gastrointestinal secretions.

Lymphocytes are produced from bone marrow. The precursor cells migrate to the lymphoid tissues like thymus and lymph node.

The committed, unipotent stem cell proliferates into lymphoblast. This precursor cell differentiates into lymphocytes. If processing takes place in the thymus, T-lymphocyte is formed. On the other hand, if the cells are processed in the spleen and liver, it develops into B-lymphocyte (Fig. 6.4).

Monocyte develops from a precursor stem cell in the red bone marrow, which is common to neutrophil (Fig. 6.3). The committed, unipotent stem cell proliferates and gives monocytoblast. This precursor cell differentiates into monocyte and released into the circulation.

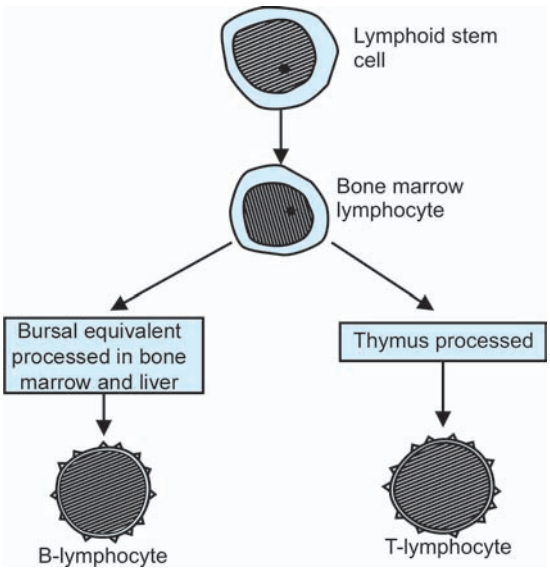


Fig. 6.4: Formation of lymphocyte

Leukemias

It is a malignant condition in which there is abnormal rise in white blood cells in the circulating blood. It can occur due to cancer of myeloid or lymphoid cell. Accordingly, the leukemias can be of two types namely, **lymphocytic leukemia** or **myelogenous leukaemia**. In acute leukemia, the undifferentiated white blood cells are more in number in the circulation. The leukaemia can be chronic, if more number of differentiated white blood cells are present in the circulation. Common effects of leukaemia include infections, anemia, bleeding tendency due to lack of platelets and tissue destruction.

Leukopenia

Fall in white cell count is called leukopenia, It occurs when bone marrow is suppressed by drugs like chloramphenicol, thiouracil, chemicals like benzene and X-ray radiation. Leukopenia can cause severe infections.

THROMBOCYTES (PLATELETS)

They are small (2 µ to 4 µ), ovalshaped and non nucleated cells. There is mitochondria in the cell and the characteristic feature is the presence of microtubular system, consisting of contractile proteins (**actin** and **myosin**) and a canalicular system, which communicates with the exterior. The cytoplasm contains two types of granules, namely, dense granules containing **serotonin**, **epinephrine**, **ADP**, other adenine nucleotides and alpha granules, consisting of several proteins like **platelet derived growth factor (PDGF)** and clotting factors.

The normal count of platelets is 250,000 to 300,000 cells/cumm of blood. Their life period is 4 to 7 days and are destroyed in the spleen. Platelets also stay in the spleen as reservoir pool. During bleeding and epinephrine secretion, this pool is released into the circulation.

Platelet count increases in the following:
Hemorrhage
Spleenectomy
Hodgkin's disease

Decrease in count is seen in the following:
Splenomegaly
Aplastic anemia
Acute infections
Thrombocytopenic purpura

Formation of platelets

The production of platelets, depends on the number of cells that are destroyed in the spleen. The substances released from lysed platelets themselves stimulate thrombopoiesis. The uncommitted stem cell in the red bone marrow becomes a committed stem cell by the stimulation of colony stimulating growth factors. Further differentiation of megakaryocytes (precursor cell) requires Interleukin (IL3). The megakaryoctyes break up into protoplasmic fragments and form thrombocytes.

Functions of platelets

- *Formation of platelet plug*

When a blood vessel is injured, the platelets undergo adhesion to the exposed **collagen**. The platelet adhesion is facilitated by **von Willebrand factor**, which is present in the wall of the platelets and also in the wall of the vascular endothelium. Once the platelets adhere to the collagen, further adhesion of platelets to the site of injury is promoted by the release of contents of platelets, like **ADP** and **platelet activating factor**. This leads to more aggregation of platelets on the damaged vascular endothelium. **Thromboxane A_2** is a potent platelet aggregator. Its formation is facilitated by the entry of Ca^{++} into the cell. The aggregation of platelets causes a plug at the site of injury and seals the wound. This arrests the bleeding. The aggregation of platelets is limited by the formation of **prostacyclin** from endoperoxides in the endothelium. The next step after aggregation is the release of platelets contents at the site of damage in the blood vessel.

Drugs like aspirin, when administered orally in low doses (75 to 100 mg/day) inhibits thromboxane A_2 without significant impairment of prostacyclin production. Thus, by altering the balance between platelet thromboxane A_2 and endothelium prostacyclin, aspirin in low doses helps to prevent myocardial infarction, unstable angina, stroke and transient ischemic attacks.

- *Release of vasoconstrictor substances*

Platelets release vasoconstrictor substances like serotonin, epinephrine, which cause vasoconstriction and reduce blood loss. Thromboxane A_2 which is formed from platelets also causes vasoconstriction of small blood vessels and minimise bleeding.

- *Platelets help in blood coagulation*

Platelets release **platelet factor 3**, which is a membrane phospholipid and facilitates the intrinsic formation of thrombin. Thrombin itself causes platelet aggregation and causes release of more platelet factor 3, thus enhancing the clotting mechanism.

- *Clot retraction*

After the coagulation of blood, the clot shrinks, expelling the serum. This is called clot retraction which is helped by platelets. The process is initiated by the thrombin acting on the platelets. It is likely that thrombin causes calcium entry into the platelets to activate contractile proteins actin and myosin, present in platelets. The contractile proteins contract like in muscle and gives retraction of clot. The retraction of fibrin threads can bring together the edges of the damaged endothelium and facilitates faster wound healing.

- *Repair of damaged endothelium*

The aggregation of platelets will also release platelet derived growth factor (PDGF). It is a growth factor, which helps in repairing of the damaged blood vessel.

Disorders of platelets function

Thrombocytopenic purpura

It is characterised by the spontaneous bleeding beneath the skin and mucous membrane. This gives purplish spots on the skin. It is a bleeding disorder, caused by the deficiency of platelets (thrombocytopenic purpura) or functional defect in platelets.

In **thrombocytosis** (increased platelet count) the tendency for intravascular thrombosis increases.

HEMOSTASIS (BLOOD COAGULATION)

Hemostasis is a process in which the bleeding is arrested by the formation of clot. Clotting of blood is an enzymatic process, which involves various plasma clotting factors, leading to the conversion of fibrinogen to fibrin.

The clotting factors are plasma proteins, which exist in inactive proenzyme form. During the process of coagulation, each inactive proenzyme is converted into active enzyme form. This activated factor inturn, activates subsequent proenzyme clotting factors to finally result in fibrin (clot). The activation of clotting begins with the blood coming in contact with the rough surface and collagen of the damaged blood vessel. At each step in the activation of clotting factor, the reaction is amplified and is known as enzyme cascade mechanism.

Broadly, the process of clotting can be described in three steps.

In the first step, the prothrombin activator is formed. It is generated in the intrinsic pathway within the blood and also in the extrinsic pathway from the damaged tissues. This, in the next step activates prothrombin to thrombin. In the last step, the thrombin converts fibrinogen to fibrin. Though it appears as a simple process, the involvement of several clotting factors makes it a complex enzymatic reaction.

Clotting factors are represented by Roman nomenclature from I to XIII.

Factor I Fibrinogen

The clot is fibrin thread, formed from fibrinogen by the enzymatic action of thrombin. Fibrinogen is one of the constituents of plasma proteins and its concentration is 0.25 to 0.3 g%. The level of fibrinogen is increased in pregnancy, inflammation, tissue damage, etc. An increased concentration of fibrinogen raises the rate of erythrocyte sedimentation by influencing its rouleaux formation.

Factor II Prothrombin

It belongs to globulin fraction of plasma proteins and it is activated by prothrombin activator (activated factor X) to thrombin. Prothrombin is synthesised from the liver, in the presence of vitamin K. Drugs like dicoumarol competitively inhibits the action of vitamin K and prevents clotting. Diseases affecting the liver, would cause deficiency of prothrombin, leading to clotting disorder.

Factor III Tissue thromboplastin

It is present in the cells. It consists of lipoprotein phospholipid complex, which is released, when the tissue is damaged. The released tissue phospholipid activates factor VII, which inturn activates factor X. Tissue thromboplastin role in the coagulation is seen in the extrinsic pathway. However, tissue thromboplastin-factor VII complex is also observed in the activation of factor IX (Christmas factor), thereby, showing that it has a role in the intrinsic pathway of coagulation also.

Factor IV Calcium

This divalent cation is required in almost all steps in the coagulation, except in the initial activation of XII Factor (Hageman Factor) in the intrinsic pathway. Chelating agents such as, EDTA, citrate, oxalate, fluoride act as anti coagulants.

Factor V Proacclerin

It is converted to active form by activated factor X (Stuart factor) in both intrinsic and extrinsic pathways of coagulation. Activated factor V is crucial in the reaction, which leads to the conversion of prothrombin to thrombin.

Factor VII Proconvertin (Serum prothrombin conversion accelerator)

It is a β globulin fraction, which is synthesised from the liver in the presence of vitamin K. Factor VII is involved in the extrinsic pathway of clotting. Tissue thromboplastin, released from the damaged tissues, activates factor VII.

Factor VIII Anti-hemophilic globulin factor (AHG)

AHG has a component called vWF (von Willebrand factor) and both are involved in the intrinsic pathway of clotting. The vWF is also present in platelets and helps in its adhesion to the damaged endothelium. Deficiency of factor VIII causes haemorrhagic disorder called **hemophilia.**

Factor IX Christmas factor

This factor is also synthesized in the liver in the presence of vitamin K. Factor IX is activated by the activated factor XI in the intrinsic pathway. Deficiency of this factor will also cause hemophilia, which is called Christmas disease.

Factor X Stuart factor

This globulin protein is involved in both the intrinsic and extrinsic pathways of coagulation. The activated factor X is also called prothrombin activator, which activates prothrombin.

Factor XI Plasma thromboplastin antecedent (PTA)

It is activated by activated factor XII. The activation requires high molecular weight kininogen and kallikrein. PTA is involved in the intrinsic pathway.

Factor XII Hageman's factor

This factor is activated by surface contact with collagen and negatively charged surface from the damaged endothelium. Activation of factor XII is the first step in the clotting process in intrinsic pathway.

Factor XIII Fibrin stabilizing factor

Activation of XIII factor is caused by thrombin. The activated factor XIII causes polymerization of fibrin, converting it into an insoluble clot.

Process of clotting

Role of platelets

Injury to the blood vessel exposes collagen and the negatively charged surface of the endothelium. This causes platelets adhesion and aggregation on the damaged endothelial surface. This is followed by the release of platelet contents, such as ADP, vasoconstrictors, platelet factor 3, etc. The adhesion and aggregation give temporary haemostatic plug. The release of platelet factor 3 activates the clotting process.

Generation of prothrombin activator

The first step in the clotting process is the formation of activated factor X, which is also called prothrombin activator. It is formed in two ways.
Intrinsic pathway (within the blood)
Extrinsic pathway (from tissues)

Intrinsic pathway (Fig. 6.5)

As said earlier, the clotting factors are proenzymes, which are activated during clotting and in each step the reaction is amplified. This is known as cascade mechanism.

The exposure of collagen and the negative charge on the injured surface, causes the activation of Hageman's factor (XII). This is the

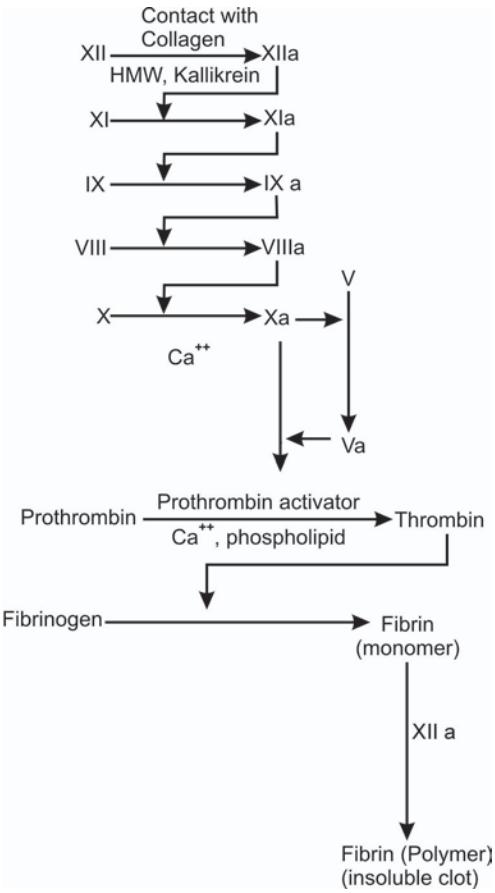


Fig. 6.5: Intrinsic pathway of clotting mechanism

beginning of sequential events in the coagulation of blood. The activated factor XII acts as an enzyme and converts factor XI to activated factor XI. This activation also requires high molecular weight kininogen and kallikrein.

The activated Hageman's factor is also involved in augmenting the activity of factor VII in the extrinsic pathway. It also activates plasminogen, which is involved in fibrinolysis. Further, it causes the formation of complement proteins which participate in the immune reactions.

The activated factor XI, in the presence of Ca^{++} activates IX into activated factor IX (activated Christmas factor). It is in this step, calcium is first required for the activation. Activated Christmas factor converts Stuart factor into its active from, through the activation of factor VIII (AHG) in the presence of Ca^{++} and phospholipid of platelet factor 3.

Activated Stuart factor is also known as prothrombin activator. It causes the conversion of prothrombin to thrombin, in the presence of activated proaccelerin (activated factor V), Ca^{++} and phospholipid.

Thrombin acts as an enzyme protease and converts fibrinogen to fibrin.

Extrinsic pathway (Fig. 6.6)

Formation of prothrombin activator in extrinsic pathway occurs, when blood comes in contact with the injured tissues. The damaged cells release phospholipid from the cell membrane. The tissue phospholipid shows, clot promoting activity through the activation of factor VII, in the presence of Ca^{++}. The activated factor VII, converts

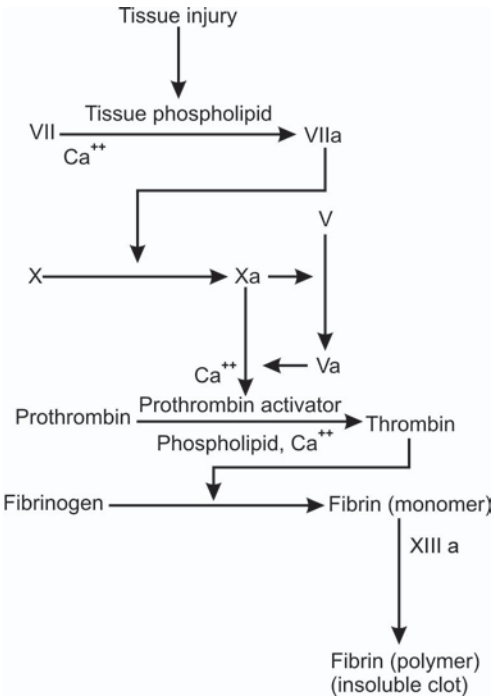


Fig. 6.6: Extrinsic pathway of clotting mechanism

factor X into activated factor X, in the presence of activated proaccelerin (V activated factor), Ca^{++} and phospholipid. From now onwards, the steps leading to the formation of fibrin are identical to those observed in the intrinsic pathway.

Formation of fibrin (Role of fibrin stabilizing factor)

Blood coagulation basically involves the conversion of soluble plasma protein fibrinogen to insoluble fibrin clot by the enzyme thrombin. Initially, fibrin is formed as soluble monomer. This is followed by the cross linking of fibrin threads into fibrin polymer by the action of XIII (fibrin stabilizing factor) and thrombin. Fibrin polymer is an insoluble clot. Calcium ions are needed for the polymerization of fibrin. The fibrin polymer appears as strands of meshwork, in which the red cells and serum are trapped. The red colour of clot is due to the trapped red cells.

Fibrinolysis

As soon as the clot is formed, the mechanism to liquefy the clot is activated. The dissolution of clot is called fibrinolysis (Fig. 6.7). The enzyme responsible for this is plasmin, which comes from the proenzyme plasminogen. The plasminogen is activated to plasmin in the natural way by substances like tissue plasminogen activators such as, urokinase, activated Hageman's factor, activated t-PA, vascular endothelium and kallikrein. There are also enzymes that are not present in the body, but shows plasminogen activation when administered. They are streptokinase and urokinase enzymes. Tissue plasminogen activators or streptokinase, are used clinically to dissolve clots, especially in coronary arteries.

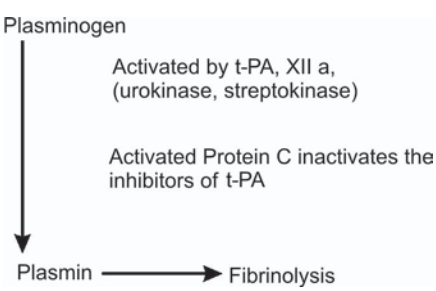


Fig. 6.7: Fibrinolytic system

Plasmin binds to the clot surface and digests fibrin, without attacking fibrinogen. As a matter of fact, the plasminogen activation occurs on the clot surface and not in the plasma. Plasmin can digest both fibrin and fibrinogen when comes in contact with them.

Inhibitors of clotting (Mechanisms which keep the blood in a fluid state)

There are mechanisms present in the blood itself to prevent clotting. The fluid state of the blood is due to the balance between factors which cause clotting and those which oppose it. The following description gives an account of inhibitors of clotting.

Antithrombin III

It is the major inhibitor of coagulation, which inhibits thrombin and activated Stuart factor (Xa). It forms a complex with heparin anticoagulant and shows enhanced activation.

Protein C

It is a proenzyme and activated by **thrombin - thrombomodulin** complex on the endothelial surface. Thromobomodulin is present on the endothelial surface and also released from platelets. It combines with thrombin and activates protein C. The activated protein C inactivates VIII and V factors and prevents clotting. It also promotes fibrinolysis.

Heparin

Heparin is a naturally occurring anticoagulant, which is secreted from mast cells and basophils. Heparin has to bind to **antithrombin III** to become an anticoagulant. There is also another factor for heparin activation. It is heparin co-factor II. This complex also inhibits thrombin activity.

Vascular endothelium

The clotting is initiated when collagen in the vessel wall is exposed due to injury. This provides the rough surface for the activation of factor XII. In normal conditions, where there is no damage to the vascular endothelium, the collagen is not exposed on the surface and hence no activation of factor XII.

Fibrinolysis

Fibrinolytic mechanism described earlier, also prevents clotting and helps to keep the blood in fluid state.

Intravascular thrombosis

It is the formation of clot within the blood vessel. There are conditions, which favour the formation of clot within the blood. They are;

- Injury to the vascular wall
- Slow rate of blood flow
- Deficiency of antithrombin III, protein C, heparin cofactor II.
- Presence of atherosclerotic plaques in the lumen of the vessel.

If the thrombus breaks up and carried away in the circulation, it gets lodged in a vessel and form an embolus. Emboli in pulmonary arteries, coronary or cerebral arteries can become severe enough to cause death.

Anticoagulants

Salts

Citrates, oxalates, fluorides and EDTA (ethylenediamine tetra acetic acid) are called chelating agents, as they bind calcium. Since calcium is required in many steps in the process of coagulation, the removal of calcium from the blood by these salts prevents clotting.

Drugs

Heparin

It is a naturally occurring anticoagulant, but, not a constituent of plasma. It is secreted from mast cells and basophils and also present in many tissues like lungs and liver. It is a negatively charged sulfated polysaccharide. Heparin requires antithrombin III for its anticoagulant activity. It also combines with heparin cofactor and shows anticoagulant action. Heparin inhibits thrombin action. It also inhibits the formation of prothrombin activator, as it blocks the action of factor IX. Heparin is widely used in clinical practice and laboratories as an anti-coagulant. Heparin action is inhibited by protamine sulphate, which is a positively charged polypeptide obtained from fish sperm. Heparin can be used as an anticoagulant both *in vivo* and *in vitro.*

Dicoumarol

It is a vitamin K antagonist, as it competitively inhibits the action of vitamin K in the liver. Vitamin K is required for the synthesis of prothrombin, factors VII, IX, and X and administration of dicoumarol, blocks the synthesis of these clotting factors. Dicoumarol can be effective as anticoagulant only *in vivo*.

Tests for clotting

Clotting time

It is usually determined by the capillary tube method, where the time taken for the appearance of fibrin threads is noted as the clotting time. The normal duration is 3 to 8 minutes. Deficiency of clotting factors leads to increased clotting time. In haemophilia, the clotting time is prolonged, but the bleeding time would be normal.

Bleeding time

This can be determined by pricking the ear lobe or the finger tip and the time taken for the arrest of bleeding is noted as bleeding time. The arrest of bleeding is due to the formation of platelet plug. Bleeding time normally ranges from 2 to 5 minutes. Bleeding time is prolonged in purpura due to platelet deficiency. However, the clotting time is normal as there is no deficiency of clotting factors.

Prothrombin time

Quick method—this test indicates the quantity of prothrombin present in the plasma. Citrated blood is taken (so that the prothrombin is not converted to thrombin). To this, tissue thromboplastin (brain extract) and calcium are added. This mixture is incubated at 37° C and the time taken for the appearance of clot denotes prothrombin time. Normal prothrombin time ranges from 10 to 14 sec. Prothrombin time increases in liver diseases and vitamin K deficiency.

Thromboplastin generation test

This test is performed to find out defects in the clotting factors involved in the formation of prothrombin activator in the intrinsic pathway. Samples of adsorbed plasma containing factors VIII, V, serum containing factors XII, XI, IX, X, calcium and platelets are added and incubated at 37° C. These three components will generate prothrombin activator. This mixture is made to act with normal plasma, which contains fibrinogen and prothrombin. The time for the clot to appear, indicates thromboplastin generation test. Normal value is 6 to 15 seconds.

Clotting disorders

Hemophilia

It is a clotting disorder in which there is a hemorrhagic tendency, due to the deficiency of clotting factors VIII (AHG) and factor IX (Christmas factor).

Haemophilia is a genetic disorder, transmitted as X linked recessive character. The males suffer from the disease and females carry the disorder. There are two types of hemophilia and they are described as follows:

Hemophilia A or classical hemophilia

It results due to the deficiency of factor VIII, antihemophilic globulin (AHG). The clotting time is abnormally prolonged.

von Willebrand's disease is also a hereditary disease, inherited as dominant trait in both sexes. Since vWF is a subconstituent of factor VIII, von Willerbrand's disease will also cause deficiency of factor VIII and symptoms of hemophilia. It also affects platelet function in forming adhesion to the injured surface of the vessel wall.

Hemophilia B or Christmas disease

Clotting factor IX (plasma thromboplastin component) is a vitamin K dependent factor, which is synthesised from the liver. Christmas disease is also an X linked disorder, resulting from the deficiency of factor IX.

BLOOD GROUPS AND TRANSFUSION

Blood groups existence is based on the two types of agglutinogens on the surface of red blood cells. These agglutinogens are A and B and they are responsible for the four types of blood groups i.e., A, B, AB and O (Fig. 6.8). The agglutinogens are **oligosaccharides**, which are not only present on the red cells, but also, in body secretions, such as, saliva, gastric juice and in tissues of liver, kidney and lungs. The red cell surface has more than 30 antigens on its surface, but they do not induce any transfusion reactions. Hence, for the purpose of blood transfusion, A, B, O and AB groups cross matching is done with donor's red cells and recipient's plasma. It should be kept in mind that there are agglutinogens other than A and B present in some individuals and the blood group is named after their family name. The red cell surface in 85% of the individuals, shows another type of agglutinogen called Rh agglutinogen. This

Blood group	*Agglutinogen (red cell)*	*Agglutinin (plasma)*
A	A	β (anti B)
B	B	α (anti A)
AB	AB	Nil
O	Nil	α and β

Fig. 6.8: Blood groups based on the agglutinogens on the RBC

is present in addition to the A, B agglutinogens and such individuals are called Rh^+. The blood group will be called Rh^-, if the Rh agglutinogen is absent on the red cell membrane.

In plasma, there are antibodies called agglutinins, which act against the blood group of corresponding agglutinogens.

Law of blood grouping

Landsteiner, the discoverer of blood groups, propounded the law of blood grouping based on the type of agglutinogens and agglutinins present in the red cell and plasma respectively.

The law states, that, when an agglutinogen is present in the RBC, the plasma will not contain the corresponding agglutinin. Conversely, when the red cell does not contain the agglutinogen, the plasma will contain the corresponding agglutinin. The second part of the law cannot be applicable to the Rh system. In Rh^- individuals, Rh agglutinins will not be present. It is produced only when Rh^- plasma is sensitized with Rh^+ cells.

Blood groups are determined by taking red cells suspension and mixing with the three types of sera namely, anti A, anti B and anti D on a glass slide and observed for agglutination reactions. The anti D sera is used for determining the Rh group.

The agglutinins are immuno globulins. The anti A and anti B agglutinins belong to IgM type. The Rh agglutinin belongs to IgG type. The presence of opposite agglutinin in plasma, can be explained, from the fact, that during infancy, there are various types of antigens including blood group antigens of non self ingested along with the food. The antibodies are developed passively in the plasma and continue to stay for the rest of life.

Inheritance of blood groups

Blood groups are inherited as mendelian allelomorphs. The dominance is seen in type A and type B genes. The O type gene does not have function and there is no agglutinogen present for it in the red cell. Hence, the inheritance of blood groups depends on any of the three genes namely A, B and O. The phenotype of A or B group person can have the homozygous or heterozygous genotypes. This is the reason, why the blood group of a child can be different from that of both the parents.

For example, if one of the parents is A group and the other is B group, then, the genotypes in the children would be AB, AO, BO, OO, and the possible blood groups in the offsprings can be any one of the groups namely, AB, A, B, O. The blood groups, besides, its role in the transfusion of blood, also helps in medicolegal cases to solve the disputed parentage. If the blood group of man, woman and child are known, then it is possible to find out that the man is not the father of the child.

Rh^+ persons will have D type gene, whereas the Rh^- person gene type is d. The Rh^+ phenotype will have, the genotypes DD, Dd and both will be Rh^+. The Rh^- phenotype will have only dd genotype.

Cross matching

Cross matching of blood groups is done to find out the compatibility of blood groups for transfusion. If transfusion of blood is carried out without cross matching of groups, the donor's red cells will be clumped by the agglutinin present in the recipient's plasma. The clumping of red cells is called agglutination.

In cross matching, the donor's red cells are matched with the recipient's plasma and observed for agglutination reactions (Fig. 6.9). Normally the donor's plasma is diluted in the recipient and hence the agglutination of recipient's red cells with the donor's agglutinin of plasma does not occur. However, in some cases, there could be agglutination reactions in the latter. To rule out such a possibility, the cross matching is also carried out, with the agglutinin of donor's plasma and the red cells of the recipient. Only if there is absence of agglutination in both the tests, the blood group is selected for transfusion.

Donor's blood (agglutinogen)	*Recipient's blood (agglutinin)*			
	A (β)	B (α)	AB (–)	O (α, β)
A	–	+	–	+
B	+	–	–	+
AB	+	+	–	+
O	–	–	–	–

– = no agglutination
+ = agglutination

Fig. 6.9: Direct cross matching of donor's blood with the recipient's plasma

It will be seen from the table that O group can be given to all other groups and AB can receive from all other groups. The O group is referred as *universal donor*, as it does not have any agglutinogen. The AB group is called as *universal recipient*, as it contains no agglutinins in the plasma. Since there is presence of Rh agglutinogen in the red cells of 85% of the individuals, it is better to avoid such terms. For transfusion of blood, Rh compatibility test should also be included. The transfusion of Rh^+ blood should never be given to Rh^- person. In emergency, where the blood typing cannot be done, O negative group can be transfused.

Rh factor

It is a type of agglutinogen called Rh antigen or D antigen present on the RBCs of 85% of the individuals. Those who have this antigen on the red cells are called Rh^+. The name is derived from the experiment performed with the rhesus monkey's cells mixed with the rabbit's serum. The rabbit's serum developed antibodies against the rhesus monkey's red cells. This immunized rabbit serum, when tested with the human RBCs, agglutination was noticed in 85% of the samples tested. This showed that the human RBC contained an antigen, similar to rhesus monkey red cell antigen. Those who show this, on their red cells, are called Rh^+.

The remaining 15% of the individuals show no such Rh antigen on the red cell and they are called Rh^-. Their plasma does not contain Rh antibodies, but produced, when Rh^+ blood is given and sensitised.

Rh incompatibility

In blood transfusion, Rh^+ blood should not be given to a Rh^- person. In the first transfusion, the Rh^- plasma becomes sensitised by the Rh^+ red cells. Subsequent transfusion of Rh^+ blood to the same person, will result in agglutination reaction, due to Rh antibodies produced by the Rh^- plasma against Rh^+ red cells. Rh incompatibility also results, when an Rh^- woman conceives Rh^+ fetus, due to the Rh antigen being inherited from the father. In the first pregnancy, the fetal red cells, which are Rh^+, enter the maternal circulation during child birth, when the placental blood vessels rupture. This causes sensitization of Rh^- plasma of the mother. In the second or third pregnancy, the Rh^- plasma, which is already sensitised with Rh^+ fetal red cells, produces enormous Rh antibodies. It crosses the placental barrier and enters the fetal circulation. The agglutination of fetal red cells by the Rh antibodies, results in **hemolytic disease of the newborn(HDN)**. The newborn will show severe jaundice and anemia. The peripheral blood shows the nucleated precursor red cells and hence the name **erythro blastosis fetalis**. Sometimes, the child dies in utero, giving still born births. If the jaundice is severe, it will lead to deposition of bile pigments in the basal ganglia, resulting in **kernicterus**. This Rh incompatibility can be treated by exchange transfusion of Rh^- blood to the child. The mother can also be effectively immunized against Rh antibodies by giving anti Rh immunoglobulin immediately after the birth of the first child.

Transfusion of blood

Transfusion of whole blood is required in severe hemorrhage, surgery, and anemia (when Hb%

falls to 40). There are conditions, where only the components of blood need to be given, such as, albumin, clotting factors, platelets concentrate, red cells, whole plasma, gamma globulins, etc.

The blood selected for transfusion should be free from diseases like, malaria, syphilis, hepatitis and acquired immune deficiency syndrome. If an individual comes to know earlier of the requirement of blood for transfusion, as in undergoing surgery, the safest way, is, to collect his or her own blood periodically at regular intervals and use it during surgery. This method of transfusion is called *autologous transfusion.*

Precautions to be taken in transfusion of blood:

- Screening of the donor, who is free from communicable diseases
- Cross matching of blood to ensure compatibility
- Storing of blood in aseptic conditions
- Transfusion should not be rapid (The rate should be 100 to 200 ml/hour).

Changes during storage of blood

The blood collected for transfusion is stored at 0 to 4°C in blood banks. During storage, the red cells become spherical, due to the inhibition of sodium pump and water moving in to the cell. Sodium pump is inhibited as the temperature is very low during storage. The size of the red cell becomes normal, when the blood is transfused, as the sodium pump is being restored. During storage of blood, acid citrate dextrose is added to provide energy to the red cell. The absence of sodium pump during storage of blood causes potassium level to be more in plasma and sodium remaining inside the red cell. The ions go back to their positions when the blood is transfused into the subject. If the transfusion is too rapid, there is a danger of hyperkalemia in the recipient.

Effects of incompatible transfusion

Incompatible or mismatched blood transfusion can occur if cross matching is not carried out. Example of mismatched transfusion would be, A group person receiving B group blood. In such conditions, the red cells agglutinate causing haemolysis. There is a shock like state, due to the hypersensitivity reaction. There is shivering, tightness of chest, fall in blood pressure, dyspnea, etc. The haemolysis leads to release of hemoglobin, hemoglobinuria and formation of increased bile pigments. This results in post transfusion jaundice. The free hemoglobin precipitates the renal tubules causing renal damage (lower nephron nephrosis). The renal failure can lead to uremia and death.

IMMUNITY

Our body is exposed to a variety of pathogenic organisms like bacteria, virus, fungi, parasites, etc. To combat these pathogens, the body shows two types of defense mechanisms. One is known as innate or non specific immunity and the other is acquired or specific immunity. Both the systems are interlinked to give an effective defense mechanism to the body.

Innate immuntiy

Barriers

It refers to the protective mechanisms, that are present to prevent the entry of pathogenic organisms. There are barriers in different regions of the body, such as, skin, and the lipid layer in it prevents entry of micro organisms.

Secretions like, tears, saliva, and acid in the stomach contain antibodies and they form barriers for pathogens.

Mucosa lining the air ways, not only contains cilia to remove the pathogens, but also, contains chemicals, which have bactericidal properties.

Lymphoid organs like tonsil, Peyer's patches, appendix, form effective barrier by trapping the bacteria, which may try to enter the body.

Phagocytosis

In spite of the body having these barriers, the micro-organisms enter the body and in such conditions, the neutrophils and macrophages are

activated to phagocytise the invader. The process of phagocytosis has been described earlier.

Complement system

In addition to phagocytois, there are enzymes called complement system present in the plasma. When activated by the micro organisms, these proteins show the following reactions.

Pathogenic organisms are coated with the complement protein, so that neutrophil and macrophages identify them easily and phagocytize them.

Some complement proteins stimulate the release of histamine from mast cells and basophils, which causes vasodilatation and migration of neutrophils to the site of infection.

There are other complement proteins, which form membrane attack complex, where the protein forms a hole in the wall of the bacteria and causes its lysis by allowing entry of sodium and water into the bacterial cell.

C - reactive protein

It is present in the plasma when there is entry of micro organisms into the body. It coats the bacteria, which inturn, activates the complement system. The complement system can promote phagocytosis.

Interferons

When virus infects the cells, it releases alpha interferons. They in turn are attached to other tissues and prevent the entry of virus.

Natural killer cells

It is a type of lymphocyte, which kills the virus without any prior sensitization. It is an important mechanism to fight against virus infections and also inhibits malignant growth of tissues.

Acquired immunity (Fig. 6.10)

Acquired immunity is specific in nature, that is, defense mechanism exists for each specific antigen. First exposure would cause sensitization (primary response) of the lymhocytes and subsequent entry of the same pathogen leads to effective immune response, by way of releasing specific antibody against it (secondary response). There is also a development of memory in the immune system to the specific antigen. The immune system is represented by the activity of lymphocytes. Functionally, there are two types of lymphocytes namely T lymphocytes and B lymphocytes.

T lymphocytes, give *cell mediated immunity.* It is responsible for delayed hypersensitivity reactions, rejection of transplants of foreign tissue, defense against viruses, fungi and bacillus

T Lymphocyte Functions

- Processed in the thymus
- Causes cell mediated immunity
- Gives delayed hypersensitivity reactions
- Causes rejection of transplants of foreign tissue
- Gives immunity against viruses, fungi and mycobacterium bacillus
- Prevents growth of tumors.

Types of T Lymhocytes and their functions

Helper T cell (CD4): Regulates B cell function. Activates B cell, through the release of cytokines. Causes proliferation and transformation of B cells into plasma cells.

Can recognise the antigen presenting cell by the presence of MHC (class II protein) on the surface.

Cytotoxic T cell (CD8 cell): Destroys antigen. Activated by interleukin 2.

Suppressor T cell: Dampens T_8 cell activity and as well as B cell activity. Responsible for turning off the immune response.

Memory T cell: Stores the immune response information.

mycobacterium. It also prevents growth of tumors. T lymphocytes are of different types namely, **helper T cells, cyto toxic T cells, suppressor T cells and memory T cells**.

B lymphocytes are responsible for *humoral mediated immunity*. It releases antibodies or immunoglobulins against antigens.

Lymphocytes are produced from bone marrow. The stem cells developing into lymphocytes go to lymphoid organs and differentiate. The lymphocytes that are processed in the **thymus** are known as **T lymphocytes**. Lymphocytes, which are processed in the **bone marrow** and **liver** forms the bursa equivalent in humans, called **B lymphocytes**. Both T and B lymphocytes can be seen in lymph nodes and bone marrow, as migration of the processed lymphocytes occurs in these regions. Although there are two types of immunity, both are interlinked for efficient functioning of the immune system.

Antigen recognition by clonal selection

Whenever an antigen first enters the body, either it is taken up by the antigen presenting cells and partially digested, or binds to the B lymphocyte receptors. The B cell after being stimulated by helper T cell, secretes antibodies by forming plasma cells. In the case of T cell, the antigen presenting cell with the antigen partially digested in it, binds to the T cell receptors. The T cell causes lysis of the antigen by forming pores (perforins) on the cell wall of the antigen. In both the types, the T cell and B cell divides to form **clones** to cells, so that, if the same antigen enters the body again, an effective immune response could be produced **(clonal selection)**.

Acquired immunity (Fig. 6.10)

In all cells on its surface, there are protein products of the gene, known as **major histocompatibility complex(MHC)** present. It consists of two class of MHC proteins namely I and II. The MHC I protein binds to peptide fragments produced within the cells and usually the viral proteins bind to it. MHC II proteins bind to extracellular antigens such as bacteria, that enters the cell by endocytosis and digested by endosomes.

Helper T cells have CD4 proteins on its surface, while the cytotoxic T cells have CD 8 proteins on its surface. The CD4 on the Helper T cells binds to MHC II proteins. The CD8 on the Cytotoxic T cell binds to MHC I proteins.

When an antigen enters it is taken by **antigen presenting cells** in the body. It includes dendritic cells of lymph nodes, spleen , macrophages, B lymphocytes, and Langerhans dendritic cells of skin.

The antigen is partially digested by the antigen presenting cell and the peptide products of the digested antigen are coupled to MHC protein II. The antigen presenting cell forms a synapse with the CD4 T cell. The digested antigen binds to the CD4 T cell receptors and activates it. The CD4 T cell secretes cytokines, which include IL 2. The interleukin 2 causes the T cell to multiply and form a clone. The activated CD 4 now either causes activation of B cell for humoral immunity by forming plasma cells or activates Cytotoxic CD8 T cell. The plasma cells formed from B cell produces immunoglobulins (antibodies).

The CD8 cell can also be activated by the formation of synapse with the MHC I antigen presenting cell. The activated CD8 T cell kills directly the antigen by forming **perforins** or pores on the cell wall, which causes osmotic movement of water and destruction of antigen

Antibodies are produced, not only, when antigen is processed by marcrophages and helper T cells, but also, without the involvement of them. The latter condition is seen, when IgM antibodies are produced in response to foreign blood group antigens. These antigens are called *thymus independent antigens.* The antigens, which stimulate the B cell through the activation of macrophage and helper T cell, are called *thymus dependent antigens.*

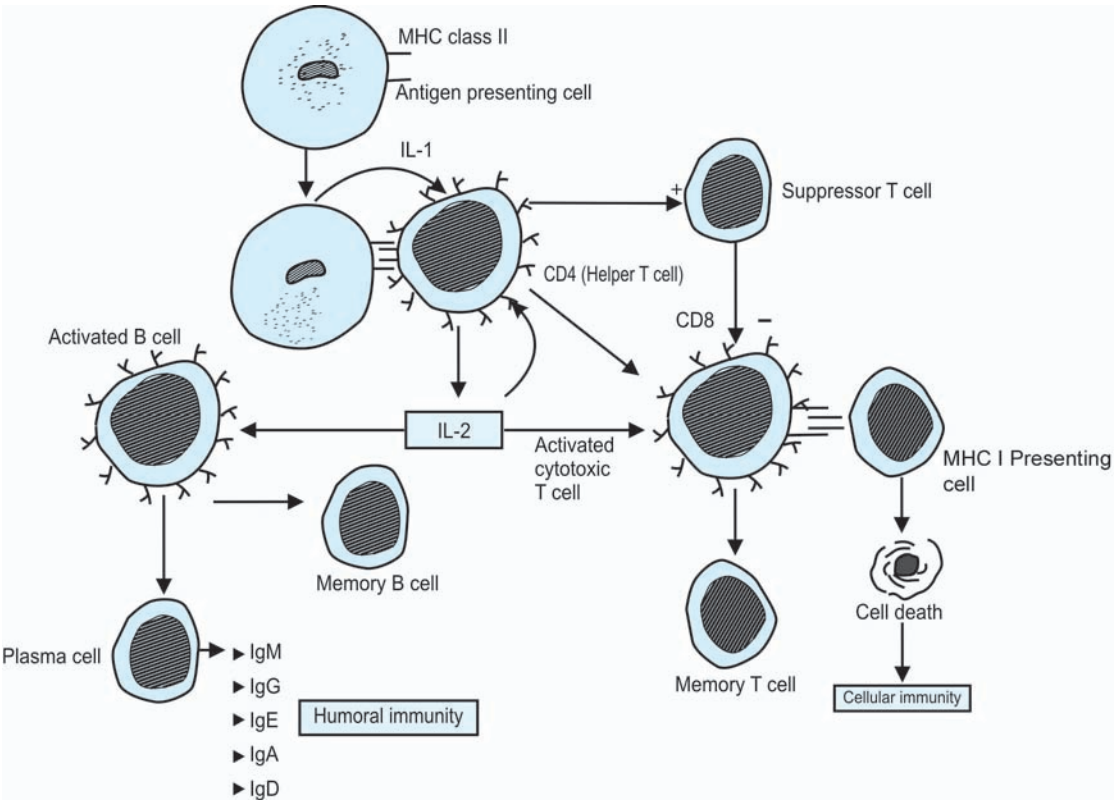


Fig. 6.10: Mechanism of acquired immunity

How does antibodies function?

There are many ways of antibodies neutralising the antigens.

1. It is observed that antigens can be directly neutralized or inactivated by the combination of antigen and antibody. Examples of this would be the inactivation of viruses and snake venom. The release of IgM antibody against the incompatible blood group antigen, producing agglutination, is also an example of direct interaction of antibody with antigen.
2. Antibodies form a coating on the antigens for the macrophages to easily phagocytize the antigen. This function of antibody is known as opsonization.
3. Antigen and antibody combination activate the complement system, involving cascade of enzyme reactions to give various complement proteins.

In the classical pathway, antigen antibody binding results in C3 formation from C1 through other components of the system. The C3 complement protein, leads to C8 and C9 proteins formation and these would cause holes in the cell membrane, producing lysis of the antigen.

The sub type of C3 namely, C3b causes opsonization and facilitates phagocytosis.

There are also subtypes C3a, C5a, C5, C6 and C7, which cause inflammatory response, leading to migration of macrophages to the site of invasion of antigen.

In the other alternative pathway, the complement system is activated without the antibody binding to antigen. The activation is due to the polysaccharides on the wall of the bacteria and facilitated by a circulating protein factor. The complement proteins that are formed, cause lysis of invading organism. This is also called *properidin pathway.*

Structure of antibody and its subtypes

Immunoglobulins consists of four polypeptide chains. There are two identical light chains (low molecular weight) and two identical heavy chains (high molecular weight) present in the molecule. Both the chains have constant and variable regions. In constant chain, the amino acid sequence is similar in all types of immunoglobulins, whereas, in variable chain, the amino acid sequence is different in each immunoglobulin. This gives specific affinity for the immunoglobulin to each type of antigen. The effector function of immunoglobuin would be present in this region.

The different types of immunoglobulins exist, due to the difference in the constant regions of heavy chains. There are five types of heavy chains namely μ, γ, α, δ, ε and the immunoglobulins are IgM, IgG, IgA, IgD, and IgE respectively.

IgG The heavy chain is γ. Its serum concentration is 7 to 16 mg/ml. It crosses placenta. Its function is anti bacterial and anti viral.

IgM The heavy chain is μ. Its concentration in serum is 0.5 to 2 mg/ml. It is produced in early immune response. It is a good agglutinator. It has antibacterial activity.

IgA The heavy chain is a. Its level in serum is 0.4 to 4 mg/ml. It is present in body secretions.

IgD Its heavy chain has δ type and its concentration in serum is 0 to 0.4 mg/ml. It is present on lymphocyte surface and involved in antigen recognition.

IgE The heavy chain is ε and its level in serum is 0.00002 mg/ml. It binds to mast cells. It is involved in hypersensitivity reactions and allergic reactions. It is also released in parasitic infestations.

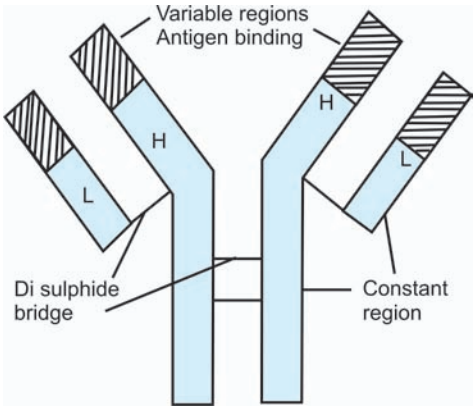


Fig. 6.11: Structure of immunoglobulin—The H and L are the heavy and light chains of amino acids respectively.There are four chains (tetramer) in the immunoglobulin molecule, two light chains and two heavy chains, joined by di sulphide bonds. The antigen is attached to the variable regions of the molecule

Regulation of immunity

Immunity is regulated by genetic, neural and endocrine factors. Recent studies have shown that regions of hypothalamus, limbic lobe, influence immune mechanism, either through neural signals or through the hormonal factors.

Abnormalities of immune mechanisms

- Immediate hypersensitivity reactions, which can lead to anaphylactic shock.
- Break down of immunological tolerance leading to antibodies being produced against self antigen and results in auto immune disease. The mechanism that eliminates antibodies against self-antigens when fails, autoimmune disease can occur. They can be T or B cell mediated. Examples are diabetes mellitus Type 1, myasthenia gravis, multiple sclerosis, Grave's disease and systemic lupus erythematosis.

Acquired immune deficiency syndrome (AIDS): It is caused by a retrovirus (human immuno deficiency virus), which binds to CD4 and helper T cells, causing their number to decrease. The loss of helper T cells results in the absence of proliferation of B cells and T cells. This results in the loss of immunity, and spread of infection from nonpathogenic bacteria. It eventually leads to death of the infected individual.

- During development, there could be a uncontrolled proliferation of B lymphocytes, which gives chronic lymphocytic leukemia. When plasma cells change into malignant growth; it results in multiple myeloma.

HEMOGLOBIN

It is a red color pigment present in the red cell. It is a chromoprotein with a molecular weight of 64,450. Hemoglobin normal concentration in males ranges from 14 to 18 g% and in females it ranges from 13 to 17g%. The function of this pigment is to carry respiratory gases and being a protein, it takes up H^+ ion and hence helps in acid base regulation.

Structure of hemoglobin

Hemoglobin contains heme and globin. The heme is made of protoporphyrin and iron. There are 4 subunits in each hemoglobin molecule. The globin consists of 4 polypetides and each one is attached to one sub unit of heme. Thus, there are 4 iron atoms in each hemoglobin. The globin polypeptides are arranged in two pairs and they are called α_2 and β_2 in adult hemoglobin. The alpha chain contains 141 amino acids and the beta chain is made up of 146 amino acid residues. Changes in the position of amino acids in the globin polypepide units, will result in abnormal hemoglobin such as sickle cell anemia. In the fetus, the hemoglobin is different from the adult hemoglobin. The β unit is replaced by γ unit in the globin molecule. The change in polypetide chain in fetal hemoglobin, has a functional advantage. That is, the fetal hemoglobin can be saturated fully at 40 to 45 mmHg of pO_2, showing greater diffusion of oxygen from maternal circulation to fetal circulation. The fetal hemoglobin is replaced by the adult type soon after birth.

Hemoglobin is synthesized in the progenitor nucleated red cells in red bone marrow and the synthesis continues even after the cells are released into the circulation, but restricted to only reticulocyte. Low level of synthesis, for a day or two can be seen in reticulocyte. In RBC, the Hb synthesis cannot occur.

The heme is synthesized separately and then conjugated, with globin molecule. The globin that is utilised in the synthesis, is usually the recycled molecule. Heme is synthesized in mitochondria and defects in heme synthesis causes porphyrias. Heme is also a constituent of myoglobin and cytochrome enzyme.

Types of hemoglobin

- Adult Hb: It contains the globin as α and β units.
- Fetal Hb: The globin has α and γ units.
- Thalassemia It is inherited as autosomal recessive character, in which, there is deficient synthesis of α and β chains.
- Hemoglobin S (sickle cell anemia) It is an abnormal Hb, formed from a mutant gene and inherited as a trait in a population. It is common in west African population.The red cells are sickle shaped and this shape provides osmotic resistance to the red cell against malarial infection. The hemolysis of sickle cells when occurs, gives sickle cell anemia.

Derivatives of hemoglobin

Oxyhemoglobin: When Hb combines with oxygen this is formed.

Deoxygenated Hb: The dissociation of oxygen from Hb gives this derivative.

Carboxy Hb: It is produced, when Hb combines with carbon mono oxide. The affinity of Hb for CO is very high. Its affinity is 200 times greater than the affinity it has for oxygen.

Methemoglobin: When ferrous (Fe^{++}) in Hb is oxidized to ferric (Fe^{+++}), it is called methamoglobin. Exposure of red cells to drugs like phenacetin, acetanilide and chemicals like nitrates, aniline dye, etc. will cause methemoglobin formation.

Sulfhemoglobin: The reaction of Hb with hydrogen sulphide gives this compound.

Fate of hemoglobin

When hemoglobin is released from red cells, as a result of hemolysis, it is taken up by a carrier protein haptoglobin in plasma. In the liver and spleen, the Hb is separated from haptoglobin and catabolised.

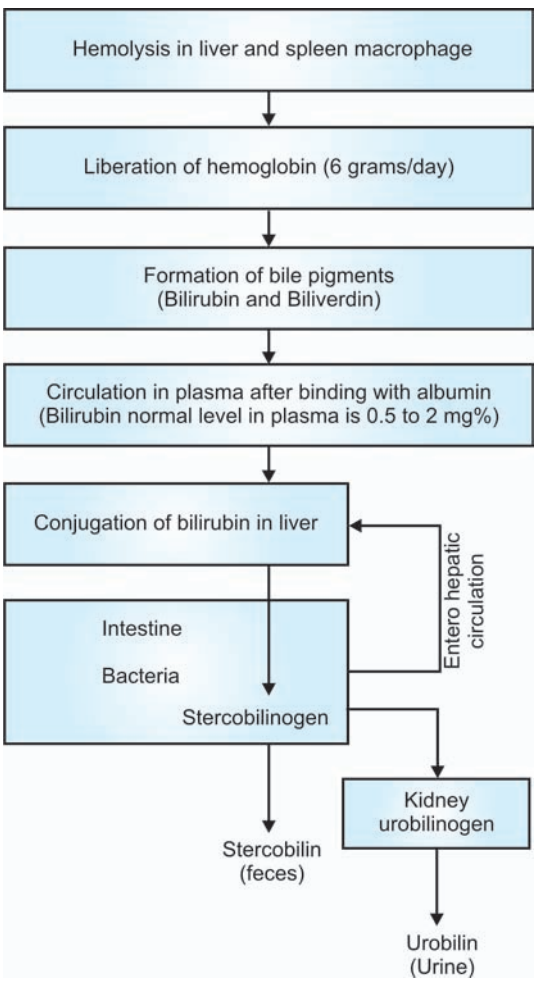


Fig. 6.12: Fate of hemoglobin in the body

Hemoglobin is also released from red cells, when hemolysis occurs in tissue macrophages in spleen, liver and bone marrow. The Figure 6.12 shows the break down of hemoglobin and formation of bile pigments (bilirubin and biliverdin). The quantity of Hb, destroyed normally in a day is 6 g, which forms around 200 mg of bile pigments (Normal concentration of bilirubin in plasma is 0.5 to 2 mg%). Increased bile pigments in the plasma is observed in jaundice.

Blood indices

A variation in the size and number of red cells or reduction in the concentration of hemoglobin assumes clinical significance. The evaluation of blood indices helps to identify the type of anemia and plan appropriate treatment. **Color index** is the ratio between the Hb% and RBC %. Its normal value is 0.8 to 1.10. The value is low in iron deficiency anemia.

Mean corpuscular volume (MCV)

It is the average volume of a single red cell and it ranges from 90 to 93 μ^3. In iron deficiency anemia, the MCV is decreased, while it is increased in megaloblastic anemia.

Mean corpuscular hemoglobin (MCH)

It indicates the average content of Hb in a single red cell. The value ranges from 28 to 32 pico grams.

In iron deficiency, MCH is decreased.

Mean corpuscular hemoglobin concentration (MCHC).

It is the relative percentage of Hb in a single red cell. Its normal value is 33%.

ANEMIAS

Anemia is defined as reduction in Hb% below normal for his or her age or decrease in the circulating red cells or combination of both the factors.

Clinically, a person is said to be suffering from anemia, when Hb% falls below 13 g% in males and 11.5 g% in females. Anemia itself is not a disease, but a symptom of some underlying disorder and its correction depends on the treatment of the underlying cause.

Classification of anemias

Anemias can be classified based on their cause. Broadly it can be classified as:

- Anemia resulting from decreased formation of red cells
- Anemia resulting from increased destruction of red cells
- Anemia resulting from blood loss.

Laboratory investigations and cell picture

To find out the type of anemia, the total red cell count, packed cell volume, Hb%, color index, MCV, MCH, MCHC, are to be carried out in a laboratory. Based on the blood indices, the cell picture shows the following.

Normocytic: Red cell volume is normal
Macrocytic: Red cell volume is increased
Microcytic: Red cell volume is decreased

Normochromic: MCHC is normal
Hyperchromic: MCHC is more than normal
Hypochromic: MCHC is below normal

The cell picture in the peripheral circulation shows characteristic features depending on the type of anemia. They are described in the discussion of various types of anemias.

I. Anemia due to decreased formation of red cells

Nutritional anemias

This type of anemia is encountered when there is deficiency of dietary factors such as vitamins, metals, and trace elements.

Lack of vit B_{12}

Vitamin B_{12} is the extrinsic factor (cyanocobalamin) and requires the intrinsic factor presence in the gastric juice, for its absorption from the terminal ileum. The lack of Vit B_{12} in the diet or autoimmune disease affecting parietal cells results in pernicious anemia. It is a megaloblastic anemia in which large red cells appear in the circulation. The cells also will be of unequal sizes (Poikilocytes), the MCHC remain normal. The cell picture shows macrocytic anemia. That is macrocytic normochromic anemia results. Vitamin B_{12} is required for the proliferation and maturation of red cells progenitors. Lack of this vitamin leads to decreased production of red cells in the red bone marrow and arrest of maturation. In this type of anemia there is also involvement of neurological deficit, affecting the myelin sheath of dorsal and lateral columns of the spinal cord. This condition is called subacute combined degeneration of spinal cord.

Folic acid deficiency

Folic acid is required for the proliferation and maturation of erythroid cells. Lack of this vitamin causes megaloblastic anemia, similar to vit B_{12} deficiency, but without any neurological symptoms.

Iron deficiency

Iron is required for the hemoglobin formation. Iron is a constituent of heme. The protoporphyrin, which is formed, combines with iron to form the haeme. The iron in the hemoglobin is necessary, as the oxygen is taken by it and transported in the blood. Deficiency of iron causes *hypochromic microcytic anemia.* This type of anemia is common in reproductive women, as there is loss of iron in the menstrual blood. During pregnancy also iron deficiency anemia is seen.

Suppressed bone marrow function (Aplastic anemia)

Suppression of bone marrow by drugs, X ray radiation, toxic agents, etc. can cause decreased production of all types of blood cells (pancytopenia).

Chronic diseases

Chronic infections, chronic inflammation or neoplasia cause depression of erythropoiesis. The blood cell picture shows normochromic normocytic anemia.

Renal disease

Chronic renal disease is usually associated with anemia due to lack of erythropoietin.

Hypothyroidism

Hypothyroidism causes anemia with the blood cell picture being normocytic and normochromic. The anemia is due to depressed metabolism of the bone marrow.

II. Anemia arising from increased destruction of RBC

They are called hemolytic anemias. Abnormal red cells are produced, when there is a hereditary defect. These abnormal red cells show a greater fragility. That is, these red cells rupture easily and undergo hemolysis, thus reducing the life span of the cells *(corpuscular defect).* It is also possible, that without any hereditary abnormality, hemolysis can take place due to external factors (*extracorpscular defect).*

Corpuscular defect

Hereditary spherocytosis

The red cells are not biconcave, but spherical. They are easily hemolyzed when they pass through the small capillaries.

Sickle cell anemia

The RBC contains abnormal hemoglobin, where the amino acid sequence in one of the globin chains is changed (valine instead of glutamic acid in the sixth position of the β chain). The hemoglobin is called HbS. HbS polymerizes at low O_2 tension which causes the red cells to become sickle shaped. The sickle shaped red cells undergo hemolysis easily. The abnormal shape of the red cells also makes it susceptible for phagocytosis. Sickle cell anemia is common in West African population and gives resistance to malarial parasite. That is, its schizont stage in the development can not be formed in the red cell.

In homozygous inheritance, sickle cell anemia is fully developed and the hemolytic anemia will be severe. In heterozygous inheritance, the sickle cell trait occurs, which gives less severe symptoms.

Thalassemia

The hemoglobin present in the red cell is an abnormal variant. It could be due to the defective synthesis of globin chain. The red cells are microcytic and have a greater fragility causing hemolysis.

G-6-P-D (glucose-6-phosphate dehydrogenase) deficiency

Deficiency of this enzyme in RBC, causes the damage of the red cell membrane by peroxidation. This is due to the absence of formation of reduced glutathione in the red cell.

Extracorpuscular defect

Hemolysis occurs, when red cells are exposed to certain chemicals, drugs, snake venoms and iso agglutinins.

Hemolytic anemia also can be seen by malarial parasite, viruses, bacterial septicemia and nonprotozoan parasites.

To detect the presence of isoagglutinins and antibodies on the surface of the red cells Coombs test is performed.

Coombs' test

This test is done to detect the presence of incomplete antibodies on the surface of RBC.

In the *direct test*, the patient's RBCs are washed and to this washed RBCs, anti human globulin serum, prepared from rabbit, is mixed. If agglutination occurs, it is a direct coombs' test. In this test, the RBCs on the surface contained incomplete antibodies, which could not be washed away.

In the **indirect test,** the surface incomplete antibodies are removed while washing the RBCs. These RBCs are mixed with the patient's RBCs containing incomplete antibodies. Now normal RBCs will be coated with the incomplete antibodies. If rabbit serum containing anti human globulin is added, agglutination of RBCs occurs. This is indirect Coombs' test.

III. Anemia resulting from blood loss

Acute blood loss, as in hemorrhage gives normocytic normochromic anemia. Chronic loss of blood as in heavy menstrual blood flow, loss of blood in peptic ulcer, piles and hook worm infestation, leads to microcytic hypochromic anemia, similar to iron deficiency.

Effects of anemia

Anemia causes compensatory changes in the respiratory and cardiovascular systems to increase the oxygen supply to the tissues. The heart rate, cardiac output, systolic blood pressure are increased. The circulation time is reduced. The increase in cardiac output and less viscosity of blood gives systolic murmur. The respiratory rate and depth are increased to carry more oxygen to the tissues. As long as the anaemic person is at rest, no difficulty is experienced. But, when the subject exerts physically, with the Hb level falling to 7.5 g%, it produces clinical signs of anemia, such as, dyspnea, palpitation, fatigue, headache, dizziness and tinnitis. If Hb falls to 3 g%, dyspnea occurs during rest and cardiac failure occurs, when Hb level reaches 2 g%.

Self-study Questions

Multiple Choice Questions

Choose the single best answer

1. **A fall in plasma albumin concentration would produce increased:**
   - **A.** Fluid exit from capillaries
   - **B.** Colloidal osmotic pressure
   - **C.** Blood volume
   - **D.** Fluid entry into the capillaries

2. **Hematocrit is increased in:**
   - **A.** Dehydration
   - **B.** Pregnancy
   - **C.** Chronic renal failure
   - **D.** Thalassemia

3. **Which of the WBCs rise in number in parasitic infections?**
   - **A.** Monocyte
   - **B.** Basophil
   - **C.** Eosinophil
   - **D.** Neutrophil

4. **Which of the WBCs is similar to mast cells in its function?**
   - **A.** Neutrophil
   - **B.** Basophil
   - **C.** Eosinophil
   - **D.** Monocyte

5. **Plasma erythropoietin level is increased in all of the following conditions *except:***
   - **A.** High altitude
   - **B.** Bone marrow tumor
   - **C.** Venous arterial shunt
   - **D.** Pulmonary disease

6. **Reticulocytosis occurs in:**
   - **A.** Bone marrow stimulation
   - **B.** Vitamin B_{12} deficiency
   - **C.** Iron deficiency
   - **D.** X ray radiation

7. **Hemoglobin structure is abnormal in:**
   - **A.** Thalassemia
   - **B.** Pernicious anemia
   - **C.** Iron deficiency anemia
   - **D.** Polycythemia

8. **Mean corpuscular volume is increased in:**
   - **A.** Iron deficiency
   - **B.** Acute blood loss
   - **C.** Hereditary spherocytosis
   - **D.** Vitamin B_{12} deficiency

9. **The immediate precursor of RBC in its development is:**
   - **A.** Stem cell
   - **B.** Proerythroblast
   - **C.** Reticulocyte
   - **D.** Late normoblast

10. **Iron is present in all of the following *except*:**
    - **A.** Heme
    - **B.** Bilirubin
    - **C.** Myoglobin
    - **D.** Ferritin

11. **Tissue macrophage is formed from:**
    - **A.** Lymphocyte
    - **B.** Monocyte
    - **C.** Neutrophil
    - **D.** Eosinophil

12. **The bleeding disorder due to platelet deficiency is:**
    - **A.** Hemophilia
    - **B.** Christmas disease
    - **C.** Von Willebrand disease
    - **D.** Purpura

13. **B lymphocytes are preprocessed in:**
    - **A.** Liver
    - **B.** Kidney
    - **C.** Bone marrow
    - **D.** A and C

14. Chronic blood loss in a person can result in which type of anemia?
- **A.** Hemolytic anemia
- **B.** Hypochromic anemia
- **C.** Megaloblastic anemia
- **D.** Aplastic anemia

15. The protein present in the vascular endothelium that combines with thrombin and prevents intravascular clotting?
- **A.** Plasminogen
- **B.** Thrombomodulin
- **C.** Thrombosthenin
- **D.** Heparin

16. The aggregation of platelets is inhibited by:
- **A.** ADP
- **B.** Prostacyclin
- **C.** Thrombosthenin
- **D.** Thromboxane A_2

17. The activation of which clotting factor is crucial for the extrinsic mechanism of blood coagulation?
- **A.** V
- **B.** VII
- **C.** VIII
- **D.** X

18. The rejection of foreign transplanted tissue is caused by:
- **A.** B lymphocyte
- **B.** T lymphocyte
- **C.** Macrophage
- **D.** Natural killer cells

19. Antigen presenting cells include:
- **A.** Dendritic cells of lymph node
- **B.** Macrophage
- **C.** B lymphocyte
- **D.** All of the above

20. Which of the following regulates immune system by the release of cytokines?
- **A.** Plasma cell
- **B.** Cytotoxic T cell
- **C.** Helper T cell
- **D.** B lymphocyte

ANSWER KEYS

1. A	2. A	3. C	4. B	5. B	6. A	7. A	8. D	9. C	10. B
11. B	12. D	13. D	14. B	15. B	16. B	17. B	18. B	19. D	20. C

Short Answer Questions

1. List the plasma proteins and their functions.
2. Enumerate the stages of erythropoiesis.
3. State the site of production of RBCs in the fetus, newborn and adult.
4. List the factors that regulate erythropoiesis.
5. State the peripheral blood cell picture in the deficiency of Iron and Vitamin B 12.
6. What are the blood cell indices done to assess anemia.
7. Describe any one hereditary haemolytic anemia.
8. Describe the fate of haemoglobin in the body.
9. Enumerate granulocytes and list their functions.
10. List the tissue macrophages and their functions.
11. Enumerate the functions of thrombocytes.
12. Describe the role of platelets in blood coagulation.
13. Describe fibrinolysis and State its importance.
14. Describe the mechanism of action of heparin.
15. List the factors that favour intravascular thrombosis.
16. Describe prothrombin time and state its importance.
17. Describe clotting disorders.
18. Describe the effects of incompatible blood transfusion.
19. State the types of T lymphocyte and their functions.
20. Describe how humoral immunity is produced in the body.

7 Cardiovascular System

ORGANIZATION

The pumping action of the heart is responsible for the circulation of blood to various organs. The perfusion of tissues depends on the efficient functioning of the circulatory system. The organization of the vascular system consists of two types and they are (Fig. 7.1):

Systemic circulation for the perfusion of various tissues and

Pulmonary circulation for the exchange of respiratory gases.

Central pumping organ

Human heart is four chambered with two atria and two ventricles. The atria have reservoir function, receiving blood from major blood vessels. Their wall is thinner than the ventricles. When ventricles themselves are compared for their thickness, we can observe that the right ventricular wall is thinner, than the left ventricle. That's why the pressure developed from right ventricle is less, which is sufficient for ensuring pulmonary circulation. The pressure developed in the right ventricle in systolic phase is 25 mmHg and during diastole, the pressure decreases to 10 mmHg. The left ventricular contraction gives rise to systemic circulation and hence the pressure developed here is greater. The peak pressure during systole is 120 mmHg and during diastole the pressure falls to 80 mmHg.

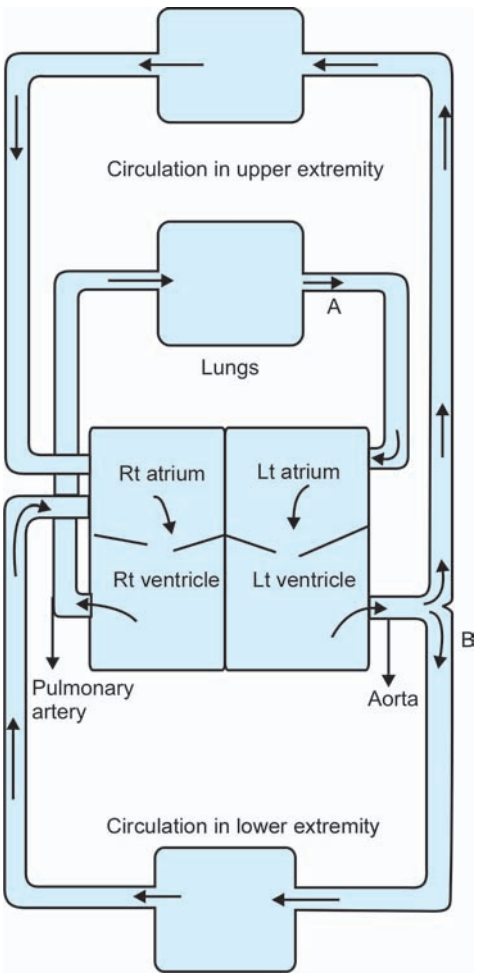


Fig. 7.1: Schematic illustration of human circulatory system, A–Pulmonary circulation, B—Systemic circualtion

Valves in the heart

To regulate the flow of blood within the chambers of the heart and between the chambers of the heart and major blood vessels, there are valves present.

Atrio ventricular valves are present between atria and ventricles. Tricuspid valve is present between right atrium and right ventricle and between left atrium and left ventricle, bicuspid or mitral valve is present.

Semilunar valves are present to regulate the flow of blood between ventricles and the major blood vessels. The aortic valve is situated between the left ventricle and aorta, while the pulmonary valve is present between the right ventricle and pulmonary artery. The valve function is to regulate the flow of blood in one direction only. That is, when they close, backward flow is prevented.

The heart is covered by a sac called pericardium, which contains a thin serous fluid. It acts as a lubricant for the mechanical activity of the heart.

Systemic circulation

The systemic circulation is the greater circulation, as the blood perfuses various organs. The arterial system includes different divisions and each division, further subdivides into many branches. Each division of the arterial system is in series with one another, whereas, the branches of each division is parallel with one another.

The arterial system includes two types of vessels namely, Windkessel and resistance. Windkessel vessels in the aorta and large arteries contain more elastic tissue in the wall and hence, they recoil when stretched. The elastic recoiling of aorta and large arteries is called Windkessel effect.

The small arteries end in arterioles, which contain thick smooth muscle in its wall. The smooth muscle is supplied by sympathetic noradrenergic fibers. The resistance offered by arterioles is called peripheral resistance and hence these vessels are also called resistance vessels.

Resistance vessels end in capillaries. The capillaries are situated between arterial and venous systems. These vessels are called exchange vessels, as the transfer of respiratory gases, nutrients, metabolic wastes, etc. takes place between these vessels and tissues.

The capillaries open into venules, which in turn end in veins. The venous blood from veins, opens into large veins and then into vena cavae. The venous blood from the lower part of the body is collected by the inferior vena cava, while the venous blood from the upper part of the body is drained into superior vena cava. The vena cavae opens into the right atrium. The venous system holds large amount of blood. The veins contain 55 to 60% of blood volume and are called capacitance vessels.

Pulmonary circulation

It is a lesser circulation, as it travels only a short distance between the right ventricle and the left atrium. The pressure developed here is less and forms the low pressure system. The deoxygenated blood from the right ventricle is pumped into the pulmonary artery. The pulmonary artery divides into arteries and arterioles. Here, the arterioles are thin walled and the peripheral resistance seen in systemic circulation is absent. The capillaries are present lining the alveoli of the lungs. The transfer of respiratory gases takes place between the capillaries and alveoli. After oxygenation, the blood from the capillaries is collected by four pulmonary veins, which open into the left atrium.

It is the haemodynamics of circulation, which ensures that the amount of blood ejected from the left ventricle is equal to the amount of blood entering the right atrium.

Windkessel vessels	-	Aorta and arteries
Resistance vessel	-	Arteriole
Exchange vessel	-	Capillaries
Capacitance vessel	-	Veins

Hemodynamics of circulation

Hemodynamics explains the influence of pressure, flow and resistance on the circulation. It is known, that the flow of blood in the vascular system is, due to the pressure gradient and resistance in the vessel will reduce the flow. This relationship is shown as:

$$\text{Flow } F = \frac{\text{Presseure (P)}}{\text{Resistance (R)}}$$

Fluid flow characteristics in a rigid tube has been explained by Poiseuille-Hagen principle. It shows that flow is directly related to pressure gradient (P) and fourth power of radius (r^4) and conversely, the flow is inversely related to viscosity (η) and length of the tube (L). It can be written as:

$$F = \frac{P\pi r^4}{8\eta L}$$

When resistance is related to the flow, then the equation will be:

$$R = \frac{8\eta L}{\pi r^4}$$

Poiseuille's principle is not strictly applicable to the blood flow in vessels, as they are not rigid tubes. Nevertheless, the relationship can be observed and hence, is discussed in the haemodynamics of circulation.

Flow in Windkessel vessels

Blood flow in aorta and arteries is pulsatile as the vessels are elastic in nature. During systole, they stretch and the force from the left ventricular contraction is stored as potential energy in the wall of these vessels. They recoil during diastole (Windkessel effect) and the potential energy in the wall is now converted into kinetic energy, which drives the blood to move forwards. When blood flow reaches the capillaries, it becomes continuous or nonpulsatile.

Flow in capillaries

There are two factors, which convert the pulsatile flow into non-pulsatile flow in the capillaries. As we know that in arterioles, there is resistance to the flow, which forms the peripheral resistance.

The elastic recoiling of Windkessel vessels and the peripheral resistance in the arterioles are the two factors responsible for converting the pulsatile flow into a laminar flow in the capillaries.

Pressure decline in the arterial system

In the aorta and arteries, the mean pressure is 100 mmHg in adults. The sharp pressure drop is seen in the arterioles, as they are resistance vessels. The pressure will be 80 mmHg when blood enters the arterioles, but reduced to 40 mmHg, when they leave the vessel (Fig. 7.2). The resistance to the flow will increase the velocity, but reduces the lateral pressure (Bernoulli's principle) (Fig. 7.3).

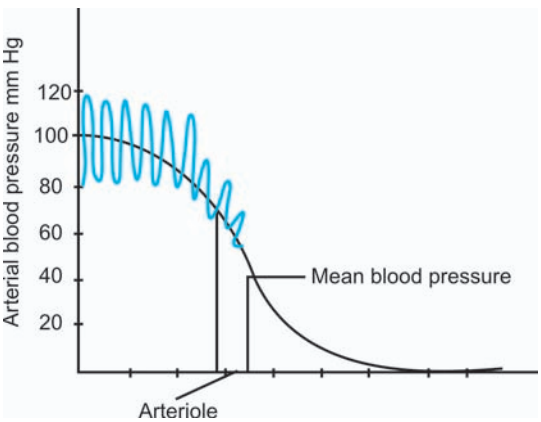


Fig. 7.2: Blood pressure in the arterial system. The pressure falls in the arteriole steeply due to resistance in the wall. The pressure declines to 40 mmHg when the blood leaves the arteriole

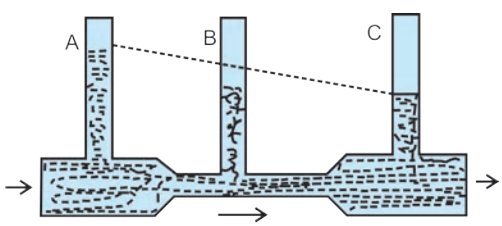


Fig. 7.3: Bernouli's principle showing the relationship between pressure, velocity and flow. In the tube B, the lumen is narrowed and hence the lateral pressure declines. This causes the velocity of flow to become greater (Bernouli's principle)

It should be remembered, that in each division of arterial system, the total cross sectional area is increased eventhough the caliber of each subdivision is lesser than the previous division. As the cross sectional area is increased in each division, the velocity of flow is reduced.

Peripheral resistance and pressure

As already discussed, the resistance in the arterioles reduces the flow, but increases the flow velocity. The peripheral resistance is a measure of diastolic pressure, as it indicates the afterload on the heart. In other words, it is the force against which the left ventricle has to contract and pump blood. If the peripheral resistance is increased, then, the elastic recoiling of the Windkessel vessels cannot be complete, as there will be more arterial blood volume. This causes the elevation of diastolic pressure.

Peripheral resistance is also determined by factors like elasticity of the vessel, viscosity and velocity of blood.

Lesser the compliance of the arterial system (stiffness of the arteries), greater the peripheral resistance. With regard to the viscosity of blood, the haematocrit is the determining factor. Increase in viscosity of blood, as in polycythemia, will increase the peripheral resistance. Blood is considered as having an anomalous viscosity, as it is a non-Newtonian fluid. It is because, the viscosity of blood changes, when the flow velocity is changed. For example, in the peripheral vessels, there is a plasma skimming effect. That is, the lumen of the peripheral vessel is just enough to allow red cells to pass through and the red cells form a pile in the center and plasma occupies the periphery of the vessel. Branches forming from these vessels will contain blood which has more plasma and less cells. This will give decreased viscosity to the blood and increased flow velocity.

Critical closing pressure

It is the lowest pressure, at which the blood vessel will collapse. Normally, the blood vessel is open because of the pressure present within, which is transmitted by the left ventricular contraction. There is also pressure acting on the vessel from outside. The difference between these two will give intramural pressure, which keeps the vessel open. If the intramural pressure drops below the outside pressure, the vessel will collapse as the flow ceases.

Radius of the vessel and pressure

Smaller the radius, lesser the distending pressure required. In capillaries, the radius is small and hence requires less distending pressure, which causes less tension in the wall (Fig. 7.4). That is why, capillaries are not prone for rupture, even though they have a small radius. The Laplace law is applied here. The distending pressure is inversely related to the radius and directly to the wall tension. Similar principle is also observed in the urinary bladder, stomach and alveoli.

$$P = \frac{2T}{r}$$

Increase in diameter of the organ or the vessel requires a greater distending pressure, which rises the tension in the wall.

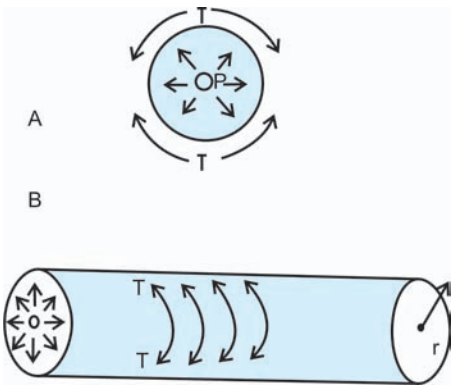


Fig. 7.4: Figure showing the relationship between radius, distending pressure and tension in the wall of the blood vessel (Laplace law). As the vessel becomes narrowed, the distending pressure to keep it open becomes less. That is, the tension in the wall is decreased. Greater the lumen of the vessel, more the distending pressure required. Laplace law states that distending pressure is directly related to twice the wall tension and inversely related to the radius of the tube (P= 2T/r)

CARDIAC MUSCLE

Structure of cardiac muscle

Cardiac muscle is a striated, involuntary muscle. The histology of the muscle shows, that it consists of interdigitating network of branches. The cell is cylindrical in shape, with all the cell organelles present in the cytoplasm. Each cell is separated from the adjoining cell by an intercalated disc (Fig. 7.5). At the sides of these discs, there is a gap junction present. Through this, ions and electrical potentials can easily pass through, facilitating the spread of excitation to the entire myocardium.

Similar to skeletal muscle, there is presence of actin, myosin, troponin, and tropomyosin. There is also a well developed sarcoplasmic reticulum, and the triad is present at the Z line and not in the A–I junction, as in skeletal muscle.

Properties of cardiac muscle

Cardiac muscle being an excitable tissue shows electrical properties, which gives rise to mechanical activity in the form of contraction.

Electrical properties

Pacemaker potential

The mammalian heart has a pacemaker, from where the impulses originate. It is present at the sinoatrial node. This region is electrically more excitable than any other region of the heart. The resting membrane potential is low in this region, which is – 60 mv. There is decreased K^+ exit in the previous repolarization of the action potential, which causes depolarization of the cell. The Ca^{++} channels open, especially the transient calcium channels to complete the prepotential. That is, the membrane potential falls from – 60 mv to – 40 mv. This phase is called prepotential (Fig. 7.6). At – 40 mv, the threshold of firing is reached, leading to opening of long lasting Ca^{++} channels. The influx of Ca^{++} through these channels produces the action potential.

Thus, we see that there is a spontaneous depolarization of the pacemaker cell due to the slow K^+ exit. The completion of prepotential and formation of action potential are due to Ca^{++} influx and not by Na^+ entry. Sympathetic stimulation increases the frequency of pacemaker potentials and hence increases heart rate. The vagal stimulation on the other hand, decreases the frequency or inhibits the formation of pacemaker potentials, depending on the intensity of vagal stimulation. This will give either decrease in the heart rate or complete inhibition of heart's action.

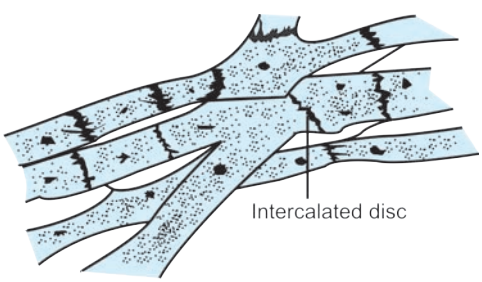


Fig. 7.5: Structure of cardiac muscle

Electrical potential from Ventricular muscle

The action potential from ventricular myocardium is different from the potential recorded in the SA node (Figs 7.7 and 7.8). First of all, the resting membrane potential is high, which is – 90 mv. The depolarization is rapid, which is caused by the opening of voltage gated Na^+ channels (phase 0). The rapid depolarization gives the overshoot and the potential reaches +20 mv. This

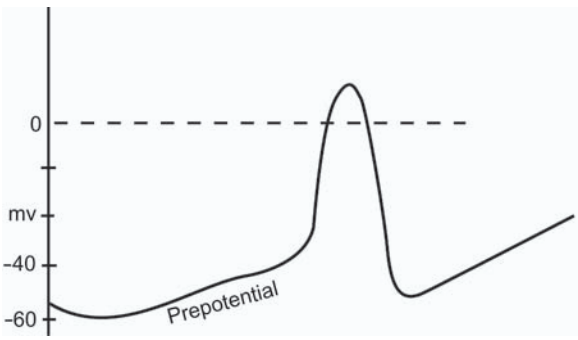


Fig. 7.6: Diagram of a pacemaker potential. Note the absence of spike and plateau in the membrane potential of the pacemaker

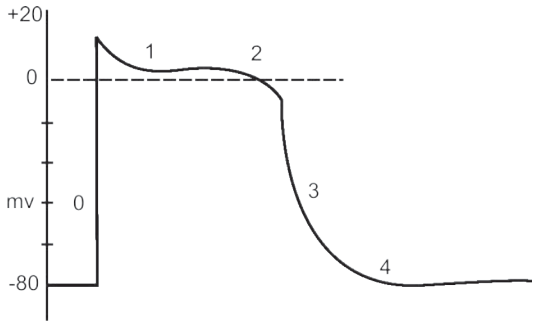


Fig. 7.7: Action potential from ventricular myocardium

0= Depolarization
1= Initial rapid repolarization
2= Plateau
3= Late rapid repolarization
4= Return of resting membrane potential

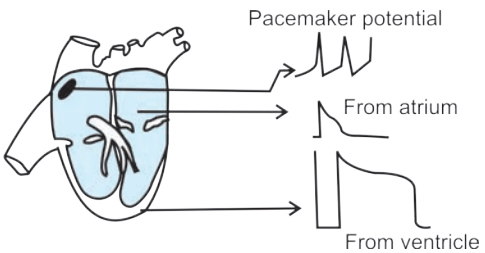


Fig. 7.8: Electrical potentials from the pacemaker, atrium and ventricle

is followed by a plateau. But before the plateau, there is occurrence of initial repolarization (phase 1), which is caused by the closure of Na^+ channels. Subsequently, the voltage gated slow Ca^{++} channels open, giving the prolonged plateau (phase 2). Repolarization begins, when the Ca^{++} channels close. The K^+ exit causes the repolarization. Potassium exit occurs through 2 types of K^+ channels, namely inward rectifier current I_{ki} and delayed rectifier current I_k potassium channels. The action potential lasts for 250 msec.

Mechanical properties

Automaticity

The pacemaker SA node is the first region to be electrically excited in the myocardium.The mechanism by which the pacemaker is excited on its own has been explained earlier. When the pacemaker is destroyed, the AV node is capable of initiating the excitation, but at a lesser rate. The ventricular muscle also can send impulses, if both SA node and AV node are destroyed. The ventricular rhythm in such conditions will be known as idio ventricular rhythm.

Refractory period

The excitability of the heart shows that during complete depolarization, plateau (phase 0, 1, 2) and about half of the repolarisation (phase 3), the tissue cannot be excited, no matter how strong is the intensity of stimulus. The tissue during this period is said to be in absolute refractory period. The duration of absolute refractory period, thus, is longer in cardiac muscle and due to this reason, the **cardiac muscle cannot be tetanized by repetitive stimuli**, unlike skeletal muscle.

All or none law

Cardiac muscle obeys all or none law. The law states that when a threshold stimulus is given, the tissue responds maximally or not at all, if the stimulus is below threshold. In cardiac muscle, there is presence of intercalated disc, which separates the individual cells. But the cardiac muscle behaves like a functional syncytium. That is, the excitation spreads through the gap junctions, which offers low electrical resistance. The impulse can easily spread to the whole myocardium, giving the maximum response.

Excitation-contraction coupling

The action potential spreads in myocardium, through the T tubular system and cell to cell, through the gap junctions. There is opening of voltage gated slow Ca^{++}channels in the sarcolemma and calcium moves along the electrochemical gradient. Calcium channels in the T tubular system **(dihydro pyridine channels)** also open and allow entry of Ca^{++} into the cell. The influx of Ca^{++}through T tubular system, triggers release of Ca^{++} from calcium stores like sarcoplasmic reticulum. This will increase the

cytosolic level of Ca^{++}. Only then, the contractile mechanism is activated.

The force of contraction of heart is increased (positive inotropic effect) by raising the cytosolic calcium concentration. For example the catecholamines show their effect by increasing the influx of calcium through increased phosphorylation of Ca^{++} channel. Cardiac glycosides like digitalis produce their inotropic effect, by raising the cytosolic Ca^{++} through the inhibition of Na^{+}-K^{+} ATP ase. The increased sodium level in the cell, will cause less calcium being removed from the cell. This raises the level of Ca^{++} in the cell and hence the increased force of contraction. Relaxation of muscle depends upon the lowering the cytosolic level of Ca^{++}. This is achieved through the uptake of Ca^{++} by the sarcoplasmic reticulum, Ca^{++} pump and $Na^{+}Ca^{++}$ exchange transport in the sarcolemma (3 molecules of Na^{+} is exchanged with one molecule of Ca^{++}).

Force-velocity relationship

Cardiac muscle shows, that as the load on the muscle increases, the velocity of shortening is decreased. This is similar to what is seen in the skeletal muscle.

Length-tension relationship

In the heart, the force of contraction is directly proportional to the initial length of the muscle fiber within physiological limits.This is the **Starling's law of the heart**. Increasing the preload to the heart increases the end diastolic volume and this in turn stretches the muscle fiber. This causes increased force of contraction (Starling's law). When sarcomere length is reached to the maximum, no more increase in force of contraction can be observed. Hence, Starling's phenomenon can be noticed only within physiological limits.

Factors affecting myocardial contraction

The contraction of the heart is affected by autonomic nerve stimulation, with vagal stimulation inhibiting the heart and the sympathetic stimulation increasing the force and rate of contraction.

Contraction is also affected by the changes in the ion concentration.

Hyperkalemia

Rise in plasma potassium causes decrease in the resting membrane potential in cardiac muscle fibers. This also decreases the intensity of action potential and hence the contraction becomes weaker. The heart is dilated and finally stops in diastole.

Hypercalcemia

Increase in plasma calcium level enhances cardiac contractility, but large amounts of calcium causes the cardiac muscle to go into spastic contraction called calcium rigor and the heart stops in systole.

The circulating hormones and neuro transmitters also affect myocardial contraction. Presence of hypoxia, acidosis and ischaemia of the tissue will cause decreased force and rate of contraction.

Conductivity

The mammalian heart shows a specialised conducting system, through which the cardiac impulse spreads to the myocardium. Any delayed conduction will result in heart blocks. The description of the conducting system and mechanism of conduction has been dealt in the next chapter.

Rhythmicity

The heart shows auto rhythmicity with SA node having the highest order (70/min). This is followed by the AV node, which shows 40/ min as the rate of rhythmicity. The last region is the ventricular muscle, which shows the lowest rate, which is 20/min (idioventricular rhythm). The property of rhythmicity can be demonstrated in an amphibian heart by Stannius ligature experiments.

CARDIAC IMPULSE

Origin of cardiac impulse

Cardiac impulses are self generated from the pacemaker present in the sinoatrial node. It is situated at the junction between superior venacava and right atrium. The histology of the pacemaker tissue shows, that the cells are spindle shaped, primitive in nature, and different from the surrounding muscle. The cells are called P cells and are rich in glycogen. The resting membrane potential is lower than the other regions of the myocardium. It is around – 60 mv and there is decreased K^+exit, causing spontaneous depolarization. The Ca^{++} transient channels open and lower the membrane potential from – 60 mv to – 40 mv. This slope in the potential is called the prepotential and at this level, the threshold level of firing is usually attained. The long lasting Ca^{++} channels open and give rise to the upstroke, causing the membrane potential to reach +20 mv. This is the action potential or the impulse. The repolarization begins after the calcium channels close. The potassium channels open (rectifier K^+ channels) and repolarization occurs. In this way, the cardiac impulse originates on its own from the pacemaker. The extrinsic autonomic nerve stimulation can modify the rate of firing of the pacemaker. From the pacemaker, the excitation spreads through a specialized conducting system of the heart.

Conducting system of the heart (Fig. 7.9)

SA node

From SA node, impulses travel through three internodal tracts, namely anterior, middle and posterior before reaching the AV node. Excitation in atria spreads in a circular fashion. The right atrial excitation occurs earlier than the left atrium. Atrial excitation is completed in 0. 1 sec. The conduction velocity in the SA node is 0.05 m/sec.

AV node

Atrioventricular node is present at the right posterior interatrial septum. The AV node is also like SA node, which shows slower conduction. The velocity of conduction in the AV node is also 0.05 meters/sec and both the SA and AV node are called slow fibers.

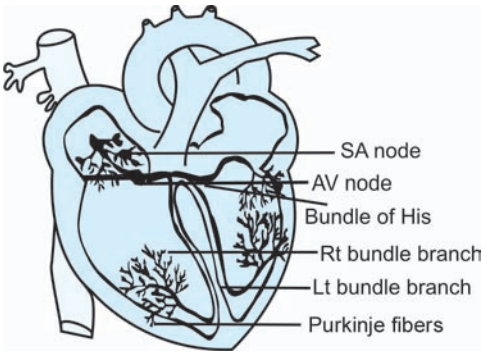


Fig. 7.9: Conducting system of heart

The delayed conduction in the AV node is called nodal delay. It lasts for 0.1 sec. This ensures completion of ventricular filling. The delay is shortened by stimulation of sympathetic nerve and lengthened by vagal stimulation.

Ventricular excitation

Bundle of His

The AV node gives rise to bundle of His, which has right and left bundle branches. They proceed on each side of the interventricular septum to their respective ventricles. The bundle of His shows conduction velocity of 1 meter/sec. If there is a conduction block in any one of the branches, the depolarization of the ventricle on that side of the block is delayed.

Purkinje fibers

The branches of bundle of His end in the Purkinje fibers which are fast fibers. The conduction velocity in this tissue is high. It is 4 meters/sec. It arborizes extensively in the ventricular myocardium.

Myocardium

The ventricular myocardium shows excitation of endocardium first and then epicardium. The depolarization of the ventricle starts with the excitation spreading from left to right, in the interventricular septum. The excitation now spreads to the apex of the interventricular septum. From the apex of the septum, excitation moves to the ventricular wall, spreading from endocardium to epicardium (Fig. 7.10). The depolarization now reaches the AV groove and the regions to be excited last are, posterobasal portion of left ventricle, pulmonary conus and upper part of the septum.

ELECTROCARDIOGRAM

Electrocardiogram (ECG) deals with the study of electrical activity of the heart. The instrument used to record the activity is called electrocardiograph. It was developed by a Dutch physiologist Einthoven in the year 1903. The recording was known as electrokardiogram (EKG). Both ECG and EKG are valid terms that can be used for the recording.

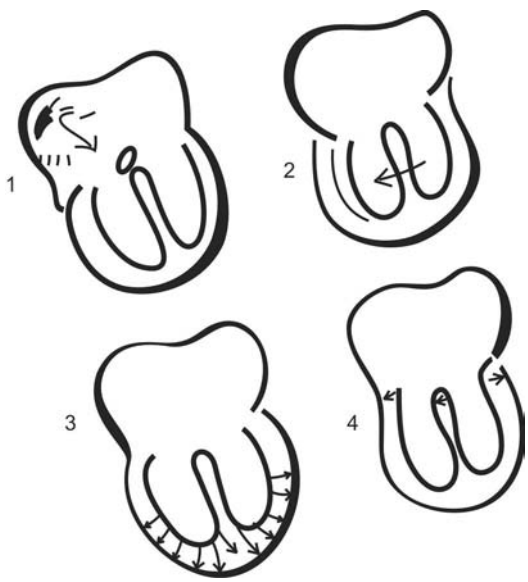


Fig. 7.10: Spread of electrical excitation in the heart

1. Excitation from SA node to AV node
2. Septal activation from left to right
3. Spread of excitation from endocardium to epicardium
4. Late activation of posterobasal portion of left ventricle and pulmonary conus

Objectives of ECG

The study of ECG, tells us the heart rate, rhythm, conduction in the heart and presence of any abnormalities in them known as arrhythmias. It is also useful to know the presence of infarction in the myocardium and the effect of drugs, electrolytes on the heart.

The ECG waves represent the sum total of tiny action potentials developed from the cardiac muscle. The electrical activity is spread to the surface of the body through the body fluid, which acts as a volume conductor. These electrical potentials from the surface of the body can be recorded by placing surface electrodes or leads, on certain conventional positions in the body. They are amplified and connected to a string galvanometer, which records them on a moving strip of paper or displayed on the screen in cathode ray oscilloscope.

Methods of recording

There are 12 leads used in the recording of ECG. Einthoven recorded the electrical activity of the heart by using bipolar limb leads. He considered right arm, left arm and left leg as the regions for surface recording and showed that, when these points are joined, an equilateral triangle could be obtained. In the center of this triangle, the heart is situated. The equilateral triangle obtained by this method is called Einthoven's triangle (Fig. 7.11).

The bipolar limb leads record the potential difference between two limbs. Accordingly, there are three types of leads present. They are (Fig. 7.12):

Lead I (between right arm and left arm)
Lead II (between right arm and left leg)
Lead III (between left arm and left leg).

In the bipolar limb leads, if we know the potentials in any two leads, the potential in the third lead can be determined. According to Einthoven's law, the sum of the potentials in lead

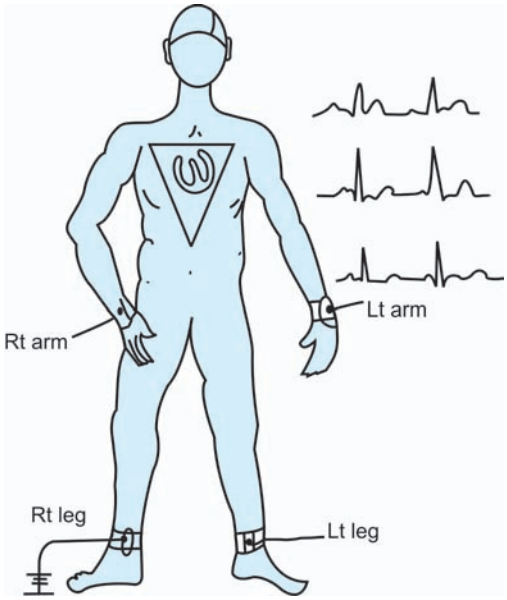


Fig. 7.11: Diagram showing the recording of ECG by using Einthoven's bipolar limb electrodes

I and lead III will be equal to the potential in lead II.

Lead I + Lead III = Lead II

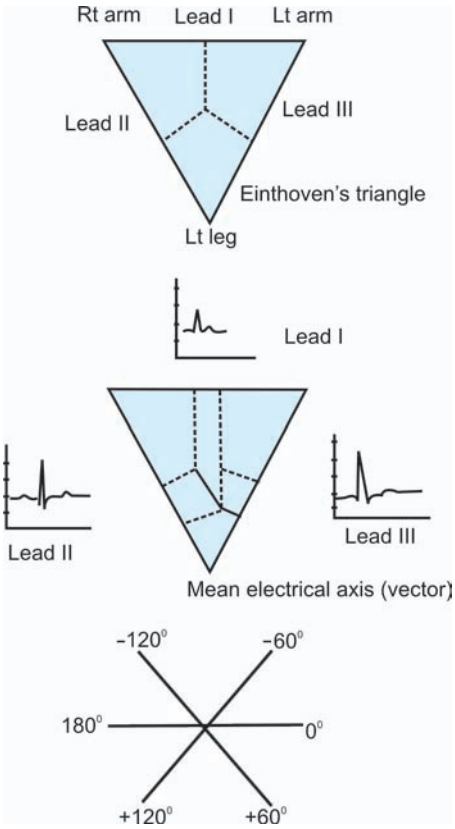
Unipolar augmented limb leads

In this method, there is an indifferent electrode (V), which is obtained by connecting the three limb leads and passing through 5000 ohms resistance to get 0 potential (Wilson's terminal). Recording between one limb and the other two limbs increases the size of the potential by 50%. The two limbs are connected through electrical resistance to the negative terminal and the other limb is connected to the positive terminal. There are three types of leads such as aVR, aVL and aVF present in this category (Fig. 7.13).

Precordial chest leads (Fig. 7.13)

There is an indifferent electrode (V) and exploring electrode is placed on the anterior chest wall in six positions. They are given numerical numbers from 1 to 6. The leads are $\mathbf{V_1}$, $\mathbf{V_2}$, $\mathbf{V_3}$, $\mathbf{V_4}$, $\mathbf{V_5}$ and $\mathbf{V_6}$.

In ECG recording, positive deflection is recorded, when the wave of excitation moves



Axial system to show the direction of the mean electrical axis. Normal range is –30° to +110°

Fig. 7.12: Bipolar limb leads and mean electrical axis determination

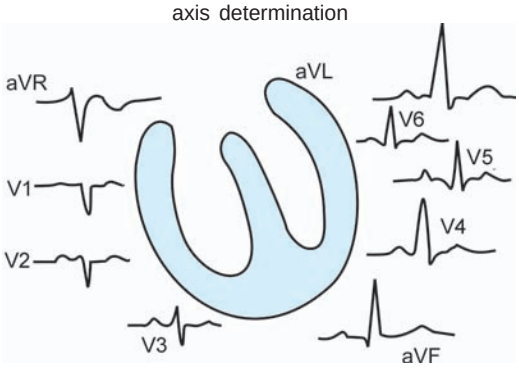


Fig. 7.13: Recording of unipolar augmented limb leads and precordial chest leads

Note aVR lead facing the cavity of the ventricle and hence the negative waves in ECG are recorded. In precordial chest leads, V1 and V2 records the right ventricular activity, while V5 and V6 records left ventricular activity. The changes in the R and S waves are due to these reasons

towards the positive or exploring electrode. If the depolarization wave moves away from the exploring electrode, a negative deflection is recorded. In **aVR** lead, the exploring electrode is facing the cavity of the ventricles and the wave of excitation moves away from the recording electrode and hence in this lead, all the deflections of ECG are negative.

ECG waves

A normal recording of ECG shows P, QRS, and T waves (Fig. 7.14). Sometimes a U wave is also recorded.

In the recording strip of paper, there are horizontal and vertical lines forming squares. There is a thick horizontal and vertical lines after every five small squares. The height of each such division gives 0.5 mv and in the horizontal direction, it gives duration of 0.2 sec. From this, it is possible to determine the magnitude of each wave and also its duration. The speed of the moving paper is 25 mm/sec (Fig. 7.15). Heart rate can be calculated from R-R intervals of two successive cardiac cycles.

$$\text{Heart rate/min} = \frac{60}{\text{R-R interval in sec}}$$

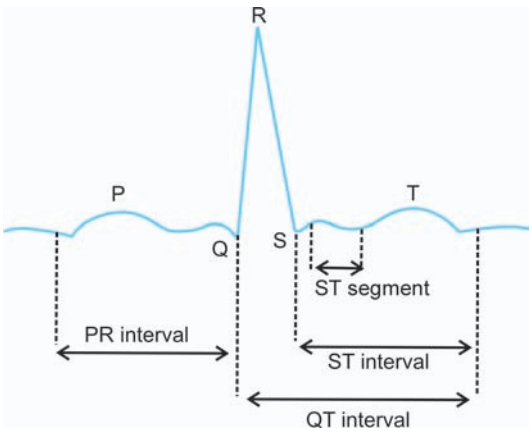


Fig. 7.14: ECG waves showing segments and time intervals

P wave

It is a positive wave except in aVR lead. It is recorded due to the depolarisation of atria. The duration of P wave is 0.1 sec. Its amplitude is 0.25 mv. P wave, when inverted, shows that the impulses originate from regions other than the pacemaker.

QRS

QRS is the ventricular complex and is caused by ventricular depolarization. The Q wave is negative and comes from the septal depolarization. The R wave is tall and positive and is due to depolarization of the ventricular wall. The S wave, is recorded as a result of depolarization of the posterior part of the ventricle and pulmonary conus, which are the last regions to be depolarized. The duration of QRS complex is 0.08 to 0.1 sec.

In precordial chest leads, V1 and V2 reflect the right ventricular activity and hence the S wave is tall and R wave is small. In leads V5 and V6, the exploring electrode is facing the left ventricle and it gives a tall R wave and a small S wave.

T wave

The repolarization of ventricle gives T wave. It is a positive wave and its duration 0.12 to 0.16 sec. The atrial repolarization is not recorded as it is obscured within the QRS complex.

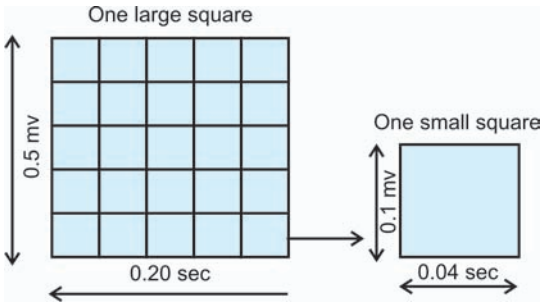


Fig. 7.15: Coordinates of the standard ECG paper used

U wave

Sometimes a positive U wave is recorded, which is due to the slow repolarization of the papillary muscle.

PR interval

It is the duration between the beginning of P wave to the beginning of R wave (Fig. 7.14). It is about 0.12 to 0.16 sec. It should not exceed 0.2 sec. PR interval indicates the time taken for the impulse to travel from atria to the ventricle. The time taken for the impulses to travel through AV node and its branches. Any delayed conduction or block in the AV node or Bundle of His will give prolonged PR interval and it is an indication of first degree heart block. Examples are excessive vagal stimulation, quinidine administration and diseases affecting the heart.

QT interval

This is the duration between the beginning Q wave and end of T wave (Fig. 7.14). It is around 0.4 sec. QT interval indicates electrical systole. The duration has an inverse relationship with the heart rate.

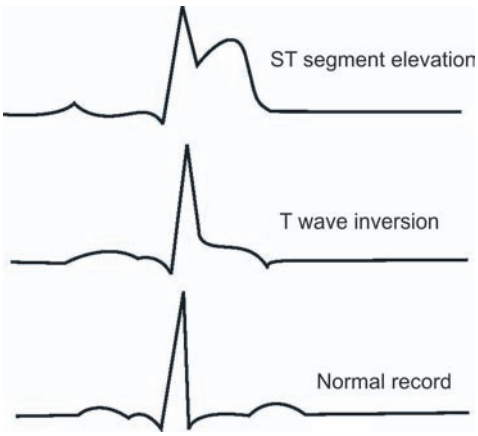


Fig. 7.16: ECG recording to show ST segment elevation in myocardial infarction. In acute stage, the elevation of ST segment occurs in the lead that is facing the infarcted region. A few days to weeks, after the acute stage, T wave inversion occurs in the same lead. Normal ECG can be recorded after complete recovery

ST segment

It is present between the end of S wave and the beginning of T wave (Fig. 7.16). It is isoelectric in the recording. If the ST segment deviates above the isoelectric line, it indicates the presence of myocardial infarction. The deviation of the ST segment will occur in the lead where the exploring electrode is facing the infarction.

J point

It is the point in the ECG recording, present at the junction between QRS complex and ST segment (Fig. 7.17). The J point signifies, the exact time at which the depolarization has been completed in the ventricles. The J point can be taken as a reference point to determine the deviation of ST segment in myocardial infarction.

Mean electrical axis

The electromotive force developed in the heart during its activity is represented by an axis, which shows a definite direction and magnitude. In Einthoven's triangle, a cardiac dipole is present at the center and it represents the mean electrical axis. It can be determined by knowing the amplitude of any two standard bipolar limb leads and drawing perpendiculars from the height of

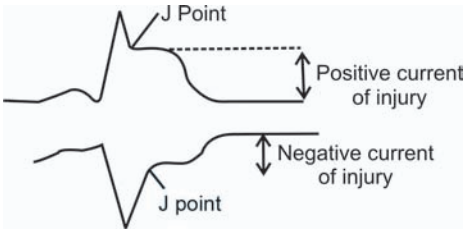


Fig. 7.17: J point in ECG helps to detect the current of injury in myocardial infarction. J point is isoelectric and signifies the end of depolarization of ventricle. In myocardial infarction, the ST segment is elevated due to current of injury. From J point it is possible to identify the current of injury whether is positive or negative. From this we can know whether the infarction is in the anterior(negative current of injury) or posterior wall (positive current of injury) of the heart

R waves (Fig. 7.13). The point of intersection of the lines will give cardiac vector or mean electrical axis. The axis, changes its direction during cardiac activity and it moves from – 30 degrees to + 110 degrees. If the axis moves to the left of – 30 degrees, it indicates left axis deviation, which is seen in left ventricular hypertrophy. If the axis moves to the right of +110 degrees, it shows the presence of right axis deviation and it occurs in right ventricular hypertrophy.

Vectorcardiography

In this, the instantaneous electrical axis is considered instead of the mean electrical axis. In the instantaneous electrical axis, the vector points developed during cardiac cycle are joined to give three loops, one each for atrial depolarization, ventricular depolarization (QRS) and ventricular repolarisation (T). The vector cardiogram is recorded in three planes namely frontal, horizontal and sagittal planes using limb leads and precordial chest leads.

Conduction defects (Fig. 7.18)

Conduction defects are observed at the AV node and bundle branches, due to ischaemia of the region. The nodal block is of three types. In the first degree heart block, there is delayed conduction in the AV node, which gives a prolonged PR interval. In the second degree heart block, the ventricle beats for every 2 or 3 atrial contraction. In the third degree heart block, the ventricular contraction is completely dissociated from the atrial contraction. The failure of ventricular beats may lead to lack of cerebral blood flow and hence fainting occurs. This condition is called Stokes-Adams syndrome.

Arrhythmias of the heart

This refers to the altered rate, rhythm and conduction of the heart. The causes of arrhythmia are:

- Re-entry phenomenon
- Presence of ectopic focus
- Multiple ectopic foci.

The re-entry is due to cardiac impulse re-exciting the area of the myocardium that has been already depolarized. This occurs when parallel pathways of depolarization having different conduction velocities and refractory periods are present (Fig. 7.19). Re-entry phenomenon is responsible for the occurrence of paroxysmal atrial tachycardia, atrial flutter, fibrillation, ventricular tachycardia, extrasystoles and ventricular fibrillation. Premature contraction or extrasystole of the ventricle can occur when there is presence of an ectopic focus especially, if it falls within the vulnerable period, which is the mid point of T wave (Fig. 7.20).

Myocardial infarction

It leads to the damage of the myocardium, which gives current of injury. That is, the current flows from the infarcted region to the healthy tissue. This causes ECG changes, giving elevation of ST segment in the lead that is facing the infarcted region (Fig. 7.16). The lead in the opposite side of the infarction will give depression of ST segment. During recovery from infarction, the lead which showed ST segment elevation, will give T wave inversion. This in the course of time disappears and a normal ECG pattern can be recorded.

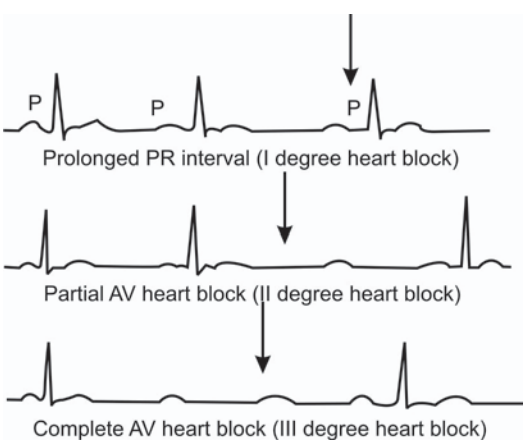
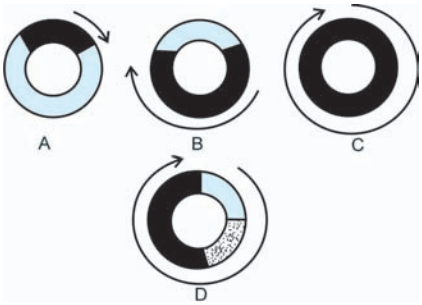


Fig. 7.18: ECG recordings to show conduction defects in the heart



Figs 7.19A to D: Diagrammatic representation of circus movement to explain arrhythmias of the heart

In the conduction pathway, if a part of the region becomes ischaemic, it shows a different refractory period and conduction velocity than the healthy tissue. Hence, the impulse when it comes to the infarcted region, finds it refractory. By the time the impulse comes around, the tissue becomes excitable and the excitation spreads over and again in a circus movement.

A. Circus movement in a strip of conducting pathway
B. Pathway taken by the impulse to form a circle
C. In a normal tissue, the excitation is extinguished when the two point of excitation meets
D. In conduction block, the re-entry of the excitation through the blocked region occurs,

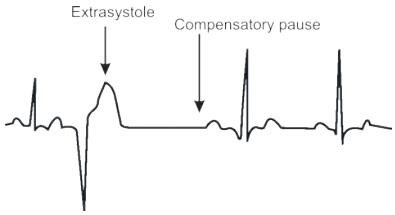


Fig. 7.20: Extrasystole from the ventricle. The premature beat is followed by compensatory pause

CARDIAC CYCLE

The electrical activity of the heart is followed by mechanical activity, which consists of contraction (systole) and relaxation (diastole). The mechanical activity shows pressure, volume changes and associated with this, there are production of heart sounds (Fig. 7.21). The mechanical events are repeated in a cyclical fashion and each cycle lasts for 0.8 sec, for the heart rate of 75/minute. Increase in heart rate (tachycardia) decreases the duration of cycle.

Each cycle has ventricular **systole** and **diastole**. The systole lasting for 0.3 sec and

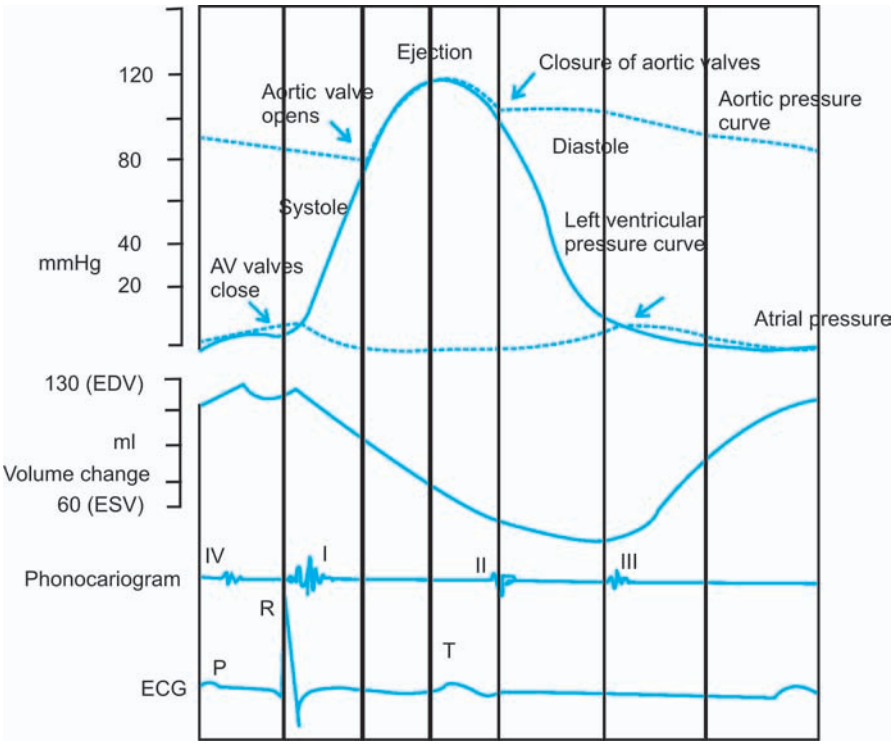


Fig. 7.21: Pressure and volume changes during cardiac cycle

diastole for 0.5 sec. The atrial systole (0.1 sec) is included in the ventricular diastole, as it occurs at the end of this phase. The atrial diastole (0.7 sec) is not considered, as it overlaps ventricular systole and diastole.

Events in ventricular systole

Systole begins with the closure of AV valves. The ventricle is a closed chamber and the contraction of ventricle becomes isometric,as the tension rises without change in volume. This **isovolumetric contraction** lasts for 0.05 sec. The increase in pressure in the ventricle leads to the opening of semilunar valves. That is, in the case of left ventricle, the pressure rises from 20 mmHg to more than 80 mmHg, forcing the aortic valve to open. The right ventricular pressure rises from 0 mmHg to more than 10 mmHg, to open the pulmonary valves. The opening of semilunar valves results in ejection. The peak systolic ejection pressure in the left ventricle reaches 120 mmHg and in the right ventricle, it attains only 25 mmHg. The right ventricular ejection gives rise to lesser circulation and hence low pressure is developed in it. The duration of ventricular ejection lasts for 0.25 sec. At the beginning of systole, the closure of AV valves produces I **heart sound**.

Ventricular diastole

The ejection of blood into the aorta and pulmonary artery causes pressure rise in these vessels and fall of pressure in the ventricles. The fall in ventricular pressure, at the end of systole is called **protodiastole**. It has a duration of 0.04 sec. These pressure changes lead to closure of semilunar valves which gives the **II heart sound**. The ventricle is again a closed chamber, with the pressure falling as the muscle relaxes. It is a phase of **isovolumetric relaxation** and lasts for 0.08 sec. As the ventricular pressure is falling, the atrial pressure becomes relatively more. It forces the AV valves to open, leading to ventricular filling. At first, there is a greater pressure gradient and this gives **rapid filling** lasting up to 0.13 sec. This is followed by **reduced filling** (diastasis) of ventricles, as the pressure gradient is reduced. The duration of reduced filling or diastasis is 0.15 sec. The end of diastole coincides with atrial systole. The **atrial systole** lasts for 0.1 sec.

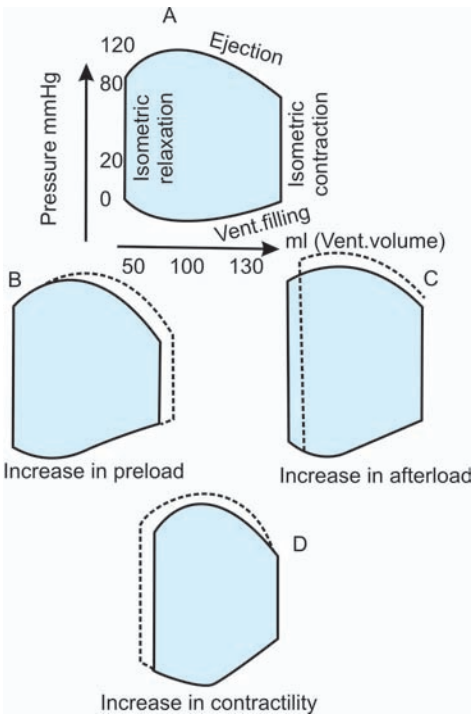
About 75% of ventricular filling occurs passively, due to fall in ventricular pressure. The atrial contraction acts as a booster pump to complete the remaining 25% of the filling. The atrial systole is not required for ventricular filling in normal heart rates. If the heart rate is higher, then the diastolic ventricular filling duration is reduced and in such cases, the atrial systolic contribution of ventricular filling becomes significant. The rapid filling phase of ventricular filling, gives the **III heart sound** and the atrial systole produces **IV heart sound**.

As said earlier, the rise in heart rate reduces the duration of cardiac cycle and fall in rate, increases the duration. For example, at 60/min rate, the duration of diastole is lengthened to 0.6 sec and the systolic duration remains same. If the rate is increased to 200/min, the duration of diastole is reduced to 0.14 sec and systole is also reduced, but not to the same extent as in diastole. It is reduced to 0.16 sec, giving the total duration of cycle 0.3 - sec.

Pressure-volume relationship in cardiac cycle

The correlation of pressure and volume during cardiac cycle gives a loop, when the graph is plotted (Fig. 7.22). The loop starts with the beginning of ventricular filling. The volume of blood in the ventricle after the ejection phase is 60 ml and it is called end systolic volume. The ventricular filling during diastole increases the volume to 130 ml, with only a small rise in pressure. The volume of blood at the end of diastole is called **end diastolic volume**.

The systole begins, with the isovolumetric contraction phase and the pressure rises. The semilunar valves open leading to ejection. The volume reduces, but in rapid ejection phase, the peak systolic contraction rises the pressure steeply. In reduced ejection, the pressure and



Figs 7.22A to D: Pressure–volume relationship in the left ventricle. Normal working heart, A. Increase in preload, B. Increase in after load, C. Increase in contractility, D

In B, the increase in pre load causes rise in diastolic ventricular volume. In C, the increase in after load causes, rise in ventricular pressure, but less blood ejection. In D, the increase in myocardial contractility (positive inotropic effect) produces greater ventricular pressure and ejection, which reduces the ventricular volume

volume both decrease. The volume of blood ejected per beat is called stroke volume, which is 70 ml. The ventricular relaxation begins with the isovolumetric relaxation, causing a sharp drop in the ventricular pressure. The end of the fall in pressure joins with the point of ventricular filling in the loop. The pattern of loop will be altered with changes in preload and afterload affecting the heart (Fig. 7.22). Increase in preload (end diastolic volume) to the heart shows the increase in the length of the filling phase of the loop. There is a larger stroke volume, but the peak systolic pressure does not change, as the afterload to the heart is not changed. When afterload (aortic pressure) is increased with constant preload, the peak systolic pressure is increased, but only less volume of blood is ejected. This causes increase in end systolic volume.

Ejection fraction

It is the ratio of the volume of blood ejected from the left ventricle per beat (stroke volume) to the volume of blood remaining in the ventricle at the end of diastole. It can also be determined as the percentage of end diastolic volume, ejected per beat. It is about 65%. Ejection fraction is a useful index of assessing ventricular function and its contractile state. Ejection fraction is increased in muscular exercise and sympathetic stimulation.

Timing of events

The events on the two sides of the heart are similar, but there are time differences in their occurrence. The right atrial contraction begins earlier than left atrium. The left ventricular contraction starts earlier to right ventricle, but the right ventricular ejection begins earlier than left ventricle. The pulmonary valve closes later than the aortic valve during inspiration, as the venous return is increased.

Location	Pressure (mmHg)	
Left ventricle:	systolic	120
	diastolic	80
at the beginning of diastole	:	< 20
Right ventricle	systolic	25
	diastolic	10
at the beginning of diastole	:	0
Right atrium	:	0 - 4
Left atrium	:	4
Aorta	:	120/80
Pulmonary artery	:	25/10

HEART SOUNDS

The mechanical activity of the heart produces heart sounds (Fig. 7.21). Sounds are produced due to vibrations caused by the closure of valves, contraction of the myocardium and movement of blood from one cavity to another within the heart. The causes of heart sounds are either all of the factors mentioned or any one of them or combination of two factors.

Heart sounds are classified, physiologically as I II III IV types. Clinical classification restricts itself to only I and II heart sounds, because, both the sounds can be auscultated with the help of a stethoscope in all subjects. The four types of heart sounds can be graphically recorded by using a phonocardiograph. The third sound is heard in young subjects and athletes during muscular work. The fourth sound is usually not heard.

First sound

It is heard at the beginning of systole, when AV valves close. The AV valves are the tricuspid and bicuspid (mitral) valves. The valves do not open completely during ventricular filling. This is due to eddy currents developed by the blood from the wall of the ventricle, which makes it partially open (Fig. 7.23). When systole begins the AV valves are shut, producing vibrations and this gives the I heart sound. The intensity of I heart sound will be greater, if AV valves are wide open and also when there is increase in the force of ventricular contraction.

The quality of I sound shows that it is low pitched, longer duration, with crescendo and diminuendo character. The duration is 0.15 sec with the frequency of 25 to 45 Hz. The first sound is heard in both mitral and tricuspid areas, but it is better heard in the mitral area.

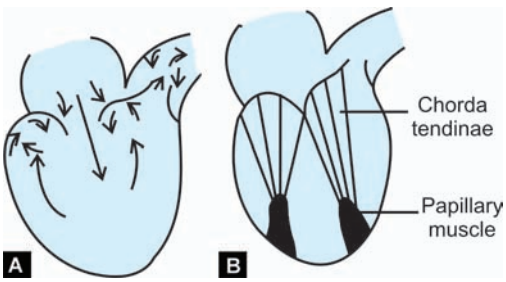
Second sound

The closure of semilunar valves at the end of systole coincides with the production of II heart sound. The semilunar valves are the aortic and pulmonary valves. These valves are also partially open, due to the eddy currents of blood developed from the wall of the vessels, which act on the valves (Fig. 7.23). The closure of these valves occurs at the end of ventricular systole and the vibrations caused by this gives the II sound. The II sound can be auscultated in aortic and pulmonary areas.

The II sound is shorter in duration (0.11sec) and high pitched. Its frequency is 50 Hz. The second sound will be louder, when the aortic or pulmonary pressure is elevated. Hence, in arterial and pulmonary hypertension, the II sound is heard louder. There is also physiological splitting of II sound, due to asynchronous closure of semilunar valves. This is observed, especially during inspiration, as the pulmonary valves close after the aortic valve closure.

Third sound

The third sound is not heard in all subjects. It can be heard in children. The duration of III sound is 0.1 sec. It is produced, due to the rushing of blood into the ventricle from atria during rapid filling phase of diastole. The rushing of blood creates vibrations in the wall of the ventricle walls, which causes the III sound to be produced. When the ventricle is distended too much, the III sound can be heard, as in cardiac patients.



Figs 7.23A and B: Diagram to show the eddy currents from the ventricle, which keep the valves partially open A.The intensity of the heart sound will be greater when the valves are wide open along with the increased force of contraction of ventricles. The diagram B shows the attachment of chorda tendinae to the valve cusps. The chordae tendinae attachment to the valve cusps does not help in the closure of valves, but prevents the valves being pushed into the atria during ventricular systole

Fourth sound

It is a low frequency oscillations seen in the phonocardiogram. It is produced, due to atrial systole. It is not usually heard. It is only graphically recorded.

Correlation of heart sounds with ECG

When heart sounds are correlated with ECG waves we can observe that the I heart sound is recorded following R wave of ECG.The II heart sound is recorded immediately after T wave of ECG (Fig. 7.24).

Murmurs

Murmurs are abnormal sounds, which can be heard in various parts of the vascular system when blood flow becomes turbulent. Murmur can occur whenever the laminar blood flow is changed to turbulent flow. The turbulent flow can result from narrowing of valves or blood vessels. Cardiac murmurs occur due to diseases of the valves. The valvular lesions can occur in rheumatic fever resulting from autoimmune disease.

In stenosis, the valves are narrowed and the turbulent blood flow through the narrowed orifice produces murmurs. In valvular incompetency, the valves do not close tightly and the blood flows backwards(regurgitation) through the narrow orifice and produce murmurs due to turbulence. Systolic murmurs can be heard in stenosis of aortic and pulmonary valves and insufficiency of AV valves. Diastolic murmurs can be heard in insufficiency of aortic and pulmonary valves and stenosis of AV valves.

The murmurs can also be heard, due to changes in velocity of blood flow. Example of this type is anemia (systolic murmur). Murmurs are also heard over aneurysmal dilatation of artery and in patent ductus arteriosus. Clinically, the functions of valves, ventricular wall and septum are assessed by echocardiography combined with Doppler technique.

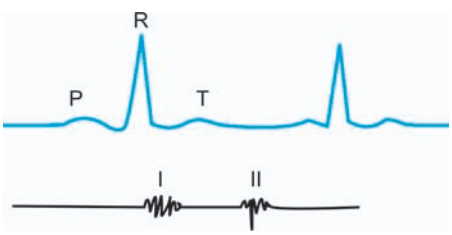


Fig. 7.24: Diagram to show the correlation between ECG and heart sounds

CARDIAC OUTPUT

The out put of each ventricle per minute is called minute volume or cardiac out put. It is about 5 lit. The amount of blood pumped per stroke or beat is known as stroke volume. It is 70 ml. The cardiac output can be calculated by multiplying pulse rate with stroke volume.

Pulse rate × stroke volume = cardiac output.

If cardiac output is expressed in relation to surface area of the body, then it is called cardiac index. It is 3.2 lit/min/sq. meter of surface area.

During sleep, the output does not change.

Cardiac output is increased in:
Muscular exercise
Emotional excitement
Sympathetic stimulation
Pregnancy
During digestion
Increased environmental temperature.

Decrease in output is observed in:
Hemorrhage
Myocardial infarction
Heart failure
Heart block

Regulation of cardiac output

The output is controlled by factors like preload to the heart, inotropic effect, chronotropic effect, and afterload to the heart.

Preload to the heart

It is the end diastolic volume of the heart. This depends on the venous return to the heart. Greater the venous return, greater will be the end diastolic volume. This stretches the muscle fiber and increases the force of contraction (Starling's law of the heart). It states that the force of contraction is directly related to the initial length of the muscle fiber within physiological limits. This type of regulation of output, which depends on the fiber length, is called heterometric regulation.

The relationship between ventricular stroke volume and end diastolic volume can be studied in Frank Starling curve.

Factors influencing venous return (preload)

The venous return to the heart is due to the pressure transmitted to the venous side from the left ventricular contraction. The rise in venous pressure increases the venous return, which inturn increases the cardiac output (Figs 7.25 and 7.26). If the heart fails to contract, the rise in venous pressure will decrease the venous return and reduce the cardiac output. The vascular curves shown in Figure 7.28 tell us this relationship. We can also learn from the vascular function curve, that decrease in peripheral resistance will increase the venous return for the same venous pressure, whereas, increase in resistance, will decrease the venous return. When vascular function curve and the cardiac out put are correlated, it will be seen that at 4 mm of Hg venous pressure, the cardiac output will be 5 liters per minute. The Figure 7.26 shows this relationship and also the effect of change of venous pressure on cardiac output.

In the vascular function curves, it can be seen that the curve is shifted to left and above in sympathetic stimulation and rise in blood volume. (Fig. 7.27).

Starling's cardiac function curves show that sympathetic stimulation, circulating catecholamines, digitalis, shift the curve above and to the left, whereas, hypoeffective heart, hypoxia, high

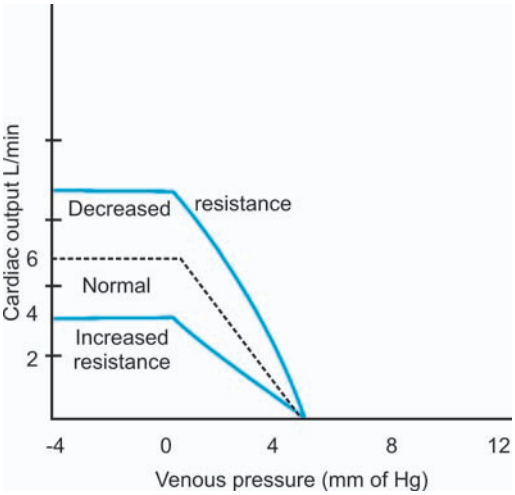


Fig. 7.25: Relationship between venous pressure and cardiac output

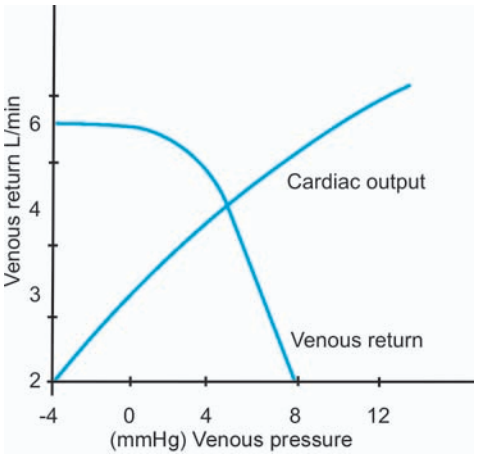


Fig. 7.26: Correlation of venous pressure and venous return. The point of intersection between venous return and cardiac output gives normal working values (At 4 mmHg venous pressure, the venous return is 5 L/ min)

venous pressure, shift the curve to the right and below (Fig. 7.28).

Determinants of venous return

Next to venous pressure, the right ventricular contraction causes, forward movement of venous return, as the base of the heart moves downwards

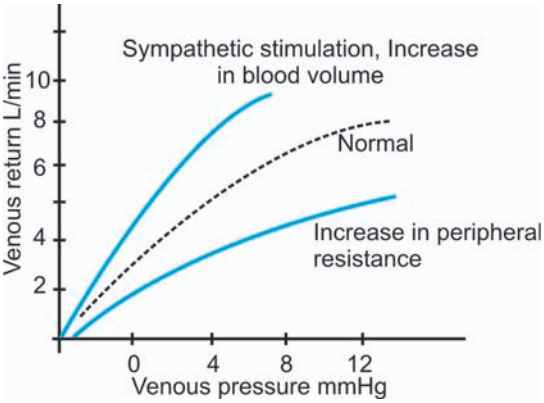


Fig. 7.27: Vascular and cardiac function curves in sympathetic stimulation, increase in blood volume and increase in aortic resistance compared with normal

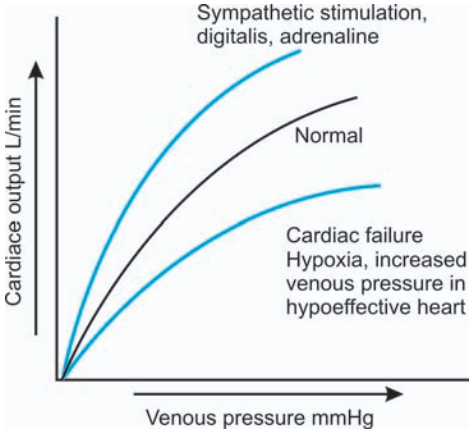


Fig. 7.28: Starling's cardiac function curves to show how positive inotropic effect on heart shifts the curve to the left and above, while the hypoeffective heart shifts the curve to the right and below

during ventricular systole. This lowers the pressure in the right atrium and facilitates venous return. Negative intrathoracic pressure gives more pressure gradient and hence more venous return. The skeletal muscle pump also augments venous return. The presence of valves in the limb veins prevents backward flow of blood, which helps venous return. Finally, the venomotor tone by the sympathetics also helps venous blood to move towards the heart.

Factors influencing preload to the heart

Left ventricular contraction
Right ventricular contraction
Negative intrathoracic pressure
Skeletal muscle pump
Valves in veins
Veno motor tone

Inotropic and chronotropic effects

The cardiac output regulation, which is independent of muscle fiber length, is called homometric regulation.

Increase in force and rate of contraction by sympathetic stimulation, increases the cardiac output. In **Frank-Starling cardiac function curve**, the sympathetic stimulation, shifts the curve above and left (Fig. 7.28). Similar effect is also seen with circulating catecholamines, digitalis and other positive inotropic agents. Hypoxia, hypercapnia, heart failure shift the Starling's curve downwards and to the right. Increase in heart rate, increases the cardiac output, if the increase in rate is upto 180/ min. Rise in heart rate after this, will reduce the ventricular filling and this leads to decrease in cardiac output.

Afterload to the heart

The aortic resistance is the after load to the heart and is related to the peripheral resistance. Increase in total peripheral resistance will increase the aortic resistance, which inturn rises the afterload to the heart. The left ventricular contractility has to overcome the afterload and pump the blood. The rise in peripheral resistance lowers peripheral run off from arterioles. This reduces the venous return and hence decreases the cardiac output. However, the increase in arterial blood volume increases the mean arterial blood pressure.

Determination of cardiac output

The cardiac output is determined by methods like echocardiography, direct Fick method and

indicator dilution technique. There are also other methods like thermal dilution, ballistocardiography, but they are not popular, as the results are not accurate.

Fick method

In this method, the pulmonary blood flow is measured, which is equal to the cardiac output, as the entire right ventricular output goes to the lungs. Blood flow is calculated by knowing the amount of substance extracted divided by the arterio venous difference in the concentration of the substance. The substance used is oxygen and the precautions that are taken are:

- The determination of oxygen consumption should be under basal conditions
- The venous sample should be from the mixed venous blood. This is obtained from the pulmonary artery, by introducing intra cardiac catheter.

$$\text{Blood flow} = \frac{O_2 \text{ utilization in ml/min}}{(A-V)O_2} \times 100$$

$$= \frac{250 \text{ ml/min}}{(20-15)} \times 100, \; = 5000 \text{ ml/min}$$

The disadvantage in this method is the collection of mixed venous blood from pulmonary artery, which involves intracardiac catheterization. Since this has to be carried out in conscious subjects, the anxiety and emotional excitement that accompany this procedure would lead to enhancement of cardiac output. Hence, the normal level cannot be determined by this method.

Indicator dilution technique

It is a popular method as the results are accurate and the procedure is simple. In this method a dye is used as an indicator. Dyes like cardio green, Evan's blue are used. A known amount of dye is taken and it is injected into the arm vein. The dye enters the circulation. From the other arm

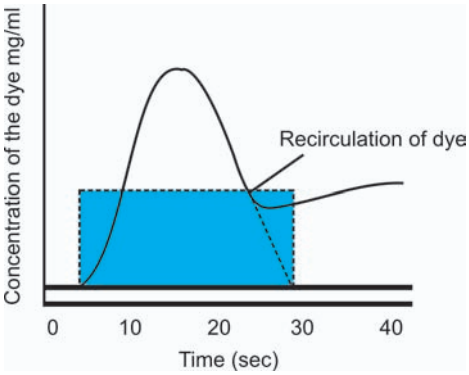


Fig. 7.29: Indicator dilution curve to determine cardiac output. The downslope of the curve is drawn to zero point to find out the concentration of the dye before recirculation and the duration also can be known from the graph after extrapolation. The rectangular area represents the concentration of the dye in the circulation for the duration before its recirculation

successive arterial samples are taken and in each sample the dye concentration is determined. A graph is plotted with concentration of the dye against time. The concentration at first increases then declines, to be followed by a second rise. The second rise in the concentration is due to the recirculation of dye. The graph is extrapolated to zero point to find out the mean concentration (Fig. 7.29).The cardiac output is determined by using the formula:

$$\text{Cardiac output} = \frac{I}{C \times t} \times 60$$

I = amount of dye injected
c = mean concentration of the dye
t = time in seconds

Distribution of cardiac output

The entire right ventricular output goes to the lungs. The left ventricle ejects blood into the aorta. From aorta, blood is distributed to various organs. There are local factors, which determine the blood flow to these organs. Table 7.1 gives distribution of cardiac output to some of the organs.

Table 7.1: Distribution of blood flow to various organs under basal conditions

Organ	Blood flow ml per minute	% of cardiac output
Heart	250	5
Kidney	1200	25
Brain	750	15
Liver	1300	27
Skin	350	7
Skeletal muscle	750	15
Other organs	300	6

REGULATION OF HEART AND BLOOD VESSELS

The maintenance of circulation depends on the control of the activity of the heart and blood vessels. It is the overall regulation of both heart and blood vessels, which ensures adequate blood flow to various organs. Hence, the best way to understand the cardiovascular dynamics is to discuss the regulations of the heart and blood vessels together.

Neural control of heart

Cardiac center

The cardiac center, is situated in the medullary reticular formation in the dorsal motor nucleus and nucleus ambiguus, which give rise to vagus (Fig. 7.30). The vagus is the parasympathetic nerve, which causes cardio inhibition. Hence, the cardiac center is also called as cardio inhibitory center.

Innervation of heart

The heart is innervated by autonomic nerves. The parasympathetic supply is by vagus and the sympathetics arise from upper thoracic segments of the spinal cord. The postganglionic sympathetic fibers supply the heart through superior, middle and inferior cardiac nerves. The sympathetic supply is more pronounced in the ventricles. Sympathetic stimulation increases the force and rate of contraction of heart. The effect is mediated by β_1-adrenergic receptors present in the myocardium.

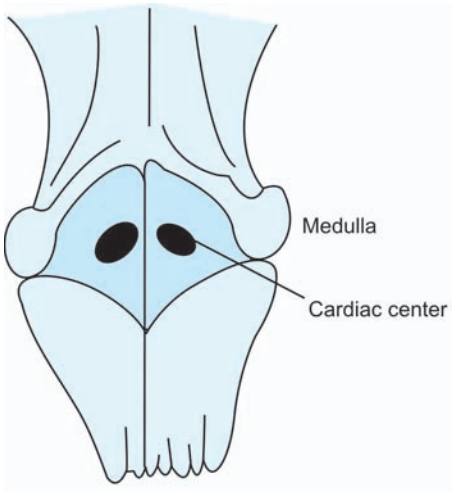


Fig. 7.30: Diagram to show the location of cardiac center

Parasympathetic innervation

The right vagus supplies the SA node, while the left vagus go to AV node. The postganglionic endings of vagus release acetylcholine and the cardiac inhibition is mediated through this transmitter. It causes hyperpolarization of the membrane by increasing the permeability for K^+. There is also decreased Ca^{++} entry as the cyclic AMP formation is reduced. All these effects of acetylcholine are produced through muscarinic receptors (M_2) and they are blocked by atropine.

Both sympathetic and parasympathetic show tonic discharge, with vagus giving a greater tonic discharge which is known as vagal tone. The predominance of vagal tone can be demonstrated by bilateral vagal sectioning or administration of atropine, which results in tachycardia.

Reciprocal relationship

There is a reciprocal relationship between sympathetics and vagus in their action on heart. When one system is stimulated the other is inhibited. The sympathetic stimulation on heart inhibts vagal activity by the release of neuropeptide Y from the sympathetic post-ganglionic endings. The neurotransmitter Y is a

co transmitter in the sympathetic endings on the heart. The neurotransmitter Y is believed to inhibit the release of acetylcholine from vagal ending.

Cardiac reflexes

There are reflexes which regulate the heart's action and they are known as cardiac reflexes (Fig. 7.31). Most of the cardiac reflexes, in addition to controlling the activity of heart, also regulate the activity of blood vessels, which help to maintain arterial blood pressure.

Bainbridge reflex

Bainbridge (1915) demonstrated that increase in venous return increases the heart rate, provided the initial heart rate is not high. The reflex is produced by stretching the right atrium and the nerve which mediates the reflex is vagus. The Bainbridge reflex is an inconstant phenomenon.

Reflex from atria

The right atrial wall contains receptors, which respond to both systole and diastole. The receptors responding to atrial systole is called type A and those responding to atrial diastole are type B. The stimulation of both the types, will cause increase in heart rate, vasodilatation and fall in blood pressure.

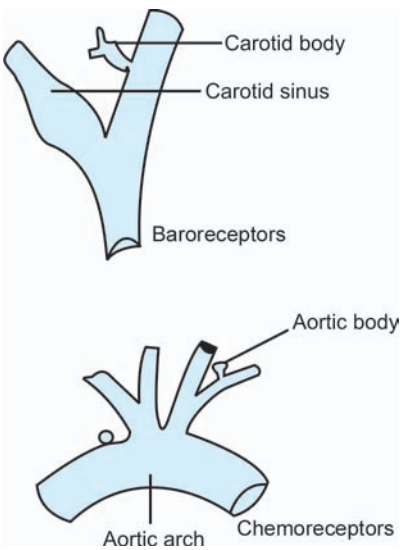


Fig. 7.31: Diagram to show the location of baroreceptors and chemoreceptors

The left atrial wall also contains stretch receptors, which respond to increase in ECF volume. The stimulation of these receptors in the left atria causes inhibition of ADH, leading to diuresis. This helps to regulate ECF volume and blood pressure.

The rise in ECF volume will also stimulate the secretion of ANP (atrial natriuretic peptide) from both right atrium and left atrium. This peptide hormone acts in the kidney and causes increased excretion of sodium and water (natriuresis). It leads to correction of fluid volume, sodium level and blood pressure.

Reflex from left ventricle

The heart rate is decreased and blood pressure falls, when there is rise in left ventricular pressure. The receptors are stretch receptors and this reflex is mediated by vagus.

Coronary chemo reflex

Administration of chemicals like serotonin, veratridine and phenyl bi guanide into the left coronary artery results in bradycardia and fall in blood pressure. The reflex is mediated by C type fiber from vagus. The reflex is also known as Bezold Jarisch reflex. Similar effects are also observed when these chemicals are injected into the pulmonary artery. The receptors are situated in pulmonary capillaries (pulmonary chemo reflex).

Sinoaortic reflex

Sinoaortic reflex is the most important one, because the regulation of blood pressure and heart rate depends on the afferents in the sinus and vagus, which mediate the reflex. The receptors are the baroreceptors, situated in the carotid sinus and aortic arch (Fig. 7.31). The rise in arterial blood pressure stimulates these receptors. These stretch receptors, when stimulated, causes stimulation of cardio inhibitory center and

inhibition of vasomotor center. This leads to reflex bradycardia and fall in blood pressure. Reverse changes occur when arterial blood pressure falls.

Chemoreceptor reflex

The stimulation of peripheral chemoreceptors (Fig. 7.31) namely, carotid and aortic bodies by hypoxia, hypercapnia and acidosis, actually produces fall in heart rate, but there is also hyperpnea. The afferents from lungs causes inhibition of the medullary cardiac center and consequently, the heart rate is increased. There is also adrenal medullary catecholamines secre tion, which also causes tachycardia.

Reflex from periphery

The peripheral nerve stimulation, or stimulation of pain fibers causes rise in heart rate. Stimulation of pain fibers when prolonged will cause bradycardia.

Reflex from higher centers

Stimulation of higher centers like cerebral cortex, hypothalamus, limbic lobe, etc, causes either tachycardia or bradycardia depending on the nature of stimuli. For example, emotional excitement, anger, anticipation of muscular exercise, will cause rise in heart rate, while, grief and fear cause decrease in heart rate. The higher centers produce their effects through the medullary cardiac center.

ARTERIAL BLOOD PRESSURE

Blood pressure is the lateral pressure exerted on the wall of the vessels by the column of blood present in it. The maximum pressure which occurs during systole is called systolic pressure and the minimal pressure produced during diastole is called diastolic pressure. The difference between the two pressures is called the pulse pressure. The average of pressure produced during a cardiac cycle is known as the mean pressure. It is calculated by taking the diastolic pressure and adding one third of pulse pressure.

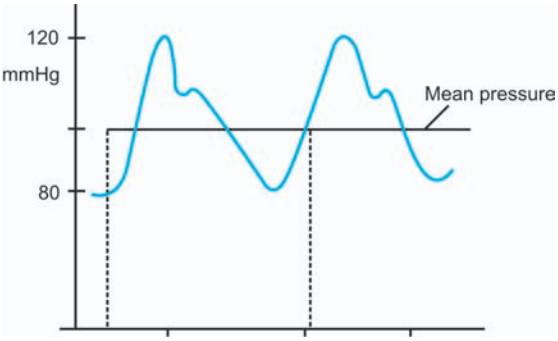


Fig. 7.32: Diagram to show the calculation of mean blood pressure from arterial pressure curve

In the graphical recording, it is calculated from the area occupied by the systolic and diastolic pressures and from it, the mean pressure is calibrated (Fig. 7.32).

Normal values

Systolic pressure ranges from 100 to 140 mmHg, with the average pressure being 120 mmHg in adults. The diastolic pressure ranges from 70 to 90 mmHg and 80 mmHg is the average pressure. The pulse pressure is the difference between the systolic and diastolic pressures and is 40 mmHg. The mean arterial blood pressure is 100 mmHg. Clinically, the diastolic pressure reading is considered important, as it indicates the resistance against which the left ventricle has to pump the blood. Rise in diastolic pressure above 90 mmHg if, occurs persistently, is an indication of hypertension.

Recording of arterial blood pressure

Indirect methods

The arterial blood pressure is recorded by indirect methods in humans. Experimentally, direct method of recording is possible by cutting the artery and inserting a canula, the other end of which is connected to a mercury manometer. In humans, the sphygmomanometer is used to record the arterial blood pressure.

Variations in values

Age

Blood pressure is low in infants and it gradually rises, as the age advances. In adults, the value of systolic pressure is 120 mmHg and the diastolic pressure is 80 mmHg. In old age, due to loss of elasticity (reduced compliance) of arteries, the blood pressure rises. The rise in systolic pressure is greater than the diastolic pressure.

Sex

Blood pressure in females is lower than males until menopause. The difference is due to the action of estrogen on the blood vessel walls. The value will become equal to males after menopause, when the estrogen secretion stops.

Posture

When posture is changed from recumbent to standing, the diastolic pressure raises due to baroreceptor mechanism. The change of posture from standing to recumbent will cause rise in systolic pressure.

Muscular exercise

There is rise in systolic pressure without any change in diastolic pressure in mild to moderate exercise,while strenuous exercise will cause fall in diastolic pressure. The reason is due to the fall in peripheral resistance caused by vasodilatation of arterioles of exercising skeletal muscles.

Emotional excitement

During emotional excitement, the systolic pressure is increased, due to sympathetic stimulation.

Digestion

The increase in cardiac output, which occurs during digestion, leads to rise in the systolic pressure.

Fall in blood pressure is seen in:

Hemorrhage

Fall in circulating blood volume (Circulatory shock).

Heart failure, myocardial infarction.

The blood pressure falls due to the failure of heart to pump blood in these conditions.

Maintenance of arterial blood pressure

The determinants of arterial blood pressure are the cardiac out put and the peripheral resistance. These two can be considered as the physiological factors, while the blood volume and the compliance of the arteries are taken as the physical factors determining the arterial blood pressure.

The systolic pressure is maintained by the cardiac output. This in turn depends upon the product of stroke volume and heart rate. The diastolic pressure is due to peripheral resistance present in the arterioles.

The mean pressure of 100 mmHg in adult depends on the balance between the amount of blood pumped into the aorta and the amount of blood leaving the arterioles (peripheral run off) (Fig. 7.33). Normally the amount of blood pumped

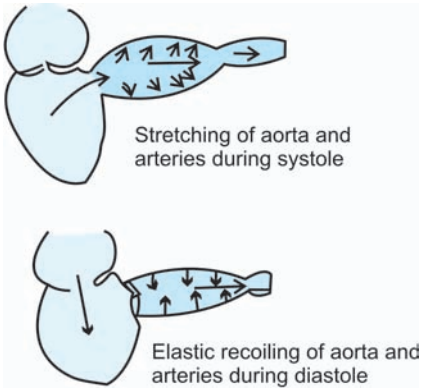


Fig. 7.33: Diagrammatic representation of how mean arterial blood pressure depends on the balance between the amount of blood that is pumped into the aorta and the amount that leaves the arterioles. Because of the elastic recoiling and peripheral resistance, the blood flow in the capillaries becomes continuous and not pulsatile

into the aorta in each beat is 70 ml. If there is a rise in stroke output, as in exercise, the amount of blood entering the arterial system increases, while the amount of blood leaving the arterioles remains the same. This will lead to stretching of arteries, which increases the systolic pressure. The rise in systolic pressure will give a greater pressure head for the extra volume of blood to leave the arterioles (Fig. 7.33).

If the amount of blood pumped into the arterial system falls, as in heart failure or in hemorrhagic shock, the mean pressure in the arterial system will decrease.

Decrease in arterial compliance (increased stiffness of arteries) will lead to rise in systolic pressure, which is much greater than the rise in diastolic pressure (Fig. 7.34).

Peripheral resistance

It is the resistance present in the arterioles due to the presence of thick smooth muscle in the wall. The resistance also causes sharp decline in the mean blood pressure in the arterioles. This peripheral vessel offers resistance to the flow of blood leaving the arterial system. It indicates the resistance against which the left ventricle has to contract. This resistance will also give afterload to the heart. It is determined as follows.

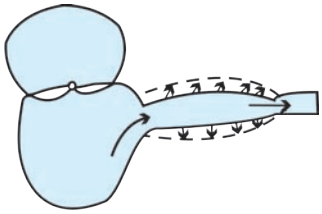
$$\text{Total peripheral resistance} = \frac{\text{blood pressure}}{\text{cardiac output}}$$

$$= \frac{100 \text{ mmHg}}{100 \text{ ml/sec}}$$

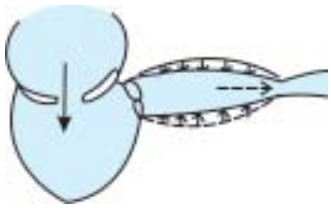
$$= 1 \text{ unit}$$

Total peripheral resistance (R) is also expressed in units. Its normal value is 1 unit. The value increases in arterial hypertension.

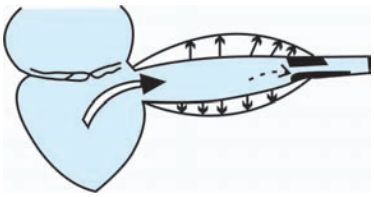
Rise in peripheral resistance reduces the peripheral run off and this increases the arterial blood volume. The balance between the blood pumped into the aorta and the amount leaving the arterioles cannot be maintained. To maintain the balance, the mean pressure in the arterial system should be increased (Fig. 7.34). There is rise in systolic and diastolic pressures and the rise in the latter, increases the workload on the heart, making it prone for infarction.



Systolic ejection in arteriosclerosis (decreased arterial compliance). The stiffness of the aorta and arteries increases the arterial blood volume



In decreased arterial compliance. The systolic pressure increases much more than the diastolic pressure. Less blood leaves the arterioles during diastole



Increase in peripheral resistance will cause decreased peripheral run off (reduced amount of blood leaving the arterioles) and this increases the arterial blood volume. The systolic and diastolic pressures are increased, but more in the latter

Fig. 7.34: Diagrammatic representation of how mean arterial blood pressure is altered in conditions like decreased arterial compliance (stiffness of arteries) and increased peripheral resistance

Peripheral resistance is also influenced by the elasticity of arteries, viscosity and velocity of blood. As described above, the loss of elasticity will increase the resistance and raises the mean pressure. Rise in viscosity of blood, as in increased hematocrit, will increase the peripheral resistance and hence the rise in diastolic pressure. Similar effect can be seen when the velocity of blood flow is decreased.

Arterial hypertension

Clinically, if the diastolic pressure shows a persistent value above 90 mmHg, it is termed as hypertension. About 95% of the subjects who suffer from hypertension have no known etiology and this condition is called essential hypertension. The other type is secondary hypertension where the etiology is known. Examples of this type are renal disease, phaeochromocytoma, Cushing's disease, Conn's syndrome. The treatment lies in the correction of the underlying disorder. The treatment of essential hypertension depends on the use of beta blockers, calcium channel blockers, ACE inhibitors (angiotensin converting enzyme inhibitors) and diuretics.

Regulation of arterial blood pressure

Arterial blood pressure regulation depends on the reflex mechanisms, which are integrated at the vasomotor center. These reflexes occur within few minutes and they are grouped under short-term regulation. There are mechanisms in the kidney, which regulate body fluids and electrolyte balance. Arterial blood pressure is also regulated by maintaining fluid and electrolyte balance. This regulation occurs over a period of time, which may take few days to several weeks. This type of regulation is called **long-term regulation**. The blood pressure is also regulated by the action of circulating hormones and transmitters, which act on the blood vessels.

Maintenance of arterial blood pressure

Systolic pressure: **Cardiac output**
- Venous return
- Myocardial contractility
- Heart rate
- Blood volume

Diastolic pressure: **Peripheral resistance**
- ↑ sympathetic tone→↑ TPR
- ↓elasticity of artery→ ↑TPR
- ↑ viscosity of blood→↑ TPR
- ↑ velocity of blood→↓TPR

$$PR = \frac{\text{Arterial blood pressure}}{\text{Cardiac output}}$$

Increase in cardiac output: ↑ systolic pressure

Increase in TPR : ↑ diastolic pressure

TPR = Total peripheral resistance

Vasomotor center

The center regulating the activity of blood vessels is present in the medullary reticular formation, close to the cardiac center. There is a **vasoconstrictor area**, situated in the lateral and rostral region, while the medial and caudal region is the **vasodilator area**. Both of them are present within the vasomotor center (Fig. 7.35). The pressor area controls the sympathetic tone of arteries and arterioles. The stimulation of this region causes rise in sympathetic discharge, resulting in vasoconstriction and rise in blood

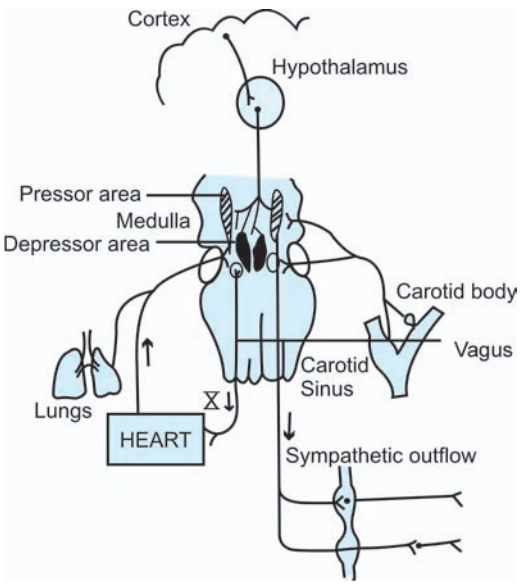


Fig. 7.35: Diagram to show the vasomotor center in the medulla and the various afferents involved in the regulation of arterial blood pressure

pressure. The vasodilatation and fall in blood pressure is produced by decreasing the sympathetic discharge caused by the inhibition of vasoconstrictor area. The stimulation of vasodilator area also will cause decrease in sympathetic tone and fall in blood pressure. In the systemic regulation of arterial blood pressure, the sympathetic vasoconstrictor fibers supplying the blood vessels are considered important. There are also vasodilator fibers present in the skeletal muscle arterioles, which is sympathetic cholinergic, but it is concerned with the regulation of blood flow to the exercising muscles. Like wise, the vasodilator fibers in the parasympathetic nerves supplying glands and smooth muscles help to regulate the local blood flow.

Short-term regulation of arterial blood pressure

The various cardiac and vascular reflexes are discussed under this type.

These reflexes occur immediately in response to changes in pressure in the heart and in the larger arteries, changes in gas tensions of blood and the state of sympathetic activity (Fig. 7.35).

Reflex from baroreceptors

There are pressure sensitive receptors present in the vena cava, pulmonary artery, which form the low pressure receptors. The receptors situated in the arch of aorta (aortic arch) and in the internal carotid artery, at the bifurcation of common carotid artery (carotid sinus) form the high pressure receptors. They are called baroreceptors. They respond to changes in the mean arterial blood pressure and pulse pressure (Fig. 7.36). They are stimulated by the rate of change of blood pressure (dynamic response) and to the steady state blood pressure (static response).

Baroreceptor stimulation occurs between 50 mmHg to 180 mmHg. At 180 mmHg mean pressure, the baroreceptors are maximum stimulated and any rise in pressure beyond this level, will give no response. The response from

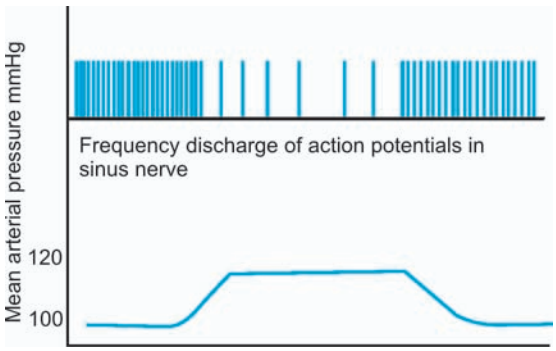


Fig. 7.36: Effect of rise in mean arterial blood pressure on the sinus nerve discharge

carotid sinus is carried by the sinus nerve, which is a branch of IX nerve, whereas the response from aortic arch is carried by vagus. These afferents carry inhibitory impulses when baroreceptors stimulation occurs by the rise in mean arterial blood pressure. The impulses end in cardio-inhibitory and vasomotor centers. The stimulation of cardioinhibitory center and inhibition of vasomotor center produces a reflex bradycardia and fall in blood pressure (Fig. 7.37). Reverse changes can be seen when the mean arterial blood pressure falls. The vagus and the sinus nerves are known as buffer nerves, as they are concerned with the adjustment of arterial blood pressure. Sectioning of both the nerves bilaterally would cause a greater rise in arterial pressure and the condition is called neurogenic hypertension.

The baroreceptors are reset, when the arterial blood pressure shows a sustained elevation, as in hypertension, since the baroreceptors are fast adapting receptors. As the mean pressure is elevated, the receptors are also reset to respond to the elevated level. That is why, in hypertension, the baroreceptors are not effective in bringing down the blood pressure.

Clinically, the baroreceptor efficiency is tested by massaging the carotid sinus and observing the fall in pulse rate in the wrist. The Valsalva's maneuver (forceful expiration against closed glottis) is also a good test to assess the baroreceptor function. Physiologically, the role

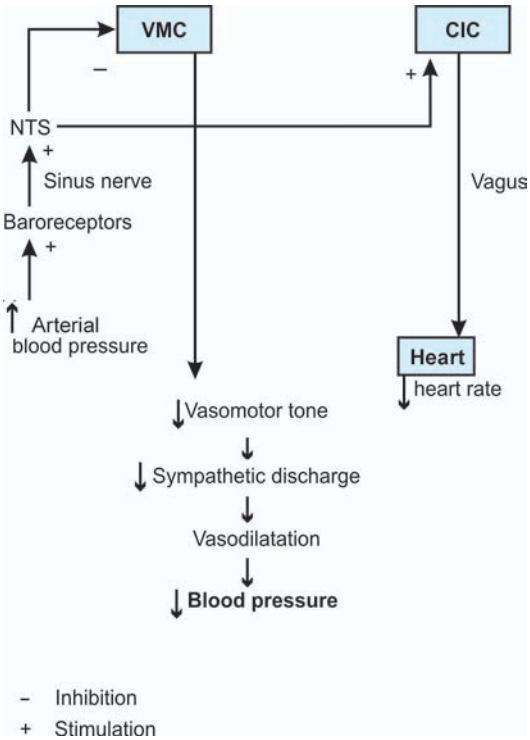


Fig. 7.37: Baroreceptor reflex

of baroreceptors in blood pressure homeostasis can be noticed, when posture is changed from recumbent to standing in an individual. The fall in blood pressure, due to pooling of venous blood in the legs, leads to less blood perfusing the brain, causing a transient giddiness. The absence of baroreceptor stimulation causes reflex tachycardia and rise in blood pressure. This restores the blood flow to the brain and also arterial blood pressure is maintained.

Chemoreceptor reflex

The stimulation of peripheral chemoreceptors by hypoxia, hypercapnia and acidosis results in vasoconstriction and rise in blood pressure. When mean blood pressure falls below 50 mmHg, as in circulatory shock, it is the chemoreceptor stimulation, which raises the blood pressure and this causes oscillations in the graphical recording called **Meyer's waves**.

Reflexes from heart

As said earlier, the reflexes from left ventricle and left coronary artery, cause fall in blood pressure, when they are distended by the rise in pressure.

Higher centers

The stimulation of higher centers such as cerebral cortex, hypothalamus and limbic lobe, by emotional excitement, anxiety and stress, etc, causes increased sympathetic discharge to the vessels, producing vasoconstriction and rise in blood pressure.

Direct stimulation of medullary center (Cushing reflex)

Rise in intracranial tension causes rise in systemic arterial blood pressure and bradycardia. The increased intracranial tension leads to medullary ischemia, which causes direct stimulation of vasomotor center, producing rise in blood pressure. The stimulation of baroreceptors, due to the rise in blood pressure causes bradycardia.

Circulating hormones

Arterial blood pressure is also influenced by the circulating chemical substances such as epinephrine, norepinephrine, serotonin, vasopressin, angiotensin, etc, which cause vasoconstriction and rise in blood pressure. They are called vasoconstrictor substances. There are also vasodilator substances namely, histamine, acetylcholine, bradykinin, etc, which produce vasodilatation and fall in blood pressure.

The atrial natriuretic peptide (ANP) is secreted when there is rise in blood volume, rise in arterial blood pressure, caused by the elevation of sodium level. The ANP helps in blood pressure regulation by causing natriuresis.

Long-term regulation of arterial blood pressure

This involves renal mechanisms. The arterial blood pressure is regulated, through the maintenance of fluid volume and electrolyte

Short-term regulation

Reflex	*Stimulus*	*Response*
Sinoaortic	↑ BP	↓BP and ↓Heart rate
	↓BP	↑ BP and ↑ Heart rate
Chemoreceptor	↓PO_2 ↑ PCO_2 ↑ H^+	↑ BP and ↑ Heart rate
From heart	↑ Lt.vent.pre	↓BP and ↓Heart rate
From lungs	Distension of vascular bed	↓BP
Higher centers	Stress, Emotion	↑ BP

Long-term regulation is carried out by

Body fluid regulation and electrolyte balance:

Body fluid balance: ↓Body fluid volume leads to ↓blood pressure and ↑ in osmotic pressure of blood. This stimulates ADH hormone secretion. Renal tubular water reabsorption in the presence of ADH causes ↑ fluid volume, ↓ tonicity and ↑ blood pressure.

Electrolyte balance: ↓ Na^+ leads to ↓ ECF volume and ↓blood pressure. Aldosterone is secreted through renin-angiotensin mechanism. Aldosterone causes Na^+ reabsorption in exchange for K^+ or H^+ secretion in the distal renal tubules. The rise in Na^+ level increases ECF volume, which inturn raises arterial blood pressure.

balance. The hormones ADH and aldosterone are involved in the homeostasis. Aldosterone is a mineralocorticoid, produced from the adrenal cortex, zona glomerulosa layer, and is responsible for the regulation of electrolyte balance. The hormone acts in the distal tubules of kidney and causes Na^+ reabsorption in exchange for potassium or hydrogen ion secretion. Aldosterone secretion is stimulated in conditions like rise in potassium, fall in blood volume, fall in blood pressure and fall in sodium level.

The hormone ADH is responsible for regulating tonicity or osmolality of plasma. This is achieved by correcting the fluid volume. Secretion of ADH from hypothalamic supraventricular and paraventricular nuclei occurs, when tonicity of blood becomes greater. The hormone acts on the distal tubules and causes water reabsorption, producing antidiuresis. This leads to rise in fluid volume, rise in blood pressure and correction of tonicity.

ARTERIAL PULSE

Pulse is defined as the pressure wave transmitted along the wall of the arterial system, which is caused by the systole of the heart. The upward movement of the peripheral artery, which is felt, as pulse, is actually due to the propagation of the pressure wave and is not due to the movement of blood flow. The velocity of pulse wave is faster than the velocity of blood flow. The speed of pulse wave in the aorta is 8m/sec, whereas in the arteries, it is still greater, which is 16 m/sec. The velocity is high in old age, due to the hardening of arteries caused by the loss of elasticity. The pulse wave configuration and velocity, shows changes as we go to the peripheral artery. First of all, the velocity of transmission is greater in the periphery. The recording of pulse (sphygmogram) shows that the dicrotic notch, which is prominent in the aorta, dampens and disappears in the periphery (Fig. 7.38). The dicrotic notch is due to the rebound effect caused by the blood striking against the closed aortic valves, at the beginning of diastole. The systolic phase of the pulse wave becomes greater in amplitude and narrower in the periphery and appearance of secondary waves after the dicrotic phase is also observed. These changes are more pronounced in young adults.

The reasons for the above described changes are due to:

a. Tapering of arteries
b. Reflections of pressure wave
c. Resonance
d. Changes of arterial compliance.

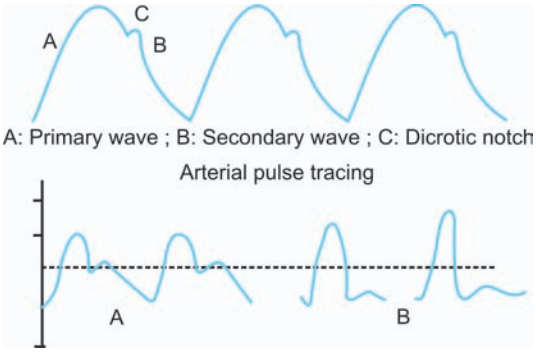


Fig. 7.38: Diagram to show the changes in the arterial pulse in the peripheral artery. Note the absence of dicrotic notch, the increase in amplitude and narrowing of pulse wave in the periphery

The peripheral artery lumen becomes narrower and this tapering gives a greater systolic pressure. The pulse wave gets reflected at the point of branching in the periphery and the pressure pulse at this point, is the algebraic sum of the incident pressure wave transmitted in forward direction and the reflected wave traveling in retrograde direction. The peripheral artery also resonates at certain frequencies of pressure wave transmission, while it shows dampening at other frequencies. The compliance of arteries is lesser at the periphery and hence gives a greater systolic pressure and also greater velocity for the pulse wave.

Clinically arterial pulse is examined in a peripheral artery like radial artery. The characteristics of pulse, which are examined, include rate, rhythm, volume, tension and condition of the vessel.

Pulse pressure is the difference between systolic and diastolic pressures. It is about 40 mmHg. The strength of pulse pressure depends upon:

a. Stroke volume
b. Arterial compliance.

Increase in stroke volume or decrease in arterial compliance will cause rise in systolic pressure and hence greater pulse pressure in such conditions occurs. In old age, arterial compliance is reduced, due to the loss of elasticity and this causes rise in systolic pressure, even though the arterial volume and the peripheral resistance are unaffected.

Increase in stroke volume will also raise pulse pressure. In aortic insufficiency, the systolic ejection will be greater, in order to overcome the blood regurgitating into the ventricle. The systolic pressure is very high and there is no dicrotic notch in the pulse wave. The heartbeat can be heard from a distance and hence it is also called water hammer pulse or Corrigan's or collapsing pulse.

VENOUS PRESSURE

Venous circulation

Venous pressure is the pressure transmitted from the arterial side, which helps in the return of blood to the right atrium. The pressure in the veins shows a gradual decrease, as we proceed from smaller venules in the periphery to larger veins in the thorax and this pressure gradient is necessary for the venous circulation. The venous circulation is also facilitated by skeletal muscle pump, which squeezes the smaller veins, as the muscle contracts. The negative intrathoracic pressure is another important factor in drawing the venous return to the right atrium. In smaller veins, especially in limbs, there is presence of valves, which prevents backward flow of blood. This also assists venous return. If there is incompetence of venous valves, then there is no sufficient venous return from limbs. This particularly occurs in lower limbs, leading to stagnation of blood in veins. This causes tortuous veins, which bulge and engorge with blood. This condition is called varicose veins. The veno motor tone caused by the sympathetic activity also contributes to the venous return. The venous return is further helped by the forward force developed by the right ventricular systole, which causes downward pulling of the base of the heart. This creates enlargement of the right atrium, providing greater pressure gradient for venous circulation.

The veins are thinner and have a larger diameter, as compared to arteries. The veins appear elliptical or oval shape, when not filled with the blood. This provides a greater volume to accommodate blood and the shape becomes spherical. This property of veins to accommodate large volume of blood without rise in pressure gives the name capacitance vessels to it. In fact, the veins can hold about 60% of the blood volume.

Venous pressure

Venous pressure varies according to posture, respiratory activity and heart's action. In standing posture, the effect of gravity can be seen as in arterial pressure. The pressure in veins below the level of right atrium is increased and above the level decreased. The venous pressure from veins in the limbs is known as peripheral venous pressure, which is 6 to 8 mmHg. The venous pressure from central veins in the thorax, near the right atrium, is less and forms the central venous pressure. Its normal value is 4-5 mmHg.

Central venous pressure is increased in the following

Congestive heart failure
Positive pressure breathing
Straining
Increase in blood volume.

Clinically the internal jugular venous pressure measurement will reflect the central venous pressure when the subject is in supine and reclines at 45°. The top of the pulsation will be visible at the top of the clavicle. The vertical height in cms between the top of the venous pulsation and the sternal angle is measured to get the jugular venous pressure. Experimentally, central venous pressure can be recorded by introducing a catheter to the thoracic vein and connecting the other end to pressure recording device or mercury manometer.

In erect posture, the venous pressure in the leg veins is 80 mmHg and it progressively decreases as the venous circulation reaches the larger veins in the thorax. The pressure in the right atrium is 0 to 4 mmHg, while in the neck vein, it is 0 mmHg and that is the reason, it is collapsed in erect posture. The venous pressure in the skull is sub atmospheric. The superior sagittal sinus shows a pressure of –10 mmHg.

Mean arterial blood pressure and venous pressure in different regions in standing posture.

Mean arterial blood pressure		
At the level of heart		100 mmHg
	leg	180 mmHg
	head	60 mmHg
Venous pressure		
Peripheral vein		8 mmHg
Leg vein		80 mmHg
Central vein		4 mmHg
Neck vein		0 mmHg
Superior sagittal sinus		–10 mmHg

Venous pulse

Venous pulse is caused by the right atrial pressure changes during cardiac cycle. The pressure wave is transmitted to the jugular vein and the pulse is known as jugular pulse. The features of the jugular pulse is similar to the right atrial pressure curve recording. The venous pulse shows three positive waves namely **a, c** and **v** (Fig. 7.39). The a wave is the first positive wave caused by the pressure rise due to right atrial systole. The **c** wave is due to pressure rise in the right atrium during

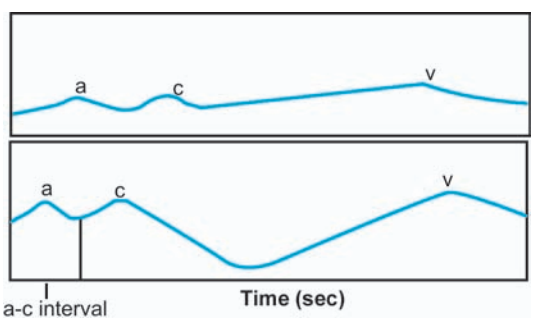


Fig. 7.39: Venous pulse tracing. The top recording is from the right atrium and the figure below is recorded from jugular vein

isovolumetric contraction phase of ventricular systole, as the tricuspid valve is pushed upwards into the cavity of right atrium. The **v** wave is caused by pressure rise in the right atrium, during atrial filling which occurs just before the ventricular filling.

REGIONAL CIRCULATION

Determinants of local blood flow

The blood flow to the tissues depends upon its metabolic needs. Greater the activity of the organ, greater the demand for oxygen, which in turn regulates the blood flow. There are also local regulatory mechanisms, which govern the blood flow to the tissues. The main mechanism involved is the change in the caliber of the arterioles brought by the action of nerves innervating it. The effect of locally released substances on the arterioles and capillaries is also important in the regulation. They are present in addition to the regulation by the heart's action. The arterioles are supplied by sympathetic noradrenergic fibers, which cause vasoconstriction and reduce the blood flow. The reduction in the sympathetic tone on the arterioles will cause vasodilatation and increase in blood flow to the tissues.

Autoregulation of blood flow

The blood flow in most of the tissues like kidney, liver, brain, heart, muscle is controlled by autoregulation. There are two mechanisms believed to be involved in the autoregulation. They are myogenic and metabolic regulatory mechanisms.

In **myogenic theory of autoregulation**, the blood flow to the organ is adjusted by changing the arteriolar resistance. When there is increase in perfusion pressure, the wall of the arteriole is stretched and this reflexly causes contraction of smooth muscle lining the arteriole, to produce constriction. This effect will bring back the normal perfusion of blood flow. The reflex contraction of smooth muscle in the arteriole is believed to be as a result of increase in wall tension, when the perfusion pressure rises.

The **metabolic theory of autoregulation** consists of vasodilatation and vasoconstriction caused by release of substances locally in the tissues (Fig. 7.40). Substances like CO_2, hypoxia, H^+ ion, lactic acid, K^+, adenosine, histamine, VIP, substance P, bradykinin, ANP, NO, etc, cause relaxation of arterioles and precapillary sphincters. This results in vasodilatation and increase in blood flow.

Vasoconstriction of the arterioles is caused by the release of substances like angiotensin II, serotonin, vasopressin, oxytocin, norepinephrine, endothelin 1, etc. The vasoconstriction produced by them will result in decrease of blood flow.

Release from endothelium

The blood flow is also regulated by substances released from the endothelium of the vessels. When there is damage to the endothelium, there is release of prostacyclin, which inhibits platelet aggregation and produce vasodilatation. The platelets on the other hand release thromboxane A_2, which causes platelet aggregation and vasoconstriction. These two substances are derived from arachidonic acid by the action of the enzyme cyclo-oxygenase during prostaglandin synthesis.

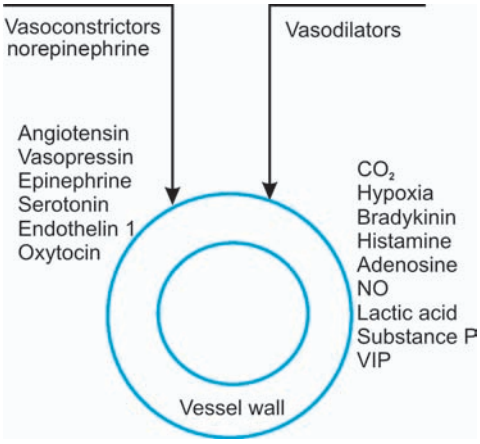


Fig. 7.40: Substances that act locally on the capillaries and arterioles to regulate the blood flow to the tissues

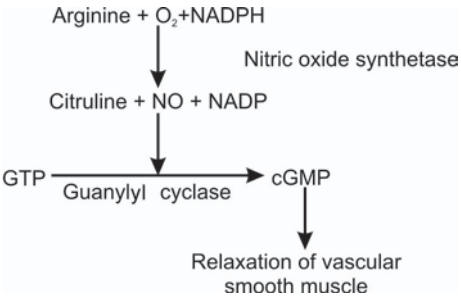


Fig. 7.41: Formation of NO and its mechanism of action through cGMP

Nitric oxide

The endothelium also releases a substance called EDRF (endothelium derived relaxing factor) which is now identified as NO (nitric oxide). It is synthesized from arginine by the enzyme NO synthetase. The NO causes relaxation of vascular smooth muscle. The action of NO is mediated by cyclic GMP (Fig. 7.41). It appears that the vasodilator effect of various substances such as acetylcholine, are due to the release of NO from the endothelium. If there is defect in the synthesis of NO, the blood pressure rises and also the vasoconstrictor effect of substances like catecholamines will be much greater. This suggests that NO is also involved in the regulation of arterial blood pressure. NO is present in other tissues also like brain, macrophages and gastrointestinal tract. Recently it has been shown that NO causes penile erection in males and drugs have been prepared based on this to treat impotence in males.

Endothelins

Endothelial cells release a potent vasoconstrictor peptide, known as endothelins. There are three forms of endothelins (1, 2, 3). It has been shown that endothelins produce a number of physiological effects, in addition to vasoconstrictor effect.

CORONARY CIRCULATION

The heart receives its blood supply from coronary arteries. There are right and left coronary arteries, with right coronary artery blood flow being dominant in 50% of humans. The left coronary artery blood flow is dominant in 20% of the individuals and blood flow is equal in the remaining 30% of the subjects. The left coronary artery supplies left ventricle and right coronary artery perfuses the right ventricle. The venous drainage from the left ventricle occurs through the coronary sinus, while from the right ventricle by anterior cardiac veins. There are also direct drainage of blood into the cavities of the heart, through arterioluminal, arteriosinusoidal and the Thebesian vessels.

Coronary blood flow can be measured by various methods. The commonly used ones are, radioisotope method using ^{133}Xe, Doppler technique, thermodilution, Fick method, etc. The coronary blood flow is 250 ml/min or 75 to 80 ml/100 gm of tissue/min in resting state in man.

Coronary oxygen uptake during rest itself is very high. It is 8 to 10 ml /100 gm/min. Hence, during muscular exercise, there is not much scope for increasing the O_2 supply. The increased supply of O_2 has to come from increased blood flow only.

Phasic blood flow

The blood flow in the left coronary artery is phasic in nature (Fig. 7.42). The left ventricular pressure developed during systole is greater and hence the coronary vessels in the left ventricular myocardium gets compressed during systole. This causes absence of blood flow to the subendocardial regions of left ventricular myocardium. The systolic pressure developed in the inner myocardium will be high during systole, which increases the extra coronary resistance. The superficial regions of the left ventricle will receive blood flow during systole, as the coronary vessels are not compressed significantly. The left ventricular subendocardial regions will get blood flow only during diastole, as the extra-coronary resistance will be absent during this phase. There are mechanisms present in the subendocardial region to overcome the lack of O_2 supply during systole. The increased

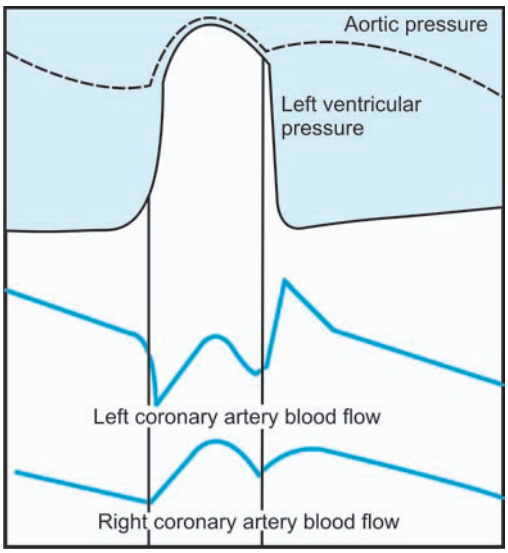


Fig. 7.42: Blood flow in coronary arteries correlated with the left ventricular pressure and aortic pressure curves. Note the phasic nature of blood flow in the left coronary artery which is more significant than right coronary artery. See text for details

myoglobin, which stores O_2 during diastole, will be released during systole. There is also increased capillary density in this region. Inspite of these, this part of the myocardium is more susceptible for myocardial infarction.

Right ventricular myocardial blood flow is not much affected during systole unlike left ventricle, as the extravascular compression is not present. The reason is that the pressure developed from right ventricular systole is very less and hence the right coronary artery receives blood flow even during systole.

Regulation of coronary blood flow

The coronary blood flow is regulated by neural and metabolic factors, with the metabolic factors taking a predominant role. The coronary blood vessels are supplied by autonomic fibers. The direct stimulation of sympathetic nerve innervating the coronary blood vessels gives vasoconstriction and increase in coronary resistance. But there is an indirect effect, which overcomes this. The increase in heart rate and force of contraction leads to formation of metabolites namely, CO_2, K^+, H^+, and adenosine, which cause coronary dilatation and increase in blood flow (metabolic effect).

The adrenergic receptors present in the coronary arteries are α_1 (vasoconstriction) and β_2 (vasodilatation). The vagal stimulation on the coronary blood vessels causes decreased coronary resistance. The aortic pressure has a parallel relationship with coronary blood flow. Increase in aortic pressure provides the pressure head for the blood flow into the coronary vessels. In cardiac failure, sufficient pressure head for coronary blood flow will not be present and hence there is decreased coronary blood flow.

Among the metabolic factors which regulate coronary blood flow, **oxygen lack (hypoxia)** or oxygen demand is considered important. As said earlier, the oxygen uptake in the resting heart itself is 80% and hence, increase in oxygen supply to the myocardium will be possible only by increasing the coronary blood flow. In this context, hypoxia in the myocardium forms a potent stimulus for coronary dilatation. The tissue hypoxia results in the formation of metabolites like CO_2, H^+, K^+, lactic acid, adenosine, etc. The release of **adenosine** from the cells gives greater coronary dilatation and increase in blood flow. Narrowing of coronary blood vessel by the atherosclerotic plaques, results in oxygen lack and results in a condition called angina pectoris, which gives substernal pain on exertion. Coronary dilators like nitro glycerine, when taken, gives relief from pain. Prolonged oxygen lack leads to myocardial ischemia and necrosis of the tissues, and the condition is known as **myocardial infarction**. It can also occur due to coronary thrombosis.

Cardiac muscle utilizes substrates for energy depending upon the availability and concentration of substrates in the arterial blood. At rest, the myocardium utilizes free fatty acids for energy production, as it is more preferred than other substrates. The myocardium also utilizes glucose, lactate, amino acids, ketone bodies for energy production.

Regulation of coronary blood flow

Direct effect of sympathetic stimulation : ↓CBF

Metabolic effect from hypoxia, Hypercapnia, H^+, ADP, etc : ↑ CBF

Adrenergic stimulants α_1 : ↓CBF

β_2 : ↑ CBF

Increase in aortic pressure : ↑ CBF

CEREBRAL CIRCULATION

Blood flow to the brain is supplied by cerebral arteries, which form the circle of Willis at the base of the brain. The venous drainage from the brain is collected by the internal jugular vein. Brain, being the vital organ, the blood flow in it more or less remains constant without any wide fluctuation. Measurements by Fick method or use of radioactive tracer substance ^{133}Xe, gives the value as 750 ml/min or 55 ml/100 gm/min. There are regional variations in the blood flow within the brain depending on the activity. But the total amount perfusing the brain remains constant.

The cerebral vessels are supplied by the autonomic fibers and sensory fibers especially those carrying pain. The sympathetic innervation causes vasoconstriction, but its regulatory role in cerebral circulation is very limited. The cerebral blood flow depends on the pressure gradient between the cerebral arteries and the internal jugular vein at the base of the brain and vascular resistance. The most important factor, which is concerned with the regulation of cerebral blood flow is **hypercapnia** (rise in carbon dioxide tension). Increase in CO_2, causes cerebral vasodilatation and increase in blood flow. Hypoxia produces vasodilatation in the brain. This is to protect the neurons, as they are very sensitive to the lack of oxygen. Neuronal damage and death occurs if hypoxia is continued beyond 12 to 15 sec. The second factor, which controls the cerebral blood flow is intracranial tension. Rise in intracranial tension causes a reflex increase in arterial blood pressure **(Cushing reflex).** The increase in intracranial tension results in ischemia of the medullary vasomotor center, as the blood vessels are compressed. The direct stimulation of ischemia on the vasomotor center causes rise in systemic arterial blood pressure.

The cerebral blood flow, as said in the beginning, does not fluctuate, when mean arterial blood pressure changes between 60 to 150 mmHg. This is possible because of the presence of **autoregulatory mechanism.**

The grey matter of the brain receives more blood, as it contains nerve cells and glia. The flow is less in the white matter, which contains mostly tracts.

The capillaries of cerebral vessels show tight junctions in the endothelial cells. There is presence of glial cell astrocytes between the neuron and the capillaries, which provide the **blood brain barrier**. The barrier helps to prevent entry of toxic substances into the neuron from blood. However, the barrier is permeable to lipid soluble substances like oxygen and carbon dioxide gases.

CUTANEOUS CIRCULATION

Blood flow through the skin is variable depending upon the body temperature regulation (flow ranges from 1 ml to 150 ml/100 gm/min.). The cutaneous blood vessels show anastomoses between arterioles and venules **(arteriovenous anastomoses)** particularly in the fingers, palms, ear lobes, face, and feet. The capillaries in the skin are closed most of the times and become patent, when the body temperature rises. The cutaneous blood vessels are directly influenced by the rise in temperature, which causes vasodilatation. The increased blood flow, occurring as a consequence to this, helps to dissipate the heat and bring down the body temperature. When body is exposed to cold environment, the hypothermia directly acts on the cutaneous blood vessels and produces vasoconstriction. The reduction of blood flow to the skin helps to conserve body heat and maintain body temperature.

Skin arteries and arterioles are supplied by sympathetic noradrenergic fibers, which cause vasoconstriction. This action is seen in hypothermia and circulatory shock. The profound vasoconstriction of the skin in hypovolemic shock, produces cold skin. There are also metabolites like bradykinin, substance P, histamine, etc released in the skin which have effect on the arterioles and capillaries of the skin. Bradykinin and histamine cause vasodilatation and increase in skin blood flow.

Cutaneous circulation shows responses to injury or trauma. These vascular responses are grouped under **triple response** (Fig. 7.43). The direct mechanical stimulation of the capillaries of the skin by a blunt object, produces dilatation which gives red line called **red reaction**. A little later, redness of the skin spreads to the surrounding area, which is due to the dilatation of arteriole caused by the **axon reflex** and called as **flare**. It is the only reflex, not mediated through the central nervous system. The impulses travel antidromically in a branch of sensory nerve and dilatation of arteriole is produced by the release of substance P or histamine at the nerve ending. After some time, there will be developement of edema in this region, caused by the release of substance P or histamine, which increases the capillary permeability. This response is known as **wheal**.

Reactive hyperemia in regional circulation

The regional circulation shows a regulatory mechanism called **reactive hyperemia**, which can be seen in coronary, skeletal muscle and cutaneous circulations. It is the mechanism which shows increased blood flow after a period of occlusion of the artery. The metabolites like CO_2, H^+, K^+, lactic acid, etc, accumulate in the interstitial fluid surrounding the arterioles and capillaries during the occlusion period. The vasodilatation produced by them increases the blood flow, when the occlusion on the blood vessel is removed. This phenomenon is called **reactive hyperemia.**

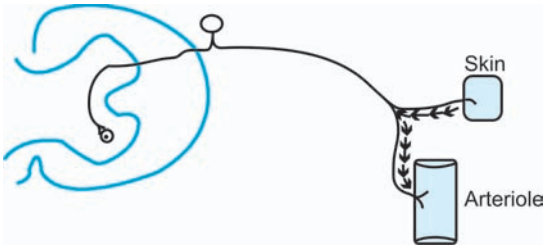


Fig. 7.43: Axon reflex. The impulses travel antidromically in a branch of sensory afferent and go to arteriole

CAPILLARY CIRCULATION (MICROCIRCULATION)

Capillaries are the exchange vessels, present between arterioles and venules. The arterioles in some places end in metarterioles, which in turn end in capillaries. The arterioles also directly end in venules, bypassing the capillaries in some places. The latter forms the arteriovenous anastomoses, which is more common in the skin. Capillaries are lined by a single layer of endothelium and there is no smooth muscle lining present in the wall. The capillary circulation is considered important for exchange of respiratory gases, nutrients, removal of waste products, etc. Besides exchange of substances, the formation of tissue fluid, lymph, glomerular filtration, etc, also occur from capillary circulation.

There are three types of capillaries namely, **continuous, fenestrated and sinusoidal**.

The *continuous capillaries* are seen in the skin, muscle and connective tissue. Its wall has a pore with a diameter of 3 to 4 nm size.

The *fenestrated capillaries* can be observed in the glomerulus of kidney, intestines and choroid plexuses. The diameter of the fenestration is larger, which is 7 nm in size.

The *sinusoidal capillaries* are present in the liver, spleen and bone marrow. The movement of fluid and substances occur through these pores. The lipid soluble substances like O_2 and CO_2 pass through the endothelium easily.

The two important transport mechanisms that are present across the capillary wall are **diffusion and filtration**.

Diffusion of substances through the endothelium of the capillary depends on its concentration gradient. The filtration of fluid, macromolecules, etc occurs through the endothelium by **Starling's mechanism**. The Starling's force gives the filtration at the arteriolar end of the capillaries and absorption of molecules into capillaries, at the venous end (Fig. 7.44). The process of filtration depends on the rate of blood flow, called vasomotion, which in turn depends on the arteriole constriction or relaxation brought by transmural pressure changes in the capillaries. Increase in transmural pressure, causes constriction of arteriole and decrease in vasomotion. The increase in vasomotion is seen, when decrease in transmural pressure causes relaxation of arteriole.

Thus we see that in filtration, small molecules equilibrate with the tissue fluid while passing through the arteriolar end of capillary, which is dependent on blood flow and this exchange is called flow limited. Conversely, molecules, which do not equilibrate with the tissue fluid, during its passage through the capillary, are called diffusion limited.

The capillary blood flow is regulated by contraction and relaxation of arterioles and precapillary sphincters. This is achieved by altering the sympathetic tone and release of tissue metabolites such as CO_2, H^+, lactic acid. The capillary dilatation is also seen due to the release of local humoral substances like bradykinin, histamine and substance P.

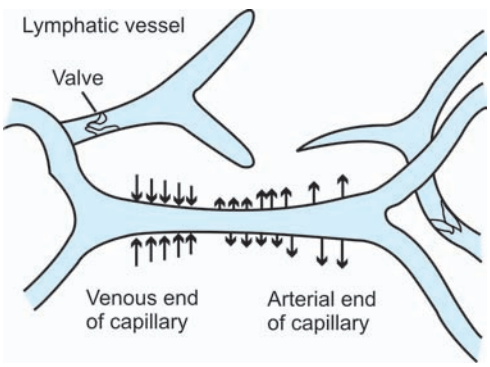


Fig. 7.44: Formation of tissue fluid due to Starling's force in the capillaries. The lymph is formed from the tissue fluid

LYMPHATIC CIRCULATION

Lymph is a tissue fluid present in the lymphatic capillaries. The tissue fluid formed in the interstial spaces as a result of Starling's mechanism, is collected by a fine network of blind ended endothelial tubes called lymphatic vessels (Fig. 7.44). These lymphatic vessels are interrupted by lymph nodes present in the regions like axilla, inguinal region, neck, etc. The lymph from left side of the body, GI tract and lower part of the body is collected by the thoracic duct, while the lymph from right side and upper part of the body is collected by right lymphatic duct. The thoracic duct and right lymphatic duct opens into left and right subclavian veins respectively.

Lymph is a colourless fluid, but in the thoracic duct, it is white in appearance after a fatty meal. Lymph contains 95% of water and 5% solids. Its concentration of substances are very identical to plasma but lesser in amount. It also contains leucocytes, lymphocytes and immunoglobulins.

Regulation of lymph flow

Lymph flow is regulated by the rhythmic contractions of the wall of large lymphatic vessels. This is the most important factor, which regulate the lymph flow. Infact, when the volume of lymph is increased, the rhythmic contractions of the wall of the lymphatics becomes greater. The contractions of the skeletal muscle also push the lymph towards the heart. The lymphatic vessels also contain valves, which prevent the backward flow. The negative intrathoracic pressure during inspiration also hastens lymph circulation towards the heart.

Factors increasing lymph flow

Lymph circulation is increased when interstitial fluid formation is greater, which in turn depends upon the Starling's force across the capillary wall. Factors such as increased capillary dilatation and permeability will result in greater lymph flow. The agents which cause this are known as lymphogogues. Example for this is histamine, kinins, substance P, etc. Lymphatic obstruction,

due to filarial parasite will lead to retention of interstitial fluid in the tissue spaces, causing edema (lymphatic edema).

Functions of lymphatic circulation

- Return of proteins from tissue fluid to the systemic circulation.
- To transport the absorbed long chain fatty acids and cholesterol as chylomicrons through the thoracic duct.
- Lymph nodes act as a filter preventing the entry of bacteria and particulate matter into the circulation.
- Lymphocytes and neutrophils present in the lymph help in defense mechanism.
- Maintenance of blood volume. The lymph flow is increased when blood volume is raised and decreased when blood volume falls.

FETAL CIRCULATION

The circulation of blood in fetus and newborn shows differences in both anatomical and physiological characteristics (Figs 7.45 and 7.46). It is the placenta which transfers respiratory gases and nutrients from maternal blood to the fetal blood. From placenta, the umbilical vein carries oxygenated blood with an oxygen saturation of 80% to the portal blood of liver. Some part of blood from umbilical vein enters inferior vena cava through ductus venosus. The portal blood from liver and venous blood coming from lower parts of the body drains into the inferior Vena cava and decreases the O_2 saturation to 65%. Thus, we see that in fetal circulation, the inferior vena cava, before draining into the right atrium, gets a mixture of oxygenated blood from umbilical vein and deoxygenated blood from lower parts of the body. After entering right atrium, the inferior vena cava blood passes into left atrium through foramen ovale. The superior vena cava, which drains deoxygenated blood from the upper regions of the body, enters the right ventricle through the right atrium.

Right ventricular ejection in fetus, pumps blood into the pulmonary artery. There is a high pulmonary vascular resistance, due to the fact

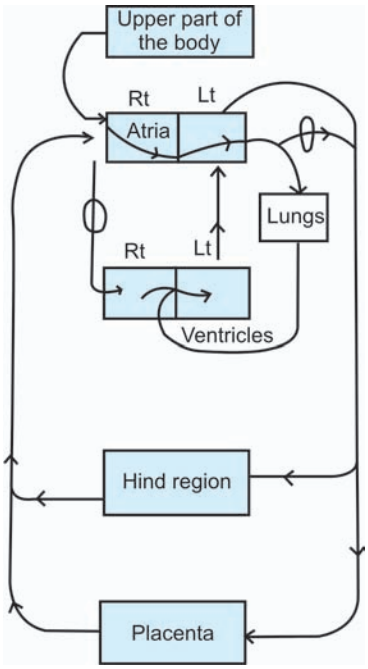


Fig. 7.45: Schematic illustration of fetal circulation

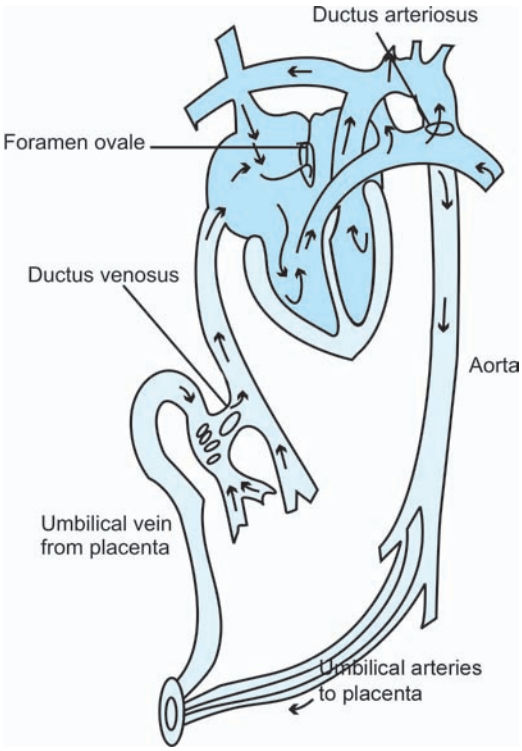


Fig. 7.46: Functional anatomy of fetal circulation

that alveoli in fetal lungs are closed. This causes the pulmonary artery pressure to be greater than the pressure in the aorta. Hence, the pulmonary circulation does not exist in the fetus. The high pressure in the pulmonary artery leads to entry of blood into the descending aorta, through ductus arteriosus. The less oxygenated blood of descending aorta, supplies the lower parts of the body, such as, trunk and limbs, while, the left ventricular ejection which has an O_2 saturation of 60%, ensures sufficient supply of oxygenated blood to the brain.

The deoxygenated blood from descending aorta drains into two umbilical arteries, which on entering placenta, forms capillaries of chorionic villi. These capillaries directly dip into the intervillous spaces, which contains maternal blood. The O_2 and CO_2 exchange takes place between maternal and fetal blood. After gaseous exchange, the umbilical vein leaves placenta and enters fetal circulation.

Fetal respiration

The PO_2 in the umbilical vein is 40 mmHg which is 35% of PO_2 present in the maternal blood. This low PO_2 in fetal blood is sufficient to give 80% O_2 saturation, because of fetal Hb (Hb F). It contains α_2 and γ_2 globin chains instead of α_2 and β_2 chains. The absence of β chains in fetal Hb, lowers its affinity for 2,3-DPG. This compound competes with O_2 for binding sites in Hb molecule. The reduced affinity of Hb F for DPG gives more nity for O_2 to bind with it. Added to this, the fetal Hb concentration is also slightly higher (17 to 18 g%) than adult Hb levels. Because of these advantages, the O_2 content of fetal blood, for a given PO_2 is higher than maternal blood. That's why, the O_2 dissociation curve of HbF is recorded to the left of HbA. The adult Hb replaces the fetal Hb within 3 to 4 months after birth.

Changes at birth

At birth, three changes occur, which give the adult type of circulation. They are:
Shutting off placental circulation
Closure of foramen ovale
Closure of ductus arteriosus.

At birth, the occlusion of umbilical cord closes placental circulation and this causes asphyxia in the fetus. Fetal chemoreceptors are well developed and hence asphyxia stimulates respiration through the chemoreceptor drive. The tactile and thermal stimuli also stimulate directly the respiratory centers. The newborn takes a few gasps and the inspiratory efforts cause distension of lungs. The negative interpleural pressure that is created in the first breath keeps the alveoli distended and facilitates inspiration. The presence of pulmonary surfactant will also ensure that alveoli do not collapse during expiration. The expansion of lungs during inspiration reduces pulmonary vascular resistance, resulting in flow of blood into the pulmonary circulation, when right ventricular ejection occurs. The return of blood from lungs causes greater pressure in left atrium and it leads to closure of foramen ovale. The closure of placental circulation raises the peripheral resistance, which in turn increases the aortic pressure and ductus arteriosus constricts usually within a few minutes after birth. The constriction of ductus arteriosus can be facilitated by drugs like bradykinin and inhibitors of prostaglandin synthesis such as indomethacin. If ductus arteriosus closes before birth, it will lead to pulmonary hypertension.

CARDIOVASCULAR CHANGES IN MUSCULAR EXERCISE

Muscular exercise, when practiced regularly, gives physical conditioning and prevents cardiovascular diseases. Hence, the study of responses produced by cardiovascular and respiratory systems in muscular exercise becomes significant. There are two types of exercises that are performed: one type involves training of muscles, leading to conditioning of cardiovascular responses. In the other type, where the exercise is not regularly done, gives acute responses in the cardiovascular functions.

Cardiac responses to exercise

The heart rate increases proportionate to the severity of exercise (Fig. 7.47). The increase in the rate varies according to the age. In old age, the cardiac response to exercise is reduced. Heart rate can go up to 180/min in strenuous exercise. The increase in the rate is due to increased sympathetic tone and withdrawal of vagal influence on the heart. There is also increased circulating catecholamines in the blood. In a trained athlete, the initial heart rate is low (bradycardia), possibly, due to the decreased beta adrenergic receptors on the heart, as a consequence to repeated sympathetic stimulation during exercise. Thus, a trained individual has a higher limit to increase the heart rate.

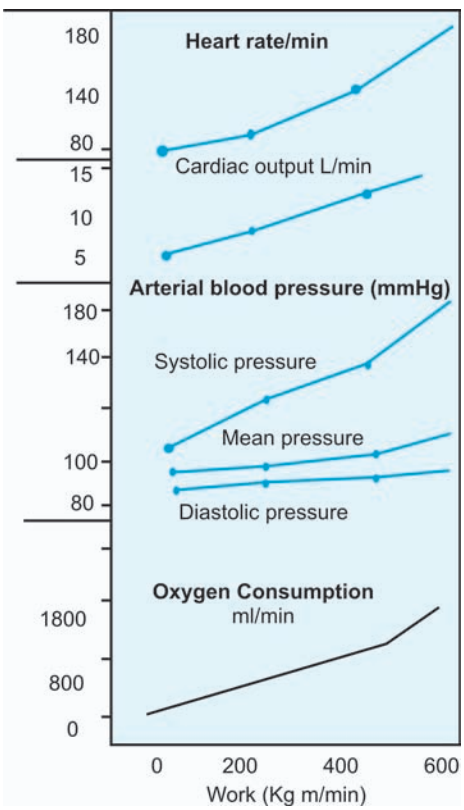


Fig. 7.47: Cardiovascular responses to muscular exercise

Stroke volume

Endurance training increases the stroke volume by increasing the end diastolic volume of the ventricles. Following Starling's law of the heart, this will increase the force of contraction and rise in stroke output. Moreover, in a trained person the cavity of the ventricles is enlarged and this in turn increases the end diastolic volume. That is why a trained athlete can raise his cardiac output from 5 lit to 25 lit/min in severe exercise (Fig. 7.47).

Circulatory responses

The mean arterial blood pressure increases during exercise. The systolic pressure increases and the diastolic pressure either does not show any change or decreases depending on the severity of exercise. In strenuous exercise, peripheral resistance falls, due to the dilatation of skeletal muscle arterioles and this is responsible for the decrease in diastolic pressure. In isotonic type of muscle contraction, heart rate, cardiac output and blood pressure are raised, whereas, in isometric type of muscle contraction, the heart rate and blood pressure are increased, but not the cardiac output, as the skeletal muscle blood flow is not greater.

Regional blood flow

The skeletal muscle blood flow is increased from 5 ml /100 gm/ min to 80 ml/100 gm/min during severe exercise. The skeletal muscle arterioles are supplied by the sympathetic cholinergic nerve fibers, which cause dilatation of the arterioles and increase in the blood flow. There is also release of vasodilator metabolites in the muscles during exercise, which also cause dilatation of arterioles and precapillary sphincters.

Arteriovenous O_2 difference

The extraction of oxygen by the muscle during exercise is greatly increased. It is increased to 15 ml/100 ml of blood in severe exercise. Maximal oxygen consumption is increased in trained atheletes.

Anaerobic threshold

This is defined as the minimal level of exercise, which shifts the metabolism from aerobic to anaerobic type. In a trained athlete, the anaerobic threshold is greater.

Respiratory changes

Respiratory minute volume is increased linearly, with the work rate, more so in a trained individual. In spite of increase in oxygen consumption and increased carbon di oxide production, the alveolar and arterial partial pressures of these gases remain the same as in a person at rest. The increase in cardiac output increases the pulmonary blood flow by opening more capillaries and this increases the perfusion of alveoli. The diffusion of oxygen from alveoli to the pulmonary capillaries is also increased, because of greater perfusion of alveoli.

Changes in muscle

The endurance training results in increase of blood volume, hemoglobin, capillary density in the muscle, myoglobin, mitochondrial enzymes, etc. These changes leads to increase in oxygen delivery and greater oxygen utilization. The muscle appearance also changes, especially, the white muscles become as red muscles due to endurance training.

After exercise the heart rate, cardiac output and oxygen consumption do not come back to normal level immediately. There is a gradual return to normal level depending upon the severity of exercise performed. The slow recovery is necessary to clear the oxygen debt, which occurs during exercise.

CIRCULATORY SHOCK

Circulatory shock includes four varieties and in all of them there is a common symptom, which is the inadequate tissue perfusion. This could be due to low blood volume (hypovolemic shock), or decreased cardiac output despite normal blood volume, as a result of expansion of vascular system (low resistance shock). Shock can also be due to failure of the heart to pump blood as in cardiac failure or myocardial infarction (cardiogenic shock). Sometimes shock occurs due to obstruction to blood flow by embolism in the lungs or heart (obstructive shock).

Hypovolemic shock

This is the shock caused by loss of blood as in hemorrhage, trauma of the muscle and bone, surgery involving loss of blood, loss of plasma as in burns, etc. The immediate effect is fall in blood pressure and decrease in tissue oxygenation. The skin becomes cold and sometimes cyanosis occurs due to stasis of blood in the capillaries. Respiration is increased and becomes irregular. There will be intense thirst stimulation. These symptoms of hypovolemic shock appears, when blood volume decreases by 15% or 10 to 15 ml/kg of body weight.

The homeostatic regulatory mechanisms take place to maintain the tissue perfusion and arterial blood pressure. There is at first, immediate compensatory reactions, which are later followed by long-term compensatory mechanisms.

Immediate compensatory reactions

The immediate compensatory reactions include tachycardia, giving a rapid thready pulse and because of decreased cardiac output, the pulse is also of low volume in character. The baroreceptor mechanism activated by the fall in blood pressure, leads to vasoconstriction and rise in mean arterial blood pressure. Tachycardia also results due to this mechanism. There is secretion of adrenal medullary catecholamines, which also is responsible for the rise in blood pressure by vasoconstriction and increase in heart rate. There is increased formation of lactic acid, due to anerobic metabolism.The respiratory stimulation occurs by chemoreceptor stimulation, caused by the tissue hypoxia, hypercapnia and acidosis.

The increased secretion of angiotensin II stimulates the thirst center. It also stimulates the secretion of aldosterone, a mineralocorticosteroid, which helps to increase the blood volume by regulating the electrolyte balance.

Causes of hypovolemic shock

Hemorrhage
Surgery
Trauma
Burns.

Effects of hypovolemic shock

↓ Arterial blood pressure
↓ Tissue oxygenation
Cold pale skin with cyanosis
Tachycardia, rapid thready pulse
Irregular respiration
Thirst stimulation
Restlessness or torpor.

The increase in the tonicity of blood stimulates vasopressin secretion and this causes antidiuresis. The secretion of ACTH causes the secretion of glucocorticoid, which increases the vascular sensitivity to the circulating catecholamines. The fall in capillary hydrostatic pressure shifts the movement of fluid from the cell into the tissue spaces and then into the capillaries, in order to increase the blood volume.

Long-term compensatory mechanisms

The red cell mass increases due to increased production from red bone marrow. The increased erythropoietin secretion is responsible for this accelerated erythropoiesis. These changes are seen within 10 days and the red cell count becomes normal in 4 to 6 weeks time. There is entry of preformed albumin into the plasma from extravascular stores. The liver starts synthesizing plasma proteins leading to replacement of all the three types of plasma proteins.

Treatment of hypovolemic shock

The treatment involves the replacement of lost fluid in the case of plasma loss and in the case of blood loss, the transfusion of blood should be given. Plasma expanders also can be given to maintain the blood volume.

Refractory shock

It refers to the state where the cardiac output is not restored to normal even after restoring the blood volume to normal levels. The mechanisms that are involved in refractory shock are:

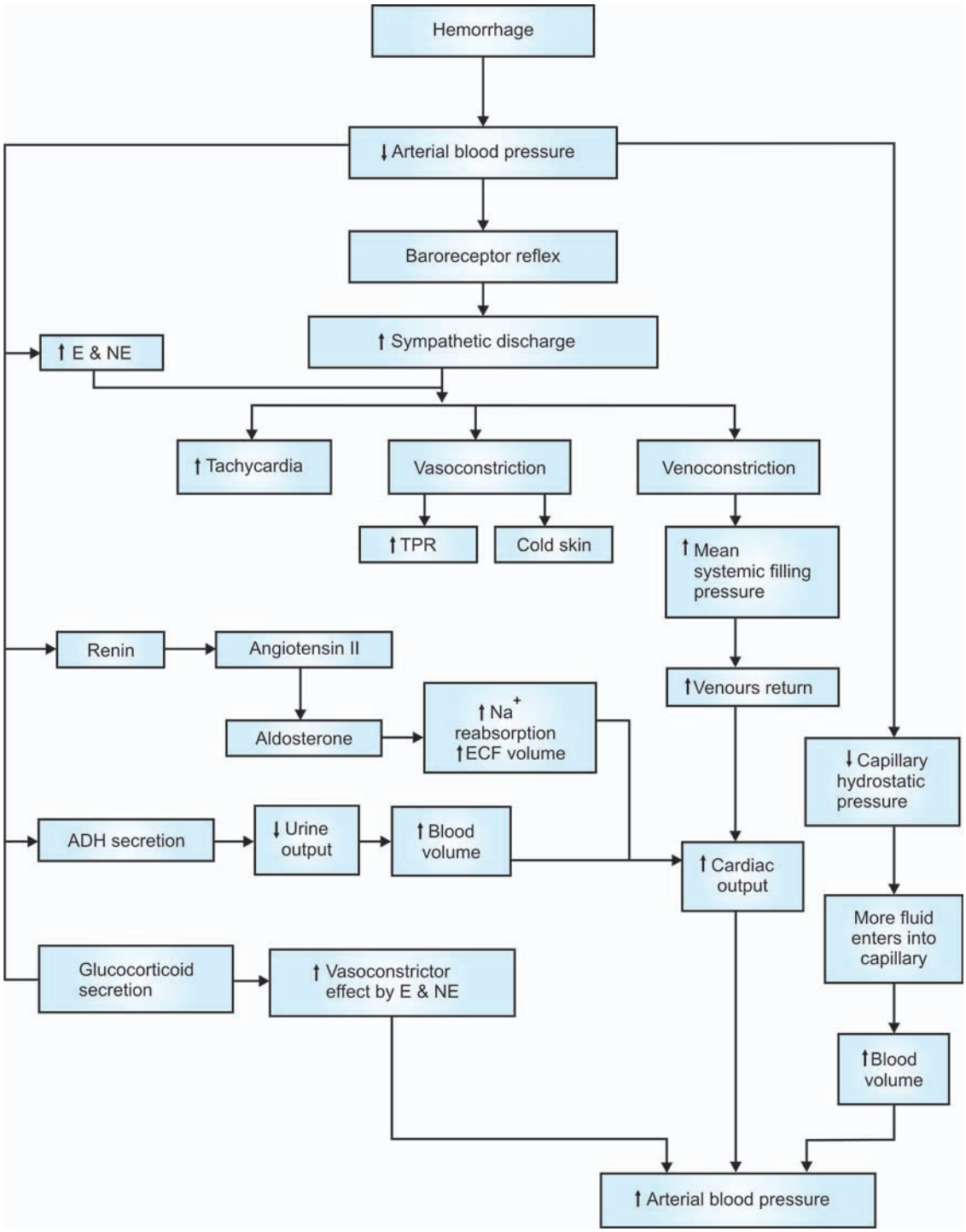
- Spasm of the precapillary sphincters and venules: This leads to the fluid leaving the vascular system in large amounts.
- Positive feedback mechanisms i.e., cerebral ischemia causing depression of vasomotor and cardiac center. Myocardial depression caused by reduced coronary flow and acidosis.
- Renal damage and renal failure caused by the reduced renal blood flow and perfusion pressure.
- Decrease in blood flow to GI tract leads to the development of endotoxic shock with the entry of bacterial toxins from the intestine.

The circulatory shock complications include **Acute Respiratory Distress Syndrome (ARDS)** or **Adult Respiratory Distress Syndrome**. When the shock becomes refractory, cytokines are released from the damaged capillary endothelial cells and alveolar epithelial cells which result in ARDS. The acute respiratory failure in ARDS can cause death. ARDS occurs in shock, severe trauma and sepsis.

Low resistance shock (Distributive shock)

It is also known as **warm shock** as there is increased vasodilatation. The common conditions for this shock include, anaphylaxis, sepsis and fainting due to neurogenic shock. The hypersensitivity reactions can result in anaphylaxis. The vascular capacity is increased due to profound vasodilatation caused by the release of histamine. This condition is also called as anaphylactic shock. The low resistance shock is also seen in septic shock, as the released bacterial toxins produce capillary dilatation and increase in its permeability. Another example of low resistance shock is neurogenic shock, where, due to sudden autonomic disturbance, there is

Compensatory mechanisms in hypovolemic shock



vasodilatation and pooling of blood in the veins leading to syncope. This is observed in strong emotional reactions such as fear or grief. The low resistance shock can be treated by administration of vasopressor agents such as epinephrine.

Cardiogenic shock

It is caused by cardiac failure as in myocardial infarction or chronic cardiac failure involving either the right or the left ventricle.

CARDIAC FAILURE

Failure of the heart occurs when the ventricle pumping action is reduced. This happens when the contractile power of the ventricle is decreased, as in myocardial infarction or increase in afterload or lung diseases. Strictly speaking, the cardiac failure refers to the ventricular failure. The ventricles have a large reserve capacity to pump blood. It can increase the output from 5 lit/min to 25 lit/min, when the demand for increased output arises, as in muscular exercise. This inherent ability of the ventricles to meet the increased demand helps the individual in the initial stages of failure to pump blood without any problem during rest, but he can not increase the output, when body activity increases. This type of failure is called compensated condition. If the ventricular contractile power is further reduced or the demand for a greater contractile action of the ventricle is increased, the cardiac failure leads to decompensated state.

Cardiac failure can start with the failure of one ventricle, which finally leads to the failure of the other ventricle and such a condition is called congestive cardiac failure.

In left ventricular failure, the backward build-up of pressure leads to pulmonary congestion and rise in pulmonary capillary pressure. This leads to pulmonary edema and orthopnea. There is tissue hypoxia and symptoms produced by this condition also will be seen. If the left ventricular failure is not treated, the backward rise in pressure from the left ventricle goes to the pulmonary circulation, which finally leads to the right ventricular failure.

Right ventricular failure occurs due to diseases affecting the lungs or mitral stenosis. The right ventricular failure, which occurs secondary to lung disease, is called cor pulmonale. The right ventricular failure shows a number of clinical signs and only a few symptoms. The signs are the edema of the dependent parts like pedal edema, increase in jugular venous pressure, enlargement of the liver and collection of fluid in the peritoneal cavity called ascites. These are due to rise in the venous pressure caused by the backward pressure transmitted from the failing right ventricle. In the long run, the right ventricular failure leads to left ventricular failure caused by the pressure build up in the pulmonary vascular bed.

The treatment of cardiac failure is aimed to correct the physiological dysfunction. The digitalis use helps to increase the contractile power of ventricles. The diuretics and restriction of salt in the diet relieves edema. The after load to the heart (arterial hypertension) is reduced by using ACE inhibitors (angiotensin converting enzyme inhibitors) or ACE receptor blockers.

GRAVITY INDUCED CHANGES IN CVS

When posture is changed from recumbent to upright, the venous pressure increases in the leg vein to 90 mmHg. In the head, the mean arterial blood pressure falls to 70 mmHg, with the venous pressure falling to zero at the base of the brain. Within the cranium, the sagittal sinus venous pressure becomes subatmospheric (–10 mmHg).

There is a compensatory reflex mechanism to maintain the normal cardiac output and perfusion pressure to the brain. The fall in arterial blood pressure in the standing posture, through the baroreceptor mechanism causes increase in heart rate and rise in arterial blood pressure. There is also increased secretion of renin and

Postural hypotension leading to syncope can be seen in a number of conditions such as:

- Sudden standing especially in old age
- Diabetes mellitus
- Autonomic insufficiency
- Syphilis
- Sympatholytic agents

aldosterone, which help to increase the blood pressure.

Prolonged standing for several hours without any movement can cause insufficient blood flow to the brain, in spite of baroreceptor compensatory reflex mechanism. This results in giddiness and unconsciousness **(syncope)**. The reason for the reduced cerebral blood flow is, due to venous pooling in the lower extremities, which reduces the cardiac output. There will also be fluid accumulation in the interstitial spaces of legs, as a result of increased hydrostatic pressure in the capillaries. These effects of gravity can be severe, if there is low blood volume.

Effects of acceleration and deceleration on CVS

Acceleration

The gravitational force is exerted from head to feet. It causes positive force (+g). Positive g up to 5 can be tolerated in humans. Beyond 5 g force, there will be black out. The vision fails and unconsciousness results. The positive g, causes reduction in cerebral blood flow, as more blood flows into the lower parts of the body.

Deceleration

The g force acts from feet to head, which is a negative g. It causes an increase in cerebral blood pressure. There is also simultaneous increase in the intracranial tension to prevent the rupture of vessels. There is more blood flow to the head region resulting in red out. The vascular congestion of the head and neck, throbbing pain in the head, ecchymoses in the eye region can be observed. Humans tolerate better, if gravity is exerted across the body (chest to back or back to chest) rather than in the axial position.

Self-study Questions

Multiple Choice Questions

Choose the single best answer

1. **The plateau of action potential recorded from ventricular muscle is caused by opening of which voltage gated channel?**
   A. Na^+
   B. K^+
   C. Slow Ca^{++}
   D. Long lasting Ca^{++}

2. **The release of acetylcholine from vagal endings on the heart increases the conductance for:**
   A. Na^+ **B.** K^+
   C. Ca^{++} **D.** Cl^-

3. **Autorhythmicity of cardiac muscle is demonstrated in:**
   A. Refractory period
   B. All or none law
   C. Starling's law
   D. Pacemaker potentials

4. **Capacitance vessels are:**
   A. Veins
   B. Arteries
   C. Arterioles
   D. Capillaries

5. **Which interval of the ECG can be used to determine heart rate?**
   A. PR **B.** RR
   C. ST **D.** QT

6. **The normal pressure in the right atrium is close to:**
   A. 0 mmHg **B.** 8 mmHg
   C. 10 mmHg **D.** 20 mmHg

7. **If preload to the heart is increased, rise in each of the following will occur *except:***
   A. Heart rate
   B. Cardiac output
   C. End systolic volume
   D. End diastolic volume

8. **The strength of pulse pressure depends on:**
   A. Stroke volume
   B. Heart rate
   C. Arterial compliance
   D. A and C

9. **Ejection fraction is increased in:**
   A. Muscular exercise
   B. Myocardial infarction
   C. Hemorrhage
   D. Myocarditis

10. **Stimulation of baroreceptors would cause a rise in:**
    A. Vasomotor tone
    B. Vagal discharge
    C. Heart rate
    D. Peripheral resistance

11. **Systolic pressure is maintained by:**
    A. Total peripheral resistance
    B. Cardiac output
    C. Venous return
    D. Windkessel effect

12. **Tachycardia which occurs due to increase in venous return is caused by:**
    A. Sino aortic reflex
    B. Cushing reflex
    C. Bainbridge reflex
    D. Bezold Jarisch reflex

13. **A positive inotropic effect on the heart causes increase in:**
    A. Preload
    B. Afterload
    C. Force of contraction
    D. Rate of contraction

14. Atrial systole produces:
- **A.** P wave of ECG
- **B.** III heart sound
- **C.** C wave of venous pulse
- **D.** IV heart sound

15. In which type of circulatory shock, warm skin is observed?
- **A.** Hemorrhagic
- **B.** Cardiogenic
- **C.** Obstructive
- **D.** Anaphylactic

16. Total peripheral resistance depends on all of the following *except*:
- **A.** Elasticity of the vessel
- **B.** End diastolic volume
- **C.** Caliber of the vessel
- **D.** Viscosity of the blood

17. Transmission of cardiac impulse is faster in:
- **A.** SA node
- **B.** AV node
- **C.** Bundle of His
- **D.** Purkinje fibers

18. In the first degree heart block:
- **A.** PR interval is shortened
- **B.** PR interval is lengthened
- **C.** ST segment is elevated
- **D.** QT interval is increased

19. Change of posture from lying down to standing shows increase in all of the following *except:*
- **A.** Blood pressure
- **B.** Heart rate
- **C.** Vagal tone
- **D.** Peripheral resistance

20. Coronary blood flow is least in which phase of the cardiac cycle?
- **A.** Ventricular filling
- **B.** Isovolumetric contraction
- **C.** Isovolumetric relaxation
- **D.** Ejection

21. Maximum fall in pressure occurs in which segment of the vascular system?
- **A.** Aorta
- **B.** Arteries
- **C.** Arterioles
- **D.** Veins

22. Blood flow in a vessel is increased by all of the following *except*:
- **A.** Increase in pressure gradient
- **B.** Decrease in viscosity of blood
- **C.** Decrease in radius of vessel
- **D.** Decrease in vasomotor tone

23. Which of the following is likely to cause bradycardia?
- **A.** Moderate exercise
- **B.** Hypovolemia
- **C.** Carotid sinus massage
- **D.** Vagal inhibition

24. A diastolic murmur is likely to occur in:
- **A.** Aortic stenosis
- **B.** Mitral stenosis
- **C.** Pulmonary stenosis
- **D.** Ventricular septal defect

25. During exercise an increase does not occur in:
- **A.** Systolic pressure
- **B.** Heart rate
- **C.** Cardiac output
- **D.** Peripheral resistance

26. R wave of ECG precedes:
- **A.** I heart sound
- **B.** II heart sound
- **C.** III heart sound
- **D.** IV heart sound

27. Vasoconstriction is caused by:
- **A.** Histamine
- **B.** Angiotensin II
- **C.** Bradykinin
- **D.** Nitric oxide

28. T wave of ECG is caused by which of the following ion currents in the ventricle?

A. K^+ influx **B.** K^+ exit
C. Na^+ influx **D.** Na^+ exit

29. Stimulation of vagus nerve to the heart would decrease the conduction of impulses in :

A. SA node
B. Bundle of His
C. Ventricular myocardium
D. AV node

30. Intravenous infusion of fluid will not improve the condition of which type of shock?

A. Hemorrhagic
B. Anaphylactic
C. Traumatic
D. Burn

ANSWER KEYS

1. (C)	2. (B)	3. (D)	4. (A)	5. (B)	6. (A)	7. (C)	8. (D)	9. (A)	10. (B)
11. (B)	12. (C)	13. (C)	14. (D)	15. (D)	16. (B)	17. (D)	18. (B)	19. (C)	20. (B)
21. (C)	22. (C)	23. (C)	24. (B)	25. (D)	26. (A)	27. (B)	28. (B)	29. (D)	30. (B)

Short Answer Questions

1. State what happens to the blood flow and pressure when the radius of the arterial blood vessels is reduced.
2. State the factors that determine peripheral resistance.
3. Explain how the blood flow is laminar in capillaries.
4. Describe the pace maker potential and state its significance.
5. Describe the action potential recorded from ventricular muscle and state its ionic basis of development.
6. List the factors that reduces the force of contraction of the heart.
7. Describe the action of epinephrine on the heart.
8. Describe the conducting system of the heart and mention the significance of nodal delay.
9. Describe PR interval of ECG and state its importance.
10. Describe the importance of ST segment in ECG recording.
11. Define ejection fraction. List the factors that reduce it.
12. Define preload and afterload on the heart. State conditions that cause increase in them.
13. State the causes of heart sounds and mention how cardiac murmurs are produced .
14. List the factors that regulate cardiac output.
15. Describe the sinoaortic reflex and its importance in the body.
16. State the factors that determine arterial blood pressure.
17. State the factors that help in the long term regulation of arterial blood pressure.
18. Describe how local blood flow is regulated.
19. Describe how coronary blood flow is regulated.
20. List the compensatory mechanisms that occur in hypovolemic shock.

8 Respiratory Physiology

The main objective of respiration is to exchange respiratory gases namely, oxygen and carbon di oxide. The exchange of gases between lungs and outside environment forms the external respiration, while the exchange of gases, between capillaries and tissues forms the internal respiration.

Functional anatomy of respiratory system

The respiratory system consists of nostrils, nasopharynx, larynx, trachea, bronchi, bronchioles, alveolar duct and alveoli. The structures included from nostrils to terminal bronchioles have 16 divisions, while the remaining structures show 7 divisions, giving a total of 23 divisions for the respiratory tract. The divisions are formed by the repeated branching of each segment of the tract.

Structures like trachea and main bronchi have cartilage in their wall and lined by pseudostratified columnar epithelial cells. The cartilage gives patency to the tract and the ciliated columnar epithelial cells perform escalator function, which pushes the dust particles towards pharynx. There are also goblet cells and mucus secreting glands, which secrete mucus. The mucus secretion helps in clearing foreign bodies and dust particles.

Trachea divides into right and left bronchus, which in turn divides into many divisions of bronchioles, to end in terminal bronchioles. The terminal bronchioles divide into respiratory bronchioles, alveolar duct and alveoli. These three form the respiratory part of the divisions, where gaseous exchange occurs.

As one proceeds from trachea to terminal bronchioles, the cartilage presence in the wall diminishes and the content of smooth muscle increases. The medium sized bronchioles contain more smooth muscle and can give greater airflow resistance. The smooth muscle of the bronchioles is supplied by the parasympathetic nerve vagus. It causes broncho-constriction. The sympathetic supply to the bronchioles is not present, but contains adrenergic receptors. The β_2-adrenergic receptor activity caused by agents like adrenaline, gives bronchial relaxation.

The smooth muscle in the bronchioles is also acted upon by peptides like *neurokinins A and B, substance P, VIP, histamine, prostaglandin* E_2. Substance P, neurokinins, histamine and prostaglandin E_2 cause bronchoconstriction, while, VIP causes bronchial relaxation.

The respiratory tract mentioned above performs the following functions:

- Conduction of inspired gases
- Filtration of dust and macromolecules
- Humidification and warming of inspired air.

Alveoli

The alveoli form the unit of respiratory system. They are air sacs, lined by two types of cells. Type I are the lining cells, while **Type II** is called **alveolar pneumocytes**. They secrete a phos-

pholipid substance, which acts as a surfactant. The alveoli are supplied by pulmonary capillaries, where gaseous exchange takes place. The alveoli also contain macrophages and mast cells. The macrophages show phagocytic action, while mast cells secrete histamine in allergic and hypersensitivity reactions.

Nonrespiratory functions of lungs

The lungs besides its respiratory function show a number of metabolic and synthetic functions. They form the nonrespiratory function of lungs. It includes:

- Activation of angiotensin I to angiotensin II by angiotensin converting enzyme (ACE),
- Inactivation of vasopressor substances like vasopressin, epinephrine, serotonin,
- Synthesis of lung surfactant and secretion of prostaglandins.

MECHANICS OF RESPIRATION

The process of respiration includes inspiration and expiration. Inspiration is an active process, whereas, expiration is passive. However, forced expiration becomes active. The muscles of inspiration are:

Diaphragm

External intercostals

The main inspiratory muscles are diaphragm and external intercostal. *Scalene and sternocleido mastoid* are accessory inspiratory muscles, which are involved in forceful inspiration.

The normal expiration is passive, as it occurs by the relaxation of inspiratory muscles. Forceful expiration involves the contraction of two types of muscles namely:

Internal intercostals

Abdominal muscles

During inspiration the thoracic cavity enlarges in three dimensions namely:

Vertical

Transverse

Anteroposterior

The contraction of diaphragm is brought by phrenic nerve activity and the downward movement of diaphragm enlarges the thoracic cavity by 70%. Hence, diaphragm is considered as an important respiratory muscle. Paralysis of intercostal muscles does not affect respiration as much as the paralysis of diaphragm.

The contraction of external intercostal muscles pushes the sternum forward and upward, which increases anteroposterior dimension (pump handle movement). The upward and outward pulling of ribs increases transverse dimension of the thoracic cavity (bucket handle movement).

Intrapleural pressure

During respiration, there are pressure and volume changes in the alveoli. The lungs are lined by the visceral pleura, whereas, the thoracic wall is lined by the parietal pleura. The space between these two is the intrapleural space. There is a thin serous fluid present in the space, which gives hydraulic traction between these two layers. This makes them inseparable. The pressure between these two layers is called intrapleural pressure (Fig. 8.1). The intrapleural pressure is **subatmospheric**.

How is intrapleural pressure created?

The first breath, which occurs at the time of birth, establishes a negative pressure in the intrapleural space. The lungs, because of its elastic structure, try to recoil, while the chest wall tends to pull

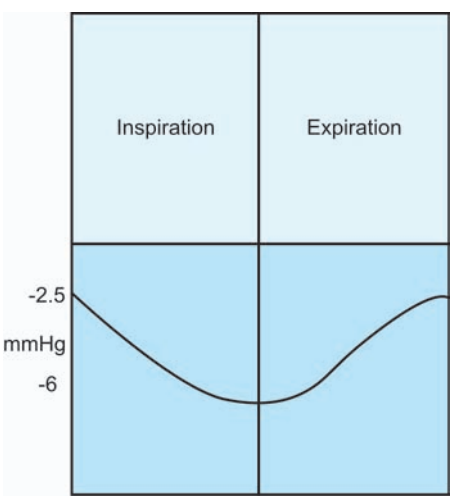


Fig. 8.1: Intrapleural pressure during respiration

outwards at the end of quiet expiration, creating a negative pressure in the intrapleural space.

Intrapleural pressure during respiratory cycle

The intrapleural pressure is – 2.5 mmHg at the beginning of inspiration and becomes – 6 mmHg at the end of inspiration. During expiration, it becomes less negative and reaches the preinspiratory level. Forceful inspiration causes intrapleural pressure to become more negative, reaching up to – 30 mmHg. The negative intrapleural pressure prevents the collapse of alveoli during expiration. Further, it makes the alveoli distended during inspiration. This is the major function of intrapleural pressure, which gives stability to the lungs. Pneumothorax refers to the entry of air into the intrapleural space. The lung collapses in such a condition.

Measurement of intrapleural pressure

The intrapleural pressure can be measured by introducing into the mouth, a catheter with an inflatable balloon at the tip and positioned in the mid esophageal region. The other free end is connected to a pressure transducer for recording the pressure changes. Since the esophageal pressure reflects the intrapleural pressure, this method is considered accurate.

Effect of gravity on intrapleural pressure

In standing posture, the effect of gravity on intrapleural pressure can be seen. There is a gradient from the basal part of lungs to the apex, with the apex showing more negative pressure (– 10 mmHg) and the basal part having less negative pressure (– 2 mmHg) (Fig. 8.2). That's why, the apical portion has more resting volume of air, but less ventilated, as compared to the basal part of lungs.

Intrapulmonary pressure

The alveoli also show pressure changes during respiratory cycle. The pressure in the alveoli is called intrapulmonary pressure. During inspiration it becomes negative (–1.5 mmHg) and hence, allows outside air to fill the alveoli. At the end of inspiration, intrapulmonary pressure becomes atmospheric. During expiration, the elastic recoiling of lung gives a positive pressure (+1.5 mmHg), which facilitates expulsion of air from lungs (Fig. 8.3).

Transpulmonary pressure

The difference between intrapleural pressure and intrapulmonary pressure is called transpulmonary pressure. This measurement is used for determining compliance of lungs. Transpulmonary pressure reflects the difference between the pressure inside and outside of the lungs. In inspiration, it goes up to 30 cms of H_2O at the maximum level (100% lung volume). The trans lung pressure becomes negative at low lung volumes.

Pressure-volume relationship in lungs

Compliance of lungs

The change in volume of lungs (ΔV) per unit change in airway pressure (ΔP) is called compliance. It is the reciprocal of elastance.

$$\text{Compliance} = \frac{\Delta V}{\Delta P}$$

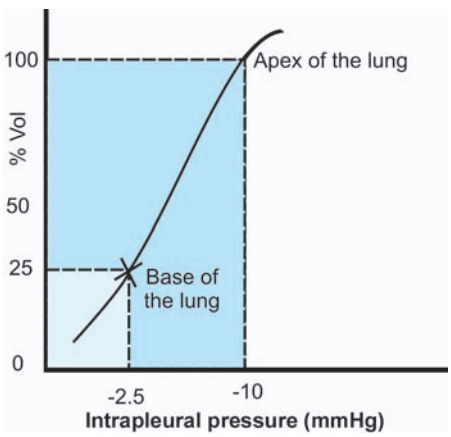


Fig. 8.2: Intrapleural pressure in the erect lung. The base of the lung has less resting volume of air and hence better ventilated. The apex of the lung has more resting volume and poorly ventilated. The intrapleural pressure also varies in respect of percentage volume of air in lungs due to gravity

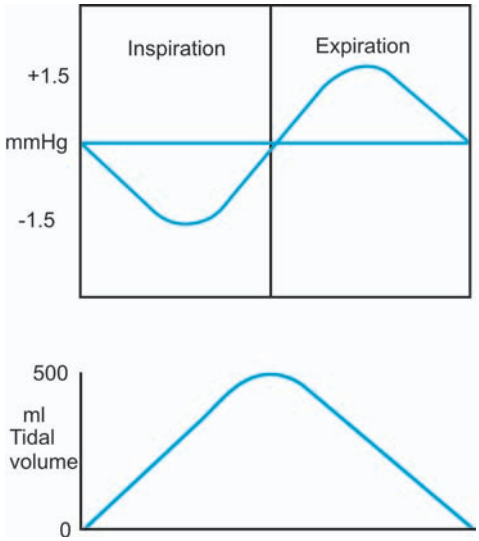


Fig. 8.3: Intrapulmonary pressure and volume changes in lungs. The amount of air that is taken in or out in a quiet respiration forms the tidal volume

Compliance refers to the stretchability of the lungs. It is the reciprocal of elastance. The compliance when measured alone for the lungs, the value is 0.2 L/cm H_2O. If total pulmonary compliance is measured, which includes lung and chest wall, the value becomes less. It is 0.1 L/cm H_2O. The latter is less because of the viscous resistance of the chest wall. When compliance is measured from the relaxation volume (functional residual capacity) it gives static compliance (Fig. 8.4). It measures the elastic recoil of lungs and chest. The slope of the curve obtained, shows, compliance being less at the top of the slope and more at the bottom.

Compliance, which measures the resistance of the lung and chest forms the dynamic compliance, which also includes the airway resistance.

The pressure volume curve in a normal respiration shows a loop called hysteresis loop (Fig. 8.5). The reason for this loop is, that inspiration and expiration curves do not meet each other. At any given transpulmonary pressure, the volume is more in expiration than inspiration. The deflation curve of the loop also shows that

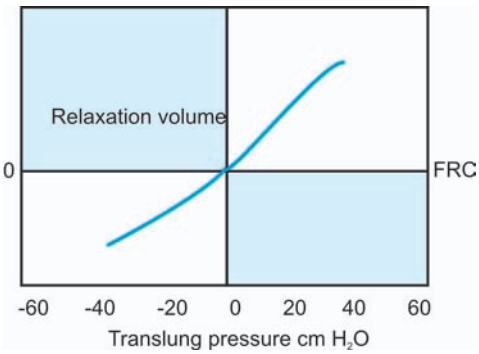


Fig. 8.4: Measurement of compliance from relaxation volume curve. Compliance is less at the top of the slope of the curve than at the bottom

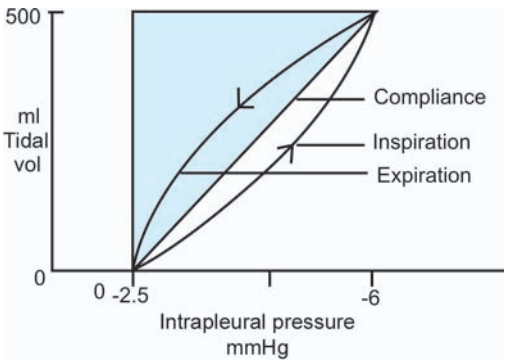


Fig. 8.5: Pressure volume changes during respiratory cycle. Hysteresis loop is obtained, showing that compliance is better in expiration than during inspiration

with a small pressure rise, large volume increase is possible and similar change is not possible during inflation, because of greater frictional resistance to airflow in inspiration. Clinically specific compliance is determined. This is the expression of compliance in relation to lung volume.

$$\text{Specific compliance} = \frac{\text{Compliance}}{\text{FRC}}$$

Decrease in compliance of lungs occurs in:
Fibrosis of lungs
Pneumothorax
Pulmonary edema
Obstruction to respiratory passages
Paralysis of respiratory muscles.

Compliance in emphysema

In emphysema the static compliance is increased, but the dynamic compliance is reduced. In emphysema, the elastic tissue is lost and hence the resting volume of the lung becomes greater.

Lung surfactant

The inner surface of the alveoli is lined by a serous fluid. The air-fluid interface gives surface tension, causing the smaller alveoli to empty into the larger ones and finally results in collapse of alveoli. This can occur especially during expiration, when the radius of alveoli becomes less and the surface tension becomes more. Such a thing does not happen, because of the presence of a surface tension reducing agent called **surfactant** in the alveoli.

The surfactant is a phospholipid with a lipoprotein moiety. It is made of dipalmitoyl phosphatidyl choline. It is secreted by **Type II cells** lining the alveoli called alveolar pneumocytes. The surfactant is present between the air-fluid interface and reduces the surface tension, produced at the air fluid interface. During inspiration, the alveoli expand and the fluid also is distributed in the expanded surface. The surface tension becomes less, because of increase in diameter of alveoli. During expiration, the radius of alveoli is reduced, the fluid is concentrated more in alveoli and can exert a greater surface tension. But the presence of surfactant, prevents this happening. The Laplace law gives the relationship between the distending pressure and radius.

$$P = \frac{2T}{r}$$

Distending pressure is directly related to the wall tension and inversely to the radius. In the smaller alveoli, the tension in the wall increases and greater distending pressure is required to keep it expanded. If there is an agent that can reduce the surface tension when the alveoli becomes smaller during expiration, the tendency of smaller alveoli opening into larger alveoli cannot occur. It also will prevent the collapse of alveoli during expiration. Surface tension in the alveoli disappears, if instead of air, fluid (saline) is filled in the lungs. The pressure volume curve for saline filled lung is to the left of the air filled lungs and no hysteresis loop occurs (Fig. 8.6).

Functions of surfactant

The presence of surfactant in alveoli ensures:

- Stability of alveoli by preventing its collapse in expiration
- Reduces work of breathing
- Keeps the alveoli dry and prevents pulmonary edema (prevents fluid exit from pulmonary capillaries)
- Reduces airway resistance
- Increases the compliance of lungs.

Deficiency of surfactant in newborn causes **respiratory distress syndrome** or hyaline membrane disease. The collapse of alveoli gives atelectasis.

Production of surfactant

Surfactant production by type II cells requires the hormone thyroxine and maturation of surfactant depends on the normal level of cortisol. Deficiencies of these hormones also can lead to respiratory distress and atelectasis.

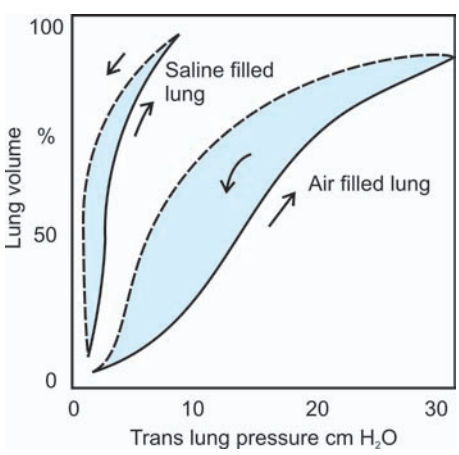


Fig. 8.6: Pressure volume relations in the lung filled with saline and air. In saline filled lung, there is no surface tension and hence at the low pressure itself, the lung is filled with the air. In the case of air filled lung, the air fluid interface causes surface tension and that's why the hysteresis loop occurs

Interdependence of alveoli for stability

The stability of alveoli is also given by inter-dependence. When collapse of alveoli occurs in one part of lungs, the neighbouring alveoli expand, preventing its collapse.

Airway resistance

Airway resistance is present in medium sized airways as the resistance depends on the diameter of the airways. The smaller airways do not show resistance, due to the fact, that the total cross-sectional area of small airways is much larger. The larger airways, because of increase in lumen size, do not show resistance. *Airway resistance depends on the rate of flow of air, lung volume and airway diameter. The latter is influenced by bronchial muscle tone, local release of chemicals and drugs*. Airway resistance is considerably increased in chronic obstructive respiratory diseases like asthma.

Work done during breathing

During respiration, work is done to overcome three types of resistance namely, **elastic resistance (65%), airway resistance (28%)** and **viscous resistance (7%)**. The viscous resistance refers to the tissue resistance coming from the chest wall. The work done during breathing is increased in muscular exercise and in conditions where compliance of lungs is reduced.

DEAD SPACE

In normal respiration the tidal volume is about 500 ml. This is the amount of air that is inspired or expired during quiet breathing. During inspiration out of 500 ml of air that is taken in, only 350 ml reaches alveoli. The remaining 150 ml, stays in the respiratory passages from nose to terminal bronchioles and this forms the **anatomical dead space**. The volume of air present in anatomical dead space does not take part in gaseous exchange.

Anatomical dead space can be determined by **single breath N_2 analysis (Fowler's method)**. In this, the subject inhales 100% O_2 and then exhales into a nitrogen meter. The dead space air will be the volume present in the first and mid point of second phase (Fig. 8.7).

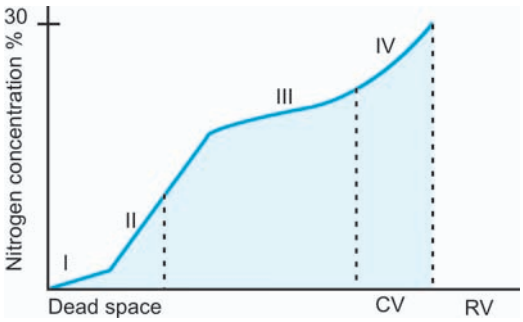


Fig. 8.7: Single breath N_2 analysis to determine anatomical dead space. CV: closing volume. It is the volume at which the alveoli in the basal part begins to close. It is 10% of vital capacity in normal subjects. RV: Residual volume

Physiological dead space

There is also alveolar dead space, which refers to the part of alveoli not perfused well. The ventilation in those areas of lungs, becomes a wasted ventilation and it is called alveolar dead space. Total dead space (physiological dead space) includes anatomical dead space and alveolar dead space. In normal health, alveolar dead space does not occur. Therefore physiological dead space is same as that of anatomical dead space. But, in conditions, where the alveoli are poorly perfused, the ventilation becomes wasted or in mismatched ventilation perfusion ratio, the physiological dead space becomes significant (Fig. 8.8). This can happen in *venous arterial shunt, pulmonary embolism, pulmonary fibrosis and pulmonary edema*.

Physiological dead space (Total dead space) can be determined by Bohr's method in which pCO_2 in arterial blood and expired air is determined.

Bohr Equation

$$\text{Total Dead Space} = \frac{V_1(PaCO_2 - PECO_2)}{PaCO_2}$$

V_T – Tidal Volume

$PaCO_2$ – Partial pressure of CO_2 in the arterial blood

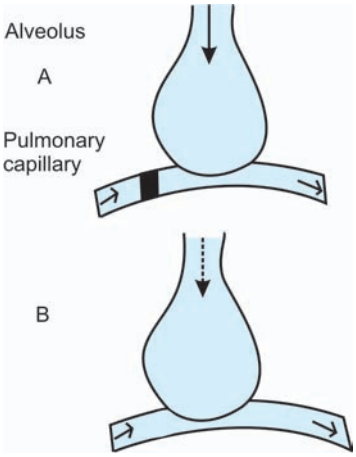


Fig. 8.8: Diagrammatic representation of mismatched ventilation perfusion in lungs. The unequal ventilation and perfusion is responsible for physiological dead space A: Ventilated but poorly perfused alveolus. The ventilation is a wasted one in this. B: Well perfused but poorly ventilated. Both the types can give rise to physiological dead space

$PECO_2$ – Partial pressure of CO_2 in the expired air

$$= \frac{500\ (40\ mmHg - 28\ mmHg)}{40\ mmHg} = 150\ ml$$

Dead space is the amount of air that does not take part in the gaseous exchange. This anatomical dead space refers to the volume of air that remains in the air passage from nose to terminal bronchioles. It is 150 ml. If the subject has to respire through a long tube connected to his nose, then his anatomical dead space will correspondingly increase.

Physiological dead space is a wasted ventilation caused by the mismatched ventilation perfusion ratio. The value becomes significant in conditions like:

Venous arterial shunt
Pulmonary embolism
Pulmonary fibrosis
Pulmonary edema.

ALVEOLAR VENTILATION

It is the amount of air that is reaching the alveoli per minute. It is calculated as

Alveolar ventilation
= (TV – Dead space) Resp. rate
(V_A) = (500–150)12
= 4200 ml/minute

Ventilation-perfusion ratio

The pulmonary blood flow that is perfusing the lungs is 5 lit/min (Q).

$$\text{Alveolar ventilation-perfusion ratio} = \frac{V_A}{Q}$$

$$= \frac{4.2}{5}$$

$$= 0.8$$

The ratio is not uniformly present in lungs, due to the effect of gravity. In an erect lung, the basal part of the lungs is well perfused, but not ventilated and hence the ventilation: perfusion ratio is low (0.6). At the apex of the lungs, the ventilation is better, than the perfusion and this zone gives a high ventilation: perfusion ratio (3.5) (Fig. 8.9).

Since the basal part of the lungs has less negative intrapleural pressure, the resting volume of air is less and in this part the smaller airways close in forceful expiration and the intrapleural pressure can even become positive in such conditions.

At the apex of the lungs the intrapleural pressure is more negative, indicating an increased resting volume of air. This region of the lungs is also poorly perfused. The oxygen rich air in this zone is conducive for the growth of *Mycobacterium bacilli*.

PULMONARY CIRCULATION

Pulmonary circulation is present between right ventricle and left atrium, giving a low-pressure circulation. The lungs receive the entire cardiac output, i.e., 5 lit/min. The right ventricular ejection occurs at a low pressure, as there are no resistance

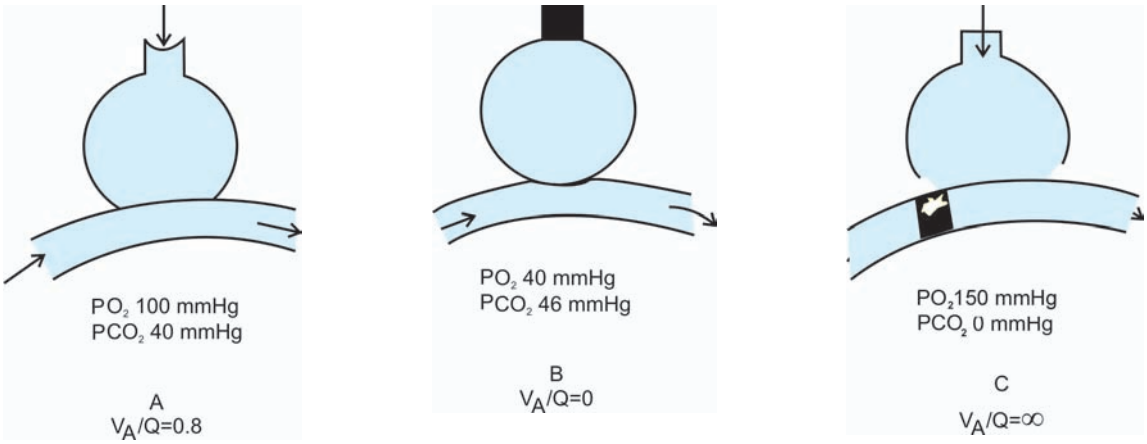


Fig. 8.9: Ventilation perfusion ratio and its effect on the gas tensions in the alveoli. A: Normal V_A/Q. B: Decreased ventilation perfusion ratio, due to block of alveolus. The PO_2 in the alveolus becomes reduced and PCO_2 rises. C: Increased ventilation perfusion ratio due to block in the capillary. The PCO_2 becomes less and PO_2 is increased

vessels similar to systemic circulation. The pulmonary artery systolic pressure is 25 mmHg and the diastolic pressure is 10 mmHg. This gives a mean pressure of 15 mmHg in pulmonary arteries. Pulmonary arteries divide and open into arterioles, which are not as thick as in systemic circulation. The hydrostatic pressure in pulmonary capillaries is around 7 mmHg and this low pressure prevents exit of fluid from the capillaries. This is necessary as fluid accumulation in the interstitial space of lungs would affect diffusion of gases and also limits its expansion. In congestive heart failure, when the pressure on the left side heart rises, the pulmonary capillary pressure also would rise to result in pulmonary edema.

The pulmonary capillary pressure can be approximately known from **pulmonary wedge pressure** determination, which also reflects left atrial pressure. Left atrial pressure ranges from 1mmHg to 5 mmHg with the average at 2mmHg.The measurement of pulmonary wedge pressure involves inserting a catheter into a peripheral vein and pushing it to the right heart, then to the pulmonary artery and finally into the small branch of an artery, where the tip of the catheter gets wedged. The pressure measured at this point will be close to the pulmonary capillary pressure and it will be slightly higher. The normal **pulmonary wedge pressure is around 5 mmHg**. In left heart failure the pulmonary wedge pressure is increased.

Blood flow in different zones of lungs

Blood flow in pulmonary capillaries in different parts of the lungs depends on the mean pulmonary arterial pressure, pulmonary vascular resistance and gravity (Fig. 8.10). In erect lung as in standing posture, the lung can be divided into three zones.

Zone I is the apex of the lung and has high ventilation-perfusion ratio. The perfusion of this zone is less, but the resting volume of air is greater. The alveolar pressure (PA) is higher than pulmonary arterial pressure (Pa) **(PA> Pa>Pv)**.

In **zone II**, the blood flow is determined by the pulmonary arterial pressure (Pa) as it is higher than alveolar pressure (PA).However, the venous pressure (Pv) is lesser than the alveolar pressure and hence the compression of capillaries reduces the blood flow in the arteries **(Pa>PA> Pv)**.

In **zone III** which is the basal part of the lung, the perfusion is high. The blood flow is determined by the arterio-venous pressure difference. **(Pa>Pv>PA)**.

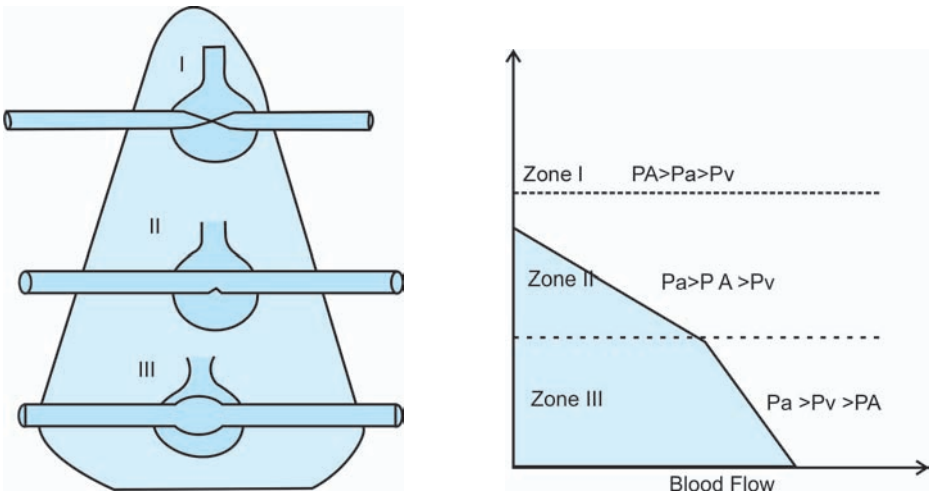


Fig. 8.10: Blood flow in different zones of an erect lung. At the apex, the alveolar pressure (PA) is greater than the arteriolar and venous pressures. It is poorly perfused. In the middle zone, the blood flow is normal as the arteriolar pressure is greater than the alveolar pressure. In lower zone, the flow is determined by arteriolar, venous pressure difference and the flow is greater here

Regulation of blood flow in lungs

Pulmonary blood flow is regulated by chemical factors. **Hypoxia** causes **pulmonary vascular constriction** and increases its resistance. This helps to divert the blood to other areas, where ventilation and oxygen are available. The alveolar hypoxia itself regulates the pulmonary blood flow. In other places, hypoxia, hypercapnia and acidosis cause dilatation of blood vessels and increase in blood flow, but, in lungs they show the opposite effect.

Release of chemicals locally such as thrombaxane causes vasoconstriction, whereas prostacyclin and NO give vasodilatation in pulmonary blood vessels.

Physiological shunt

The bronchial blood vessels supply blood to lung parenchyma. The venous blood from these, by passes pulmonary circulation and drains directly into left atrium. There is also thebesian vessel in coronary circulation, which directly drains into the left atrium. The direct draining of venous blood into the left atrium, gives physiological shunt and is about 2% in systemic arteries. The result of this is a slight fall in arterial PO_2. Instead of 100 mmHg, the partial pressure of oxygen in the arterial blood is 98 mmHg.

Pulmonary blood volume

In pulmonary circulation, 500 ml blood is present as blood volume. In erect posture, it is drained into the systemic circulation and in supine position, the volume is increased. The increase in pulmonary blood volume, lowers the vital capacity of the lungs.

DIFFUSION OF GASES

Gas diffusion across the pulmonary capillaries follows Fick's law of gas diffusion.

$$V = \frac{K\ (P_1 - P_2)\ A}{D}$$

V is the volume of gas that diffuses from one region to another.

K is the solubility coefficient of the gas, which is directly related to solubility of the gas in solution and inversely to the molecular weight of the gas.

P_1 and P_2 are the pressure gradient for the gas.

A refers to the surface area across which the transfer occurs.

D is the distance through which the gas diffuses.

The surface area of the alveoli is 70 m^2 and the thickness of the alveolar capillary membrane is 0.5 µ. PO_2 in the pulmonary capillaries is 40 mmHg and in the alveoli, it is 100 mmHg. This gives 60 mmHg pressure gradient for the diffusion of oxygen from the alveoli into the pulmonary capillaries (Fig. 8.11). The duration at which the diffusion of oxygen in the lungs takes place is 0.8 sec, and is reduced during exercise. The diffusion capacity of oxygen is **20 ml/min/mmHg** surface area.

Diffusion capacity of CO_2 is 20 times greater than oxygen. Carbon dioxide diffuses from the pulmonary capillaries into the alveoli. The PCO_2 in the alveolar capillaries is 46 mmHg and in alveoli 40 mmHg. The pressure gradient of 6 mmHg for CO_2 is sufficient for transfer of the gas, as CO_2 has more solubility in the body fluids, which facilitates faster diffusion of the gas. The diffusion capacity of CO_2 is **400 ml/min/mmHg**.

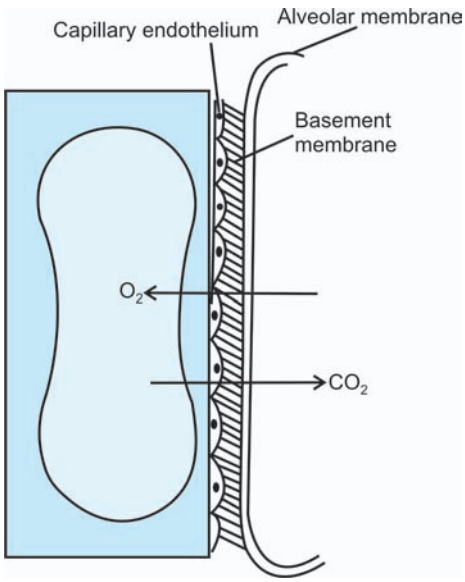


Fig. 8.11: Diagram to show the alveolar capillary membrane for the diffusion of gases

Factors affecting diffusion of gases

Diffusion of gases is affected in conditions where the alveolar capillary membrane is thickened **(alveolar capillary block)**. Examples of this can be seen in asbestosis and pneumoconiosis. Formation of pulmonary edema also affects the diffusion of gases. When diffusion of oxygen is reduced, it leads to hypoxemia.

Composition of gases

INSPIRED AIR

	Partial pressure	%Conc
Oxygen	153 mmHg	20.5
Carbon dioxide	0.5 mmHg	0.05
Nitrogen	596 mmHg	79
Water	4.5 mmHg	0.5

ALVEOLAR AIR

	Partial pressure	%Conc
Oxygen	100 mmHg	15
Carbon dioxide	40 mmHg	5
Nitrogen	573 mmHg	74
Water	47 mmHg	6

EXPIRED AIR

	Partial pressure	%Conc
Oxygen	116 mmHg	16.5
Carbon dioxide	28 mmHg	3.5
Nitrogen	569 mmHg	74
Water	47 mmHg	6

ARTERIAL BLOOD

	Partial pressure	%Conc
Oxygen	98 mmHg	14
Carbon dioxide	40 mmHg	5
Nitrogen	575 mmHg	75
Water	47 mmHg	6

MIXED VENOUS BLOOD

	Partial pressure	%Conc
Oxygen	40 mmHg	5.5
Carbon dioxide	46 mmHg	6.5
Nitrogen	575 mmHg	82
Water	47 mmHg	6

Collection of gases

Alveolar air is collected by Haldane-Priestly sampling tube and in that the last portion of air is collected and analysed for the alveolar air.

Expired air is collected by using Douglas bag. The gas tensions and composition are determined by Van Slyke gas apparatus.

TRANSPORT OF RESPIRATORY GASES

Transport of oxygen

Oxygen diffuses from the alveoli into the pulmonary capillaries along the pressure gradient. The gas diffuses into RBCs, where it combines with the Hb to form oxyhemoglobin. Oxygen as dissolved form in the plasma is negligible and the major form of transport is oxyhemoglobin.

Reactions between Hb and oxygen

The oxygen enters the Hb and binds to the iron present in the heme. There are four heme in each Hb molecule and each iron atom can take one molecule of oxygen. When oxygen enters the Hb, there are molecular interactions between iron and oxygen and the reactions proceed in a step wise fashion. The hemoglobin, which is partially saturated with oxygen, will show greater affinity for further oxygen uptake to become fully saturated. Oxygen affinity is greater for Hb molecule, which is partially saturated. Hence, oxygen moves towards such Hb molecules, rather than binding to a fresh Hb molecule. This affinity of Hb for oxygen to get fully saturated at the pulmonary capillaries is responsible for the 'S' or sigmoid shaped curve of oxygen dissociation curve (Fig. 8.12).

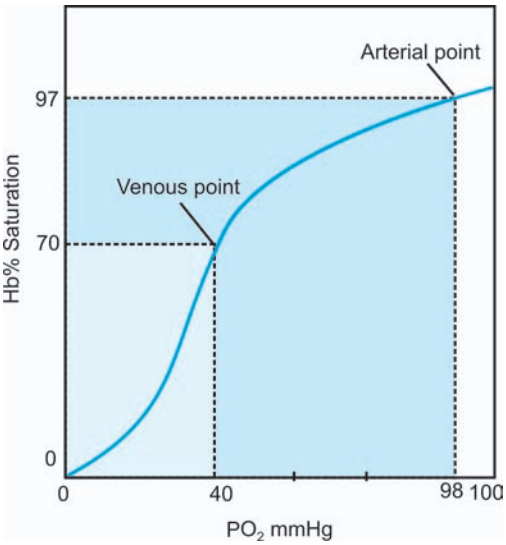


Fig. 8.12: Oxygen dissociation curve showing arterial and venous % saturation of Hb and PO_2

Oxygen dissociation curve

Percentage saturation of hemoglobin with the oxygen at different partial pressures gives the oxygen dissociation curve (Fig. 8.12). At 60 mmHg of PO_2, the Hb is already 90% saturated and thereafter, the curve is flat. At 98 mmHg, the Hb is 97% saturated and this represents the arterial point. The curve from 60 mmHg to 98 mmHg of PO_2, is called loading part. Below 60 mmHg of PO_2, the curve steeply declines in the % saturation of Hb. The declining part of the curve is known as unloading of oxygen, which occurs in the tissues. At 40 mmHg of PO_2, the saturation of Hb is 70 %, which is the venous point.

P_{50} in oxygen dissociation curve

The PO_2 required for 50% saturation of Hb is called P_{50}. It occurs at 27 mmHg (Fig. 8.13). Greater

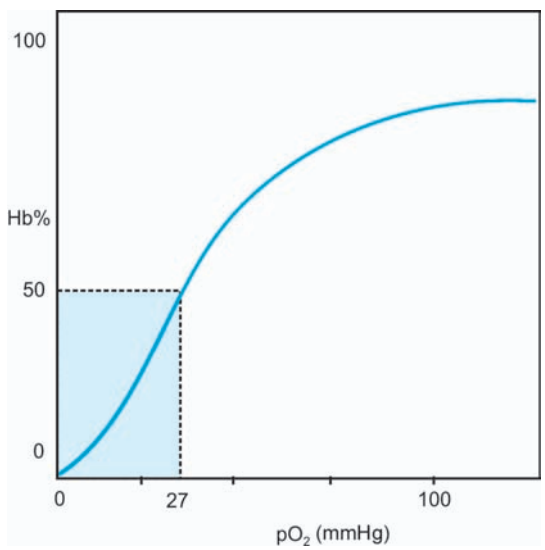


Fig. 8.13: Oxygen dissociation curve showing P_{50}. It refers to the partial pressure of oxygen required for 50% saturation of hemoglobin

the affinity of Hb for oxygen, lesser the P_{50} value. At the tissue capillaries the Hb affinity for oxygen is reduced and hence P_{50} is increased.

P_{50}: It refers to the pressure of O_2 required for 50% saturation of hemoglobin. Its normal value is 27 mmHg.

$P_{50} \uparrow$ = indicates less affinity of Hb for O_2

$P_{50} \downarrow$ = shows greater affinity of Hb for O_2

Increase in P_{50} is observed in

Tissue capillaries

High altitude

- 2, 3-DPG

Bohr's effect (Fig. 8.14)

At the tissue capillaries, the oxygen dissociation from Hb is facilitated by the increase in PCO_2, which is produced by the metabolism of tissues. The dissociation of O_2 shifts the curve to the right and P_{50} is increased. The other factors like rise in temperature, fall in pH or rise in H^+ ions, increase in 2, 3-DPG (diphosphoglycerate) also shift the oxygen dissociation curve to the right (Figs 8.15 and 8.16).

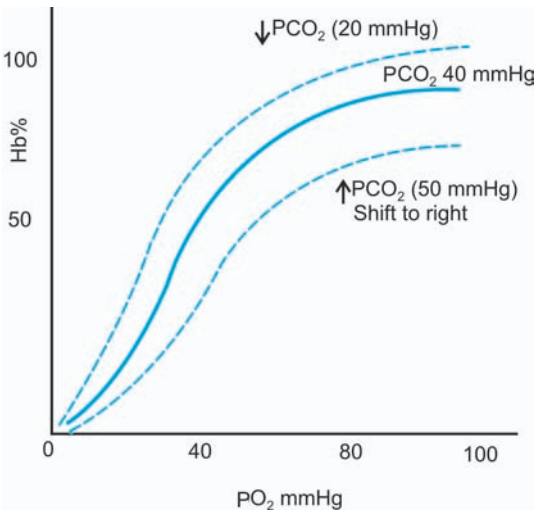


Fig. 8.14: Bohr's effect in the tissues. The rise in carbon di oxide tension shifts the oxygen dissociation curve to the right

2, 3-DPG is formed in RBCs in greater amounts, than in any other tissues. It is due to anaerobic glycolysis in RBCs as there is absence of mitochondria. The 2, 3-DPG is formed as a side reaction. It reduces the affinity of Hb for oxygen and it binds strongly with the reduced Hb.

In hypoxia and rise in pH, this compound is formed more, whereas, its production is reduced in stored blood.

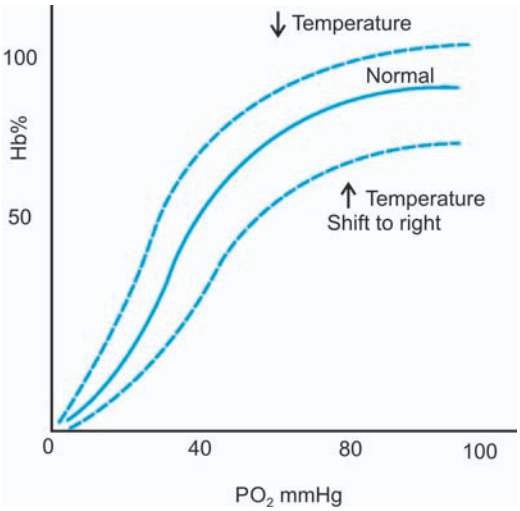


Fig. 8.15: Bohr's effect due to rise in temperature

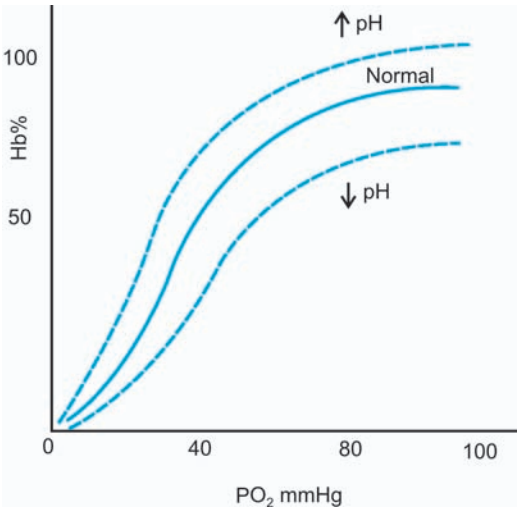


Fig. 8.16: Bohr's effect due to fall in pH

Oxygen carrying capacity

One gram of Hb can combine with 1.34 ml of oxygen and 100 ml of blood which contains 15 gm of Hb, can carry 20 ml of oxygen.

Oxygen content of blood

Arterial blood contains 19.5 ml of oxygen per 100 ml of blood, 0.5 ml is in dissolved form in plasma. The tissues take up 5 ml of oxygen per 100 ml of blood and hence the venous blood contains 14.5 ml of oxygen. In man, the cardiac output in the resting state is 5 lit/min and this will give 250 ml of oxygen consumption for the whole body/min under basal conditions. This value increases during muscular exercise.

Hb with CO

The affinity of Hb for carbon monoxide is 250 times greater than oxygen. At 0.5 mmHg of PCO, the Hb is 50% saturated and this greatly affects oxygen transport. The COHb gives a cherry red colour, which masks the cyanosis. The CO poisoning is improved by 100% hyperbaric oxygen therapy.

Oxygen transport in methemoglobin formation

The transport of oxygen is also affected when Fe^{++} is oxidized to Fe^{+++} by agents such as nitrates, and phenacetin, which cause methemoglobinemia. Oxidized Hb cannot bind and transport oxygen. Normally, the reducing compounds formed in the RBC during metabolism can reduce ferric to ferrous and prevent methemoglobin formation.

Transport in sickle cell anemia

In sickle cell the Hb is present as HbS and causes shift of oxygen dissociation curve to the right, as the sickle cell produces increased 2, 3-DPG.

Dissociation curve of fetal Hb

Fetal Hb shows the oxygen dissociation curve shifted to the left as the Hb in fetal RBC is different from adult Hb (Fig. 8.17). In HbF, the γ polypeptide chain poorly binds to 2, 3 DPG but shows greater affinity for oxygen and hence the shift to the left occurs. Moreover, it has an advantage in the fetus, where the PO_2 is low (40 mmHg).

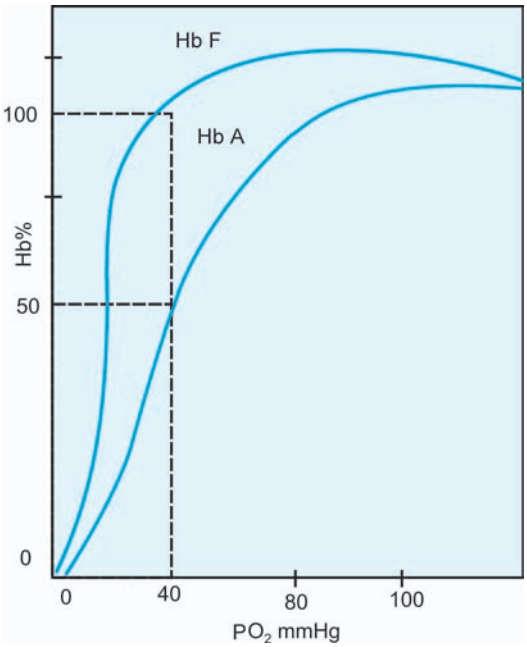


Fig. 8.17: Oxygen dissociation curves of adult Hb(Hb A) and fetal Hb (Hb F) are compared. Note that in HbF, the Hb% saturation is 90% at PO_2 of 40 mm Hg. That is the reason for the Hb F dissociation curve to move to the left and above of Hb A

Transport of carbon dioxide

Carbon dioxide is formed in the cell as a result of metabolism. In man the CO_2 that is produced per minute is 200 ml in resting condition, which may go up during physical activity. Carbon dioxide from tissues diffuses into the capillaries along the pressure gradient. In tissues the PCO_2 is 46 mmHg and in the capillaries, it is 40 mmHg. This small pressure difference facilitates CO_2 diffusion for the reasons mentioned earlier. Carbon dioxide is transported to lungs by three ways.

Simple physical solution in plasma
Carbamino compounds
Bicarbonate

Transport as simple solution

CO_2 is freely diffusible in plasma and is 20 times more soluble than O_2. Carbon dioxide on entering the tissue capillaries forms carbonic acid by combining with plasma.

$$CO_2 + H_2O \leftrightarrow H_2CO_3$$

Since the enzyme carbonic anhydrase is absent in plasma, the carbonic acid that is formed can shift the reaction to the left. Hence the transport of CO_2 in physical solution is limited to only 5%.

Transport as carbamino compounds

CO_2 combines with NH_3 terminal group, present in the protein molecule and form NH_2–COOH. The reaction occurs with the plasma proteins and also in RBCs by combining with Hb. The transport of CO_2 as carbamino compounds forms 20% of the total transport of the gas.

Transport as bicarbonate

The major form of carbon dioxide transport occurs as bicarbonate (75%) CO_2 is freely diffusible in RBC membrane and the red cell has carbonic anhydrase enzyme, which catalyzes the hydration of CO_2 to form carbonic acid.

$$CO_2 + H_2O \leftrightarrow H_2CO_3$$

Chloride shift

The carbonic acid that is formed in the RBC is a weak acid and dissociates into H^+ and HCO_3. The H^+ ion is taken up by the deoxygenated Hb which is a lesser acid and forms HHb. The bicarbonate from the red cell diffuses into plasma in exchange for Cl^- ion (Fig. 8.18).

$$H_2CO_3 \rightarrow H^+ + HCO_3^-$$

$$Hb\ O_2 + H^+ \rightarrow HHb + O_2$$

The red cell membrane is freely diffusible to bicarbonate and chloride ions. Since the exit of bicarbonate disturbs Donnan's ionic

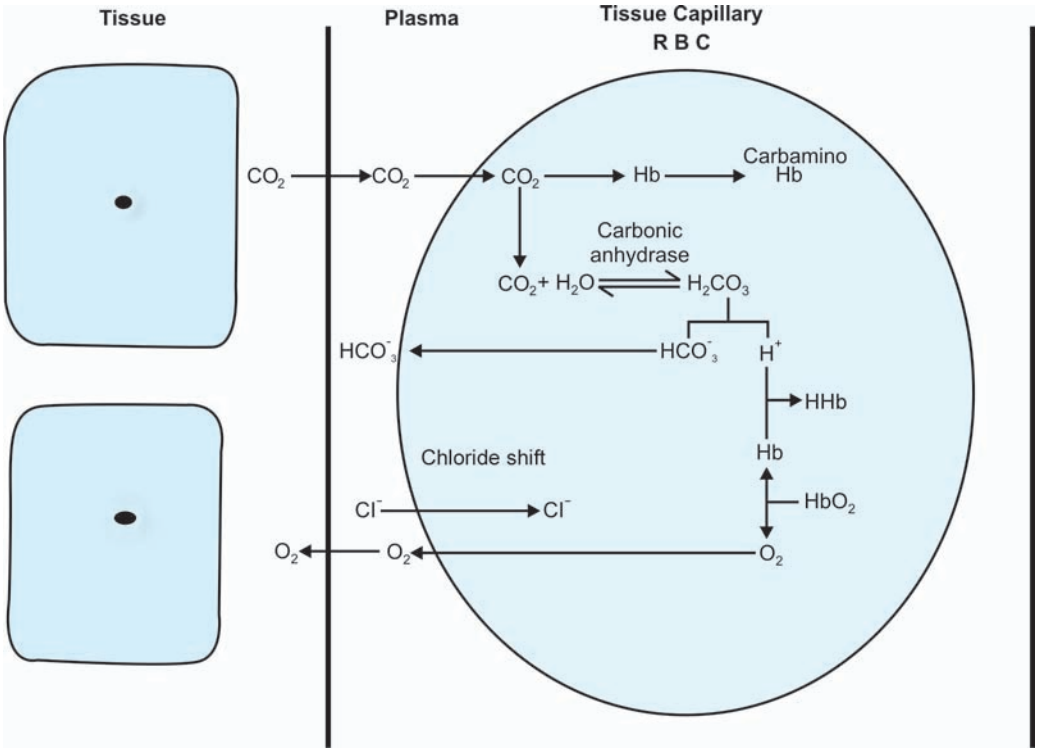


Fig. 8.18: Diagram showing carbon dioxide transport as bicarbonate and chloride shift in the tissue capillaries

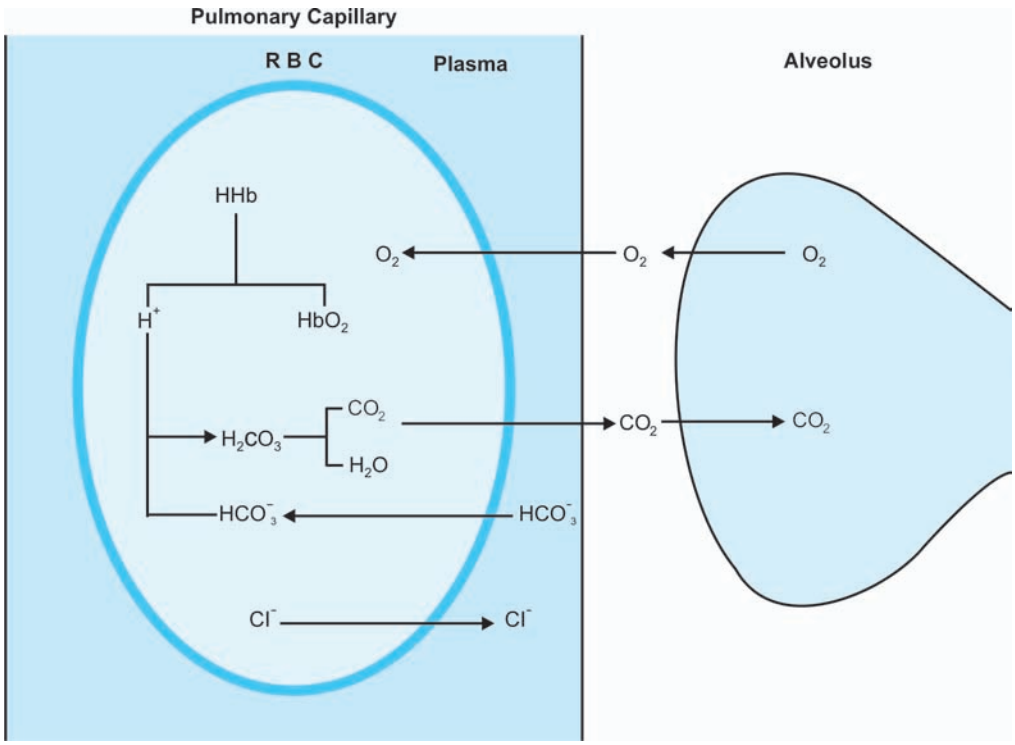


Fig. 8.19: Changes in the lungs in the transport of carbon dioxide. In the lungs Haldane's effect occurs, which facilitates expulsion of carbon dioxide

electrochemical equilibrium, another anion Cl^- diffuses into the red cell. This is called the chloride shift (Hamberger's phenomenon). The CO_2 that enters the red cell is transported as bicarbonate in plasma.

The red cell in the venous blood is slightly spherical, due to the entry of water caused by electrolyte movement into the cell. The pH of venous blood (7.38) is also slightly less, as compared to the arterial blood (pH 7.40), due to more carbon dioxide content.

Haldane's effect

In the lungs the diffusion of O_2 into the pulmonary capillaries leads to chemical reactions, resulting in the expulsion of CO_2 (Fig. 8.19). This is known as Haldane's effect. Haldane effect helps to reduce CO_2 retention and pH change in the venous blood.

The diffusion of O_2 in the pulmonary capillaries, releases H^+ from Hb.

$HHb + O_2 \rightarrow Hb\ O_2 + H^+$

The free H^+ ion in the red cell causes the diffusion of HCO_3^- from plasma. This movement occurs in exchange for Cl^- ion (reverse chloride shift). HCO_3^- combines with H^+ to form H_2CO_3 which dissociates into CO_2 and H_2O.

$H^+ + HCO_3 \rightarrow H_2CO_3 \rightarrow CO_2 + H_2O$

The CO_2 diffuses into the alveoli along the pressure gradient and expelled in the expired air.

Carbon dioxide content in blood

Arterial blood PCO_2 is 40 mmHg and has a volume of 48%. In venous blood, the PCO_2 is 46 mmHg and volume is at 52%. The dissociation of CO_2 takes place between 46 and 40 mmHg (Fig. 8.20).

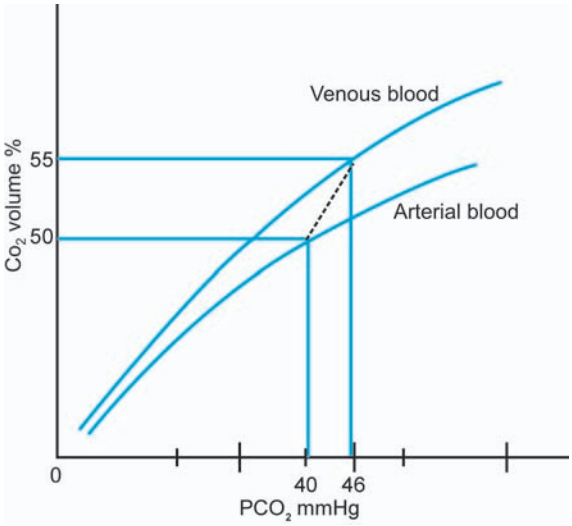


Fig. 8.20: Carbon dioxide dissociation curve. The dissociation of CO_2 occurs between the venous and arterial partial pressures

REGULATION OF RESPIRATION

Respiration takes place involuntarily depending upon the body metabolic needs. The involuntary breathing can be overridden by voluntary breathing. The voluntary control of breathing comes from the activity of cerebral cortex. The automatic breathing is due to a group of nerve cells in the medulla, which functions as pacemaker. These cells are known as **pre-Botzinger complex**. It is situated between the nucleus ambiguous and lateral reticular nucleus. These neurons in the medulla are responsible for **rhythmic respiration**. Lesions of dorsal and ventral group of neurons in the medulla do not stop respiration as they are connected to pre-Botzinger complex neurons. The rate and depth of breathing are controlled by the neural and chemical stimuli, reaching the central nervous system. Accordingly, the regulation of respiration is discussed under neural and chemical controls.

Neural control of respiration

The respiratory centers controlling the respiration are present in the medulla and pons. The medullary reticular formation contains the dorsal and the ventral group of respiratory neurons (Fig. 8.21).

Dorsal group

They are situated in the nucleus tractus solitarius in the medullary reticular formation. It contains mostly inspiratory neurons, as they are stimulated during inspiration. The connections from this nucleus go to the contralateral spinal motor neurons supplying phrenic nerve, intercostal nerves and medullary ventral group of neurons.

Ventral group

The ventral group contains both inspiratory and expiratory neurons. The neural connections from this supply the abdominal muscles and accessory muscles of inspiration.

The medullary centers receive impulses from pontine, and pneumotaxic centers. They also receive chemoreceptive and stretch receptor impulses, which help in the rhythmic respiration.

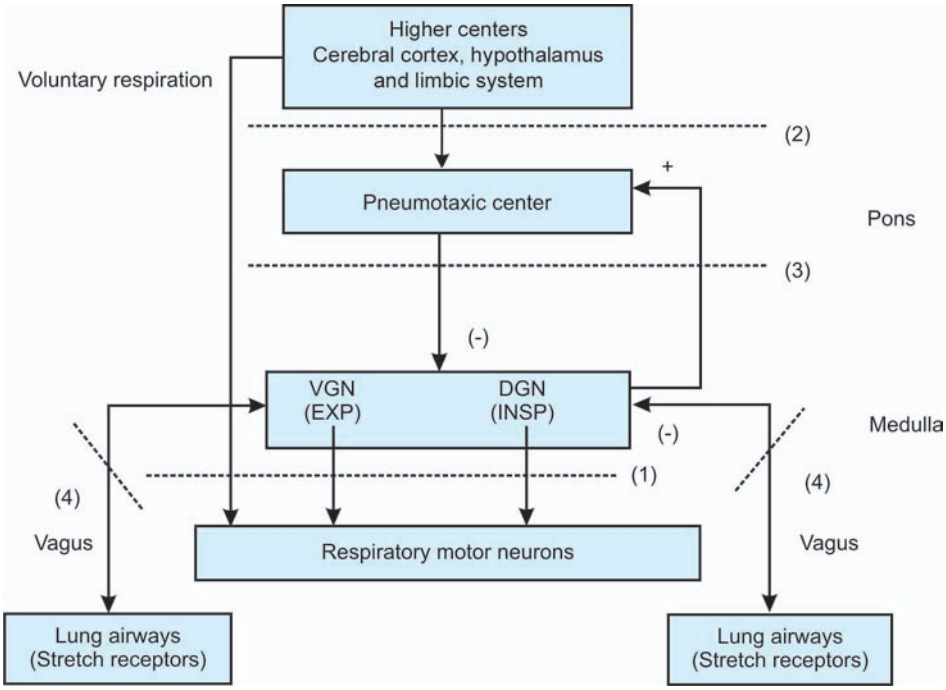
Pontine center

Pneumotaxic center

It is situated in the upper part of the pons and checks the activity of inspiratory neurons and stimulates expiration. The connection between inspiratory and pneumotaxic centers helps in cutting off inspiration. This in turn sets the tidal volume and the rate of breathing. Lesion of pneumotaxic center casues slowing of respiration and the tidal volume increases. If vagi are cut after lesioning the pneumotaxic center, prolonged inspirations called **apneusis** occurs. If only vagi are cut, the depth of inspiration is increased.

Mechanism of rhythmic breathing

The frequency and intensity of firing of inspiratory neurons are determined by the PCO_2 in the arterial blood. The discharge of impulses along



1. Section of brainstem below medulla – respiration stops.
2. Section of brain stem above pons –automatic respiration intact
3. Mid pontine section – slow respiration
4. Bilateral Vagotomy – ↑ depth of breathing
   Midpontine section and bilateral vagotomy – apneusis

Fig. 8.21: Respiratory centers showing neural control of breathing. The effect of lesion of brainstem at various levels has been shown

the inspiratory motor neurons causes inspiration. As the inspiratory neurons fire, recurrent collaterals go to the pneumotaxic center and stimulate it. The medullary respiratory centers also receive stretch receptor impulses through the vagi from the lungs. These inhibitory impulses and pneumotaxic center discharge on the inspiratory neurons, lead to the inhibition of inspiration and stimulation of passive expiration. In this way, the rhythmic respiration is carried out.

Respiratory reflexes (Fig. 8.21)

There are number of reflexes that modify the rhythmic respiration. The receptors are stretch receptors, irritant receptors and chemoreceptors.

Hering-Breuer reflex

There are two types of reflexes, which can be observed. One is present during distension of the lungs and another during deflation.

Hering-Breuer inflation reflex

The hyperpnea of the lungs causes the stimulation of smooth muscle of airways present in the walls of the bronchi and bronchioles. The afferents are the myelinated fibres of vagi, which go to the medullary respiratory centers and inhibit the inspiratory neurons. Hence it is called inspiration inhibitory reflex. The inhibition of inspiration leads to the stimulation of expiration, which prevents the excessive distention of the lungs. Hering-Breuer inflation reflex can be seen in the newborn.

Hering-Breuer deflation reflex

This is the inhibition of expiration and stimulation of inspiration reflex. The receptors are present in the bronchioles. The forceful expiration, extreme deflation of the lungs and pneumothorax stimulate the receptors. The impulses are carried by the vagi to the medullary respiratory centers to cause inhibition of expiration and stimulation of inspiration.

Reflex from lung irritant receptors

Inhalation of chemicals, smoke and toxic gases stimulates the irritant receptors present in the airways. The stimulation causes hyperpnea, bronchoconstriction, mucus secretion and coughing.

Reflex from J receptors

These are the juxtapulmonary receptors present in the wall of the alveoli, close to the capillaries. These receptors are stimulated by the increase in the pulmonary capillary pressure, which occurs in high altitude, congestive cardiac failure and due to chemicals (veratridine, phenyl bi guanide, serotonin), pulmonary edema, and emboli. The response obtained is apnea followed by rapid shallow breathing. The afferents are the unmyelinated C nerve fibers present in the vagi.

Reflex from proprioceptors of respiratory muscles

Stimulation of the proprioceptors of the respiratory muscles in the chest wall, reflexly modify the spinal motor neuron discharge to the respiratory muscles. This helps to decrease the airway resistance and maintain the tidal volume.

Chemical regulation of respiration

Reflexes from chemoreceptors

The chemical control of breathing helps to maintain the alveolar levels of PCO_2, PO_2 and arterial H^+ ion concentration constant. The respiratory adjustments in response to changes in blood gas tensions and H^+ concentration occur due to the activity of chemoreceptors.

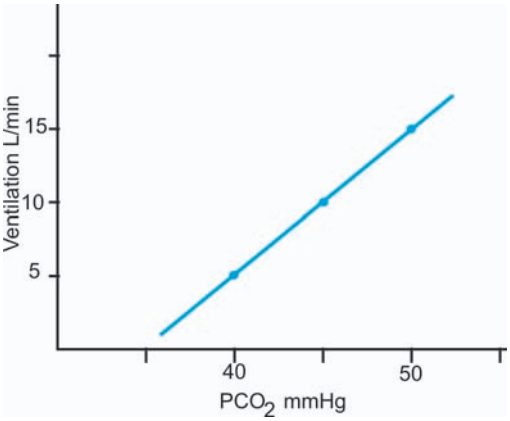


Fig. 8.22: Carbon dioxide response curve at alveolar PO_2 100 mmHg

Peripheral chemoreceptors

The **peripheral chemoreceptors** are the **carotid body** and **aortic body**. Carotid body is present at the bifurcation of common carotid artery and aortic body in the arch of aorta. They are innervated by the IX and X nerves respectively. The carotid body receives very high blood flow and utilises very little oxygen. The receptors are highly sensitive to change in PO_2.

Central chemoreceptor

Central chemoreceptor present in the ventral surface of the medulla is sensitive to the rise in PCO_2. The receptors are however stimulated by the H^+ ion, which is formed from CO_2. The CO_2 being freely permeable to the blood brain barrier, diffuses into the brain interstitial space and enters the CSF. The enzyme carbonic anhydrase present in the CSF, hydrolyzes CO_2 to carbonic acid, which dissociates into H^+ and HCO_3^-. The H^+ ion diffuses into the ventral surface of the medulla and stimulates respiration. The rate and depth of breathing is increased by this stimulation.

Peripheral chemoreceptors

Peripheral chemoreceptors are most sensitive to hypoxia, although the receptors are stimulated by PCO_2 and H^+ ion. The hypoxic stimulation of peripheral chemoreceptors can be noticed when PO_2 in the alveoli falls to 60 mmHg. The fall in PO_2 from 100 mmHg to 60 mmHg, does not stimulate chemoreceptors, as the % saturation of Hb is already 90% and above, in that range of PO_2. At PO_2 60 mmHg, the % saturation of Hb steeply declines and this stimulates the carotid body. The rate and depth of ventilation are more than doubled. This kind of hypoxic stimulation of carotid body can be seen in high altitude. When PO_2 falls below 35 mmHg in the alveoli, damage to the neurons in the medulla occurs. This can lead to unconsciousness, coma and death.

Presence of hypoxia together with rise in PCO_2

The stimulation of respiration is greatly accentuated, when both hypoxia and hypercapnia coexist. The respiratory stimulation from PCO_2, requires only a slight rise, whereas PO_2 decrease has to be substantial (from 100 mmHg to 60 mmHg). The presence of both hypoxia and hypercapnia potentiates each other's effect.

Respiration during exercise

Respiration during muscular exercise is stimulated to meet the metabolic demand. The increase in rate and depth of ventilation facilitates supply of O_2 to the exercising muscles and removal of excess CO_2 that is produced. It should be known that the stimulation of respiration does not come from hypoxia and hypercapnia that can be expected to exist in this condition. Actually the arterial PO_2 and PCO_2, are nearly normal during muscular exercise, so the question of hypoxic and hypercapnic stimulation of ventilation does not arise at all.

Stimulation of ventilation during muscular exercise comes from two mechanisms. They are as follows.

Influence of higher centers on respiratory centers

During muscular exercise the higher centers like cerebral cortex sends impulses to the respiratory centers and stimulate them. This is similar to the cortex stimulating vasomotor center to raise the arterial blood pressure during stress.

Proprioceptive input from muscles, tendon and joints

The second mechanism, which stimulates respiration during exercise is the proprioceptive impulses coming from the exercising muscles and joints. These afferents go to respiratory centers and stimulate them.

Type of metabolism

The rise in the level of lactic acid during exercise depends on the anerobic threshold. Trained athletes have greater anerobic threshold and hence work output is greater in them.

Respiration during sleep

Sleep apnea syndrome

There are two types of sleep apnea syndrome. One is called obstructive and the other is called as nonobstructive.

Obstructive type is due to the loss of tone of pharyngeal muscles, which occurs during deep stages of REM sleep. The loss of tone of pharyngeal muscles leads to the obstruction of pharynx and prevents air flow. If partial obstruction occurs, then snoring results. In complete obstruction, apnea occurs, which causes hypoxemia and hypercapnia. The rise in PCO_2 and decrease in PO_2 awakens the subject. This helps the affected individual to get respiration.

The **nonobstructive type of sleep apnea** is due to low sensitivity of peripheral chemoreceptors to hypoxia and hypercapnia, resulting in inhibition of respiration and apnea during sleep.

Sudden infant death syndrome is a form of sleep apnea.

Abnormal breathing

Periodic breathing

Periodic breathing refers to apnea alternating with hyperpnea. There are two types of periodic breathing namely Cheyne-stokes breathing and Biot's breathing.

Cheyne stokes breathing is characterised by waxing and waning ventilation which ends in apnea. This type of breathing is seen immediately after hyperventilation, in high altitude and during sleep. Pathologically, it occurs in congestive heart failure and uremia.

Biot's breathing shows hyperpnea alternating with apnea. There is no waxing and waning pattern of ventilation. This type of periodic breathing occurs in tumors of brain and meningitis.

HYPOXIA

Hypoxia is a condition in which there is decreased oxygen supply to the tissues. It can occur due to any of the following conditions.

- Decreased PO_2 in the arterial blood
- Reduced blood flow to the tissues
- Reduced oxygen carrying capacity of blood
- Inactivation of respiratory enzymes in the tissues by poisons.

Decreased PO_2 in arterial blood (Arterial hypoxia or hypoxic hypoxia)

In this type of hypoxia the partial pressure of oxygen is reduced in arterial blood, which can be seen in high altitude, venous arterial shunt, pulmonary fibrosis, pulmonary edema, emphysema, pneumothorax, respiratory muscle paralysis, obstruction to the respiratory passage and depression of respiratory center.

In arterial hypoxia the rate of blood flow and oxygen carrying capacity are normal. Cyanosis is present in this type.

Decreased rate of blood flow (stagnant hypoxia)

Hypoxia can occur when the rate of blood flow to the tissues is reduced as in cardiac failure (pump failure) and aneurysm of the aorta. In this type, the PO_2 and oxygen carrying capacity are normal. The presence of cyanosis is also characteristic of this hypoxia.

Reduced oxygen carrying capacity (anemic hypoxia)

Anemic hypoxia is caused by reduced hemoglobin content or hemoglobin present as COHb or formation of methemoglobin (Fe^{++} in Hb is oxidised to Fe^{+++}). The PO_2 in the arterial blood and the rate of blood flow to the tissues are normal. Since the quantity of Hb available is lower than normal, cyanosis does not occur in this type of hypoxia.

Inactivation of respiratory enzymes in the tissues (histotoxic hypoxia)

When respiratory enzymes like cytochrome oxidases are inactivated by cyanide, the oxygen that is delivered to the tissues cannot be utilised and hence hypoxia results.

Effects of high altitude on respiration

In high altitude the barometric pressure is reduced, but not the composition of gases in the atmosphere. As one climbs the mountains, the partial pressure of oxygen is decreased proportionate to the ascent. The reduction in the arterial blood oxygen partial pressure, occurs in hypoxic hypoxia.

At 12000 feet height, the barometric pressure is reduced to 380 mmHg and alveolar PO_2 decreases to 60 mmHg. If the exposure to this altitude is sudden, as occurs in loss of cabin pressure in an aircraft, acute hypoxia can result.

The **effects of acute hypoxia** are due to hypoxic effects on the medullary neurons. The symptoms like disorientation, euphoric states similar to alcohol drinking, poor judgment, mental confusion, headache, palpitation and dyspnea are observed in the affected subjects.

Mountain sickness

When subjects go to high altitude as in mountain climbing, on exposure to hypoxia at 12,000 feet height, symptoms of mountain sickness occurs within 24 hours. It is characterised by nausea, vomiting, headache, palpitation, dyspnea and mental confusion. At high altitude, if one engages in physical work or climbing the mountain too rapidly without rest, it can lead to **high altitude pulmonary edema**. This is caused by the increased pulmonary capillary pressure, leading to fluid exit into the pulmonary interstitial space. The symptoms of mountain sickness occur, probably due to the cerebral edema caused by the cerebral dilatation of capillaries and fluid exit, in the presence of hypoxia. Administration of large amounts of glucocorticoids, resulted in relief from mountain sickness. There are reports that mountain sickness does not occur, if there is diuresis. However, use of diuretics does not prevent mountain sickness from developing.

Acclimatization to high altitude

Acclimatization to high altitude (12000-15000 feet height) occurs after 4 to 5 days of exposure. In acclimatization, the physiological adaptation to high altitude occurs. The physiological changes are as follows (Table 8.1):

- Stimulation of ventilation from peripheral chemoreceptor stimulation, caused by the fall in PO_2. The rate and depth of breathing is increased. The increase in ventilation lowers PCO_2 as HCO_3 is formed. The renal compensation of HCO_3, helps to keep the level of PCO_2 normal, so that, the chemoreceptor stimulation of respiration continues. There is also active transport of H^+ ions into the CSF and this lowers the HCO_3 level. The respiratory stimulation continues for 4 to 5 days and thereafter, normal breathing occurs.
- Increase in the heart rate and cardiac output occurs, due to the chemoreceptor stimulation by hypxoia and sympathetic activation.
- Polycythemia occurs due to increased erythropoietin secretion from the kidney.
- Red cell 2, 3-DPG production increases and the oxygen dissociation curve shifts to the right, which raises the P_{50} value for Hb% saturation.
- The tissues show increase in cytochrome enzymes content and its activity for greater oxygen utilization.
- The increase in capillary density and mitochondrial number also helps more oxygen supply and its utilization.

The acclimatization changes disappear after a few days, when an individual comes back to the plains at sea level.

The acclimatization to high altitude can be observed up to 18000, feet height. Beyond this level, oxygen has to be supplied in the respired air, as the alveolar PO_2 falls to 35 to 40 mmHg. This is the critical level of PO_2 in the alveoli, at which the damage to CNS neurons occur leading to death.

RESPIRATORY DISORDERS

Asphyxia

The presence of acute hypercapnia and hypoxia as in obstruction to the respiratory passage leads to asphyxia.

At first, the respiration is intensely stimulated by the hypoxia and hypercapnia acting on the chemoreceptors. This is the **stage of hyperpnea** which gives violent respiratory efforts. The hypoxia and hypercapnia also cause the secretion of catecholamines. This leads to the **stage of excitation** lasting for 2 to 3 minutes. The heart rate and blood pressure are increased.

Table 8.1: Physiological changes during acclimatization to high altitude

Physiological variables	Response
Alveolar PO_2	↓
Arterial PO_2	↓
Ventilation	↑ (chemoreceptor stimulation)
Arterial pH	Alkaline, but compensated (The alkalosis which occurs due to stimulation of ventilation is compensated by renal excretion of alkaline urine. Hence pH does not change)
Heart rate	↑
Cardiac output	↑
Arterial Blood Pressure	↑
Circulation time	↓
RBC count	↑ (Polycythemia due to hypoxic stimulation of erythropoietin release)
2,3, DPG in RBC	↑
Hb dissociation curve	Shift to right, ↑ P_{50}
Pulmonary capillary resistance	↑ (due to hypoxia)
Capillary density in the tissues Tissue myoglobin, mitochondria and respiratory enzymes	↑

The pupils dilate and CNS reflexes are exaggerated. The sphincter control in bladder and rectum are lost. During this stage, resuscitation of heart and lungs, if given helps the subject to survive. The prolonged hypoxia and hypercapnia lead to depression of cardiac and respiratory centers in the medulla. **(stage of depression)**. This causes slowing of the heart, fall in blood pressure and absence of CNS reflexes. Finally the respiration and heart stops. This stage lasts for 2 minutes. The total duration of asphyxia lasts for 4 to 5 minutes before death occurs.

Cyanosis

The accumulation of reduced Hb causes bluish discolouration of the skin and mucous membrane, especially, the nail bed, finger tips, lips and ear lobes. For cyanosis to occur, the reduced Hb (deoxygenated Hb) should be above 5 g%. Hence hypoxic hypoxia and stagnant hypoxia give rise to cyanosis. This is absent in the anemic and histotoxic hypoxia. In anemic hypoxia, the quantity of Hb itself is low and hence the reduced Hb level never goes above 5 g%.

Cyanosis depends on:

Amount of Hb
Degree of unsaturation of Hb
State of blood flow in capillaries.

If cyanosis occurs due to heart failure or venous arterial shunts, it is called **central cyanosis**, while cyanosis occurring in the limbs, due to local obstruction of blood flow gives **peripheral cyanosis**. Exposure to cold does not induce peripheral cyanosis in the limbs as the blood flow is significantly reduced by vasoconstriction. This keeps the amount of reduced Hb below 5 g%. In carbon monoxide poisoning, the COHb gives a cherry red color, which masks the bluish color of cyanosis. In anemic hypoxia, the amount of deoxygenated Hb falls below 5 g%, as the quantity of Hb available itself is less and hence cyanosis does not occur. The common conditions for cyanosis to occur are, the development of hypoxic hypoxia and stagnant hypoxia.

Dyspnea

Dyspnea is the term used, when the subject is conscious of the increased respiratory effort. In

other words, it is the difficulty in breathing. It occurs in various cardiopulmonary disorders. It occurs in severe muscular exercise, acidosis, respiratory muscle paralysis, obstruction to respiratory passage, pneumothorax, pulmonary edema, pulmonary fibrosis and in congestive cardiac failure. In the latter condition, the difficulty in breathing is observed in recumbent posture as there is increase in pulmonary blood volume which limits the expansion of lungs. The dyspnea of congestive cardiac failure is relieved, when the subject assumes sitting posture and hence it is known as **orthopnea**.

The development of dyspnea can be known from dyspneic index. The normal value is 90%. If it falls below 70%, dyspnea results.

$$\text{Dyspneic index} = \frac{(MVV - PV)}{MVV} \times 100$$

MVV = maximum voluntary ventilation
PV = pulmonary ventilation

Decompression sickness

When a person goes to a high barometric pressure, N_2 gas, which is inert at atmospheric pressure, dissolves in the body fluids. It also dissolves in the fatty tissues like the myelin sheath of neurons. These changes, which are observed in high barometric pressure give the condition called **dysbarism** or **nitrogen narcosis**. For every 33 feet depth one atmospheric pressure is added. If a person goes down to say 30 meters, the pressure exerted would be 4 atmospheres.

Decompression sickness occurs, when the subject rapidly ascends from a high barometric to a low barometric pressure. This condition is prevalent in mine workers, tunnel workers in sea bed (Caisson's workers) and in deep sea divers.

Rapid ascent to the low barometric pressure from the high barometric pressure causes decompression sickness. The nitrogen bubbles block the minute capillaries and form emboli. This can lead to chokes (dyspnea), pain in the joints, sensory disturbances (pins and needles), myocardial infarction (if emboli are in coronary vessels), stroke and paralysis (if emboli occur in cerebral vessels). Decompression sickness occurs only if the ascent is rapid. It can be prevented by wearing decompression suite or inhaling 100% O_2 with helium (helium has low solubility in high barometric pressure as compared to N_2). The decompression sickness can be treated by recompression followed by slow decompression.

Emphysema

This a chronic obstructive pulmonary disease. Excessive smoking of cigarettes can cause emphysema.

The chronic smoking causes inhibition of escalator action of ciliated pseudocolumnar epithelial cells lining the respiratory tract. This leads to mucus secretion not being cleared. The alveolar macrophage activity is also depressed, which leads to the spread of infection in lungs. The increase in leucocytes count causes the release of elastase from lysosomes. Normally there is secretion of α_1 antitrypsin enzyme, which counteracts against these agents. But in emphysema, the secretion of α_1 antitrypsin is absent and hence there is destruction of elastic tissue in lungs. The septa break down and smaller alveoli join and form larger alveoli. This gives a barrel shaped chest.

The resting volume becomes more and hence the fall in FEV_1. The functional residual capacity consequently is greater. Since the FEV_1 is reduced, the respiration becomes laboured. This causes decrease in compliance of lungs. The blood level of PO_2 is reduced and PCO_2 is increased. The rise in PCO_2 is so high, which depresses the medullary neurons. In such a condition, it is the hypoxia that stimulates respiration and hence administration of 100% O_2 will remove the stimulus coming from hypoxia.That is why pure oxygen administration to emphysematous subjects to relieve hypoxia is not recommended.

Oxygen therapy

Oxygen therapy is useful in all conditions of hypoxic hypoxia except in venous arterial shunt.

In emphysema

Loss of elastic tissue in lungs
More resting volume
FEV_1 is reduced
$\downarrow PO_2$ and $\uparrow$ PCO_2
Work of breathing is increased
Static compliance is greater but dynamic compliance is reduced.

Administration of 100% oxygen helps to increase the arterial O_2 content. Pure oxygen therapy is not useful in stagnant and anemic hypoxia.

Hyperbaric oxygen therapy

Oxygen administration under high pressure is called hyperbaric oxygen therapy. Usually 3 to 4 atmospheres for 30 minutes duration is given to treat carbon monoxide poisoning and gas gangrene. It is also useful in cardiac surgery.

Prolonged hyperbaric oxygen administration, causes release of free radicals or superoxide anions. These free radicals normally are inactivated by superoxide dismutase enzyme. Hyperbaric oxygen can lead to poor activity of this enzyme and results in symptoms like irritation of respiratory passage, cough, bronchopulmonary cysts, retrolental fibroplasia in premature newborn, loss of surfactant, muscle twitchings, convulsions due to inhibition of GABA secretion in CNS, unconsciousness and death.

ARTIFICIAL RESPIRATION AND LUNG FUNCTION TESTS

Artificial respiration is employed in both acute and chronic respiratory failures. Acute failure can result due to drowning, strangulation, electrocution, etc. Chronic failure can arise due to pneumothorax, respiratory muscle paralysis, depression of respiratory center, etc.

In acute respiratory failure, manual methods like mouth to mouth breathing and back pressure arm lift methods are attempted (Holger Nielsen method). Chronic respiratory failure requires mechanical methods like Drinker's respirator, ventilators, etc.

Mouth to mouth breathing, is a first aid procedure and effective for acute respiratory failure. The victim is laid down in supine and the rescuer places his hand on the forehead and another hand below the neck and lifts up. This will extend the neck and open the airways. The mouth of the victim is cleaned and the rescuer closes the nostrils of the victim. The rescuer takes a deep breath (twice the tidal volume) places his mouth airtight on the victim's and blows into his mouth. This expands the lungs of the victim, giving inspiration. The rescuer withdraws his mouth and allows passive recoiling of lungs to occur as expiration. The process is repeated for 12 to 15 times until normal breathing starts. If there is cardiac failure, then cardiac resuscitation (cardiac massage) should be attempted alternatingly with mouth to mouth breathing.

Lung function tests

Lung function tests are done to assess the functional efficiency of lungs. Tests are conducted based on lung volumes, capacities and blood gas tensions. Spirometer is used to record lung volumes and capacities (Fig. 8.23).

Tidal volume

The amount of air inspired or expired in a quiet breathing is called tidal volume and is 500 ml.

Rate of breathing

The rate of breathing in a quiet state ranges from 12 to 15 per minute. Increase in rate and depth of breathing is known as hyperpnea. Rapid shallow breathing is called tachypnea, which occurs in fever.

Inspiratory reserve volume

From the end of normal inspiration, maximum inspiration can be taken, which forms the inspiratory reserve volume. It ranges from 2 to 2.5 lit.

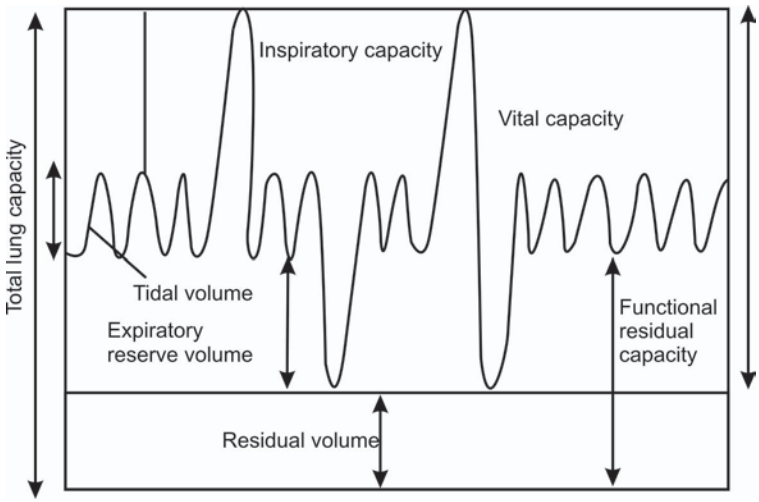


Fig. 8.23: Lung volumes and capacities

Inspiratory capacity

The amount of air that is maximally inspired from the end of normal expiration forms the inspiratory capacity. Its value is 3 to 3.5 lit.

Expiratory reserve volume

From the end of normal expiration, it is possible to maximally expire, which forms the expiratory reserve volume. Its value ranges from 1000 to 1500 ml.

Residual volume

Even after a maximal expiration, the lungs are not completely empty. There is some amount of residual air present, which is 1000 ml.

Functional residual capacity

The volume of air that remains in the lungs after a normal expiration is called functional residual capacity. It includes the expiratory reserve volume and residual volume. The value of FRC is 2 to 2.5 lit. It cannot be recorded by using spirometer. It is indirectly measured by inhaling 100% O_2 and determining the nitrogen concentration before and after inhalation of pure oxygen. The degree of dilution of nitrogen in the expired air gives FRC. In old age and emphysema, its value is increased.

Vital capacity

It is the amount of air that is maximally expired after a forceful inspiration. It includes inspiratory capacity and expiratory volume. The value ranges from 3.5 lit to 4 lit.

In females it is slightly lower. In children and old age vital capacity decreases. The value also decreases in lying down posture.

Vital capacity is expressed in relation to age, height and weight. More the surface area, greater the vital capacity. In cigarette smoking, vital capacity is reduced due to reduced compliance. Vital capacity is reduced in both obstructive and restrictive respiratory diseases.

FEV_1

The expression of vital capacity in relation to time is called timed vital capacity or forced expired volume (FEV) (Fig. 8.24). The FEV value is taken in the Ist second (FEV_1) to find out if any obstructive respiratory disease is present. The normal value of FEV_1 is 85%. In the II second, it is 90% and in the III second, the value becomes 95%. The recording of FEV_1 is helpful in distinguishing

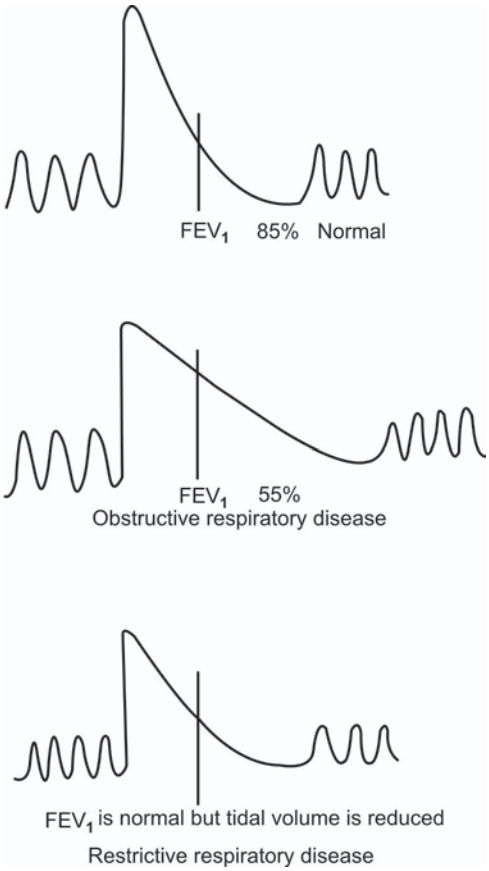


Fig. 8.24: Forced expiratory volume in the first second recorded in normal, obstructive and restrictive respiratory diseases

respiratory disease as obstructive or restrictive types. In obstructive respiratory disease like bronchial asthma and emphysema, the FEV_1 is reduced to 55%. In restrictive type like pulmonary fibrosis, the tidal volume is less, but FEV_1 is normal.

Total lung capacity include vital capacity and residual volume and its normal value is 6 L/minute.

MVV (maximum voluntary ventilation)

Maximum voluntary ventilation (MVV) is the volume of air that can be maximally respired per minute. It is recorded for 15 sec and expressed for 1 minute. The value ranges from 100 to 180 lit per minute. The value is less in females and more in atheletes . MVV is reduced in bronchial asthma, emphysema, pulmonary edema, pulmonary fibrosis, etc.

Breathing reserve and dyspneic index

Breathing reserve (BR) forms the difference between pulmonary ventilation and maximum voluntary ventilation (MVV – PV).

Dyspneic index can be obtained from BR.

$$DI = \frac{MVV - PV}{MVV} \times 100$$

= DI normal value is 90%. If the value falls below 70%, dyspnea can occur.

PEFR

The determination of peak expiratory flow rate (PEFR) is useful clinically in detecting obstructive respiratory diseases like bronchial asthma. The test is done by using a peak flow meter (Wright's) and the subject is asked to expire maximally for 10 milliseconds and the value is expressed for 1 minute. The value is 400 to 450 lit/min. In obstructive respiratory diseases, it is reduced.

Closing volume

It is the volume of the lungs at which the smaller airways in the basal part of the lungs begin to close. Its value is 10% of vital capacity. In old age or due to cigarette smoking, it is increased.

Closing volume is determined by recording single breath nitrogen analysis. The subject takes 100% O_2 and expires into a nitrogenmeter. In the expired air the concentration of nitrogen is determined. There will be four phases. The closing volume will be at the end of the third and beginning of the fourth phase.

Important clinical tests for pulmonary function

Tests	Normal value	Changes in disease
FEV_1	85%	Reduced in obstructive respiratory disease
PEFR	400 lit/min	As above
MVV	150 lit/min	Reduced in both restrictive and obstructive respiratory diseases
Arterial blood	PO_2 98 mmHg	Reduced in cardiopulmonary diseases
	PCO_2 40 mmHg	Increased in emphysema, asphyxia

Terms used in respiratory physiology

Eupnea - normal breathing
Apnea - temporary stoppage of respiration
Hyperpnea - increase in rate and depth of ventilation
Tachypnea - rapid shallow breathing
Hypoxia - reduced oxygen supply to tissues
Hypercapnia - increase in carbon dioxide tension
Asphyxia - increase in PCO_2 and fall in PO_2
Cyanosis - bluish coloration of skin and mucous membrane
Dyspnea - difficulty in breathing
Orthopnea - dsypnea of congestive cardiac failure

Self-study Questions

Multiple Choice Questions

Choose the single best answer

1. **Stability to alveoli is given by all of the following *except:***
   A. Intrapleural pressure
   B. Lung surfactant
   C. Surface tension
   D. Lung elastin

2. **Airway resistance is increased by all of the following *except:***
   A. Lung Surfactant
   B. Histamine
   C. Prostaglandin
   D. Leucotriene

3. **Chloride shift shows all of the following *except:***
   A. Occurs in the lung capillaries
   B. Occurs to maintain ionic equilibrium
   C. Rises chloride level in the RBC
   D. Necessary for CO_2 transport

4. **At what level of alveolar oxygen pressure the peripheral chemoreceptors are activated? (Pressure in mmHg)**
   A. 150 **B.** 100
   C. 90 **D.** 60

5. **The affinity of hemoglobin for oxygen is increased if there is an increase in:**
   A. pH
   B. temperature
   C. 2,3 DPG
   D. PCO_2

6. **The region which initiates rhythmical breathing is present in:**
   A. Dorsal group of neurons in the medulla
   B. Ventral group of neurons in the medulla
   C. Pneumotaxic area in the pons
   D. Pre- Botzinger complex in the medulla

7. **Thickening of alveolar capillary membrane causes:**
   A. Emphysema
   B. Pulmonary edema
   C. Hypoxemia
   D. Atelectasis

8. **During inspiration, all of the following show an increase *except*:**
   A. Intrapleural pressure
   B. Stroke volume
   C. Venous return
   D. Intrapulmonary pressure

9. **The stimulation of ventilation during exercise is mainly caused by:**
   A. Hypoxia
   B. Hypercapnia
   C. Proprioceptive afferents
   D. Acidosis

10. **If the intrapleural pressure becomes atmospheric, the changes in the lungs would include:**
    A. Increased compliance
    B. Decreased work of breathing
    C. Decreased air way resistance
    D. Collapse of alveoli

11. **Rise in capillary hydrostatic pressure at the base of lungs can result in:**
    A. Pulmonary embolism
    B. Pulmonary edema
    C. Dyspnea
    D. B and C

12. **Which of the following relaxes bronchial muscle?**
    A. Acetylcholine
    B. Leucotrienes
    C. Histamine
    D. Epinephrine

13. Voluntary hyperpnea can lead to:
- **A.** Respiratory acidosis
- **B.** Respiratory alkalosis
- **C.** Metabolic acidosis
- **D.** Metabolic alkalosis

14. The volume of air that is present in the lungs after a forceful expiration is called:
- **A.** Tidal volume
- **B.** Residual volume
- **C.** Expiratory reserve volume
- **D.** Functional residual capacity

15. Automatic respiration stops after:
- **A.** Bilateral vagotomy
- **B.** Transection above pons
- **C.** Transection below medulla
- **D.** Lesion of pneumotaxic center

16. Alveolar surface tension is caused by:
- **A.** Air in the alveoli
- **B.** Fluid in the alveoli
- **C.** Air fluid interface
- **D.** Lung surfactant

17. Physiological dead space is increased in all of the following *except*:
- **A.** Venous arterial shunt
- **B.** Emphysema
- **C.** Hyperpnea
- **D.** Pulmonary edema

18. In chronic obstructive pulmonary disease an increase in which of the following can not occur?
- **A.** Work of breathing
- **B.** Air way resistance
- **C.** Vital capacity
- **D.** Residual volume of lungs

19. Maximum voluntary ventilation is reduced in:
- **A.** Old age
- **B.** Obstructive lung disease
- **C.** Restrictive lung disease
- **D.** All of the above

20. In which of the following the residual volume of lungs is included?
- **A.** Tidal volume
- **B.** Vital capacity
- **C.** Functional residual capacity
- **D.** Inspiratory capacity

21. Pulmonary edema mostly occurs at the base of the lungs because of:
- **A.** Less PO_2 at the base
- **B.** More PCO_2 at the apex
- **C.** Increased capillary hydrostatic pressure at the base
- **D.** Increased vascular resistance at the base

22. A decrease in arterial blood oxygen content could occur by:
- **A.** An increase in 2, 3 DPG
- **B.** Rise in pH
- **C.** Decrease in PCO_2
- **D.** Decrease in temperature

23. 100% oxygen administration is useful in all of the following *except*:
- **A.** Loss of cabin pressure in airplane
- **B.** Hypoventilation
- **C.** Pulmonary edema
- **D.** Venous arterial shunt

24. Respiratory acidosis occurs in:
- **A.** Pneumonia
- **B.** Diarrhoea
- **C.** High altitude
- **D.** Hyperpnea

25. In obstructive pulmonary disease there is a high:
- **A.** Forced expiratory flow rate
- **B.** Ventilation perfusion ratio
- **C.** Functional residual capacity
- **D.** Vital capacity

ANSWER KEYS

1. (C)	**2. (A)**	**3. (A)**	**4. (D)**	**5. (A)**	**6. (D)**	**7. (C)**	**8. (D)**	**9. (C)**	**10. (D)**
11. (D)	**12. (D)**	**13. (B)**	**14. (B)**	**15. (C)**	**16. (C)**	**17. (C)**	**18. (C)**	**19. (D)**	**20. (C)**
21. (C)	**22. (A)**	**23. (D)**	**24. (A)**	**25. (C)**					

Short Answer Questions

1. List the agents that cause narrowing of airways.
2. State the importance of intrapleural pressure.
3. List the functions of lung surfactant.
4. State the normal compliance of lungs and list four conditions where it is reduced.
5. List the conditions that cause increase in physiological dead space.
6. State the normal pulmonary wedge pressure and list two conditions where it is increased.
7. State the normal carbon di oxide tension in the alveoli and mention a condition for its rise.
8. Explain Bohr effect and Haldane effect in respiratory gas transport.
9. Describe chloride shift.
10. State the neural center for rhythmic breathing.
11. List the ventilatory responses to hypoxia and hypercapnia.
12. Describe the changes in lung volume and capacities in chronic obstructive pulmonary disease.
13. Describe the importance of FEV_1 and PEFR.
14. Describe how carbon monoxide poisoning affects respiration and state how can the condition be improved.

9 Renal Physiology

Introduction

There are two kidneys, situated retroperitoneally deep in the abdominal cavity. Kidneys are not just the organs of excretion, but regulatory organs, which help to maintain **homeostasis**. The functions of kidney can be summarised as follows:

- Forms urine and excretes waste products of metabolism
- Maintains body fluid volume constant
- Regulates the composition of body fluid
- Maintains tonicity of body fluid
- Maintains arterial blood pressure
- Regulates the pH of blood
- Secretes hormones such as erythropoietin, renin and Vitamin D_3

Structure of kidney shows a cortex and a medulla. The nephrons in the cortex and medulla finally open into renal pyramids, which in turn join to form the renal papillae and pelvis. The latter continues as ureter of the kidney (Fig. 9.1)

NEPHRON

The structural and functional unit of kidneys is the nephron (Fig. 9.2). There are more than a million nephrons in each kidney. There are two types of nephrons present, namely **cortical** and **juxtamedullary** nephrons.

Cortical nephrons are more in number (85%)and present within the cortex of the kidney. They have short loop of Henle and are involved in the formation of urine. Juxtamedullary nephrons are few in numbers (15%) and arise from the corticomedullary junction with long loops of Henle.These nephrons are involved in the concentration of urine.

The structure of a nephron shows, a vascular region and a tubular part. The vascular region is the **glomerulus,** which consists of tuft of capillaries invaginating into the Bowman's capsule (Fig. 9.2). The visceral layer of Bowman's capsule and the glomerulus forms the filtering surface. The glomerular capillaries are situated, between the afferent and efferent arterioles, which give a high hydrostatic pressure.

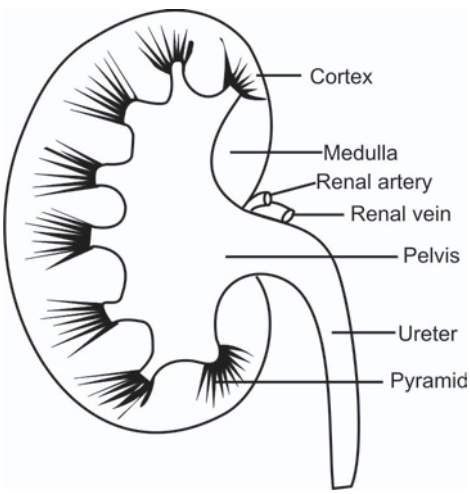


Fig. 9.1: Vertical section of kidney to show the internal structures

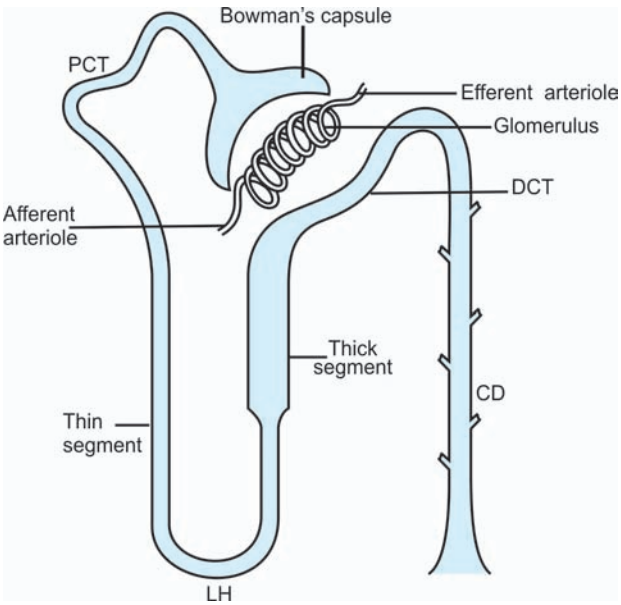


Fig. 9.2: Structure of nephron

Tubular part of nephron (Fig. 9.2)

It begins with a dilated Bowman's capsule, into which, 20 to 30 capillary loops of glomerulus invaginate. The Bowman's capsule ends in **proximal convoluted tubule (PCT).** The PCT cells show a brush border at the luminal surface, and infoldings at the basal and lateral surface give a basolateral labyrinth (Fig. 9.3). There are a number of mitochondria at the basal surface, which show a greater metabolic activity.

The Bowman's capsule and PCT are situated in the cortex of kidney. The PCT cells are supplied by the peritubular capillary network formed from efferent arteriole. In the case of juxtamedullary nephrons, the PCT cells are supplied by the branches from vasa recta.

Loop of Henle

The PCT ends in loop of Henle, which is shorter in cortical nephrons and very much longer in juxtamedullary nephrons. The descending limb tubular cells are flat and contain only a few mitochondria and less brush border.

The descending limb of loop of Henle has a thin segment. The descending limb goes down into the inner medulla. The ascending limb begins with the thin segment and ends in the thick

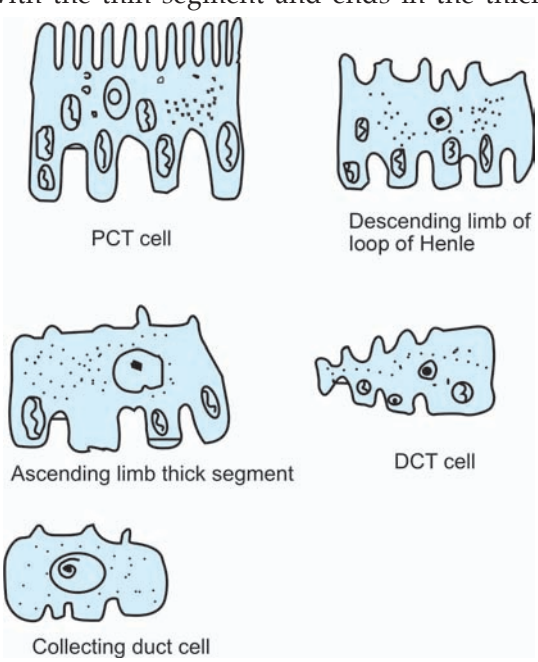


Fig. 9.3: Structure of cells in different segments of nephron. In the regions where there is active reabsorption process, the cells show brush border and more mitochondria at the basal infoldings

segment. The thick segment has many mitochondria at the baso lateral surface. The loop of Henle is supplied by vasa recta, which is a U shaped blood vessel formed from the efferent arteriole.

DCT

The thick segment of loop of Henle continues as DCT in the cortex. The distal convoluted cells face glomerulus and form the **macula densa** (Fig. 9.4). The structure formed by macula densa, juxtaglomerular cells of afferent and efferent arteriole and lacis cells give **juxtaglomerular apparatus.**

The DCT cells are similar to the PCT in their extensive infoldings at the basolateral surface and presence of more mitochondria.

Collecting duct

The DCT cells end in collecting duct, which goes into outer and inner medulla. There are two cell types in the collecting duct. One is *principal cells* and the other is *intercalated cells*. The principal cells contain few mitochondria, while the intercalated cells have plenty of them. Collecting ducts continue and end in renal papillae. This eventually leads to pelvis and ureter of the kidney (Fig. 9.1).

RENAL BLOOD FLOW

Both the kidneys together receive 1.2 lit/ minute as blood flow, which are about 25% of cardiac output. The cortex receives 90% of blood flow, while the medulla receives only 10%. The inner medulla receives only 1 to 2% of the renal blood flow. The sluggish blood flow of the inner medulla helps in maintaining the high osmolality of that region.

The renal circulation begins with the renal artery breaking into interlobar arteries. These in turn open into arcuate arteries, which run along the base of the papillae of the kidney. From arcuate artery, interlobular arteries arise and go to cortex of the kidney. From interlobular artery, afferent

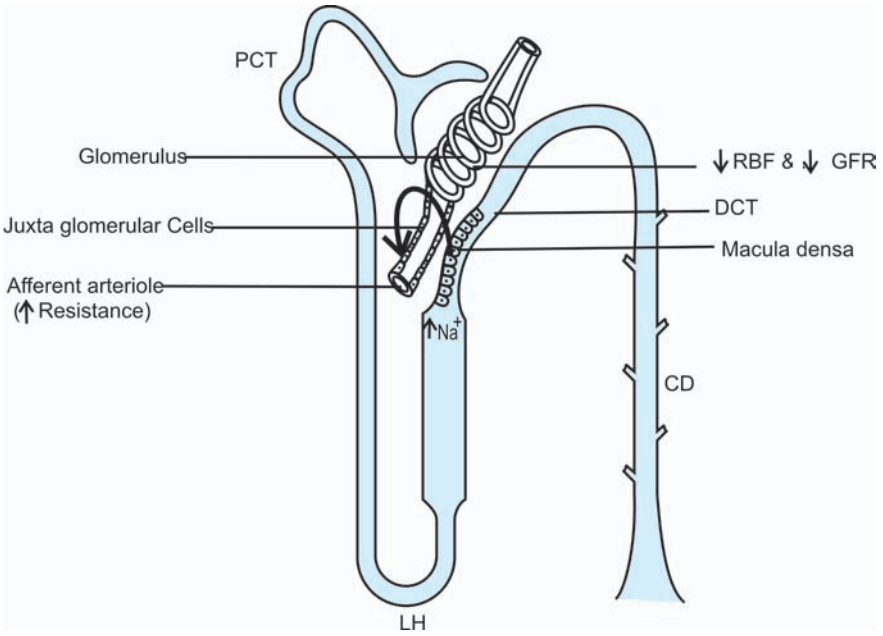


Fig. 9.4: Tubulo glomerular Feedback
Changes in the flow rate in the DCT, alters GFR, by changing the resistance of afferent and efferent arterioles. The increase in the filtered load of Na^+ in DCT, reduces GFR by constricting the afferent arteriole

arteriole is given off and from which arises glomerular capillary tuft. They unite and form the efferent arteriole. It continues as the vasa recta in the case of juxtamedullary nephrons and peritubular capillary in the cortical nephrons. The vasa recta supplies the loop of Henle, DCT, collecting duct and the neighbouring nephrons.

The venous drainage begins with the vasa recta opening into the interlobular vein and then into the arcuate vein. This in turn drains into the interlobar vein, which finally opens into the renal vein.

Measurement of renal blood flow

The renal blood flow can be measured by the **Fick's principle**. The substance used to measure is **PAH** (para-aminohippuric acid) or Diodrast. These substances are secreted from the peritubular capillaries into the lumen of the PCT. In one circulation, about 90% is secreted from the renal blood flow.

Renal PAH clearance measure renal plasma flow. In the determination of PAH clearance, the arterial plasma concentration of PAH is considered. Since the renal venous blood PAH concentration is not measured, the PAH clearance gives only the effective renal plasma flow (ERPF).

$$ERPF = \frac{UV}{P}$$

U = concentration of PAH in urine mg/ml
V = volume of urine in ml/ min
P = Plasma concentration of PAH in mg/ml

The **actual renal plasma flow** can be determined by knowing the extraction ratio. The normal renal extraction ratio for PAH is 0.9.

$$RPF\ (Renal\ plasma\ flow) = \frac{ERPF}{Extraction\ ratio}$$

The RPF ranges from 600 to 700ml/min

The renal blood flow can be determined if hematocrit value is known.

$$RBF = \frac{RPF}{(1\text{-}Hct)}$$

Normal RBF is close to 1200ml/min

Autoregulation of renal blood flow

The unique feature of the renal blood flow is its autoregulation. The RPF and GFR remain constant inspite of variations in the mean arterial blood pressure from 90 to 200 mmHg (Fig. 9.5). There are *two mechanisms*, which can explain the autoregulation.

Myogenic

The reflex constriction of afferent arteriole, when renal perfusion pressure is increased, leads to restoration of normal RBF and GFR. The mechanism is independent of extrinsic nerve supply of the afferent arteriole and is due to the property of the smooth muscle lining the afferent arteriole.

Tubuloglomerular feedback (Fig. 9.4)

It refers to the feedback from the renal tubules, which helps to maintain GFR. Whenever the rate of flow is decreased in the tubule, due to fall in GFR, the sodium reaching the first part of the distal tubule called macula densa will be reduced. This is sensed by the macula densa cells, which in turn causes dilatation of the afferent arteriole and at the same time, the release of renin from juxta glomerular cells, leads to the formation of **angiotensin II**. This causes **constriction of the efferent arteriole**. The net effect is increase of

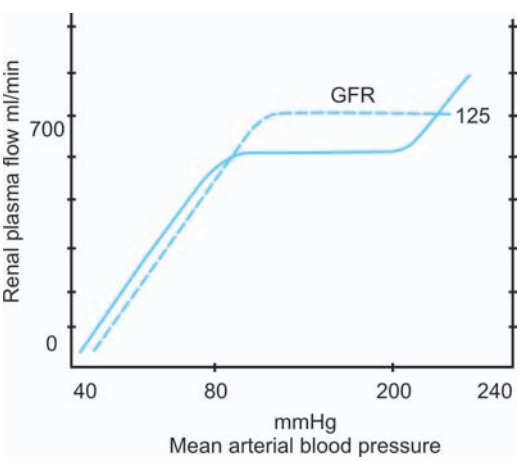


Fig. 9.5: Autoregulation of blood flow in kidneys

glomerular hydrostatic pressure and GFR. Conversely, when the rate of flow is increased due to rise in GFR, the GFR is reduced by the vasoconstriction of the afferent arteriole. The arteriolar constriction may also be due to the release of thromboxane A_2.

Renal oxygen consumption

Renal blood flow to the renal cortex is high but oxygen consumption is low whereas the blood flow to the medulla is low but the oxygen utilisation is high as the ascending loop of Henle is involved in active Na^+ reabsorption.

Sympathetic stimulation

Renal blood vessels are supplied by the sympathetic nerves and its stimulation causes reduction in renal blood flow and GFR. The sympathetic stimulation to the kidneys also causes release of renin.

GLOMERULAR FILTRATION

The first step in the formation of urine is filtration at the glomerulus. Glomerulus is a tufted network of capillaries, enclosed in the Bowman's capsule (Fig. 9.6). The renal plasma filters through the capillary endothelium and enters the Bowman's capsule. The ultra structure of the glomerulus, reveals, the presence of three layers for the filtration barrier (Fig. 9.7). They are:

Capillary endothelial layer
Basement membrane
Visceral layer of Bowman's capsule.

The capillary endothelial cells have fenestrae with 8 nm size. The solutes pass through this, depending on their molecular size and their electrical charge.

The basement membrane is made of proteoglycan material, which acts as a sieve, allowing the solutes to easily pass through.

The visceral layer of the Bowman's capsule is called the podocytes, as the apical border shows the presence of foot like processes, which encircle the endothelium. There are slits or gaps between the foot like processes, through which, water and solutes filter through and enter the lumen.

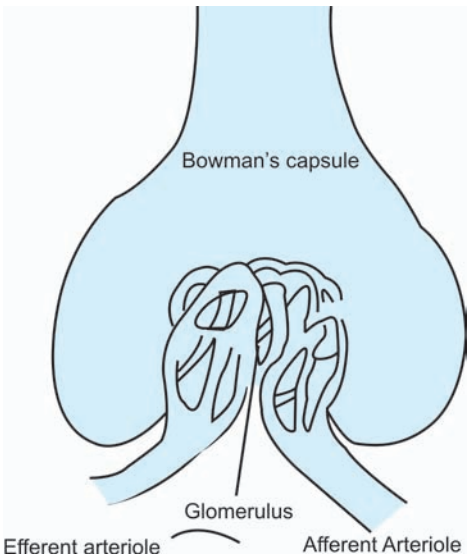


Fig. 9.6: Structure of a glomerulus

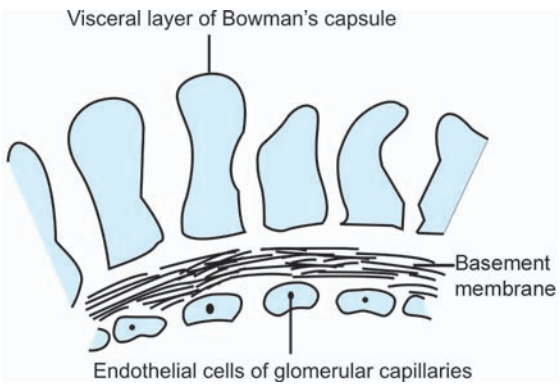


Fig. 9.7: Ultrastructure of glomerulus showing the three layers for filtration

The glomerular membrane *per se* includes all these three layers for filtration.

Mesangial cells and GFR

There are mesangial cells present between the capillary loops and between basement membrane and capillaries. These cells show contractile function. It can cause obliteration of the capill ary lumen, reducing the renal blood flow and GFR. Agents like angiotensin II, vasopressin, etc, can cause mesangial cell contraction. Mesangial

cells are also present outside the glomerulus and they are called *extraglomerular mesangial cells.* They are present in the JGA.

Permeability of charged particles

The filtration of solutes depends on their size and electrical charge. Anions up to 4 nm show filtration and at 4 nm size, the filtration is only 50% of the neutral substances of same size. The anions are negatively charged and the cell membrane also has sialoproteins, which are negatively charged. The two negatively charged particles repel each other and that's why, albumin, which is around 7 nm is not filtered, eventhough, the pore size of the endothelium is slightly greater than 7 nm. When glomerular membrane is damaged by inflammation or infections, i.e., glomerulonephritis, the negative charge on the membrane is lost and allows albumin to appear in the urine.

Neutral substances are filtered freely up to 4 nm and between 4 nm to 8 nm size, the filtration is inversely related to the size. At 8 nm size, the permeability is almost nil.

Cations show filtration similar to neutral ions, but greater than it.

Factors determining GFR (FIG. 9.8)

Glomerular filtration is an **ultra filtrate of renal plasma,** as the plasma proteins are not filtered. The filtrate composition is similar to plasma except for proteins.

The ultra filtration depends on the Starling's *Forces* namely:

Glomerular capillary hydrostatic pressure (50 mmHg)
Colloidal osmotic pressure (25 mmHg)
Tubular hydrostatic pressure (15 mmHg)
Filtration pressure= $k_f(P_{GC} - \pi_{GC}) - (P_T - \pi T)$

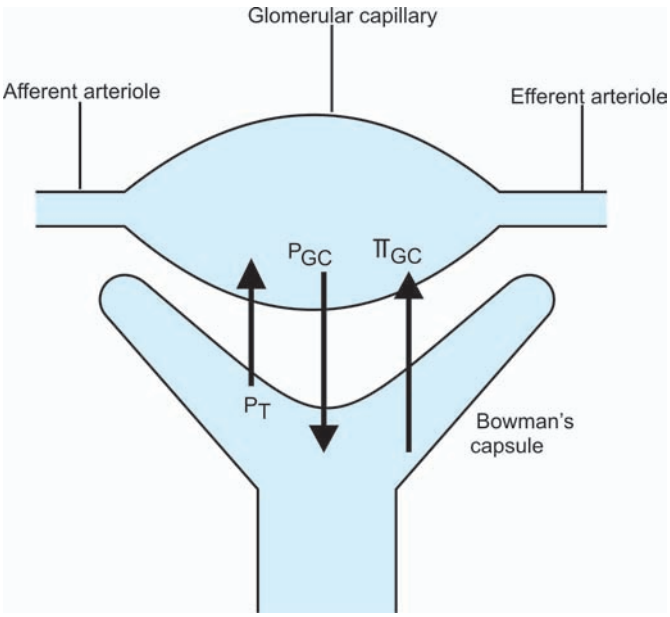


Fig. 9.8: Starling's force determining GFR

P_{GC} : Glomerular capillary hydrostatic pressure
P_T : Tubular hydrostatic pressure
π_{GC} : Colloidal osmotic pressure

k_f = filtration coefficient which is the product of surface area of glomerular membrane and its permeability.
π_{GC} = glomerular capillary hydrostatic pressure
p_{GC} = colloidal osmotic pressure of glomerular capillaries
P_T = tubular hydrostatic pressure
π_T = osmotic pressure of tubular fluid (since there is no filtration of proteins, this pressure is nil)

Net filtration pressure = 50 – 25 – 15 = 10 mmHg.

The rate of glomerular filtration is 125 ml/min for an adult having 1.72 sqmt of surface area. In female, it is 10% lower and in old age the GFR is less.

Determination of GFR

GFR is determined by the clearance of inulin or creatinine. The latter is an endogenously produced substance. Its clearance measurement is very convenient and less cumbersome. We should keep in mind that the plasma measurement of creatinine will not reflect any renal dysfunction.

$$\text{Inulin clearance} = \frac{UV}{P}$$

U = urine concentration of inulin (mg/ml)
V = volume of urine in ml/min
P = plasma concentration of inulin (mg/ml)

Inulin is a fructopolysaccharide, obtained from the dhalia tubers. Its molecular weight is 5200. It is innocuous and filtered freely at the glomerulus. It shows neither reabsorption nor secretion in the renal tubules. Hence, the rate of excretion will be equal to the rate of filtration.

Filtration fraction (FF)

It is the ratio between the GFR and RPF. Its value is 0.2.That means 20% of renal plasma is filtered in one circulation. The FF value is increased in conditions that lowers blood volume, i.e. hemorrhagic shock.

Factors that affect GFR

- Renal blood flow
- Renal perfusion pressure
- Colloidal osmotic pressure
- Tubular hydrostatic pressure
- Surface area of glomerular membrane
- Number of active glomerular capillaries.

GFR is reduced in:

- Fall in renal blood flow
- Fall in blood pressure
- Increase in tubular hydrostatic pressure
- Rise in colloidal osmotic pressure
- Decrease in surface area of glomerular membrane
- Decrease in the number of active glomerular capillaries
- Constriction of afferent arteriole by sympathetic stimulation, angiotensin II.

The GFR and RBF remain constant within normal limits over a wide range of arterial blood pressure change (90 to 200 mmHg). This is due to the autoregulation mechanism described earlier. The GFR is affected, if the mean arterial blood pressure does not occur within the autoregulatory range (Table 9.1).

Table: 9.1: Conditions which can alter FF in the kidney

Condition	Changes in GFR	Changes in RPF	Change in FF $\frac{GFR}{RPF}$
Afferent arteriole constriction	↓	↓	No change
Efferent arteriole constriction	↑	↓	↑
↑ Colloidal osmotic pressure of plasma	↓	No change	↓
Ureteral obstruction	↓	No change	↓

TUBULAR FUNCTION

Formation of urine

The first step in the formation of urine is ultrafiltration of renal plasma into the lumen of Bowman's capsule. The filtrate is formed at the rate of 125 ml/ min and only 1 to 2 ml is excreted as urine, while the rest is reabsorbed in the various segments of nephron.

The tubular functions are **selective reabsorption** and **secretion** (Fig 9.9). These two processes modify the volume, composition, tonicity and pH of filtrate that pass through the lumen of nephron (Fig. 9.10). These changes ensure the constancy of the internal environment.

PCT

In the PCT, about two third (65%) of the filtrate volume is reduced and the tonicity remain isotonic to the plasma. The substances that are completely reabsorbed in the PCT include glucose and amino acids. They are called threshold substances, as they are completely reabsorbed in the normal plasma concentrations.

Reabsorption of glucose and amino acids

The renal threshold of glucose is 180 mg%. It is the plasma level at which glucose appears in the urine. The reabsorption of many solutes in the PCT depends on the primary active transport of Na^+. From the lumen Na^+ transport occurs by facilitated diffusion. Glucose and amino acids are cotransported (symport) with Na^+. The Na^+ from the cell moves into the lateral intercellular space, by the active transport involving Na^+–K^+ ATPase. The transport of Na^+ from the cell gives chemical gradient for the further movement of Na^+, glucose and amino acids. That's why, the transport of glucose and amino acids are known as **secondary active transport** (Fig. 9.11). Substances like *phosphate, lactate, sulphates, citrates* are also transported in PCT by secondary active transport.

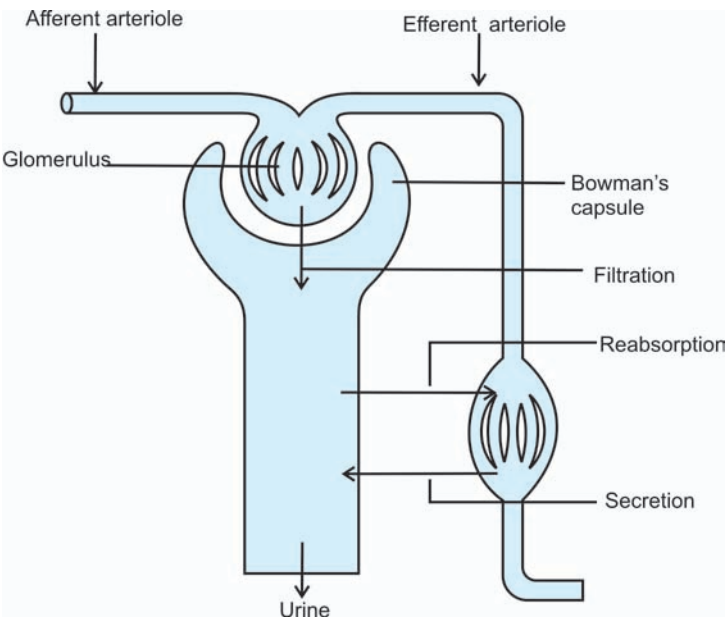


Fig. 9.9: Processes in the formation of urine

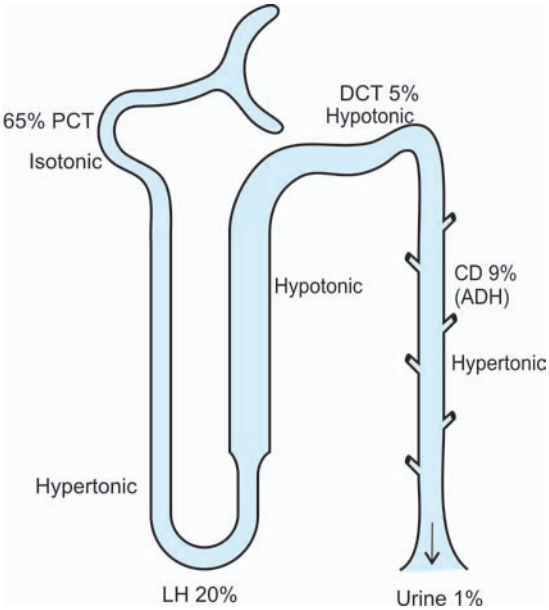


Fig. 9.10: Changes in the volume and tonicity of the filtrate in the nephron

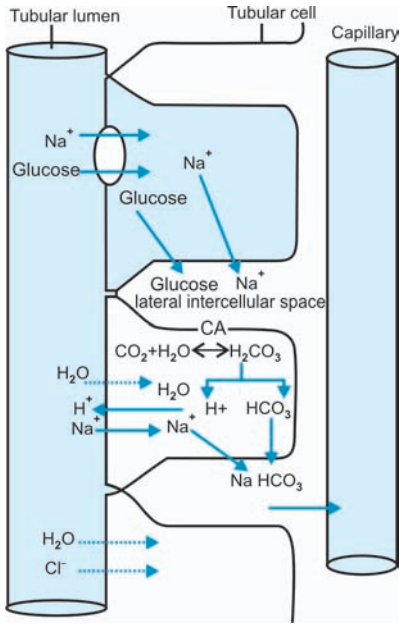


Fig. 9.11: Diagram to show the reabsorption and secretion in the PCT. The sodium and glucose are cotransported from the tubular lumen to the cell by facilitated diffusion. Inside the cell, sodium is actively transported by the enzyme $Na^+ – K^+$ ATPase at the basolateral border. This primary active transport of sodium is responsible for the chemical gradient, which facilitates further glucose entry into the cell. This type of transport is called secondary active transport. Substances like amino acids, sulphate, phosphate are also transported by the same mechanism. There is also $Na^+ – H^+$ counter transport in the PCT. The hydrogen ion comes from the hydration of carbon dioxide catalyzed by the enzyme carbonic anhydrase. The sodium combines with the bicarbonate inside the cell and enters the capillary through the basolateral interstitial space. The active solute reabsorption also causes the passive movement of water into the PCT cell

TmG

Glucose transport in the PCT cells show maximum reabsorptive capacity known as **Transport maximum (Tm).** The TmG value in men is 350 mg/min and in women 300 mg/min. For this Tm, the threshold level in plasma should have been 300 mg%

$$\frac{375}{125} \times 100$$

but, it is 180 mg%. This deviation of actual value from the ideal is known as **splay** and it can be seen in the glucose titration curve (Fig. 9.12) The reason for the splay is that all nephrons do not show uniform GFR and TmG values.

The secondary active transport of glucose can be inhibited by the metabolic poisons like phlorhizin. Glucose clearance in the normal health is taken as zero, although it is seen in traces. It appears in the urine *(glycosuria)*, when plasma glucose level is more than renal threshold level (180 mg%) as in diabetes mellitus.

Water reabsorption

In the PCT, water is reabsorbed passively along the osmotic gradient created by the active transport of the solute Na^+. In PCT about 65% of the filtrate is reabsorbed and equal amount of water from the filtrate is also reabsorbed. The PCT tubular cells have water channels called

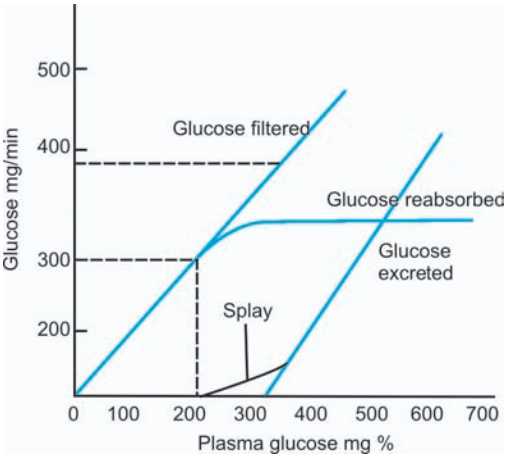


Fig. 9.12: Graph showing relationship between plasma level of glucose and its excretion in the urine. The splay in the graph is the deviation of the actual plasma level and renal reabsorption from the theoretical values

aquaporin- I. The filtrate at the end of the PCT is **isotonic** to plasma.

Potassium

Potassium is filtered, reabsorbed and secreted in the renal tubules. In PCT 65% of the filtered Potassium is actively reabsorbed. In the asecending limb of loop of Henle about 25% reabsorbed. There is secretion of K^+ which takes place in the distal tubules and collecting duct. The amount of K^+ secreted depends on flow rate of the filtrate in the distal tubules, amount of Na^+ reaching the distal segments and acid base status of blood. Thus the daily excretion of K^+ can vary depending on the amount secreted from DCT and collecting ducts.

Cl^- and HCO^-_3

In the PCT there is also passive diffusion of Cl^- and water through the paracellular pathway. The HCO^-_3 in the filtrate is reabsorbed as CO_2. The secreted H^+ combines with the bicarbonate and forms CO_2 and water (Fig. 9.14). HCO_3^- excretion in the urine depends on its plasma concentration. When plasma HCO_3^- is in its normal level (25 mEq/L), almost all filtered HCO_3^- is reabsorbed in the PCT. When plasma HCO_3^- decreases, there will be more H^+ ions present in the filtrate showing that secretion of H^+ is far more than the amount of HCO_3^- available for reabsorption. The increased H^+ ions in the filtrate also causes NH_3 secretion in the DCT. This will finally give an acidic urine with increased NH_4 level. When the plasma HCO_3^- level is increased, the excretion of HCO_3^- in the urine is also increased and the urine becomes alkaline.

Secretion in PCT

The substances that are secreted in the PCT include H^+, urate, hippurate, 5HIAA, penicillin, salicylates, probenecid, PAH, Diodrast, etc. The secretion in the tubules involves active transport with Tm and gradient related passive secretion. The secretion of PAH and creatinine is by active transport.

Starling's force in lateral intercellular space causes the movement of solutes and water into the peritubular capillary(Fig. 9.13).

The movement of solutes and water into peritubular capillary depends on the plasma

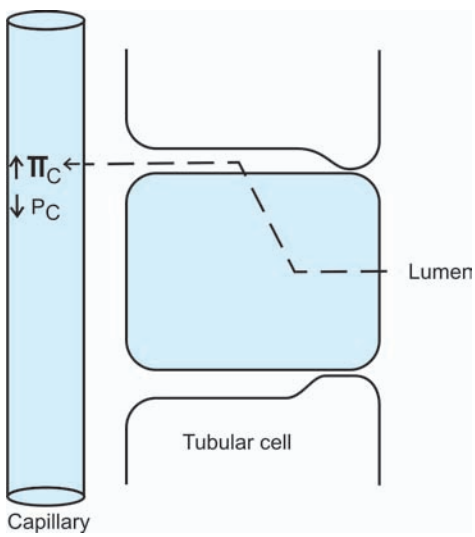


Fig. 9.13: Starling force determining reabsorption in the peritubular capillary

π_C : Colloidal osmotic pressure
P_C : Capillary hydrostatic pressure

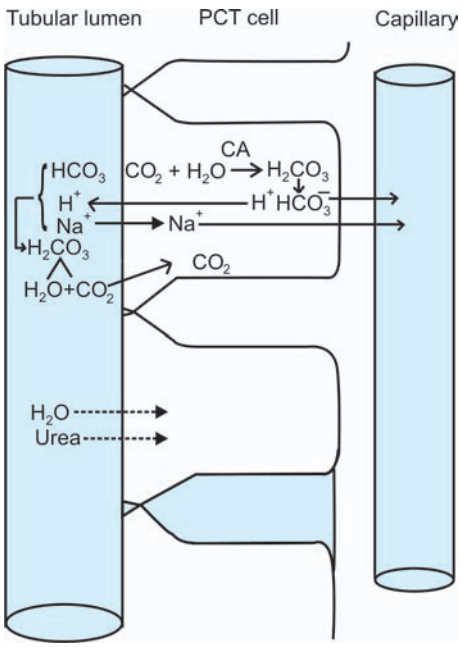


Fig. 9.14: Reabsorption of Na^+ in the PCT and the mechanism by which the secreted H^+ ions are neurtalized in the lumen. Urea is reabsorbed passively, following the passive movement of water. Whenever water is passively reabsorbed, the concentration gradient for the urea becomes more, which facilitates its passive movement into the cell

osmotic pressure of peritubular capillary and hydrostatic pressure of fluid in the lateral intercellular space.

Glomerulotubular balance

The PCT cells reabsorb a constant fraction of Na^+ and water from glomerular filtrate, instead of a fixed quantity. This is due to the presence of glomerulotubular balance. It means, that the reabsorption of sodium from the PCT is related to the rate of glomerular filtration. There will be an increased reabsorption of Na^+ and water, when the filtrate that is formed is more. Conversely, the reabsorption of Na^+ and water is reduced, when the glomerular filtration is less. This happens, when the Na^+ balance in the body is normal and the GFR spontaneouly changes. The mechanism of glomerulotubular balance can be explained by the changes in the peritubular capillary oncotic pressure.

Changes in loop of Henle

In the loop of Henle, about 20% volume reduction occurs. In the descending limb, the tubular cells are permeable to water and there is existence of hyperosmolality of the medullary interstitium, due to counter current multiplier mechanism. The reabsorption of water changes the tonicity of the filtrate from isotonic to **hypertonic** at the end of descending limb (Fig. 9.8).

The ascending limb is permeable to solutes **Na^+ K^+** and **$2Cl^-$**. The reabsorption of solutes from the ascending limb, especially in the thick segment, changes the tonicity of the filtrate to **hypotonic** at the end of ascending limb. The reabsorption of Na^+, $Cl^-$and K^+ from the thick ascending limb is inhibited by the loop diuretics like furosemide.

The major function of LH is to generate hypertonicity of the medullary interstitium, which is maintained by the counter current exchanger system, produced by the vasa recta. The counter current multiplier and exchanger systems are necessary for the concentration of the urine.

Changes in DCT

The filtrate, which is entering the DCT is always **hypotonic**, irrespective of the tonicity of the final urine that is excreted. About 5% of volume reduction takes place in this segment. The early part of DCT cells are the continuation of thick segment of ascending limb of LH and these cells show reabsorption of Na^+, Cl^-. These cells are not permeable to water and urea. The later part of DCT has two types of cells, namely **principal** and **intercalated cells**. In the former type, reabsorption of Na^+ occurs in exchange for K^+ in the presence of the hormone aldosterone. This hormone causes the transport of Na^+ from the cell into the lateral intercellular space in exchange for K^+ entry into the cell. This is achieved by increasing the activity

of Na^+-K^+ ATPase enzyme at the lateral intercellular border (Fig. 9.12). The Na^+ reabsorption is also continued in the collecting ducts in the presence of aldosterone. Aldosterone action is best seen in the cortical segment of collecting ducts.

Water reabsorption in DCT

The water reabsorption in the DCT is dependent on the presence of vasopressin hormone, which changes the permeability of the distal tubular cells for water. This occurs in the later part of the DCT, which continues with the cortical segment of collecting duct.

The hormone **vasopressin(ADH)** which is released from the posterior pituitary gland acts on the late DCT and CD. The action of ADH in CD is important for changing the final volume and tonicity of urine. Vasopressin acts on the V_2 receptors present in the late DCT and CD cells. The receptor activation causes insertion of **Aquaporin 2** water channel proteins into the cell membrane to make the cells permeable to water.

The intercalated cells in the DCT secretes H^+ and the formation of H^+ ion from CO_2 hydration by the carbonic anhydrase gives HCO_3^- ion. This moves into the peritubular capillary along with Na^+. The entry of bicarbonate into the capillary helps acid base balance.

Renal tubular cells secrete both NH_4 and NH_3. The NH_3 is secreted only from CD, whereas NH_4 is secreted from PCT, thick segment of ascending limb and DCT. In both the secretions there is addition of HCO_3 to the plasma which helps to regulate pH (Fig. 9.15).

The tonicity of the filtrate in the DCT is hypotonic in the absence of ADH and if ADH is present the filtrate first changes to isotonic and then to hypertonic as it enters CD.

Changes in collecting duct

Collecting duct cells are permeable to water in the presence of vasopressin hormone. About 9% of volume reduction occurs in this segment. The presence of hyperosmotic medullary interstitium,

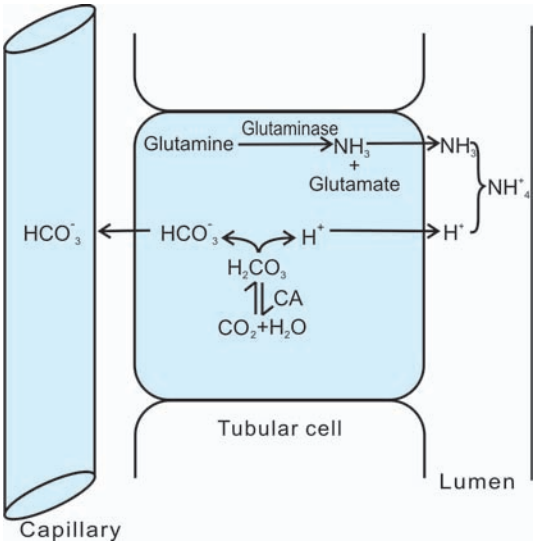


Fig. 9.15: NH_4^+ secretion in the tubular cells

gives osmotic gradient for the movement of water out of collecting ducts (Fig. 9.16). The tonicity of the filtrate becomes hypertonic (antidiuresis) in the collecting ducts. In the absence of vasopressin, the water reabsorption cannot occur and the filtrate becomes hypotonic and water diuresis is produced.

Urea reabsorption

Urea reabsorption is seen passively, when water moves out of the tubular lumen. The removal of water out of the tubular lumen gives concentration gradient for urea and this facilitates the passive reabsorption. In the PCT, DCT, and collecting ducts, the reabsorption of urea can be seen. The clearance of urea depends on the urine flow rates. If the urine flow rate is 2 ml/min, the clearance of urea is 75 ml/ min (maximum urea clearance). Lesser the urine flow, greater the urea reabsorption in the tubules and lesser will be its clearance.

URINE CONCENTRATION

Concentration mechanism is one of the homeostatic processes to maintain the body water balance. In our daily life, it is commonly

experienced, that, whenever, there is dehydration arising from, excessive vomiting, diarrhea sweating, etc, the urine output is reduced and concentrated. Conversely, when there is excess fluid intake, there is a regulatory response from the kidneys by excreting a dilute urine called water diuresis.

The juxtamedullary nephrons are involved in the urine concentration. They have long loop of Henle, extending up to the inner medulla. The advantage of this structure is that the medullary interstitium can become hyperosmotic in greater depth, which is a prerequisite for producing a concentrated urine.

The segments of nephron that are involved in the creation of medullary hyperosmolality are:
Loop of Henle
Collecting duct
Vasa recta.

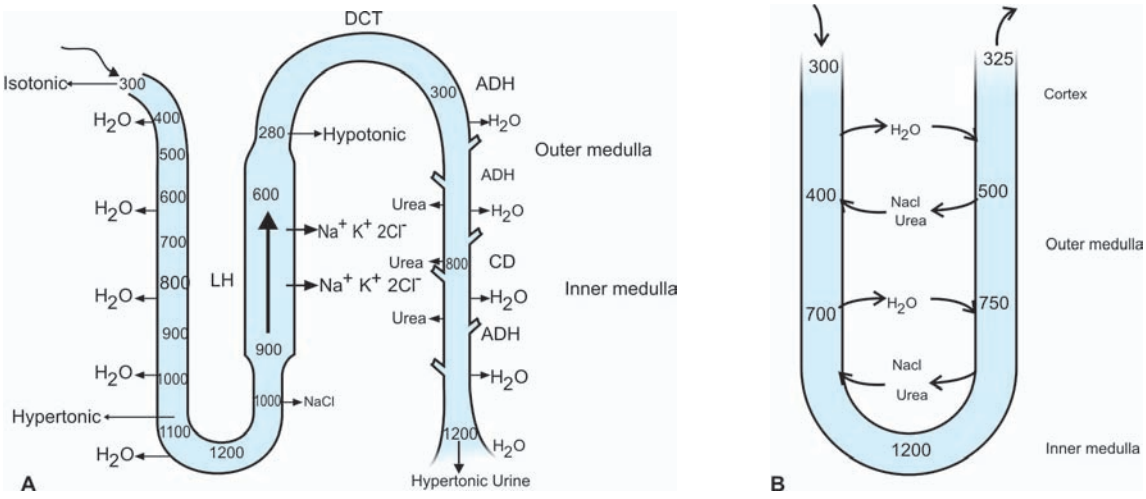
The hormone, which is taking part in the concentration of urine is **ADH (vasopressin**).

It should be known, that greater the hypertonicity of inner medulla, greater the concentration of the final urine that can be excreted. From the above statement, we can learn that there are two mechanisms required for this process. One to create hypertonicity of the medulla and the other to maintain it. These two processes are:
Counter current multiplier system
Counter current exchanger system.

Counter current multiplier system

The loop of Henle of juxtamedullary nephrons are longer and extends deep into the inner medulla. The U shaped arrangement of the tubule, enables the fluid to flow in opposite directions (counter current) in the two limbs and during this flow, the ascending limb loses its tonicity to the descending limb (Fig. 9.16). The descending limb fluid shows tonicity, which is identical to the tonicity present outside. The tonicity of the filtrate in both the descending limb and medullary interstitium is multiplied in a vertical direction. The tonicity begins at 300 milliosmole/liter of water (isotonic) at the corticomedullary junction and gradually increases to reach 1200 milliosmoles/liter of water **(hypertonic)** at the hairpin bend of loop of Henle.

Since the ascending limb loses its tonicity due to the solute removal, there is a gradual decrease in the osmolarity of both the filtrate and the medullary interstitium. From 1200 milliosmole/liter of water, it decreases vertically upwards to reach 270 milliosmoles/liter of water



Figs 9.16A and B: Counter current multiplier (A) and counter current exchanger (B) systems

(hypotonic). The filtrate that is entering the distal tubule is always **hypotonic** irrespective of the tonicity of the final urine that is excreted.

The loop of Henle has a descending limb, where the cells are permeable to water and not to solutes. The ascending limb shows a thin and a longer thick segment.

The thin segment of ascending limb shows movement of Na^+ and Cl^- along the concentration gradient from the lumen into the tubular cells and from the cells into the medullary interstitium, the Na^+ moves by active transport.

The thick segment is permeable to the solutes, sodium, chlorides, potassium and urea. These cells are not permeable to water. This part of the segment shows reabsorption of 1 Na^+, 1 K^+ and 2 Cl^-. The reabsorption of 1 Na^+1 K^+ 2Cl^- from the tubule of the thick segment into the tubular cells is by **secondary active transport** as it depends on the chemical gradient created by the active transport of Na^+ from basolateral border of the tubular cell into the interstitium. The purpose of the thick segment of the ascending limb removing the solutes is to make the medullary interstitium hyperosmotic. More the hypertonicity of medulla, more the water can leave by the osmotic force from the descending limb. This will give greater solute load in the thick segment of ascending limb, which in turn ensures greater tonicity of the medullary compartment. Unless there is hypertonic medullary interstitial fluid, the collecting duct cells cannot have osmotic gradient for water reabsorption in the presence of ADH.

Counter current exchanger system

The hypertonicity of the medulla created by the loop of Henle is maintained by the vascular system, vasa recta. It is also a U shaped structure with the descending and ascending limbs. The descending limb of vasa recta gains tonicity by the removal of water and entry of solutes. The ascending limb loses tonicity, due to the removal of solutes and entry of water (Fig. 9.16). The final tonicity of blood leaving the ascending limb is slightly hypertonic to the plasma. The vasa recta facilitates recirculation of solutes within the medullary interstitium and thereby maintains hypertonicity.

Counter current multiplier mechanism creates hyperosmolality of the medullary interstitium, by the reabsorption of solutes from the thick ascending limb of loop of Henle.

Counter current exchanger mechanism maintains the hyperosmolality of the medulla, by recirculating the solutes within medulla.

Role of collecting duct

ADH hormone is secreted, whenever, there is increase in the osmotic pressure of the blood, caused by the dehydration or decrease in the ECF volume. ADH acts on the distal tubules and collecting duct. These cells are not permeable to water unless ADH is present. In the presence of ADH, the permeability is changed for water and the osmotic gradient present in the inner medulla causes the removal of water from the filtrate and the urine becomes concentrated **(antidiuresis)** (Fig. 9.16).

Role of urea in medullary hypertonicity

The reaborption of water from collecting ducts in the presence of ADH creates concentration gradient for urea. Urea is transported by urea transporters by facilitated diffusion into the medullary interstitium. The urea concentration in the medullary interstitium also adds to the hyperosmolality, which can facilitate more movement of water from collecting duct in the inner medulla to make the urine concentrated.

When there is fall in osmotic pressure of blood or rise in ECF volume, ADH hormone secretion is inhibited. The renal medulla also in such conditions, does not produce hypertonicity. This is mainly due to the increase in flow rate of the filtrate in the loop of Henle and faster rate of blood flow in vasa recta. The latter can remove the solutes from the inner medulla. In the absence of both the medullary osmotic gradient and the permeability of the distal segments, the water reabsorption does not occur in these segments, resulting in **water diuresis**.

Free water clearance

The amount of water gained or lost in the urine can be known from the free water clearance measurement.

$C_{H_2O} = V - C_{osmol}$

V = volume of urine

C_{osmol} = osmolar clearance.

Free water clearance in antidiuresis, measures the amount of water to be added to the urine to make it isotonic with the plasma. The value is negative (–1 to –2 ml/min).

In water diuresis, the free water clearance measures the amount of water that should be removed from the urine to make it isotonic with the plasma. It is 12 to 14 ml/min (Fig. 9.17).

The concentration or dilution of urine can be determined by measuring the specific gravity of the urine. Normal urine has a specific gravity 1010. In antidiuresis, it can go maximally up to 1030 and in water diuresis it can come down to 1002 (Fig. 9.18).

Factors affecting concentrating ability of kidney

- Absence of vasopressin hormone secretion from the hypothalamus (pituitary diabetes insipidus)

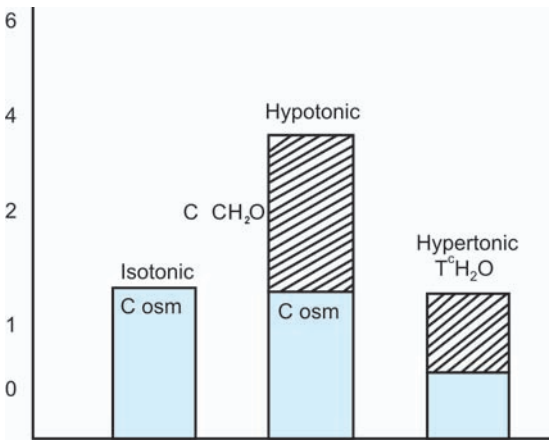


Fig. 9.17: Free water clearance in hypertonic and hypotonic urine excretion. In hypotonic urine the free water clearance depends on the amount of solute free water that has to be removed from urine to make it isotonic to plasma. In hypertonic urine, it is the amount of solute free water that has to be added to the urine to make it isotonic to plasma

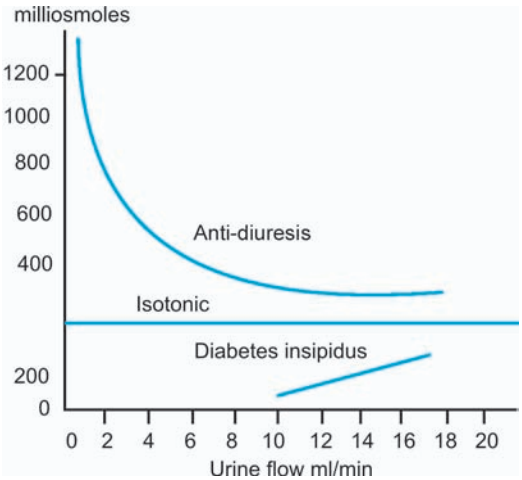


Fig. 9.18: Relationship between urine osmolality and urine flow rate

Note the higher urine osmolality in maximum vasopressin secretion (antidiuresis) and lower urine osmolality in diabetes insipidus

- Failure of the renal tubule to respond to vasopressin (nephrogenic diabetes insipidus)
- Inappropriate secretion of vasopressin (vasopressin secretion from the tumor tissues as occurs in lungs)
- Damage to the functional nephrons
- Drugs and diuretics.

Water diuresis

It is the excretion of increased amounts of water in the urine. The urine volume is greater and the specific gravity decreases, reaching the value upto 1002. Water diuresis occurs, when the body water volume is more. The dilution of plasma by the water, decreases the osmotic pressure, which inhibits the ADH release from the hypothalamus. The absence of ADH, produces a hypotonic urine called water diuresis.

Osmotic diuresis

It is the excretion of increased volume of urine with the solutes like sodium chloride, glucose, manitol, etc. These osmotically active solutes, when filtered at the glomerulus in greater amounts, the PCT reabsorbs at their Tm values and the remaining unreabsorbed solutes retain

Contd...

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water with it. These solutes along with water, pass through the loop of Henle, distal tubules and are excreted as osmotic diuresis. Polyuria in diabetes mellitus, is due to the increased filtered load of glucose which is excreted as osmotic diuresis. When the unreabsorbable, but osmotically active solutes like mannitol is present in the filtrate, then, also, osmotic diuresis is produced. The osmolality of the urine approaches the plasma osmolality in osmotic diuresis.

In water diuresis, the amount of water reabsorbed in the PCT is normal, but in osmotic diuresis, because of the greater concentration of the solutes, water reaborption is less. Even when ADH is present, water reabsorption in the distal segments does not occur, as there is no osmotic gradient in the medullary interstitium.

REGULATION OF BODY WATER BALANCE

In man, the total body water is about 60% of body weight and in 70 kg body weight, it is around 40 lit. This is the total body water **(TBW).** It is distributed between intracellular **(ICF)** and extracellular fluid **(ECF)** compartments. ICF in the total body water forms **two third** and the remaining **one third** is the ECF.

The ECF contains the plasma and interstitial fluid. Interstitial fluid includes another compartment called **transcellular fluid,** consisting of CSF, aqueous humor, synovial fluid, and the secretions of GI tract.

The water moves from one compartment to another following the osmotic force and in normal conditions, the volume of water in each fluid compartment is kept within the normal range.

Determination of TBW and ECF

Fluid volume can be determined by injecting a substance which can be distributed in the space that is being selected and finding out its mean concentration in the plasma.

$$\text{Fluid volume} = \frac{\text{Amount of substance injected} - \text{amount excreted in the urine}}{\text{Mean concentration in the plasma}}$$

Substances like Tritium oxide, antipyrine can be used for measuring TBW while substances such as inulin, sucrose, sodium, thiosulphate will measure ECF.

ICF is determined from TBW and ECF

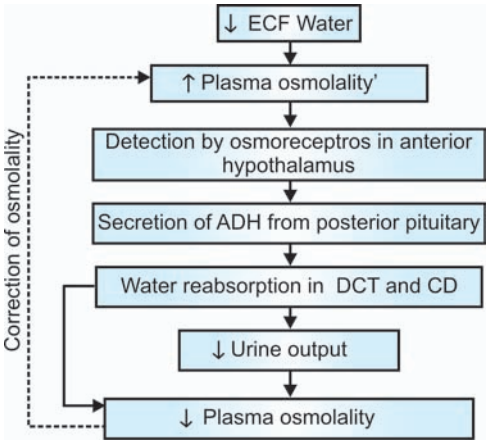
ICF = TBW- ECF

Osmolality and body fluid volume

The fluid compartment that is more susceptible for the fluctuation is the ECF and kidneys show regulatory mechanisms to maintain its volume constant. The ECF volume is regulated, mainly by Na^+ and Cl^- ions, which are present in higher concentration in the extracellular compartment. The tonicity or osmolality of the ECF is also due to NaCl concentration. Changes in the sodium level in ECF, thus, can greatly affect ECF volume and its tonicity.

Whenever, ECF volume is changed, it affects blood volume, which in turn affects the cardiovascular function. For example, the fall in ECF volume can lead to fall in the blood volume and fall in the arterial blood pressure. If the fall in blood pressure is significant, it can lead to circulatory shock and death ensues, if immediate remedial measures are not taken. Loss of water from ECF is commonly observed in dehydration states, as in burns, vomiting, diarrhea and excessive sweating. The volume of the ECF can increase, if there is excess water or fluid intake. The ECF volume is also subjected to fluctuations, due to osmolality changes of plasma. Increase in the plasma osmolality, as in excess NaCl (salt retention), leads to retention of water, which raises ECF volume. Loss of salt from the body leads to decrease in plasma osmolality and also decrease in ECF volume. From the aforesaid description, it can be understood that ECF volume can change, due to changes in the plasma osmolality. So it becomes necessary to discuss both the regulations of water and Na^+ balance to understand the total body water balance.

Regulatory responses to fall in ECF volume



Fall in ECF volume leads to

- Stimulation of thirst
- Stimulation of vasopressin secretion
- Stimulation of sympathetic nervous system
- Secretion of renin-angiotensin
- Secretion of aldosterone
- Inhibition of atrial natriuretic peptide (ANP)

Stimulation of thirst

Thirst center is situated in the hypothalamus near the third ventricle, very close to the neurons secreting the ADH hormone. Rise in the plasma osmotic pressure, fall in the ECF volume, drying of pharyngeal mucosa stimulate the thirst center. The hormone angiotensin II directly acts on this center and stimulates thirst. The stimulation of this center leads to the drinking behaviour.

Secretion of ADH (vasopressin)

Fall in ECF volume, rise in osmotic pressure of plasma are the important stimuli for the supraoptic and paraventricular nuclei of the hypothalamus to secrete ADH. This hormone is transported along the hypothalamohypophyseal tract and released from posterior pituitary. ADH acts on the distal tubules and changes the permeability of the tubular cells for water. The reabsorption of water from these segments leads to rise in the concentration of the final urine that is excreted. This is a regulatory mechanism to maintain the water balance.

Stimulation of sympathetics

The immediate response to fall in the ECF volume is increase in the sympathetic activity. The decrease in ECF volume causes fall in blood volume and arterial blood pressure. The absence of baroreceptor stimulation causes the reflex stimulation of the sympathetic nervous system. The sympathetic stimulation of the renal blood vessels causes, decrease in renal blood flow, which in turn reduces GFR. This reduces the urine flow rate, which regulates ECF volume.

Stimulation of renin-angiotensin

Renin-angiotensin secretion is stimulated, whenever there is a fall in ECF volume, fall in blood pressure, fall in sodium level and rise in K^+. Angiotensin II acts at the proximal, distal and collecting segments and causes Na^+ reabsorption. It also causes the stimulation of aldosterone secretion from the adrenal cortex. The formation of angiotensin II, helps to raise arterial blood pressure by its vasoconstrictor action and also through Na^+ reabsorption. The body water raises, due to reabsorption of sodium and increased intake of water arising from the stimulation of thirst by the action of angiotensin II.

Secretion of aldosterone

Aldosterone is a mineralocorticoid, which acts on the P cells **(Principle cells)** of the collecting ducts and causes Na^+ reabsorption in exchange for K^+ or H^+ secretion. Aldosterone secretion from adrenal cortex occurs, due to the stimulation of angiotensin II and rise in Plasma K^+ concentration. Reabsorption of Na^+ leads to the retention of water and rise in ECF volume.

The fall in ECF volume causes fall in blood pressure due to decrease in circulating blood

volume. This inhibits the secretion of ANP from the atria and prevents natriuresis.

Peritubular capillary oncotic pressure

Rise in the filtration fraction at the glomerulus, results in the increase of peritubular capillary oncotic pressure. This will lead to greater entry of solutes and water from the lateral intercellular spaces, due to the Starling's force, which decreases the urine flow rate.

Regulatory mechanisms when ECF volume is increased

Increase in ECF volume gives rise to regulatory mechanisms such as

- Inhibition of ADH and thirst
- Absence of sympathetic stimulation
- Inhibition of renin-angiotensin formation
- Inhibition of aldosterone secretion
- Secretion of ANP, BNP
- Secretion of PGE_2, endothelin and IL_1.

Inhibition of thirst

Rise in ECF volume, as well as, fall in plasma osmolality inhibits vasopressin secretion from the hypothalamic nuclei. Absence of ADH leads to water diuresis. The elimination of excess water in the urine corrects the body water level.

The thirst center in hypothalamus is also inhibited by the same stimuli.

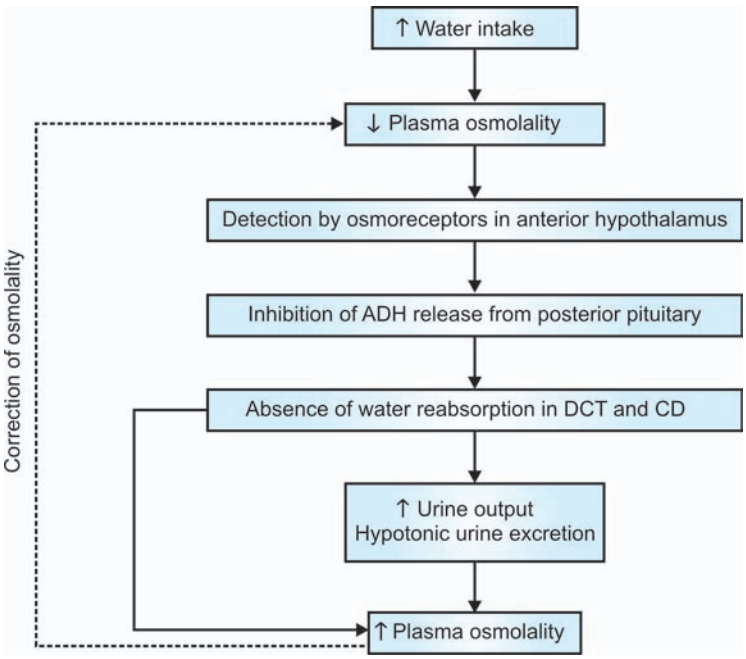
Absence of sympathetic stimulation

The rise in blood volume and arterial blood pressure reflexly inhibits sympathetic activity, through the baroreceptor stimulation. The absence of sympathetic stimulation in the kidney leads to the rise in RBF, which in turn increases the GFR. The rise in GFR causes greater urine flow rate.

Inhibition of angiotensin and aldosterone

Increase in the blood volume and blood pressure inhibits the formation of renin from JGA. The

Regulaory responses to rise in ECF volume



absence of renin - angiotensin II secretion inhibits aldosterone secretion. The combined absence of angiotensin II and aldosterone inhibit Na^+ reabsorption from the proximal and distal tubules. The net result is increased sodium excretion in the urine and along with it, more water is also excreted. This helps to correct both the Na^+ and water balance.

Secretion of ANP and BNP

Rise in ECF volume, stimulates **ANP** secretion from the atria and BNP (brain natriuretic peptide) from the brain. These hormone, besides inhibiting aldosterone action, increas GFR and inhibit Na^+ reabsorption in the PCT. These effects cause **natriuresis**, which help to regulate Na^+ and water balance.

Secretion of PGE_2

There is also secretion of prostaglandin **PGE_2, endothelin** and **interleukin 1** in the kidney, which promotes natriuresis through the inhibition of angiotenisn II and aldosterone actions.

The fall in the filtration fraction in the glomerulus decreases the plasma oncotic pressure of peritubular capillary. This prevents the entry of solutes and water from the lateral intercellular space into the peritubular capillary, due to the Starling's force.

REGULATION OF SODIUM BALANCE

Sodium is a cation present extracellularly and its concentration influences both tonicity and volume. Sodium is present in the ECF together with the anion chloride. There is no regulatory mechanism to control the dietary intake of sodium chloride, but the kidneys, by regulating its excretion in the urine, maintain the plasma level constant. Plasma level of sodium is 145 mEq/L ± 5/lit. The urinary excretion of sodium varies, depending on the dietary intake of salt. It ranges from 1 mEq/day to 350 mEq/day, on a low salt intake to high intake respectively. Since changes in the sodium chloride concentration alter the tonicity and volume of ECF, the regulation of sodium balance becomes important to maintain both the volume and tonicity. The regulation of sodium balance is mainly carried out by the kidneys.

The renal mechanisms in the regulation of sodium balance include:

- Changes in GFR
- Glomerulotubular balance
- Tubulo glomerular feed back
- Starling's force in peritubular capillaries
- Secretion of angiotensin II
- Secretion of mineralocorticoid
- Release of ANP and BNP

Regulatory mechanisms in increased salt intake (Fig. 9.19)

Changes in GFR

Rise in sodium level in the body leads to increase in the tonicity of ECF, which stimulates **thirst**. The secretion of ADH from the hypothalamic nuclei is also stimulated by the increase in the tonicity of ECF. The water intake from the thirst stimulation and water reabsorption in the distal tubules of nephron by ADH, cause expansion of ECF volume. The increase in the circulating blood volume and arterial blood pressure caused by these mechanisms, stimulate baroreceptors and reflexly inhibit the sympathetic effect on the blood vessels including kidney. The absence of sympathetic effect on the renal blood vessels, causes the dilatation of glomerular afferent and efferent arterioles. This increases the renal blood flow, which in turn raises the GFR.

Starling's effect in PCT

Pressure diuresis and Pressure Natriuresis

Increase in GFR gives more filtered load of sodium in the PCT. The GFR is increased by the arteriolar dilatation caused by the inhibition of sympathetic effect. This increases the hydrostatic pressure in the peritubular capillaries. The dilution of plasma by water retention, lowers the colloidal oncotic pressure in the peritubular capillaries.

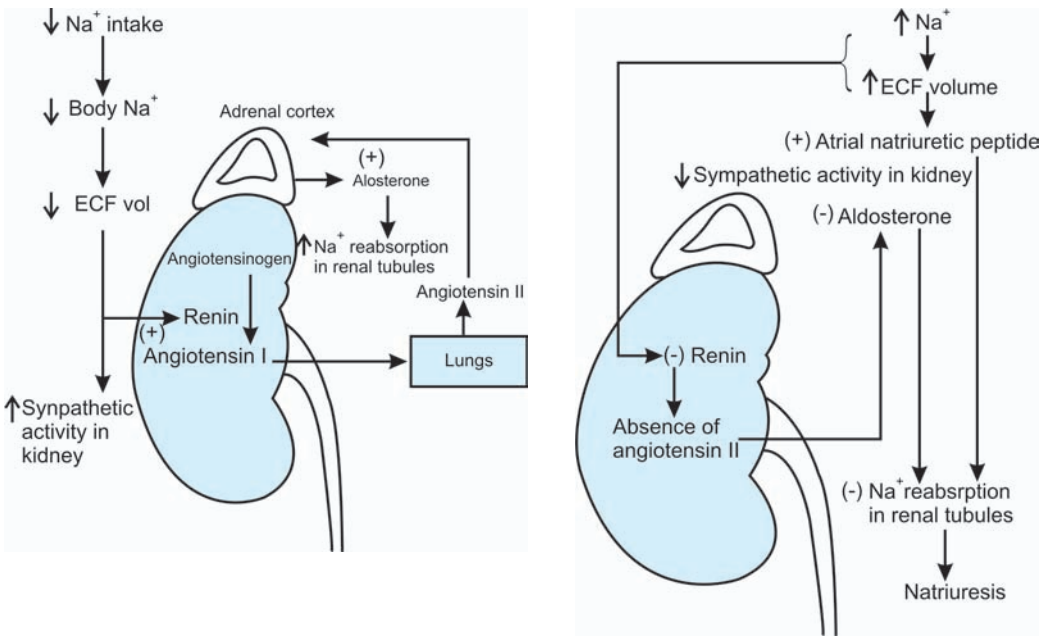


Fig. 9.19: Sodium homeostasis

The rise in the peritubular hydrostatic pressure and decrease in the plasma oncotic pressure, prevent the entry of Na^+ and water from the lateral intercellular space. This leads to more Na^+ in the filtrate. The kidney is able to regulate the rise in ECF volume and blood pressure intrinsically as described above and excretes more water **(pressure diuresis)** and Na^+ **(pressure natriuresis)** in the urine. These effects are independent of changes in GFR and hormone actions.

Inhibition of angiotensin and aldosterone secretion

Rise in the circulating blood volume and blood pressure inhibits renin-angiotensin formation from JGA. This prevents aldosterone secretion from the adrenal cortex (Fig. 9.19). The absence of angiotensin II, causes inhibition of sodium reaborption in the PCT. Absence of aldosterone causes inhibition of sodium reabsorption from the collecting ducts. The net result is the presence of more sodium in the tubular fluid and also more water, which is retained with it. The excess sodium is eliminated in the urine as osmotic diuresis.

Secretion of ANP and BNP (Atrial natriuretic peptide) (Brain natriuretic peptide)

When circulating blood volume and blood pressure are increased by excess sodium intake, the atria in the heart brain tissue secrete a peptide hormone ANP and BNP respectively. *These peptides cause*:

- Increase in GFR
- Inhibition of angiotensin II action in PCT which prevents sodium reabsorption
- Inhibition of aldosterone action in the collecting ducts, preventing the sodium reabsorption

These effects promote natriuresis.

The elimination of excess sodium in the urine corrects the tonicity and volume of ECF. This in turn normalises the blood pressure.

Regulatory mechanisms when salt intake is low

Fall in GFR

The regulatory mechanisms help to minimise the loss of sodium in the urine. It is well known that loss of sodium leads to the fall in the circulating blood volume and blood pressure. The decrease in the arterial blood pressure causes, absence of baroreceptor stimulation and reflex sympathetic stimulation of blood vessels. The renal vasoconstriction of glomerular arterioles results in the reduction in renal blood flow and GFR.

Starling's effect

The peritubular capillary hydrostatic pressure falls and the plasma colloidal oncotic pressure rises, due to the reduction of renal blood flow, caused by the sympathetic stimulation of afferent and efferent arterioles. The decrease in hydrostatic pressure and increase in the plasma oncotic pressure of the peritubular capillaries allows more entry of Na^+ and water into it from the lateral intercellular space. This causes more reabsorption of sodium in the PCT.

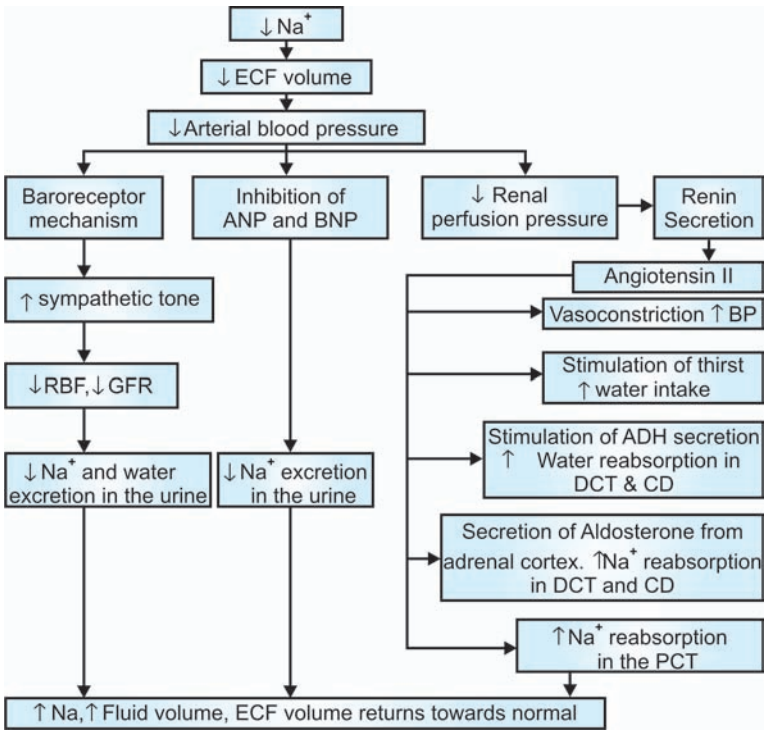
Sympathetic stimulation

Sympathetic stimulation of the kidney increases the sodium reabsorption in the PCT.

Glomerulotubular balance

When sodium intake is less, the glomerulotubular balance ensures a proportionate reabsorption of sodium from the filtrate in the PCT. This helps to minimise the loss of salt in the urine.

Regulatory mechanisms to fall in Na^+ level in the ECF



Tubuloglomerular feedback

In conditions where GFR is increased, the filtered load of sodium in the DCT becomes high. The macula densa senses the increased luminal content of Na^+ which leads to increased afferent arteriolar resistance. This will reduce RBF and GFR. Conversely, when GFR is reduced, the macula densa cells receive less Na^+ and GFR is increased by the dilatation of afferent arteriole and increased resistance of efferent arteriole. The DCT cells of the juxtamedullary nephrons (macula densa) sense increase in the filtered load of sodium that enters the distal segment. The afferent arteriole constricts, which reduces renal blood flow and GFR. This helps to prevent loss of sodium in the urine.

Secretion of angiotensin II and aldosterone

The fall in blood volume and blood pressure stimulate the renin-angiotensin secretion from JGA. The release of angiotensin II, also promotes sodium reabsorption in the PCT. The angiotenisn II stimulates aldosterone secretion from adrenal cortex (Fig. 9.19). This mineralocorticoid acts in the collecting ducts and causes sodium reabsorption in exchange for potassium or hydrogen secretion.

Inhibition of ANP

The fall in blood volume and blood pressure inhibits ANP secretion from the atrial cells. This facilitates more sodium reabsorption and prevents natriuresis.

Diuretics and sodium balance

Administration of diuretics corrects the fluid volume and blood pressure, by excreting more sodium in the urine. The main action of diuretics is the inhibition of reabsorption of sodium in the renal tubules.

Renal regulation of potassium balance

The plasma level of K^+ is maintained by adjusting its excretion in the urine. Normally the K^+ that is excreted in urine is equal to the intake in food.

Contd...

Contd...

Potassium is filtered , reabsorbed and secreted in the renal tubules. In PCT, 65% is reabsorbed and another 20 % in the thick segment of ascending limb of LH. In DCT and CD, potassium secretion occurs in exchange for Na^+ reabsorption by the action of aldosterone.

Factors affecting K^+ secretion in collecting duct

1. Greater the **tubular flow rate,** more the Na^+ delivered to the distal segments and greater the K^+ secretion.
2. Greater Na^+ reabsorption results in the **greater negativity of the tubular fluid**, which stimulates K^+ secretion.
3. **Aldosterone** facilitates K^+ secretion in exchange for Na^+ entering into the cell.
4. In **metabolic acidosis,** the intracellular K^+ in the collecting duct will be less and hence more H^+ is secreted .
5. In **metabolic alkalosis**, the intracellular K^+ is more and H^+ ion is less. Hence, more K^+ is secreted in the urine.

Loop diuretics such as *furosemide* inhibit the sodium reabsorption from the thick ascending limb of loop of Henle.

Thiazide diuretics like *chlorathiazide* act on the early part of the distal tubule and inhibit the reabsorption of sodium.

Aldosterone antagonists like *spiranolactone* inhibits the aldosterone effect in the collecting tubules. This prevents the sodium reabsorption and spares potassium.

Renal regulation of Ca^{++}, PO_4^- and Mg^{++}

These three are together considered, as they form the constituent of bone mineral. Hence, the regulation of these ions, show not only adjustment in the input and output, but also their deposition in bone.

Seventy percent of filtered Ca^{++} is reabsorbed in the PCT by secondary active transport. The reabsorption also occurs in the thick ascending limb of loop of Henle and DCT, in the presence

Contd...

Contd...

of parathyroid hormone (25%). The parathyroid hormone's main action is to maintain the Ca^{++} balance.

Urinary excretion of Ca^{++} is increased in:
Hyperparathyroidism
Chronic adrenocorticoid therapy
Vitamin D excess
Increased Mg^{++} in plasma
Chronic metabolic acidosis.

PO_4 is also regulated by the parathyroid hormone. In the absence of PTH, 75% of filtered PO_4 is reabsorbed. Presence of PTH, inhibits PO_4 reabsorption in the PCT and more than 40% of filtered PO_4 is excreted in urine.

PTH also regulates Mg^{++} ion balance. The presence of this hormone, inhibits the excretion of Mg^{++} in urine.

ACID BASE BALANCE

Acid base regulation helps to maintain the pH of blood. The pH of arterial blood is 7.40, while that of mixed venous blood is 7.38. The slightly lower pH in mixed venous blood, is due to the higher carbon dioxide present in it. The pH of 7.40, reflects H^+ ion concentration of 40 mmol/L. (range is 38 to 42 mmol/L.). The constant pH of blood, ensures normal activity of cellular processes. Normally, the alteration of pH results from the addition of acids to ECF. They are the acids produced during metabolic reactions in the normal way. The blood buffers, neutralizes these metabolic acids and keep the pH of blood within the normal range. The buffer, which is effective in neutralizing the acid is bicarbonate. This is called the **alkali reserve**. Its concentration in the plasma is 25 mEq/L. The pH regulation depends on the maintenance of bicarbonate level in the plasma. The kidneys are important in replenishing the plasma bicarbonate. This is achieved by making the urine acid by the secretion of H^+ ion. The acidification mechanism generates the bicarbonate from the tubular cells, which is added to the blood. The reabsorbed bicarbonate helps to keep the pH of blood constant.

Types of metabolic acids added to the ECF

Volatile acids

It is formed from CO_2 by its hydration. The respiratory process prevents its level going up in plasma. Normal level of PCO_2 in arterial blood is 40 mmHg. In respiratory failure, the pH decreases, due to the retention of CO_2 and conversely, in hyperventilation, the pH increases, due to the lowering of PCO_2 in the arterial blood.

Nonvolatile acids

Fixed acids (Nonvolatile) Sulphuric acids formed from the metabolism of sulphur containing amino acids and phosphoric acids formed from the metabolism of phospholipids, phosphoproteins, etc. are examples of nonvolatile acids. The addition of these acids is not as severe as CO_2 retention in altering the pH of blood.

Organic acids

Lactic acid, acetoacetic acid, β hydroxy butyric acid are the organic acids that are added to the ECF. In hypovolemic shock, lactic acidosis develops, while in uncontrolled diabetes mellitus, ketoacidosis is produced, which alter the pH of blood.

Buffer systems in body fluids

The buffers are weak acids and hence they accept H^+ ions. The *Henderson-Hasselbach equation* tells how a weak acid can function as a buffer.

$$pH = pk + \log \frac{\text{Base } [A^-]}{\text{Acid } [HA]}$$

The optimal buffer is the one that has pK value close to pH of blood and present in greater concentration. Accordingly,

Hb: It is a major buffer in the blood. The deoxygenated Hb is especially a weak acid, as it can accept more H^+.

$$\frac{[Hb]^-}{[HHb]}$$

PO_4: The weak acid is H_2PO_4. It is more effective intracellularly than in the ECF.

$$\frac{[HPO_4]^-}{[H_2PO_4]}$$

HCO_3: Even though it is considered not as a major buffer, yet it is regarded as the most effective buffer as the concentrations of HCO_3 and H_2CO_3 can be independently regulated.

$$\frac{[HCO_3]^-}{[H_2CO_3]}$$

Respiratory mechanisms in pH regulation

Respiratory mechanisms help to maintain the level of CO_2 in the plasma. The CO_2, which is added to the blood from metabolism stimulates the respiration and keep the level of CO_2 within normal limits. In hyperventilation, the washing away of CO_2, depresses respiration, which helps to elevate the CO_2 level. The respiratory mechanisms in the acid base regulation show immediate response, but not as effective as renal mechanisms, although, the response comes after some time.

Renal mechanisms in acid base regulation

One of the chief functions of the kidneys is to maintain the pH of blood constant. This homeostatic regulation involves, generation of HCO_3 from the renal tubule cells, which can replenish the bicarbonate ion in the plasma. This process makes the urine acid by the secretion of H^+ ions. The tubular filtrate itself has buffers, which take up these H^+ and limits the urine pH. Thus, we see that the kidneys help to maintain the plasma HCO_3.

Mechanism in PCT

About 2/3 of filtrate is reabsorbed in this segment. The major solute that is reabsorbed is Na^+. The tubular cells have carbonic anhydrase enzyme, which catalyses the hydration of CO_2. The carbonic acid is a weak acid and dissociates into H^+ and HCO_3^-. The H^+ is secreted from tubular cells, in exchange for Na^+ entry **(antiport).** The reabsorbed Na^+ combines with HCO_3 and enter the peritubular capillaries as sodium bicarbonate (Figs 9.11 and 9.14). In the PCT, maximum number of H^+ ion is secreted, yet the tubular filtrate pH shows little change.

The secreted H^+ ion is taken up by HCO_3 present in the filtrate and forms H_2CO_3. This dissociates into CO_2 and water. CO_2 diffuses into the tubular cells and utilised for further HCO_3 generation. The bicarbonate in the filtrate forms the first buffer, which neutralises the secreted H^+ ions. Infact, the bicarbonate that is filtered is reabsorbed as CO_2 and not bicarbonate.

In DCT and collecting duct

There is further reabsorption of Na^+ in the distal tubules and collecting ducts. The distal tubules show Na^+ reabsorption as in PCT. The collecting duct reabsorption of Na^+ is in exchange for K^+ or H^+ secretion, in the presence of aldosterone hormone. There is a competition between K^+ and H^+ ion secretion for Na^+ reabsorption in the collecting ducts. Even though, the secretion of H^+ ion is not that high as compared to PCT, yet, the pH change of the tubular filtrate here becomes significant. The filtrate becomes more acidic.

The secreted H^+ and free acids are taken up by HPO_4, present in the filtrate of DCT. The H_2PO_4 that is produced as a result of buffer action, become titratable acids in the urine. This also includes creatinine, β hydroxy butyric acid and acetoacetic acid and not ammonium. HPO_4 is another buffer present in the filtrate to limit the pH of urine.

In the distal segment of the nephrons, the presence of free H^+ ion in the filtrate stimulates the tubular cells to secrete NH_3 (Fig. 9.15). Ammonia is formed from glutamate in the presence of glutaminase enzyme. NH_3 is lipid soluble and hence, its secretion occurs freely. The free H^+ combines with the NH_3 and form NH_4, which is lipid insoluble. The glutamic acid is converted to α ketoglutaric acid, which in turn generates NH_4 by the activity of glutamic

dehydrogenase enzyme. The conversion of NH_3 to NH_4 creates gradient for further secretion of NH_3 and this is called **nonionic diffusion**.

The secretion of NH_3 in response to the free H^+ is called adaptation which is characteristic of the DCT cells. This mechanism helps to limit the pH of urine.

Ammonium Secretion

Ammonium is secreted from PCT thick segment of ascending limb and CD whenever H^+ concentration in ECF rises. NH_4 is formed from glutamine in the tubular cells. During the synthesis, for each NH_4, there is also formation of 2 molecules of HCO_3. The ammonium is secreted in exchange for Na^+ reabsorption, which combines with HCO_3 in the cell and enters the capillary. In this way, the secretion of NH_4 helps to generate new HCO_3 which is added to plasma to regulate the pH. The secretion of NH_4 in the renal tubules is increased in acidosis.

Factors affecting renal H^+ secretion

PCO_2 level

The secretion of H^+ from the tubular cells depends directly on the pCO_2 concentration in the plasma. If the level falls down, less H^+ is secreted while in excess pCO_2, more H^+can be secreted in the urine.

Carbonic anhydrase

This enzyme catalyses the hydration of CO_2 in renal tubular cells. The inhibition of this enzyme by *acetazolamide(diamox)* prevents H^+ secretion and no Na^+ reabsorption can occur. This causes the diuretic effect.

Mineralocorticoid

The hormone aldosterone acts on the collecting ducts and reabsorbs Na^+ in exchange for K^+ or H^+ secretion. The secretion of K^+ is promoted in metabolic alkalosis and H^+ in metabolic acidosis. Excess secretion of this hormone causes K^+ loss in the urine and metabolic alkalosis.

Plasma K^+

When plasma contains more K^+, H^+ secretion is inhibited, as the intracellular concentration of K^+ will be greater and that of H^+ less. Lowering of the plasma K^+ causes more H^+ secretion, as the intracellular concentration of K^+ will be less and that of H^+ greater.

Clinical evaluation of acid base balance

pH of arterial blood

The pH should be within the narrow range of 7.38 to 7.42. The fall in the pH occurs in acidosis (> 7.35) and rise of pH is seen in alkalosis (> 7.45).

Examples of clinical disturbance of the acid base balance are as follows.

Clinical measurement of acid base disorders include

Arterial blood analysis of pH (normal 7.40)
Arterial blood PCO_2 (40 mmHg)
Arterial blood level of HCO_3 (25 mEq/L)

When acid base disturbance occurs due to change in HCO_3^- level, it becomes a metabolic disorder. In metabolic acidosis , the HCO_3^- level is decreased and in metabolic alkalosis its level is increased.

If acid base disturbance is due to change in PCO_2 level, it is categorised as respiratory disorder. Increase in PCO_2 causes respiratory acidosis and a decrease in PCO_2 result in respiratory alkalosis.

Compensation in acid base disorders

In order to minimize the pH changes, compensatory mechanisms take place involving lungs and kidneys. In metabolic acid base disorders, the compensation is produced by lungs, while, kidneys give compensation for respiratory acid base disorders (Table 9.2).

In metabolic acidosis, the respiratory stimulation causes fall in PCO_2 which helps to rise the pH towards the normal level.

The compensated acid base status occurs finally by the kidney excreting more H^+ in the

Table 9.2: Compensation in acid base disorders

Disorder	*Causes*	*Primary disturbance*	*Compensation*	
			Respiratory	*Renal*
Metabolic acidosis	Ketoacidosis Lactic acidosis Diarrhea Shock Chronic Renal failure Renal tubular acidosis	↓ HCO_3^-	↓ PCO_2 stimulation of ventilation	—
Metabolic alkalosis	Vomiting Hyperaldosteronism Ingestion of alkali	↑ HCO_3^-	↑ PCO_2 Slowing of respiration	—
Respiratory acidosis	Respiratory failure caused by drugs and anesthetics,Airway obstruction adult respiratory distress syndrome COPD	↑ PCO_2	—	↑ HCO_3^- ↑ Reabsorption and generation of HCO_3^- ↑ H^+ excretion
Respiratory alkalosis	Hyperventilation	↓ PCO_2	—	↓ HCO_3^- ↑ HCO_3^- excretion in the urine (alkaline) ↓ H^+ excretion

Normal values (arterial blood) : pH: 7.4, PCO_2: 40 mmHg, HCO_3^- : 25 mEq/L, H^+ : 40 nEq/L

urine, reabsorbing and generating more HCO_3 fromthe renal tubules.

In metabolic alkalosis, the respiratory depression will increase PCO_2 to lower the pH.

The final compensation comes from the kidney by excreting more HCO_3 in the urine.

In respiratory acidosis, kidneys reabsorb and generate more HCO_3^- and secrete more H^+. Respiratory acidosis results due to depression of respiration and PCO_2 rises .

In respiratory alkalosis, the kidney excrete more HCO_3^-in the urine. Respiratory alkalosis is caused by hyperventilation which lowers PCO_2

Metabolic acidosis

NH_4Cl ingestion
Renal failure
Severe exercise (lactic acidosis)
Diabetic ketoacidosis
Shock
Diarrhea

Metabolic alkalosis

Vomiting of gastric contents
Intake of excess alkali
Hyperaldosteronism

It should be kept in mind that for respiratory acid base disorders, it is only the renal compensatory mechanisms that help to regulate the pH. It should also be understood that for both metabolic and respiratory acid base disorders, the normal acid base status can be achieved only with the clinical intervention to correct the underlying disorder.

Measurement of anion gap

It refers to the difference between the concentrations of cations excepting Na^+ and anions other than Cl^- and HCO_3 in plasma. The difference is mainly due to the proteins as anions, PO_4, SO_4 and organic acids. Its normal value is 8-16 mEq/L. It

is increased in metablolic acidosis (ketoacidosis and lactic acidosis), methanol poisoning and chronic renal failure. The value falls when the plasma albumin concentration is low.

JUXTAGLOMERULAR APPARATUS (JGA)

It is a structure consisting of macula densa, juxtaglomerular cells and mesangial cells (extraglomerular) (Fig. 9.20). Macula densa are the DCT cells facing its own glomeruli. The mesangial cells are present between afferent arteriole and macula densa. These cells secrete prostaglandins and also the constriction of these cells regulates blood flow in the arteriole.

The functions of JGA are in the regulation of Na^+ balance and maintenance of ECF volume. The tonicity and arterial blood pressure are also regulated consequent to the electrolyte and fluid volume regulation.

Renin-angiotensin

The JGA cells (afferent arteriole endothelial cells) contain renin, which acts as an enzyme on angiotensinogen present in the plasma. The renin is secreted from the juxtaglomerular cells in the following conditions.

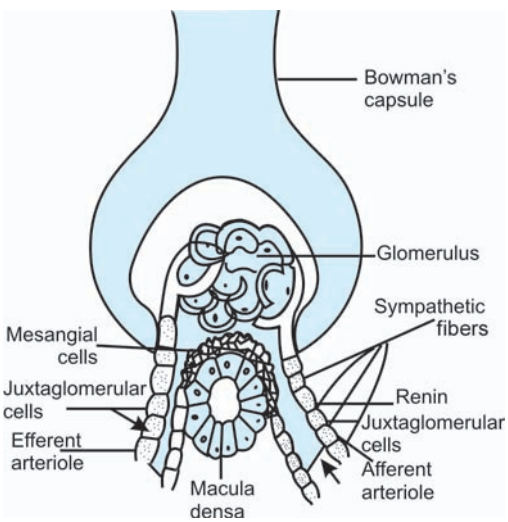


Fig. 9.20: Juxtaglomerular apparatus

- Fall in renal perfusion pressure
- Fall in blood volume
- Fall in Na^+ level in plasma
- Rise in plasma K^+
- Decrease in the luminal Na^+ load in macula densa
- Sympathetic stimulation of JG cells.

Renin acting on the plasma protein α_2 globulin angiotensinogen, produces angiotensin I which is inactive. The angiotensin I is converted to angiotenisn II by the ACE (angiotensin converting enzyme) present in the lung and kidney tubules and endothelial cells. Angiotensin II is active and it is a powerful vasoconstrictor in action, besides stimulating aldosterone secretion from the adrenal cortex. The secretion of aldosterone and angiotensin II, both combined, produce Na^+ reabsorption and water retention. This expands ECF volume and raises the arterial blood pressure. The arterial blood pressure rise also occurs, due to the vasoconstrictor action of angiotensin II on blood vessels (arterioles).

MICTURITION

The process of voiding of urine from the bladder is called micturition. It is a reflex phenomenon.

The filling of the bladder occurs by a pair of ureters, which arise from the pelvis of the kidney. The urine is transported in the ureter by the peristaltic waves, which are generated from the pelvicalyceal junction. The distention of this region by the presence of urine forms the stimulus for the initiation of peristalsis. The urine as it flows along the ureter, does not show any change in its composition, as the ureter is lined by transitional epithelium. The ureter ends in the bladder and the junction is known as ureterovesical junction. About 2 to 3 mm length of ureter at its termination, is embedded within the bladder muscle, which ends obliquely at the posterolateral angle in the bladder. This anatomical arrangement prevents reflux of urine into the ureter, when bladder contracts during micturition. The contraction of bladder raises the resistance at the termination of ureter and hence no reflux of urine into it takes place.

The bladder contains a smooth muscle called detrusor. Its arrangement in the bladder forms the trigone. The detrusor continues as bladder neck and forms the internal sphincter. The posterior urethra at the urogenital diaphragm, shows a somatic muscle, which forms the external sphincter.

The bladder and the urethra are supplied by the autonomic nerves (Fig. 9.21). The sympathetics arise from T12 , L1, L2 and travel in the presacral nerve. The ureter, bladder and the internal urethral sphincter are innervated by it. The sympathetic stimulation causes inhibition of the bladder and contraction of internal urethral sphincter. The pain impulses from the bladder and urethra is also carried by the sympathetics. The parasympathetic innervation arises from S2, S3 and S4 segments of the spinal cord and the fibres travel in the pelvic nerve. The stimulation of the pelvic nerve causes bladder contraction and relaxation of internal urethral sphincter. The external urethral sphincter is supplied by a somatic nerve called pudendal nerve, which arises from S2, S3 and S4 segments of the spinal cord.

The process of micturition although a reflex one, involves social and cultural factors. The bladder is normally under the central inhibition and during micturition this central inhibition is removed. The lowest center is the sacral segments. Higher centers are present in the midbrain, pons, hypothalamus and cortex. The sacral center can produce the reflex, only when the higher centers inhibition is removed.

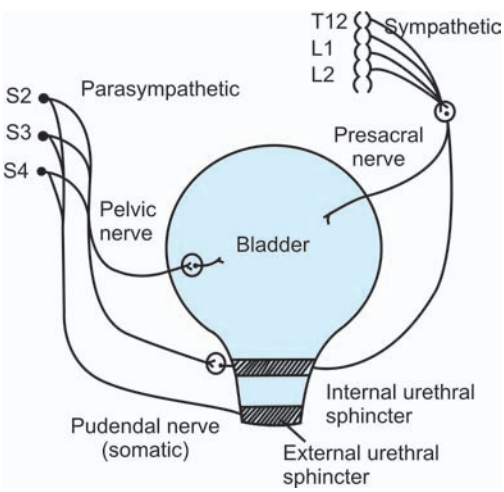


Fig. 9.21: Innervation of urinary bladder

Bladder filling causes stretching, which forms the stimulus for the reflex. The afferent and efferent impulses are carried in the pelvic nerve. Rise in volume up to 100 ml, raises the pressure in the bladder to 10 cm of H_2O. Further rise in the volume, i.e, from 100 to 400 ml, does not show a corresponding rise in the intravesical pressure, due to the property of **adaptation.** The plasticity of the bladder muscle is responsible for accommodating the large volume with little rise in pressure. If the volume rises above 400 ml, the adaptation mechanism fails and the reflex contraction of the bladder occurs causing rise in the pressure. Experimentally, this relationship between volume and pressure in the bladder can be studied and the graph obtained is known as **cystometrogram** (Fig. 9.22).

The desire to void urine occurs around 200 to 250 ml of volume. If a suitable place is found to void urine, the central inhibition is removed and

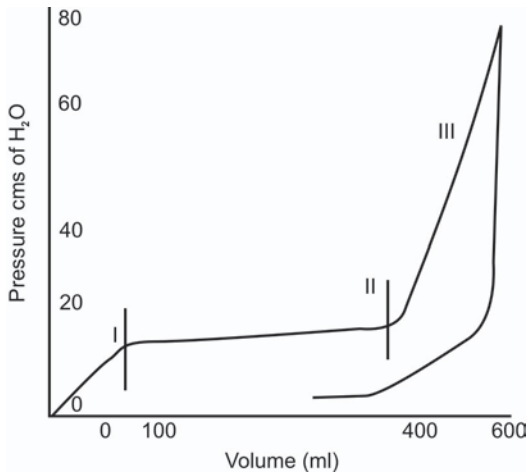


Fig. 9.22: Cystometrogram—The pressure-volume relationship in urinary bladder shows three phases. In the I phase, there is a initial pressure rise to the volume rise of 100 ml. The II phase is due to adaptation of bladder. The property of plasticity can be seen in this phase. In the III phase, the micturition reflex occurs as the adaptation fails with the rise in volume

the sacral segments produce the reflex contraction of the bladder. The contraction of the bladder and the relaxation of the internal sphincter are caused by the pelvic nerve. The presence of urine in the urethra reinforces the reflex mechanism. That is, the bladder contracts continuously as long as the urine is in the urethra. This ensures complete emptying of the bladder. The presence of urine in the urethra also causes inhibition of pudendal nerve and the external sphincter relaxes. Since this is a somatic muscle, it is under voluntary control. It is possible to stop micturition voluntarily by its contraction.

The inhibition of external sphincter allows urine to be expelled outside. During micturition, respiration stops. The straining efforts increase the intraabdominal pressure, which augments the intravesical pressure, facilitating micturition.

Sectioning of pelvic nerve, or spinal lesion involving sacral segments, results in paralysis of detrusor muscle. The micturition reflex will be absent in such conditions. Ability to hold the urine in the bladder is lost (incontinence). Overflow incontinence results. The bladder contracts with smaller volumes, on its own, which is known as **automatic bladder.**

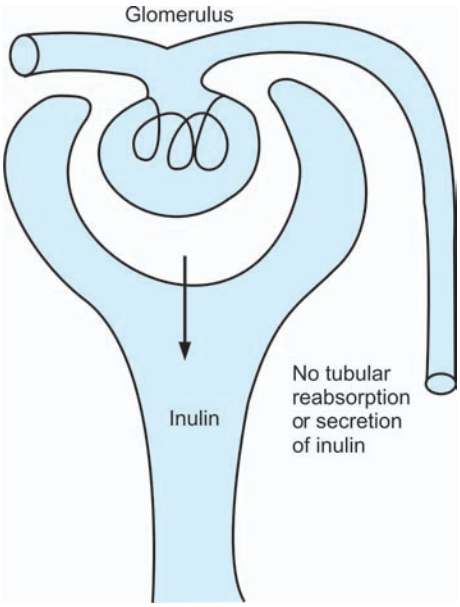


Fig. 9.23: Diagram to illustrate inulin clearance by the kidney

Rate of inulin extretion is equal to the ratae of filtration = U x V. Since plasma contain 10% of inulin after filtration in the glomerulus, the clealrance of inulin will be:

$$\frac{U \times V}{P}$$

TESTS TO ASSESS RENAL FUNCTION

Clearance studies

Clearance is defined, as the amount of plasma required to clear off a substance in a period of time. Clearance of inulin and creatinine measures GFR, while the clearance of PAH and Diodrast measures RPF.

Inulin clearance (Fig. 9.23)

Inulin is a fructopolysaccharide with a molecular weight of 5200 and obtained from the Dhalia tubers. Inulin when injected into the body, is almost (90%) filtered in the glomerulus in one circulation. Since it is neither reabsorbed nor secreted in the renal tubules, the rate of excretion of inulin will be equal to the rate of filtration. The rate of excretion can be calculated from U×V, where U is the amount of inulin in the urine in mg/ml and V is the volume of urine in ml/min. Since a small amount of inulin, still remains in plasma, the plasma concentration is also taken into account (P).

$$\text{Inulin clearance} = \frac{U \times V}{P} = \text{GFR}$$

Normal value is 125 ml/min. In females it is 10% lower. In old age, the GFR is decreased. The value is also decreased in glomerulonephritis and acute renal failure.

Endogenous creatinine clearance

Creatinine is produced in the body from the metabolism of creatine present in muscle. Creatinine is both filtered and secreted. However, the GFR value is close to inulin clearance.

Urea clearance

Urea clearance can also be measured, but it depends on the urine flow rate. If the urine flow is 2 ml/min, maximum urea clearance is present, which is 75 ml/min. If the urine flow is less than 2 ml/min, then standard urea clearance will be present and its value is 54 ml/min.

PAH clearance

PAH when injected is secreted (90%) in the PCT. Its clearance value measures the renal plasma flow.

$$\text{PAH clearance} = \frac{U \times V}{P} = \text{RPF}$$

Normal value is 600 to 700 ml/min. Renal blood flow can be calculated from RPF and knowing the hematocrit value. Normal RBF is 1200 ml/min.

$$\text{RBF} = \frac{\text{RPF}}{(100 - \text{Hct})}$$

Filtration fraction (FF)

It is the ratio between GFR and RPF. Its normal value is 0.19 to 0.20 (20% of renal plasma is FF).

$$\text{FF} = \frac{\text{GFR}}{\text{RPF}}$$

It is more common to observe decrease in RPF, in conditions like hemorrhage, where FF is increased.

Renal concentration test

The ability of kidneys to concentrate the urine is affected in renal failure. The test can be done by asking the subject to stop fluid intake for 12 hrs. The urine excreted thereafter is collected (4 samples) and its specific gravity is determined. Normal specific gravity of urine is 1010 and in maximum antidiuresis it goes up to 1030.

Renal diluting ability

The ability of kidney to dilute the urine (diuresis), when fluid volume in the body is increased can be tested by asking the subject to drink 3 to 4 lit of water. Four samples of urine are collected after 40 minutes duration and their specific gravity are determined. One of the samples should show specific gravity of 1002, which indicates maximum diuresis.

Urine analysis

Urine is analyzed for its volume, reaction, specific gravity and for the presence of any abnormal substances such as RBC, pus cells, casts, blood, glucose, albumin, increased ketone bodies and bile pigments.

Blood examination

The estimation of blood urea nitrogen (BUN), (20 to 40 mg%) creatinine (1.5 to 2 mg%) K^+ and reaction of blood, will indicate renal pathology, if there is any change from their normal values.

Effects of renal disorders

Proteinuria
Loss of concentrating and diluting ability
Polyuria (increased urine output)
Oliguria (reduced urine excretion)
Anuria (absence of urine formation)
Uremia
Acidosis
Hyperkalemia
Edema
Hypertension
Anemia
Osteomalacia

Intravenous pyelography

Infusion of radio opaque substance (iodinated radioactive compound) gives X-ray shadow of renal blood vessels, pelvis of kidney and ureter. Presence of renal stones and vascular lesions if any, can be visualised by this procedure.

Renal biopsy and scanning of kidneys are also undertaken to detect the presence of any malignant tumor.

Self-study Questions

Multiple Choice Questions

Choose the single best answer

1. **Concentrated urine is excreted in response to the administration of:**
   - **A.** Angiotensin II
   - **B.** Vasopressin
   - **C.** Aldosterone
   - **D.** Renin

2. **Secondary active transport is shown by all of the following *except:***
   - **A.** Glucose
   - **B.** Urea
   - **C.** Aminoacids
   - **D.** Phosphate

3. **Which of the following does not show secretion in the renal tubules?**
   - **A.** Potassium
   - **B.** Urea
   - **C.** Uric acid
   - **D.** Creatinine

4. **In which segment of nephron water reabsorption does not occur when concentrated urine is excreted?**
   - **A.** PCT
   - **B.** DCT
   - **C.** Ascending limb of LH
   - **D.** Descending limb of LH

5. **Urine volume is increased by all of the following except:**
   - **A.** Sympathetic stimulation
   - **B.** Infusion of mannitol
   - **C.** Diabetes insipidus
   - **D.** Diabetes mellitus

6. **Dehydration causes the stimulation of all of the following *except:***
   - **A.** Vasopressin
   - **B.** Aldosterone
   - **C.** Atrial natriuretic peptide
   - **D.** Angiotensin II

7. **Tonicity of ECF is regulated by which hormone?**
   - **A.** Vasopressin
   - **B.** Aldosterone
   - **C.** Atrial natriuretic peptide
   - **D.** Angiotensin II

8. **The solute reabsorption in the thick ascending limb causes:**
   - **A.** Hypertonicity of medullary interstitium
   - **B.** Hypertonicity of tubular fluid
   - **C.** Hypotonicity of tubular fluid
   - **D.** A and C

9. **The actions of angiotensin II does not include:**
   - **A.** Vasoconstriction
   - **B.** Na^+ reabsorption
   - **C.** Water reabsorption
   - **D.** Thirst stimulation

10. **Polyuria occurs in:**
    - **A.** Diabetes insipidus
    - **B.** Acute renal failure
    - **C.** Dehydration
    - **D.** Hemorrhage

11. **The concentration of which substance will be greatest at the end of PCT?**
    - **A.** Glucose
    - **B.** Sodium
    - **C.** Phosphate
    - **D.** Creatinine

12. **Both RPF and GFR will be reduced when:**
    - **A.** Both afferent and efferent arterioles are constricted
    - **B.** Both afferent and efferent arterioles are dilated
    - **C.** Constriction of efferent arteriole and dilatation of afferent arteriole
    - **D.** Constriction of efferent arteriole only

13. **In renal failure the rise in blood urea is due to:**
    - **A.** Increased production from liver
    - **B.** Increased reabsorption from tubules
    - **C.** Decreased GFR
    - **D.** Decreased secretion in renal tubules

14. **In metabolic acidosis there is an increase in:**
    - **A.** Plasma pH
    - **B.** Anion gap
    - **C.** Arterial PCO_2
    - **D.** Plasma HCO_3^-

15. **H^+ secretion from DCT increases in all of the following conditions *except:***
    - **A.** Hyperkalemia
    - **B.** Respiratory acidosis
    - **C.** Metabolic acidosis
    - **D.** Increased level of aldosterone in plasma

16. **An increase in anion gap occurs if there is an increase in the plasma concentration of which of the following?**
    - **A.** Sodium
    - **B.** Potassium
    - **C.** Bicarbonate
    - **D.** Lactate

17. **In the excretion of concentrated urine , which of the following cannot occur?**
    - **A.** Decrease in urine flow rate
    - **B.** Decrease in urine volume
    - **C.** Increase in urine tonicity
    - **D.** Increase in urine flow rate

18. **Prolonged diarrhea causes:**
    - **A.** Metabolic acidosis
    - **B.** Metabolic alkalosis
    - **C.** Respiratory acidosis
    - **D.** Respiratory alkalosis

19. **Filtered bicarbonate in the renal tubules is reabsorbed by interaction with:**
    - **A.** Na^+
    - **B.** K^+
    - **C.** H^+
    - **D.** CO_2

20. **Decrease in plasma HCO_3^- and pH occur in:**
    - **A.** Metabolic acidosis
    - **B.** Metabolic alkalosis
    - **C.** Respiratory acidosis
    - **D.** Respiratory alkalosis

ANSWER KEYS

1. (B)	**2. (B)**	**3. (B)**	**4. (C)**	**5. (A)**	**6. (C)**	**7. (A)**	**8. (D)**	**9. (C)**	**10. (A)**
11. (D)	**12. (A)**	**13. (C)**	**14. (B)**	**15. (A)**	**16. (D)**	**17. (D)**	**18. (A)**	**19. (C)**	**20. (A)**

Short Answer Questions

1. Enumerate the factors that are necessary for glomerular filtration.
2. List the conditions that can cause fall in GFR.
3. Describe the changes in volume and tonicity of the filtrate in PCT, LH, DCT and CD of nephron.
4. Describe water reabsorption in renal tubules.
5. List the factors that are necessary for urine concentration.
6. State the differences between water diuresis and osmotic diuresis.
7. List the compensatory responses to fall in ECF volume.
8. Enumerate the factors that help to regulate sodium balance.
9. Describe the compensatory regulatory responses to metabolic acidosis.
10. State the importance of clearance tests.

10 Skin and Body Temperature

SKIN

Skin is the outermost covering of the body, which is also known as integument. The structure of skin shows epidermis and dermis.

Epidermis

Epidermis contains five layers namely, stratum corneum, stratum lucidum, stratum granulosum, stratum spinosum and stratum germinativum. Stratum corneum is the outermost layer of epidermis and consists of dead cells filled with keratin. These cells are shed off from the surface of the skin and new cells from the base migrate and occupy this layer. Source of sterols and fatty acids that exist in the surface of the skin comes from the dead cells of the stratum corneum.

The basal layer stratum germinativum is active in mitosis and the new cells that are produced, move upwards. The cells of the basal layer also have the pigment melanin, which gives dark colour to the skin. The change of blood flow to the skin also, alters the color of the skin. Exposure to UV rays of the sunlight causes more distribution of melanin, which helps to protect the skin against cancer. Stratum germinativum also gives rise to skin appendages like nail. Nail is formed by the proliferation of this basal layer, which becomes keratinized.

Dermis

The dermis of the skin contains, glands, hair follicle, smooth muscle, blood vessels, nerve fibers, sensory receptors, collagen and fibroblasts.

Glands

Three types of glands are present in the dermis. They are sweat glands, sebaceous gland and ceruminous gland.

Sweat glands

Apocrine and eccrine are the two types of sweat glands present.

Apocrine glands

Apocrine is concentrated in the axilla, pubis and areola of the breast. The secretion from the gland directly reaches the skin surface through a straight duct. The gland cells are innervated by the sympathetic adrenergic fibers. Its secretion starts at the time of puberty and the adrenal androgens stimulate the secretion. The apocrine secretion that comes to the surface of the skin is colorless and odourless. But the bacterial flora present on the skin surface, acts on it and gives the characteristic odour. The gland cells are stimulated by emotional excitement and

mediated through the sympathetic adrenergic innervation. The apocrine glands are classified under sweat glands, but they do not help to regulate the body temperature.

Eccrine glands

The eccrine glands have a coiled duct system, which opens into the skin surface. They are distributed more in the palm, hand, chest and forehead. The cells of eccrine glands are supplied by **sympathetic cholinergic fibers**. The eccrine gland is stimulated in response to the increase in the body temperature. The sweat that is secreted cannot bring down the body temperature, but the vaporisation of sweat does it. The duct cells reabsorb Na^+ in exchange for K^+ and hence the flow rate can alter the composition of sweat. More the flow rate, more the Na^+ concentration in the sweat. Aldosterone also acts in the duct cells of eccrine gland and causes sodium reabsorption. Sweat contains 99% water and 1% solids, which includes inorganic salts, urea and lactate. Excess sweat secretion can lead to dehydration and electrolyte loss.

Ceruminous glands line the lumen of the external auditory meatus and secrete cerumen (wax).

Sensory receptors that are present in the dermis, include mechanoreceptors, nociceptors and thermoreceptors. They are supplied by the sensory afferents going to the spinal cord. They convey to the nervous system about the changes in the external environment.

Hair follicle is the downward invagination of the epidermis and opening into the hair bulb, is the sebaceous gland. The secretion of **sebaceous gland** is under the influence of sex steroids. Androgens stimulate the secretion, while the estrogen inhibits it. The secretion, on reaching the hair follicle, is acted upon by the bacteria and clogs the follicle to form **acne.**

Situated near the base of the hair follicle is the **erector pili smooth muscle**. The contraction of this muscle raises the hairs (goose flesh) and facilitates trapping of air between the hairs. This provides insulation to the skin against cold.

Functions of skin

Protective

The skin covering protects the inner visceral structures against the entry of germs and foreign particles.

Body temperature regulation

The distribution of blood flow to the skin surface regulates the body temperature. More blood flow to the skin causes more heat loss and less blood flow helps to reduce it. The secretion of sweat and its vaporisation is an important mechanism to maintain the body temperature. The insensible perspiration from the skin also causes heat loss.

Sensory function

The cutaneous sensory receptors convey the changes in the external environment to the central nervous system, which not only perceives the stimuli, but also responds in the form of motor behaviour.

Synthesis of vit D

The UV rays from sun promote the synthesis of vit D from 7-dehydrocholesterol.

Storage function

The subcutaneous layer stores glycogen and fat.

Protection against UV rays of sun

The colour of the skin depends on the distribution of melanin in the basal layers of epidermis and its presence protects the skin against the harmful effects of UV rays from the sun.

BODY TEMPERATURE REGULATION

Humans are homeothermic, because the body temperature remains constant irrespective of changes in the environmental temperature. It is the core of the body, representing the visceral organs that show constant temperature and not the periphery (shell). The periphery normally

reflects the external temperature. The core temperature should be kept constant, as it is necessary for the normal cell function.

Variations of body temperature

Normal body temperature in humans is 37° C and shows variations, depending on the activity, sleep-wake cycle, and due to hormonal effects. The temperature has a circadian rhythm, which shows the highest in the evening and lowest in the early morning. In infants the body temperature can increase, if there is a bout of crying. In reproductive women, during mid cycle, the basal body temperature rises by 0.5°C following ovulation, due to the progesterone effect. Hyperthyroidism raises the body temperature, while hypothyroidism lowers it. Pathologically, during fever, the body temperature is increased, due to the resetting of thermostat by cytokines and interleukins 1, 2, 6.

Recording of body temperature

The body temperature is usually measured with the help of a clinical thermometer. In adults, oral temperature can be recorded and in infants, recording can be done from the axilla. Rectal temperature is also recorded, which reflects the core temperature. It is 0.5 to 1°C more than the oral temperature. The temperature recorded from the axilla is 0.5°C lower than oral temperature. Oral temperature recording will not give the normal value, if cold or hot drinks are taken prior to recording. The normal body temperature that is recorded, reflects the balance between the heat production and heat loss in the body.

Sources of heat production

The sources of heat production come from the metabolism of food in the liver and skeletal muscle contraction. Heat is also gained, by *radiation, conduction* and *convection,* if the ambient temperature is higher than the body temperature. Normal heat production in the resting state is about 1600 cal/day in an adult. This is increased, when there is muscular exercise or physical activity.

Heat loss from the body

Heat loss from the body occurs in several ways. If the ambient temperature is lesser than the body temperature, heat is lost from the body by **radiation, conduction** and **convection.**

Radiation is caused by the electromagnetic waves radiating to the surrounding air and cold objects. **Conduction** is by molecular collision, where the heat is transferred to the cold objects to which the body is in contact with and also to the surrounding air. The air current facilitates greater transmission of heat in conduction. **Convection** refers to the movement of air, which removes heat from the surface of the body, thereby causing more heat dissipation.

The other mechanism for heat loss is by the formation of sweat. It is the **vaporisation of sweat** that removes heat from the body and not the sweat that is formed. Vaporization of water in the sweat dissipates heat and each g of water, when vaporises, can dissipate 0.54 kcal of heat. Air current and dry air influence the vaporization of sweat. If humidity is more, the vaporization of sweat is less and causes sultriness. Heat is also lost from the surface of the skin as **insensible perspiration**. The other routes for heat elimination are the **expired air, urine and feces**. The elimination of heat in the expired air is an important mechanism to regulate the body temperature in animals like dogs. Panting in these animals helps to remove the excess heat in the expired air.

Regulatory mechanisms (Table 10.1)

In man, temperature regulatory mechanisms include autonomic, somatic, endocrine, behavioural changes.

Temperature regulating centers

The autonomic regulatory mechanism is effected through the temperature regulating center present in the hypothalamus. The regulatory centers of hypothalamus are also known as **thermostat**. The center for heat loss is situated in the *anterior hypothalamus* and *preoptic area*, while, the heat gain center is present in the *posterior hypothalamus.*

Table 10.1: Body temperature homeostasis

Temperature regulatory mechanism

25° 30° 35° 37° 38° 39° 40° 41° 42°C

↓

Response to hypothermia		Normal	Response to hyperthermia	
Reduction of heat loss	Cutaneous vasoconstriction		**Heat loss**	Cutaneous vasodilatation Sweating and vaporization ↑ Respiration
Increased heat production	Shivering ↑ Metabolism ↑ Calorigenic effect by Epinephrine and Thyroxine ↑ Appetite		**Reduction of heat production**	Reduced physical activity Reduced appetite Decreased metabolism
Somatic	↑ Physical activity		**Behavioral Effects**	Resting in cool place Use of fans, room cooler
Behavioral effects	Curling up of body Use of room heaters Use of woollen clothes			

These centers are stimulated by the temperature changes of the blood , which is perfusing them.

Body responses to rise in temperature

The regulatory mechanism shows two ways of reducing the body temperature. One is to eliminate more heat from the body and the other is to reduce the heat production from the body.

Ways of dissipating heat from the body

Increase in body temperature stimulates cutaneous warm receptors and as well as thermostat. The latter is stimulated through the blood perfusing the preoptic and anterior hypothalamus. The cutaneous stimulation of the warm receptors causes dilatation of blood vessels, which increases the skin blood flow. This facilitates heat loss from the surface of the skin.

The eccrine sweat glands are stimulated by the activity of heat loss centers present in the hypothalamus. The increase in cutaneous blood flow causes more sweat to be formed. As said earlier, the vaporisation of sweat from the surface of the skin eliminates heat. The rate of sweat secretion can vary from 30-90 ml/hour depending upon the rise in the body temperature.

Increase in respiratory rate can also facilitate more elimination of heat. This is especially more pronounced in dogs, which does it through panting.

Behavioral response to excess body heat can be noticed in humans, in the avoidance of physical activity, use of fans, coolers, cold shower bath and less clothing.

The heat production is reduced by lowering SDA (specific dynamic action) through loss of appetite and apathy.

Body response to decrease in body temperature

When the body temperature is lowered, the regulatory mechanisms aim at raising the temperature of the body, by reducing the heat loss and increasing the heat production.

Mechanisms for reducing the heat loss

The peripheral cutaneous cold receptors are stimulated locally by the fall in temperature. This causes vasoconstriction of cutaneous blood vessels. The stimulation of heat gain center in the hypothalamus also will cause cutaneous vasoconstriction, which reduces the blood flow.

This reduces the heat loss from the surface of the skin. There is also stimulation of the smooth muscle erector pili, present at the base of the hair follicle in the dermis of the skin. The contraction of this muscle produces raising of hair (pilo erection), which helps trapping of air between hairs. The air trapped between the hairs gives insulation to the skin, preventing heat loss. The presence of adipose tissue is another factor that gives insulation to the skin. Wearing of woolen clothes in humans, presence of fur and feathers in lower animals also prevents heat loss by similar mechanism.

Ways of increasing heat production

During exposure to cold environment, **shivering** of the body is seen, which is nothing but the involuntary contraction of skeletal muscles, which raises the heat production.

Increase in appetite and increased metabolism are other factors that cause rise in body heat.

Somatic changes include the increased physical activity, which helps to increase the heat production.

The heat production is also increased by stimulating metabolism. This is achieved by increased secretion of catecholamines and thyroxine, which show calorigenic effect. The rise in BMR from this effect, increases the body temperature.

In infants, brown fat in the body is utilised for increased metabolism, to raise the body temperature when there is cold exposure.

Behavioural response to the cold exposure shows curling of the body, wearing of woolen clothes and use of room heaters.

Effects of hyperthermia

When the body temperature is raised more than 102°C, the symptoms of hyperthermia can be seen. Chronic exposure to hyperthermia causes heat stroke. The symptoms are due to dehydration and salt loss. The circulatory shock that develops can lead to death if immediate correction of the fluid and electrolyte loss is not done.

Fever

Hyperthermia is also seen in fever. The rise in body temperature during fever is believed to be a physiological adaptation, because, the high temperature is not conducive for the growth of bacteria. The mechanism for the rise in body temperature during fever is due to the action of bacterial toxins on macrophages, which secrete cytokines and interleukins (IL 1, IL 6). These in turn stimulate the formation of prostaglandins, which raises the set point of the thermostat, and hence the body temperature rises. Antipyretics stop the production of prostaglandin, which causes the set point of thermostat to come back to normal.

Hypothermia

Fall in body temperature between 30-25°C can lead to hypothermia. The oxygen consumption of the tissues is reduced, so also, the heart rate and blood pressure. Hypothermia is used in the surgery of heart and brain to reduce the blood flow in these organs. The body temperature is brought to 25°C before doing the surgery.

11 Gastrointestinal System

Gastrointestinal system includes alimentary canal, which is a hollow muscular tube extending from posterior pharynx to anus. The system also has accessory organs like salivary glands, liver, pancreas, etc. The main function of this structure is to ingest, digest and absorb food materials, so that the tissues in the body can assimilate them and survive. The various parts of alimentary canal and the accessory organs show secretion of digestive juices, which digest complex foods into simpler ones and facilitate absorption.

Organization of digestive tract

The digestive tract includes mouth, pharynx, esophagus, stomach, small intestine, large intestine, rectum and anus. The histological structure is mostly similar in all regions except esophagus and rectum. In these two regions, serous coat and attachment to peritoneum are not present.

The basic structure of digestive tract shows three layers namely from outwards, *serous coat*, middle *muscular layer* and inner *mucous layer*. The muscular coat is made of three layers namely, two layers of longitudinal and one layer of circular smooth muscle (Fig. 11.1).

The wall of the GI tract has intrinsic nervous system, which consists of Auerbach's plexus or myenteric plexus and Meissner's plexus or submucous plexus.

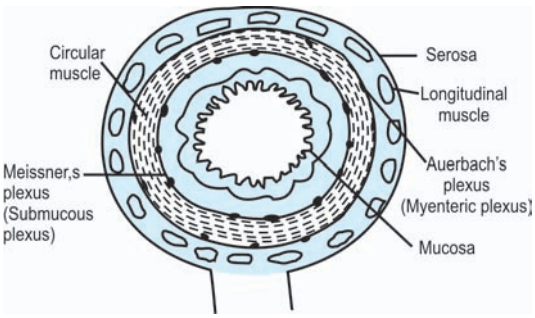


Fig. 11.1: Diagram showing the different layers of the gut wall

Between longitudinal and middle circular layer of smooth muscle, Auerbach's plexus is present. These neurons are motor in nature and supply the smooth muscle. They also make connections with the neurons of Meissner's plexus. The myenteric plexus is mainly concerned with controlling the motor activity of the gut.

Situated in the submucous layer (between middle circular layer of smooth muscle and inner mucous layer) there is another intrinsic nerve plexus called Meissner's plexus. These neurons supply the secretory glands present in the wall of the gut. There is also interconnection between these two neuronal systems and together they are as **enteric nervous system.** The function of

myenteric plexus is to produce peristalsis, by the contraction of the smooth muscle, whereas, the Meissner's plexus is concerned with the sensing of osmolar changes, pH changes and chemical composition of food. In response to this stimulation, the Meissner's plexus causes, secretion of digestive glands present in the wall of the gut. They are also sensitive to stretch, which is characteristic of these neurons.

The enteric nervous system action is independent of the extrinsic nerve supply to the gut. The extrinsic innervation can modify the activity of enteric nervous system. The extrinsic supply comes from autonomic nerves. The sympathetics give relaxation of smooth muscle and vasoconstriction of blood vessels, while, the parasympathetic (vagus and pelvic nerves) innervating the gut, causes contraction of smooth muscle, vasodilatation and secretion of digestive juices.

Neurotransmitters in enteric nervous system

The actions of enteric nervous system are mediated through the secretion of transmitters. The released transmitters, besides, showing effects locally, act on surrounding cells (paracrine) and also on distant glands (endocrine) through circulation in the blood. In addition to norepinephrine, acetylcholine, serotonin, GABA secreting neurons, the enteric nervous system also has NO, substance P, VIP, somatostatin, CCK, GRP secreting neurons. NO secretion causes vasodilatation and relaxation of smooth muscle. VIP secreting neurons show, secretion of intestinal juice and relaxation of sphincters. The neurons secreting substance P, gives contraction of intestinal smooth muscle and stimulation of gastric acid secretion. The CCK secreting neurons show inhibition of the gastric motility and emptying, while somatostatin causes the inhibition of gastric and intestinal secretions.

DIGESTIVE SECRETIONS

Salivary secretion

The digestive secretion in the mouth comes from salivary glands. There are many salivary glands present in the buccal cavity, but three pairs of glands are considered significant in their secretion of saliva. These glands are parotid, submaxillary (submandibular) and sublingual. These are exocrine glands, as the secretion is carried by a duct and poured into the mouth.

Each gland shows in common, secretory cells arranged in acini and duct cells, which continue from the lumen of the acini. In the acini, two types of secretory cells are found. One is **serous**, which secrete watery juice rich in the enzyme ptyalin and other is, **mucous**, which gives a thick viscous secretion rich in mucin. The *parotid gland* mainly consists of *serous cells* and the *submaxillary gland* has *mixed cells (seromucus)*. The *sublingual gland* predominantly contains *mucous cells*. About 70% of salivary secretion comes from submaxillary gland, which has mixed cells.

Innervation of salivary gland

Salivary glands are supplied by both the sympathetic and parasympathetic nerves. The sympathetic action gives vasoconstriction, which causes the secretion to be viscous and scanty. The parasympathetic effect causes vasodilatation and copious secretion of saliva. The parasympathetic fibers in the VIIth nerve, through the chorda tympani branch, supply the submaxillary and sublingual glands. The parotid gland receives parasympathetic supply from the IX cranial nerve.

Composition

The secretion of saliva per day ranges from 1000 to 1500 ml. It has a pH 6.8 and ranges from 6.7 to 8. Saliva contains 99.5% water and 0.5% solids. The latter consists of inorganic and organic

substances. Na^+, K^+, Cl^-, HCO_3^-, are the major inorganic constituents. The enzyme *salivary-a amylase, mucin, lysozyme, IgA, blood group antigens* are some of the organic substances present in the saliva.

Duct cells function

The saliva that is secreted in the lumen of the gland is isotonic, whereas the secretion in the mouth is **hypotonic**. It is due to the changes that take place in the lumen of the ducts (Fig. 11.2). The duct epithelial cells show active reabsorption of Na^+ in exchange for K^+. There is also exchange of Cl^- with HCO_3^-. Chloride is reabsorbed in exchange with bicarbonate secretion from the duct cells. Since the duct epithelial cells are impermeable to water, the removal of Na^+ and Cl^- ions makes the saliva hypotonic. Aldosterone, which is a mineralo corticoid, acts on the salivary duct cells and causes sodium reabsorption in exchange for K^+ secretion. The concentrations of Na^+ and K^+ in saliva depend on the flow rates. At high flow rates, less time is allowed for transfer of ions and hence Na^+ is more and K^+ is less (Fig. 11.3). Inspite of greater Na^+ concentration in the saliva, it remains isotonic in the mouth at high flow rates.

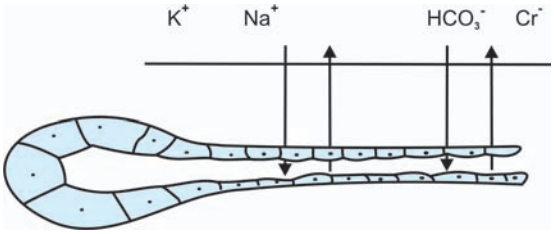


Fig. 11.2: Duct cells function in the salivary gland

The saliva that is secreted in the gland is isotonic to plasma, but the secretion in the mouth is hypotonic, due to the changes in the ionic composition, as the saliva passes through the duct

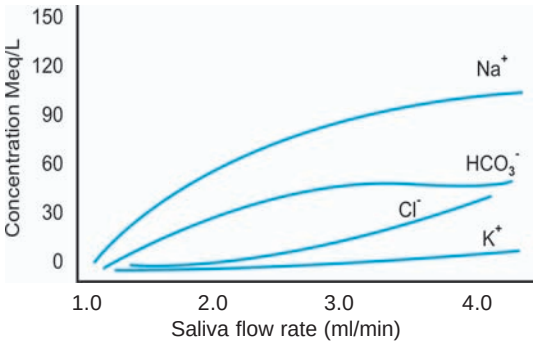


Fig. 11.3: Relationship between ionic composition and the rate of salivary secretion

Regulation of secretion

Salivary secretion is regulated by mainly neural mechanism. It shows both **conditioned** and **unconditioned reflexes**. Conditioned reflex is established by learning and the secretion of saliva can be seen from sight, smell and thought of food. The unconditioned reflex comes from the presence of food in the mouth. The secretion of saliva in both types of reflexes is caused by the activity of parasympathetic nerves (VII and IX cranial nerves) supplying the gland.

Functions of saliva

- The enzyme **α amylase** acts on boiled starch and converts it to maltose. Thus carbohydrate digestion begins in the mouth.
- The mucin present in the saliva lubricates the food, which helps mastication.
- Saliva is necessary for swallowing of food.
- It helps in taste perception of food materials by dissolving them.
- It facilitates speech and in dry mouth speech will be difficult.
- Lysozyme and IgA present in the saliva give bactericidal and immunity functions respectively.
- Saliva is the route for the excretion of heavy metals like lead, mercury, drugs and virus.
- The proline rich proteins present in saliva protect the enamel and in its absence as in low secretory rates, dental caries are common.
- It neutralizes the gastric acid that refluxes into the duodenum and relieves heartburn.

Gastric secretion

Gastric juice is secreted by the gastric glands, present in the gastric mucosa of fundus and body of the stomach. The glands are long coiled tubular structures, which arise from the gastric pit, from the surface mucosa (Fig. 11.4). The gland has three types of cells, namely *neck cells, chief cells* and *parietal cells*. The **neck cell** and the surface epithelial cells secrete mucus. The **chief cells** contain zymogen granules and secrete enzymes. The **parietal cells** or oxyntic cells are the source of HCl and intrinsic factor.

Composition of gastric juice

The volume of secretion per day is around 2 lit and the pH varies from 1 to 2. The low pH is due to the secretion of concentrated HCl. The inorganic constituents include, Na^+, K^+, Cl^-, PO_4^- and SO_4^-. The organic substances present in the secretion are digestive enzymes, pepsinogen, rennin, lipase, mucin and intrinsic factor.

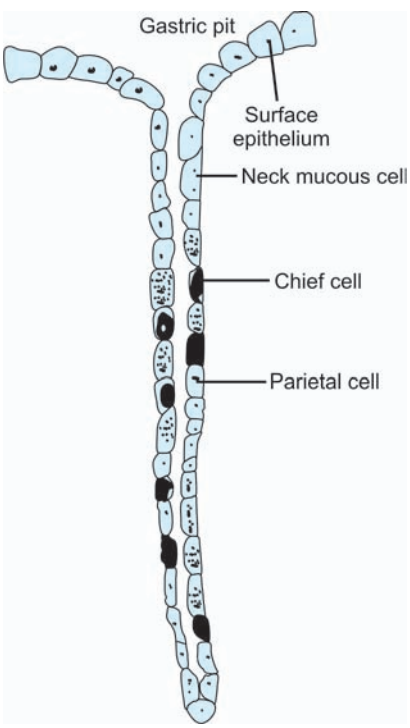


Fig. 11.4: Structure of the gastric gland showing different types of cells

Functions of HCl

- The concentrated HCl in the gastric juice, is necessary to activate pepsinogen to pepsin and the optimum pH that is needed for this action will be 2. The extremely acidic pH acts as bactericidal and also it converts cane sugar to monosaccharides. The acidic pH in the upper part of duodenum facilitates iron absorption.

Functions of gastric juice

The **beginning of protein digestion** takes place in the stomach. Pepsin acts on protein and converts it to peptones. The enzyme is secreted from the chief cells as inactive pepsinogen.

Gastric rennin is a *milk curdling enzyme* which is absent in humans. It is present in calves. The gastric lipase is a weak fat splitting enzyme.

Intrinsic factor is a glycoprotein and secreted from parietal cells of the body and the fundus of stomach. Intrinsic factor is one of the important constituents of gastric juice which is required for the absorption of vit B_{12} (extrinsic factor). The absorption of vit B_{12} occurs in the terminal ileum.

Mucus in the gastric juice comes from two sources. Besides the surface epithelium of the gastric mucosa, the neck cells of the gastric glands also secrete mucus. The surface epithelium of the mucosa in addition to mucus, release HCO_3^-. The mucus and the bicarbonate form a gel in the lining of gastric mucosa. The presence of this gel and the mucus from neck cells, protect the gastric mucosa from the action of acid. Infact, there is a pH gradient from the lumen to the mucosal wall of the stomach. The pH in the mucosa is 7, while in the lumen, it is between 1 and 2. The presence of bicarbonate and mucus forms the **acid mucosal barrier**. In certain conditions like *chronic stress*, and substances like *alcohol, vinegar, aspirin and other nonsteroidal anti inflammatory drugs(NSAID)*

have a tendency to erode the acid mucosal barrier. Loss of this barrier leads to gastric ulcer.

HCl secretion (Fig. 11.5)

Hydrochloric acid in the gastric juice is secreted from parietal cells. The parietal cells show canaliculi within the cell. The canaliculi open into the duct, which drains the secretion into the gastric pit.

The secretion of HCl is an active process and is against the electrochemical gradient. The concentration of H^+ ion in the juice is 150 mEq/L, whereas, in plasma its concentration is 0.00004 mEq/L. The source of H^+ ion is from the dissociation of H_2CO_3. Carbonic acid is formed from the hydration of CO_2 in the presence of carbonic anhydrase enzyme. H_2CO_3 dissociates into H^+ and HCO_3^-. The H^+ that is formed, is exchanged with K^+ and the transport carrier is H^+-K^+ ATPase. The energy released from the breakdown of ATP, is utilized for the active transport of H^+. Cl^- is transported down the electrochemical gradient through Cl^- channels activated by cAMP. The H^+ ion couples with Cl^- to form HCl. The active transport of HCl is followed by passive movement of water into the lumen of canaliculi.

The secretion of H^+ ion leaves bicarbonate ion within the cell. HCO_3^- is extruded into the interstitial space from the basolateral surface of the membrane of the parietal cell in exchange for Cl^-. From here it enters the blood. It is known that during digestion, the alkali level in blood and urine raises and is called *postprandial alkaline tide.*

Agents causing secretion of acid and its inhibition (Fig. 11.6)

Parietal cells have receptors for **gastrin** (G receptors). **Acetylcholine** acts on $\mathbf{M_3}$ receptors present on the cell and **histamine** produces its

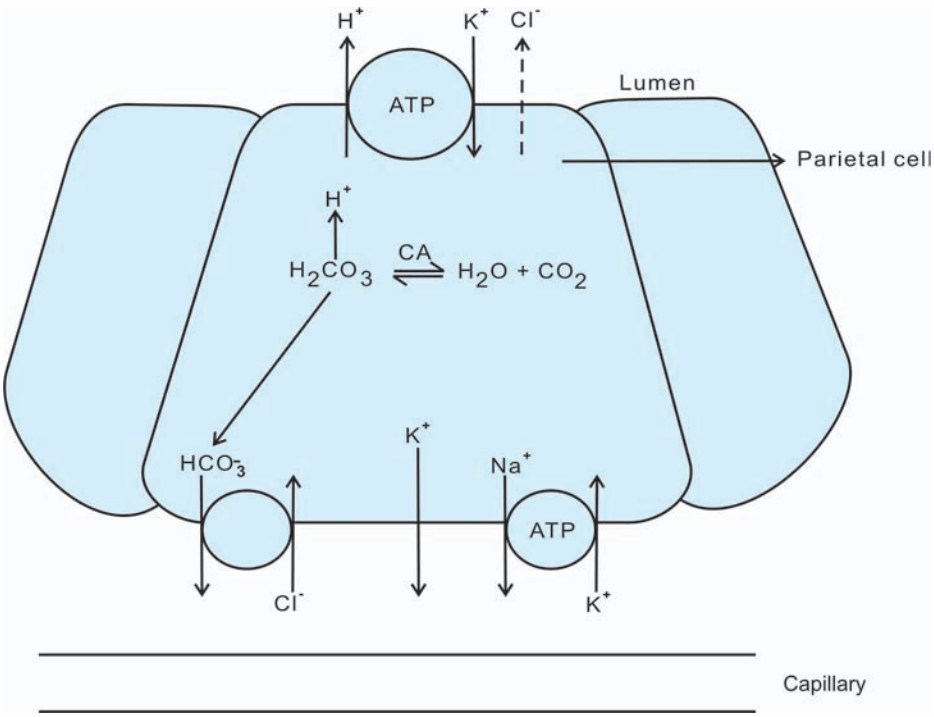


Fig. 11.5: HCl secretion from the parietal cell of gastric gland

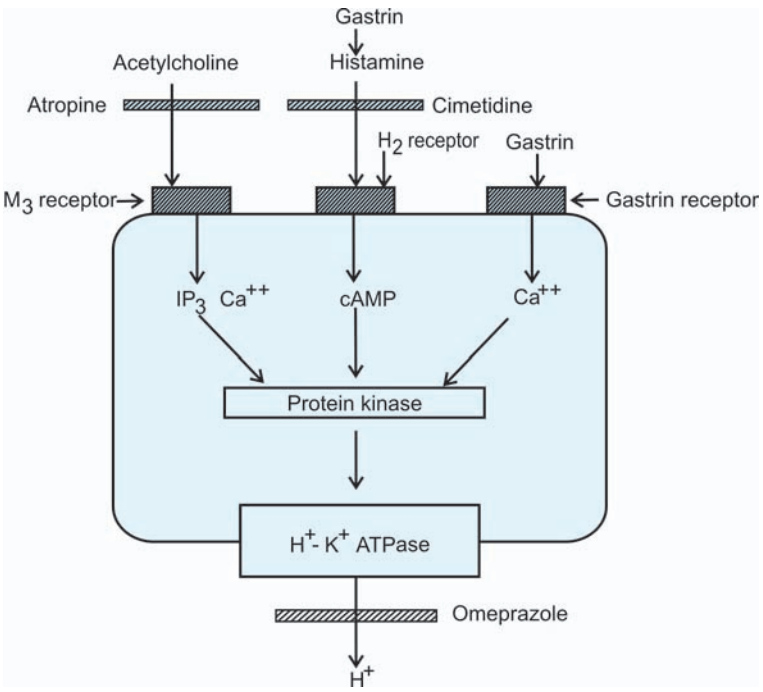


Fig. 11.6: Parietal cell showing the action of agents that cause secretion of Hcl. The agents which inhibit secretion are also shown

effect by acting on H_2 receptors. Gastrin is released from pyloric antrum and acetylcholine is secreted from the vagal ending. The histamine comes from the enterochromaffin cells lining the gastric mucosa. When vagus is activated, it causes release of acetylcholine. This stimulates the release of **gastrin releasing peptide** (GRP), which causes secretion of gastrin. Gastrin also stimulates enterochromaffin cells to release histamine. All the three agents cause secretion of HCl. In the case of peptic ulcer, the blocking of **H_2 receptor** by **cimetidine** causes suppression of acid secretion. Acid secretion is also inhibited in cases of peptic ulcer by giving **H^+- K^+ ATPase** enzyme blocking agent **omeprazole**.

Regulation of gastric secretion

Gastric juice secretion is regulated by both nervous and hormonal mechanisms.

Neural regulation

Neural regulation is mediated by the vagus. Acetylcholine secreted from the postganglionic endings of vagus acts on the parietal and chief cells and stimulates secretion. Acetylcholine activates phospholipase C enzyme, which in turn raises intracellular $Ca.^{++}$ The intracellular rise in calcium promotes secretion of gastric juice.

Hormonal regulation

Gastrin is a peptide hormone and is secreted mainly from the pyloric antral mucosa. This region has G cells and they are known as APUD cells amine (precursor uptake and decaroxylase). There are three forms of gastrins secreted and they are G34, G17, and G14. The numbers represent the amino acid residues in the molecule. The G17 is the principle form and active. The G cells also receive the vagal endings, but they release a

peptide GRP (gastrin releasing peptide). This causes the release of gastrin.

The secretion of gastrin also occurs in the following conditions

Caffeine
Products of protein digestion
Ca^{++}
Distention of pyloric antrum
Circulating adrenaline

Gastrin is able to produce gastric secretion by raising the intracellular Ca^{++}, similar to acetylcholine.

The secretion of gastrin is inhibited by the acid in the pyloric antrum, VIP, secretin, GIP and somatostatin.

Phases of gastric secretion

There are three phases namely:

Cephalic
Gastric
Intestinal.

Cephalic phase

Conditioned reflexes like sight, smell, thought of food causes secretion of the gastric juice. Presence of food in the mouth also causes secretion in the stomach. The cephalic phase occurs by the activity of the vagus. Sham feeding experiments in animals like dogs, gives a good example for the cephalic secretion of gastric juice. The quantity of juice secreted in this phase is less when compared to gastric phase.

Gastric phase

The arrival of food and distention of stomach causes the secretion of gastric juice. The secretion in this phase involves the activity of vagus and the hormone gastrin. During this phase, maximal secretion of gastric juice occurs.

Intestinal phase

The arrival of food and products of food digestion in the small intestine also stimulates gastric juice secretion. The quantity of juice secreted is very less. However, the presence of products of food digestion and acid in duodenum inhibits the secretion of gastric juice. This kind of inhibition is mediated through the enterogastric reflex. The presence of acid releases **secretin** and fat in the duodenum releases **CCK**. There is also secretion of **GIP, VIP** hormones from small intestine. All of them cause inhibition of gastric juice.

Peptic ulcer

The eroding of mucosa in the stomach or in the duodenum by HCl and pepsin is called peptic ulcer. It occurs in various conditions and the important ones are:

Breakdown of acid mucosal barrier due to
- infection by *Helicobacter pylori*
- ingestion of aspirin, NSAID (nonsteroidal anti-inflammatory drugs)
- Zollinger-Ellison syndrome (gastrinomas especially from pancreas)
- Chronic alcoholism
- Chronic exposure to stress.

Treatment of peptic ulcer involves administering H_2 blockers cimetidine, ranitidine, etc.

Blocking of H^+- K^+ ATPase by omeprazole

Pancreatic secretion

Pancreas has both exocrine and endocrine functions. The digestive enzymes are secreted from the exocrine pancreas.

The exocrine pancreas consists of acini with the duct system beginning from the centroacinar cells. The acinar cells are rich in zymogen granules, which are the enzyme precursors.

Composition

Pancreatic juice has a volume 1500 ml/day and the pH of the secreted juice is around 8. The alkalinity is due to HCO_3^- secreted from duct epithelial cells. The enzyme carbonic anhydrase is present in the duct epithelial cells, which hydrates CO_2 to carbonic acid. The carbonic acid immediately dissociates into $H^+$and HCO_3^-. The

H^+ ion is exchanged for Na^+ at the basal membrane by Na^+-H^+exchanger. Higher the flow rate, more the HCO_3^- present in the secretion. The HCO_3 in pancreatic secretion, neutralizes the acid present in the small intestine. Absence of HCO_3^- in the pancreatic secretion can lead to duodenal ulcer. The other inorganic substances are, Na^+, K^+, Ca^{++},Cl^-, PO_4^- and SO_4^-. Organic constituents that are present in the pancreatic juice include, digestive enzymes, which are of four types.

Peptidases

Lipases

Amylases and

Nucleases

Peptidases

Trypsin, chymotrypsin and elastase

It is secreted in an inactive form as trypsinogen and activation occurs by enterokinase. The trypsin formed from trypsinogen, itself can activate trypsinogen.

Trypsinogen $\xrightarrow{\text{Enterokinase}}$ Trypsin

Chymotrypsinogen $\xrightarrow{\text{Trypsin}}$ Chymotrypsin

Trypsin and chymotrypsin are endopeptidases and act on native proteins and convert them to peptides.

Procarboxypeptidase It is inactive and converted to carboxypeptidase by trypsin. It converts protein to peptides and aminoacids.

Nucleases

Ribonuclease and deoxyribonuclease—They act on RNA and DNA and convert them to nucleotides.

Lipase

Pancreatic lipase is an important fat digestive enzyme. It requires the presence of bile salts for its action.

Lipase converts fat into glycerol, mono-and diglycerides and fatty acids in the presence of bile salts.

Phospholipases

They act on phospholipids and convert them to lysolecithin, fatty acids and lysophopholipids.

Amylase

Pancreatic amylase acts on starch and converts it to maltose, which is a disaccharide.

Trypsin inhibitor

It is also secreted by the exocrine pancreas and its function is to protect pancreas from auto-digestion by proteases.

Regulation of pancreatic secretion
Control in cephalic phase

The regulation of pancreatic secretion is mainly from hormones, although, the vagal activity during the cephalic and gastric phases causes pancreatic secretion. It is a low volume secretion in this phase.

In cephalic phase, the sight, smell, thought of food causes pancreatic secretion and is mediated through vagal activity.

There is also enteric plexus causing the secretion of pancreatic juice, which is mediated through the release of acetylcholine and VIP. Acetylcholine acts on acinar cells and VIP controls the secretion of centroacinar cells.

Regulation in gastric phase

In **gastric phase,** the distention of pyloric antrum gives **vagovagal reflex**, which causes low volume secretion. The gastrin that is released, will also stimulate the pancreatic secretion, with high enzyme content, but less in volume.

Regulation in intestinal phase

The main secretion occurs in this phase and the secretion is regulated by hormones. There are two hormones, regulating two types of secretion from the exocrine pancreas.

Secretin

It is the first hormone discovered and the name hormone is given after its discovery. It is a polypeptide produced from the duodenal mucosa. This peptide hormone acts on the duct epithelial cells and causes pancreatic secretion rich in water and bicarbonate. The stimulus for the hormone secretion is the presence of acid chyme in the duodenum. The secretion of watery fluid rich in bicarbonate helps to neutralize the acid pH. The action of secretin is potentiated by CCK.

CCK (pancreozymin)

Cholecystokinin (CCK) is a peptide hormone which acts on the acinar cells and causes secretion of pancreatic juice rich in digestive enzymes. CCK also acts on gallbladder. It causes its contraction and expulsion of bile. The hormone is produced more from duodenal mucosa and the stimulus for its release is the products of food digestion entering the duodenum.

Clinical importance

Acute pancreatitis caused by the inflammation of pancreas results in the release of digestive enzymes into the circulation. Estimation of **serum amylase** shows significant rise which is of diagnostic importance.

In chronic pancreatitis, arising due to bacterial infection, the digestive enzymes are not sufficiently secreted. This results in poor digestion and especially, the digestion of fat is affected. The stool contains more fat and gives a foul smell. The excretion of more fat in the feces is known as **steatorrhea.** In chronic pancreatitis, there is also poor absorption of fat soluble vitamins and this leads to malnutrition.

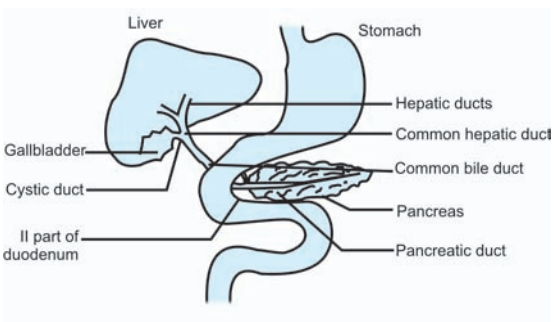


Fig. 11.7: Relationships and connections of ducts of liver, gallbladder and pancreas

Bile

Bile is secreted from the liver. Liver contains lobules and each lobule shows rows of hepatocytes radiating centrifugally from a central vein. At the other end in the connective tissue, a **triad** is present. It includes *portal vein, hepatic artery and bile duct* (Fig. 11.8). The portal vein opens into the sinusoids, which drain into the central vein. The sinusoids are present at the basolateral surface of hepatocytes. The blood from the central vein is ultimately drained into the hepatic vein. Between the rows of hepatocytes, at the apical border, bile ductules are present, which drain bile into the bile duct. Bile from right and left lobes of liver is collected by right and left hepatic ducts respectively. These two ducts join with cystic duct from gallbladder and form the common bile duct (Fig. 11.7). Pancreatic duct joins with the common bile duct before opening into the second part of duodenum, where the orifice is guarded by the sphincter of Oddi.

Composition of bile

The volume that is secreted per day is 500 to 1000 ml and secretion occurs, when chyme enters the duodenum. The secretion is alkaline in nature with pH ranging from 7.6 to 7.8. The inorganic constituents consist of Na^+, K^+, Cl^-, and HCO_3^- The organic substances include:

Bile salts
Bile pigments
Phospholipids.
Cholesterol
Lecithin
Fatty acids

Bile salts

They are the secretory products of the liver. They are formed from cholesterol, which gives rise to **primary bile acids.** These are **cholic** and **chenodeoxycholic** acids. The conjugation with sodium or potassium gives **taurocholate and glycocholate,** which are the bile salts. In the colon, the primary bile acids are converted to **secondary bile acids** by the action of bacteria. The secondary bile acids are **deoxycholic** and **lithocholic acids**.

Bile pigments

The breakdown of Hb gives bilirubin and biliverdin, which are the bile pigments. They are the excretory products of liver. The golden yellow colour of the bile is due to the presence of bile pigments.

Phospholipids

Lecithin is the chief phospholipid present in the bile. It is insoluble in water and in the micelles form, it is solubilized. If micelles contains lecithin together with bile salts, the solubilization of fat soluble substances becomes efficient.

Cholesterol

The cholesterol in bile is solubilized by the bile salts present in the micelles. The presence of bile salts keeps cholesterol in solution and prevents its precipitation to form stones.

Functions of bile

The bile salts have digestive function. They are necessary for fat digestion and absorption. The digestion and absorption of fat and fat soluble vitamins depend on bile salts presence. The fat is lipid soluble and the digestive enzyme lipase is water soluble. In order to facilitate the action of lipase on lipids, bile salts show the following effects.

- The bile salts produce **emulsification** of fat. By this the large molecules are broken down into smaller ones.
- They show **hydrotropic effect**. This action of bile salts enables lipase enzyme to digest fat.
- Bile salts **reduce surface tension**. This effect facilitates the lipase enzyme action
- The **micelles** that is formed after fat digestion, promotes absorption. The micelles consists of digested glycerides combined with bile salts.

Enterohepatic circulation of bile salts

The bile salts that are secreted into the duodenum are reabsorbed and recirculated. About 90% of bile salts that enter the small intestine are absorbed from terminal ileum and enter the liver through portal circulation. From liver, they are recirculated into the duodenum. This forms the enterohepatic circulation.

The bile salts in the circulating pool are only 3.6 gm. But the quantity required for fat digestion is 4 to 8 gm. So, by recirculating the limited quantity available, normal fat digestion and absorption are able to take place. The total circulating pool of bile salts recirculates twice during the digestion of each meal and this amounts to 6 to 8 times per day.

The rate of synthesis of bile salts depends on the rate of its return to the liver. Normally 0.2 to 0.4 g/day is lost in the feces and this quantity is replaced by the synthesis in the liver. Any condition that affects the enterohepatic circulation, as in ileal resection, small intestine diseases like, sprue, Crohn's disease, results in decreased bile acid pool, which causes malabsorption of fat and fat soluble vitamins. This leads to steatorrhea and nutritional deficiency.

Regulation of bile secretion

Bile secretion is controlled by hormones. There are two mechanisms in its regulation. They are:

Bile independent fraction of biliary secretion

This regulation gives volume with more fluid and bicarbonate, but less bile salts. This is caused by

the hormone, secretin produced from duodenum when acid chyme enters. **Secretin** acts on the biliary duct cells and causes increased secretion of water, electrolytes and bicarbonate. This helps to neutralize the acid chyme in the duodenum. This action of secretin is known as **hydrocholeretic** action.

Bile dependent fraction of biliary secretion

This regulation refers to the amount of bile salts secreted by the liver. The **bile salts** in bile itself cause stimulation of liver to secrete bile. This action of bile salts is called **choleretic effect.** This effect ensures normal enterohepatic circulation of bile salts.

Release of bile from gallbladder

The hormone **CCK** (cholecystokinin) acts on the gallbladder smooth muscle and causes its contraction, resulting in the expulsion of bile. This action of CCK in causing the release of bile from the gallbladder is known as **cholagogue** effect. CCK also acts on the sphincter of Oddi and inhibits it. The relaxation of the sphincter facilitates entry of bile into the duodenum. Vagal stimulation also causes gallbladder contraction and relaxation of sphincter of Oddi.

Gallbladder

The gallbladder releases the bile, whenever food containing fat enters the duodenum. In addition to storage (capacity is 20 to 50 ml) and release of bile, the gallbladder makes the bile concentrated. This is done by the active reabsorption of Na^+, HCO_3 and passive reabsorption of water. The third function of the gallbladder is to release bile, when fat reaches duodenum. The hormone CCK, produces this effect. The release of bile from the gallbladder, also occurs, by the vagal activity.

Gallstones (cholelithiasis)

Gallstones are of two types. Cholesterol stones (common in the western population) and pigment stones (calcium bilirubinate) are the two types.

Normally, cholesterol and lecithin are kept in solution in the bile, through the micelles formation with the help of bile salts. When the proportion of cholesterol, lecithin and bile salts is altered, cholesterol crystalizes to form stones. Cholesterol stones are radiolucent. Pigment stones are formed due to infection of biliary tree. The deconjugation of bilirubin makes it insoluble causing precipitation. The pigment stones are radio opaque.

Cholecystography

The gallbladder and biliary tree are X-ray visualized by injecting an iodinated radio opaque compound. The gallbladder is visualized before and after giving a test meal containing fat. The gallbladder size should reduce by one third after the fat test meal within 30 minutes. If gallstones are present, this decrease in the size of the gallbladder will not occur.Now it is possible to visualize gall bladder by CT scan, utrasonography and nuclear cholescintography.

Cholecystectomy

It is the surgical removal of gallbladder. After the removal of gallbladder, bile empties slowly but continuously. Normal fat digestion and absorption will not pose any problem. Only excess fat intake in the diet should be avoided.

Differences between liver and gallbladder bile

	Liver bile	*Gallbladder bile*
Water	97%	89%
Solids	3%	11%
Bile salts	0.7%	4.5%
Bile pigments	0.2%	2.1%
Cholesterol	0.06%	0.2%
pH	7.8 to 8	7 to 7.4

Intestinal secretion

The small intestine consists of duodenum, jejunum and ileum. The mucosa of small intestine contains finger like projections called villi. They

are 0.5 to 1 mm long and lined by columnar epithelial cells, which show brush border at the apical surface. They form the microvilli. The presence of mucosal folds, villi and microvilli increase the surface area for absorption. The villus also shows the presence of blood capillaries and lymphatic vessel called lacteal. The apical cells show constant removal and replaced by the new cells produced by the basal cell mitotic activity. The basal part of the villi has a long tubular structure which is the intestinal gland **(crypts of Lieberkühn).** It contains cells that secrete mucus and electrolytes. The intestinal mucosal cells also show enterochromaffin cells, which secrete serotonin. The goblet cells in the glands secrete mucus. The intestinal glands of duodenum are called **Brunner's glands,** which mainly secrete mucus. The mucosa of ileum contains Peyer's patches, which are aggregates of lymphatic nodules. This region serves immunity function. The mucus secreted by the intestinal glands gives protection to mucosa against mechanical damage and also gives lubricative function. The small intestine secretion has mainly water, mucus and electrolytes. It is alkaline in reaction with a volume ranging from 1000 ml to 1500 ml/day.

Digestive enzymes of small intestine are not secreted into the lumen. The enzymes are present at the apical surface of the villi cells. The products of food digestion namely, tri, di peptides, disaccharides are digested at the apical surface of the cells, where the enzymes are located. These enzymes are as follows.

Peptidases which include tripeptidase and dipeptidase.

Peptidases act on the peptides and convert them to amino acids, which are the end products of protein digestion.

Nucleases act on the nucleotides and convert them to pentoses, purine and pyrimidine bases.

Disaccharidases such as sucrase, maltase and lactase. They act on disaccharides and convert them to monosaccharides.

$$\text{sucrose} \xrightarrow{\text{sucrase}} \text{fructose} + \text{glucose}$$

$$\text{maltose} \xrightarrow{\text{maltase}} 2\ \text{glucose}$$

$$\text{lactose} \xrightarrow{\text{lactase}} \text{galactose} + \text{glucose}$$

Although most of the digestive enzymes are located at the surface of the villi cells, peptidases are also present in the cytosol. It has been observed that molecules of tri and di peptides enter the cell and undergo digestion in the cytosol. The desquamated villi cells also release certain enzymes into the lumen, which include enterokinase and lipase.

Regulation of secretion

Neural

Vagal nerve stimulation or its activity during digestion causes secretion of intestinal glands.

Hormonal

The peptide hormone vasoactive intestinal polypeptide **(VIP)** increases secretion of intestinal glands.

GASTROINTESTINAL HORMONES

The secretion and motility of gastrointestinal tract are regulated by hormones besides neural mechanisms. Chemically, the GI hormones are made of peptides. They produce paracrine and endocrine effects. Most of the GI hormones are secreted from the pyloric part of stomach and proximal part of the small intestine.

Gastrin

This is secreted from the G cells of pyloric antral mucosa. This region has a neural crest origin and is capable of taking amine precursors and decarboxylate them. That's why these cells are called APUD cells (amine precursor uptake and decarboxylation). Gastrin secreting cells are also found in other regions like stomach and intestine which is not physiologically significant. The gastrin that is secreted from G cells of pyloric antrum has 17 amino acid residues (G17). It is

the principle form of gastrin, which stimulate gastric acid secretion and pepsin. The other gastrins are G34 and G14 secreted from the other regions. Gastrin secreting tumors (gastrinomas) can be found in pancreas. G17 has a half life of 2 to 3 minutes in the circulation.

Actions of gastrin

Stimulates enterochromaffin like cells(ECL) to secrete histamine

Stimulates growth of mucosa in the stomach and small intestine

Causes secretion of gastric acid and pepsin

Stimulates gastric motility

Stimulates insulin secretion after a protein meal.

Regulation of gastrin secretion

Secretion of gastrin occurs due to

- Distention of stomach
- Presence of protein and products of protein breakdown
- Amino acids especially tryptophan and phenylalanine
- Stimulation of postganglionic parasympathetic vagal endings supplying the pyloric antrum. The transmitter that is released is not acetyl choline, but GRP (gastrin releasing peptide). This peptide causes the secretion of gastrin.
- Blood borne factors like Ca^{++}, epinephrine.

Inhibition of gastrin secretion

Presence of acid in the stomach has a negative feed back control, which inhibits gastrin secretion.

Blood borne substances like secretin, GIP (gastric inhibitory peptide), VIP, glucagon and calcitonin inhibit gastrin secretion.

CCK-pancreozymin

It has many forms, similar to gastrin, ie, CCK 8, CCK 12. Its half life in the circulation is 5 minutes.

CCK is cholecystokinin, which causes the gallbladder contraction and release of bile. The same hormone acts on the acinar cells of the exocrine pancreas and produces pancreatic secretion rich in enzymes. Hence, CCK is also called pancreozymin. Since both are one and the same, it is commonly referred as CCK- pancreozymin.

CCK is secreted from the mucosa of the upper part of small intestine, from nerve endings in distal ileum, colon and neurons in brain.

Actions of CCK-pancreozymin

- Stimulates secretion of pancreatic juice rich in digestive enzymes
- Augments secretin action to produce pancreatic secretion rich in HCO_3^-.
- Produces contraction of gallbladder and release of bile
- Causes inhibition of gastric emptying
- Shows trophic effect on pancreas
- Increases motility of small intestine and colon
- Stimulates secretion of enterokinase
- Both gastrin and CCK are called gut factors which stimulate glucagon secretion.

Regulation of CCK secretion

Secretion is stimulated by the entry of products of food digestion into the duodenum, especially by polypeptides, amino acids and fat.

Secretin

It was the first chemical messenger discovered by Bayliss and Starling in the year 1902. The historical significance is that the term hormone for chemical messengers has been coined by them after the discovery of secretin.

It is secreted from the S cells of intestinal glands in the mucosa of the upper part of small intestine. The structure is very close to glucagon, VIP and GIP. Its half life in the circulation is 5 minutes.

Actions of secretin

Stimulates HCO_3^- secretion from the duct cells of pancreas and biliary duct

Causes watery alkaline pancreatic secretion

Augments the action of CCK on pancreatic acinar cells

Decreases gastric acid secretion

Causes contraction of pyloric sphincter.

Regulation of secretin

Secretion of secretin is stimulated by the acid chyme entering the duodenum. The alkaline secretion produced by secretin, helps to neutralize the acidity.

Products of protein digestion in the upper part of small intestine stimulate secretin release.

GIP (gastric inhibitory peptide)

It is produced from K cells in the mucosa of duodenum and jejunum.

Actions of GIP

- In large doses it causes inhibition of gastric secretion and motility
- In smaller doses it does not show this action
- Stimulates insulin secretion and forms one of the important β cell stimulating GI hormones.

Regulation of GIP

Secretion of GIP is caused by the presence of glucose and fat in the duodenum.

VIP (vasoactive intestinal polypeptide)

It has 28 amino acid residues and found in the nerves of GI tract, brain and in autonomic nerves as cotransmitter with acetylcholine.

It's half life in the circulation is 2 minutes.

Actions of VIP

- Stimulates intestinal secretion of electrolytes and water
- Relaxes intestinal smooth muscle including sphincters
- Causes dilatation of peripheral blood vessels
- Inhibits gastric acid secretion.

VIP which exists as cotransmitter with acetylcholine in autonomic nerves, shows potentiation of acetylcholine action.

VIP-omas (VIP secreting tumors) produces severe diarrhea.

VIP is regulated by the presence of products of food digestion and nerve stimulation in the small intestine.

Enterogastrones

These are hormones which cause inhibition of gastric secretion and motility. They are secreted from the mucosa of the upper part of small intestine in response to the presence of acid chyme and fat in the lumen. The hormones belonging to this category are:

Secretin

GIP

CCK

Other GI hormones

Motilin

It is a polypeptide and secreted from the duodenal mucosa. It causes contraction of intestinal smooth muscle and regulates interdigestive motility (migrating motor complex).

Neurotensin

It is found in the mucosa of ileum. The secretion is stimulated by fatty acids. Its actions include:

Inhibition of GI motility and increase in ileal blood flow.

Substance P

It is found in the endocrine cells of GI tract and it stimulates motility of small intestine. This hormone may enter the circulation.

GRP (Gastrin releasing peptide)

It has 27 amino acid residues and the first 10 amino acid residues resemble amphibian bombesin. It is secreted from vagal endings on the G cells and causes gastrin release.

Somatostatin

It is secreted from the D cells of GI tract, D cells of islets of pancreas and hypothalamic neurons. There are two forms S14 and S28. In GI tract, it causes inhibition of gastrin, secretin, VIP, GIP and motilin. The secretion of somatostatin in the GI tract is stimulated by the acid in the lumen.

It inhibits gastric acid secretion and motility. It also inhibits pancreatic exocrine secretion. The gallbladder contraction, absorption of glucose, amino acids and triglycerides are inhibited by this hormone.

Glucagon

Glucagon secretion from GI tract in diabetes mellitus assumes significance in accentuating hyperglycemia.

Enkephalins

These are opioid peptides and the important among them is dynorphin. Its secretion helps to regulates GI tract secretion and motility.

Peptide YY

This peptide gut hormone has been recently discovered. It is secreted from the jejunum of the small intestine in response to fat in the diet. Its main action is to inhibit gastric secretion and motility. It acts on the hypothalamus and inhibits appetite. The peptide YY hormone has been considered as a candidate for gastric inhibitory peptide **(GIP).**

Ghrelin

It a peptide hormone secreted from the stomach. It is also involved in the control of food intake. It stimulates appetite and increases food intake. Its level in the blood is increased during fasting and reduced in obese individuals. The hormone ghrelin has receptors in the anterior pituitary and stimulates the secretion of growth hormone secretion.

LIVER FUNCTIONS

Functional anatomy

Functional unit of liver consists of lobules. Each hepatic lobule has cells radiating centrifugally from a central vein. The periphery of the lobule shows branches of hepatic artery, portal vein and bile duct. These three structures form the portal triad. There are large sinusoids present between the cells, which contain blood from portal vein and hepatic artery. The sinusoids drain blood into the hepatic vein. The endothelial cells lining the sinusoids have Kupffer's cells and tissue macrophages. Biliary canaliculi are present

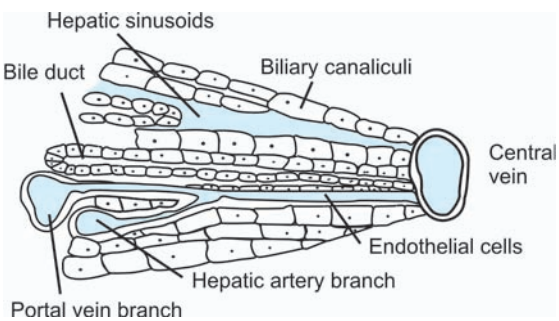


Fig. 11.8: Diagram showing portal triad in the liver

between adjacent layers of cells. They collect bile and drain into the bile duct.

Each lobe of liver is drained by one hepatic duct. The right and left hepatic ducts join together to form the common hepatic duct. It joins with the cystic duct from gallbladder and forms the common bile duct. This opens at the second part of duodenum, together with the pancreatic duct (ampulla of Vater). The opening at the second part of duodenum is guarded by the sphincter of Oddi.

Liver has many functions which include metabolic, synthetic, storage, excretory, and detoxification.

Metabolic functions

Carbohydrate metabolism Liver helps in glucose homeostasis. It shows glycogenesis, gluconeogenesis and glycogenolysis. It is the site for the conversion of galactose to glucose and fructose to glucose.

Protein metabolism The formation of urea from ammonia, deamination and transamination reactions take place in the liver.

Fat metabolism Lipogenesis, lipolysis, β oxidation of fatty acids, synthesis of lipoproteins, synthesis and esterification of cholesterol, formation of bile acids from cholesterol, ketogenesis, take place in the liver.

Synthetic

Formation of blood coagulation factors, prothrombin synthesis in the presence of vit K, synthesis of plasma proteins, formation of bile salts takes place in the liver.

Excretory

Liver excretes bile pigments, drugs, metals and dyes (BSP).

Storage

Liver stores glycogen, vit A, D, B_{12} and iron.

Detoxification

Inactivation of hormones, drugs, toxic substances occurs in the liver.

Destruction of blood cells

The Kupffer cells and macrophages in the liver destroy blood cells.

Formation of blood cells

In the early foetal stage, liver is the site of hemopoiesis.

Jaundice

It is the yellow colouration of skin and mucous membrane, due to increased bile pigments level in plasma. This condition occurs, when bilirubin level in the plasma exceeds 2 mg%. Depending on the cause, three types of jaundice can occur (Table 11.1). They are:

Hemolytic (prehepatic)

Hepatic

Obstructive (posthepatic)

The **hemolytic jaundice** is caused by increased destruction of red cells, arising from intrinsic and extrinsic defects in RBCs.

Hepatic jaundice occurs, due to hepatitis caused by virus. There are several forms of this type, i.e. Hepatitis A, B, C, etc.

Obstructive type is caused by gallstones in the bile ducts and tumors of biliary tree.

Liver function tests

There are several tests to assess the functional integrity of the liver and to diagnose the type of jaundice produced. The commonly conducted clinical tests are as follows:

BSP excretion test

In liver disease, the dye excretion after 45 minutes, fails to clear 90% of the level from plasma.

Table 11.1: Comparison of the three types of jaundice

	Hemolytic (prehepatic)	*Hepatic*	*Obstructive (posthepatic)*
Cause	Intrinsic and extrinsic defects causing large scale destruction of RBC's	Diseases of liver and hepatitis (viral)	Obstruction to biliary passage from gallstones, tumors
Serum bilirubin (0.5 to 2 mg%)	Increased	High	Very high
Type of bilirubin	Unconjugated	Both	Conjugated
van den Bergh's reaction	Indirect or delayed +	Biphasic	Direct
Urine bilirubin	Nil	Present (+)	More in the urine (+++)
Urobilinogen *(0-4 mg/day)*	Increased	Decreased	Decreased or absent
Fecal stercobilinogen *(40-300 mg)*	Increased	Decreased	Decreased or absent
Prothrombin time	Normal	Increased	Increased
Alkaline phosphatase	Normal	Increased	Increased
SGOT and SGPT	Normal	Markedly increased	Increased

van den Bergh's reaction

In this test, the type of jaundice can be identified.

Estimation of transaminases and isocitrate dehydrogenase

In liver disease the SGOT, SGPT and isocitrate dehydrogenase levels in plasma are increased. The damaged liver cells liberate these enzymes.

Prothrombin time

In liver disease, it is elevated.

Estimation of serum bilirubin

The level is increased in liver damage.

Galactose tolerance test

The level of galactose in plasma, after its infusion remains elevated in liver damage.

In clinical laboratory, liver scanning and biopsy are also done to detect the extent of degeneration.

GI MOTILITY

Deglutition

It refers to the act of swallowing. It consists of oral, pharyngeal and oesophageal stages. The first stage is voluntary, second and third stages are involuntary and reflex in nature.

Oral stage

It is voluntary. The food is masticated by mixing with the saliva. The solid food is converted into a soft bolus and positioned on the dorsum of the tongue to be pushed to the oropharynx. In the oral stage, the bolus passes through the oral cavity towards the pharynx, assisted by the tongue pressing against the hard palate.

Pharyngeal stage

The presence of food at the entry of pharynx stimulates the receptors in the fauces of tonsil, and epiglottis which initiate the reflex. This stage is involuntary. The afferent and efferent impulses are carried by V, IX , X and XII cranial nerves. The center is the swallowing center, situated in the medulla and lower pons. The second stage of swallowing is important, as the reflex mechanisms allow the bolus to enter the esophagus and not the other openings. The exit of bolus from the mouth is prevented by its closure and the tongue pressing against the hard palate, creating high resistance. The entry of bolus into the nasopharynx is prevented by the soft palate raising and pressing against the posterior pharyngeal wall. The entry of bolus into the trachea is avoided by the upward and forward movement of larynx, which causes the glottis approximating with epiglottis. This seals entry into the larynx. The vocal cords also approximate, inhibiting speech. Respiration stops during this phase of swallowing. With the reflex mechanisms preventing entry into these openings, the bolus is directed to enter esophagus through the pharynx.

Esophageal stage

The bolus enters the esophagus from pharynx through the upper esophageal sphincter and the entry into the stomach occurs through the lower esophageal sphincter. The upper esophageal sphincter is supplied by the vagus and the distention of the sphincter, causes the inhibition of vagus and relaxation of sphincter. The distention of upper esophageal sphincter initiates a wave of peristalsis, which spread along the length of esophagus, pushing the bolus forwards. These are the **primary peristaltic waves**, traveling at the speed of 3 to 4 cm/sec. The force of gravity helps in faster rate of movement of bolus. Liquids travel faster than solids to reach the stomach. When the bolus reaches the lower esophageal sphincter (cardiac sphincter), it relaxes and allows the bolus to enter the stomach. After allowing the food entry into the stomach, the lower esophageal sphincter closes and prevents regurgitation of food into the esophagus. The lower esophageal sphincter is regulated by noncholinergic and nonadrenergic nerve endings coming from the intrinsic myenteric plexus. The transmitter that is released here is **VIP or NO.** Lower esophageal sphincter(LES) contracts by acetylcholine released from vagal endings and relaxes by VIP and NO. If the primary peristalsis does not completely empty the esophagus, then one or more **secondary peristalsis** arises from the distal part of esophagus, due to distention and empty the food from esophagus.

Disorders of swallowing

Dysphagia is difficulty in swallowing.

Achalasia

It is the difficulty in emptying the food from esophagus into the stomach, due to absence of peristalsis in the lower third of esophagus and failure of lower esophageal sphincter to relax. It has been shown that in achalasia, the **VIP** secreting neurons of **Auerbach's plexus,** supplying the lower esophageal sphincter degenerate, causing distention of lower part of esophagus. There is dysphagia present for the solid food. When lower esophageal sphincter does not contract and close the opening with the stomach as in LES incompetence, regurgitation of gastric contents into esophagus occurs to produce heart burn and esophagitis.

Gastric motility

The stomach shows fundus, body, antrum and pylorus as anatomical divisions (Fig. 11.9). The wall of the stomach has intrinsic enteric nervous system, which consists of myenteric (**Auerbach's plexus)** (between outer longitudinal and inner circular layers of smooth muscles) and **Meissner's** or submucous plexus (between circular smooth muscle and mucous layer). The

motility is initiated by this intrinsic nerve plexus and the stimulus comes from the distention of the wall by food. The activity of the intrinsic nerve plexus can be modified by the extrinsic nervous system, which consists of parasympathetic (vagus) and sympathetic divisions. The parasympathetic stimulation causes, increased motility, while the sympathetic stimulation, inhibits motility.

Functions of stomach

- *Storage organ:* Stomach can store large quantities of food as it shows receptive relaxation.
- Beginning of protein digestion by the pepsin enzyme occurs here.
- Makes the food into a chyme by propulsive, mixing and retropulsive movements
- The acid in the stomach has bactericidal effect
- The gastric secretion has intrinsic factor, which is necessary for the absorption of vit B_{12}.

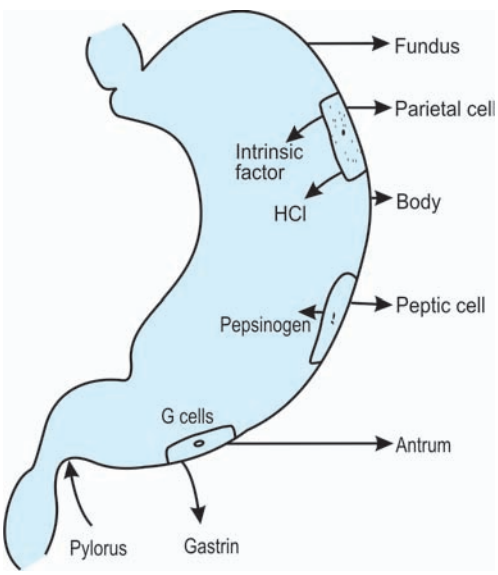


Fig. 11.9: Regions of stomach showing secretion of HCl, intrinsic factor, pepsinogen and gastrin

- The acid in the stomach converts cane sugar to fructose and glucose
- The acid chyme in the duodenum facilitates absorption of iron.

Movements of stomach

Receptive relaxation

The stomach shows **receptive relaxation**, accommodating large volume of food. The receptors for this are present in the wall of pharynx and esophagus.

The function of fundus and body of the stomach is to store the food (storage function). The afferent and efferent impulses for receptive relaxation are carried by the vagus (**vagovagal reflex)** and causes the myenteric plexus to secrete **VIP**. This transmitter causes relaxation of the wall of the stomach. Vagotomy decreases the receptive relaxation, though, not completely abolishes, because, the intrinsic nerve plexus is responsible for the receptive relaxation.

Mixing of food (digestive peristalsis)

The distal part of the stomach shows digestive peristalsis. The distention of the wall of the distal part of body and antrum stimulates the intrinsic plexus. The smooth muscle in the wall, shows slow waves, which are nonpropagatory depolarization waves. They are also called **basic electrical rhythm (BER).** The distention of the wall or the activity of vagus causes development of trains or spikes on the peak of slow waves. They are action potentials, developed, when the slow waves reach the threshold level of firing. The entry of Na^+ and Ca^{++} into the cell causes depolarization. Once the action potential spikes are developed, it becomes propagatory in the form of peristalsis (Fig. 11.10). Vagal stimulation, acetylcholine, gastrin, cause development of spikes or action potentials on the peak of slow waves, which results in peristalsis.

The digestive peristalsis travel towards the pylorus, pushing the food forwards. The peristalsis is the wave of contraction followed by

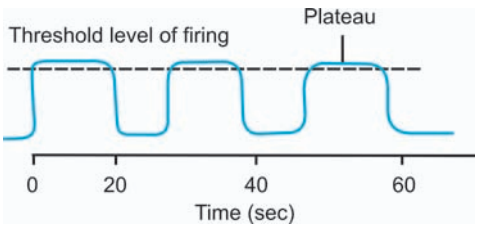
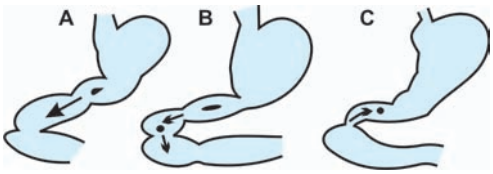


Fig. 11.10: Slow wave potentials recorded from the stomach. The action potential is developed in the depolarizaton phase of the slow waves, when the membrane potential reaches the threshold level. The action potential superimposes on the slow wave. Note the long plateau in the action potential



Figs 11.11A to C: Sequence of events in the stomach during digestive peristalsis

A: Peristalsis moves towards pylorus(propulsion)

B: Mixing of food and mechanical digestion occurs in the pylorus. A small quantity squirts into the duodenum.

C: Retropulsion of food as the pyloric sphincter closes

relaxation. The frequency of digestive peristalsis in the stomach is 3 to 5/min (20 sec rhythm). The food when reaches the pylorus is retropulsed into the antrum, due to the pyloric sphincter closure. The sphincter closes as the peristalsis arrives at the pylorus. This is necessary to prevent the entry of food into the duodenum without thorough mixing and forming acid chyme. The **propulsion, mixing** and **retropulsion** in the pylorus breaks down the food into smaller particles (**chyme**) and helps thorough mixing with the gastric juice (Fig. 11.11). Each time the peristalsis arrives at the pylorus, only 2 to 3 ml of chyme is emptied into the duodenum.

Gastric emptying

Gastroduodenal pressure cycle

Gastric emptying occurs mainly due to the gastroduodenal pressure cycle caused by the digestive peristalsis of stomach. As said earlier, the digestive peristalsis coming from the distal part of the stomach when reaches the pylorus, results in the rise of duodenal bulb pressure. The pyloric sphincter closes and allows the food to be retropulsed. The peristalsis allows only about 2 ml of chyme to enter the dudodenum before the closure of pyloric sphincter. The transit time for the gastric emptying of food is 3 to 4 hrs.

Factors influencing emptying

Consistency, chemical composition, tonicity, volume of food, pH of gastric contents, etc. influence emptying.

Solids leave the stomach later than liquids. The carbohydrates can be emptied faster than proteins and the fat is emptied last. Increase in acid pH inhibits emptying. Isotonic fluids leave the stomach earlier, than either hypertonic or hypotonic fluids.

Duodenal factors influencing gastric emptying

Enterogastric reflex

Presence of **fat, acid and hyperosmolar solutions** in the duodenum inhibits gastric emptying. The inhibition is mediated by both neural and hormonal mechanisms. The neural mechanism involves inhibition of vagus (**enterogastric reflex**) and the hormonal mechanism includes the release of **secretin, CCK, GIP**. These hormones are called **enterogastrones**. They cause inhibition of gastric motility and gastric secretion.

Vomiting

Vomiting is a protective reflex, which helps to relieve the gastric and intestinal contents. The reflex occurs, if there is any irritation of gastro-intestinal tract especially duodenum or its over distension. The vomiting center is situated in the medulla, which integrates the reflex. The afferent impulses are carried in the X cranial nerve and sympathetics, while, the efferents are carried in the V, VII, IX, X and XII cranial nerves. Vomiting

also occurs, due to other stimuli coming from genitourinary tract, psychic stimuli, touching the pharyngeal mucosa, stimulation of vestibular organs (motion sickness) pregnancy (morning sickness), tumors of brain, poisons, severe pain and certain chemicals. Drugs like apomorphine directly acts on the area close to the vomiting center and causes vomiting. This region or area postrema of the brain stem is called **chemo-receptor trigger zone.** Vomiting produced by the direct stimulation of this center is called central vomiting.

Symptoms like nausea, increased salivation, rapid heart rate, sweating, dilatation of pupil, and retching are noticed earlier to vomiting. **Retching** refers to the involuntary contractions that precede vomiting. The vomitus is not expelled, because the abdominal and thoracic pressures are insufficient to open the upper esophageal sphincter.

During vomiting, the contents of the upper part of small intestine are regurgitated into the stomach or the contents of the stomach itself can cause its distention. The abdominal muscles contract, which increase the intra-abdomonal pressure. This forces the gastric contents to reflux into the esophagus. A deep inspiration is taken, which brings the contents from the lower esophagus to the upper esophageal sphincter. The upper esophageal sphincter relaxes, glottis closes, respiration stops and the soft palate raises to expel the vomitus through the mouth.

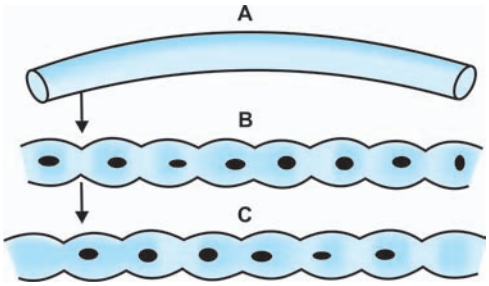
Though vomiting is a protective reflex to relieve the effect of the toxins and other irritants in the GI tract, prolonged vomiting, from any cause can result in loss of gastric acid, leading to metabolic alkalosis. This disturbs the fluid and acid base regulations.

Small intestine motility

Small intestine includes three fourths of GI tract and it consists of **duodenum, jejunum** and **ileum**. The movements that are seen here are as follows:
Rhythmic segmental contractions
Peristaltic contractions
Pendular contractions
Villi movements.

Segmental peristalsis

The frequently occurring movement in the small intestine is segmental contractions (Fig. 11.12). It occurs in a small segment, due to the contractions of circular smooth muscle. The

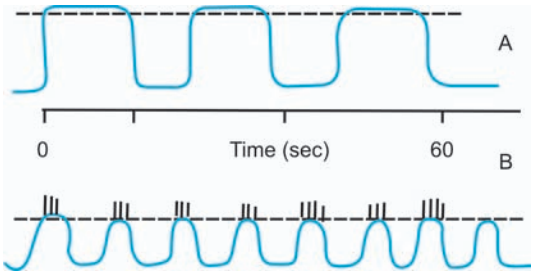


Figs 11.12A to C: Segmental peristalsis in the small intestine

contraction is a result of **slow waves (BER)** developed in the wall of the intestine. As described earlier, the slow wave is formed due to the intrinsic nerve plexus. The frequency and amplitude of the slow wave is increased by the **parasympathetic stimulation, acetylcholine, gastrin, CCK** and **motilin.** Sympathetic stimulation, secretin and glucagon inhibit the slow wave development. The segmental contractions result, when slow wave depolarisation reaches the threshold level.

The slow waves show a gradient in its frequency, with the highest rate at the duodenum (12/min) and lowest at the ileum (8/min) (Fig. 11.13). This facilitates the bolus to be propelled aborally (**law of intestine).**

The segmental contractions involve ring like regular constrictions along the length of a segment of intestine. The constricted part later relaxes and relaxed part constricts. This process is repeated over and again. This will cause the bolus to move back and forth within the lumen of the intestine. The functions of segmental contractions in the small intestine are as follows:



Figs 11.13A and B: Slow waves in the stomach (A) and small intestine (B). The dashed lines show the threshold level of firing. The action potentials in the case of small intestine is in the form of spikes appearing at the peak of slow waves. The frequency of slow waves in the small intestine is more than stomach. In this figure the slow waves from the ileum is shown. In the small intestine itself, there is a gradient in the frequency of slow wave potentials, with the duodenum showing the highest (12/min) and lowest in the ileum (8/min)

- The bolus mixes well with the digestive enzymes and facilitates the completion of digestion.
- The segmental contractions also cause exposure of the digested food to the villi surface for absorption.
- Finally, the occurrence of segmental contractions in the proximal segment and inhibition in the distal segment, facilitates propulsion of bolus towards colon.

The small intestine shows propulsive peristalsis only for a few cms of length and it rarely occurs. It is not significant in small intestine, as segmental movements carry out the propulsive function.

Sometimes the longitudinal muscle contractions give pendular movements, which facilitate mixing of bolus with the digestive enzymes. The intestinal villi shows forward and backward movements, due to the contractionsof the smooth muscle in the villi.The hormone villikinin stimulates the villi movements and they facilitate absorption of digested food.

MMC (migrating motor complex)

It is the inter digestive peristalsis occurring in the stomach and small intestine. It occurs between meals at every 70 to 90 min time intervals. Each peristalsis lasts for 10 min.It is developed, due to the activity of intrinsic myenteric nervous system. It helps in sweeping the contents of the stomach and small intestine towards colon during interdigestive periods. The peptide hormone **motilin** secreted from the duodenal mucosa, is the hormonal candidate responsible for MMC.

Functions of small intestine

- Contains pancreatic, bile and intestinal secretions
- Completion of digestion and absorption of digested food occur in small intestine
- Presence of villi, facilitates absorption of digested food
- Upper part of small intestinal mucosa secretes GI hormones like secretin, CCK, VIP, glucagon, motilin, etc.
- Regulate GI secretion and motility
- Peyer's patches in the ileum are a lymphoid organ which helps in immunity.

Gastroileal reflex

Distention of stomach by the food, causes relaxation of ileocaecal sphincter and allows emptying of ileal contents into the caecum, which is the first part of colon. Distention of ileum also will cause relaxation of ileocaecal sphincter and ileal emptying. The distention of caecum on the other hand, results in the contraction of the sphincter and prevents reflux of caecal contents into the ileum. The activity of the ileocaecal sphincter is controlled by the myenteric plexus.

Large intestine

It includes caecum, ascending colon, transverse colon, sigmoid colon, rectum and anus. The mucosa secretes mucus, but there is no villi in it. Hence, there is no absorption of food and also no digestive enzymes are present in the colon. However, the colon, has some important functions. They are:

Functions of large intestine

- Absorption of water and electrolytes
- Formation of feces
- Secretion of mucus to lubricate feces
- The bacterial flora synthesise B group vitamins and vit K.
- Folic acid and short chain fatty acids produced by bacteria are absorbed significantly in the colon

Motility of large intestine

The large intestine shows:

Haustral shuttling (segmental contractions)
Peristalsis
Mass peristalsis.

The wall of the colon has enteric nervous system. It has both excitatory and inhibitory effects on the smooth muscle. But the overall **neural effect is inhibitory on colon**. If enteric nervous system is destroyed in colon, the segments remain contracted, due to the increase in colonic tone **(e.g., Hirschsprung's disease).** The extrinsic nervous system to the colon has both parasympathetic and sympathetic controls. The parasympathetic innervation of proximal colon is by the vagus and the distal colon is innervated by the pelvic nerve. The stimulation of parasympathetic nerves to the colon increases the motility. The sympathetic nerves to the colon cause inhibition of motility.

Haustral contractions

It is similar to small intestine segmental contractions. In large intestine, the longitudinal muscle gives three bands forming **teniae coli.** The enteric nerve plexus below this region is greater. The region adjacent to the teniae coli has a thin wall and hence, when segemental contractions occur, it gives rise to sac like or pouches along the length of segment of colon. They are called **haustra.** The back and forth movements, cause the chyme to be exposed for absorption of water and electrolytes. Out of 1500 ml of fluid chyme that enters the colon per day, all but 50 to 100 ml are absorbed in the colon. The haustral shuttling also faciltate propulsive movement of feces to the distal colon. The transit time in the colon is very slow, that is 5 to 10 cm/ hr.

The digestive peristalsis also sometimes occurs in the colon, but is insignificant in the propulsion of feces.

Mass peristalsis

It occurs in a large segment of colon and these contractions are powerful enough to cause, the colon to be in a contracted state for a long period of time. The intrinsic myenteric plexus activity is responsible for mass peristalsis. The mass peristalsis sweeps the feces along the long segment of colon. That is, from the ascending colon to the transverse colon and from transverse colon to the sigmoid colon the feces is propelled. The mass peristalsis occurs 3 to 4 times in a day and it usually leads to defecation. The arrival of feces into the rectum by the mass peristalsis gives the desire to defecate. The gastrocolic reflex forms the stimulus for initiating mass peristalsis. The distention of stomach by the food, stimulates the intrinisic myenteric plexus to initiate mass peristalsis. The vagal activity and the release of gastrin and CCK are said to be responsible for gastrocolic reflex.

Defecation

It is a reflex process and the lowest center is in the sacral segments of spinal cord (S2, S3 and S4). The higher centers are in the pons, hypothalamus and cerebral cortex. The act of defecation involves social and cultural factors and hence the inhibition from higher centers should be removed for defecation reflex to occur. The distal segments of colon are innervated by the parasympathetic pelvic nerves. It also supplies the internal anal sphincter. The pudendal nerve which is a somatic nerve supplies the external anal sphincter. It is a striated muscle and is under voluntary control.

During defecation reflex, the rectum receives the feces from the sigmoid colon. The activity of

pelvic nerve is responsible for the motor activity in distal colon, rectum and relaxation of internal anal sphincter. The distention of the wall of the rectum, stimulates the stretch receptors and sends afferent impulses, through the pelvic nerves to the spinal cord. If the central inhibition is not present, the internal anal sphincter is inhibited, causing it to relax. The pudendal nerve is also inhibited, which relaxes the external anal sphincter. The contraction of abdominal muscles, caused by straining efforts and descent of diaphragm, increases the intra abdominal pressure, which augments the defecation reflex.

DIGESTION AND ABSORPTION OF FOOD

Carbohydrates

In man, 50 to 60% of diet contains carbohydrates, which includes starch, sucrose and lactose. The daily intake of carbohydrate in humans varies from 250 to 800 g. The major form is starch, which includes, both straight chain (amylose) and branched chains (amylopectin). The cellulose in the vegetables is not digested and cannot be absorbed in humans. It is excreted in the feces unused.

Digestion of starch

Starch is a polysaccharide. It is hydrolysed by salivary amylase (ptyalin) and pancreatic amylase enzymes. Both hydrolyze the 1:4, α linkages in the starch molecule and convert it to oligosaccharides namely maltose (disaccharide) maltotriose (tri saccharide) and α limit dextrins. Further digestion of these oligosaccharides takes place in the brush border of villi of the small intestine. The α-dextrinase, hydrolyze α-dextrins, while, maltase acts on maltose and maltotriose to give 2 molecules of glucose.

Digestion of Sucrose and Lactose

Both of them are disaccharides and they are hydrolyzed by disaccharidases, sucrase and lactase present in the brush border of the villi of the small intestinal mucosa. Sucrase, acts on sucrose and converts it to fructose and glucose, while lactase acts on lactose and converts it to glucose and galactose.

The end products of digestion of starch, sucrose and lactose are monosaccharides.

Absorption of glucose and galactose

The transport of glucose across the intestinal mucosal cell requires a carrier protein **SGLT 1,** which carries both Na^+ and glucose (symport or cotransport). It is a **secondary active transport**. The active transport of Na^+ across the lateral intercellular surface supplies energy for the glucose entry into the cell and also creates concentration gradient for Na^+ to move into the cell. If Na^+ entry is facilitated, then glucose also is transported into the cell. The transport does not depend on insulin presence and is similar to the reabsorption in PCT of kidney. Transport from the cell into the interstitial fluid occurs, through the lateral intercellular border and the carrier protein involved is **GLUT 2.**

Galactose

Galactose absorption is similar to glucose. Deficiency or absence of cotransporter in the mucosal surface of the villi, leads to mal-absorption of glucose and galactose.

Fructose absorption

Fructose absorption is different from glucose and galactose in the sense that it is independent of Na^+ absorption. The transport across the brush border of villi, occurs by facilitated diffusion with the help of transporter **GLUT 5**. From the cell to the lateral intercellular space, the transport is helped by the transporter **GLUT 2**. Some amount of fructose is converted into glucose in the mucosal cell itself.

The absorption of pentoses shows simple diffusion.

Deficiency of disaccharidases enzymes results in diarrhea, increased gas formation (flatulence) and bloating of abdomen after ingestion of sugar. Lactase enzyme activity is greater at birth and

decreases as the age advances. In adulthood, low lactase levels are reported and are responsible for the lactose intolerance. Deficiency of lactase in infants also can cause lactose intolerance and the symptoms described for the lack of disaccharidases are observed.

Digestion and absorption of proteins

Proteins for digestion come from two sources. One is from the diet and the other is from the digestive secretions and desquamated epithelial cells. The daily requirement of proteins in adult is 0.5 to 0.7 g/kg of body wt. In children (1 to 3 years), the requirement is more, i.e. 4 g/kg of body wt.

The digestion of protein begins in the stomach. The enzyme **pepsin** hydrolyzes proteins to peptones and peptides. It is secreted as inactive pepsinogen and activated by HCl at pH 1 to 2. In the duodenum, the alkaline pH inhibits the action of pepsin.

In small intestine, the pancreatic juice contains important proteolytic enzymes. The pancreatic exocrine secretion contains **trypsin chymotrypsin, carboxypeptidases** and **elastase**. Trypsin is secreted as trypsinogen and is activated by enterokinase. The trypsin which is formed, itself can activate trypsinogen, chymotrypsinogen and procarboxypeptidase. These proteolytic enzymes hydrolyze proteins and give peptides.

The peptides that are formed, undergo further digestion in the small intestinal cells brush border surface. The enzymes involved are **tripeptidases** and **dipeptidases**. They hydrolyze the tripeptides and dipeptides respectively and convert them to amino acids. The cytosol of the cell also contains peptidases. Those peptides, that enter the cell without hydrolysis at the brush border surface, undergo digestion by the cytosolic peptidases and form amino acids. The peptides that have more than 3 peptides are called polypeptides. They are poorly absorbed in the intestinal mucosal cell.

Absorption of amino acids

The amino acids that are formed at the brush border surface of the villi by the hydrolytic action of peptidases, show three types of transporters, one each for basic, neutral and acidic amino acids. They are transported together with Na^+ as cotransport. The entry into the cell occurs by secondary active transport, similar to glucose. The amino acids from cytosol to the basolateral space are transported by facilitated diffusion.

Digestion and absorption of fat

Dietary intake of fat consists of triglycerides, sterols, sterol esters and phospholipids. The daily intake of fat in man ranges from 25 to 150 g.

Lipids are absorbed by passive diffusion, but they should be made water soluble to enter the cell.

Digestion of lipids

There are three lipolytic enzymes involved in the digestion of fat. They are present in the pancreatic secretion. These enzymes are:

Pancreatic lipase
Cholesterol esterase
Phospholipase

The pancreatic lipase cleaves fatty acids from triglycerides, from 1 and 1' positions and leaves 2 monoglycerides.

Cholesterol esterase cleaves fatty acids from cholesterol ester and gives free cholesterol.

Phospholipase cleave fatty acids from phospholipids.

The lipase enzyme is water soluble and to act on the lipids, it requires the assistance of bile salts. The bile acids and lecithin, first of all **emulsify fat**, which gives smaller molecules of lipids. This provides large surface area for the enzyme action. The combination of bile salts with the lipid, makes the lipase enzyme hydrolyze the lipid much easier, as the bile salts show **hydrotropic effect**. The lipase alone, when present is inactivated by bile salts. To avoid this another enzyme **colipase** from pancreatic secretion combines with the lipase. The products of triglyceride digestion are fatty acids and 2 monoglycerides. They combine with the bile salts

and form micelles with a diameter of 5 nm. The interior of micelles contains the hydrophobic chains such as 2 monoglycerides, fatty acids and lysophosphatides, while, the polar water soluble ends face the exterior. The fat soluble vitamins and cholesterol, are also present in the interior of micelles. The micelles are formed, only if bile salts concentration in the duodenum is above the critical level.

Absorption of digested fat

The long chain fatty acids and monoglycerides are resynthesized into triglycerides after absorption.

Absorption of fat involves simple diffusion, but to make it water soluble, the digested products of fat form micelles, by combining with bile salts. The micelles transports fatty acids, 2 monoglyceride, cholesterol, fat soluble vitamins etc across the brush border of the villi and after their transport, bile salts come back to the lumen of the small intestine. Bile salts are absorbed from the terminal ileum by a Na^+ dependent active transport.

In the cytosol of the intestinal mucosal cell, the absorbed products of fat digestion are reconstituted in the smooth endoplasmic reticulum as follows:

- Two monoglycerides combine with fatty acids and form triglyceride
- Lysophosphatides combine with fatty acids and form phospholipid
- The combination of cholesterol with fatty acids give cholesterol ester.

The reconstituted triglyceride, phospholipid and cholesterol ester, coalesce to form **chylomicrons** within the smooth endoplasmic reticulum. These are small lipid droplets with 1 nm size. The chylomicrons leave the cell by exocytosis. Before the exit, the chylomicron is covered by a β-lipoprotein (apoprotein) coating. Without this covering by the apoprotein, exocytosis of chylomicron cannot occur from the cell. After exit from the cell, chylomicrons join to form a large droplet, with the size varying from 50 to 500 nm. The large droplet enters the lacteal of the villi and then through the lymphatic circulation enters the systemic circulation.

The *short chain fatty acids* (containing less than 10 carbon atoms) are relatively more soluble in water and hence they diffuse into the mucosal cell of the small intestine. From the cell, they enter the liver through the portal vessel.

Malabsorption of fat, usually comes from the deficiency of pancreatic lipase and insufficiency of liver to produce bile acids. In malabsorption of fat, the feces contains more fat (steatorrhea) and smells foul. In tropical sprue, the intestinal epithelial cells are flattened, causing the number of villi to become reduced. This leads to malabsorption of fat, due to the decreased surface area available.

Absorption of water and electrolytes

Daily intake of water in man is 1.5 lit and to this about 7.5 lit of GI secretions are added in the GI tract. The excretion of water in the feces is only 100 to 200 ml and the remaining 8.9 lit of water is absorbed in a day from the GI tract.

Water is absorbed passively, due to the osmotic gradient. The movement of water usually follows the active absorption of solutes like electrolytes and nutrients, as it creates osmotic gradient. The movement of water will go on, until osmotic equilibrium is reached. That is why, the intestinal fluid is always kept iso osmotic to plasma.

Water absorption can be seen in stomach, small intestine and colon. Maximum absorption occurs in the jejunum (5.5 L/day), next comes the ileum and last is the colon (1.3 L/day).

NaCl absorption

Sodium is abosorbed from the intestinal luminal cells by facilitated diffusion as cotransport with glucose or aminoacids. Sodium from the intestinal cells is transported into the lateral intercellular space by active transport involving the carrier Na^+- K^+ ATPase.

Chloride moves passively from the lumen into the cell following the electrochemical gradient. There is also cotransport of Na^+ and Cl^-.

Chloride secretion

Chloride is secreted from the epithelial cells of crypts of Lieberkuhn. Chloride enters the intestinal epithelial cells from the interstitial fluid by Na^+- K^+ - 2 Cl^- Cotransporter. From enterocytes the chloride enters the lumen via Cl^- channels regulated by cAMP.

Cl^- and HCO_3^- absorption

In jejunum, both Cl^- and HCO_3^- are absorbed in greater amounts, whereas, in ileum, Cl^- is absorbed and HCO_3^- is secreted. In the colon, similar process occurs for Cl^- and HCO_3^-. In severe diarrhea, HCO_3^- is lost and this can lead to metabolic acidosis.

Absorption of vitamins

Fat soluble vitamins A, D, E and K form micelles with the bile salts and absorb like any other lipids from the proximal part of the small intestine.

Water soluble vitamins namely vitamin C, B group vitamins are transported across the mucosal cell by Na^+ dependent active transport. Vit B_{12} is absorbed from the terminal ileum, after forming a complex with the intrinsic factor. Folate and Vit B_{12} absorption is Na^+ dependent.

Absorption of Ca^{++}

The absorption of calcium occurs to maintain its balance. About 25 to 80% of the ingested dietary calcium is absorbed. The absorption is through the membrane bound carrier, which is activated by vit D. The active form of vit D, 1, 25 DHCC (calcitriol), induces the formation of calcium carrier at the luminal surface of the intestinal epithelial cell and facilitates Ca^{++} absorption. From the cell into the lateral intercellular space, Ca^{++} is transported by the active transport involving Na^+-Ca^{++}exchange system or Ca^{++} ATPase carrier. Presence of phosphates, oxalates, fatty acids inhibit Ca^{++} absorption from the lumen of the intestine, as they form water insoluble compounds with Ca^{++}. Mg^{++} is transported similar to calcium.

Absorption of iron

Iron is absorbed from duodenum and jejunum. It is absorbed either as free form or as haem. Fe^{++} is absorbed more efficiently than Fe^{+++}. Vitamin C causes reduction of ferric to ferrous and promotes iron absorption. The acid from the gastric juice, prevents the formation of insoluble complexes and facilitates absorption.

There are four steps involved in the iron absorption.

1. Transport across the luminal surface of the small intestine by a specific carrier protein.
2. Binding of apoferritin by the iron in the cell and formation of ferritin.
3. Dissociation of iron from ferritin and binding to another protein at the basolateral surface. The iron is transported out of the cell to the capillaries.
4. Binding to β-globulin (transferrin) in the plasma and transported in the circulation.

The daily intake of iron is 15 to 25 mg and 0.5 to 1mg is absorbed in the adult male and 1 to 1.5 mg is absorbed in reproductive women. Hemorrhage leads to iron loss and hence, iron absorption from the intestine is increased in such conditions. Growing children and pregnant women also show increased iron absorption from the intestine.

ENERGY BALANCE

Energy balance refers to the equilibrium between the caloric input and output. The caloric input to the body comes from the oxidation of food substances namely carbohydrate, protein and fat, while, the energy output forms the energy expended for the mechanical work of the muscle, synthetic reactions, excitation of nerve and muscle, transport of substances, secretory activity, etc. The energy expended is expressed as joules or kilocalories/gram. Normally the caloric input

is equal to the energy output in our body. If the caloric input is greater than the energy output, a positive balance is obtained, leading to the weight gain or obesity. On the other hand, if the caloric input is lesser than the output, then a negative balance occurs. In such states, the body stores of glycogen, fat and protein are utilized for the energy production, leading to weight loss in the body. The amount of energy liberated depends on the type and the relative amount of food ingested. Although the respiratory quotient varies for carbohydrate, protein and fat, an approximate value as 4.82 kilocal of energy production for 1 liter of oxygen consumed has been considered .

Basal metabolic rate

The amount of energy liberated per unit of time is taken as the metabolic rate. In the resting conditions (basal state), its measurement forms the **resting metabolic rate** or basal metabolic rate. The energy output under basal conditions in an adult is 2,500 kcal per day. Out of this, 75% is due to the BMR, 8% of the energy release comes from the **dietary thermogenesis** (energy release from the digestive and metabolic processes) and 17-18% is due to the physical activity. The energy output also includes the **non-shivering thermogenesis**, (heat production in cold) which is negligible under basal states. As said earlier, the energy output is due to the combustion of food that we ingest. The carbohydrates form the major source of energy production. The metabolism shows the anaerobic and aerobic cytoplasmic glycolysis, leading to the formation of pyruvate. If pyruvate is formed anaerobically from 1 molecule of glycogen, 4 molecules of ATP are formed, but the net gain is only 3 mole of ATP. On the other hand if pyruvate is formed anaerobically from 1 molecule of blood glucose, 2 molecules of ATP are produced. The glycolysis, if occurs, through the trioses, it is known as Emden Meyerhof pathway, whereas the pathway through 6-phosphogluconate and pentoses give HMP (hexose monophosphate shunt). The energy production through the glycolysis forms only 8% of the total energy production. In the continued absence of oxygen the pyruvate is converted to lactate, which also is utilised for the energy production through its conversion to glycogen in the liver. The aerobic glycolysis and TCA give more ATP molecules per molecule of blood glucose, which is 38 in number. The major energy liberation comes from the oxidation of acetyl CoA, which is formed from pyruvate. This is the Kreb's cycle, which occurs in the mitochondria.

Fatty acids when oxidised provide greater amount of energy than the carbohydrates and proteins. The fatty acids undergo β-oxidation in the mitochondria, which gives acetyl CoA. This enters the TCA cycle and produces ATP molecules. When the production of acetyl CoA becomes greater, then the extra acetyl CoA enters the liver for ketoacids formation. These compounds also provide energy when oxidised in the other tissues.

Proteins at first are hydrolysed to amino acids, which give rise to intermediates of TCA cycle, through different pathways. The formation of acetyl CoA from these intermediates, leads to the TCA cycle, producing energy.

Measurement of basal metabolic rate

There are two ways of measuring the energy output under basal conditions, which include the direct and the indirect methods. In the direct method, the measurement of heat liberated by using a bomb calorimeter reflects the energy output (direct calorimetry). In the indirect method, the energy output is measured by estimating the amount of oxygen consumed per unit time. Since the energy production depends on the combustion of food, the oxygen consumption will be equal to the energy liberation. The subject reports after 12 hours of gap from the last meal taken. The subject should be kept in a basal state without any physical and mental activities. The apparatus used is Benedict Roth spirometer, which has a provision for measuring the oxygen consumed and to absorb the carbon dioxide released. The subject is asked to breath from the spirometer through the mouth piece for 6 minutes. The amount of oxygen consumed is measured from the spirometer. The value is converted for

1 hour. From this the caloric equivalent of oxygen per liter is calculated. The value obtained is expressed in relation to the body surface area, height and weight by using the Dubois and Dubois formula.

$S = 0.0007184 \times W^{0.425} \times H^{0.725}$

S = Surface area m^2

W = Body weight in Kg

H = Height in cms

The surface area can also be calculated from the nomogram.

Normal value of BMR is 40 kcal/m^2/hour. ± 15% variation is considered normal.

Physiological variations of BMR

Age: In children the BMR is higher due to the growth activity. As the age advances the value declines. In old age the BMR is low as the lean body mass is less in the old age.

Sex: In females the BMR is low as compared to men due to the female sex hormones and less lean body mass.

Ambient temperature: Environmental temperature is quite important when measuring the BMR. Fall in the ambient temperature (cold) will cause shivering and increase heat production, raising the metabolic rate. The rise in ambient temperature, more than the body temperature also will cause rise in BMR, due to the stimulation of metabolism. Hence the BMR should be measured in the neutral zone temperature (25 to 27°C).

Sleep: During sleep the BMR is reduced by 10 to 15%.

Body composition: Increase in surface area increases the BMR. If the fat content is more in the body, then the BMR is less, as the lean body mass is decreased.

Anxiety: Increase in BMR occurs due to the adrenaline secretion from the adrenal medulla.

Prolonged starvation: Decrease in BMR can be seen.

Circulating hormones of thyroxine and adrenaline: Increase in BMR occurs, whenever these hormones are secreted in more quantities, as they have calorigenic action.

Pregnancy, muscular exercise and fever: show increase in BMR as the metabolism is accelerated in these conditions.

Self-study Questions

Multiple Choice Questions

Choose the single best answer

1. **Receptive relaxation of stomach facilitates:**
   A. Mixing **B.** Storage
   C. Propulsion **D.** Emptying

2. **Presence of acid in the duodenum stimulates the secretion of:**
   A. Gastrin
   B. Secretin
   C. CCK
   D. VIP

3. **Gastric emptying rate increases with the increase in:**
   A. Fat in the duodenum
   B. Intraduodenal volume
   C. Intragastric volume
   D. Acid in duodenum

4. **Acid pH in the duodenum causes:**
   A. Decrease in gastric motility
   B. Increase in gastric acid secretion
   C. Decrease in pancreatic bicarbonate secretion
   D. Increase in intestinal motility

5. **If terminal ileum is removed:**
   A. Bile and fat content in the feces will increase
   B. Iron absorption decreases
   C. Vitamin B_{12} absorption decreases
   D. A and C

6. **Gastric acid secretion is stimulated by all of the following *except*:**
   A. Acetylcholine
   B. Norepinephrine
   C. Histamine
   D. Gastrin

7. **Internal anal sphincter can be relaxed when there is distension of:**
   A. Stomach **B.** Distal colon
   C. Rectum **D.** Proximal colon

8. **Gallbladder contraction is stimulated by:**
   A. Secretin
   B. Fat in duodenum
   C. Acid in duodenum
   D. Hyperosmolality of duodenum

9. **Vagal stimulation produces the least effect on:**
   A. Saliva
   B. Gastric secretion
   C. Pancreatic secretion
   D. Bile

10. **Mucus in the GI tract is secreted by all of the following *except:***
    A. Surface epithelial cells of stomach
    B. Brunner's gland
    C. Goblet cells
    D. Enterochromaffin cells

ANSWER KEYS

1. (B) 2. (B) 3. (C) 4. (A) 5. (D) 6. (B) 7. (C) 8. (B) 9. (A) 10. (D)

Short Answer Questions

1. list the factors that decrease acid mucosal barrier.
2. Explain the significance of parietal cell function in the gastric gland.
3. Explain the mechanism of action of any two agents that inhibit gastric acid secretion.
4. Describe the regulation of pancreatic secretion.
5. State the importance of cholesterol and lecithin in the bile.
6. Describe the functional importance of gastrin and secretin hormones.
7. Describe enterogastric reflex.
8. Describe segmental peristalsis .
9. Describe gastrocolic and gastroileal reflexes.
10. Describe how the digested fat is absorbed.

12 Endocrine System

GENERAL CONSIDERATIONS

Endocrine glands are ductless glands. Their secretions are directly released into the circulation, producing their effects on a distant target organ. The term hormone has been given to the chemical substances secreted from endocrine glands. In addition to hormones, there are also other types of secretions namely:

Neurocrine: The substance is secreted from the axonal end into the blood stream, forming **neural hormones.**

Paracrine: The secretion shows the effects of the hormone on the adjacent cells.

Autocrine: Cell's own secretion acts on it, producing actions similar to the hormone.

Hormones are involved in the chemical communication of tissues, which helps in the metabolic regulation. This is not the only function of hormones as believed earlier. They are also responsible for growth, maturation, development, reproduction and response to environmental stimuli in the form of behavior.

Nervous system and endocrine system are the two important regulatory systems in our body to maintain the steady state or homeostasis. Both respond to stimuli, but the rate at which it occurs differs. The nervous system responds by faster nerve signals to give appropriate motor response in the form of behavior. Endocrines also respond to stimuli by chemical secretion, but the response is much slower, as it is achieved through metabolic regulation. This cannot be strictly said for all endocrines, because, adrenal medullary response to the environmental stimuli is quicker and dependent on the nervous system activity. Thus, nervous system and endocrine system respond to stimuli from internal and external environments and their activities help to integrate the functions of various organs, thereby, maintaining the constancy of the internal environment. In so many ways, both the systems are interrelated to each other in fulfilling this objective. There are endocrine glands like adrenal medulla, which depends on the nervous regulation for its activity and there are nerve cells in hypothalamus, which depend on hormones for their function.

Chemical nature of hormones

Hormones are of three types chemically (Tables 12.1 and 12.3). They are proteins, polypeptides amines and steroids. Their chemical nature determines their half life, solubility in plasma, transport and mode of action.

Synthesis and secretion of protein hormones

Protein hormones are water soluble and hence they are carried in unbound form in plasma. Examples of protein, peptides and glycoprotein

Table 12.1: Chemical nature of hormones

Polypeptides	*Glycoproteins*	*Amines*	*Steroids*
Growth hormone	TSH	Norepinephrine	Cortisol
Prolactin	FSH	Epinephrine	Aldosterone
ACTH	LH	T3	Estradiol
Hypophysial hormones	HCG	T4	Testosterone
Parathyroid hormone			Progesterone
Calcitonin			1,25, Dihydroxycholecalciferol
Insulin			
Glucagon			
Somatostatin			
Vasopressin			
Oxytocin			

Table 12.2: Half life of hormones in the circulation

Steroids	*Proteins/ Polypeptides*	*Thyroid hormones*
Cortisol 70 to 100 min	ADH, Oxytocin <1 min	T3 = 24 hrs
		T4 = 7days
		Amines
Aldosterone = 30 min	Insulin = 7 min	
	ACTH = 15 to 25 min	NE = 15 sec
	Prolactin = 12 min	E = 10 sec

The shortest half life is present for epinephrine, while thyroxine has the longest half life in the circulation.

hormones are listed separately. The synthesis of these hormones is little different from proteins synthesized for intracellular activity. Protein hormones are synthesized as a large precursor molecule called **preprohormone** in the endoplasmic reticulum. Cleavage into prohormone from preprohormone occurs in the endoplasmic reticulum. The prohormones are transferred to Golgi apparatus for packaging into secretory vesicles. Within the secretory vesicle, another cleavage occurs, separating the hormone from prohormone. Insulin is synthesized as prohormone with 3 peptide chains. Cleavage in the secretory vesicle gives insulin proper with 2 peptide chains. Sometimes, a large prohormone complex such as pro-opiomelanocortin secreted from the anterior pituitary and adrenal medulla gives several peptide hormones on cleavage, such as, ACTH, MSH, lipotropin, endorphins and enkephalins.

The secretion of protein hormones involves exocytosis, requiring Ca^{++} entry and expenditure of energy. The secretion also requires cytoskeleton (microtubules and microfilaments) for the transport of secretory vesicle to the cell membrane.

Half life of protein hormones

The half life of protein hormones is short, because they are mostly carried in the plasma in unbound form. Protein hormones, peptide hormones and catecholamines are water soluble and in the dissolved form in plasma they are transported to the target tissues, where they diffuse out from capillaries. Some protein hormones like HCG (human chorionic gonadotrophin) appear in the urine in an active form, as they are not bound to the plasma proteins.

Protein hormones cannot be administered orally, as they are hydrolyzed by the digestive enzymes. Hence they have to be given through the parenteral route for therapeutic purposes.

Receptors for protein hormones are present on the surface of the cell membrane.

Half life of hormones: The time at which the hormone's 50% of the activity is lost in the circulation (Table 12.2).

Table 12.3: Physiological characteristics of hormones

Characteristics	*Protein hormone/polypeptide*	*Amines*	*Steroids*
Synthesis	As prehormone or prohormone	From tyrosine amino acid	From cholesterol
Storage	Stored as membrane bound granules	Stored in membrane bound granules	Not stored
Solubility and transport in plasma	Soluble and unbound	Bound and unbound	Protein bound
Half life	Short	T3-1 day, T4-7 days	Short
Receptor location	Cell membrane	T3-nucleus Catecholamines- cell membrane	Cytoplasm and nucleus
Route of administration	Cannot be given orally	Thyroid hormone can be given orally. Catecholamines, through parenteral route.	Can be given orally

Synthesis and secretion of amines

They include **thyroid hormones** and **catecholamines**. Both are derived from the amino acid tyrosine and they are also known as phenolic derivatives.

Thyroid hormones are T4 (thyroxine) and T3 (triiodothyronine). These hormones are synthesized outside the secretory cell in the thyroglobulin, present in the lumen of the gland. The synthesized hormone is also stored here. The hormones are lipid soluble and hence they are bound to the plasma proteins (thyroid binding globulin) and transported. Because of their protein binding, they have a long half life (T4 has 7 days, while, T3 has 1 day only). Receptors for these hormones are present in the nucleus. Thyroid hormones can be orally administered, as they are absorbed intact in the intestine.

Catecholamines are epinephrine and norepinephrine secreted from adrenal medulla. They are stored in the membrane bound granules in the secretory cell. Catecholamines exist in both bound and free form. Their half life is very short. (epinephrine has 10 sec and norepinephrine has 15 sec) and hence their oral administration is ineffective, even though, they can be absorbed intact. Receptors for catecholamines are present on the cell surface.

Steroid synthesis and secretion

Steroids are synthesized from cholesterol and they have cyclopentanoperhydrophenanthrene ring Examples of steroid hormones are given in the list. Steroid hormones are not stored in the secretory cell. They are lipid soluble and hence are bound to plasma protein globulin and transported. Their half life is in minutes (aldosterone has 30 min and cortisol shows 90 to 100 min). Receptors for steroids are present intracellularly in the cytoplasm and nucleus. They are readily absorbed intact from the GI tract and hence they can be given orally.

Mechanism of action of hormones

Hormones acting on the membrane receptors

Receptors for hormones belonging to proteins, peptides and catecholamines are situated on the cell surface. These receptors are membrane proteins and specific to the ligands. Their number is regulated in relation to the hormone concentration. If the hormone level is high in the plasma, the receptor number decreases **(down regulation)** and when the level of hormone is low, the receptor number increases **(up regulation)**. The membrane receptors are reduced in number

Table 12.4: Mechanism of hormone action

cAMP	*IP_3*	*Transcription and Translation*	*cGMP*	*Tyrosine kinase*
ACTH	GnRH	Cortisol	ANP	Insulin
LH & FSH	TRH	Estrogen	NO	IGF-I
ADH (V_2)	GHRH	Progesterone		
HCG	Angiotensin II	Aldosterone		
MSH	ADH (V_1)	Vitamin D_3		
CRH	Oxytocin	Thyroxine		
β_1 β_2 Adrenergic receptors	α_1 Adrenergic receptor			
Calcitonin				
PTH				
Glucagon				

by *internalization*. The endocytosis process brings the receptors from the membrane into the cytoplasm, where the receptor protein is degraded and internalized.

Receptor diseases

The actions of hormones depend not only on the specific receptors, but also, on their functional status. Sometimes, the receptors will be present on the target tissue, and if it is resistant to the hormone action, the effects of hormone deficiency occur, even though the hormone secretion is normal. In some other cases, the receptors are present, but occupied by the antibodies produced by the immune system to its own receptor protein. Examples for this type are myasthenia gravis, Graves' disease, diabetes mellitus, etc.

Second messengers (Table 12.4)

Hormones which have receptors on the cell surface bind to it and form hormone-receptor complex. This initiates intracellular production of compounds called second messengers, through which the hormone effects are mediated. The hormone themselves will be the first messengers. The second messenger systems are:

- cAMP and cGMP.
- Ca^{++} - Calmodulin system
- IP_3
- Tyrosine kinase system.

Adenylyl cyclase system

It consists of three membrane proteins namely the receptor, G protein and the catalytic unit of adenylyl cyclase. The hormone receptor complex stimulates G stimulatory protein (Gs), which catalyzes GTP to GDP. This reaction stimulates adenyl cyclase enzyme, which converts ATP into cAMP. If the hormone receptor complex gets attached to Gi protein, then the adenylyl cyclase enzyme is inhibited and cAMP is not formed.

Cyclic AMP is the second messenger and it activates protein kinase A (Fig. 1.14). This enzyme causes phosphorylation of protein, leading to formation of new proteins and enzymes. The physiological effect of the hormone is brought by these new proteins. The cAMP is inactivated by phosphodiesterase, which converts cAMP to 5' AMP. This inactivation can be inhibited by caffeine and theophylline, as these agents inhibit phosphodiesterase. Hence administration of these agents potentiates the action of cAMP.

Phosphatidyl inositol system (IP_3)

Hormone receptor complex acts on the G protein Gq and activates **phospholipase C** (PLC) enzyme. It causes breakdown of phosphotidyl inositol diphosphate (PIP_2) to inositol triphosphate (IP3) and diacyl glycerol (DAG). The IP_3 is a second messenger, which causes mobilization of Ca^{++}

from the intracellular stores such as endoplasmic reticulum and mitochondria. The Ca^{++} that is released acts as second messenger. It binds to the calcium binding protein calmodulin and the activation of which gives the physiological response. The DAG activates calcium dependent **protein kinase C**, which causes phosphorylation of protein, leading to the formation of new enzymes (see Fig. 1.15).

Calcium-calmodulin system

Hormone receptor complex opens up Ca^{++} channels, and allows more entry of calcium into the cell. The intracellular Ca^{++} level is also increased by IP_3. The calcium binding protein calmodulin takes up 4 molecules of Ca^{++} and alters the enzyme activity (see Fig. 1.16).

Tyrosine kinase system

Insulin, IGF_I, EGF, PGDF, have receptors with α and β subunits. The α is on the exterior of the cell membrane and contains the binding site. The β subunit passes through the membrane and contains enzyme tyrosine kinase on the cytoplasmic side. The hormone receptor complex activates this enzyme causing phosphorylation of intracellular protein, which regulates the enzyme activity.

Transcription and translation effects

Steroids and thyroid hormones act through this mechanism (Table 12.4). Steroids have receptors in the cytosol or in the nuclear membrane, whereas, the thyroid hormone has receptor in the nucleus. The hormone receptor complex modulates the transcription in the nuclear chromatin to cause *mRNA* synthesis and secretion. The specific mRNA enters the cytoplasm and directs the synthesis (translation) of specific proteins (see Fig. 1.17). The new proteins and enzymes give the physiological effect of the hormone.

Circadian rhythm

Endocrine secretion shows diurnal variation as a result of sleep-wake cycle, environmental and internal stimuli. The hormones showing the rhythms are ACTH, corticosteroids, growth hormone and melatonin.

Regulation of secretion

Regulation of secretion shows feedback mechanism caused by the free level of hormones in the circulation. Regulation is also observed by the blood levels of substances, which are produced by the hormone itself. The feedback regulation includes negative feedback, which causes inhibition of secretion. Negative feedback occurs in response to the increased blood level of hormones.

Measurement of hormones

Since hormones are secreted in minute quantities like nanogram (ng) and picogram (pg), the measurement in the body fluids should be accurate. Radioimmuno assay (RIA) is the method which is sensitive to measure even small quantity and this method is commonly employed to measure hormone levels in the blood.

Radioimmuno assay

Plasma is taken and mixed with the antibody specific to the hormone that is being measured. To this, purified hormone tagged with radioactive isotope is added. The natural hormone in the plasma and hormone tagged with the isotope compete with each other for binding to antibody. The hormone antibody complex is separated and the quantity of radioactive hormone bound to antibody can be known by using radioactive counters. If a large amount of radioactive hormone is bound to the antibody, it gives a low plasma concentration of the natural hormone. Likewise, if a small amount of radioactive hormone is bound to the antibody, then the natural hormone level

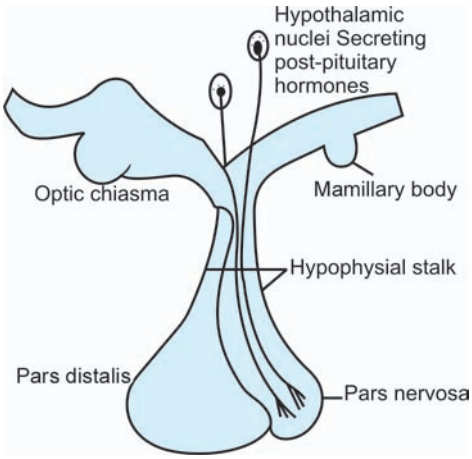


Fig. 12.1: Structure of pituitary gland

in the plasma will be high. The ratio of bound to free form will give the concentration of the natural hormone in the plasma.

Pituitary gland

It is situated at the base of the brain and attached to the hypothalamus through a stalk. It consists of anterior lobe and posterior lobe in humans (Fig. 12.1). But in lower animals, in addition, there is an intermediate lobe present.

ANTERIOR PITUITARY (ADENOHYPOPHYSIS)

It includes pars distalis, pars tuberalis and pars intermedia. The pars distalis contains a glandular structure, which shows secretory cells. With immunocytochemical techniques, the different types of secretory cells have been identified and they are:

Somatotrophs	(50%)
Lactotrophs	(10 - 30%)
Corticotrophs	(10%)
Thyrotrophs	(5%)
Gonadotrophs	(20%)

These cells secrete anterior pituitary hormones namely, somatotrophic hormone, prolactin, adrenocorticotrophic hormone (ACTH), thyroid stimulating hormone (TSH) and gonadotrophins (FSH and LH). The somatotrophic hormone, prolactin and ACTH are peptides, while the TSH and gonadotrophins are glycoproteins.

Control of anterior pituitary secretion

The secretion from the anterior pituitary gland is regulated by the hypothalamic releasing and inhibitory hormones. The median eminence of hypothalamus receives superior hypophysial artery, which breaks up into capillary network (Fig. 12.2). These regions are outside the blood brain barrier. The capillaries join to form a portal vessel and run through pars tuberalis to reach pars distalis. Here, the portal vessel opens into sinusoids lining the anterior pituitary cells. The hypophysial portal vessel carries the secretion from the median eminence of hypothalamus and releases into the anterior pituitary gland. These releasing hormones are peptides and released in episodic bursts due to the intrinsic activity of neural oscillator of nerve cells that produce the releasing hormones. The actions of hypothalamic releasing and inhibitory hormones are:

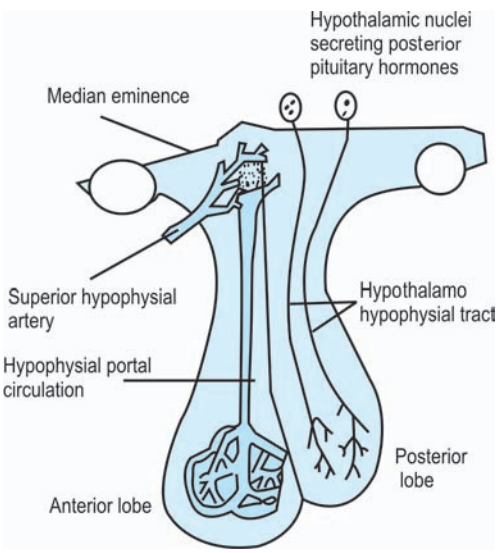


Fig. 12.2: Pituitary gland and its regulation

The anterior lobe shows hypophysial portal circulation, through which the regulation of its hormone secretion occurs. The posterior lobe receives hypothalamo hypophysial nerve tract, which transports posterior pituitary hormones from hypothalamic nuclei

Table 12.5: Actions of hypothalamic releasing and inhibitory hormones on the anterior pituitary

Releasing hormone	*Actions*
CRH	+ ACTH
Growth hormone releasing hormone	+ Growth hormone
Somatostatin	– Growth hormone and – TSH
TRH	+ TSH
GnRH	+ FSH and LH
PIF (dopamine)	– Prolactin
PRF	+ Prolactin

+= stimulation ; – = inhibition.

- They act on the secretory cells and increase their size.
- They either stimulate or inhibit secretion (Table 12.5).
- The releasing hormones also promote increased synthesis of anterior pituitary hormones by acting at the translational level.
- They can modify the biological action of anterior pituitary hormones by acting at the post translational level (the addition or removal of chemical groups to the molecule can increase or decrease the activity of the hormone).

Hypothalamus receives multiple inputs from various regions of CNS, carrying information from visual and somatic regions. Hypothalamic nuclei such as supraoptic, paraventricular and arcuate send their axons to the median eminence. The posterior pituitary hormones secreted from supraoptic and paraventricular nuclei also reach median eminence. The arcuate nucleus secretion (GnRH) is influenced by norepinephrine secreting neurons which stimulate it, while, dopamine and endorphins inhibit its secretion. The median eminence sends its own secretion to the hypophysial portal circulation and as well as the secretions received from other hypothalmic nuclei, as described above.

Regulation of anterior pituitary gland secretion by feedback mechanisms

There are three feedback regulatory loops namely: long loop feedback, short loop feedback and ultra short loop feedback, which help in the regulation of anterior pituitary secretion.

Long loop feedback: This mechanism is mostly inhibitory in nature. The increased blood level of target gland hormones causes inhibition of anterior pituitary gland and hypothalamic releasing hormone secretion.

Short loop feedback: Trophic hormones from anterior pituitary gland reaches hypothalamus and inhibit the secretion of releasing hormones.

Ultra short loop feedback: The hypothalamic releasing hormone secretion is inhibited by its own secretion.

Hormones of anterior pituitary

Adrenocorticotrophic hormone (ACTH)

It is secreted from a prohormone **pro-opiomelanocortin (POMC)**. This is secreted from anterior pituitary gland and adrenal medulla. It gives rise to β **LPH, ACTH, β endorphin, MSH** and **enkephalins** on cleavage. The corticotrophs of anterior pituitary gland are basophilic and secrete ACTH. It is a 39 amino acids peptide hormone. The first 13 amino acids are identical to MSH (melanocyte stimulating hormone). That is why, darkening of skin occurs whenever ACTH secretion is elevated, e.g. Addison's disease, Cushing syndrome caused by the tumor of ACTH secreting cells. In human beings, MSH secretion does not occur, as ACTH molecule itself contains the MSH moiety.

Actions

ACTH stimulates the growth and steroidogenesis of adrenal cortex. The mechanism of action is mediated through **cAMP**.

The secretion of ACTH shows circadian rhythm, which is parallel to the cortisol secretion. The peak level of secretion occurs in the morning (8 am) and 8 pm samples gives the lowest level.

Regulation of secretion

Increased ACTH secretion is seen during stress. Immune products like cytokines also stimulate its secretion. ACTH secretion is inhibited by cortisol and opioids.

Thyroid stimulating hormone (TSH)

It is a large glycoprotein hormone and regulates the growth and metabolism of thyroid gland. It stimulates the synthesis and secretion of thyroid hormones. These effects are mediated by the second messenger cAMP. TSH secretion is regulated by TRH. Fasting, cold, stimulates TRH secretion. Increased level of TRH causes down regulation of TRH receptors in thyrotrophs.

Gonadotrophins

It includes **FSH** and **LH**. Both are glycoproteins. During pregnancy human chorionic gonadotrophin **(HCG)** is secreted which is also a glycoprotein. These hormones have α and β subunits and the hormone action resides in the β subunit. FSH and LH are secreted in a cyclical pattern in reproductive women and in men they are secreted in a continuous pattern. The functions of these gonadotrophins include the growth and development of gonads, gametogenesis, secretion of sex hormones from gonads. The gonadotrophins secretion is regulated by GnRH produced from the arcuate nucleus and preoptic area of hypothalamus.

Prolactin

It is a protein hormone having 199 amino acids. Its half life in the circulation is 20 minutes and it circulates in unbound form in plasma. Prolactin is secreted by the acidophil cells (lactotrophs) of anterior pituitary. The basal secretion of this hormone in both male and female is similar. The chief action of prolactin is lactation, which involves mamogenesis, lactogenesis and galactopoiesis. The maternal behaviour observed in animals is due to this hormone. Prolactin causes inhibition of GnRH and hence during lactation, menstrual cycle is absent. Excess secretion of prolactin gives metabolic effects of growth hormone and produces glucose intolerance and hyperinsulinemia.

Prolactin secretion is regulated by the hormone PIF. It is dopamine and during nursing and breast stimulation, PIF is inhibited. Thus, the secretion of prolactin occurs by a neuroendocrine reflex. The secretion also occurs during stress fear, arousal and excitement. The estrogen hormone and TRH stimulate prolactin secretion. Administration of dopamine antagonists stimulates prolactin secretion and dopamine agonists inhibit it. Hyperprolactinemia can occur due to the tumor of lactotrophs or drugs interfering with dopamine secretion. In women, this condition causes oligomenorrhea or amenorrhea and infertility. In men, loss of libido and infertility occurs. Tumors of lactotrophs in men rarely produce galactorrhea.

Growth hormone (somatotrophin)

It is a protein hormone with 191 amino acids and its molecular weight is 22,000. The structure is similar to human placental lactogen and prolactin. These two hormones show the metabolic effects of growth hormone, without the growth promoting effect. The synthesis of growth hormone is under the influence of growth hormone releasing hormone. Since it is a protein hormone, species specificity will be present. Hence, replacement therapy of growth hormone depends on the hormone synthesized by using recombinant genetic engineering technique.

Actions of growth hormone

The promotion of growth and metabolic effects are the two important actions of growth hormone.

The growth hormone has a half life of 6 to 20 min in the circulation. The hormone shows diurnal rhythm in its secretion, which is related to sleep-wake cycle and not to light-dark cycle. The peak level of secretion is noted 1 –2 hr after the onset of deep sleep. The measurement of hormone level in the plasma is of little value clinically, as the levels show wide variation in the pulsatile secretion and presence of diurnal rhythm. The measurement of IGF_I in plasma is useful in knowing the growth hormone activity in humans.

Actions of growth hormone

1. The effect of growth hormone on growth, cartilage, protein metabolism depends on the growth factor IGF- I in adults
2. On bone it ↑ chondrogenesis, ↑ width of epiphysial plates at the ends of long bones, ↑ bone matrix, ↑ linear growth of bone
3. ↑ Growth of soft tissues (↑ visceral growth) by ↑ protein synthesis
4. Causes hyperglycemic effect by ↓peripheral utilization of glucose, ↑ glucose output from the liver (gluconeogenesis) and anti-insulin effect in the skeletal muscle.

Metabolic effects

Protein anabolic effect

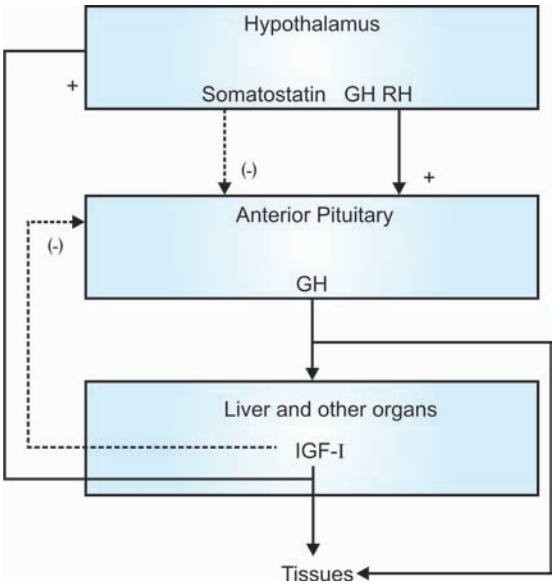
It is a protein anabolic hormone, showing positive nitrogen balance. Growth hormone increases transport of amino acids into the cell. The increased protein synthesis is due to the hormone influence in the transcription and translation mechanisms in the cell.

Lipolytic effect

It is a lipolytic hormone as it causes mobilization of fat from adipose tissue. The FFAs are taken up by the skeletal muscle and liver, where they undergo oxidation. Excess secretion of growth hormone can be ketogenic.

Hyperglycemic effect

Growth hormone has hyperglycemic action, due to decreased uptake and utilization of glucose in the skeletal muscle and adipose tissue. There is also increased output of glucose from the liver by gluconeogenesis. Growth hormone antagonises the action of insulin at the post receptor levels in the skeletal muscle and liver. It reduces insulin sensitivity and in excess secretion, it can become diabetogenic.



Regulation of growth hormone secretion

Growth hormone stimulates secretion of IGF-I from liver and the tissues. IFG-I stimulates secretion of somatostatin from hypothalamus. Somatostatin inhibits growth hormones secretion from anterior pituitary. IFG-I can directly act on the anterior pituitary and inhibits GH secretion

Regulation of growth hormone secretion

Growth hormone stimulates secretion of IGF-I from liver and other tissues. IGF-I stimulates secretion of somatostatin from hypothalamus. Somatostatin inhibits Growth hormone secretion from anterior pituitary. IGF-I can directly act on the anterior pituitary and inhibit GH secretion

Growth effects

The growth promoting effects are due to growth factor IGF- I (insulin growth factor), produced from liver and other tissues. In adult the IGF- I is significant and in fetal stage, the IGF- II causes growth effects. The IGFs are similar to insulin in structure. Earlier they were known as somatomedins, because of their action on the skeleton. But now we know that they also have their effects on the metabolism and soft tissue growth. The secretion of IGF- I is regulated by the

growth hormone and insulin is necessary for the growth hormone to produce IGF- I. The secretion of IGF- I is increased during puberty. IGF- I acts on the hypothalamus and releases somatostatin releasing hormone, which leads to the inhibition of pituitary growth hormone secretion.

Bone growth

IGF- I increases linear growth of bones by acting on epiphysial cartilage. The width of epiphysial plate is increased which helps in the linear growth. Increased bone matrix synthesis increased osteocytic activity and increased chondrogenesis are the other effects of IGF-I.

Visceral growth

The growth of viscera is due to the anabolic effect of growth hormone. The soft tissue growth also depends on IGF- I. There are interrelationships between insulin, growth hormone and IGF- I secretion. The presence of increased amino acids, glucose stimulates growth hormone and insulin secretions. Both growth hormone and insulin, cause secretion of IGF- I, which brings about growth. Hence, for the normal growth, the diet should be rich in protein and carbohydrates. The IGF- I influences transcription at the genetic level in the nucleus and translation at the endoplasmic reticulum for promoting protein synthesis.

Regulation of growth hormone secretion

Hyposecretion of growth hormone

Hyposecretion of growth hormone in children produces **Dwarfism**

Can occur due to deficiency of GHRH, GH, IGF-I or other causes.

Short stature is a characteristic feature

If GH secretion is deficient it is called **pituitary dwarfism**

If GH secretion is normal, but GH receptor in the target tissues is insensitive, it is known as **Laron dwarfism**

In African pygmy, the IGF-I secretion is deficient at the time of puberty but GH secretion is normal

Hypersecretion of growth hormone

Acromegaly

Hypersecretion of growth hormone after the closure of epiphyses produces acromegaly.

Causes: Tumor of acidophilic cells of anterior pituitary

Linear growth of bone is not possible and hence disproportionate growth of bones and soft tissues occur

Enlargement of hands and feet, ↑ growth of nose, ears and frontal bones, and protrusion of lower jaw (prognathism) occur

↑ Visceral growth,

Bitemporal hemianopia

Hypersecretion of growth hormone before the epiphysial closure causes **Gigantism**

↑ Linear growth of bones produces ↑ height.

↑ Visceral growth, Glucose intolerance

Growth hormone secretion is regulated by a number of factors. Secretion is stimulated by amino acids, stress, slow wave sleep, exercise, dopamine agonists, GHRH and TRH Ghrelin. The secretion of growth hormone is inhibited by somatostatin, dopamine antagonists and hyperglycemia IGF-I, REM sleep, glucose, Cortisol and FFA. Growth hormone has a feedback control mechanism. Growth hormone causes release of IGF- I . IGF- I acts on the hypothalamus and stimulates the secretion of somatostatin. Somatostatin directly acts on the anterior pituitary and inhibits growth hormone secretion.

Disorders of growth hormone secretion

The usual causes of growth hormone dysfunction are *hypothalamic diseases, pituitary tumor, failure of IGF secretion and receptor disease.*

Dwarfism

Hyposecretion of growth hormone before the onset of puberty results in dwarfism. This is called **pituitary dwarfism.** The disease is characterized

by stunted skeletal growth giving a short stature. In **Laron type dwarfism**, the growth hormone level is normal and the defect is due to a genetic disorder in the receptor expression. In **African pygmy,** the growth hormone secretion is normal, but the IGF rise from increased growth hormone will not be present at the time of puberty.

Gigantism

Hypersecretion of growth hormone before the fusion of epiphysis of long bones causes gigantism. It is usually due to a pituitary tumor. The subject grows to a height of 8 feet or more. There is glucose intolerance, hyperinsulinism and finally leads to diabetes mellitus. The viscera increase in size and there is also cardiac hypertrophy. Susceptibility to infections also is increased in this disorder.

Acromegaly

Excess secretion of growth hormone in adults after the epiphyses of long bones have fused with the shaft leads to acromegaly. The hand and feet are enlarged. The visceral growth is increased. The growth of nose, ears and frontal bones are increased. The enlargement and protrusion of mandible is called *prognathism.* The excessive bone and cartilage growth can produce carpal tunnel syndrome and joint problems. People with gigantism can also get acromegaly, if the condition is not corrected before puberty.

Hypopituitarism

Panhypopituitarism is commonly seen with infarction of pituitary gland. The example for this is postpartum necrosis **(Sheehan's syndrome).** The target glands are affected as the trophic hormones are deficient. *Hypothyroidism, hypogonadism, hypoadrenalismic growth disorders, low BMR, hypoglycemia, low blood volume, fall in blood pressure, poor resistance to stress are some of the symptoms of panhypopituitarism.*

Physiology of growth

Role of other hormones

Growth is a complex process which involves the action of several hormones, in addition to the growth hormone. Growth refers to the increase in the linear growth and size. It is not the increase in weight that can be called growth. The action of growth hormone on growth promotion requires the presence of thyroxine, insulin and cortisol. The sex hormones androgens and estrogens interact with growth hormone in promoting growth and development of sex organs.

Growth pattern when plotted against age, shows two spurts. The first spurt during infancy (2 years) and second one, at the time of puberty. If comparison is made between sexes, it will be seen that the pubertal spurt occurs earlier in girls than boys (Fig. 12.3).

Thyroxine

Thyroxine has a permissive effect on growth hormone action. It is likely that thyroxine enhances the secretion of IGF-I. In

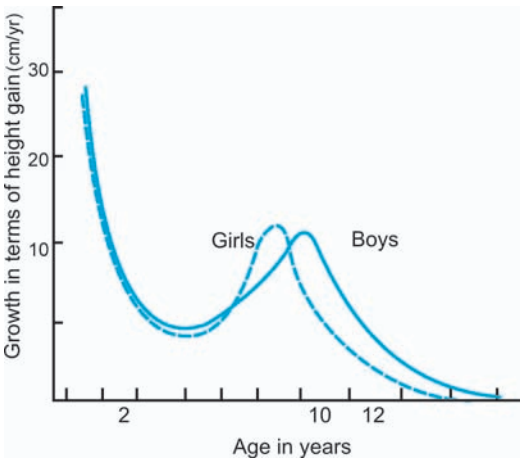


Fig. 12.3: Growth pattern in boys and girls

Two spurts in growth can be observed. First during infancy and second during puberty. Pubertal growth occurs earlier in girls than boys

hypothyroidism, the response of growth hormone to hypoglycemia is not present. This shows that thyroxine may be necessary for the normal rate of growth hormone secretion, although the basal secretion is not affected in the absence of thyroxine. Thyroxine has a role in the growth and development of brain, ossification of cartilage, growth of teeth, contours of face and proportions of the body. Hypothyroid children show infantile features with stunted skeletal growth and they are called cretins with mental retardation.

Factors promoting normal growth

Normal rate of secretion of growth hormone and normal levels of IGF I

Adequate diet with proteins, vitamins and calories.

Absence of chronic diseases

Normal secretion of thyroxine, insulin, cortisol, and sex steroids.

Absence of genetic disorders

Normal psychosocial milieu.

Insulin

It is a growth hormone. Growth hormone increases IGF with parallel rise in insulin. If insulin is deficient, increased IGF to growth hormone is not present. It is commonly observed that diabetic young adults (juvenile diabetics) are short statured. Insulin is also an anabolic hormone, as it causes increased protein synthesis.

Cortisol

Adrenocortical steroids' role in growth is only in providing the milieu for proper growth. That is, it maintains water balance and blood pressure. The excess secretion of glucocorticoids shows catabolic effect, with a negative nitrogen balance and retards growth.

Sex steroids

Androgens and estrogens have direct effect on the growth and development of sex organs. At the time of puberty, their secretion is increased, which is responsible for the growth of reproductive organs. The sex hormones probably produce growth by the increased growth hormone and IGF secretion. The androgens have anabolic effect and promote protein synthesis. Sex steroids directly act on the bone and cause fusion of epiphysis, leading to stoppage of linear growth. That is why, sexual precocity gives short stature, while hypogonadism gives rise to tall individuals.

Growth factors

Growth factors are peptides in nature and promote the growth of soft tissues and skeleton. Some are also involved in the regulation of the activity of immune cells and macrophages.

IGF (insulin-like growth factor)

There are two types of insulin like growth factors. They are IGF I and IGF II. The IGF I is secreted in adults and IGF II is present in the fetus. IGF shows endocrine, paracrine and autocrine actions depending on the place of secretion. Though liver is the chief site of production of IGF, other tissues also secrete this growth factor. IGF I acts through tyrosine kinase receptors on the membrane. It stimulates the uptake of glucose and amino acids into the cell. Inside the cell, it promotes the protein synthesis. On the bone, it causes chondrogenesis, stimulates osteoblasts replication, increases bone matrix and collagen formation. The rise of IGF by the growth hormone runs parallel to the insulin secretion.

PDGF (platelet derived growth factor)

It is a polypeptide secreted from many tissues. Earlier it was believed to be secreted from platelets. The main action of this growth factor is cell proliferation necessary, for wound healing. This is achieved by inducing fibroblast growth. It also acts a chemotaxic agent for macrophages.

FGF (fibroblast growth factor)

It is secreted from bone and other tissues. It has a mitogenic action on fibroblasts and increases

collagen synthesis. It is also a mitogen for vascular endothelial cells and helps in new vascular connections (neovascularization) during fetal growth and wound healing.

EGF (epidermal growth factor)

It is originally detected from mouse salivary glands. Now it is known that it is produced from other tissues. It stimulates the proliferation of epidermal and epithelial cells.

NGF (nerve growth factor)

Similar to EGF, NGF also is secreted from salivary glands and other tissues. It is necessary for promoting growth of sympathetic neurons and facilitate synapse formation from sensory neurons. Its overall effect is in CNS neuronal growth and synapse formation.

TGF (tumor growth factor)

It is present in normal and neoplastic tissues. It is similar to EGF. It simulates and inhibits cell proliferation depending on the local conditions in the tissue. It also stimulates growth of osteoblasts and fibroblasts.

Inhibins and activins

They are synthesized from ovaries, testes, pituitary and other tissues as well. On the pituitary gland, inhibins inhibit FSH secretion, while activins stimulate it. On the gonads, inhibins stimulate steroid synthesis and activins produce its inhibition. Both of them belong to the family of growth factors.

POSTERIOR PITUITARY (NEUROHYPOPHYSIS)

It includes pars nervosa and infundibular stalk. The pars nervosa contains glial cells called pituicytes. Rich capillary network with fenestrations is present in the gland. The blood supply is from inferior hypophyseal artery. The posterior pituitary receives axons from supraoptic and paraventricular nuclei (Fig. 12.4). There are

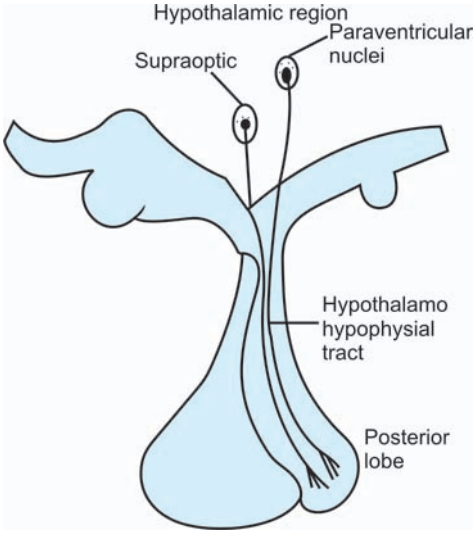


Fig. 12.4: Posterior pituitary with its hypothalamic neural connection. The hormones of posterior pituitary are synthesized in the supraoptic and paraventricular nuclei. They are transported along the axons of the hypothalamohypophyseal tract and reach the posterior pituitary for release into the circulation

two peptide hormones secreted from these nuclei, namely **vasopressin (ADH) and oxytocin**. The magnocellular part of these two nuclei secretes both the hormones. The parvocellular cell sends its axons to median eminence, where ADH influences CRH secretion during stress.

Synthesis

These two peptide hormones are synthesized as prohormone and it contains a segment called neurophysin. Vasopressin prohormone contains neurophysin II and oxytocin prohormone contains neurophysin I. Cleavage of prohormone into the hormone and neurophysin occurs during transport along the axons to the posterior pituitary. The prohormone together with the neurophysins is packaged into a granule and then transported along the axon. When these granules are stained, they appear as **Herring's bodies**. The cleavaged hormone proper diffuses into the sinusoidal capillaries and enters the circulation.

Both the hormones are nonapeptides (9 amino acids) and only two amino acids are different between them. That is why, there is overlapping of activity between vasopressin and oxytocin.

Vasopressin (antidiuretic hormone)

In humans arginine vasopressin is secreted and it is transported in plasma unbound. Its half life in circulation is 15 to 20 minutes.

Actions

Reduces free water clearance in kidney

ADH acts on the distal segments, collecting ducts of nephron and causes water and urea permeability of the cells. The action is mediated by cAMP. The change in permeability results in decrease in urine flow as water is reabsorbed. The urine osmolality reaches upto 1200 milli osmoles/kg water. This action of vasopressin on the kidney is mediated through V_2 receptors. Absence of vasopressin results in increased urine flow, decreased osmolality and increased free water clearance of kidney.

Actions of vasopressin (ADH)

1. Vasopressin acts on the collecting ducts of kidney and increases permeability of the tubular cells to water. Increased reabsorption of water in collecting ducts produces a concentrated urine.
2. The action of ADH on the CD cells is mediated by V_2 receptors, which moves **aquaporin 2** water channels from the interior of the cell to the luminal membrane.
3. Vasopressin is the hormome which maintains plasma osmolality
4. Acts on the V_1 receptors present in the smooth muscle cells of arterioles and causes its contraction and this effect occurs in high concentration of the hormone.
5. Stimulates secretion of CRH and ACTH.

Disorders of vasopressin secretion

Deficiency of Vasopressin causes **Diabetes insipidus.**

Two types, Pituitary diabetes insipidus and nephrogenic diabetes insipidus

I Pituitary diabetes insipidus

Causes:

Deficiency of Vasopressin secretion
Neoplastic lesions of hypothalamus
Vascular lesions and Post traumatic conditions affecting hypothalamus
Surgical removal of posterior pituitary

II Nephrogenic diabetes insipidus.

The kidney does not respond to vasopressin
Plasma ADH level will be normal in this type.

Symptoms of diabetes insipidus

Polyuria, polydipsia,
Rise in plasma osmolality

Administration of ADH corrects plasma osmolality in pituitary diabetes insipidus, but not in nephrogenic diabetes insipidus.

Increased secretion of ADH

SIADH (Syndrome of inappropriate secretion of ADH)

Occurs in:
Carcinoma of lung
Trauma
Anesthetic pain

Effects in the body

↑ Water retention, ↑ ECF volume
Inhibition of Aldosterone, loss of salt in the urine

Contraction of mesangial cells

They are interstitial cells present in JGA. Vasopressin causes contraction of these cells and reduces GFR. This action is mediated by V_1 receptors.

Inhibits renin secretion

Vasopressin acts on JG cells and inhibits renin secretion to compensate for the increased plasma ECF osmolality. This action is mediated by V_1 receptors.

Arteriole constriction

Vasopressin by acting on the V1 receptors brings about arteriole constriction and rise in the blood pressure. This action is seen with high concentrations of vasopressin.

Secretion of ACTH

Vasopressin causes secretion of CRH and ACTH during stress. The parvocellular cells send axons to median eminence and stimulate CRH secretion, which in turn causes ACTH secretion from anterior pituitary.

Regulation of vasopressin secretion

The normal osmolality of plasma is 290 milliosmoles/L of water. Rise of osmolality above this level stimulates ADH secretion. There are osmoreceptors present in the hypothalamic supraoptic and paraventricular nuclei (around magnocellular cells). Increase in osmotic pressure of ECF causes dehydration and shrinkage of osmoreceptors. This stimulates ADH secretion. Fall in osmotic pressure of ECF causes swelling of osmoreceptors which inhibit ADH secretion (Fig. 12.5). The increase in osmotic pressure produced by NaCl, glucose and manitol are effective stimuli in stimulating vasopressin secretion. The rise in urea concentration in ECF is less potent in stimulating vasopressin secretion, as urea is freely permeable to cell.

Regulation by blood volume

There are volume receptors present in the atria. When blood volume increases, the atrial wall is stretched and stimulates volume receptors. This leads to inhibition of ADH secretion, causing water diuresis. In this way excess fluid volume is eliminated and the ECF volume comes back to normal (Fig. 12.5).

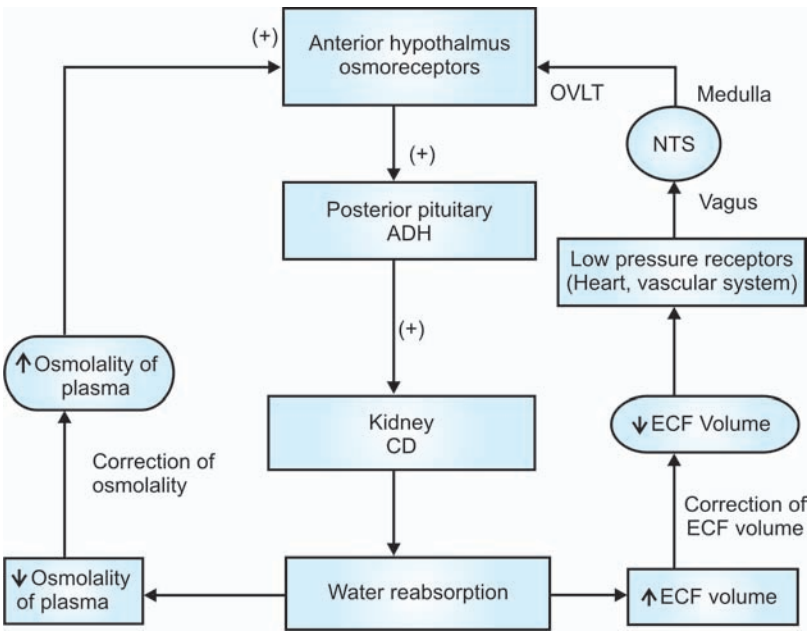


Fig. 12.5: Regulation of plasma osmolality by ADH

When blood volume decreases, there is absence of stimulation from the left atrial volume receptors and this stimulates the ADH secretion. The ADH presence in this condition helps to increase the fluid volume.

Other factors

ADH secretion is stimulated in stress, pain, nausea, emotion, morphine, nicotine and the secretion is inhibited by alcohol and opiate antagonists.

Disorders of ADH secretion

Diabetes insipidus

Usually it includes two types namely, **pituitary** and **nephrogenic**. In *pituitary diabetes insipidus*, ADH secretion is absent. The plasma osmolality is increased. Polyuria and polydipsia are characteristic features of this disease. Administration of ADH corrects urine and plasma osmolality.

In *nephrogenic diabetes insipidus*, plasma level of ADH is normal or more. The nephron is insensitive to circulating ADH. Administration of ADH also has no effect in correcting the urine and plasma osmolality. This disorder is probably caused by the receptor deficiency or tissue resistance to the action of hormone.

In certain diseases like pulmonary carcinoma, tumors of tissues, pulmonary tuberculosis, trauma, anaesthetic pain, etc, there is high concentration of ADH in plasma. The condition is called *syndrome of inappropropriate secretion of antidiuretic hormone (SIADH).*

Oxytocin

It is also a nonapeptide with half life in the circulation ranging from 3 to 5 minutes. The hormone is degraded in the liver, kidney and also in mammary gland and uterus.

Actions

Contraction of uterine myometrium

Oxytocin causes contraction of uterine muscle and this action is especially seen during labour. The secretion of this peptide occurs, by positive feed back stimulation and the stimulus for its release is the stretching of cervix. The sensitivity of uterine muscle to oxytocin is increased by estrogen and inhibited by progesterone. The oxytocin receptors in the uterine myometrium are increased before the onset of labor.

In nonpregnant women, the contraction of uterine muscle facilitates sperm transport in the female genital tract and helps fertilization. The stimulus for secretion includes genital stimulation in the female during coitus.

Actions of oxytocin

1. Causes contraction of smooth muscle of uterus
2. Contraction of pregnant uterus facilitates labor
3. Contraction of nonpregnant uterus facilitates sperm transport in the genital tract
4. Causes milk ejection in lactating woman by causing contraction of myoepithelial cells lining the ducts of mammary gland

Milk ejection

Oxytocin acts on the myoepithelial cells lining the ducts and alveoli of mammary gland. The hormone causes contraction of myoepithelial cells and expulsion of milk. The secretion of oxytocin involves neuroendocrine reflex. The stimulation of nipple initiates the reflex. The hormone is also secreted by the conditioned response. The sight of crying of baby stimulates oxytocin in the nursing mother. The secretion of oxytocin is blocked by pain, fear and stress.

Regulation of oxytocin secretion

This secretion is regulated by neuroendocrine reflex. The suckling reflex and distention of cervix are the stimuli initiating the reflex. The afferent impulses are carried by somatic sensory tracts and reach hypothalamus via reticular formation.

THYROID GLAND

It is a bilobed structure with isthmus connecting the two lobes. It extends across the ventral surface of trachea below the larynx. The gland receives rich blood supply and the autonomic nerves regulate the blood flow.

The histology of the gland shows follicles or acini with cuboidal cells lining them in a normal gland. When stimulated, the cells become columnar. Between the follicles, there are parafollicular cells (C cells), which secrete calcitonin. The lumen of the follicle contains a colloid called **thyroglobulin**. It is a glycoprotein with a molecular weight of 660,000. It contains thyroid hormones.

Thyroid hormones

T_4 : Thyroxine or tetraiodothyronine. It constitutes 90% of the secretion from the gland.

T_3 : **Tri iodothyronine** (3, 5, 3′T3). It constitutes 9% of the secretion.

r T_3 : **Reverse T_3** (3, 3′5′rT3) It is not biologically active and forms 1% of the secretion.

Synthesis (Fig. 12.6)

Iodide trapping

The follicular cell actively transports inorganic iodide from plasma against electrochemical gradient. The **thyroid; serum ratio of iodide** gives the effectiveness of iodide trapping. Normal value of T/S ratio is 30. That means, the thyroid gland has iodide concentration 30 times more than the plasma. TSH hormone regulates T/S ratio for iodide. Fall in TSH secretion reduces the ratio and increase in TSH level raises it. The carrier which transports iodide, has greater affinity for perchlorate and thiocyanate. Iodide transport into the gland follicular cells is inhibited when these substances are administered.

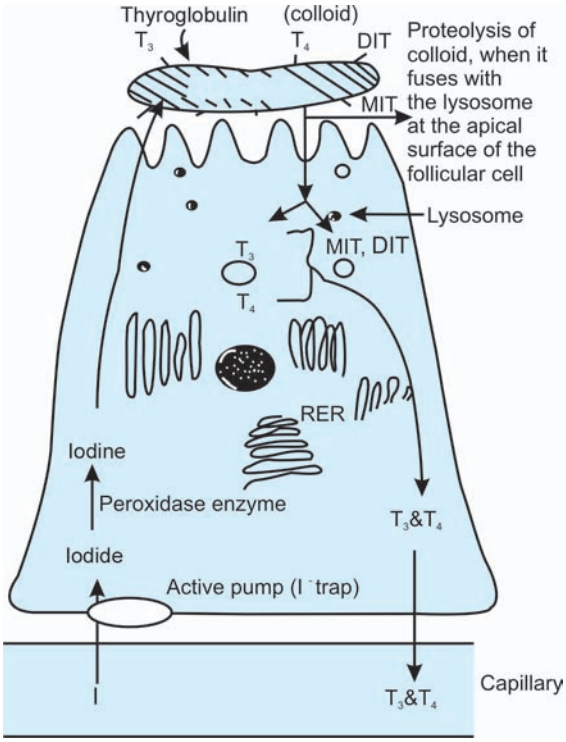


Fig. 12.6: Thyroid follicular cell showing synthesis and secretion of thyroid hormones. The colloid contains the tyrosine residues. Iodine enters the colloid and undergoes coupling reactions to give MIT, DIT, T_3 and T_4.The colloid droplets at the apical border fuse with the lysosome and release the hormones by proteolysis . T_3 and T_4 are released into the circulation as thyroid hormones, while MIT and DIT are deiodinated by deiodinase enzyme. The iodine released from this enzyme action is reutilized

Thyroglobulin

It is a large glycoprotein containing 123 tyrosine amino acid residues. They are iodinated and coupled to form thyroid hormones. Thyroglobulin is synthesized from rough endoplasmic reticulum of follicular cells and glycosylated in the Golgi body before being packaged into vesicle. It is secreted into the lumen by exocytosis.

Oxidation of iodide

Iodide is oxidized to iodine by the thyroid peroxidase enzyme, which is present at the apical surface of the follicular cells.

Organification

Iodination of tyrosine residues present in thyroglobulin produces MIT (mono iodo tyrosine) and DIT (di iodo tyrosine).

Coupling

One molecule of MIT couples with one molecule of DIT and forms T_3. Condensation of DIT with MIT will give RT_3.

Two molecules of DIT couple to form T_4. Thyroxine peroxidase enzyme catalyzes these reactions. For each molecule of thyroglobulin, 3 residues of T_3 or T_4 will be present. On the whole, T_4 concentration is 10 times more than T_3.

Secretion

TSH stimulates endocytosis of colloid droplets into follicular cytoplasm. The droplets are membrane bound and the lysosome fuses with it to form the phagosome. The proteolytic enzymes present in the phagosome hydrolyzes the colloid droplet into **T_4, T_3, rT_3, MIT** and **DIT**. MIT and DIT are not biologically active. The follicular cell has deiodinase enzyme, which acts on MIT and DIT and releases iodine. This is subsequently reutilized for the thyroid hormone synthesis. T_4 and T_3 are secreted into the capillaries from the cell, probably by simple diffusion.

Plasma levels of T_3 and T_4

The radioactive measurement of thyroid hormones in plasma shows T_4 level at 8 µg% and T_3 at 0.18 µg%. The hormones are transported in the plasma as free and bound form. The protein which carries them is called **thyroid binding globulin (TBG).** The next protein to bind is prealbumin, which has higher capacity, but low affinity for T_3 and T_4. TBG level is increased by estrogen and reduced by glucocorticoids. During pregnancy TBG level is raised, but the free level of thyroid hormones remains normal. Hence hyperthyroidism does not result.

Extra thyroidal pool

Roughly one third of body's T_4 is present in the liver and kidney. Whenever secretion of thyroid hormones is decreased, this extra thyroidal pool can maintain normal level in the plasma. The extra thyroidal pool for T_3 is small. When T_4 is administered, only 5% rise in the serum level can be seen, as T_4 pool is large. Even though T_3 is more potent, for replacement therapy in hypothyroidism, T_4 is preferred for the reasons mentioned above.

Metabolism

T_4 is converted to T_3 by 5' monodeiodinase or deiodinated to form inactive rT_3. T_3 and rT_3 are further deiodinated to T_2, T_1 and T_0. There is also conjugation with sulphates and glucuronic acid and secreted in the bile. The hormones also show decarboxylation and deamination during metabolism.

Agents inhibiting synthesis of thyroid hormones

Wolf-Chaikoff effect

Excess iodine, when given to individuals with normal thyroid gland function, organification and coupling reactions are inhibited, leading to the blockade of synthesis, which is known as Wolf-Chaikoff effect. This blockade is not permanent, as escape occurs normally within several days inspite of increased iodine being present in the diet.

Inhibition of iodide transport

Agents like perchlorate and thiocyanate competitively bind to the carrier protein and block the iodide transport from the plasma into the follicular cell.

Goitrogens

They are substances which block the synthesis of thyroid hormones and also produce goiter. Goitrogenic substances are naturally present in cabbage and turnip. Thiourea and thiouracil are also examples of goitrogens, which inhibit thyroid peroxidase, preventing oxidation of iodide and organification. These substances also block the conversion of T_4 into T_3 in the peripheral tissue.

Major actions of thyroxine

T_3 is more potent and acts more rapidly than T_4

At the target tissue, T_4 is converted to T_3 by deiodinase enzyme

Causes calorigenesis, ↑ O_2 consumption, ↑ BMR

Promotes growth and development of skeleton and brain

↑ Glucose absorption from intestine, Increased insulin resistance

Stimulates lipolysis, ↓cholesterol by ↑ LDL receptors in the liver.

↑ Adrenergic receptors on the heart, produces positive inotropic and positive chronotropic effect. ↑ Cardiac output, ↑ pulse pressure

↑ response of the heart to the circulating catecholamines.

Regulation of thyroid gland function

TSH is involved in each step in the biosynthesis and secretion of thyroid hormones. It stimulates iodide uptake, oxidation of iodide, organification and coupling. TSH secretion is inhibited by high plasma level of T_4 and lesser extent by T_3. In anterior pituitary cells (thyrotrophs), 80% of T_4 is deiodized to T_3 by 5' monodeiodinase enzyme. The TSH secretion is regulated by TRH (Fig. 12.7). If T_3 concentration in pituitary thyrotrophs is increased, TRH inhibits TSH secretion. This is done by the down regulation of TRH receptors and diminished response of pituitary thyrotrophs to TRH. If the plasma level of T_4 falls, intracellular T_3 concentration in thyrotrophs decreases, which results in TRH mediated TSH release.

In hypothyroid, the TSH basal level is increased and the TSH secretion in response to TRH would be greater in this condition.

TSH acts on the thyroid follicular cells and increases the formation of cAMP. The actions of TSH are mediated through this second messenger. TSH receptors are also present in other tissues. In normal secretion of thyroid hormones, their functions are not known.

Thyroid hormone receptors

The thyroid receptors show more affinity to T_3 than T_4. The hormone enters the cell by diffusion and carrier mediated transport.

The hormone binds to the receptors in the nucleus and attached to the thyroid responsive element of DNA. This causes regulation of gene transcription and produces mRNA. The translation of mRNA at the endoplasmic reticulum occurs leading to protein synthesis. Thyroid receptors are also present in the mitochondria.

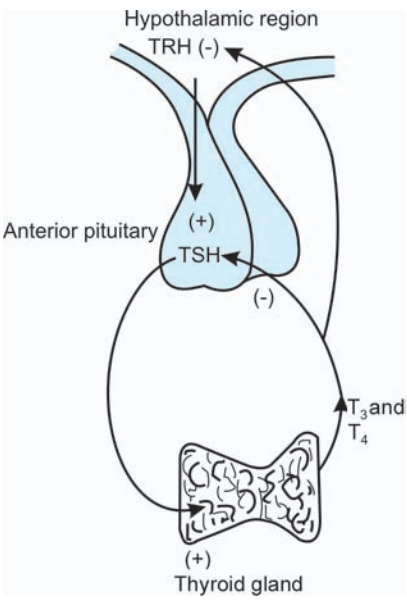


Fig. 12.7: Regulation of thyroid gland by the pituitary and hypothalamus (hypothalamo hypophyseal thyroid axis)

Note the negative feedback inhibition of TSH and TRH by the blood level of T_3 and T_4

Actions of thyroid hormones

At the peripheral tissues, T_4 is converted to T_3 and then the actions are produced.

Metabolic effects

Calorigenic action

Thyroid hormone shows metabolic effect and causes increased oxygen consumption. The heat production from the oxidation of food causes BMR. In the cell the mitochondria number, its size, oxygen consumption and oxidative phosphorylation are increased by the thyroid hormone. Thyroxine increases the activity of **Na^+- K^+ ATPase** which is necessary for Na^+ pump in the cells. Thyroid hormone stimulates sodium pump and causes 15 to 40% of basal energy utilised by this activity. Thyroid hormone stimulates all metabolic pathways. In moderate concentration of the hormone, protein synthesis is stimulated and in increased concentration protein breakdown occurs. Similarly, the metabolism of fat and glycogen depends on the rate of secretion of thyroid hormone.

Growth and development

Thyroid hormone is necessary for the normal growth and development. Thyroid hormone may have a permissive role in the action of growth hormone. It is also necessary for the secretion of growth factors. Development of tissues depends on the presence of thyroid hormone. The myelination of neuron and development of cartilage into bone are examples of thyroid horomone action on development. Deficiency of thyroid hormone in children causes stunted skeletal growth with mental retardation. The mile stones in development are also delayed.

Carbohydrate metabolism

Excess secretion of thyroid hormone causes abnormal glucose tolerance test. After the infusion of glucose, the level rises more rapidly than normal. The glucose absorption from the GI tract is increased and insulin resistance also becomes more.

Fat metabolism

Thyroxine is a lipolytic hormone and also causes lipogenesis. The cholesterol level is decreased in an euthyroid person, but the effects are transient. The LDL receptors in the liver are increased and hence the cholesterol is cleared as LDL from plasma.

Neurological effects

Thyroid hormone is necessary for brain development. Neuronal proliferation, differentiation, myelin formation, neuronal outgrowth and synapse formation are influenced by the thyroid hormone. In adults, excess secretion of the hormone, causes increased neuronal excitability, restlessness and decreased reflex time. On the other hand, the insufficiency of thyroid hormone causes less excitability of the nervous system. The individual is dull, lethargic and the CNS reflexes are slow.

On sympathetic nervous system

Thyroid hormone causes an increases the number of β-adrenergic receptors in tissues like heart. In other tissues, increased affinity for catecholamine is present in the presence of thyroxine. There is increased sympathetic activity in excess secretion of this hormone, which causes increased heart rate, increased cardiac output and rise in blood pressure. The outstretched hands show fine tremor, which is abolished by β-blockers.

On CVS

Thyroid hormone on the heart shows positive inotropic and chronotropic effects. The cardiac output increases and pulse pressure rises, as the peripheral resistance is decreased. The hormone increases Na^+-K^+ATPase and myosin ATPase activities in the myocardium. In hyperthyroidism, myocardial hypertrophy can be seen. The peripheral resistance decreases due to increased metabolism and the cutaneous vasodilatation gives warm and moist skin. Hypothyroidism causes fall in heart rate and cardiac output.

Skeletal muscle

In both skeletal and cardiac muscles, thyroid hormone causes increase in myosin heavy chain (MHC) protein. In hyperthyroidism, myopathies are present. Muscle weakness, wasting and fatigability are common in hyperthyroidism. In hypothyroidism, muscle stiffness, delayed muscle contraction and relaxation are observed.

Other actions

Causes secretion of lung surfactant
Converts β-carotene to vit A
Increases GFR and RBF in kidney
Necessary for milk secretion
Helps in erythropoiesis.

Disorders of thyroid gland secretion

Hypothyroidism

Cretinism

It is a condition of hypothyroidism in children. It is caused by:

- In utero *iodide deficiency* (endemic cretinism)
- *Congenital hypothyroidism* resulting from dysgenesis and defects in hormone biosynthesis.
- *Autoimmune disease* of thyroid gland in the mother. The thyroid blocking antibodies from the mother cross placental barrier and cause neonatal hypothyroidism.

Symptoms

1. Stunted skeletal growth with delayed milestones in development
2. Mentally retarded
3. Absence of sexual maturity
4. Poor feeding, hoarse cry, umbilical hernia
5. Respiratory distress syndrome.

Hypothyroidism in adults

Myxedema

Hypothyroidism in adults causes myxedematous skin, due to the accumulation of muco polysaccharides in the interstitial spaces, giving a nonpitting edema. *Low BMR, hypothermia, cold intolerance, dry cold skin, lethargy, somnolence, dullness, mental slowness, increased reflex time, increase in blood cholesterol, weight gain, amenorrhea in female, sterility in male, psychosis, papilledema, are the symptoms of hypothyroidism in adults.*

Hyperthyroidism

Thyrotoxicosis

It is a condition of hyperthyroidism and **Grave's disease** is included in this type. Grave's disease is caused by the **autoimmune disease**. The thyroid stimulating immunoglobulin (TSI) mimics the action of TSH on the gland. The immunoglobulin belongs to the IgG type. The hypersecretion of the gland caused by TSI does not show feedback regulation. The hypertrophy and hyperplasia of the gland result in goitre (Fig. 12.8). TSI acts on the retro-orbital tissues and causes retention of mucopolysaccharides, which gives **exophthalmos**. The upper lid retraction and stare occur as a result of protrusion of the eye ball.

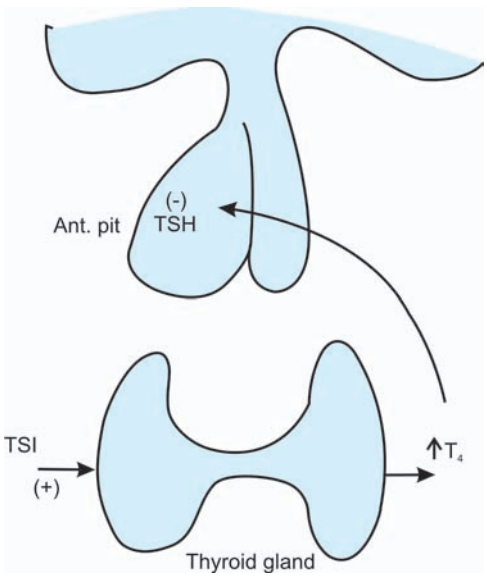


Fig. 12.8: Mechanism of goitre in grave's disease.The stimulation of the gland comes from the thyroid stimulating immunoglobulin (TSI) and the elevated levels of T_4 inhibits TSH secretion from anterior pituitary

Hypothyroidism

Myxedema

Hypothyroidism in adults causes myxedema

↓BMR, intolerance to cold
Myxedematous skin
Dry, yellowish skin,
Sluggish activity, poor mentation, poor memory, psychosis
Slow and husky voice

↑ Plasma cholesterol, weight gain
Sterility in male and amenorrhea in female

Cretinism

Hypothyroidism in children from birth or before produces cretinism

Causes:

In utero iodide deficiency, congenital hypothyroidism, autoimmune disease of thyroid gland in the mother during pregnancy

- Stunted skeletal growth with mental retardation
- Pot belly with tongue protrusion
- Milestones in development are delayed
- Absence of sexual maturity

Hyperthyroidism

Causes:

Graves' disease, Toxic adenoma, Toxic goiter, Hashimoto's thyroiditis, Tumor of TSH secreting cells of anterior pituitary.

↑ BMR, heat intolerance,

Palpitation, ↑ pulse pressure, warm moist skin

Nervousness, increased excitability of neurons,

↓reaction time of reflexes

Muscle weakness and fatigue

In Graves' disease exophthalmos and goiter also occur.

Causes of Hypothyroidism

Surgical removal of the gland
Irradiation
Autoimmune disease
Cancer
Dietary iodide deficiency
Inhibition of hormone synthesis (enzyme defects in biosynthesis)
Hypothalamus and pituitary disorders
Receptor resistance to hormone

Other symptoms of Grave's disease

High BMR, heat intolerance, tremor of the out stretched hands, decreased reaction time of reflexes, palpitation, increased cardiac output, warm moist skin, muscle weakness, and fatigue.

Thyroiditis

It is an inflammatory condition of the gland, resulting from viral infection. Fever, tenderness of the gland and symptoms of hyperthyroidism are observed in this condition.

Hashimoto's thyroiditis

It is a common cause of acquired hypothyroidism. It is an autoimmune disease, where the immunoglobulins damage the gland cells. In early stages, hyperthyroidism may occur and as the disease progresses, hypothyroidism results. Goitre also will be present in this condition.

Goitre

It is the enlargement of thyroid gland occurring in a variety of conditions. The hypertrophy and hyperplasia of the gland are caused by either the elevated level of TSH or **TSI (thyroid stimulating immunoglobulins).**

Endemic goitre: Endemic deficiency of iodide causes goitre. It is prevented by the dietary intake

of common salt, fortified with potassium iodide.
Antithyroid drugs: Drugs which interfere with iodide trapping. Thiocyanate and perchlorate produce their effects by this way.

Drugs which block organification of iodine: Thiocarbamides group, i.e. thiourea, thiouracil, and methimazole belong to this category.

Goitrogens: Naturally occurring goitrogens such as cabbage, turnip contain progoitrin, which is converted to goitrin. It causes increased growth of the gland producing goitre.

Goitre in hypothyroidism occurs due to the increased TSH secretion.

Goitre in grave's disease is caused by TSI.

ADRENAL GLAND

A pair of glands, one on the top of each kidney is present. Each gland has an outer cortex and an inner medulla. The cortex occupies 80% of the gland and secretes corticosteroids, while the medulla is only 20% of the gland and secretes catecholamines (Fig. 12.9).

During embryonic development, the cortex, which develops from mesoderm, shows an outer neocortex (15%) and an inner fetal zone (85%). After birth (3 to 12 months postpartum), the fetal zone involutes and the outer neocortex forms the adult cortex. During fetal life, 100 to 200 mg of steroids are secreted per day and most of them are sulfate conjugates. DHEA sulfate is one such steroid secreted during fetal stage.

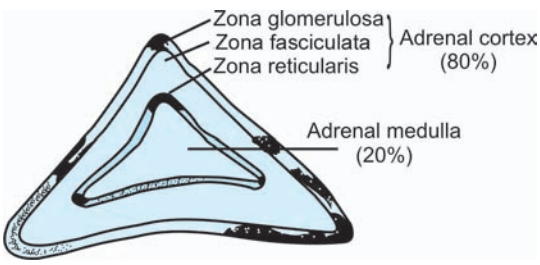


Fig. 12.9: Structure of the adrenal gland. Adrenal cortex secretes steroids and the medulla secretes catecholamines

Histology of the gland

Adrenal cortex has three layers, namely, *zona glomerulosa, zona fasciculata and zona reticularis.* The outermost layer is zona glomerulosa which secretes the mineralocorticoid aldosterone. This layer cannot produce the glucocorticoid and sex steroid, due to the absence of 17 α-hydroxylase enzyme. The zona fasciculata and zona reticularis contain 17α-hydroxylase and hence, they synthesize the glucocorticoid and androgen.

Synthesis of corticosteroids

The precursor of all steroids is cholesterol. It is present in LDL in the circulation. Adrenal cortical cells have LDL receptors and hence they take up LDL. The cholesterol in LDL is in the ester form and the enzyme cholesterol ester hydrolase in the cortical cells converts ester cholesterol to free cholesterol.

The next step is conversion of cholesterol to pregnenolone in the mitochondria by 20, 22-desmolase enzyme and it is the rate limiting step in the synthesis of steroids. Pregnenolone is the common precursor for all steroid hormones.

The third step involves the conversion of pregnenolone to progesterone in the endoplasmic reticulum (microsomes) by 3 β-hydroxysteroid dehydrogenase and Δ^5 isomerase.

Finally, hydroxylation reactions produce various steroid hormones.

Hormones of adrenal cortex

Adrenal cortex secretes

Glucocorticoid	(C_{21})	**cortisol**
Mineralocorticoid	(C_{21})	**aldosterone**
Androgen	(C_{19})	**DHEA**

Synthesis of aldosterone shows hydroxylation at C_{21}, C_{11}, C_{18} positions. 18 hydroxydehydrogenase is a mitochondrial enzyme present in the zona glomerulosa only (Fig. 12.10).

Cortisol formation involves hydroxylation at C_{17}, C_{21} and C_{11} positions (Fig. 12.11).

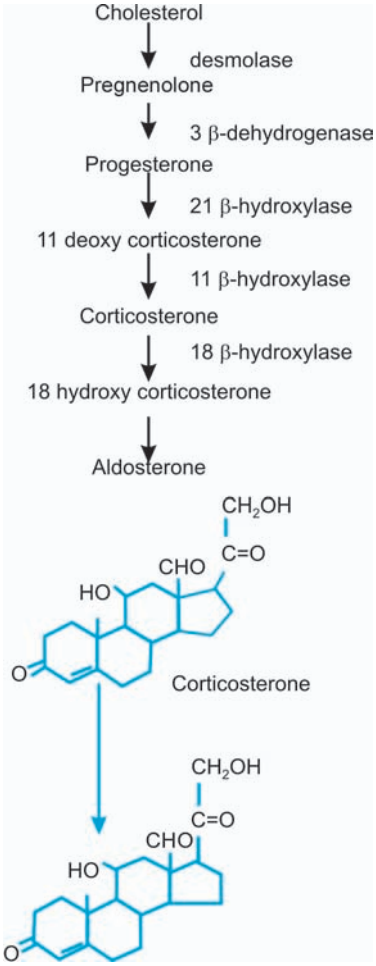


Fig. 12.10: Synthesis of aldosterone in zona glomerulosa layer of adrenal cortex

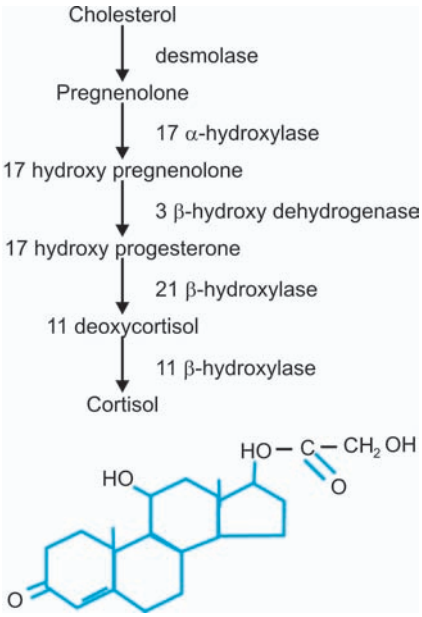


Fig. 12.11: Synthesis of cortisol

Androgen synthesis shows hydroxylation at C_{17} by 17 α-hydroxylase enzyme (Fig. 12.12). Both 17α-hydoxy progesterone and 17 α-hydroxy pregnenolone can give rise to androgen or glucocorticoid.

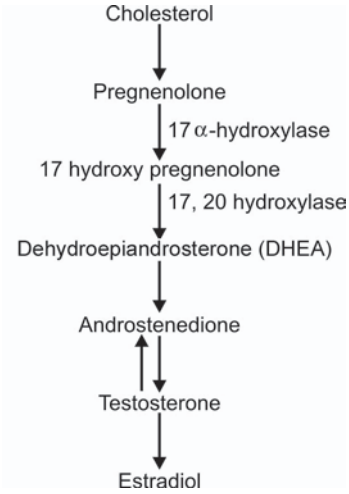


Fig. 12.12: Synthesis of adrenal androgens

In man, the main glucocorticoid is cortisol and in rodents, it is corticosterone.

Transport in blood

The steroid hormones are bound to **transcortin,** which constitute 90% of the hormone in circulation. The free form is 10%. The bound form serves as a reservoir for the free hormone.

Metabolism

The corticosteroids are metabolized in the liver. Cortisol is converted to dihydrocortisol and then to tetrahydrocortisol. It is conjugated with glucuronic acid and excreted in the urine. Aldosterone is excreted as tetrahydro aldosterone glucuronide.

Adrenal androgens are excreted as 17 keto steroids, out of which, 5% is derived from cortisol. The normal level of urinary 17 ketosteroids in males is 8 to 20 mg/day and in females, it is 5 to 14 mg/day.

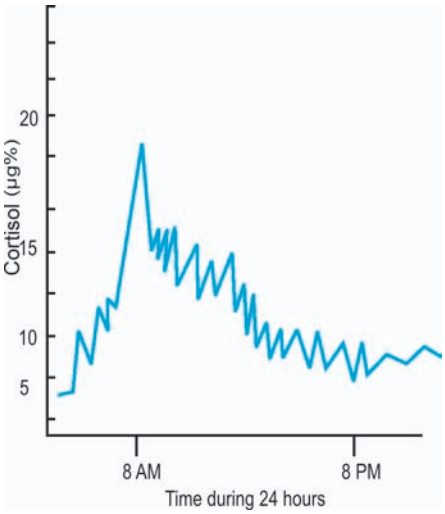


Fig. 12.13: Cortisol secretion during 24 hours day–night cycle. The cortisol secretion shows diurnal variation, with peak levels in the morning and lowest in the evening

Diurnal variation of cortisol secretion

The secretion of cortisol is related to sleep wakefulness pattern, and light-dark cycle. Hence, the level is at its peak in the morning and lowest in the evening (Fig. 12.13). The normal mean level of cortisol in the plasma in a day is 13 µg%.

Mechanism of action of ACTH

ACTH acts on the zona fasciculata and zona reticularis and causes the formation of cAMP. The second messenger cAMP influences steroid biosynthesis.

Mechanism of action of cortisol

Glucocorticoid receptors are bound to the heat shock proteins (HSP). Cortisol binds to the receptor-HSP complex, which results in the formation of HSP and activated hormone receptor complex. The latter causes transcription of DNA to give specific mRNA. The mRNA enters the cytoplasm and goes to endoplasmic reticulum for translation of mRNA. Newer proteins that are formed as a result of this produce the physiological response.

Regulation of cortisol secretion

ACTH is the trophic hormone for the secretion of cortisol from adrenal cortex. The ACTH secretion from anterior pituitary is regulated by the CRH secreted from the median eminence of hypothalamus. CRH secretion is stimulated by the cholinergic neurons, psychological stress, physical stress (trauma, exercise, hemorrhage, pain, surgery and infections) and vasopressin.

CRH inhibition occurs when cortisol level is increased in the blood, which is a negative feed back inhibition (Fig. 12.14). The CRH secretion caused by the stress stimuli is not under the negative feedback regulation of cortisol. GABA-ergic neurons and adrenergic neurons that end on the median eminence inhibit CRH release.

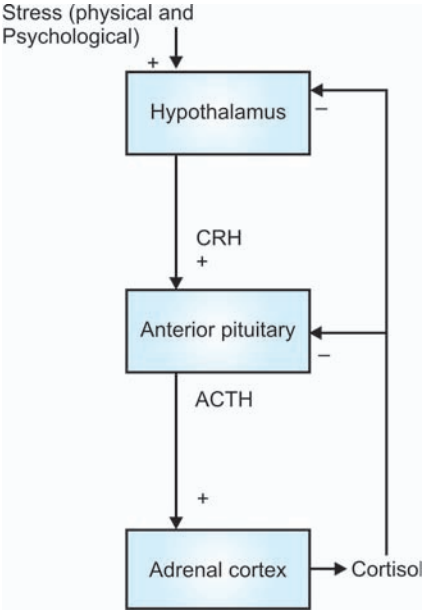


Fig. 12.14: Diagrammatic representation of hypothalamo hypophyseal adrenal axis to show the regulation of cortisol

Physiological effects of cortisol

Metabolic actions

Carbohydrate sparing effect

It is a carbohydrate sparing hormone and therefore exerts an anti insulin effect which leads to hyperglycemia. The insulin resistance is seen in skeletal muscle and adipose tissue, where it blocks the transport of glucose. It also causes increased production of glucose by gluconeogenesis in the liver. The synthesis of enzymes that are involved in gluconeogenesis is stimulated by cortisol in the liver, whereas, in muscle, it causes catabolism of proteins. The increased blood glucose level causes steroid diabetes. The excess glucose is also stored in the liver as glycogen (glycogenesis).

Lipolytic effect

Cortisol has a permissive action on the catecholamine's lipolytic effect. It also potentiates lipolytic action of growth hormone, glucagon and T3. On its own, cortisol has a weak lipolytic action. Excess secretion of glucocorticoids causes centripetal distribution of fat, giving a pendulous abdomen and subclavicular fat retention (buffalo hump). When cortisol is more, the appetite is increased which causes increase in glucose level in the blood. This leads to stimulation of insulin secretion, which in turn causes lipogenesis. This could possibly results in obesity which is characteristic of Cushing's syndrome.

Protein catabolic effect

Cortisol causes protein catabolism in the skeletal muscle and increases amino acids level in the blood. The glycogenic amino acids like alanine, leucine are transported to the liver for the formation of glucose by gluconeogenesis.

Antigrowth effects

Cortisol causes negative nitrogen balance and hence retards growth. Cortisol suppresses growth hormone secretion and inhibits the somatic growth in chronic administration of exogenous glucocorticoids. The other antigrowth action is seen in delayed wound healing due to the inhibition of fibroblast proliferation.

On bone

On bone, it causes increased resorption, causing demineralisation of bone (osteocytic osteolysis). The intestinal absorption of calcium is inhibited and also decreases the renal reabsorption of calcium. The glucocorticoids directly act on osteoblasts and inhibit bone formation. Therapeutically, glucocorticoids are useful in the treatment of inflammatory conditions associated with arthritis, but excessive use can cause osteoporosis.

On muscle

Glucocorticoids in large dose cause muscle weakness and atrophy.

Stimulation of gastric secretion

Cortisol stimulates gastric secretion and excess level in the blood can lead to peptic ulcer. Absence of cortisol causes decreased GI secretion and motility.

Facilitates renal free water clearance

Cortisol inhibits ADH secretion and promotes free water clearance. Deficiency of cortisol causes potentiation of ADH action. The free water clearance in response to water load becomes difficult, resulting in water intoxication.

Cortisol also has a weak mineralocorticoid action and increases GFR.

Stimulates erythropoiesis

Cortisol stimulates erythropoiesis by increasing the secretion of erythropoietin from kidney. Lack of cortisol causes anemia.

Increases neutrophils in the circulation

Cortisol causes eosinopenia, lymphocytopenia and fall in basophil. The neutrophils increase in number due to the reduced migration from vessels and increased release from the bone marrow. The reduction of circulating eosinophil, basophil, monocytes and lymphocytes is due to the sequestration of these cells in the lymphoid tissue like spleen.

Permissive effect on blood vessels

The vasoconstrictor action of catecholamines is due to the permissive effect of cortisol. The absence of cortisol causes low peripheral resistance and orthostatic postural hypotension.

Stress adaptation

Resistance to stress is due to the activation of hypothalamo pituitary adrenal axis. The stimulation of adrenal gland occurs when the organism is exposed to physical and psychological stress. Deficiency of corticosteroids leads to poor adaptation of stress.

Actions of glucocorticoids

Cortisol is the glucocorticoid secreted from the adrenal cortex
↑ Blood glucose by↑ hepatic glycogenolysis and gluconeogenesis.
Anti-insulin effect in the peripheral tissues
↑ Protein catabolism
↑ Mobilization of fat, ↑ FFA level
↑ Bone resorption
Required for vascular responsiveness to circulating catecholamines
Required for resisting stress
↑ Neuronal excitability
Causes eosinopenia, lymphocytopenia and neutrophilia
Facilitates renal free water clearance in response to water load
Pharmacologically used for anti-inflammatory, anti-allergic and immunosuppressive effects.

Increases excitability of neurons

Cortisol in excess amounts causes neuronal excitability, leading to euphoria, paranoid and steroid psychosis. Insomnia, reduced REM sleep are also observed in the hypersecretion of cortisol. Deficiency of cortisol causes depression, apathy and irritability.

PHARMACOLOGICAL ACTIONS

Anti-allergic effects

Cortisol inhibits the mediators of allergic response like histamine. The release of histamine from mast cells and basophils is inhibited by cortisol and hence prevents allergy and hypersensitivity reactions.

Glucocorticoids in therapeutic doses relieve bronchial asthma.

Anti-inflammatory effect

Glucocorticoids in pharmacological doses cause anti-inflammatory effects. It inhibits the mediators of inflammation by inhibiting the key enzyme Phospholipase A_2. This prevents the formation

of prostaglandins, leucotrienes and thromboxane. The release of proteolytic enzymes is prevented by stabilizing the lysosomes. The migration of leucocytes is reduced by decreasing the capillary permeability. This also prevents the edema formation.

Immunosuppressant effect

Glucocorticoids suppress the immune response by reducing the circulating T lymphocytes. This leads to the absence of cell mediated immunity and hence these agents are used as immunosuppressants to prevent tissue rejection in transplantation of organs.

Involution of lymphoid tissue

Cortisol in excess causes involution of lymphoid tissue, lymph nodes and thymus. The antibody level in the blood decreases and fibroblasts proliferation is inhibited. This leads to the spread of infections and that is why antibiotics are necessary adjunct to the steroid therapy.

Adrenal androgen

In both male and female the adrenal cortex secretes **DHEA** and **androstenedione**. These can be converted into testosterone or estrogen in the peripheral tissues.

Actions of adrenal androgen

Adrenal androgen causes development of pubic and axillary hair at the time of puberty in both the sexes. In male, the adrenal androgen can also cause male secondary sexual characteristics, including increased muscle mass and positive nitrogen balance.

Actions of adrenal androgens

DHEA and androstenedione are the androgens secreted in both sexes
Growth of pubic and axillary hair at the time of puberty
Protein anabolic effect and increases muscle mass
In males, the secretion is more

Adrenogenital syndrome

Occurs in congenital adrenal virilizing hyperplasia caused by deficiency of 11β hydroxylase and 21 β hydroxylase enzymes.
Characterized by ↑ masculanization effect
In 11β hydroxylase deficiency, virlilization with hypertension occurs
In 21β hydroxylase deficiency virilization with salt loss occurs
Adrenogenital syndrome in adult female causes
Growth of hair in the face **(Hirsutism)**, chest and trunk, baldness
↑ Muscle mass, atrophy of breasts, enlarged clitoris
↑ Urinary excretion of 17 ketosteroids.
In prepubertal boys it causes **precocious pseudo puberty**
Adreno genital syndrome in genetic female in the fetal stage causes **female pseudo hermaphroditism**

Congenital adrenal hyperplasia

It is a disorder in which excess adrenal androgen is secreted due to the congenital defect in the enzymes involved in the synthesis of cortisol and aldosterone. The deficiency of 21β-hydroxylase and 11 β-hydroxylase leads to this disorder. The deficiency of 21 β-hydroxylase is common and causes adrenal virilizing hyperplasia of salt losing form of hypotension, whereas deficiency of 11β-hydroxylase causes virilizing hyperplasia with hypertension.

The disorder when occurs in adult female is known as **adrenogenital syndrome**. It is characterized by **virilism**, with growth of hair in the face, hand and trunk. Increase in muscle mass, deepening of voice, atrophy of breasts, enlargement of clitoris and amenorrhea are other masculinisation changes that occur in adrenogenital syndrome.

In young boys, if this disorder occurs, it causes **precocious puberty**. In adult male, adrenogenital syndrome causes exaggeration of existing male characteristics.

Aldosterone

It is a C_{21} steroid produced from the zona glomerulosa of adrenal cortex. It is the main mineralocorticoid secreted in man. It regulates electrolyte balance and body fluid volume.

The hormone is bound to albumin and transcortin and transported in the blood. Inactivation of aldosterone takes place in the liver. The metabolite tetrahydro aldosterone conjugates with glucuronic acid and excreted in the urine.

The synthesis and secretion of aldosterone are stimulated by angiotensin II, which is formed from JGA. The renin is secreted from the JG cells due to the fall in the renal perfusion pressure and it converts angiotensinogen to angiotensin I. This is inactive and gets converted in the lungs to active form angiotensin II. The enzyme that is involved in this activation is **ACE (angiotensin converting enzyme)**

Actions of angiotensin II

Angiotensin II has multiple actions. It is a vasoconstrictor on blood vessels. The secretion of ADH and ACTH is stimulated by this hormone. It acts on the lateral hypothalamus and stimulates thirst. The secretion of catecholamines is also stimulated by angiotensin II.

Factors causing release of aldosterone

- Angiotensin II is a trophic hormone for the secretion of aldosterone. It causes the growth and increased vascularity of zona glomerulosa.
- The synthesis and secretion of aldosterone is also stimulated by the rise in K^+ concentration. About 10% rise in K^+ concentration in the plasma will cause direct effect on the zona glomerulosa and increases the secretion of aldosterone. It is likely that increase in the plasma K^+ causes depolarisation of zona glomerulosa cells by opening Ca^{++} channels. This stimulates the aldosterone secretion.
- ACTH though not a major factor in causing aldosterone secretion, chronic administration of ACTH can produce its secretion.
- Fall in the Na^+ level (10% decrease) in the plasma stimulates aldosterone secretion.

Actions of aldosterone

Na^+ reabsorption

Aldosterone maintains sodium balance, which in turn regulates the body fluid volume. The ECF volume is maintained by promoting Na^+ reabsorption in exchange for secretion of either K^+or H^+ ions. This action is observed in the late DCT and CD. The Na^+ reabsorption and K^+ secretion, due to the action of aldosterone can be seen in other tissues also, like sweat glands, gastric glands and salivary glands.

Actions of mineralocorticoids

Aldosterone is the major mineralocorticoid secreted from the adrenal cortex

Acts on principal cells of collecting ducts in the renal tubule

Aldosterone action is also present in salivary gland, sweat gland and intestine

Causes Na^+ reabsorption in exchange for K^+ and H^+ secretion in the kidney

Maintains ECF volume by regulating Na^+

Regulates arterial blood pressure by the regulation of blood volume

Major hormone to maintain ECF volume

Hyperaldosteronism

Primary hyperaldosteronism – Conn's syndrome
Causes:
Tumor of Zona glomerulosa layer of adrenal cortex
↑ ECF volume, ↑ Arterial blood pressure
Hypokalemia, muscle paralysis
Hypokalemic alkalosis, tetany,
Polyuria, ↓Plasma renin level,
Peripheral edema absent
Secondary hyperaldosteronism
Occurs due to ↑ plasma **renin**
Causes:
Liver cirrhosis, heart failure, nephrosis
Symptoms are similar to primary hyperaldosteronism but peripheral edema is present.

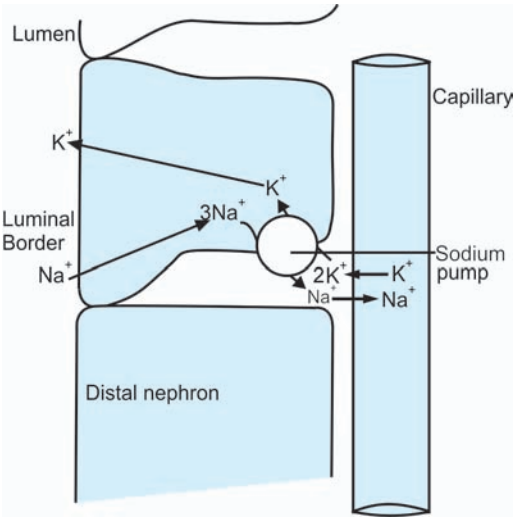


Fig. 12.15: Action of adlosterone in the DCT and CD of renal tubules. The hormone causes increased protein synthesis through transcription and translation processes in the tubular cells. The synthesized proteins are used for the formation of ENaC (epithelial sodium channels) and inserted in the cell membrane. Aldosterone also causes the synthesis of Na^+-K^+ATPase exchanger for sodium pump

Regulation of body fluid volume

The reabsorption of Na^+ leads to retention of water and this helps to maintain body fluid volume. At the luminal membrane, increased permeability of Na^+ occurs. The increase in intracellular concentration of Na^+ stimulates basal border Na^+–K^+ ATPase enzyme activity, which facilitates sodium pump (Fig. 12.15).

Aldosterone escape

Continuous aldosterone secretion leads to aldosterone escape phenomenon in 2 or 3 days. That is, as the ECF volume is increased, the GFR is also increased, which increases the sodium delivery to the renal tubules. This causes decreased reabsorption of sodium and increased excretion in the urine, even though aldosterone is present **(aldosterone escape).** In such situations, the secretion of ANP (atrial natriuretic peptide) will also be stimulated, leading to natriuresis. However K^+ or H^+ does not show escape from the effect of aldosterone. Hence, continued presence of aldosterone can not stop metabolic alkalosis being developed.

Aldosterone in acid base balance

The acid base balance is disturbed in disorders of aldosterone secretion. In hypersecretion, the increased K^+ and H^+ secretion leads to metabolic alkalosis. In deficiency of aldosterone secretion, there is impaired renal secretion of K^+ and H^+, which results in metabolic acidosis.

Aldosteronism

It is caused by excess secretion of aldosterone and it includes primary and secondary types. The primary aldosteronism is called **Conn's syndrome** and occurs due to a tumor of zona glomerulosa layer. The plasma aldosterone level is elevated. Increased sodium reabsorption and retention of water leads to hypertension, polyuria and hypokalemic alkalosis. Peripheral edema is absent in this type.

Secondary hyperaldosteronism

It usually occurs due to diuretics, nephrosis, cirrhosis of liver and congestive cardiac failure. The increased sodium and water rises the ECF volume which results in edema. Hypertension, hypokalemic alkalosis, muscle weakness are the other symptoms of secondary hyper-aldosteronism. In this type, there will be an elevated renin-angiotensin II in the circulation.

Uses of aldosterone antagonist

Spiranolactone competitively blocks the sodium retaining and potassium excreting effects of aldosterone at the late DCT and CD of the renal tubules.

In hyperaldosteronism, if the potassium level and arterial blood pressure are returned to normal after administering spiranolactone, it is primary

hyperaldosteronism. If only potassium level comes to normal, but not the arterial blood pressure, then it is secondary hyperaldosteronism.

Disorders of adrenocortical secretion

Addison's disease

It is the insufficiency of adrenal cortex caused by autoimmune disease or tuberculosis of cortex. There is deficiency of cortisol secretion. This leads to the elevated level of ACTH which causes skin darkening. Loss of mineralocorticoid leads to the fall in ECF volume and blood pressure. The absence of cortisol causes decreased vascular response to the circulating catecholamine. The peripheral resistance is lowered, blood pressure falls and can lead to circulatory shock. The response to stress is poor and fasting leads to hypoglycemia. When water load is present, the kidneys cannot show the normal water clearance and hence results in water intoxication. Muscle weakness, anemia, decreased GI motility, decreased appetite, nausea, vomiting, weight loss, emaciation, disturbance in the mood and behavior are other characteristic features of Addison's disease.

Addison's disease - hypoadrenalism

Causes:

Atrophy of adrenal cortex by autoimmune disease or tuberculosis lesion.

Primary adrenal insufficiency

↓ECF volume, hyponatremia, hyperkalemia

↓Cardiac output, ↓arterial blood pressure,

↓Blood glucose (hypoglycemia),

inability to excrete water load

↑ ACTH level in the circulation, tanning and

↑ pigmentation of skin

Muscle weakness, anemia

↓Metabolic functions

Inability to cope with stress

The reactions of stress and the physical debility causes Addisonian crisis leading to death.

Hypersecretion of adrenal corticosteroids

Causes:

Cushing disease

ACTH dependent - adenoma of ACTH secreting cells of anterior pituitary, ACTH secreting tumors from other organs (lungs)

Cushing syndrome

ACTH independent – adenoma of adrenal cortex

↑ Protein catabolism, muscles poorly developed

Central distribution of fat, pendulous abdomen, purple striae

Round face, supraclavicular fat retention, buffalo hump

Hyperglycemia, insulin resistant diabetes mellitus

↑ Salt and water retention, hypertension

↑ K^+ loss in the urine, Hypokalemia

Loss of bone mass, osteoporosis, ↓immunity, ↑ susceptibility to infections,

Poor wound healing

↑ Excitability of neurons, psychosis

Darkening of skin in ACTH dependent hypersecretion

Cushing's syndrome

It is caused by excess secretion of adrenocorticosteroids. It is caused by the adrenocortical tumor or pituitary adenoma. The latter, when occurs is known as Cushing's disease. The ACTH level is elevated and skin darkening is present. When exogenous corticosteroids is administered, the ACTH is suppressed and hence skin pigmentation does not occur. Cushing's syndrome is characterized by the weight gain, centripetal distribution of fat giving buffalo hump, pendulous abdomen with purple striae, moon like face and thin limbs.

Polycythemia, muscle weakness, due to hypokalemia and proteolysis are also present. Osteoporosis, poor wound healing, glucose intolerance, hyperglycemia, insulin resistance, steroid diabetes, suppressed immunity, susceptibility to infections, elevated ECF volume, rise in arterial blood pressure, increased capillary fragility and echymoses are features of Cushing's syndrome.

Adrenal medulla

The medullary tissue contains chromaffin cells which secrete **catecholamines** namely **dopamine**, **norepinephrine** and **epinephrine**. The medulla is considered as a component of the sympathetic nervous system because, the medullary cells are homologous to sympathetic ganglia. The splanchnic nerve which supplies these cells forms the preganglionic nerve.

Synthesis and secretion of catecholamines

Norepinephrine is synthesized from the hydroxylation and decarboxylation of tyrosine (Fig. 12.16). Epinephrine is formed by the methylation of norepinephrine by the enzyme (PNMT). This enzyme is present in the brain and adrenal medulla. In the medulla, NE and E are stored in granules with ATP. The granules contain chromogranin A and the ratio of catecholamine to ATP in the granules is 4:1. The secretion is triggered by the release of acetylcholine from preganglionic nerve endings (splanchnic nerve). Acetylcholine opens up cation channels and Ca^{++} enters the cells from ECF. The exocytosis resulting from this, releases catecholamines, ATP and protein. The medullary cells also secrete metencephalin, which is an opioid peptide.

In plasma 95% of DA, 75% of NE and E are conjugated to sulphate. These sulphate conjugates are inactive forms. Plasma DA free level is 35 pg/ml and 50% of this, comes from the adrenal medulla. The remaining comes from the sympathetic ganglia and ANS. The half life of catecholamine is very short, which is 2 minutes.

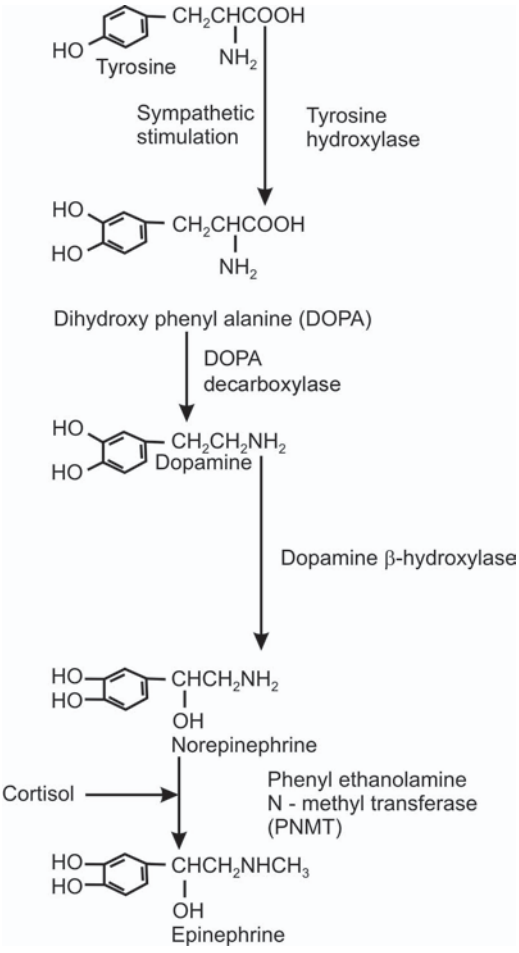


Fig. 12.16: Steps in the synthesis of catecholamines

Metabolism of catecholamines

The catecholamines are methoxylated by **COMT** and then oxidised by **MAO** to *o*-methylated and deaminated derivative. The *o*-methylated deaminated product of epinephrine and norepinephrine is 3-methoxy-4 hydroxy mandellic acid (**vanillyl mandelic acid: VMA).** In humans, 30 µg of NE, 6 µg of E and 700 µg of

VMA are excreted in the urine per day. Catecholamines are also metabolized to metanephrine and normetanephrine and excreted. Most of the urinary VMA and metanephrine are derived from neuronal, rather than adrenal catecholamines.

Physiological effects

The effects of catecholamines are mediated through adrenergic receptors. They are of two types namely **α** and **β**, which are again subdivided into α_1, α_2, β_1, and β_2. The following table gives their distribution and the mechanism by which they produce their effects (Table 12.6).

Table 12.6: Types of adrenergic receptors, their distribution in the tissues and mechanism of action

Receptor type	*Mechanism*	*Site of distribution*
α_1	IP_3 DAG	Postsynaptic terminals
α_2	cAMP	Presynaptic terminals
β_1	cAMP	Heart
β_2	cAMP	Liver

Norepinephrine predominantly acts on the α receptors, whereas, epinephrine acts on both α and β receptors.

On CVS

On the heart the positive inotropic and chronotropic actions are mediated by β_1 receptors. Vasoconstriction of blood vessels is mediated by α_1 receptors. Norepinephrine increases the peripheral resistance and raises arterial blood pressure. This stimulates the baroreceptors producing a reflex bradycardia, which overrides the direct effect of NE on the heart. Hence, the cardiac output decreases. With epinephrine administration, the pulse pressure widens and the baroreceptor stimulation is not sufficient to obscure the direct effect of epinephrine on the heart. Hence, the heart rate and cardiac output increase.

Metabolic actions

Glycogenolysis in the liver and skeletal muscle is stimulated. The increased glucose in the blood stimulates insulin secretion. Insulin facilitates glucose entry into the skeletal muscle and adipose tissue. Epinephrine stimulates glucose formation from lactate, glycogenic amino acids (gluconeogenesis) and glycerol in the liver.

Epinephrine is a ketogenic hormone. It causes the mobilization of fat from the adipose tissue. The increased free fatty acids enter the liver and undergo β-oxidation. The acetyl CoA that is formed gives rise to ketone body formation.

The catecholamines show calorigenic effect as they stimulate the metabolism. This action is present only when there is normal secretion of T_3.

Plasma K^+ level is regulated by catecholamines. Whenever the level of K^+ in the plasma is increased, its transport into the skeletal muscle is stimulated which brings down the level of K^+ in the plasma.

Catecholamines, especially the epinephrine causes alertness, anxiety and fear.

Regulation of secretion

The secretion of catecholamines is through the nervous system especially, the sympathetic nervous system. When the organism is exposed to any emergency situation or stress, the adrenal medullary secretion is stimulated. The action of catecholamines during this period prepares the individual for flight or fight reaction. Exposure to physical stress, anxiety, hypoglycemia, extremes of cold and heat, hemorrhage and muscular exercise are some of the stimuli that stimulate the catecholamine secretion.

Selective secretion

Hemorrhage stimulates epinephrine secretion and not norepinephrine, whereas, emotional stress, which is familiar to the individual, stimulates NE. The unfamiliar emotional

stimulate epinephrine secretion. Anger and active aggression cause NE secretion, while, anxiety causes E secretion.

Disorder of adrenal medullary secretion

Pheochromocytoma

It is caused by the tumor of the chromaffin tissue. The catecholamines secretion is greatly increased with more of NE. The symptoms of this disorder are sporadic rather than continuous. The symptoms noticed are palpitation, hypertension, headache, sweating, anxiety, chest pain and orthostatic hypotension. The elevated urinary excretion of VMA is an important finding in the diagnosis.

HORMONAL REGULATION OF CALCIUM AND PHOSPHORUS METABOLISM

There are three important hormones involved in the regulation of calcium and phosphorus metabolism. They are:

Parathyroid hormone
Calcitonin
1,25, Dihydroxy cholecalciferol (DHCC)

Besides these, there are also other hormones such as growth hormone, glucocorticoids, thyroxine and sex hormones which influence calcium and phosphorus metabolism.

Role of calcium

Calcium is needed for:

- Formation of bone and teeth
- Cell membrane integrity
- Coagulation of blood
- Nerve muscle excitability
- Contraction of muscle
- Secretion of neurotransmitters and hormones.

Calcium metabolism

Adult human contains 1000 mg of calcium. Of this 99% is present in the bone as salts of apatites. The remaining 1% is in soft tissues and ECF.

The ECF contains 9 to 11 mg% of calcium and exists in diffusible and nondiffusible forms. The diffusible form consists of ionic form (50%). The non-diffusible form includes calcium as complex (10%) and bound form (40%). The former exists by combining with citrates and oxalates, while the latter is bound to plasma protein albumin. The free form of calcium is biologically active and the regulatory mechanisms help to maintain this level.

Absorption of calcium from the intestine is stimulated by 1, 25-DHC. The formation of 1, 25, DHC is controlled by parathyroid hormone. Increase in the level of DHC and high protein diet increases calcium absorption. The absorption is inhibited by agents that form insoluble salts with calcium, e.g. oxalates and phosphates.

The sources of calcium and phosphorus in the diet are milk and diary products, egg, meat, fish and wheat. The requirement of calcium in man per day is 0.7 to 1 gm.

When 1000 mg/day of calcium is taken in the diet, 300 mg is absorbed from the upper part of small intestine. Calcium is also secreted in the digestive juices, which constitute 125 mg. This gives the net absorption of calcium as 175 mg/day. The feces excrete 825 mg/day (Fig. 12.17).

The calcium in ECF is around 9 to 11 mg%. Of this, 50% exchanges with calcium present in the bone. The total ECF may contain 900 mg of calcium, which is in dynamic equilibrium with the calcium in bone. The calcium in the bone is present in stable form (99%) as hydroxyapatites, the remaining 1% is readily exchangeable with the ECF calcium. About 500 mg of calcium is deposited into and mobilized from the bone in a continuous process of remodeling.

Calcium excretion in the kidney is related to the regulation of calcium by the parathyroid hormone. Calcium that is filtered at the glomerulus is reabsorbed at the PCT and DCT. The DCT reabsorption is regulated by PTH. Usually, the amount of calcium that is excreted in the urine is equal to the net amount of calcium absorbed from the gut.

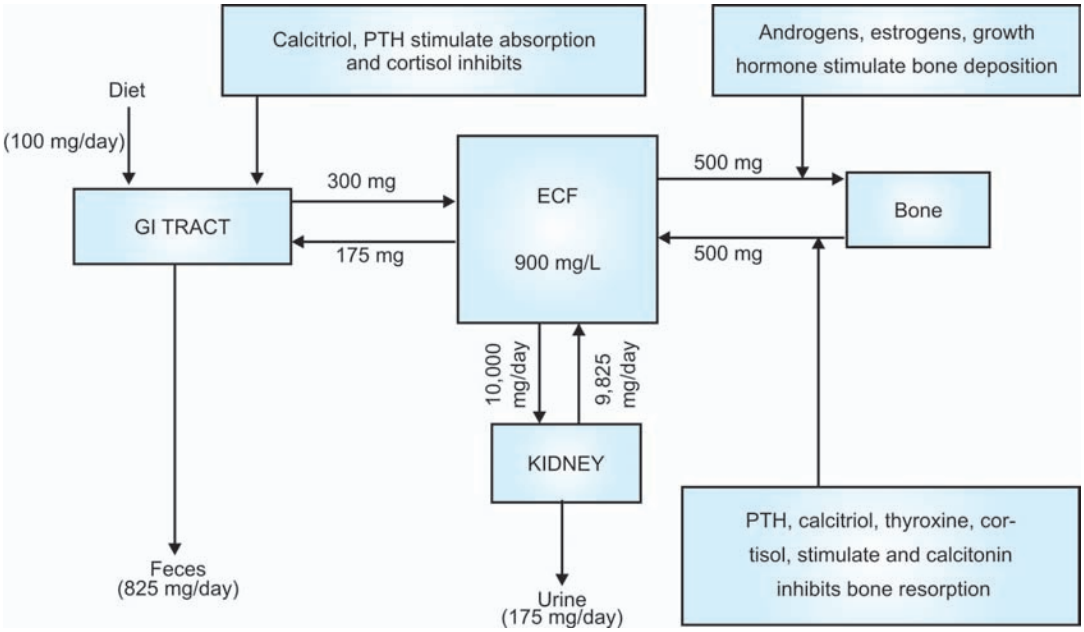


Fig. 12.17: Calcium homeostasis. The calcium balance is maintained by hormones PTH, calcitonin and others, by regulating calcium absorption in the GI tract, renal excretion and bone calcium flux

Phosphorus metabolism

Importance of phosphorus

- Phosphorus helps in the formation of bone and teeth similar to the calcium action.
- It forms part of several compounds such as phosphoproteins, phospholipids and nucleotides.
- Storage of energy in the cells as ATP, ADP and GTP and creatine phosphate.
- Helps in the activity of enzymes.

Total phosphorus in the body is 500 to 800 g and 90% is present in the bone. The plasma contains 12mg%, which includes both inorganic and organic forms. The level of inorganic phosphate in serum is 3 to 4.5 mg%. The regulation is related to the maintenance of this inorganic phosphate (Fig. 12.18).

Absorption from the intestine is facilitated by 1, 25-DHC. Excessive iron binds phosphorus and reduces its absorption. The dietary intake of phosphorus is 800 to 1000 mg/day and out of this 600 mg enters the plasma.

The level of inorganic phosphorus is regulated by PTH, calcitonin and DHCC. The inorganic form is in dynamic exchange with phosphorus present in the bone and muscle. The urinary excretion of phosphorus is related to the regulation of calcium by PTH and calcitonin.

Physiology of bone

It is a dynamic structure which is remodeled throughout life. It is also a highly vascular tissue receiving about 10% of the cardiac output. The organic matrix in the bone is called collagen. The bone mass depends on the relative balance between bone formation and breakdown (resorption). There are two types of cells called **osteoblasts** and **osteoclasts** present in the bone.

Osteoblasts

These bone cells synthesize organic matrix, which consists of collagen and osteocalcin. These cells have receptors for PTH and calcitriol. The enzyme alkaline phosphatase is produced by the

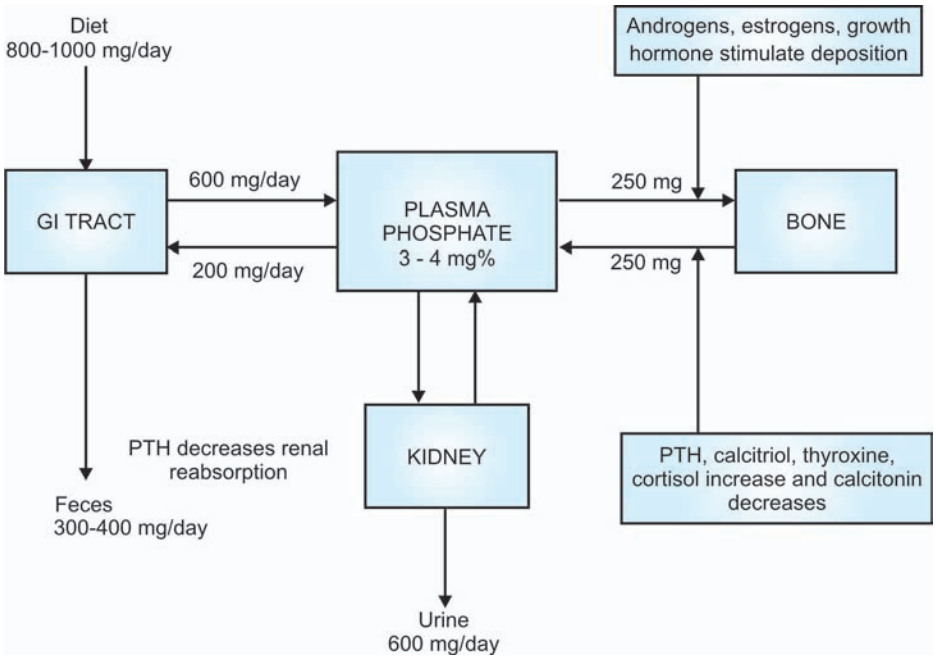


Fig. 12.18: Hormonal regulation of phosphate balance in the body

osteoblasts. It hydrolyzes the phosphate esters and increases the phosphate levels. As the phosphate levels are increased, the tendency of calcium-phosphate salts to precipitate is increased. The osteoblasts, when entrapped in the bone matrix, form osteocyte. These are present in the lacunae and interconnected to one another. The main function of osteocyte is to regulate the flux of minerals in and out of the adjacent bone matrix (Fig. 12.19).

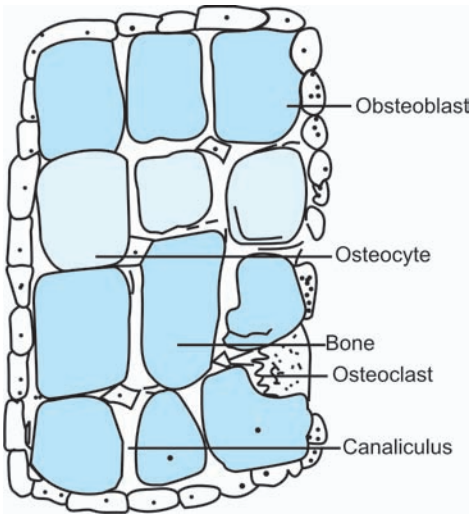


Fig. 12.19: Structure of the three types of bone cells. The canaliculi provide functional communication between osteocytes and osteoblasts

Bone mineral salt

The bone is made of **hydroxy apatite** [$Ca_{10}(PO_4)_6(OH)_2$]. The minerals of bone are continuously exchanged with ECF and a dynamic equilibrium exists between bone and ECF Ca^{++} and PO_4^{--}.

Osteoclasts

They are large multinucleate cells derived from hematopoietic stem cells. They are present external to the bone lining layer of surface osteoblasts. Osteoclasts penetrate bone lining and get attached to bone matrix. This results in bone

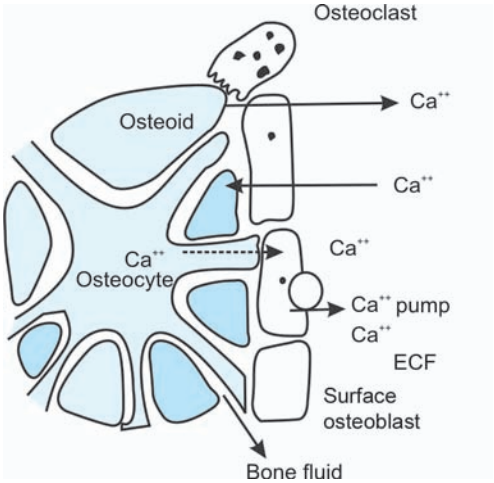


Fig. 12.20: Transport of calcium between ECF and bone. The calcium that enters the osteoid comes to the osteocytes, from where it goes to the surface osteoblasts. From there, the calcium is actively pumped into ECF

resorption (Fig. 12.20). Mature osteoclasts lack receptors for PTH and calcitriol, but have calcitonin receptors. The hormone PTH acts on osteoblast and stimulates the formation of osteoclast activating factors like cytokines.

Parathyroid hormone (PTH)

It is a protein hormone having 84 amino acids. The gland contains chief cells, which secrete the hormone. The hormone is synthesized as preprohormone, which is converted to prohormone. At the time of secretion, prohormone is converted to PTH. The hormone has a short half life in the circulation and degraded in the liver and kidney.

Actions of PTH

PTH acts mainly on three tissues namely, bone, kidney and intestine. It is a hormone, which raises blood calcium level **(hypercalcemic effect).**

Resorption of calcium from bone

PTH increases osteoclast number and its activity. The latter function is mediated through cytokines formed from osteoblasts. PTH also causes the differentiation of osteoclasts precursors into osteoclasts. The resorption of calcium from the bone paves the way for bone remodeling.

The process of resorption is carried out as follows. PTH increases calcium permeability of osteocytes and osteoblasts and calcium enters from bone fluid into these cells. Then, the calcium is transported through the gap junctions from osteocytes to the surface osteoblasts and from there to the ECF (Fig. 12.20). Whenever calcium in the bone fluid is decreased, bone demineralisation is stimulated to attain equilibrium, between calcium in bone and bone fluid. This process is called **osteocytic osteolysis.**

The stimulus for PTH secretion is the fall in serum calcium level. When PTH secretion is deficient, the total serum calcium level falls. If the level decreases below 6 mg%, tetany results. Now, PTH will set a new equilibrium between serum calcium and bone calcium. This will enable serum calcium to be higher and bone calcium to be less.

Increased reabsorption of Ca^{++} from distal tubules of kidney

PTH causes reabsorption of calcium from thick ascending limb of loop of Henle. Although bulk reabsorption occurs in PCT, it is not regulated by PTH. The hormone regulates only 10% of Ca^{++} reabsorption in distal tubules. As the calcium reabsorption is facilitated in these segments of nephron, the phosphate reabsorption is inhibited and its excretion is increased. The net effect is the rise in serum Ca^{++} and fall in phosphate levels. The PTH action on the kidney is mediated through cAMP.

PTH stimulates the renal 1, α-hydroxylase enzyme, which activates vit D_3 to calcitriol (1, 25-dihydroxy cholecalciferol).

Major actions of Parathyroid Hormone (PTH)

Shows hypercalcemic effect

Acts on both osteoblasts and osteoclasts of bone.

Its action on osteoblasts causes release of cytokines which activates osteoclasts.

↑ Bone resorption, ↑ plasma Ca^{++} and ↓plasma PO_4^{--} (osteocytic osteolysis)

↑ Ca^{++} reabsorption in DCT of kidney,

↑ PO_4^{--} excretion in urine

↑ Formation of 1,25 Dihydroxycholecalciferol (DHCC) from kidney, which ↑ absorption of calcium and phosphate from intestine

Absorption of calcium from the intestine

The calcitriol formed from the kidney by the action of PTH, facilitates absorption of both calcium and phosphorus from the intestine.

Regulation of PTH secretion

Blood level of calcium regulates the secretion of parathyroid hormone. Fall in serum calcium level stimulates, while high level of calcium does not abolish the secretion, but decreases the level (Fig. 12.21).

Calcitonin

It is a polypeptide hormone with 32 amino acids. It is secreted from the C cells of thyroid gland.

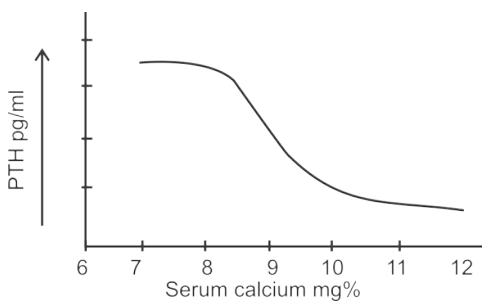


Fig. 12.21: Regulation of PTH by the serum calcium. When blood level of calcium level is high, PTH secretion is inhibited. Fall in blood level of calcium raises PTH level

The C cells are also known as parafollicular cells. Its main actions are seen in the bone and kidney. Since calcitonin is inactivated in the kidney, renal failure increases calcitonin level.

Actions of calcitonin

On bone, it inhibits bone resorption and lowers serum Ca^{++} and PO_4^{--} levels. The resorption of bone is inhibited by the inhibition of osteoclast differentiation from its precursors and the activity of the existing osteoclasts. The osteoclasts have receptors for calcitonin and the action is mediated by cAMP.

On kidney

Calcitonin increases the renal clearance of both calcium and phosphate.

Regulation of calcitonin occurs by the serum level of calcium. Rise in serum calcium level stimulates the secretion of not only calcitonin, but also the secretion of GI hormones like glucagon, secretin and CCK.

In humans the role of calcitonin is not very clear because, there has been no report of complications due to its deficiency or hypersecretion. However, administration of calcitonin to patients with high bone turnover disorder, like Paget's disease has shown improvement in reducing the bone turnover.

Calcitriol

Vit D_3 (cholecalciferol) is obtained from the diet and synthesized in the skin in the sunlight from cholesterol derivative 7-di hydrocholesterol. Vit D_3 is not the active form. It is 25- hydroxylated in the liver to 25-hydroxycholecalciferol and then a hydroxylated by PCT in the kidney to 1,25 dihydrocholecalciferol (DHCC). The formation of DHCC in the kidney is catalyzed by the enzyme 1α hydroxylase. Serum PTH and phosphate directly regulate 1α-hydroxylase activity. Fall in serum phosphate and secretion of PTH stimulates this enzyme activity. Fall in the serum Ca^{++} indirectly stimulates α-hydroxylase enzyme activity through PTH secretion.

Calcitriol is transported in the blood in bound form with **transcalciferin**. Hence, its half life in the circulation is long (5 to 24 hrs).

Actions

Intestine

Calcitriol increases absorption of Ca^{++} and PO_4^- from the intestine. This action is produced by increasing the formation of calcium binding proteins calbindins, especially **calbindin- D**. Calbindin D protein formation in the intestine facilitates Ca^{++} transport across the intestinal epithelium. From the intestinal cells Ca^{++} is pumped by Ca^{++}- H^+ ATPase which is also facilitated by calcitriol.

Bone

Calcitriol stimulates synthetic activity of osteoblasts which is needed for normal calcification of bone matrix. The stimulation of osteoblasts causes secondary increase in the activity of osteoclasts. Deficiency of vitamin D impairs bone mineralization leading to rickets in children and osteomalacia in adults.

Kidney

Calcitriol causes increased Ca^{++} reabsorption in the kidneys.

Calcitriol receptors are also present in other tissues which include skeletal and cardiac musles, skin, lymphocytes, monocytes, breast, anterior pituitary gland. DHCC is believed to be involved in the regulation of growth and production of growth factors.

The level of calcitriol is increased in pregnancy, which helps to increase the absorption of calcium from the intestine.

Other hormones acting on bone

Estrogen

Estrogen receptors are present in osteoblasts and hence a direct action on these cells is seen by estrogen. After menopause, fall in the estrogen level causes demineralization and administration of estrogen in such cases, reduces the urinary excretion of hydroxy proline levels, showing the estrogen effect in reducing bone loss, though, not capable of stimulating formation. Androgens also have similar effect.

Both estrogen and androgen cause fusion of epiphysis of long bones and stop longitudinal growth.

Glucocorticoids

Glucocorticoids decrease the absorption of calcium from intestine and increase its excretion in the urine. The fall in serum calcium level, as a consequence to this leads to a mild secondary hyperparathyroidism. Cortisol receptors are present in osteoblasts and the hormone decreases its activity. The synthesis of bone matrix and bone protein osteocalcin is also reduced by excess cortisol. The hypersecretion of cortisol leads to osteoporosis.

Thyroxine

Excess secretion of thyroid hormone causes osteoporosis, because of increased bone turnover. The bone resorption becomes greater than bone formation.

Disorders of Ca^{++} and PO_4 balance

Hyperparathyroidism

Primary hyperparathyroidism It is caused by the increased secretion of PTH due to the tumor of parathyroid gland. The serum level of Ca^{++} is elevated and PO_4 level decreased as the kidneys excrete more phosphate in the urine. The changes in the serum Ca^{++} level is due to the increased bone resorption. The symptoms are related to hypercalcemia and hypercalciuria. The symptoms are reduced neuromuscular excitability, fatigue, mental confusion, psychological depression, decreased muscle tone and muscle weakness. The ECG shows decreased QT interval and in severe cases, cardiac arrest also can occur. The renal calculi are another

complication of this disorder. The bone shows osteoporosis, bone pain and multiple bone cysts **osteitis fibrosa cystica**. The plasma level of alkaline phosphatase and osteocalcin are elevated and urinary hydroxyproline excretion is increased. The latter finding indicates high bone resorption, as hydroxy proline is present typically in type I collagen.

Hyperparathyroidism

Causes:

PTH secreting adenoma (primary hyperparathyroidism, Chronic renal disease, Rickets (secondary hyperparathyroidism)

↑ PTH secretion

↑ Serum Ca^{++} and ↓$PO4^{--}$ (↑ osteoclastic activity in the bones)

Osteitis fibrosa cystica develops

↑ Alkaline phosphatase in the plasma

Renal calculi, muscle weakness, constipation, lack of appetite, ↓QT interval in ECG, cardiac arrest and death

Hypoparathyroidism

Produces hypocalcemia and tetany

Causes:

Surgical removal of parathyroid gland, hypomagnesemia, tissue resistance to PTH

Deficiency of PTH and calcitriol

↓Serum Ca^{++} and ↑ $PO4^{--}$ (osteocytic osteolysis in the bone is inhibited due to lack of PTH)

↓Serum Ca^{++} causes latent tetany

If the serum Ca^{++} falls below the critical level, frank tetany occurs

↑ Neuromuscular excitability

Chvostek's sign
Trousseau's sign
Laryngeal spasm
Choking and death

Pseudohypoparathyroidism

It is a rare familial disorder, which is characterised by tissue resistance to PTH secretion.

Hypoparathyroidism (Tetany)

It is caused by the deficiency of PTH and calcitriol. The serum Ca^{++} is decreased, while the PO_4^{--} level is increased. There is inhibition of osteoclastic resorption and osteocytic osteolysis, due to the lack of PTH. The urinary HCO_3^- excretion is reduced and this leads to alkalosis. This further lowers the Ca^{++} level in the serum.

The symptoms of hypoparathyroidism are due to the fall in serum Ca^{++} level. If the calcium level falls below the critical level, then **frank tetany** occurs. Some times serum Ca^{++} is not low enough to produce frank tetany, but a **latent tetany** can be demonstrated as in carpopedal spasm **(Trousseau's sign)** and **Chvostek's sign.**

Frank tetany can give rise to increased neuromuscular excitability, muscle cramps, tingling in the fingers or toes (paresthesia), laryngeal spasm and biliary colic. Laryngeal spasm can cause choking and death.

Treatment of tetany includes high Ca^{++} diet, calcitriol administration, sometimes thiazide diuretics, which can increase reabsorption of Ca^{++} in the thick ascending limb of LH. Acute hypocalcemia is treated with intravenous calcium gluconate injection.

Hypoparathyroidism can also occur due to **hypomagnesemia**. Fall in magnesium level due to malabsorption or chronic alcoholism inhibits secretion of PTH and reduces the biological response to PTH presence.

ENDOCRINE FUNCTION OF PANCREAS

The hormones of pancreas are secreted from islets of Langerhans, which constitute about 2% of pancreatic tissue. The islets have four types of cells. The cell types, the extent of their distribution in the Islets and their secretion are as follows:

A	(25%)	Glucagon (α)
B	(60%)	Insulin (β)
D	(10%)	Somatostatin (δ)
F	(5%)	Pancreatic polypeptide

Autonomic fibers comprising sympathetic and parasympathetic nerves end close to α, β and δ cells, and modulate their secretions through the release of neurotransmitters. Vagal stimulation and acetylcholine administration stimulate insulin release, when glucose level is high in the plasma. The stimulation of sympathetic fibers or administration of norepinephrine causes, inhibition of insulin release and stimulation of somatostatin secretion. These effects are mediated through α-adrenergic receptors.

The islets cells show functional syncytium. The secretion from one type of islet cell diffuses into the another type of islet cell and regulates its secretion. This is a paracrine control system, which regulate islet cell function. Accordingly, insulin inhibits α cell and glucagon stimulates β cell. Somatostatin inhibits both α and β cells secretions. Pancreatic polypeptide inhibits β cell and δ cell secretions.

Insulin

It is a protein hormone secreted from β cells of islet of Langerhans. It is a hypoglycemic hormone. Human insulin consists of 51 amino acids, having molecular weight 5808. Insulin shows antigenic property, as it is a protein and hence injections of insulin from one species into another species develop antibodies to the injected hormone. Human insulin is now prepared by the recombinant DNA technology, which does not have any antigenic property.

Synthesis and secretion

Insulin is synthesized in the β cell as preproinsulin, which is converted to proinsulin. Proinsulin is a single chain polypeptide with 86 amino acids. There are three chains namely A, B and C chains present in the proinsulin molecule (Fig. 12.22). In the Golgi body, cleavage of proinsulin to insulin occurs, which involve the separation of C chain. The insulin molecule contains A and B chains connected by disulfide bonds. The A chain has intradisulfide ring. The separated C chain contains 31 amino acids and β cell secretes equimolar concentrations of insulin and C chain into the circulation. Even though, the C chain has no biological activity, its measurement in the plasma reflects the β cell activity.

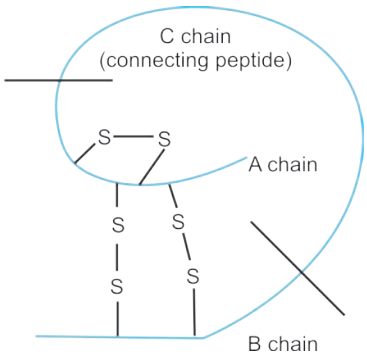


Fig. 12.22: Structure of proinsulin. The connecting peptide C chain is necessary for the disulphide bridges formation. The C chain is cleavaged at the time of insulin secretion, but together with insulin, it is secreted from the β cell of the islets

Synthesis and secretion of insulin are closely linked. The synthesized insulin is stored in the β cell, as a crystalline hexamer with two atoms of zinc per hexamer. The β cell has on its membrane, glucose transporter called GLUT 2. This causes facilitated diffusion of glucose into the β cell. The enzyme glucokinase in the β cell phosphorylates glucose and this is the rate limiting reaction for further entry of glucose into the β cell. This in turn determines the rate of insulin secretion.

The K^+ channels in the β cell membrane close, due to ATP formation and the resulting depolarization opens up Ca^{++} channels. The intracellular Ca^{++} concentration increases which helps the movement of secretory granules towards the cell membrane. The entry of Ca^{++} finally causes exocytosis and the hormone is released into the circulation.

The secretion of insulin from the β cell also occurs through cAMP and protein kinase C formation.

The secreted insulin in the circulation is transported as monomer and its half life in plasma is 5 minutes.

Regulation of secretion

Carbohydrate

The main stimulus for the release of insulin is hyperglycemia. Rise of plasma glucose more than 80 mg% stimulates insulin synthesis and secretion. The administration of monosaccharides (hexoses and trioses) that can be utilised by the β cell, stimulates insulin release. Infusion of 2 deoxy glucose, which is not metabolized by the β cell does not cause insulin secretion.

GI hormones

Plasma insulin is increased after oral administration of glucose than after IV glucose administration. It is due to the release of GI hormones like GIP, gastrin, secretin and CCK in response to oral glucose intake which cause direct stimulation of β cell secretion.

Amino acids

Essential amino acids like arginine, lysine, and phenylalanine stimulate insulin secretion. Similar to glucose, oral administration of amino acids causes increase in insulin secretion mediated by GI hormones.

Islet hormones

Glucagon stimulates insulin secretion while somatostatin inhibits its secretion.

Autonomic nerves stimulation

Stimulation of vagus causes insulin secretion, when plasma glucose level is normal and stimulation of sympathetic nerves inhibits insulin secretion.

Free fatty acids

Increase in free fatty acids and ketone bodies in the plasma stimulate insulin secretion.

cAMP

cAMP stimulates insulin secretion.

Ions

Ions like K^+ and Ca^{++} are necessary for the normal insulin and glucagon responses to glucose.

Pattern of insulin secretion in response to rise in plasma glucose

When glucose level in plasma rises, insulin secretion shows a biphasic response. First, there is an immediate rise in insulin secretion, which falls to rise again slowly in the next 1 hour giving the biphasic response. The first rise may be due to the release of performed insulin and the late phase may represent the secretion of newly formed insulin.

Metabolism of insulin

Insulin is metabolized in the liver and kidney. Specific enzyme splits the disulphide bonds separating A and B chains.

Mechanism of action of insulin

Insulin receptors have α and β subunits. The α unit is extracellular and binds to insulin, while the β unit spans the membrane and contains tyrosine kinase. When insulin binds to αsubunit, the tyrosine kinase activity of β subunit is stimulated causing autophosphorylation of tyrosine residues on β subunit. The phosphorylated receptor acts by either phosphorylating or dephosphorylating other proteins and enzymes inside the cell. This eventually leads to metabolic effects (Fig. 12.23).

Actions of insulin

Receptors for insulin are present in the liver, muscle, adipose tissue, lymphocyte, monocytes

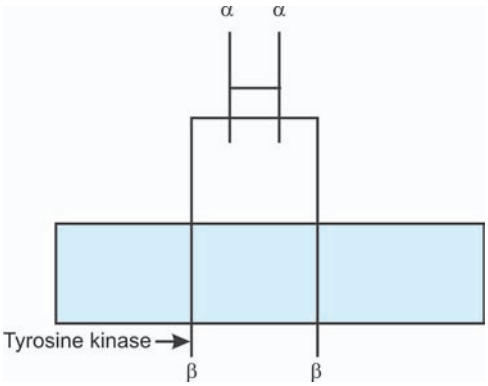


Fig. 12.23: Insulin receptor. There are two alpha and two beta subunits, with alpha units present on the outside of the membrane and contain the hormone binding sites. The beta subunits are situated on the inner cytosolic side and contain tyrosine kinase activity. The hormone receptor complex causes phosphorylation of tyrosine residues of beta units and cytoplasmic proteins. It is likely that insulin mediates its action by the phosphorylation of tyrosine residues of cytoplasmic proteins

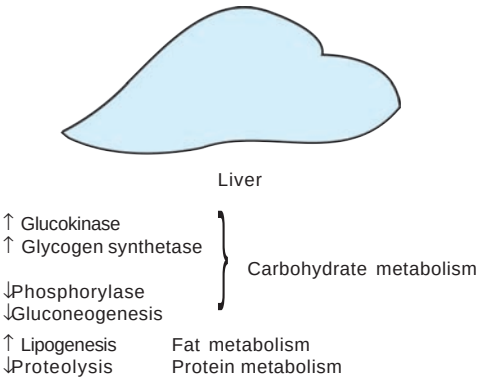


Fig. 12.24: Summary of actions of insulin in liver

and granulocytes. Insulin independent tissues are RBC, lens, mucosa of small intestine, renal tubular brush border, brain regions except for pituitary, ventromedial and lateral nucleus of hypothalamus.

Transport of glucose into the cell

Insulin promotes glucose entry into the liver, muscle and adipose tissue. The glucose is transported across the membrane, by binding to transporter protein called GLUT and diffuses into the cell (facilitated diffusion). The type of transporter in liver is GLUT 2 and in muscle and adipose tissue is GLUT 4. These transporters are regulated by insulin.

On carbohydrate metabolism

In the liver, glucose can enter the cells even when insulin is absent. In liver, glucose is phosphorylated to glucose-6-phosphate by glucokinase. Insulin stimulates glucokinase and activates glycogen synthetase, which promotes glycogenesis. Insulin inhibits gluconeogenesis and glycogenolysis, which decreases the output of glucose from the liver (Fig. 12.24). Insulin also promotes conversion of glucose into fatty acids and very low density lipoproteins in the liver.

Actions of insulin on liver

↑ glucose uptake when plasma glucose is high
↑ glycogenesis, ↓glycogenolysis
↑ glycolysis, ↓gluconeogenesis
↑ fatty acid synthesis and very low density lipoprotein formation, ↓ketogenesis.

In the adipose tissue, glucose entry into the cell is followed by its conversion to α-glycerophosphate. This is utilized for the esterification of FFA.

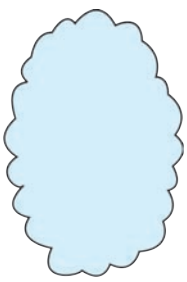
In the muscle, the glucose uptake is increased and inside the cell, glucose undergoes glycolysis and glycogenesis.

Fat metabolism

Insulin is lipogenic and anti lipolytic. In the liver, insulin causes lipogenesis. Most fatty acid synthesis occurs in the liver. The synthesized fatty acids are esterified with a glycerophosphate to produce triglycerides and then transported to the storage depot adipose tissue. The transport is

predominantly as very low density lipoprotein (VLDL) to the adipose tissue.

In the adipose tissue membrane, insulin activates lipoprotein lipase, which hydrolyzes triglyceride in VLDL to fatty acids and glycerol (Fig. 12.25). The entry of glucose into the adipose tissue leads to the formation of α-glycerophosphate, which is utilized for the esterification of fatty acids to triglyeride. The short chain fatty acids, present into the chylomicrons also are removed from the circulation in a similar fashion. Insulin, while favouring lipogenesis, inhibits lipolysis in the adipose tissue, by inhibiting hormone sensitive lipase enzyme. Deficiency of insulin leads to the activation of this enzyme, causing lipolysis and from acetyl CoA that is formed, ketogenesis is stimulated. Deficiency of insulin would also cause rise in VLDL level in serum, as they are not effectively cleared from the circulation.



Adipose tissue

↑ glucose transport
↑ glycerol synthesis
} carbohydrate metabolism

Fat metabolism

↑ Triglyceride
↑ Fatty acid synthesis
Activates lipoprotein lipase which hydrolyses triglycerides in VLDL to fatty acids and glycerol
↓ Lipolysis

Fig. 12.25: Actions of insulin on adipose tissue

Action of insulin on adipose tissue

↑ glucose uptake, ↑ glucose utilization,
↑ glycolysis, ↑ formation of α-glycerophosphate
↑ esterification of fats, ↓lipolysis.

Protein metabolism

Insulin is an anabolic hormone and is necessary for the normal growth. It increases the amino acid uptake in the muscle and stimulates protein synthesis (Fig. 12.26). Deficiency of insulin causes proteolysis.

Skeletal muscle

Carbohydrate metabolism
↑ Glucose transport
↑ Glycolysis
↑ Glycogen synthesis

Protein metabolism
↑ Amino acid uptake
↑ Protein synthesis

Fig. 12.26: Action of insulin on skeletal muscle

Action of insulin on muscle

↑ glucose uptake, ↑ glycogenesis,
↑ glycolysis, ↓glycogenolysis,
↑ amino acid uptake, ↑ protein synthesis,
↓proteolysis

Electrolyte metabolism

Insulin lowers serum K^+ level by stimulating its uptake by the muscle and hepatic tissue. Deficiency of insulin leads to K^+ leaving the cell to enter ECF and excreted via kidney.

Action on ion permeability

Insulin lowers membrane permeability to Na^+ and K^+. The permeability of Na^+ is much more reduced than K^+. This results in hyperpolarisation of the muscle cell.

Diabetes mellitus

It is a pathological disorder caused by an absolute or relative deficiency of insulin. The major types of this disorder are:

Type 1 (IDDM): Insulin dependent diabetes mellitus: Usually caused by the autoimmune disease. The insulin level in the plasma is very low and not detectable. Majority of the cases occurs in childhood and hence the term juvenile diabetes to this disorder is used. Ketosis and acidosis are common in this type.

Symptoms of diabetes mellitus include hyperglycemia, glucosuria, polyuria, polydipsia, polyphagia, weight loss, ketosis and acidosis. The untreated and refractory cases can lead to coma, convulsion and death. Long-term problems associated with diabetes mellitus are neuropathies, myopathies, retinopathies, hyperlipemia, nephropathies, atherosclerosis, hypertension, coronary artery disease, sterility and loss of libido.

Type 2 (NIDDM): Non-insulin dependent diabetes mellitus: In this disorder, plasma insulin level is normal or elevated, but the tissue response to insulin is subnormal. This disorder is late in onset. It occurs usually after the age of 40 years. Ketosis and acidosis are not common in this type. This type does not require insulin to maintain life.

Glucose tolerance test will tell whether the individual is diabetic or not.

Preparations of insulin

Different forms are available such as crystalline insulin, insulin complex with protamine and zinc insulin. The long-acting one is zinc insulin. Insulin has to be given only by injection, since it is a protein hormone.

Oral hypoglycemic agents

Oral hypoglycemic agents are available and they are given, when some insulin secretion is present. These agents act by either increasing the secretion of insulin or delay the absorption of glucose from the GI tract. Sulfonylureas (tolbutamide, chlorpropamide) belong to the former category, while biguanides (phenformin, metformin) fall under the latter category.

Glucagon

It is a protein hormone with 29 amino acids. It is a hyperglycemic hormone and its major actions are seen in the liver. Glucagon is produced from α cells of islets of Langerhans pancreas.

Actions

Carbohydrate metabolism

It stimulates hepatic glycogenolysis and gives hyperglycemia. The increase in blood glucose is not from the inhibition of peripheral utilization of glucose.

Glucagon promotes gluconeogenesis in the liver. The hyperglycemic action of epinephrine is amplified by the glucagon secretion. The hyperglycemic action of glucagon is also enhanced by its effect in inhibiting Insulin secretion.

Fat metabolism

Glucagon is a lipolytic hormone, as it stimulates hormone sensitive lipase enzyme in the adipose tissue. The free fatty acids and glycerol rise in the blood. Glycerol is utilized as a glyconeogenic substrate in the liver. The fatty acids are utilized as energy substrate, sparing glucose.

Glucagon also promotes ketogenesis and in the absence of insulin, glucagon can accelerate ketogenesis, leading to metabolic acidosis.

Protein metabolism

Glucagon has a proteolytic effect in the liver. It promotes gluconeogenesis and has an anti anabolic effect.

Mechanism of action

Glucagon binds to the extracellular receptors that have Gs proteins. The hormone receptor complex finally leads to the formation of cAMP. The increase in the cAMP levels initiates metabolic changes associated with the enzyme phosphorylation.

Regulation of glucagon secretion

Hypoglycemia, amino acids especially arginine, alanine, fall in free fatty acids are the potent stimuli for the glucagon secretion.

GI hormones such as gastrin and CCK also stimulate glucagon secretion.

Glucagon secretion is inhibited by the rise in blood glucose, insulin and somatostatin.

Somatostatin

It is a tetradecapeptide (14 amino acids) produced from the delta cells of islets of Langerhans. It is also secreted from the other locations such as GI tract and hypothalamus.

Actions

The pancreatic somatostatin diffuses into the α and β cells, causing inhibition of insulin, glucagon and pancreatic polypeptide secretions.

The gut hormone causes slowing of the gut motility and inhibits the release of gastrin, secretin, VIP and CCK. The intestinal absorption of glucose is decreased.

Somatostatin secreted from the hypothalamus is a growth hormone inhibiting hormone. It acts on the anterior pituitary and inhibits the secretion of growth hormone and TRH. Somatostatin secretion is increased by glucose, amino acids and CCK.

Pancreatic polypeptide

It is a polypeptide with 36 amino acids. It is secreted from the F cells of the islets. The hormone is secreted by:
Ingestion of protein meal,
Hypoglycemia,
Severe exercise.

The secretion is also mediated by the vagus nerve stimulation. Pancreatic polypeptide inhibits the exocrine function of the pancreas and gallbladder contraction.

REPRODUCTIVE SYSTEM

DEVELOPMENT OF GONADS AND GENITALIA

In humans, there are 22 pairs of autosomes and 1 pair of sex chromosomes. XY and XX, are the sex chromosomes in males and females respectively. If a sperm containing X chromosome fuses with the female germ cell, the resulting genetic sex is XX which is a female. If the sperm containing Y chromosome fuses with the female germ cell, the genotype would be XY and this gives male genetic sex.

Gonadal sex

Until 6th week of gestation, the gonads are bipotential. The primitive gonad contains cortex and medulla. The medulla develops into testes, if the XY genotype is present. The short arm of Y chromosome secretes a gene material called *testis determining factor* from the **sex determining region of Y chromosome (SRY).** This is different from the H-Y antigen, which is also secreted from the short arm of Y chromosome. H-Y antigen formation is essential for the normal spermatogenesis. The differentiation of medulla of the primitive gonad into testes causes the cortex to regress.

The presence of XX genotype leads to the cortex developing into ovaries and the medulla regresses.

Genitalia development

Until the 8th week, the primordial ducts, which develop into internal genitalia, are bipotential. That is, the primordial ducts contain a pair of Wolffian and Mullerian ducts. The male internal genitalia is differentiated from the Wolffian duct in the presence of fetal testosterone and Mullerian regression factor. In the genetic male, the fetal testis actively secretes testosterone, by the trophic action of human chorionic gonadotrophin. The

female internal genitalia are differentiated from Mullerian ducts due to the absence of testosterone and Mullerian regression factor. The internal genitalia are developed by 10 weeks of gestation.

The external genitalia in male are developed by the action of dihydrotestosterone (DHT), which is a metabolite of testosterone. The enzyme 5 α reductase converts testosterone to dihydrotestosterone. The absence of dihydrotestosterone in female gives the development of female external genitalia. By about 12 weeks of gestation, the external genitalia are normally developed.

Wolffian duct is stimulated by ***fetal testosterone*** to develop into **male internal genitalia,** which include:

- Epididymis
- Vas deferens
- Seminal vesicle
- Ejaculatory ducts.

The presence of **dihydro testosterone (DHT),** develops ***external genitalia in male***. They are:

Prostate gland

- Penis
- Scrotum

Mullerian duct in the absence of testosterone and Mullerian regression factor, develops into **female internal genitalia** which include:

- Fallopian tube
- Uterus
- Cervix
- Upper one-third of vagina

The **absence of dihydro testosterone** develops ***female external genitalia*** which include:

- Lower portion of vagina
- Clitoris
- Labia majora
- Labia minora

Sex chromatin (Barr body)

In the somatic cells of adult females, one of the X chromosomes condenses and appears as Barr body or sex chromatin. It can be seen when a buccal smear is taken and its cells are examined. The Barr body appears at the nuclear membrane. The sex chromatin also appears in females in the polymorphonuclear leucocytes (5 to 25%) as drumstick from the nucleus. The X chromosome to be condensed is selected randomly. The importance of sex chromatin test is to find out the sex of the person as female by examining the somatic cells.

Abnormalities of sex development

Genetic disorders

The development of sex organs becomes abnormal if there is a genetic disorder. The nondisjunction of sex chromosomes can cause abnormalities such as *gonadal dysgenesis and seminiferous tubule dysgenesis.* During meiotic division of ovum, one of the daughter cells receives both the X chromosomes and the other none. If fertilization occurs, between X sperm and ovum having no sex chromosomes (O), the resulting chromosomal pattern will be **XO.** This is known as **gonadal dysgenesis or Turner's syndrome**. In this condition, the ovaries are absent and menstrual cycle does not occur. The individual is short stature and mentally retarded.

If fertilization occurs between Y sperm and ovum with XX chromosomes, the resulting karyotype **is 47 XXY.** There is an additional X chromosome in genetic male. This gives rise to **seminiferous tubule dysgenesis,** which is also known as **Klinefelter's syndrome.** This is the most common genetic disorder in the sex development. The testes are small and the testosterone secretion shows low to normal pattern. There is also possibility of gynecomastia. The genitalia are also normal, but the individual is sterile because of the defect in the seminiferous tubules. The spermatogenesis is affected and hence sterility.

True hermaphroditism

When the sex chromosomal pattern forms XX/XY, then both the gonads develop.

Pseudohermaphroditism

This is the condition in which the gonads of one sex and the genitalia of opposite sex develop.

In **female pseudohermaphroditism**, the gonads are ovaries, but the external genitalia are that of male. This can occur in the following conditions:

- Administration of androgens to the mother during 5th to 12th weeks of pregnancy.
- Presence of congenital adrenal virilizing hyperplasia in the mother.
- Tumors of androgen secreting cells of adrenal gland in the mother during pregnancy.

Male pseudohermaphroditism refers to the condition in which the gonads developed are that of male, but the external genitalia are that of female. It can occur due to:

- Absence of testosterone secretion from the defective fetal testis or
- Deficiency of enzymes in the synthesis of testosterone (17 α-hydoxylase enzyme deficiency).
- Androgen resistance
- Deficiency of 5 α-reductase enzyme

Puberty

The puberty refers to the maturation of reproductive organs where reproduction becomes possible. The mechanism of onset of puberty is explained by the gradual decline in the sensitivity of hypothalamic neurons to the circulating sex hormones. This leads to the removal of feedback inhibition on gonadotrophin releasing hormone secretion (GnRH). The pulsatile release of GnRH from the hypothalamus is believed to be responsible for the onset of puberty. The rise in pituitary gonadotrophins causes increase in gonadal activity with the secretion of sex hormones and gametogenesis. In girls, the gonadotrophin secretion occurs cyclically, which is responsible for the menstrual cycle. In boys, the gonadotropin secretion is noncyclical.

In boys and girls, much earlier to the onset of puberty, i.e., from 7 to 10 years of age, the adrenal androgen secretion increases and is known as **adrenarche.** In girls, the breast development first occurs **(thelarche)** and then the development of pubic and axillary hair **(pubarche).** This is followed by the onset of first menstrual bleeding **(menarche).** In girls, menarche occurs by 12 to 14 years of age. In the first year, the reproductive cycle is usually anovulatory and also the cycle is irregular. In boys, the growth of pubic and axillary hair starts around 10 years of age. The onset of puberty is seen by 13 to 14 years of age.

Precocious puberty

In this condition, the sex organs mature earlier than the normal age. In **true precocious puberty**, the sex organs are matured and reproduction is possible. In **pseudoprecocious puberty**, there is only adult type of genitalia development present without the ovarian or testicular development. **True variety** can occur in the tumor of gonads or pineal gland. Constitutional form of true precocious puberty is also present and is more common in girls. The **pseudo type** is observed in congenital virilizing hyperplasia.

TESTIS

During the 7th month of intrauterine life, the testes descend through the inguinal canal into the scrotum, facilitated by the presence of androgen and Mullerian regression factor. The temperature of the testes is normally maintained at 32°C which is 5°C lower than the core body temperature. The lower temperature is necessary for spermatogenesis.

Adult testis performs two important functions namely, **production of gametes and secretion of sex steroids**. The gamete production is known as spermatogenesis and occurs in seminiferous tubules. The steroid secretion takes place in the Leydig cells. Seminiferous tubules comprise about 80% of testicular volume and the remaining 20% are occupied by the Leydig cells.

Epididymis

Seminiferous tubules open into rete testis and then into efferent ductules. This finally

coal-esces into a single duct called epididymis. Epididymis drains into vas deferens. Sperms which enter the epididymis are immature and nonmotile. During their stay in epididymis, sperms mature. The sperms during their transit through the ducts, are bathed by the seminal fluid, secreted from the seminiferous tubules, seminal vesicle, prostate and bulbourethral glands.

Sertoli cells

Seminiferous tubules consist of peritubular cells, a basement membrane, the germinal epithelium, developing germ cells and Sertoli cells. The Sertoli cells line the basement membrane and extend into the developing germ cells. These cells form the tight junctions and form the **blood testis barrier**. The barrier restricts the transport between the blood and the developing germ cells. The developing germ cells are in adluminal compartment and are separated from the basal compartment, which is in contact with extracellular fluid (Fig. 12.27). The blood testis barrier prevents the entry of matured germ cells into the circulation, as they are immunogenic.

The other functions of Sertoli cells include mechanical support to the developing germ cells and spermiogenesis. The latter process helps maturation of spermatid into spermatozoan. The Sertoli cells secrete a protein called *androgen binding protein,* which binds androgen and maintain its level higher in testis. During intrauterine life, the embryonic testis secretes a protein called *Mullerian regression factor,* which regresses Mullerian duct, facilitating the development of male genitalia. In adult testis, Sertoli cells also secrete **inhibin, activin, follistatin, IGF$_I$** and **cytokines**. Inhibin released from the Sertoli cells regulates FSH secretion, by inhibiting it. Sertoli cells have receptors for androgens and FSH, but not for LH.

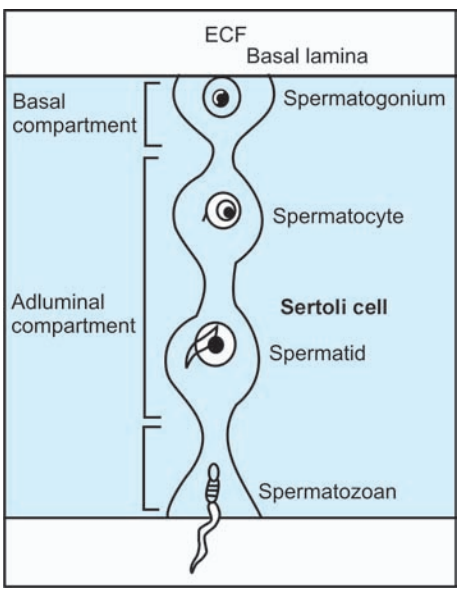


Fig. 12.27: Spermatogenesis from the seminiferous tubules and the formation of blood testis barrier by the Sertoli cells. The Sertoli cells line the basal lamina and extend deep into the seminiferous tubule. The Sertoli cell and the developing germ cells form tight junctions to give blood tests barrier. See text for other functions of Sertoli cells

Spermatogenesis

The process of spermatogenesis begins at the onset of puberty and continues for the rest of the life. The spermatogonium is present in the basal layer of the seminiferous tubule. It divides mitotically into primary spermatocytes. It gives two quick successive meiotic divisions to produce secondary spermatocyte and spermatid (Fig. 12.28). The last stage germ cells will have haploid number of chromosomes. The spermatids undergo spermiation with the help of Sertoli cells and form spermatozoa. The germ cells, after primary spermatocyte, are linked to one another by intercellular cytoplasmic extensions. Each

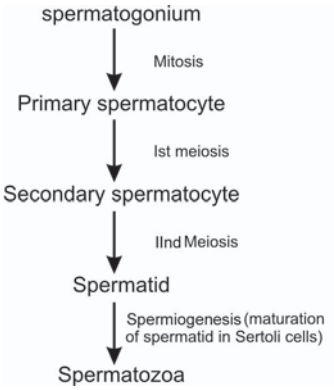


Fig. 12.28: Stages of spermatogenesis

spermatogonium gives rise to 64 sperm cells and spermatogenesis requires 74 days for completion in man.

The sperms enter the epididymis, where in its head region, they undergo maturation, which requires adequate testosterone secretion. By the time the sperms reach the tail of epididymis, they are capable of directional motility and capacitation. The sperms approximately spend 10 to 14 days in the epididymis for maturation. The movement of sperms in the ductile system is due to combination of fluid pressure, ciliary motion and peristaltic contraction.

Spermatogenesis requires FSH and testosterone, as they directly act on the seminiferous tubules.

Spermatogenesis is affected when the scrotal temperature is high. The counter current mechanism in the pampiniform plexus carries away the heat and reduces the scrotal temperature by 5°C. Presence of varicocele abolishes this effect and hence, spermatogenesis is affected in such conditions. Exposure to X-ray radiation, malnutrition, working in high environmental temperature, can reduce sperm count. Non descent of testis, as in cryptorchidism, causes failure of spermatogenesis.

Factors inhibiting spermatogenesis

- Lack of FSH and testosterone
- Cryptorchidism
- Varicocele
- Exposure to X-ray radiation
- Malnutrition
- Working in high environmental temperature.

Functions of Sertoli cells

- Secrete Mullerian regression factor in embryonic testis
- Give mechanical support to germ cells in adult testis
- Cause maturation of spermatid to spermatazoa
- Give blood testis barrier, which restricts transport of substances from the blood to the germ cells (Fig. 12.27). The entry of matured spermatozoa into the circulation is also prevented by this barrier, as the sperms are immunogenic.
- Secrete inhibin which regulates FSH secretion
- Produce androgen binding protein
- Produce seminiferous tubular fluid.

Endocrine function of testis

The Leydig cells secrete androgen and the major androgen produced by testis is testosterone. It is a C_{19} steroid.

Testosterone is not stored in the testis. Cholesterol esters are the precursors and are stored as lipid droplets in the Leydig cells. The Sertoli cells and Leydig cells secrete limited quantities of estrogen in men and occurs by the aromatisation of androgen. Even in the tissues like brain, skin, liver and adipose tissue, aromatisation of testosterone to estrogen takes place. The daily secretion of testosterone in adult is 4 to 9 mg/day.

Androstenedione is also secreted from the testis, but at a lower rate. It is also an important steroid precursor for the circulating estrogen in men.

The other androgen is DHEA secreted mainly from the adrenal gland.

DHT

Dihydrotestosterone (DHT) is formed from testosterone by the action of 5α-reductase enzyme. Only 20% of DHT is synthesized from the testis. The remaining is derived from the peripheral conversion of testosterone (skin, prostate gland, seminal vesicle). DHT is more effective physiologically than testosterone.

Biosynthesis of testosterone

The conversion of cholesterol to pregnenolone by 20, 22-desmolase is the rate limiting step. This reaction is regulated by the LH. The enzyme

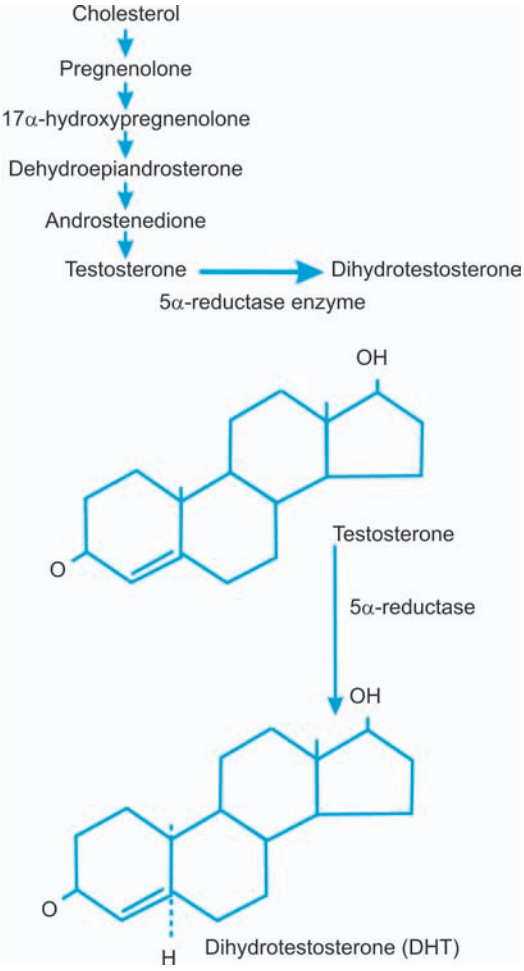


Fig. 12.29: Biosynthesis of testosterone and formation of dihydrotestosterone

17 α-hydroxylase converts pregnenolone to 17-hydroxy pregnenolone. Some pregnenolone is also converted to 17-hydroxy progesterone. This gives rise to androstenedione through the Δ^5 pathway. Androstenedione is the precursor for testosterone synthesis (Fig. 12.29).

Transport of androgens

The circulating androgens are transported by binding to plasma proteins, especially the globulins.

Metabolism

Testosterone is converted to androsterone and etiocholanolone and excreted in the urine as its metabolites.

Testosterone is also metabolized to 17 keto-steroids and excreted in the urine. The urinary excretion of 17 ketosteroids will also contain metabolite of adrenal androgens. Thus, the testis contributes only 30% for the urinary 17 ketosteroids. That's why, the measurement of urinary 17 ketosteroids will not reflect the testicular function.

The second metabolic pathway for testosterone is the conversion to DHT by 5 α-reductase. The testosterone is also converted to estrogen by aromatase enzyme.

Mechanism of action of testosterone

Testosterone enters the target cell where it binds to the androgen receptor in the nucleus and releases the heat shock protein (HSP) from the receptor. This increases binding sites in DNA. The ligand receptor complex acts as transcription factor to regulate the gene expression. The mRNA released as a result enters the cytoplasm. In the endoplasmic reticulum, mRNA causes translation which leads to protein synthesis.

Actions of testosterone

The major action of testosterone is in the regulation of male reproductive organs growth and their maintenance.

Development of genitalia

In fetus, testosterone causes differentiation of Wolffian duct into male internal genitalia and DHT causes the differentiation of external genitalia. In the seventh month of intrauterine life, testosterone helps in the descent of testis into the scrotum through the inguinal canal.

Puberty changes

Testis remains inactive from birth until the onset of puberty. At the time of puberty, the testis

secretes more amount of testosterone, which is responsible for the secondary sexual characteristics. The growth of male type of pubic hair, hair growth in axilla, trunk, face, and limbs, deepening of voice, increased muscle mass are due to testosterone action. The genitalia enlarge in size and the accessory sex organs (prostate, seminal vesicle), become secretory in nature by the action of testosterone.

Spermatogenesis

Spermatogenesis depends on the high level of testicular testosterone and FSH. Deficiency or fall in the testosterone level leads to the arrest of spermatogenesis. Testosterone causes increase in androgen binding protein in Sertoli cells, which helps to keep the level of testosterone in the testis high. Raising the plasma level of testosterone cannot influence spermatogenesis. It is the local high concentration of testosterone which is responsible for the normal sperm production.

Anabolic effect

Testosterone is an anabolic steroid and hence it causes retention of nitrogen, calcium, phosphorus and sodium. The anabolic effect increases the protein synthesis and muscle mass becomes greater.

Action on skeleton

Testosterone causes growth of bones at the time of puberty, but at the same time it causes fusion of epiphysis and stops linear growth. That is why, hypogonadism individuals are abnormally tall.

Effect on sebaceous glands

On the skin, it causes the secretion of sebaceous glands and produces acne at the time of puberty, when the secretion is greater.

Effect on scalp hair

It causes recession of hair at the temples and gives male type baldness of the scalp.

Stimulation of erythropoiesis

Testosterone influences erythropoiesis and males have increased red cell mass by this effect.

Male behavior and libido

Testosterone acts on the brain and produces male type of behavior and the conversion of androgen to estrogen is responsible for libido.

Actions mediated by DHT

Testosterone is converted to DHT by 5α-reductase in some of the peripheral tissues and then the actions are mediated. The receptors for DHT are present in the scrotum, prostate and skin.

Development of penis, penile urethra, scrotum, and prostate gland during fetal period is due to the action of DHT. At puberty, the DHT causes the growth of scrotum, prostate gland, pubic hair and sebaceous glands. The prostatic secretion in adulthood is caused by the action of DHT.

Endocrine regulation of testis

The seminiferous tubules and Sertoli cell functions are regulated by the FSH and testosterone. LH acts on the Leydig cells and stimulates steroidogenesis. The androgen and FSH by synergistic action stimulates seminiferous tubules to produce germ cells. The androgen also stimulates the synthesis of androgen binding protein in the Sertoli cells, which helps to keep the level of androgen in the testis high. The rise in plasma testosterone concentration inhibits GnRH which in turn inhibits the LH secretion from the anterior pituitary (negative feedback inhibition) (Fig. 12.30). Testosterone also inhibits LH secretion by direct action on the anterior pituitary. The exogenous administration of testosterone cannot influence spermatogenesis if the testis concentration of testosterone is low. Thus, the Leydig stimulation by LH is necessary for raising the intratesticular concentration of testosterone for normal spermatogenesis to take place.

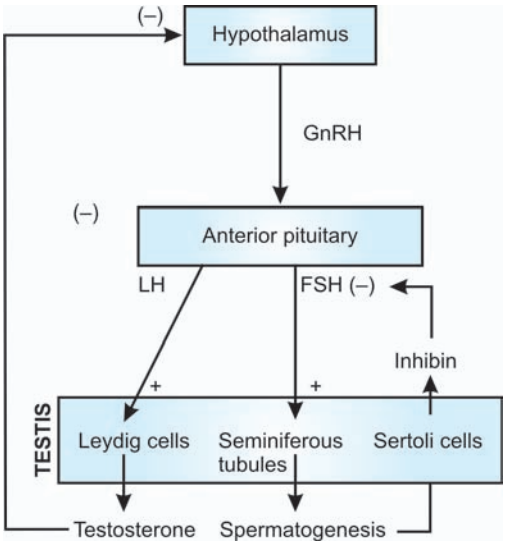


Fig. 12.30: Endocrine regulation of testis
FSH acts on the seminiferous tubules and causes spermatogenesis. This inturn causes the secretion of Inhibin from Sertoli cells, which inhibits FSH secretion from anterior pituitary. The LH secretion is regulated by the plasma level of testosterone

The plasma testosterone level has little effect in regulating FSH secretion. The FSH secretion is regulated by a peptide produced from Sertoli cells known as **inhibin**. This peptide hormone inhibits FSH secretion from the anterior pituitary.

Semen

It is a fluid, ejaculated during coitus, at the time of orgasm in male. It has a volume of 3 to 5 ml per ejaculation. It consists of secretions from:

- Seminal vesicle (60%)
- Prostate (20%)
- Sperms
- Seminiferous tubules
- Bulbourethral glands (Cowper's glands).

The semen not only provides bulk to sperm, but also, supplies nutrients, hormones and capacitation factors. The pH of seminal fluid is alkaline (7.7 to 7.9) which is due to prostatic secretions and buffers. The buffers in the semen are bicarbonates and phosphates which help to neutralise the vaginal acidity.

Seminal vesicle secretion

It contains fructose, prostaglandins, ascorbic acid, phsophorylcholine and ergothionine. The fructose is the energy substrate for sperms. The prostaglandins in the semen stimulate female reproductive tract motility and this helps sperm movement towards the fallopian tube.

Prostatic secretion

It contains an alkaline secretion, which is rich in zinc, citrate, spermine and acid phosphatase.

The secretions of Cowper's glands are rich in mucus which help to lubricate the urethra.

Sperms

The normal sperm count is 100 million per ml of semen. Infertility results if the count is below 20 million per ml or 50% of sperms are not motile.

FEMALE REPRODUCTIVE SYSTEM

Ovary

Ovaries are the primary sex organs in female. There are two ovaries present and each ovary has a cortex and medulla. The cortex contains; a number of immature germ cells called primordial follicles, a few developing primary and secondary follicles, connective tissue and blood vessels. The major functions of ovary are gametogenesis and hormone secretion.

Oogenesis

The formation of oocytes is completed before birth, in midgestation itself. Before birth, the number of oocytes is 7 million and after birth, it is reduced to 2 million. At the time of puberty, the number is further reduced to 400,000. During the reproductive phase of a woman, only around 400 oocytes are needed. The rest undergo atresia.

The first meiotic division starts during gestation, but it stops at prophase. The first meiotic division is completed, when ovulation occurs during first reproductive cycle. There are two daughter cells formed, with secondary oocyte

and a smaller cell called first polar body. The latter does not receive any genetic material and it regresses. The secondary oocyte starts its second meiotic division, but stops at metaphase. The completion of second meiosis occurs during fertilization, when sperm fuses with the ovum. The completion of second meiotic division, leads to ovum receiving haploid number of chromosomes (Fig. 12.31). The second polar body which is formed undergoes degeneration.

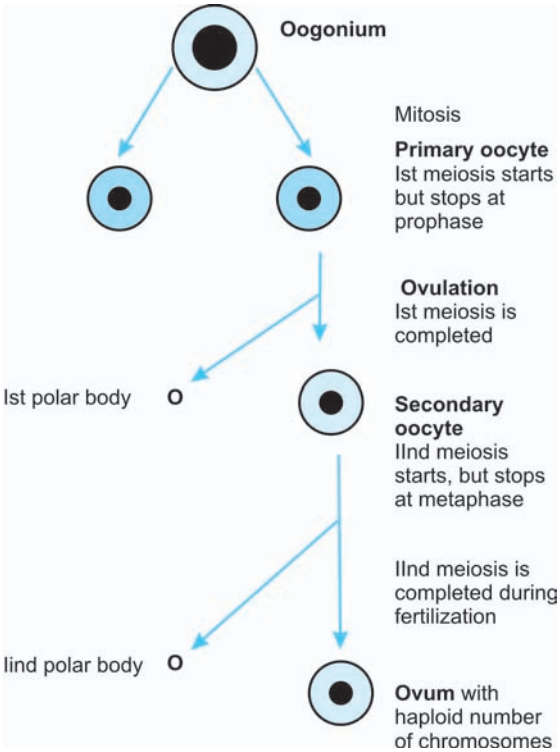


Fig. 12.31: Oogenesis

Follicular development (Fig. 12.32)

In females, the follicular development and maturation occur with the onset of reproductive cycle. The process is under the control of FSH and sex steroid estrogen. The immature follicle is called primary follicle, which is smaller in size with a single oocyte and a surrounding single layer of granulosa cells. There are a number of these primary follicles, but most of them degenerate. A few of them develop, by the action of FSH into a secondary follicle. It is larger in size and the follicular cells divide into many layers of granulosa cells. The oocyte also is covered by a layer called zona pellucida. The connective tissue surrounding the follicle differentiates into two layers namely, theca interna and theca externa. These two layers are vascularized. Theca interna has endocrine

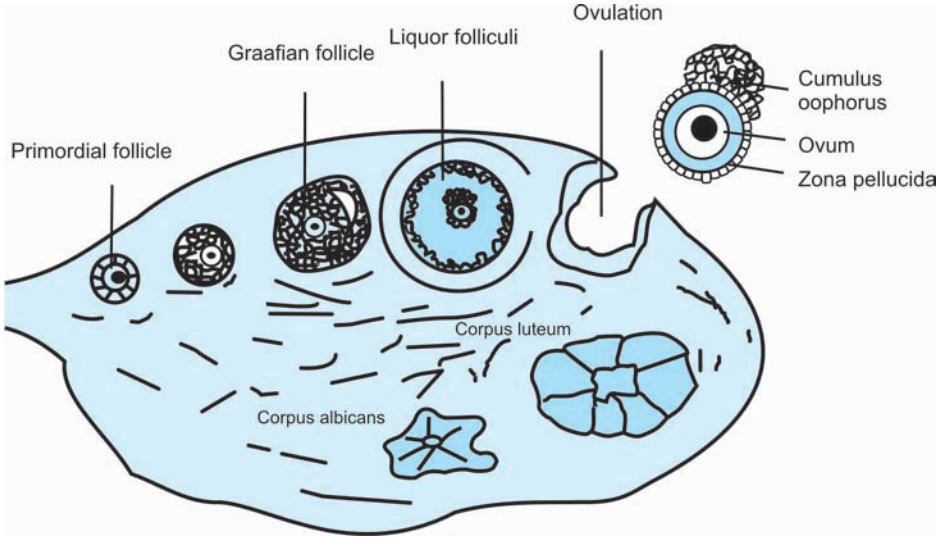


Fig. 12.32: Ovarian changes during reproductive cycle. The diagram shows the development and maturation of primordial follicle, ovulation, formation of corpus luteum and corpus albicans

function. It contains receptors for FSH and enzymes for estrogen synthesis. The estrogen that is secreted in reproductive women is 17β-estradiol. The granulosa cells and oocyte receive their nutrients, hormones and electrolytes by diffusion.

The secondary follicle, which is formed, develops into graafian follicle. The granulosa cells fuse and form antrum. The fluid collects around the oocyte and this follicular fluid contains estrogen, progestin, growth factors, cytokines and electrolytes. The buoyancy created by the fluid (antrum) pushes the graafian follicle to the surface of the ovary.

The ovum is surrounded by a single layer of granulosa cells, called corona radiata. The ovum is supported by a projection of granulosa cells, which extend into the antrum and form cumulus oophorus.

These follicular developments and maturation occur in the first half of the reproductive cycle, by the action of FSH and estrogen. Around mid cycle (14th day), the LH surge occurs, which causes ovulation. The LH reduces the blood flow in the thecal layers and activates plasminogen activator in the granulosa cells. Prostaglandins also are released, which make the follicular wall thin. The formation of plasmin and collagenase, facilitate the rupture of the follicular wall including ovary surface. The ovum, which is surrounded by corona radiata, cumulus oophorus and follicular fluid, is released into the peritoneal cavity.

After the release of ovum, the theca cells and granulosa cells become lutein cells and the graafian follicle now is called corpus luteum. It has an endocrine function and it secretes progesterone and estrogen. The lutein cells are acted upon by LH. If fertilization does not occur, corpus luteum survives for only 14 days and then degenerates to become corpus albicans. If fertilization occurs, corpus luteum in the ovary persists, until the placenta is developed.

Hormonal interactions in ovary

- FSH stimulates follicular development and maturation which is synergistic with estrogen action.
- FSH not only increases its own receptors, but also of estrogen, on the theca and granulosa cells.
- Towards mid cycle, FSH will also increase LH receptors in the follicle. This will cause secretion of both estrogen and progesterone.
- Rise in estrogen level causes LH surge, which results in ovulation.
- In the postovulatory phase, LH causes more of progesterone secretion, than estrogen from the corpus luteum.
- Towards the end of postovulatory phase, both FSH and LH levels decrease.
- In the menstrual phase, two days after menses, FSH level begins to rise and the next cycle starts again.

Ovarian steroids

Ovary secretes estrogens and progesterone. The estrogens are estrone and estradiol. The estrogens are C_{18} steroids, while progesterone is C_{21} steroid.

Estrogens (Figs 12.33A and B)

Estrone: It is a weak estrogen formed from ovary and peripheral tissue like liver. In the periphery, the conversion of androstenedione gives estrone.
Estradiol: It is the principal circulating estrogen and biologically active. 17 β-estradiol is secreted in reproductive women.
Estriol: It is the weakest of all naturally occurring estrogens. It is synthesized in the placenta and liver and not in ovary. It is secreted more in pregnant women. In nonpregnant women, estriol can be formed from estradiol and estrone in the liver.

Synthesis

The theca cells can form androstenedione and testosterone and these androgens enter granulosa cells, where they are converted into estrogens. Androstenedione gives rise to estrone and

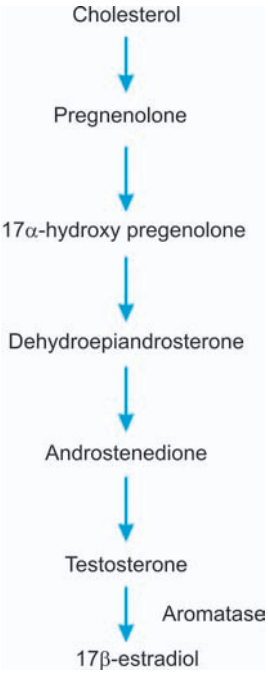


Fig. 12.33A: Biosynthesis of estrogen

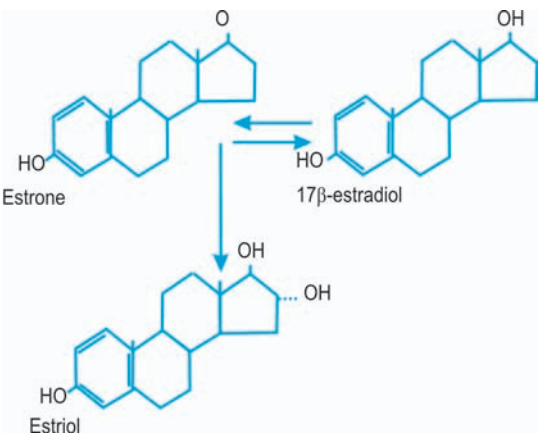


Fig. 12.33B: Estrogen types and their structures. Metabolism of estradiol gives rise to estrone, which further metabolizes to estriol

testosterone is converted to estradiol (Fig. 12.31). The granulose cells can also synthesize directly estradiol. The synthesis of estrogens occurs by the tropic action of FSH and LH. Estrogen synthesis and secretion are stimulated during follicular phase of reproductive cycle.

Estrogens bind to plasma proteins and transported in the circulation. About 2% is in free form. 60% is transported by binding to albumin and the remaining by binding to gonadal steroid binding globulin.

Estradiol is metabolized in the liver to estrone and estriol and excreted in the urine as soluble conjugates of glucuronides and sulphates.

Actions of estrogen

Estrogens are responsible for the development and maintenance of genitalia and secondary sex characters in females. It is also responsible for puberty changes in girls.

On ovary

Estrogen causes follicular development and maturation by its synergistic action with FSH.

Effect on uterine myometrium

Estrogens increase the amount of contractile proteins and increase the excitability of smooth muscle in the uterus. They sensitize the muscle for the action of oxytocin, in causing uterine contraction.

Uterus size

Estrogen causes increase in the size of the uterus. This is especially seen during pregnancy.

On endometrium

The endometrial cell shows proliferation under the influence of estrogen. The superficial layer of endometrium called stratum functionalis is shed during menses and in the proliferative phase, this layer is replaced by the action of estrogen. The thickness of endometrium in the secretory phase reaches up to 5 mm.

Action on cervix

Estrogen causes cervical secretion to be copious and watery. This occurs in the preovulatory phase of the cycle. When placed on a glass slide, fine long threads from the fluid can be drawn.

This is called **spinnbarkeit** and used as an index of estrogenic activity. The cervical mucus, if allowed to dry on a glass slide, ferning pattern appears. This is known as **fern test** and normally occurs, before the onset of ovulation, when estrogen level is at its peak. The fern test is also used as an index of estrogen activity.

On vagina

The vaginal epithelial cells become cornified by the action of estrogen. The vaginal secretion, stimulated by estrogen, becomes acidic by the action of bacteria. The acidic pH has a bactericidal effect.

Mammary gland

The growth of mammary gland begins well before the onset of menstrual cycle in girls and it is due to the action of estrogen. It promotes the growth of duct system of the gland. Esterogen also causes growth of stromal cells and deposition of fat.

On bone

Estrogen shows osteoblastic activity, causing growth spurt at the time of puberty. The same hormone causes closure of epiphysis of long bones and stops the linear growth.

Estrogen promotes the retention of calcium and phosphates on the bone matrix. After menopause, the fall in estrogen level leads to demineralization and osteoporosis.

Other actions of estrogen

1. Estrogen promotes retention of sodium and water in the kidney.
2. On the skin, it inhibits sebaceous gland secretion and prevents acne formation.
3. Increases high density lipoprotein level and lowers the serum cholesterol level.
4. Increases the synthesis of transport globulins in the liver, such as thyroid binding globulin (TBG) and transcortin.
5. Increases the progesterone receptors.

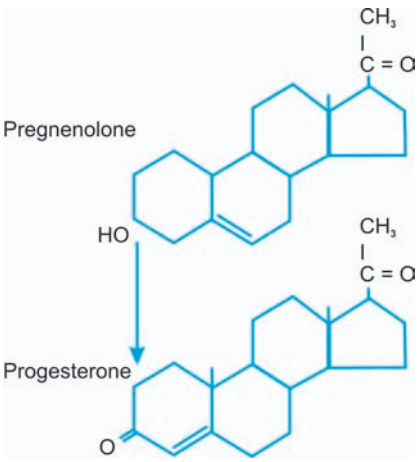


Fig. 12.34: Structure of progesterone

Progesterone

It is a C_{21} steroid produced from ovary and placenta. It is synthesized from cholesterol and its precursor steroid is pregnenolone (Fig. 12.34). The steroid is mainly transported by binding to transcortin and albumin. Progesterone is catabolized in the liver to pregnanediol, which is conjugated with glucuronides and sulphates, and excreted in the urine.

Actions

On uterine myometrium

Progesterone causes hyperpolarization of the smooth muscle and inhibits its contractility. That is why, it is called the hormone of pregnancy.

On endometrium

Progesterone produces secretory changes in the uterine endometrium. The uterine glands become secretory and secrete a fluid rich in glycogen. This is the source of energy for the implanted embryo. The action of progesterone on the endometrium causes decidual changes when implantation occurs.

On cervix

Progesterone causes the cervical secretion to be thick and viscid. This prevents the free movement of sperms.

Thermogenic effect

Progesterone raises the set point of thermostat in the hypothalamus. That is why, following ovulation the body temperature rises by 0.5°C. The rise in basal body temperature in the mid cycle reflects that ovulation has occurred.

On mammary gland

Progesterone together with estrogen promotes the growth of mammary gland. The specific action of progesterone on the mammary gland is, that it produces alveolar development. Progesterone causes development of lobules and alveoli of mammary gland.

Other actions of progesterone

Stimulates respiration
Shows natriuretic action in kidney.

Other hormones from ovary

Ovary secretes a number of peptide hormones, such as **inhibin, activin, growth factors, relaxin**, and **cytokines.**

Regulation of ovarian endocrine function

The endocrine function of the ovary is under the regulatory control of pituitary gonadotrophins, which in turn is regulated by the gonadotrophin releasing hormone (GnRH). In reproductive women, the pituitary and ovary show cyclical changes in the hormone secretion. The GnRH is secreted in a pulsatile pattern and the medial hypothalamus is considered as pulse generator for GnRH secretion. As a result of this, the pituitary gonadotrophins are also secreted in pulses.

In the first half of the cycle **(preovulatory phase),** FSH and LH are secreted from the anterior pituitary. FSH acts on granulosa cells and causes aromatisation of androgen to estrogen (Fig. 12.35). The LH acts on the theca cells and causes secretion of androgens, which are the precursors of estrogen. FSH and estrogen together promote follicular growth and increase the LH receptors in the granulosa cells. The granulosa cells secrete inhibin, which directly acts on the anterior pituitary and inhibits FSH. The increase in LH receptors in midcycle also causes inhibition of FSH and stimulation of LH release. The LH surge results in ovulation. The most effective inhibition of FSH comes from elevated plasma levels of estradiol by negative feedback inhibition. The inhibition of FSH, by this mechanism will also have a positive feedback stimulation of LH secretion, through GnRH release. Elevated level of estrogen has a positive feedback effect on LH secretion to produce LH surge. During this preovulatory phase, the concentration of estrogen is high, while the concentration of progesterone is low.

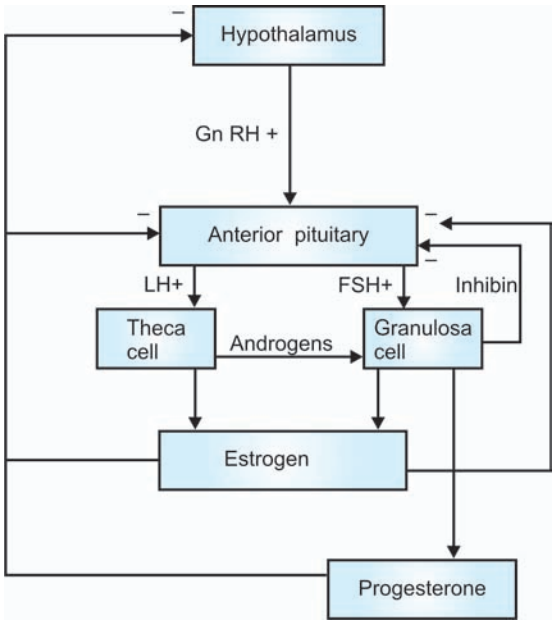


Fig. 12.35: Endocrine regulation of ovarian function

In the **postovulatory phase**, the corpus luteum secretes progesterone and small amounts of estrogens. The LH is the gonadotrophin which causes the secretion of these steroids from corpus luteum. Towards the end of post ovulatory phase, the corpus luteum degenerates into corpus albicans and the blood levels of progesterone and estrogens fall. This fall in the blood level of sex steroids results in menstrual bleeding. The GnRH pulsatile secretion once again occurs for the next cycle.

Menstrual cycle

The reproductive cycle in women is called menstrual cycle, which is characterized by the cyclical endometrial bleeding. The first menstrual cycle starts at the time of puberty **(menarche)** and continues until 45 to 50 years of age, when cessation of menstrual cycle occurs **(menopause).**

The duration of cycle is 28 ± 3 days and the average is 28 days. The duration of the cycle varies from woman to woman and in the same woman the duration can vary from cycle to cycle.

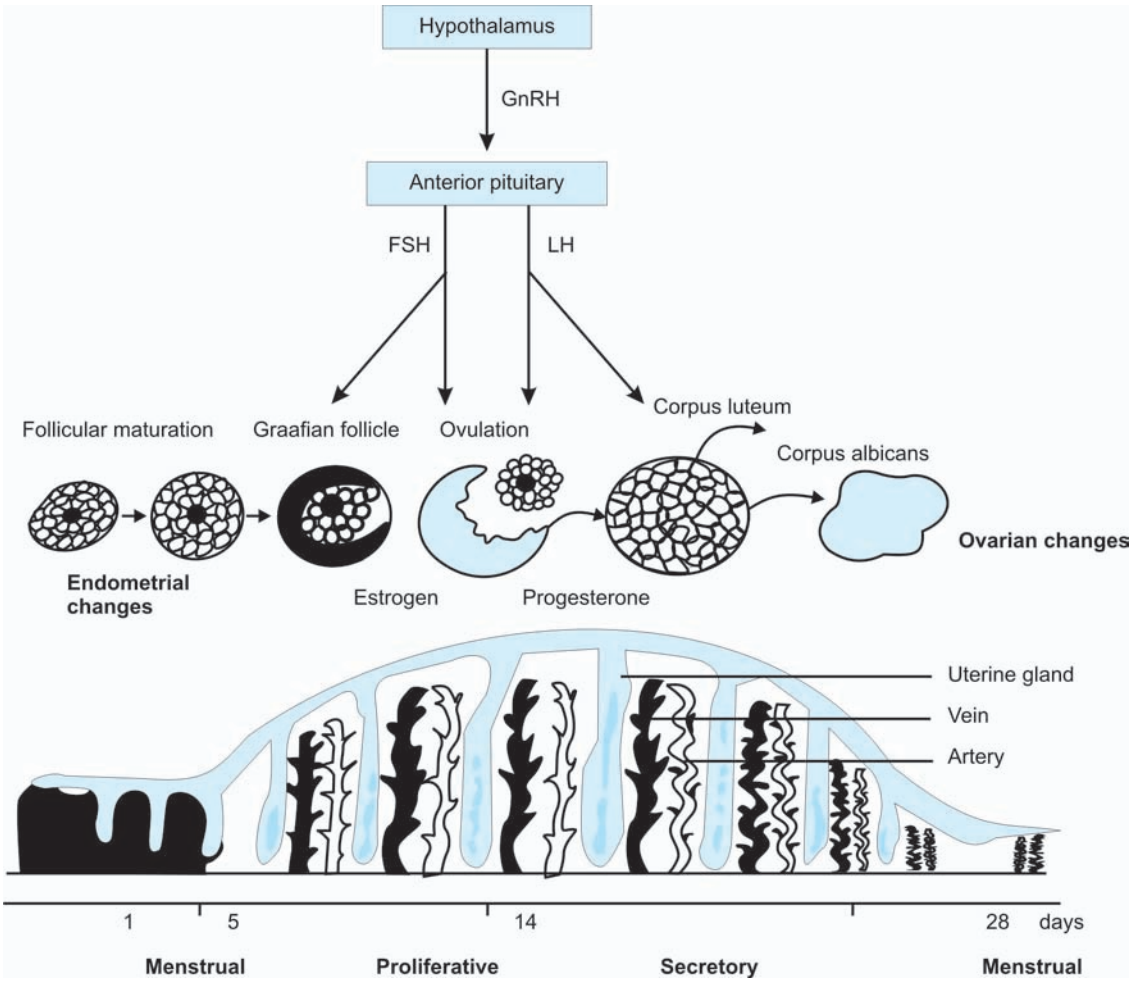


Fig. 12.36: Endometrial and ovarian changes during menstrual cycle

Phases of menstrual cycle

Proliferative (follicular phase) 6th to14th day
Secretory phase (luteal phase) 15th to 28th day
Menstrual phase (1 to 5 days).

The cycle duration can be measured from the beginning of the menses as day one to the onset of next menstrual bleeding.

The cycle can also be divided into *preovulatory, postovulatory and menstrual phases.*

The menstrual cycle is characterized by the cyclical changes in ovary, uterine endometrium and pituitary (Fig. 12.36).

Preovulatory phase

Changes in ovary

In the first half of the cycle, the ovary shows follicular development and maturation. The primordial follicle becomes graafian follicle by the influence of FSH and estrogen. The maturation of graafian follicle results in ovulation, which is caused by the LH surge during midcycle.

Changes in uterine endometrium

The inner layer of uterus is called endometrium. The superficial two third is called stratum functionalis and the inner third is called stratum basalis. The outer two third is supplied by coiled spiral arteries, while the inner one third is perfused by the straight basal arteries. The outer two third of the endometrium is the one which is shed during the menstrual phase and replaced by the proliferation of stratum basal layer.

The proliferative action is shown by estrogen. The thickness of the endometrium increases along with the blood vessels and glands development (Fig. 12.36). The proliferative changes, which start from day one of the cycle, continues into the second half of the cycle, when the endometrium becomes secretory.

Hormonal changes

In the first half of the cycle the LH secretion is held in check by estrogen. In the first half of the cycle, the pituitary gonadotrophins, FSH and LH are secreted in a pulsatile fashion, synchronising with the GnRH secretion in pulses from hypothalamus. The FSH causes the thecal and granulosa cells of the graafian follicle to secrete estradiol, estrone and progesterone. The secretion of estradiol is in greater amounts than other steroids. The increase in estradiol which occurs towards the midcycle, causes inhibition of FSH and stimulation of LH surge (Figs 12.37 and 12.38). The FSH is also inhibited by inhibin, secreted from granulosa cells.

Ovulation

The release of ovum usually occurs during mid cycle caused by the LH surge. The timing of

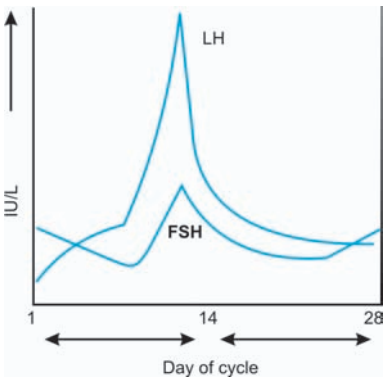


Fig. 12.37: Circulating pituitary gonadotrophins level during menstrual cycle. The peak secretion of LH (LH surge) occurs during midcycle, which causes ovulation

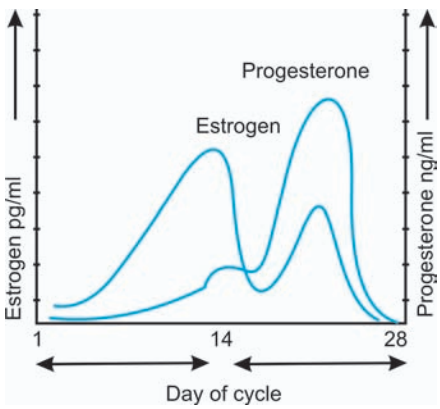


Fig. 12.38: Plasma concentration of estrogen and progesterone during menstrual cycle

ovulation is unpredictable, as it varies in the same person from cycle to cycle. The indicators of ovulation are:

Basal body temperature test

Recording of basal body temperature shows a rise by 0.5°C, which indicates that ovulation has taken place. The progesterone, which is secreted by the action of LH on lutein cells is responsible for the thermogenic effect. The temperature rise is continued in the second half of the cycle until the onset of menstrual phase.

Spinnbarkeit effect of cervical mucus

The stretching of cervical mucus into long thin strands indicates peak estrogen secretion, which occurs prior to ovulation.

Fern test

The drying of cervical smear gives a fern like pattern, which indicates the peak estrogen secretion occurring prior to ovulation.

Estimation of urinary pregnanediol

The metabolite of progesterone, pregnanediol is excreted in the urine in greater amounts after ovulation and estimation of this level indicates ovulation has occurred.

Postovulatory phase

Changes in ovary

After the release of ovum, the graafian follicle becomes the corpus luteum. The lutein cells secrete progesterone by the action of LH. The corpus luteum stays for 14 days, if fertilisation does not occur. If fertilization occurs, corpus lutem persists until delivery by which time, the placenta is developed fully.

When fertilization does not occur, after 12 to 14 days, corpus luteum degenerates into corpus albicans.

Changes in endometrium

The endometrium grows further in size, reaching up to 5 mm thickness. The spiral arteries cannot cope with the proliferation of outer third layer and hence become coiled. The uterine glands become secretory in nature by the action of progesterone. The fluid is rich in electrolytes, enzymes and glycogen. This provides nutrition to the embryo in the initial stages of implantation. The secretory changes can occur better in the endometrium if estrogen has already acted on the tissue, since estrogen stimulates the production of progesterone receptors.

Endocrine changes

After ovulation, the LH secretion will be greater than FSH. These gonadotropins act on the corpus luteum and cause the secretion of progesterone and estrogen. The estrogen secretion is in lower amounts than progesterone. However, around 7 days after ovulation, there is another peak secretion of estrogen, which is lesser in concentration, than the preovulatory level and is broader. With the corpus albicans formation towards the end of the second half of the cycle, the concentrations of progesterone and estrogen fall in the blood. This triggers the onset of menstrual flow.

Menstrual phase

The fall in the blood level of progesterone and estrogen, causes the spasm of spiral arteries in the outer two third of endometrium. This causes ischemia of the tissue and necrosis. There is release of vasodilator substance from the necrotic tissue causing blood vessels to dilate and rupture. The menstrual fluid consists of blood, necrosed endometrial cells and mucus. The release of blood occurs in bouts lasting for 3 to 5 days. Each cycle results in loss of 30 to 50 ml of blood. The blood which is released, at first clots and then liquefied by the fibrinolysin. Excess clot, if present in the menstrual blood, indicates excessive blood loss **(menorrhagia).**

Premenstrual syndrome

In reproductive women, a few days prior to the onset of menstrual flow, symptoms such as irritation, depression, bloating of stomach, tenderness of breast, weight gain, constipation, etc are experienced. These symptoms are called premenstrual syndrome. The weight gain is believed to be due to the retention of water, caused by the estrogen action.

Abnormalities of menstrual cycle

Oligomenorrhea: It refers to reduced frequency of periods.

Hypomenorrhea: It indicates scanty menstrual flow.

Menorrhagia: Excessive menstrual flow.

Dysmenorrhea: Painful menstruation due to the release of prostaglandins from endometrium.

Amenorrhea: Absence of menstruation. It has primary and secondary types:

Primary amenorrhea: It is caused by disorders of ovary, hypothalamic disease and pituitary disorders. In this type, the menstrual cycle has never occurred.

Secondary amenorrhea: In this type, menstrual cycle is present for some period, but subsequenty stops. Physiologically, secondary amenorrhea occurs in pregnancy. Disorders of ovary and diseases of other endocrine glands can also lead to secondary amenorrhea.

Fertilization

The viability of sperm in the female genital tract is 48 hours and for ovum, it is 24 hours. For conception to occur, the coitus should overlap these periods. Fertilization occurs in the ampulla of the fallopian tube. Immediately after the zygote formation, the cell division starts. First the morula stage is formed which enters the uterus. The blastocyst stage is formed (8 to 16 cell stage) after 3 to 4 days of fertilization. That is, on the 21st day of the cycle, if fertilization has taken place on the 14th day.

Postmenopausal syndrome (PMS)

Menopause is caused by the loss of ovarian function, which occurs at the age of 45 to 50 years in women. The fall in the blood level of estrogen and progestrone, leads to symptoms, such as, hot flashes in face and irritability. The estrogen deficiency causes vaginal epithelium to atrophy and dry. The bone demineralization is also accelerated. The postmenopausal women are susceptible to high blood level of LDL and atheroscelerosis. The incidence of hypertension and coronary artery disease are not uncommon in them. The blood level of pituitary gonadotrophins will be high, as estrogen and progesterone levels are low.

Pregnancy test

Immunological test is done to detect pregnancy. A sample of urine is taken and to this antisera of HCG is added. This is followed by the addition of latex particles coated with HCG. If agglutination is present, it indicates negative test for pregnancy. Absence of agglutination is a positive test for pregnancy.

Implantation

The implantation of the blastocyst occurs, in the posterior wall of the uterus (Fig. 12.39). The endometrium is primed with progesterone for this effect. The endometrium at the site of implantation shows decidual reaction. That is, the stromal cells of endometrium enlarge and thicken. These decidual cells of the endometrium give rise to the maternal part of placenta. Implantation is affected, if progesterone level falls in the blood, resulting in abortion. The blastocyst has an inner embryo and an outer trophoblast.

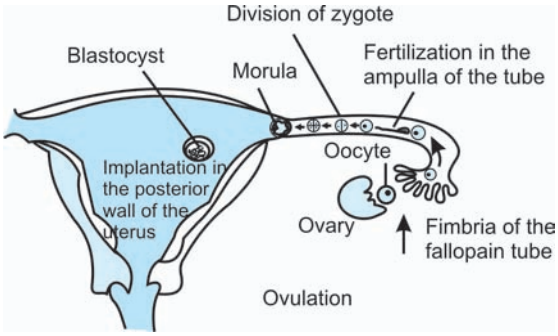


Fig. 12.39: Details of fertilization and implantation

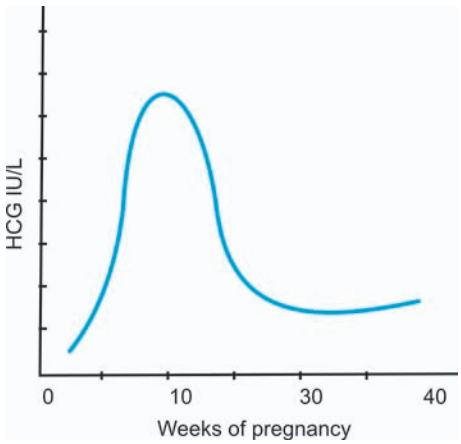


Fig. 12.40: Pattern of HCG secretion during pregnancy

Note the peak level reaching by 8 weeks of gestation and thereafter the level in the plasma declines

PLACENTA

The link between the fetus and the mother during pregnancy occurs through the placenta, which has a fetal part and a maternal part. The trophoblast layer forms the fetal part, while, the decidual layer of the endometrium becomes the maternal part of the placenta. The trophoblast layer differentiates into outer syncytiotrophoblast and an inner cytotrophoblast layer. The syncytiotrophoblast layer penetrates the decidua of the endometrium and gives rise to finger like projections called chorionic villi. It is through these villi, the fetal and maternal circulations are established.

Functions of placenta

Placenta performs respiratory, nutritive, excretory, storage and endocrine functions. The endocrine function of placenta is considered important, as the hormones secreted from it help to maintain pregnancy.

Hormones of placenta

Placenta secretes the following hormones,
HCG (Human chorionic gonadotrophin)
HPL (Human placental lactogen)
Progesterone, Estrogen.

HCG

It is a glycoprotein hormone with α and β subunits. The α subunit of HCG is similar to α subunit of LH, FSH and TSH. The excretion of HCG can be seen in the urine 12 to 14 days after conception. Its secretion occurs from the syncytiotrophoblast cells of placenta. The secretion of HCG reaches the peak level at 8 weeks of pregnancy and thereafter the level declines (Fig. 12.40).

The action of HCG is similar to LH. It has lutenizing and luteotrophic actions. It maintains corpus luteum in the early weeks of pregrancy. It acts on the corpus luteum, during the first 8 to 10 weeks of pregnancy and causes the secretion of 17 α-hydroxy progesterone and lesser amounts of progesterone. After 10 weeks period, the placenta is fully developed and progesterone secretes from it. HCG also acts on the fetal zone of adrenal cortex and causes the secretion of DHEA. The other important function of HCG is, its urinary excretion forming the basis for the pregnancy detection tests.

HPL (human placental lactogen)

HPL is also known as human chorionic somatomammotrophin. It is a protein hormone, secreted from the syncytiotrophoblast cells of placenta. Its structure is similar to growth hormone and prolactin. Its secretion can be seen throughout pregnancy (Fig. 12.41). Its actions are:

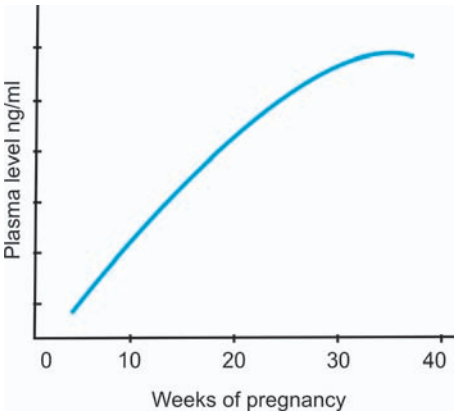


Fig. 12.41: Plasma level of human placental lactogen in the mother during pregnancy

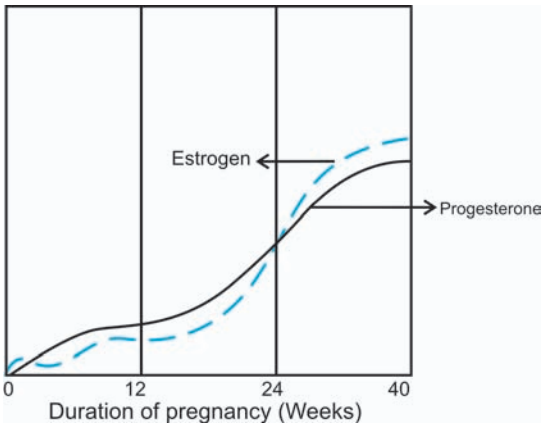


Fig. 12.42: Pattern of estrogen and progesterone secretion during pregnancy

- Mobilises nutrients from the mother to the fetus.
- Lipolytic and antagonizes insulin action.
- Stimulates mammary gland growth and its development in the mother.

Progesterone

Progesterone in the first trimester of pregnancy, is secreted from the corpus luteum of ovary by the trophic action of HCG. After 8 weeks of pregnancy, the placenta is developed and can secrete progesterone. The increased progesterone secretion is reflected in the urinary excretion of its metabolite pregnanediol, which is excreted in greater amounts during pregnancy. The level of progesterone falls lower than the estrogen towards the end of pregnancy (Fig. 12.42). The actions of progesterone during pregnancy are:

- Maintains pregnancy by inhibiting uterine myometrial excitability.
- Causes increased development of alveoli in the mammary gland and prepares the gland for lactation.
- Acts as a precursor for fetal adrenal secretion of corticoids and DHEA.
- Progesterone promotes increased excretion of Na^+ in the urine, which compensates for the increased secretion of aldosterone, that occurs during pregnancy.

Estrogen

During pregnancy there is secretion of **estradiol**, **estrone** and **estriol.** The estriol secretion is much higher than other estrogens during pregnancy. In the first trimester of pregnancy, the source of estrogen is from the corpus luteum secreted by the action of HCG. The estrogens that are secreted include estradiol and estrone. After the formation of placenta, estriol is the major estrogen that is secreted. The formation of estriol from the placenta requires the participation of fetal adrenal cortex and fetal liver, as the placenta does not have certain enzymes, i.e., involved in steroidogenesis, such as, 17 α-hydroxylase, 17, 20, desmolase and 16 α-hydroxylase. Since the estrogen is synthesized with the involvement of fetal adrenal cortex and fetal liver, all the three together form the **fetoplacental unit** (Fig. 12.43).

Placenta can synthesized cholesterol, but not in greater amounts. Cholesterol coming from the maternal blood into the placenta is much greater. The cholesterol is converted to pregnenolone and then to progesterone. Since placenta lacks 17 α-hydroxylase and 17, 20 desmolase enzymes, pregnenolone enters fetal adrenal cortex, where in the presence of these enzymes pregnenolone is converted to DHEA. This is converted by 16 α-hydroxylase to 16 α-hydroxy DHEA. In the fetal liver, it is sulfate conjugated, so that its biological

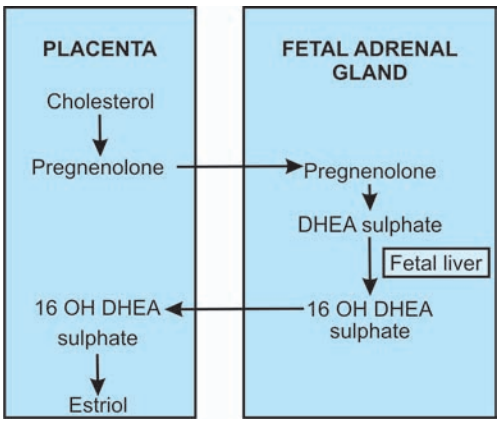


Fig. 12.43: Fetoplacental unit in the synthesis of estrogen

activity cannot occur. The sulfate moiety is removed in the placenta and the androgen is aromatised by the trophoblast cells to estriol.

Actions of estrogen during pregnancy

- Causes increase in uterine size to accommodate the growing fetus.
- Promotes the increase in the size of mammary gland and stimulates the duct development.
- In the liver, it increases the synthesis of thyroxine binding globulin, transcortin, angiotensinogen proteins. Renin and angiotensin II secretions are also increased by estrogen.

Relaxin

It is a polypeptide hormone, secreted from the corpus luteum in early pregnancy and placenta in the later stages of gestation. The structure is similar to proinsulin. The peak level of relaxin is seen in early pregnancy. Its actions are:

- Relaxation of pubic symphysis
- Softening of pelvic ligaments and cervix
- Inhibits uterine myometrium

These actions facilitate easy expulsion of fetus during parturition.

The placenta also secretes other hormones like:

ACTH
TSH
Acetylcholine
Chorionic adrenocorticotrophin.

PHYSIOLOGICAL CHANGES DURING PREGNANCY

During pregnancy there are a number of physiological changes in the mother, which help to maintain pregnancy. The changes are seen in the endocrine, renal, respiratory, blood, cardiovascular and digestive systems.

Endocrine glands

As already mentioned in the endocrine function of placenta, the mother shows enlargement of thyroid, adrenal and pituitary glands. But there are no symptoms of hypersecretion as the plasma proteins (transcortin, thyroid binding globulin) that carry the hormones are produced in greater amounts in the liver. The blood level of estrogen, progesterone are high and the pituitary gonadotrophins are inhibited. The absence of gonadotrophins secretion during pregnancy inhibits the menstrual cycle.

The body weight of the mother increases due to the developing fetus. At full-term, the weight gain in the mother can go up to 12 kg.

Renal system

The renal system shows increased GFR and urine output. There may also be glucose excretion in the urine due to increased GFR. There is increased salt and fluid retention due to steroid hormones secreted from placenta and adrenal cortex. But the fluid volume rise during pregnancy is only marginal as increased GFR during pregnancy increases solute and water excretion in the urine.

Respiration

During pregnancy the increased progesterone stimulates respiration. The fall in PCO_2 may lead to a mild alkalosis. The increase in ventilation causes a fall in functional residual capacity. The

vital capacity is not reduced during pregnancy, since the upward displacement of diaphragm by the developing fetus is compensated by the increase in horizontal dimension of thoracic cavity.

Blood

Blood volume is increased due to the rise in plasma volume. In the later stages of pregnancy, inspite of the stimulation of erythropoiesis, there is a relative fall in Hct, as the plasma volume is increased. This results in anemia. The iron requirement is greatly increased in the mother during pregnancy as the growing fetus draws iron from the mother. There is also increased synthesis of clotting factors like fibrinogen, VII, VIII and IX.

CVS

The heart rate and cardiac output are increased. The cardiac output is increased by 30 to 40%. The blood pressure stays at normal levels and some times the diastolic pressure may show a fall due to the drop in the total peripheral resistance. If rise in arterial blood pressure occurs in the mother during pregnancy, it is not a good sign. It is seen in conditions such as preeclampsia. The compression of abdominal veins by the growing fetus increases the venous pressure in the lower extremities in standing posture. The rise in venous pressure can lead to edema of the legs.

Digestive system

Gastric emptying and intestinal transit time are increased during pregnancy. This could be due to the action of increased progesterone inhibiting the smooth muscle of GI tract. Reflux of gastric secretion into the esophagus causing heart burn can also be observed. The decreased GI motility during pregnancy causes constipation in the mother.

Morning sickness

In the first trimester of pregnancy, the metabolic changes produce morning sickness which is characterized by vomiting. The prolonged vomiting which occurs in hyperemesis gravidorum requires immediate medical attention. The increased energy demand which occurs during pregnancy is met by the action of human placental lactogen.

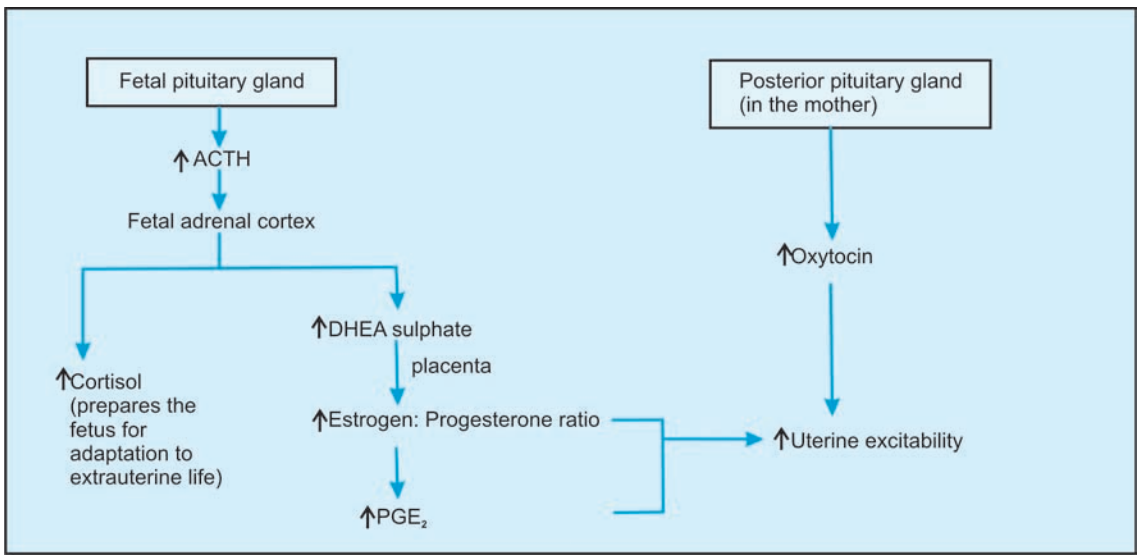


Fig. 12.44: Possible factors in the triggering of parturition

PARTURITION

The duration of pregnancy is 40 weeks or 280 days ± 10 days. In the last weeks of pregnancy itself, weak ineffective uterine contractions occur. Actual labor contractions take place during parturition. The actual mechanism that triggers this process is not exactly known. However, the secretion of cortisol and estrogen from the fetal adrenal cortex is believed to be the stimuli for parturition (Fig. 12.44). The other factors that also contribute to the initiation of labour are:

- Fall in progesterone
- Rise in estrogen
- Secretion of oxytocin from posterior pituitary
- Secretion of prostaglandin (PGE_2) from placenta.

During pregnancy, the high progesterone and estrogen ratio keeps the uterus quiescent and maintains pregnancy. In the last weeks of pregnancy the ratio is reversed, with estrogen level rising and progesterone level falling in the plasma (Fig. 12.42). But, before parturition, the levels of estrogen and progesterone fall. The fall in progesterone level increases the uterine excitability. Also increase in fetal cortisol secretion and placental secretion of prostaglandin PGE_2 increases the uterine excitability. The stretching of cervix, by the head of the baby initiates the neuroendocrine reflex, leading to the secretion of oxytocin from the posterior pituitary. The oxytocin and prostaglandins act on the uterine muscle and increase its contraction. The contractility of uterus is enhanced by the catecholamines secretion, which occur during labour. The α-adrenergic receptors on the uterine muscle cause its contraction. Further distention of cervix, leads to a greater secretion of oxytocin, which in turn causes a stronger contraction of the uterus. This is a positive feedback stimulation, which helps uterine contractions and expulsion of the baby. However, delivery can occur, even in the absence of oxytocin.

The catecholamines and prostaglandins may help in parturition, in such conditions. The whole process occurs in three stages. In the first stage, the fixing of the baby in the cervix occurs, while in the second stage, the baby is delivered through the vagina, as the uterine contractions push the baby out of the uterine cavity and cervix. This stage may last for one hour. The third stage, which occurs immediately after the delivery of the baby leads to the expulsion of placenta.

LACTATION

The process of lactation involves lactogenesis and galactopoiesis. **Lactogenesis** is the process of milk synthesis, while **galactopoiesis** deals with the maintenance of milk secretion.

The preparation of milk secretion includes the development of breast. At the time of puberty, the gland is developed by the action of estrogen and progesterone. Estrogen is responsible for the size of the gland and duct development, while progesterone causes the alveoli development. The duct and alveoli development of the breast take place during menstrual cycle. During pregnancy, the high estrogen and progesterone levels cause increase in the size of the gland, greater development of the duct and alveoli lobular system. The alveolus is the functional unit of the gland in milk secretion. The cells are surrounded by the myoepithelial cells. When these cells contract, the milk from alveoli is expelled into the ducts.

During pregnancy, prolactin, together with estrogen, prepare the alveoli of the gland for milk secretion. The actual milk flow can take place only if estrogen level declines. The flow of milk from the mammary gland occurs after 1 or 2 days of delivery when the level of estrogen decline.

The hormone necessary for the milk secretion is prolactin, which is a lactogenic hormone in humans. Prolactin is secreted from the anterior pituitary and continuation of its secretion depends on the neuroendocrine reflex, produced by the suckling of the nipple by the baby. Cortisol, thyroxine, growth hormone and insulin are also necessary for milk production, through their effect on metabolism. The milk let down or milk ejection

occurs due to the action of oxytocin secreted from the posterior pituitary. The same reflex also causes milk secretion, through prolactin release.

Hormones acting on mammary gland

Estrogen
Causes the development of breast at the time of puberty and produces duct developement.

Progesterone
Produces the alveoli development of the gland.

Prolactin
Lactogenic hormone, which is responsible for the milk synthesis.

Cortisol, Insulin, Thyroxine, Growth Hormone
Not having a direct action on the gland, but necessary for the milk synthesis due to their effect in the metabolism.

Oxytocin
Causes milk ejection or milk let down.

During nursing, menstrual cycle is absent in the mother due to the absence of pituitary gonadotrophins which are inhibited by prolactin. The absence of menstrual cycle during lactation is called *lactational amenorrhea* and it can not be considered as a reliable contraceptive method. Normal reproductive cycle starts with the weaning of the baby.

CONTRACEPTION

In female, the temporary methods of contraception include:

Oral contraceptives (pill)
Intrauterine contraceptive device (IUCD)
Safe period (rhythm method)
Mechanical barriers
Spermicidal agents
Norplants

Permanent method of contraception in the female includes tubectomy.

Oral contraceptives are synthetic estrogen and progestins. The examples of synthetic estrogens are ethynyl estradiol and stilbestrol. Synthetic progestins include norethindrone and norethinodrel. The modern pill consists of a combination of estrogen and progestin, which is called combined pill. The combined pill showed less adverse side effects.

Mechanism of action of oral contraceptives

Oral contraceptives produce contraception by:

- Inhibiting LH surge and preventing ovulation
- Making the cervical mucus thick which is unfavorable for sperm penetration.
- Changing the endometrial development. It interferes with implantation.
- Decrease in the fallopian tube motility.
- Suppression of gonadotrophin secretion.

IUCD

Intrauterine contraceptive device is the fixing of foreign bodies like copper T, Lippes loop etc into the uterine endometrium.

These foreign bodies prevent the implantation by causing inflammatory response in the endometrium. These agents also inhibit the sperm viability and their transport in the female genital tract. Copper containing IUCD shows spermicidal effect . The IUDs that slowly release synthetic progestins produce thickening of cervical mucus and impedes the entry of sperms.

Rhythm method

It is also known as safe period, where conception is prevented, by avoiding sexual intercourse during ovulation time. Coitus is avoided before and after the ovulation. It is not a reliable method as the timing of ovulation varies in the same woman from cycle to cycle. The timing of ovulation can be known by recording the basal body

temperature and noticing the change in the nature of cervical mucus secretion. Since the timing of ovulation, cannot be accurately known, the safe period for sexual intercourse can be 8 days before and after menstruation.

Mechanical barriers include use of diaphragm, cervical cap etc. The method is cumbersome, since it involves positioning of the device in the vagina before the coitus. The mechanical barriers prevent the semen from entering the upper part of the vagina. Spermicidal creams and jellies can be used in the mechanical barriers for the best result in contraception.

Tubectomy is a laproscopic sectioning and ligation of fallopian tube on both sides. This method prevents fertilization.

Norplant

It consists of capsules filled with synthetic progestins(levonorgesterol). These capsules are injected under the skin on the inner side of the woman's arm. Norplants work by thickening cervical mucus, changing the endometrium and reducing sperm transport. They provide contraception for up to 5 years.

Methods in male

Condoms

In male the commonly used method is condoms. It is a rubber sheath, which is unrolled over the erect penis before coitus. It is a reliable method and safe for both the partners against the spread of sexually transmitted diseases and AIDS. The use of condom prevents sperms entering the female genital tract.

Coitus interruptus

In this method the penis is withdrawn from the female genital tract before the ejaculation. It is not a reliable method of contraception.

Vasectomy

It is the bilateral sectioning and ligation of vas deferens. This method prevents the transport of sperms from epididymis to the ampulla of the vas deferens. The individual can have active sex life, but cannot reproduce as the ejaculate will not contain sperms.

Self-study Questions

Multiple Choice Questions

Choose the single best answer

1. **Growth hormone shows increased metabolic effect in all of the following *except:***
   A. Protein synthesis
   B. Glucose utilization
   C. Mobilization of fatty acids
   D. Utilization of fatty acids

2. **Which of the following peptide hormones is not formed from pro-opiomelanocortin in humans?**
   A. ACTH
   B. β LPH
   C. β endorphin
   D. MSH

3. **Which of the following hormones is not stored in secretory vesicles?**
   A. Vasopressin
   B. Glucagon
   C. Cortisol
   D. Thyroxine

4. **Tyrosine is the precursor for all of the following hormones synthesis *except*:**
   A. Epinephrine
   B. Norepinephrine
   C. Thyroxine
   D. Vasopressin

5. **Which of the following hormones increase aquaporin channel protein in the cell membrane?**
   A. Cortisol
   B. Vasopressin
   C. Aldosterone
   D. Angiotensin II

6. **Plasma fatty acids level is increased by all of the following *except*:**
   A. Growth hormone
   B. Cortisol
   C. Insulin
   D. Glucagon

7. **Deficiency of iodine in the diet causes:**
   A. Rise in plasma TSH level
   B. Rise in plasma thyroxine level
   C. Hypertrophy of the thyroid gland
   D. A and C

8. **Which of the following is not involved in the synthesis of thyroxine?**
   A. TSH
   B. Iodide
   C. Thyroid binding globulin
   D. Thyroglobulin

9. **The uptake of iodide by the thyroid gland cells is inhibited by:**
   A. Thiouracil **B.** Thiocyanate
   C. TSH **D.** T_3

10. **The hormone which increases the number of β adrenergic receptors on the heart includes:**
    A. Epinephrine
    B. Cortisol
    C. Thyroxine
    D. Norepinephrine

11. **The secretion of growth hormone is stimulated by:**
    A. Hypoglycemia
    B. Growth hormone
    C. Increase in free fatty acids
    D. Cortisol

12. **Thyroxine increases all of the following *except*:**
    - **A.** Plasma cholesterol
    - **B.** Glycolysis
    - **C.** Insulin secretion
    - **D.** Fatty acids level in plasma

13. **Hyperpigmentation of the skin can occur due to increased secretion of:**
    - **A.** ACTH
    - **B.** TSH
    - **C.** Cortisol
    - **D.** Androgen

14. **Dopamine antagonists causes increased secretion of:**
    - **A.** Oxytocin
    - **B.** Prolactin
    - **C.** Cortisol
    - **D.** Glucagon

15. **Aldosterone secretion is increased in:**
    - **A.** Hypernatremia
    - **B.** Hyperkalemia
    - **C.** Hypervolemia
    - **D.** Hypertension

16. **Which of the following does not use cAMP as second messenger system?**
    - **A.** Vasopressin
    - **B.** ADH
    - **C.** Parathormone
    - **D.** Insulin

17. **An Acidophilic tumor of anterior pituitary causes:**
    - **A.** Cushing syndrome
    - **B.** Acromegaly
    - **C.** Myxedema
    - **D.** Hypogonadism

18. **17 α hydroxylase enzyme is required for the biosynthesis of all of the following steroids *except*:**
    - **A.** Cortisol
    - **B.** Aldosterone
    - **C.** DHEA
    - **D.** Estrogen

19. **Fall in ECF volume stimulates the secretion of:**
    - **A.** Vasopressin
    - **B.** Aldosterone
    - **C.** Angiotensin II
    - **D.** All of the above

20. **Inositol triphosphate is the second messenger for:**
    - **A.** ACTH
    - **B.** Insulin
    - **C.** Cortisol
    - **D.** Angiotensin II

21. **Prolactin secretion in lactating females causes:**
    - **A.** Stimulation of milk secretion
    - **B.** Stimulation of gonadotrophin secretion
    - **C.** Inhibition of gonadotrophin secretion
    - **D.** A and C

22. **Calorigenic effect is shown by all of the following *except*:**
    - **A.** Cortisol
    - **B.** Epinephrine
    - **C.** Thyroxine
    - **D.** Glucagon

23. **Stimulation of erythropoiesis occurs by:**
    - **A.** Testosterone
    - **B.** Vasopressin
    - **C.** Erythropoietin
    - **D.** A and C

24. **Actions of insulin include all of the following *except*:**
    - **A.** Ketogenesis
    - **B.** Lipogenesis
    - **C.** Glycogenesis
    - **D.** Protein synthesis

25. **Which of the following is least likely to influence the growth of bone?**
    - **A.** Parathyroid hormone
    - **B.** Testosterone
    - **C.** Estrogen
    - **D.** Cortisol

26. In Cushing syndrome all of the following features are present *except*:
- **A.** Diabetes mellitus
- **B.** Hypotension
- **C.** Osteoporosis
- **D.** Obesity

27. Stimulation of specific mRNA forms the mechanism of action of:
- **A.** Insulin
- **B.** Oxytocin
- **C.** Thyroxine
- **D.** Epinephrine

28. Calcitriol formation in the kidney is stimulated by:
- **A.** Cortisol
- **B.** Vitamin D
- **C.** Calcitonin
- **D.** Parathyroid hormone

29. The pressor effect of epinephrine on the blood vessels require the presence of:
- **A.** Vasopressin
- **B.** Angiotensin II
- **C.** Cortisol
- **D.** Aldosterone

30. Urinary excretion of 17- ketosteroids is increased in:
- **A.** Conn's syndrome
- **B.** Adrenogenital syndrome
- **C.** Cushing's syndrome
- **D.** Sheehan's syndrome

31. Tetany can occur in all of the following conditions *except*:
- **A.** Acidosis
- **B.** Alkalosis
- **C.** Hypocalcemia
- **D.** Hypomagnesemia

32. Neuronal excitability is depressed in:
- **A.** Hyperthyroidism
- **B.** Hyperadrenalism
- **C.** Hypoparathyroidism
- **D.** Hyperparathyroidism

33. Inhibition of mediators of inflammation is produced by:
- A. Prostaglandins
- **B.** Bradykinin
- **C.** Cortisone
- **D.** ACTH

34. Hypersecretion of prolactin in men is associated with:
- **A.** Inhibition of gonadotrophins
- **B.** Impotence
- **C.** Hypogonadism
- **D.** All of the above

35. Increased resistance to insulin occurs in the hypersecretion of:
- **A.** Growth hormone
- **B.** Cortisol
- **C.** Thyroxine
- **D.** A and B

36. The secretion of growth hormone is increased by:
- **A.** Hyperglycemia
- **B.** Exercise
- **C.** Growth hormone
- **D.** Free fatty acids

37. The actions of insulin include:
- **A.** Converting glycogen to glucose
- **B.** Stimulation of gluconeogenesis
- **C.** Increasing plasma free fatty acids
- **D.** Increasing potassium entry into cells

38. Hyperthyroidism would show increase in all of the following *except*:
- **A.** Pulse pressure
- **B.** Cardiac output
- **C.** Food intake
- **D.** Reaction time of reflexes

39. Which of the following hormones should not be administered orally?
- **A.** Thyroxine
- **B.** Progesterone
- **C.** Parathyroid hormone
- **D.** Estrogen

40. Plasma renin and angiotensin II levels are increased in all of the following conditions *except*:
- **A.** Conn's syndrome
- **B.** Nephrotic syndrome
- **C.** Cirrhosis of liver
- **D.** Congestive cardiac failure

41. The action of luteinizing hormone in males includes:
- **A.** Stimulation of spermatogenesis
- **B.** Secretion of testosterone
- **C.** Secretion from accessory sex organs
- **D.** Maturation of sperms

42. Progesterone actions include the inhibition of all of the following *except*:
- **A.** LH secretion
- **B.** Sperm motility
- **C.** Uterine myometrium
- **D.** Uterine gland secretion

43. Spermatogenesis is affected by the decrease in all of the following *except*:
- **A.** Testosterone
- **B.** Scrotal temperature
- **C.** FSH
- **D.** LH

44. The secretion of which hormone is least in the third trimester of pregnancy?
- **A.** Progesterone
- **B.** Estrogen
- **C.** HCG
- **D.** Prolactin

45. The rise in basal body temperature following ovulation is due to the action of:
- **A.** LH
- **B.** FSH
- **C.** Estrogen
- **D.** Progesterone

46. Prior to the onset of ovulation which hormone will be at its peak level in the blood?
- **A.** LH
- **B.** FSH
- **C.** Estrogen
- **D.** Progesterone

47. Alveoli development of mammary gland is caused by:
- **A.** Estrogen
- **B.** Prolactin
- **C.** Progesterone
- **D.** Growth hormone

48. In males inhibin inhibits:
- **A.** FSH
- **B.** LH
- **C.** Testosterone
- **D.** Spermatogenesis

49. Ovulation coincides with rise in the serum levels of which of the following?
- **A.** FSH and LH
- **B.** Estrogen and LH
- **C.** LH and progesterone
- **D.** FSH and estrogen

50. Lack of 5 α reductase causes the absence of:
- **A.** Male external genitalia
- **B.** Male internal genitalia
- **C.** Testosterone secretion
- **D.** Spermatogenesis

ANSWER KEYS

1. (B)	2. (D)	3. (C)	4. (D)	5. (B)	6. (C)	7. (D)	8. (C)	9. (B)	10. (C)
11. (A)	12. (A)	13. (A)	14. (B)	15. (B)	16. (D)	17. (B)	18. (B)	19. (D)	20. (D)
21. (D)	22. (A)	23. (D)	24. (A)	25. (D)	26. (B)	27. (C)	28. (D)	29. (C)	30. (B)
31. (A)	32. (D)	33. (C)	34. (D)	35. (D)	36. (B)	37. (D)	38. (D)	39. (C)	40. (A)
41. (B)	42. (D)	43. (B)	44. (C)	45. (D)	46.(C)	47.(C)	48.(A)	49.(B)	50.(A)

Short Answer Questions

1. Explain how adenylyl cyclase system functions as second messengers in the cell signal transduction process.
2. Describe the mechanism of action of steroid and thyroid hormones.
3. Explain how posterior pituitary hormones secretion is regulated.
4. List the hormones that cause growth of bones
5. State the factors that cause secretion of IGFI
6. Describe the action of thyroxine on the nervous system.
7. Describe the hormonal regulation of blood glucose.
8. List the factors that cause goiter.
9. Describe hypothyroidism in children
10. List the therapeutic actions of gluco-corticoids.
11. State the effects of hypersecretion of cortisol.
12. Explain how aldosterone secretion is regulated?
13. Describe the effects of adrenal insufficiency.
14. Explain the hormonal regulation of calcium in the body.
15. Describe the effects of deficiency of parathyroid hormone secretion.
16. Describe the actions of insulin in adipose tissue and liver.
17. List the factors that inhibit spermatogenesis
18. List the actions mediated by dihydro-testosterone in males.
19. Describe the hormonal changes that leads to ovulation.
20. List the hormones that act on the mammary gland and state their actions.
21. Describe the mechanism of action of intrauterine device.

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